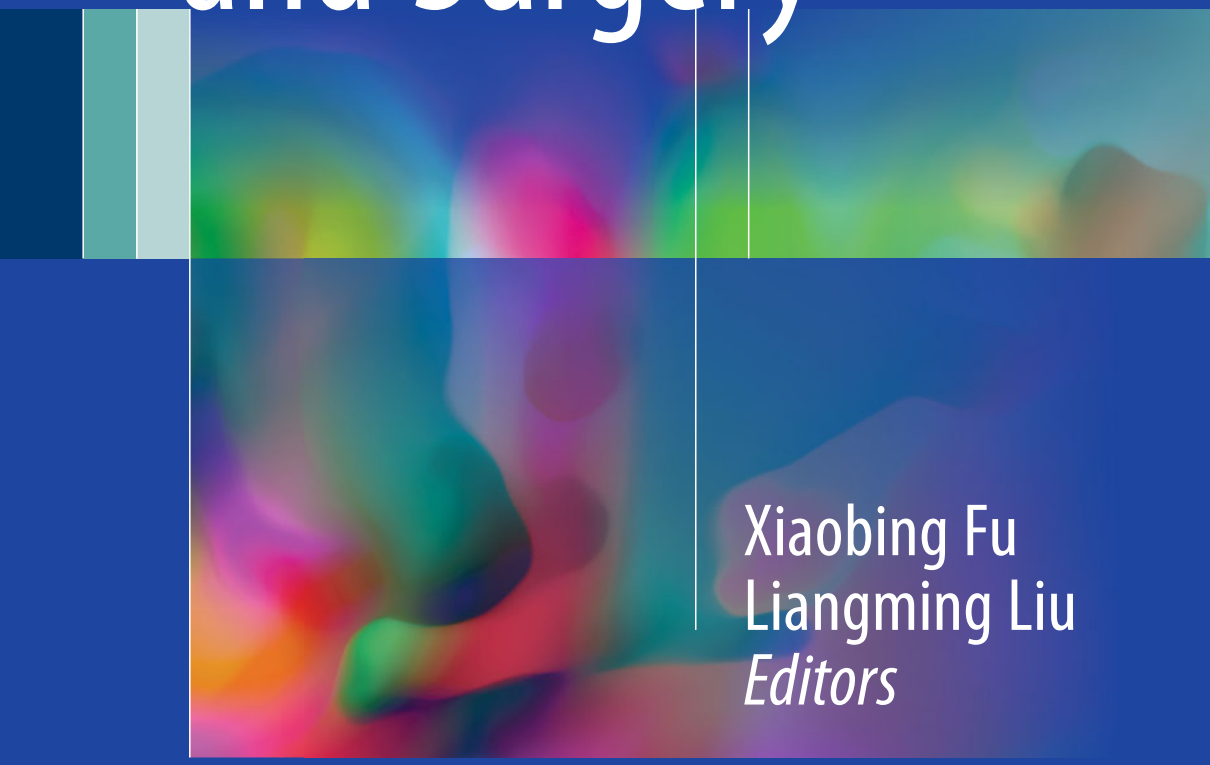
Advanced Trauma and Surgery



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Preface

As we know that trauma and accident injury are one among the main causes of death in human, and they are the first cause of death in youth below 45 years. Effective and precise prevention and management of serve damage in tissues and organs will benefit not only in reducing the early mortality, but also in improving the quality of life after survival in later. Advances in basic research and clinical management in trauma and surgery have confirmed these conceptions.

In this monograph titled Advanced Trauma and Surgery, recently advanced knowledge and skills in early wound care, traumatic or burn shock, pathogenesis in sepsis and tissue repair and regenerative medicine are offered. The authors pay much attention to the cellular, molecular, and gene research and their relationship with the pathogenesis in trauma and injury. Also, how to translate application of these advanced theories and skills in management of trauma patients is highlighted.

We would like to say that this book is not served as the sophisticated instruction manual to guide those who have less experience through difficult experiences in the management of trauma and surgery. It is our hope that this book can be served as the advanced textbook to help the young scientists and clinicians to know the new knowledge and technology in these fields, which will benefit their work in research and clinic through multidisciplinary collaboration.

We would like to express our gratitude to all authors who contribute his knowledge and time for this book. We thank Prof. Zengang Wang and Prof. Zhiyong Sheng in particular, for their continued support and guide in the fields of trauma and burn. Both of them are very famous experts in trauma, burn, field surgery, and traffic medicine. Their professional contribution is not only in China, but also in the world. Their ideas have been responsible for influencing the ideas in young generation, and we appreciate them very much for the great contribution.

Beijing, China — Xiaobing Fu
Chongqing, China — Liangming Liu

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Editors and Contributors

About the Editors

Prof. Xiaobing Fu, M.D., Ph.D., He is an academician (Chinese Academy of Engineering, Division of Health and Medicine); president of the College of Life Science, the General Hospital of PLA; director of the Key Laboratory of Wound Repair and Regeneration of PLA, Trauma Center of Postgraduate Medical College.

He has made great contributions on trauma, especially in tissue repair and regeneration. His works were supported in part by the National Basic Science and Development Program (973 Program), National High-Technique Program (863 Program), Grant for National Distinguished Young Scientists, and Grant for National Natural Science Foundation of China. He has published more than 500 scientific papers, including papers published in the *Lancet* (1998, 2001), and 20 books as the editor in chief, and won the 25 international and national prizes of Sci-Tech Progress from 1989 to 2015.

He was the member of the Scientific Committee of the Third Joint Meeting of the European Tissue Repair Society and the Wound Healing Society held in Boudreaux in 1999; he was also a member of the Scientific Committee, advisor or member of the International Faculty from the First World Union of Wound Healing Societies (WUWHS) Congress to 5th WUWHS Congress, held in Melbourne in 2000, Toronto in Canada in 2008, Yokohama in Japan in 2012, and Florence in Italy in 2016. He was the vice chairman of Trauma and Burn Section of the International Conference on Life Science and Clinical Medicine in 2000.

Now, he is the chairman of the Asian Wound Healing Association (AWHA), the president of the Chinese Tissue Repair Society (CTRS), the president of the

Chinese Tissue Repair and Regeneration Society (CTRRS), and the member of National Natural Science Foundation of China.

Professor Fu was awarded the International Lifetime Achievement Award in Wound Repair and Regeneration in 2008. He was selected as the academician (Chinese Academy of Engineering, Division of Health and Medicine) in 2009.

Liangming Liu, M.D., He is a research professor, doctoral supervisor, director of Shock Research Lab, Research Institute of Surgery, Third Military Medical University. He is also a shock research specialist; a distinguished young scholar of national science foundation of China; the committee member of International Federation of Shock Society and Chinese Trauma Society; the vice chairman of Shock Society of China and War Wound and Trauma Committee of PLA; the standing member of Cardiovascular Committee of Chinese Pharmacology Society; the editorial board member of Annals of Cardiology and Cardiovascular Diseases, Frontier of Medicine, Chin J Traumatology, and Military Medical Research; the invited reviewer of Intl J Cardiology, Cell Biol Intl, J Endocrinology, Exp and Ther Med, Pharmacol Reports, and so on; and the evaluation expert of National Natural Science Foundation of China and Yangtse River Scholars of Education Ministry in China.

He has long been undertaking the studies of pathogenesis, prevention, and care of traumatic shock. His main grants comprise the state and military key and major projects such as the key project of the national natural science foundation of China, the national major new drug development plan, and the national major research program of basic science (973 plan). In the flied of shock research, he raised the calcium desensitization mechanism of vascular hyporeactivity in critical illness such as severe trauma and shock and raised the effective prevention and treatment measures; he raised the new concept that permissive hypotensive resuscitation is all needed for uncontrolled hemorrhagic shock before and after bleeding controlled; and he developed a series of emergent care devices for war wound and trauma, which play important roles in combat casualty care, disaster rescue, and military training. These studies published over 350 papers including in Ann Surg, Cardiovasc Res, Crit Care Med, Crit Care, and Anesthesiology and Shock and obtained 12 national and provisional science and technology progress awards.

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Damage Control Theory in Treatment of Multiple Injuries

Xiangjun Bai

Abstract Lethal triad of death is the most serious complication of multiple injuries, which often manifests as metabolic acidosis, hypothermia and coagulation disorder, and all lead to multiple organ dysfunction syndrome (MODS). During the treatment process, early effective control of all kinds of primary injuries and bleeding could maintain the stability of the internal environment for avoiding exhausting of patients' physiological potential, then making it possible for patients to get definitive surgeries after resuscitation, which depends on the damage control theory (DCT).

Keywords Trauma · Lethal triad · Multiple injury · Damage control

As modernization of nowadays society progresses, trauma has become the primary cause of death in patients under 40 years old, therefore a major public enemy of society. High energy severe multiple injuries can easily cause serious physiological disorders in patients, which often manifests as metabolic acidosis, hypothermia and coagulation disorder [1], known as the lethal triad of death, followed by secondary strike such as severe infection, systemic inflammatory response syndrome (SIRS) and sepsis, which can all lead to multiple organ dysfunction syndrome (MODS). During the treatment process, because of multiple injury sites and complex associated injuries, early stage inappropriate treatment and definitive surgery could result in aggravating the injury and patients often die of late stage exhaustion of physiological storage. Therefore, how to effectively control the primary injury in early stage of treatment and to actively prevent secondary injury have become an urgent problem for emergency and trauma surgeons. The main purpose of damage control treatment of multiple injuries is: early effective control of all kinds of primary injuries and bleeding, maintaining the stability of the internal environment, as well as avoiding exhausting of patients' physiological potential, so that patients,

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especially those suffering from severe multiple injury, can get through the acute stress period safely and stably, making it possible for patients to get definitive surgeries after resuscitation. The time gap between the onset of the injury and the occurrence of lethal triad is critical to improve the success rate of severe multiple trauma treatment and improve the prognosis of patients. Therefore, this period is also defined as the new gold hour (NGH).

1 Damage Control Theory and Relative Concepts

Damage control theory (DCT) is the core of damage control. DCT is an emergency surgical staging principle, which emphasizes on temporary control of bleeding and further contamination instead of taking definitive surgery for anatomical repair, contemporaneously conducting resuscitation to ensure minimal tissue perfusion, avoiding excessive, low temperature, ineffective liquid infusion. DCT contains two primary concepts: damage control surgery (DCS) and damage control resuscitation (DCR) [2].

1.1 Damage Control Surgery

As a surgical strategy for periodic recovery of severe trauma patients, DCS aims to avoid the irreversible physiological damage caused by "lethal triad" [3]. Stone first raised the concept of DC on the Ann-Surg in 1983. In 1993 Rotondo established a standardized procedure regarding DCS, namely the three stage principle of DCS: early simplified surgery, resuscitation in ICU and late definitive surgery. The reasonable application of DC surgery can effectively reduce the mortality of patients with complicated trauma. DCS was first applied in abdominal trauma patients. In recent years, DCS has been gradually applied to the department of orthopedics, the department of Neurosurgery, the vascular surgery, especially the treatment of multiple injuries, and has made certain progress.

1.2 Damage Control Resuscitation

Derived from DCS, DCR was first pointed out by the American military trauma surgical consultant colonel HoLcomb in 2006 [4]. The basic principle of damage control resuscitation is to quickly identify patients with high risk of abnormal coagulation mechanism, and to reverse the abnormal coagulation, hyperthermia and metabolic acidosis by fluid resuscitation. It has provided people with insights into certain clinical medical problems. DCR is now becoming more and more important in the treatment of severe multiple trauma [5].

1.3 Secondary Strike

Patients with Severe multiple injuries often die from secondary strike such as MODS and MOF. This theory suggests that as a direct result of trauma, the first strike develops early. Secondary strike is a series of inflammatory reactions from SIRS, sepsis to MODS and MOF, resulting from inappropriate treatment and definitive surgery of the first strike. The purpose of DCT is to minimize the impact of secondary strike on patients.

1.4 New Golden Hour

With the systematic establishment of trauma organizations, development of trauma center, application of standard resuscitation method and improvement of modern blood bank technology, the ability to resuscitate trauma patients with extreme trauma has improved. The concept of "Gold hour" is understood to be the fastest speed and the effectiveness of resuscitation, and its ultimate goal is to reduce injury-to-incision time. This change does not simply indicate the transportation of the severe injured patients from the scene of the accident to the emergency room, rather than applying resuscitation in the operation room and eventually the ICU. The most appropriate meaning of "the new golden hour" is the critical time period before patients develop lethal triad in the operation room. ICU nurses must understand the importance of this triad, because it indicates the establishment of the concept of DC or DCS, as well as the medical team's emphasis on damage control in trauma patients.

1.5 The Dual Meaning of DCT

The proposal and clinical practice of DCT has played an important role in the development of medicine [6, 7]. It is a milestone in the development of multiple injuries treatment. In recent years, DCT continues to improve and ripen. DCT consider surgeries to be a part of resuscitation, rather than an end in itself [8], and that the prognosis depends much on patients' physiological limit, rather than surgeon's efforts to restore anatomical structure. Therefore, there is a secondary meaning of DCT [9]: (1) to control, decelerate or prevent blood loss and infection caused by original trauma; (2) to reduce the damage brought by surgery and invasive procedure in order to stabilize patients, creating opportunities for following treatment.

2 Pathophysiological Basis of DCT

2.1 Metabolic Acidosis

When blood pH is below 7.25, the body is in a continuous hypoperfusion state. Normal pathway of glycometabolism changes, anaerobic glycolysis will replace aerobic metabolism under normal physiological state, resulting in the accumulation of lactic acid in the body. Therefore, the level of blood lactic acid can reflect the severity of acidosis in patients with multiple injuries. Previous studies show that there is a clear correlation between blood lactic acid level and mortality [10]. The survival rate is 100 % in patients who can clear lactic within 24 h, and the survival rate in patients who can clear lactic within 48 h is only 14 %. If the duration of surgery is too long, the patient will develop continuously and repeatedly low perfusion and hypoxia, resulting in excessive accumulation of lactic acid in the body, which leads to increased mortality.

2.2 Hypothermia

Blood loss, large number of cryogenic liquid perfusion and exposure of body cavity all lead to acceleration of heat loss. Thermogenesis dysfunction and loss of peripheral vasoconstriction during anesthesia can cause patients' core temperature to drop under 35 °C. If the continuous hypothermia reaches patients' body limit (below 32 °C for more than 90 min), the damage become irreversible [11], and death is inevitable.

2.3 Coagulation Disorder

Acidosis and hypothermia can cause reduction in thrombin, platelet count and synthesis of clotting factor V, VIII, as well as activation of fibrinolytic. Blood dilution caused by fluid resuscitation can further aggravate coagulation disorders, which can develop into uncontrollable DIC, seriously endangering patients' lives. Therefore, patients can die of physiological exhaustion during or after invasive definitive surgery or procedures, which leads to application of DCT. Surgeons should not only tend to control the blood loss and infection, but also control damage caused by emergency surgeries and invasive procedures, keeping patients stable enough for follow-up treatment [12].

3 Indications of DCT

Grasping indications is very important in DCT application. Studies show that pH level, hypothermia and blood transfusion are sensitive index of patients' prognosis, therefore important index of choosing DCS [13]: (1) patient status: severe multiple injuries, ISS ≥ 25, associated severe hemorrhagic shock, diastolic pressure <70 mmHg on admission, in need of emergency operation; (2) lethal factors: severe acidosis, hypothermia, non-mechanical coagulation disorder, pH <7.25, BE ≤ -8 mmol/L, T <35 °C, PT >16 s, APTT >50 s or 50 % of normal value; (3) surgery: estimated resuscitation and operation time is more than 90 min, in need of large amount of blood transfusion (>10 u); (4) conflicts in treatment: hard to make priorities in case of severe and complicated injuries; (5) medical condition: in low level hospitals or when restricted by technical conditions, or dealing with large number of patients, definitive surgery can not be performed, precious time need to be saved for transportation. When meet the first requirement and any of the rest 4 requirements, DCS should be applied.

4 Three-Phases Principle of DCT

The physiological potential of severe multiple injury patients is often on the verge of exhaustion. Even if surgeons can perform complex surgery despite of technicality, patient would eventually die of exhaustion of physiological potential. Therefore, surgery ought to be considered part of the whole resuscitation progress. The outcome of treatment does not depend on surgically restoring anatomic relations, but on timely correction of severe internal environment disorder [14]. Inappropriate, unbearable operation will accelerate patients' death. DCT reduces secondary strike to patients. Blood loss is reduced by methods of ligation and packing hemostasis. In the mean time reducing blood transfusion can lower the possibility for transmitted inflammatory factors and toxic substances [15]. Providing a better foundation for rapid resuscitation can significantly reduce patient mortality.

The three-phases principle of DCT includes [16, 17]: (1) simple surgery: during the initial operation, the surgeon carries out only the absolute minimum necessary to rapidly control blood loss and infection. Close the wound immediately to avoid further damage, keeping operation time within 90 min. (2) Secondary resuscitation in traumatic intensive care unit [18, 19]: this phase includes correction of coagulation and acidosis, rewarming, ventilatory support and full body examination. Improve tissue perfusion and microcirculation in order to reduce lactic production; avoid heat loss and blood loss by shortening operating time; avoid excessive infusion of low temperature liquid, and take measures to keep patients' body temperature to reduce the possibility of developing DIC or Coagulation disorder [20]. (3) Elective surgery (definitive surgery): further surgery to remove package and to repair injuries organs after fully surgical exploration.

4.1 Bleeding and Contamination Control

Wadding and pressure hemostasis are most simple and effective methods for superficial bleeding, and also one of the most applied methods for treating trauma patients. For deep hemorrhage with deep plugging part, temporary vascular ligation or interventional therapy can be applied. Catheter drainage can be used to prevent the spillage of intestinal contents and urine into the peritoneal cavity. After keeping bleeding and contamination under control, vacuum sealing drainage can be used for thoracic or abdominal cavity closure.

4.2 Rewarming and Resuscitation in TICU

Measures should be taken to rewarm the patient at the beginning of treatment, such as keep the patient away from the cold environment, raise room temperature, preserve body temperature, etc. Infusion and peritoneal lavage of warm liquid is also effective. Patient's body temperature should be monitored during rewarming process. We've discovered in practice that the key to bleeding control and to avoid coagulation disorders is to stop the bleeding, rather than fluid infusion. Blood perfusion of vital organs can be maintained while the systolic pressure is over 70 mmHg. Aggressive and rapid fluid resuscitation on post operation patient can cause diluted coagulation disorder, which aggravates patient's critical condition [21]. Damage control fluid resuscitation doesn't request fully blood pressure recovery. With less fluid infusion, preoperative preparation and TICU resuscitation can be completed much sooner: (1) maintain systolic blood pressure around 90 mmHg in case of rebleeding caused by high blood pressure; (2) use colloid solution as resuscitation fluid. Component blood transfusion is better than concentrated red blood cells transfusion. Platelets and cryoprecipitation transfusion to improve coagulation should also be considered when necessary. For patients in critical conditions, whole blood transfusion can also be used. The amount of crystal liquid infusion should be minimized. Crystal liquid should be used only for preparation of necessary first aid drugs or to maintain smooth flow of the catheter after blood transfusion.

4.3 Definitive Surgery

The purpose of reoperation is to remove the stuffing in body cavity, to stop active bleeding, and to treat secondary injury and explore oversighted injury, including the repair, resection and partial resection of parenchyma organs, repair or resection of cavity organ damage, repair of vascular injury, permanent fixation of fracture, and closure of the thoracic abdominal incision, etc. Definitive surgery is generally performed within 48–72 h after initial surgery. Fixation of bone fracture is usually

performed two weeks after injury, when patient's condition is suitable for further surgery and invasive treatment.

5 DCT in Multiple Injury

5.1 Preparation

Patient prognosis improves as time from injury to DCT shortens. So the decision of whether to perform DCT should be made before or soon after surgery begins. The decision is based on patient's initial physiological conditions and traumatic conditions. Do not wait until after the appearance of metabolic disorder. Prehospital emergency medical care time and preparation time should be minimized. After initial patient estimation, procedure of diagnosis and treatment should be made and all non-necessary examination should be avoided. For example, if diagnostic abdominocentesis is positive and patient presents symptom of hemorrhagic shock, immediate exploratory laparotomy should be performed. Under this circumstance, radiographic examination is not necessary. Consent forms for examination, treatment and surgery should be prepared in advance for time saving. Standard procedures of diagnose and treatment for critical patients should be established by paramedics, surgeons and the operation room, and should be followed. Volumetric resuscitation can aggravate hypothermia and coagulation disorders, therefore is not recommended.

5.2 Primary Estimation of Trauma Injury

Whether to apply DCT should depend on type of trauma. Patient's physiological function parameters only serve as a reference. The type of trauma is the decisive factor: (1) the mechanism of injury: high energy blunt trauma, multiple penetrating injury; (2) the nature of injury: major vessel injury with multiple organ damage and fatal bleeding. (3) major organ damage: major thoracic vascular injuries and heart injury, severe liver and peripancreatic vascular injury, severe pancreatic duodenal injury, pelvic hematoma and open pelvic fracture.

6 DCT Principle in Multiple Injury

6.1 Brain Injury

The main principle is to actively treat primary brain injury and prevent secondary brain damage and other complications, such as cerebral edema, brain swelling,

hypoperfusion of brain tissue and ischemia reperfusion damage. The initial estimation of brain damage patients is based on trauma history and GCS. In the early stage, any unnecessary definitive surgeries can aggravate patients CPP, therefore aggravate secondary strike. For patients with unstable hemodynamics, especially those in need of emergency craniocerebral operations, diastolic pressure during surgery need to be maintained around 90 mmHg to ensure organ hemoperfusion. In summary, primary surgeries are aimed to stop intracranial hemorrhage, to evacuate intracranial hematoma and to prevent infection. Treatment at this stage emphasizes on quick diagnosis and immediate surgeries, while treatment at later stage emphasizes on preventing cerebral edema and intracranial infection: (1) Large amount of scalp avulsion: treatment focuses on controlling bleeding and contamination. (2) Skull fracture: restoration of open fracture and depressed fracture should be performed after early debridement and suture, when risk of infection decreases. (3) Basilar fracture with active bleeding: first apply intubation to ensure airway potency. Meanwhile, use gauze packing, hemostasis injection or interventional therapy to control bleeding, as well as blood transfusion to correct hypovolemia. (4) Intracranial hemorrhage: conservative treatment should be applied for patients in critical condition. For patients with intracranial mass lesion resulting from epidural, subdural or intracranial hematoma who can not tolerant major surgery while in need of intracranial decompression, surgeons should consider trepanation and drainage. After improvement, patient should be treated with decompressive craniotomy. (5) When suffering from brain stem damage, patient is in risk of coma and cardiac arrest and should be treated with tracheotomy.

6.2 Thoracic Injury

The main principle is to immediately treat life-threatening tension pneumothorax, hemathroax and cardiac vessel injury to ensure patient's respiratory function and control bleeding. Precautions should be taken to prevent pulmonary edema, ARDS and infection. Analgesic therapy should be applied to relief active breathing reduction due to fear of chest pain. Thoracic close drainage and chest fixation can be used for most thoracic injuries. Disturbance of circulation due to mediastinal flutter and late-presenting diaphragmatic hernia should not be ignored. Patients with cardiac tamponade and bleeding caused by thoracic vessel injury should be operated immediately. Severe lung and bronchus injury can be treated with emergency pneumonectomy. In case of diaphragm injury, diaphragmatic repair should be performed. Resuscitation and life support are necessary after surgery. Definitive surgeries such as internal fixation of rib fracture, esophagus repair, vessel damage restoration should be performed after patient's improved. (1) Chest wall injury: chest wall injuries mainly consists of rib fracture, sternum fracture and paradoxical respiration due to flail chest. Treatments include external fiction of chest wall and

external tractive fixation. Treating lethal associated injury and shock before internal fixation can significantly improve patient's prognosis and reduce complications. (2) Lung and bronchus injury: lung injuries include contusion of lung, laceration of lung, traumatic wet lung, atelectasis and hydropneumothorax, all of which can further develop into ALI and ARDS. Excessive infusion of crystalloid and rapid reexpansion should be avoided. Patients with trachea injury and complex injury should be treated with intubation, distal inflation or bronchial fistula. Definitive surgery should be performed after the patient is stabilized. (3) Major cardiac vessel injury: cardiac vessel injury mainly include cardigan tamponade, aortic arch injury and traumatic aortic arch dissection. Treatment should focus on early diagnosis and immediate emergency surgery.

6.3 Abdominal Injury

Main principle is to contain contamination, to temporarily close the abdominal cavity, and to apply resuscitation and life support within 48 h after initial surgery. Definitive surgery should be performed after resuscitation to repair damage discovered during the initial surgery. (1) Parenchymal organs: use packing and vascular ligation method to control bleeding and contamination (bile, etc.). Packing can be used for all the abdominal organs and retroperitoneal tissues, such as liver, pancreas, kidney, spleen, pelvis, retroperitoneal vascular and other organs. Bleeding caused by tissue damage, including arterial, venous bleeding and wound errhysis. Simple, safe and effective measures can be adopted for complex vascular damage, such as repair of rupture, ligation, temporary cavity shunt. (2) Hollow organs: Once the bleeding is under control, surgeons should focus on containing contamination. Simple intestine perforation can be closed using one-layer suture. Complex intestine damage such as colon damage or extensive small bowel injury should be treated with resection and secondary anastomotic. Ileostomy or colostomy is not recommended, neither is conventional resection and anastomosis. Duodenal, biliary, pancreatic injury can be treated with drainage and packing. Gallbladder stoma drainage can be performed for biliary injury. Pancreaticoduodenectomy can be performed for duodenal papilla injury, but restoration is not recommended. Apply intubation and drainage for ureteral injury instead of direct suture. Urethral drainage and suprapubic drainage can be applied in bladder injury. Distal pancreatic injury with extensive tissue damage, including pancreatic duct damage, can be treated with distal pancreatic resection. Severe pancreatic and duodenal injury should only be treated with debridement and drainage, because of patient's intolerance of complex surgery such as pancreatic and duodenal resection. Small duodenal injury can be fixed by single layer suture, while debridement and secondary surgery are required for major duodenal injury.

6.4 Bone Fracture

The main principle is to stabilize fracture ends by temporary external fixation. Vascular shunt can ensure the blood supply of the distal end of the limb. Close open fracture to prevent infection and bone necrosis. Definitive surgery should be performed after stabilization. (1) Limb fracture: For elderly patients or patients with primary cardiac or pulmonary diseases and who can not tolerant major surgery, bone traction and external fixation should be considered. Precautions should be taken to prevent thrombosis and thrombosis associated complications. Anatomical reduction is not required. Patients with intra-articular fracture or bone defect should be treated with second operation such as internal fixation and bone grafting. (2) Pelvic fracture: Using pelvic external fixator and sacral iliac screw technology to stabilize the pelvic ring. Packing and intervention can be used to reduce pelvic bleeding, as well as internal iliac artery ligation. Damage control in orthopedics department is mainly used in 2 occasions, one of which is the joint injury associated with head, chest or abdominal injury, the other is when patient can't tolerant complex surgery. (3) Spinal fractures: Spinal fractures often combines with nerve injury, resulting in paraplegia or high paraplegic. Prone position is often used during surgery. Therefore, the priority is to guarantee respiratory and circulatory function. Fixation of bone fracture should wait until secondary surgery.

6.5 Vessel

The main principle is to use temporary intravascular shunt technology instead of vascular transplantation and repair. The procedure can be performed safely by medical instruments, with no rely on vascular surgeons. Definitive surgery of restoration must be completed within 24 h. For patients under unstable condition who can not tolerant surgery, amputation is necessary to prevent necrosis induced sepsis, which can develop into life threatening MODS.

6.6 Skin and Soft Tissue

The main principle is to deride damaged tissue as much as possible. Using VSD technology to contain contamination and to promote wound healing, eventually to improve wound condition for the secondary suture and skin grafting. (1) Soft-tissue defection; (2) burn: Using VSD can prevent patient from further deterioration. Fixation of skin graft and skin substitute is necessary. topical application of antibiotics. The physiological advantages and the same point of other indications are: to remove the wound edema, infection and inflammatory substances, and to increase the perfusion of the wound. Especially in the prevention of further burns in

the skin layer depth. Partial administration of antibiotics to prevent infection. Taking cautions to prevent wound swelling, infection and inflammation benefits tissue recovery. (3) Skin graft: The survival of skin graft depends on sufficient fixation during the critical period of 2–5 days after transplantation. The graft is likely to shift and exude on the irregular surface, which results in separation and eventually graft failure. VSD technique can be applied to reduce the defect, to increase granulation tissue formation, to control infection and to clear exudate, therefore to keep the graft and recipient bed laminated. VSD can also stimulate neovascularization, increasing the successful rate for skin graft.

6.7 Resuscitation

Damage control resuscitation (DCR) is an integrated recovery measures. It starts immediately after the initial rapid assessment of patients in the emergency room, and carries out during following surgeries and ICU monitoring. The main principles are: (1) Systolic blood pressure should be maintained under 90 Hg mm (1 Hg = 0.133 kPa mm), in case of rebleeding due to high blood pressure. (2) Use blood products as resuscitation fluid. The amount of crystal liquid infusion should be minimized. Crystal liquid should be used only to maintain smooth flow of the catheter after blood transfusion. (3) Restoration of blood volume mainly relies on fresh frozen plasma (FFP). FFP should be infused together with concentrated red blood cells at the ratio of 1:1. (4) For severe critical patients, whole blood transfusion can be used for resuscitation. (5) Other blood products such as platelet and recombinant activated factor VIIa. (6) When to terminate resuscitation: (1) Blood level and duration time of lactic are closely related with patient's prognosis, so the lactic acid level could be used as a parameter for ending DCR treatment. (2) Lack of alkali can reflect the degree of systemic tissue acidosis and is closely related with prognosis. The level of alkali lack should be monitored during DCR process, as well as be used as a parameter for ending DCR treatment.

Patients with multiple injuries are often in critical conditions. Therefore, indications of DCT on severe multiple injury patients should be extended. In addition, there are conflicts when dealing with severe multiple injury. Surgeons should establish priorities. Severe injuries such as intracranial hematoma with intracranial hypertension, severe lung and bronchus injury, thoracic-abdominal injury, parenchyma organ injury such as liver and spleen combined with hemorrhage etc. should be treated immediately. Surgeons can divide into different groups and deal with each injured site simultaneously if necessary. The less lethal injuries can be treated with secondary definitive surgeries after resuscitation. Operation plan is also necessary for definitive surgery. Restoration of each tissues and organs can be settled one by one. Therefore, in future treatment of multiple injuries, it is important to pay attention to every step of treatment. Application of DCT can effectively

sustain first strike during early time of trauma, and actively prevent secondary strike. Applying customized and integrative treatment according to each patient's individual injuries and conditions is an important developing direction of multiple injury treatment [22].

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Open Abdomen Treatment for Severe Trauma

Lianyang Zhang

Abstract Open abdomen (OA) has been proposed for more than 20 years, which is a type of surgery to save the lives of critically ill patients and also is controversial on its use since it appeared. In 2012, Chinese Medical Association issued "Diagnosis and Treatment Norms for Trauma-induced Intra-abdominal Hypertension/Abdominal Compartment Syndrome". In 2013, the World Association of Abdominal Compartment Syndrome (WSACS) issued new treatment guidelines and expert consensus. Based on the field progress and authors' experience, this chapter induces the concept of open abdominal surgery, focuses on inducing key open abdomen technologies, including skin closure techniques, fascial closure techniques (FCTs) and VSD assistive technology, as well as open abdominal surgical site and incision. Also, this chapter describes perioperative management techniques at the acute stage, the middle stage and the reconstruction stage.

Keywords Open abdomen · Severe trauma

The management of open abdomen (OA) for saving critically ill patients is a surgery of continuous debate since its first appearance 20 years ago. Despite the benefit, maintenance of an open abdomen creates numerous management challenges, including ① despite of decompressive laparotomy, the mortality rate of patients with abdominal compartment syndrome (ACS) is still up to 50 % [1]; ② OA may bring with potential major complications such as fluid loss, infection, fistulas, and large ventral hernias [2]; ③ patients require one or more surgeries; ④ it is named 'nightmare' by paramedics for high costs and long stay in hospital. On the other hand, OA can effectively reduce mortality and early postoperative morbidity, so it is one of the surgical hotspots [3]. This surgical technique has made remarkable progress since the mid-1990s and gradually becomes related to the technique of the

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negative pressure wound therapy [4]. OA system equipments, such as ABThera™ has been developed. These methods can significantly remedy the shortage of the original open abdominal cavity and ensure complete definitive abdominal closure for most patients in 2–3 weeks, so that the OA surgery gradually mature. According to a review of 2012, the total number of OA cases reported was 5248 by 2011 [3]. Reasonable application of this technique will certainly improve outcomes of abdominal critically ill patients [5]. Given that IAH/ACS associated with trauma is more and more common, the traumatology branch of Chinese Medical Association has published a guideline to its diagnosis and treatment (A guideline to the diagnosis and treatment of IAH/ACS associated with trauma) in 2012 [6], hoping to effectively decrease its occurrence and delayed diagnosis, to manage it properly and to improve the outcome of the patients [7].

1 Definition of OA

OA is a planned management strategy in which the abdominal wall layer is temporarily closed with skins or artificial materials (also called temporary abdominal closure, TAC) other than conventional layered suture closure method when the intra-abdominal operation is completed [8].

OA is not to expose abdominal organs to the air. The abdominal wall layers still need the skin or artificial materials to complete TAC. Because the abdominal wall is temporarily closed, relaparotomy or another surgical procedure is often necessary to complete definitive abdominal closure. This surgical technique requires not only to open the abdominal cavity, but also should take the following affairs into account: reconstruction of the abdominal wall barrier to avoid abdominal pollution, convenience to drain and quantify peritoneal effusion, convenience to enter the abdominal cavity again, mini-invasiveness to each layer of the abdominal wall, and less abdominal wall fascia and skin retraction.

2 Indication and Effect of OA in the Treatment of Severe Trauma

2.1 Indication of OA

In the treatment of severe trauma, OA is mainly used for [4, 9, 10] post traumatic peritonitis, including missed diagnosis of gastrointestinal tract injury, traumatic pancreatitis, purulent abdominal infection caused by gastrointestinal fistula, etc.; abdominal trauma, patients on whom damage control laparotomy (DCS) need to be performed, or those with abdominal wall damage and can not receive primary abdominal closure; patients with primary or secondary intra-abdominal

hypertension (IAH), or ACS with the need for intra-abdominal volume increment (IAVA) or decompressive laparotomy.

2.2 *Effect of OA*

Effect of OA on survival rate: Regner et al. [3] reported 6 pieces of literature including 399 trauma patients performed OA and DCSwith the survival rate of 65–90 %, and the mortality rate of 915 cases of ACS underwent OA was 43–75 %, and 493 cases of emergency general surgery underwent OA, with the survival rates of 25–70 % in pancreatitis patients and 53–73 % in patients with abdominal infection caused by perforation.

We retrospectively analyzed the clinical data of 33 multiple trauma patients who received damage control laparotomy from 2009 to 2015. Twenty four cases were males, and 9 cases were females, with an age range from 19 to 68 years (mean 41.0 years). The trauma causes included traffic injuries in 21 cases, falling injury in 6 cases, crashing object injury in 5 cases, and detonator blast injury in 1 case. Injury severity score (ISS) ranged from 14 to 64 (mean 27.0). All patients had abdominal injury. They were complicated with other kind of injuries, including brain injury in 10 cases, chest injury in 23 cases, pelvic and limb injury in 21 cases. After abdominal operations, all patients underwent TAC assisted by vacuum sealing draining (VSD), and 12 cases developed IAH or ACS underwent IAVA assisted by VSD. Hospital stay time was 21–70 days (mean 31.4 days). The time for primary fascial closure (PFC) after surgery was 5–12 days in 29 cases (87.88 %). Four cases unable to complete definitive abdominal wall reconstruction within the short term underwent skin grafts to form planned ventral hernia. All patients were safely discharged from hospital [11] (Fig. 1).

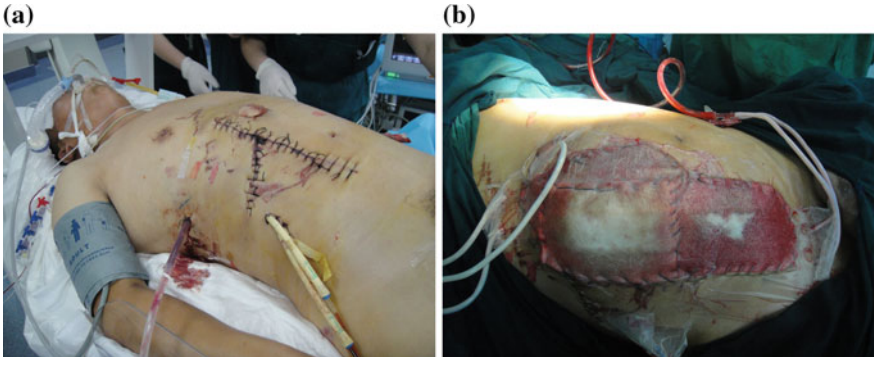

Fig. 1 A male patient of 44 years injured from a traffic accident was transferred into our hospital 2 days after undergoing exploratory laparotomy, partial resection of right liver, diaphragmatic repair, and duodenal repair, with an intra-bladder pressure of 24 mmHg. **a** Abdominal swelling; **b** the patient underwent IAVA assisted by VSD through downward extension of the original incision

3 Key Techniques of OA

3.1 Temporary Abdominal Closure (TAC)

Skin closure techniques is to use skins or other materials to keep the integrity of the abdominal wall, mainly including continuous skin suture, towel clips, silo technique, three-litre bag and silicone diaphragm placement. This technique is prompt, economic and easy to implement. However, its disadvantages are also remarkable. It may increase skin necrosis, abdominal contamination, ascites leak, evisceration and cannot reverse the process of abdominal wall retraction. The incidence of ACS is 13–36 %, primary fascial closure is no more than 30 %, and the incidence of intestinal fistula varies considerably, ranging from 0 to 14.4 % [12]. Therefore, the techniques mentioned above are mainly used in the early stage of TAC development while rarely employed nowadays. Based on 549 cases of 10 articles, the mortality is 17–58.4 %. The mean incidence of intestinal fistula from 3 articles is 7.41 %.

Fascial closure techniques (FCTs) is to insert grafting materials and suture them to the fascia [13, 14]. Materials once used clinically include three-litre bag for parenteral nutrition or peritoneal dialysis, various kinds of surgical dressings, nonabsorbable meshes including polypropylene mesh (Marlex mesh), Wittmann mesh, expanded polytetrafluoroethylene (ePTFE) mesh, polypropylene and ePTFE composite mesh, and absorbable meshes including hydroxy acetic acid polyester mesh (Vicry1), polyglycolic acid mesh (Dexon). In order to prevent intra-abdominal hypertension, redundant materials are often used to keep the abdominal wall relax, and gradually tightened in postoperative stage [15–17]. The greatest advantage is the achievement of reversible and tension-free TAC, facilitating reoperations, especially for cases with limited opportunity of definitive closure for open abdomen within 1 week [18]. However, this kind of technique is not able to evacuate peritoneal fluid and lack the ability of wound drainage, which may lead to recurrent IAH or wound impregnation. Nonabsorbable mesh may accompany with a relevant high incidence of intestinal fistula (6–18 %) [19], even up to 75 % [20]. Other disadvantages of nonabsorbable mesh include incapability to prevent adhesion between viscera and anterior abdominal wall, low primary closure rate, increasingly expensive materials and are hardly available in domestic. Nowadays, Velcro-like Wittmann mesh is usually applied in clinical practice. Five articles with 365 cases reported the mortality, incidence of fistula and primary fascia closure rate are 7.7–67 %, 1.3 % and 78–100 %, respectively [21].

Vacuum-assisted closure is a technique in which the intestine is covered by omentum majus underlying the wound. Polyvinyl alcohol and gelatin sponge composite material is tailored to proper size and sutured with abdominal wall with/without sheet in order to fully accommodate the viscera, provide physical environment and avoid desiccation of the viscera. Besides, it is the way of abdominal wall reconstruction, may help to prevent mechanical damage of the viscera, avoid abdominal cavity contamination, evacuate peritoneal fluid, as well as

reduce and maintain IAP. Biological membrane is set to seal the foam and wounds (3–4 cm over the edge of incision), then the silicone tube is connected with negative pressure equipment (45–60 mmHg). The vacuum-assisted closure system is sealed by biological membrane and the abdominal cavity is separated with the outside environment. This may help to expand the abdominal volume considerably, reduce IAP and prevent bacterial invasion. Besides, it may reduce postoperative workload due to unnecessity of regular dressing change. The continuous vacuum drainage is benefit for alleviation of inflammation and edema, as well as wound healing. This is the most commonly used technique with multiple choices [15, 16, 22]. The author applied vacuum-assisted closure system to manage 20 open abdomen cases since 2008 and all patients had satisfactory outcome. Among the 20 patients, 12 cases received primary abdominal wall reconstruction within 5–9 days, 8 were conducted skin grafting to cover the wound after 2 weeks (Fig. 2a–c) to develop planned ventral hernia (Fig. 2d). A review of vacuum-assisted closure in the treatment of open abdomen in 1744 cases from 20 articles showed a mortality of 17–60 % and the mean incidence of fistula of 7.26 % [3].

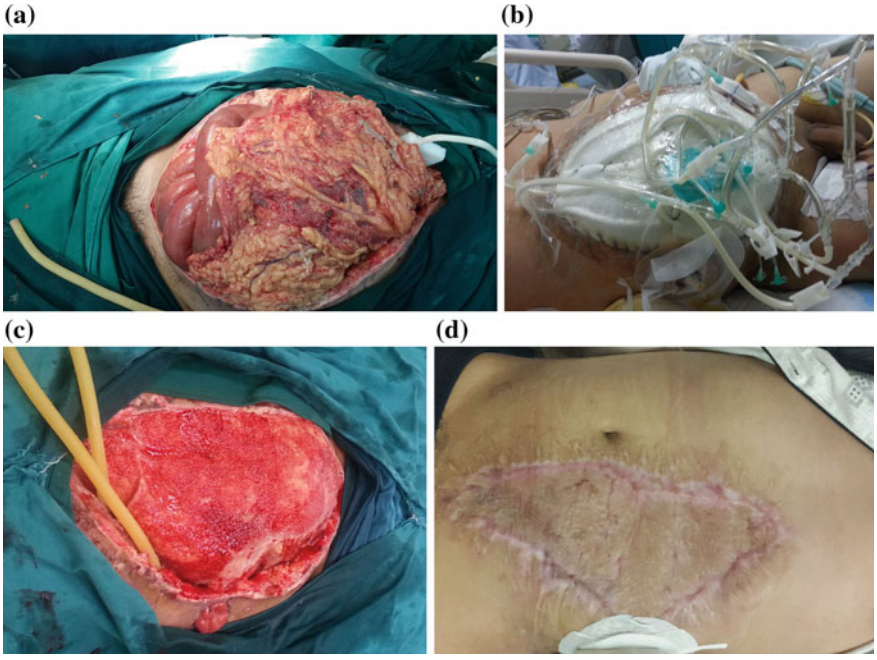

Fig. 2 A male patient of 28 years old with abdominal stab wound was transferred to our hospital 3 days after primary laparotomy with transverse colon repair and ileostomy. The temperature was 39.2 °C and the IVP was 18 mmHg. Intra-abdominal volume increment was applied after admission. **a** abdominal viscera bulging after sutures removal, **b** temporary abdominal closure with VSD, **c** granulation under the foam 10 days after open abdomen, **d** planned ventral hernia after skin grafting

3.2 *Locations and Incisions of Open Abdomen*

Patients who need to be performed open abdomen are often with unstable vital signs. They even cannot be transferred to the operation room and the operations may be conducted at the ICU bedside [23] (Fig. 3). However, if the operation room is able to supply relevant equipments, vacuum device, staff and sterile condition, it is the best choice.

The history of laparotomy may result in different operative incisions. The original incision in ACS patients with the history of laparotomy should be completely opened and the length of the incision should be extended if needed. The midline incisions(from the sword to the pubic symphysis) is usually chosen for primary surgery in ACS patients without the history of laparotomy. Midline incision is convenient for the application of rectus push migration in secondary definitive abdominal closure.

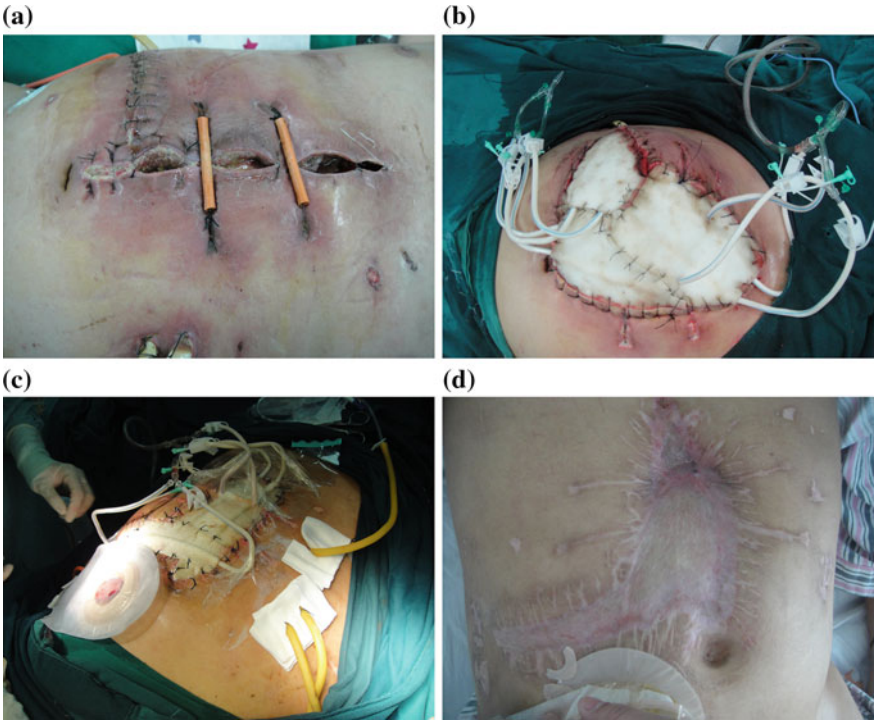

Fig. 3 A male patient of 36 years old with multiple injuries by traffic accident. Emergency exploratory laparotomy, liver repair, cholecystectomy, transverse colon repair and peripancreatic drainage were performed. The patient had persistent fever up to 39 °C after surgeries. **a** incision infection and dehiscence when transferred to our hospital, **b** open abdomen via the original incision, **c** transverse colon fistula on postoperative day 5, applied ileostomy at the ICU bedside, **d** planned ventral hernia after skin grafting

3.3 Peri-operative Management

Acute phase refers to the 24–72 h after surgery. The patient whoever goes through the open abdominal surgery would be classified as being critical. Based on that, the priority of care should be given to the stable hemodynamics, the caridio-, respiratory, renal and coagulatory functions [1] as well as to reverse hypovolemia, and to prevent the lethal triad of major trauma which includes the hypothermia, coagulopathy and acidosis as quickly as possible.

Intermediate phase refers to the 72 h-Day 10 after surgery. In this phase, the effectiveness of the open abdominal surgery should be obtained. According to the goal of treatments, the clinical investigations should be focused on the peritonitis, the intraabdominal infection, the perfusion of gastrointestinal track as well as the intra-abdominal pressure. The amount of the resuscitation fluid of the patients who develop the secondary ACS should be restrained to reduce the intestinal edema so as to decrease the intra-abdominal pressure and achieve definitive abdominal closure as early as possible (within the first 7 days). In order to decrease the intra-abdominal hypertension during the open-abdominal surgery, additional dressings will be applied to reduce the tension of the abdominal wall as much as possible. With the visceral edema being alleviated, the dressing could be tailored and re-sutured every 1–2 days to pull the fascia of the abdominal wall towards each other as close as possible till the gap decreases to 2–4 cm when the abdominal wall could be sutured directly. In addition, management of severe trauma patients should follow the principle of damage control. The bleeding should be controlled aggressively, the misdiagnosed injuries should be handled, the complications should be intervened, and the staged operation strategies should be employed.

Reconstruction phase refers to the Day 10 after surgery to totally being healed. Open abdominal surgery is only a temporal approach during the trauma care and the definitive abdominal closure should be achieved as soon as possible. The prolonged definitive closure usually associates with increased potential risks of complications, which includes bleeding, infection, recurrent ACS, reperfusion syndrome, intestinal fistula, fascia retraction and planned abdominal hernia. So long as the open abdominal surgery is employed, the criteria of definitive abdominal closure are: (1) IAP <15 mmHg for 48 h; (2) unnecessary retention suture; (3) no signs of local infection; (4) no need of re-operation in a short term. Definitive closure should be achieved if the above conditions are met 1–2 weeks (Fig. 4); otherwise, negative wound pressure therapy should be applied to stimulate the growth of granulation tissue to form the frozen abdomen so that the skin graft could be performed, which could help with the early reverse of the metabolism and decrease the risks of fistula by covering the abdominal organs as soon as possible. The reconstruction of abdomen wall will be completed after 6–12 months [1] (Fig. 5).

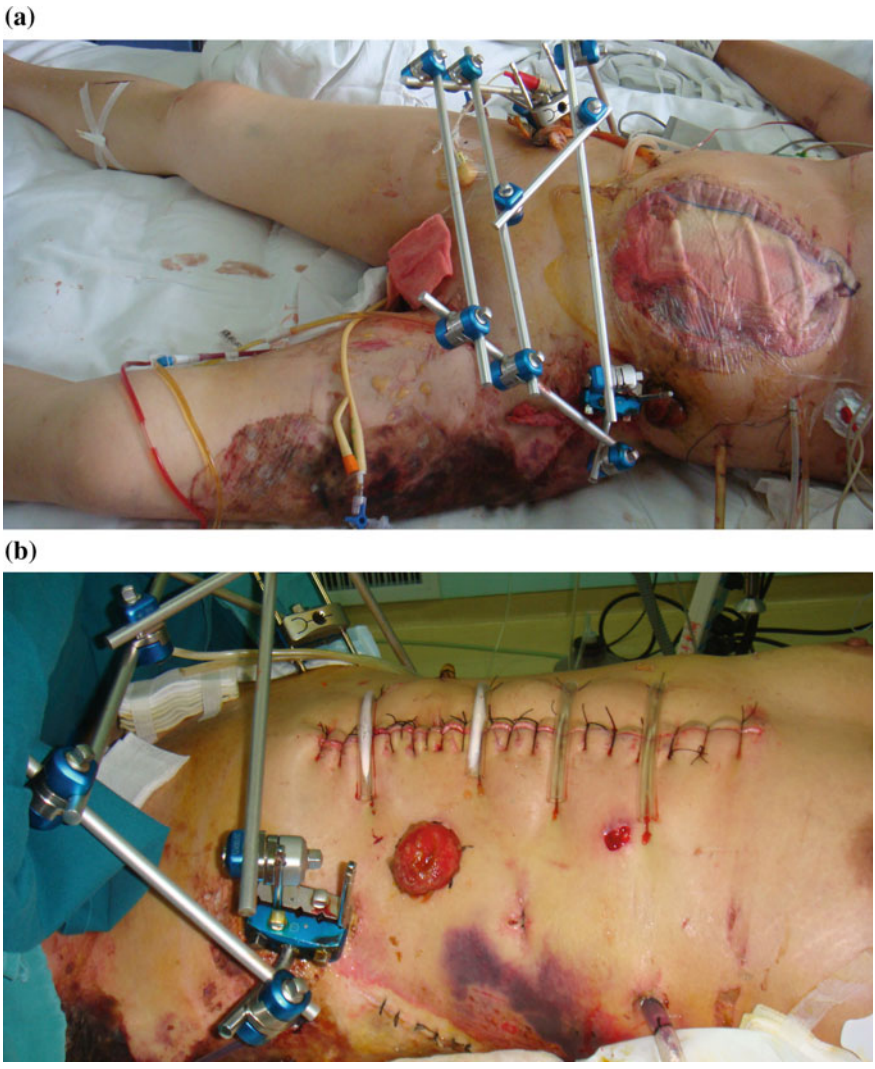

Fig. 4 A female patient of 46 years old was admitted because of multiple trauma caused by motor vehicle crash (MVC). She was performed emergent laparotomy, diaphragmatic repair, resection of the rectum, colostomy and pelvic external fixation. **a** The postoperative incision split on Day 6 after abdominal surgery; **b** early definitive abdominal wall closure on Day 10 after abdominal surgery

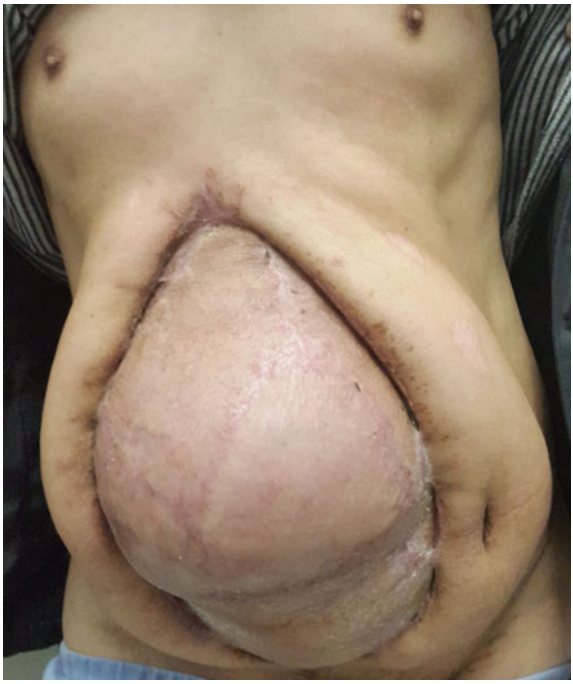

Fig. 5 A male patient of 45 years old with planned abdominal hernia 6 months after skin graft in the aftermath of open abdominal surgery

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Advances in the Management of Thoracic Trauma

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Abstract Thoracic trauma is the second most common cause of mortality of all traumatic injuries, accounting for about 25 % of traumatic deaths. The present review focuses on the advances of the relevant literature regarding the most appropriate approach and definitive treatment of thoracic trauma. The universal clinical application of focused assessment with sonography for trauma (FAST) has greatly enhanced the tool for traumatic diagnosis, monitoring, and interventional procedural guidance. FAST has been used as an efficient triaging tool in blunt thoracic trauma patients. Additionally, video-assisted thoracoscopic surgery (VATS) has been established as safe and effective for managing thoracic trauma in hemodynamically stable patients. VATS for specific indications in thoracic trauma is associated with improved outcomes, decreased morbidity and mortality, and shortened hospital stay. Emergency resuscitative thoracotomy (ERT) is an accepted intervention for penetrating cardiothoracic trauma patient in extremis. However, its role in blunt trauma patient in extremis has been challenged and has been a subject of considerable debate. The treatment of flail chest injuries has undergone dramatic evolution over the last hundred years. Surgical stabilization of severe rib fractures offers several theoretical advantages, but the use of surgery for the treatment of flail chest is still controversial. For the treatment of deep pulmonary lacerations, the techniques that achieve hemostasis while preserving the maximal amount of pulmonary parenchyma are desirable. Lung-sparing technique is an less extensive surgical techniques of repair and resection surgical including suture pneumonorrhaphy, stapled and clamp pulmonary tractotomy with selective vessel ligation, and non-anatomic resection. The management of traumatic aortic injury has been transformed through the application of thoracic endovascular aortic repair (TEVAR). TEVAR has been increasingly used for definitive treatment and its outcomes appear to be at least equally safe and effective as those of open repair. Finally, extracorporeal organ support is becoming more commonplace in trauma critical care management as clinical evidence for the merits of this approach builds.

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Thoracic trauma is the second most common cause of mortality of all traumatic injuries, accounting for about 25 % of traumatic deaths [1]. Thoracic injury as a result of motor vehicle collisions throughout the world is extremely prevalent. Thoracic trauma is associated with high morbidity and mortality rates due to it can affect oxygenation, ventilation, and maintenance of circulation and may result in significant respiratory distress and shock. Many thoracic injuries cause death can be immediate or within the first minutes or hours after injury. Immediate deaths usually involve disruption of the heart or great vessel injury. Early deaths are frequently caused by airway obstruction, tension pneumothorax, pulmonary contusion, or cardiac tamponade. Injuries from chest trauma ranged from rib fractures requiring only pain control to cardiac lacerations with tamponade or exsanguinating hemorrhage. Given the range of injuries, acuity and clinical presentation, multiple diagnostic modalities, and treatment options, exist for thoracic trauma. Initial resuscitation is performed according to advanced trauma life support with immediate attention to airway, breathing, and circulation. During the primary survey, tracheal intubation, mechanical ventilation, closed chest tube thoracostomy and shock resuscitation can be crucial, time-sensitive therapies. Recently, much progress in the science of technologies in trauma critical care management and research-based advances improve outcomes for patients with major chest trauma.

1 Focused Assessment with Sonography for Trauma (FAST)

The injured patient should be rapidly evaluated and the treatments are prioritized based on the mechanism of injury, the patient's presentation and physical examination. The first priority is to treat patients with unstable condition or dying patients. The principle of the initial assessment and resuscitation is according to the beginning of resuscitation while performing concurrent diagnostic procedures. It is important to avoid performing time-consuming auxiliary examination for severe unstable thoracic injury or dying patients due to the rapid changes of these patients condition. Currently, diagnostic thoracic/abdominal cavity puncture, FAST and emergency room bedside X-ray radiograph are three key component of primary diagnostic tool for unstable patients in most large institutions.

Primary assessment and management of thoracic trauma should follow the standard advanced trauma life support principles, starting with control of the

airway. While it is obvious that a chest injury may affect breathing, the major effect of a tension pneumothorax and haemothorax is on circulation. A chest drain will be both diagnostic and therapeutic. During the initial hospital phase, the injured patient is rapidly assessed and the treatments are prioritized based on the mechanism of injury and the patient's vital signs. The goal of the resuscitation is to improve organ and tissue perfusion by rapidly identifying and simultaneously treating life-threatening conditions.

Continued advances in imaging technology and the application of existing techniques are at the forefront of the initial evaluation of the trauma patient, particularly in patients who are critically injured. Radiographic diagnostics must be rapid and accurate. The initial radiographic evaluation screens for immediate life-threatening conditions. More sophisticated diagnostics then identify organ-specific injury and characterize its severity.

FAST and chest X-ray (CXR) are both rapid and repeatable bedside auxiliary examination and can help determine if indications for immediate operative intervention are present. Since its inception in the 1990s, the use of FAST scanning has assumed a key role in the rapid non-invasive assessment and subsequent management of patients suffering thoracoabdominal trauma [2]. The evolution of FAST has seen its incorporation into American College of Surgeons Advanced Trauma Life Support (ATLS) training and it is currently used by emergency physicians in resuscitation rooms worldwide to assist timely decision-making. FAST has proved to be a promising tool for pneumothorax. CXR and computed tomography (CT) scan are the two important investigations commonly used for the diagnosis. However, CXR has been shown to be an insensitive examination. The CT scan is the gold standard for the detection of pneumothorax, but it requires severely injured patients to be transported to the CT room, is time consuming and results in delayed diagnosis. FAST is easily performed at the bedside in the trauma resuscitation room and is used to perform rapid evaluation for severely injured patients. The use of FAST to detect pneumothorax has been studied by several trials to have a higher sensitivity and specificity compared to CXR [3–5]. Three meta-analyses provided a comprehensive analysis of the current literature evaluated the diagnostic accuracy of transthoracic ultrasonography for the diagnosis of pneumothorax in comparison CXR. Ding et al. [6] showed that pooled sensitivity and specificity were 0.88 and 0.99 respectively for ultrasonography, and 0.52 and 1.00 respectively for CXR. Alrajhi et al. [7] reported that ultrasonography was 90.9 % sensitive and 98.2 % specific for the detection of pneumothorax. Alrajab et al. [8] indicates that ultrasonography had a pooled sensitivity of 78.6 % and a specificity of 98.4 %. CXR had a pooled sensitivity of 39.8 % and a specificity of 99.3 %. These meta-analyses demonstrated that bedside ultrasonography performed by clinicians had higher sensitivity and similar specificity compared with CXR in the diagnosis of pneumothorax.

2 Minimally Invasive Techniques in Thoracic Trauma

Approximately 10–20 % of patients who sustain chest trauma will eventually need operative intervention [9, 10]. Although the majority of hemodynamically stable patients with chest trauma can initially be treated with closed tube thoracostomy, it may be ineffective, leading to an increased risk of conversion to open thoracotomy or a prolonged duration of hospitalization [11]. Open thoracotomy is a major surgical procedure and its large incisions are associated with a long and painful recovery [12]. Thoracoscopic evaluation of penetrating thoracic injuries was first described by Branco in Brazil in 1946 [13]. Video-assisted thoracic surgery (VATS) was then subsequently described by Jackson and Ferreira in 1976 to diagnose diaphragmatic injuries incurred by penetrating trauma to the left lower chest [14]. In 1981, Jones, et al. reported the performance of emergency thoracoscopy with local anesthetic in patients with ongoing bleeding following tube thoracostomy placement for traumatic hemothoraces [15, 16]. Over the past 20 years, the advent and increasing expertise in VATS has made it an effective and safe alternative in the assessment and management of thoracic surgery. VATS is associated with decreased morbidity and mortality, and shortened hospital stay [17].

Indications for VATS in stable patients are based upon initial findings and the patient evolution. The immediate indications including significant hemothorax (>1500 ml at chest tube insertion), continuous bleeding (>300 ml/h within the first 3 h after chest tube insertion), suspected diaphragmatic injury, suspicion of a penetrating heart wound, and withdrawal of a stab in situ under direct vision [18–20]. Delayed (up to several days after the trauma) indications including retained or clotted hemothorax, prolonged air leak and/or recurrent pneumothorax, secondary empyema, thoracic duct injuries, and foreign body extraction such as bullets, wires, etc. [21–24]. In multiply-injured patients, VATS is used in a more delayed fashion; as other general surgical, neurosurgical and orthopaedic issues take priority before consideration is given to residual haemothorax [16]. Furthermore, ongoing chest drainage post-thoracostomy placement in the stable patient can be investigated and occasionally treated by VATS [25]. Expanded uses of VATS include: control of intercostal arterial bleeding, pulmonary resection, bronchoplasty, thoracic duct ligation, pericardial window creation, foreign body removal, evaluation and repair of diaphragmatic injury, evaluation of esophageal injury, and chest wall repair [26, 27]. The major and most frequent contraindication for using the VATS approach to addressing thoracic trauma is hemodynamic instability. Other relative, but not absolute, contraindications include suspected injuries to the heart or great vessels, inability to tolerate prolonged single lung ventilation, inability to tolerate lateral decubitus position, an obliterated pleural space, prior thoracotomy, other injuries with indication for emergency thoracotomy or sternotomy, and coagulopathy. In general, rules of patient safety and beneficial outcome should always be paramount to any dogma or focus on the type of surgical approach.

The most common urgent use of VATS following trauma is the drainage of residual hemothoraces greater than 500 ml or collections that result in the

opacification of one-third of a hemithorax [28]. In many cases, the source of hemorrhage is either a bleeding intercostal artery or vein secondary to ribcage trauma or fracture. Other common causes of intrathoracic bleeding include parenchymal injuries such as pulmonary lacerations or pulmonary vascular injury from ballistic trauma. Many of these sources of bleeding can be managed thoracoscopically via direct coagulation, the placing of clips, or via suture. In cases of parenchymal injury, endoscopic surgical staplers have made the resection of segments or entire lobes of the lung reasonably facile.

Recently, a meta-analysis of randomized control trials and cohort studies comparing the perioperative outcomes of VATS with open thoracotomy for chest trauma patients demonstrated that VATS is an effective and even better treatment for improving perioperative outcomes of hemodynamically stable patients with chest trauma and reduce the complications [17]. Pooled analyses showed significant reductions in the incidence of postoperative complications, chest tube drainage volume, duration of tube drainage, duration of hospitalization, operation time, amount of bleeding and transfusion volume in chest trauma patients treated with VATS compared with open thoracotomy. The perioperative mortality rate was not significantly different between patients received VATS and open thoracotomy. In addition, VATS was also decrease the total cost of hospitalization when compared with tube thoracostomy in patients with blunt chest trauma [29].

3 Emergency Resuscitative Thoracotomy

A total of 72.4 % of patients of penetrating cardiac injuries die before reaching the hospital [30], and resuscitative thoracotomy becomes the only hope for the survival of traumatic cardiac arrest patients. Emergency department thoracotomy (EDT), also known as emergency resuscitative thoracotomy (ERT), has been considered a heroic, high-risk procedure for patients in extremis since its introduction in 1967, and over the last four decades, ERT has become well established technique for managing life-threatening thoracic trauma, the technique has been used with increasing selectivity.

A left anterolateral thoracotomy (LAT) is typically considered the standard incision for ERT, because it provides rapid access to the heart and the descending aorta, ability to perform in a supine patient, and ability to be extended to the right hemithorax (clamshell) or laparotomy if clinically warranted [31, 32]. Many surgeons have demonstrated that a stepwise approach to ERT incisions based on clinical presentation may be a reasonable approach, starting with LAT and extending the incision to clamshell if necessary [33, 34]. Others have suggested that the clamshell incision may instead be the standard initial ERT incision [35, 36]. Median sternotomy provided better access to intrathoracic structures than left and right anterior thoracotomies. Definitive control of the origin of the left subclavian artery was difficult with left 2nd or 3rd intercostal space incisions. Bilateral anterior thoracotomy, the clamshell incision, was easy to perform and gave superior access

to all intrathoracic structures. In severe thoracic trauma, specific injuries are unknown, even if they can be anticipated. The best incision is therefore one that provides the most rapid and definitive access to all thoracic structures for assessment and control. While the right and left anterolateral incisions may be successfully employed by surgeons with extensive experience in ERT, the clamshell incision remains the superior incision choice [34].

The goals of an EDT are draining of pericardial tamponade, controlling intrathoracic vascular or cardiac hemorrhage, cross-clamping the pulmonary hilum after massive air embolism or bronchopleural fistula, cross-clamping the descending aorta, performing open cardiac massage, and confirming proper endotracheal tube placement [33–35, 37]. Other objectives include vascular control for intra-abdominal hemorrhage and managing acute bronchovenous air embolism.

Emergency thoracotomy is an accepted intervention for penetrating cardiothoracic trauma patient in extremis. However, its role in blunt trauma patient in extremis has been challenged and has been a subject of considerable debate [38]. The challenge for today's surgeon lies in determining whether a patient would benefit from EDT, a radical procedure that offers a chance of survival for patients who present in extremis. ATLS course states that patients with penetrating thoracic injuries arriving pulseless with myocardial activity should undergo immediate EDT, while those sustaining blunt trauma are not candidate for EDT based on extremely low survival rates.

In 2004, Powell and colleagues established indications for EDT based on 26 years of consecutive data [39]. The authors made three general recommendations for performing an EDT: EDT is indicated when there is witnessed penetrating chest trauma and <15 min of prehospital cardiopulmonary resuscitation (CPR); witnessed non-penetrating chest trauma and <5 min of prehospital CPR; or witnessed blunt trauma and <5 min of prehospital CPR. EDT should also be performed for severe hypotension (systolic blood pressure < 60 mmHg) due to cardiac tamponade, intrathoracic hemorrhage, air embolism, or active intra-abdominal hemorrhage. Contraindications for ERT include penetrating trauma and >15 min of CPR and no signs of life, and blunt trauma with >5 min of CPR and no signs of life or asystole [39]. Signs of life include detectable blood pressure, respiratory or motor effort, cardiac electrical activity, or pupillary activity. In 2011 the Western Trauma Association (WTA) paper, Moore et al. report survivors of blunt torso injuries with pre-hospital CPR up to 9 min and penetrating torso wounds up to 15 min, and recommended broadening the indications for EDT. Furthermore, the WTA multicenter experience suggests that resuscitative thoracotomy in the EDT is unlikely to yield productive survival when patients (1) sustain blunt trauma and require >10 min of prehospital CPR without response, (2) have penetrating wounds and undergo >15 min of prehospital CPR without response, or (3) manifest asystole without pericardial tamponade [40]. However, this multicenter study's conclusion might have been limited because it does not provide the total number of EDTs performed, patient CPR time, or the characteristics of non-survivors [41].

According to the American College of Surgeons Committee on Trauma guidelines, EDT should rarely be performed on patients suffering blunt trauma [42]. This

point may be disputed by data from a Scandinavian study that reported a survival rate of 12.2 % for blunt trauma EDT [43]. Lustenberger et al. and Kandler et al. reported similarly higher survival rates for blunt trauma (7.7 and 20 % respectively). Based on these studies and Moore et al.'s survivor analysis, the declaration of futility of EDT in blunt traumas should be reconsidered. Guidelines published by WTA in 2012 stating that patients undergoing CPR on presentation to the hospital should be stratified based on injury and transport time to ascertain whether EDT is advisable [44].

In 2015, with the support of recent data, Dayama et al. suggested revision to the guidelines for performing EDT as following: (1) EDT should be performed selectively in patients sustaining cardiopulmonary arrest secondary to blunt trauma after SOLs are lost in the emergency department or at the scene with transportation time equal to or less than 10 min. (2) EDT should be performed in patients sustaining penetrating thoracic injuries with loss of SOLs witnessed in the emergency department or at the scene with transportation time equal to or less than 15 min. Finally, as in all studies, the completeness and accuracy of the prehospital information is in question. In summarizing these data, EDT should be offered to patients who arrive in asystole to the hospital and are suspected to have a cardiac injury and tamponade [45].

The role of EDT continues to be debated due to these data are largely derived from retrospective analyses of trauma registries that have not been specifically designed to evaluate the critical factors predictive of survival after ED thoracotomy. EDT is best applied in patients with penetrating cardiac injuries, and should be applied with penetrating cardiac injuries, and should be applied in patients with penetrating non-cardiac thoracic injuries and exsanguinating abdominal vascular injuries in accordance with the level II recommendations of the American College of Surgeons [42]. In general, patients who require EDT after penetrating trauma mechanisms have better outcomes than those who have suffered blunt trauma (comes than those who have suffered blunt trauma). The mechanism of injury is usually the strongest predictors of survival post-EDT [46]. Recently, in a meta-analysis performed by Dayama et al. [41], the overall survival rate in patients with EDT for penetrating traumas was 9.8 % (169 of the 1719, range 0–45.5); the survival rate in patients with EDT for blunt injuries was 5.2 % (24 of the 460, range 0–12.2). These data are compared with analysis by the American College of Surgeons Committee on Trauma, which demonstrated patients with penetrating injuries had a survival rate of 11.2 and 1.6 % in those with blunt trauma. Furthermore, an interesting finding in this review was a notable difference in the survivor outcome of US experience versus non-US experience with only 6.5 % surviving in the US as compared with 11.4 % outside the US. And also, this review shows a better survival rate of EDT in military (11.9 %) and in pre-hospital settings (16.3 %). Seamon et al. [47] investigated whether EDT versus resuscitation without EDT improves outcomes in patients who present to the hospital pulseless after critical thoracic injuries. Patients presenting pulseless after penetrating thoracic injury have the most favorable EDT outcomes both with (survival, 21.3 %; neurologically intact survival, 11.7 %) and without signs of life (survival, 8.3 %; neurologically intact survival, 3.9 %). In patients

presenting pulseless after penetrating extrathoracic injury, EDT outcomes were more favorable with signs of life (survival, 15.6 %; neurologically intact survival, 16.5 %) than without (survival, 2.9 %; neurologically intact survival, 5.0 %). Outcomes after EDT in pulseless blunt injury patients were limited with signs of life (survival, 4.6 %; neurologically intact survival, 2.4 %) and dismal without signs of life (survival, 0.7 %; neurologically intact survival, 0.1 %).

Although most survival studies of EDT focus on neurologic outcome, a novel study by Keller et al. examined the long term social, cognitive, functional, and psychological consequences, and determined that of the 8.3 % of patients who survived hospitalization after EDT, the majority of EDT survivors had no evidence of cognitive, functional, or psychological long-term impairment. Seamon and colleagues strongly recommend that patients who present pulseless with signs of life after penetrating thoracic injury undergo EDT. Furthermore they conditionally recommend EDT for patients who present pulseless and have absent signs of life after penetrating thoracic injury, present or absent signs of life after penetrating extrathoracic injury, or present signs of life after blunt injury. Lastly, they conditionally recommend against EDT for pulseless patients without signs of life after blunt injury [47].

4 Surgical Fixation for Flail Chest

Flail chest cause chest wall instability, asynchronous movement of the flail segment, and paradoxical chest motion, which is result in deformity of the chest wall, loss of thoracic volume, decreased lung volume, atelectasis, chest tightness, dyspnea, and chronic pain [48–50]. Flail chest is a life-threatening injury, and is associated with significant morbidity and mortality [51]. Respiratory insufficiency as a result of paradoxical chest movement, lung collapse, and pulmonary contusion are the main factors leading to serious morbidity in patients with flail chest. Of all the above, pulmonary contusion is by far the most important single factor, along with paradoxical motion disrupts the mechanics of ventilation, which contributes to intra-alveolar hemorrhage and flooding resulting in ventilation/perfusion (V/Q) mismatch and hypoxemia. The treatment of flail chest injuries has undergone dramatic evolution over the last hundred years [52]. In the first half of the twentieth century, treatment was focused on mechanical stabilization of the chest wall. At the time, chest wall stabilization was performed by bracing or adhesive strapping or traction of the chest wall. In the second half of the century, the concept of internal pneumatic stabilization use of positive pressure mechanical ventilation became a critical treatment strategy for stabilization of flail chest injuries. Kirschner wires and Judet struts used for stabilization of flail chest injury are outdated modes of fixation compared to current modern technique of plates and screw fixation [53, 54]. The current modern treatment of flail chest injuries includes nonsurgical and surgical treatment strategies including mechanical ventilation, tube thoracostomy, pain control, chest physiotherapy and surgical fixation.

Mechanical ventilation is necessary which is usually for hypoxemia due to pulmonary dysfunction and gas exchange abnormalities, rather than treat chest wall instability [50, 55]. Mechanical ventilation should be weaned from the ventilator at the earliest time possible. Prolonged mechanical ventilation has been reported to leads to barotrauma and increases the risk for pneumonia, sepsis, extended time in the intensive care unit, and death [48–50, 56–58]. Decreasing the number of days on mechanical ventilation may result in decreased morbidity and mortality and may dramatically decrease medical costs [50]. However, patients with head injury and pulmonary contusion may require long-term mechanical ventilation and do not attain the benefits of early extubation [50]. Noninvasive ventilation (NIV) for patients with blunt chest trauma, which demonstrated that early use of NIV in appropriately identified patients with chest trauma and without respiratory distress may prevent intubation and decrease complications and ICU length of stay [59]. NIV may be considered in patients with blunt chest trauma who are neurologically intact, hemodynamically stable and not in respiratory distress. There is no apparent benefit of NIV in the prevention of intubation in patients with respiratory decompensation. In fact, delaying intubation in these patients leads to harm. Future studies need to be methodologically sound and focus on the use of NIV in patients with blunt chest trauma early in the course of the disease, prior to overt respiratory failure [59].

Surgical stabilization of severe rib fractures offers several theoretical advantages (Fig. 1), but the use of surgery for the treatment of flail chest is still controversial [60]. There have been a number of studies demonstrated that surgical fixation of flail chest injuries improved outcomes including fewer days on mechanical ventilation, decreased length of ICU stay, fewer chest infections and less chronic pain, and reduce mortality [61–64]. However, there are limited randomized controlled trials on this area [48, 58, 65]. These randomized controlled trials remain limited by antiquated fixation system, lack of prospective study design and small sample size, outdated methods of surgical fixation and vague study criteria. In 2015, Pieracci

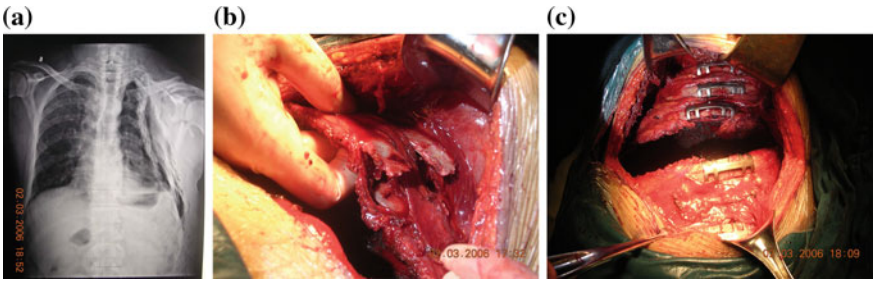

Fig. 1 Surgical stabilization of severe rib fractures for a 53-years-old male patient with flail chest. The chest X-ray film showed severe ribs fractures on the *left side* (**a**); the surgical finding showed severe multiple ribs fractures (**b**). Surgical internal ribs fixation showed that shape memory alloy of nickel and titanium encircle rib bone plate is one of the best suitable choose for correction of chest wall floating (**c**)

et al. conducted a prospective, controlled clinical evaluation of surgical stabilization of severe rib fractures. In this clinical evaluation found that surgical stabilization of rib fractures, as compared to best medical management, was associated independently with a 76 % decreased likelihood of respiratory failure and an 82 % decreased likelihood of tracheostomy, as well as 5 day decreased duration of mechanical ventilation, and significantly improved spirometry readings among extubated patients [66]. Surgical stabilization of severe rib fractures offers several advantages, however, there remains a lack of consensus regarding both indications and technique [67]. High-quality large, multicenter randomized controlled trial in this area are still need to better assess the benefits of surgical fixation versus non-operative care for trauma patients with flail chest injuries.

There are no absolute indications for operative repair of a flail chest injury. Indications for surgical stabilization of severe rib fractures by Denver Health Medical Center were included: (1) Acute respiratory insufficiency despite optimal medical therapy: either need for mechanical ventilation or ≥ 2 of the following: tachypnea, hypercarbia, hypoxia, uncontrolled secretions, incentive spirometry <75 % predicted. (2) Uncontrolled pain despite optimal medical therapy: ≥ 2 of the following: numeric pain score $\geq 4/10$, splinting, lung hypoexpansion on imaging. (3) Anticipated chronic pain/impaired pulmonary mechanics: ≥ 1 of the following: flail chest, ≥ 3 severely displaced fractures, hemithorax volume loss ≥ 30 % [67]. Whereas most reviews have listed four or five categories based mostly on anatomic diagnoses, the Denver Health Medical Center uniquely lists three categories based on the clinical situation. The indications emphasize the goal that the surgery is trying to achieve, but this categorization may promote overuse of this procedure [68].

The most common indication for surgical fixation of flail chest, and that with the strongest evidential support, is for respiratory failure with an anterolateral flail segment without severe underlying pulmonary contusion [58, 65, 69–71]. When contemplating surgical fixation of a chest wall injury, the absence of severe underlying pulmonary contusion (PC) may be particularly important. PC in turn is the most common injury identified in the setting of blunt thoracic trauma, occurring in 30–75 % of all cases [72]. Flail chest is typically accompanied by PC [55]. 54 % of the flail chest injuries patients had lung contusions [50]. Voggenreiter et al. [73] demonstrated that surgical fixation permits early extubation in patients with flail chest and respiratory insufficiency without pulmonary contusion, while patients with pulmonary contusion do not benefit from operative chest wall stabilization. These authors concluded that flail chest and respiratory insufficiency without underlying PC is an indication for surgical fixation. However, in recent, Zhang et al. retrospectively analyzed a study comparing the clinical efficacy of surgical fixation and nonsurgical management of flail chest and PC. These authors concluded that surgical fixation for flail chest with PC could reduce the hospital length of stay (38 vs. 60 days, $p = 0.049$) [74]. Those studies were a single-center, uncontrolled and retrospective and involved a small sample size. Consequently, although surgical fixation clearly corrects the anatomic chest deformity, the mortality and short term morbidity of flail chest combined with PC entity have not improved. Additional

larger, multiple-center, prospective randomized controlled studies are needed further evaluation.

With the increasing technological advancements available in the trauma critical care management, conservative management, has become more common [71]. The optimal nonoperative treatment of patients with flail chest includes adequate pain management, via use of epidural catheters, intercostal nerve blocks, or patient-centered analgesia [55]. The use of epidural catheters seems to be the most preferred method, with improved outcomes and lower complications compared with other methods [52, 55, 75]. Compared with intravenous narcotic use, epidural catheters allow for improved subjective pain perception, pulmonary functions tests, lower rate of pneumonia, as well as decreased length of time on a mechanical ventilator or ICU stay [52, 55, 76]. They also have lower rate of complications such as respiratory depression, somnolence, and gastrointestinal symptoms [55]. Epidural catheters have also been compared with intrapleural catheters in a previous randomized controlled trial and have shown to decrease pain and improve tidal volume and negative inspiratory pressures [75]. Other modes of pain management include use of oral and intravenous narcotic administration and patient-centered analgesia [52, 55].

5 Deep Pulmonary Lacerations

Pulmonary lacerations secondary to either blunt trauma usually as result of displaced rib fractures puncturing the lung parenchyma or penetrating injuries directly to the lung. The natural history of blunt trauma is usually spontaneous resolution, as most are small and superficial and heal without any intervention. The vast majority of lung injuries requiring surgery are caused by penetrating trauma. Deep pulmonary laceration is typically associated with rupture of the visceral pleura and is a critical condition. Deep pulmonary laceration accounts for about 50 % of patients with intrathoracic hemorrhage and often results in death. If early and appropriate treatment is based on accurate diagnosis by rapid assessment of the pathology and by diagnostic imaging (Fig. 2), deep pulmonary laceration is a treatable condition in which the lives of these patients can be saved [77, 78]. VATS has been demonstrated to be an accurate, safe and reliable alternative method for the direct evaluation of the lung injuries.

In the past, major lung resection (lobectomy and pneumonectomy) when performed after traumatic lung injuries has been associated with high morbidity and mortality rates. Simple superficial oversewing of deep penetrating lung injuries potentially lead to postoperative lethality of hemoptysis [79, 80]. Therefore, techniques that achieve hemostasis while preserving the maximal amount of pulmonary parenchyma are desirable (Fig. 3). A major advance in lung-sparing techniques for the treatment of pulmonary penetrating injuries was introduced by Gao et al. [81], and Wall et al. [82] respectively in 1994. Lung-sparing technique is an less extensive surgical techniques of repair and resection surgical including suture pneumonorrhaphy, stapled and clamp pulmonary tractotomy with selective vessel

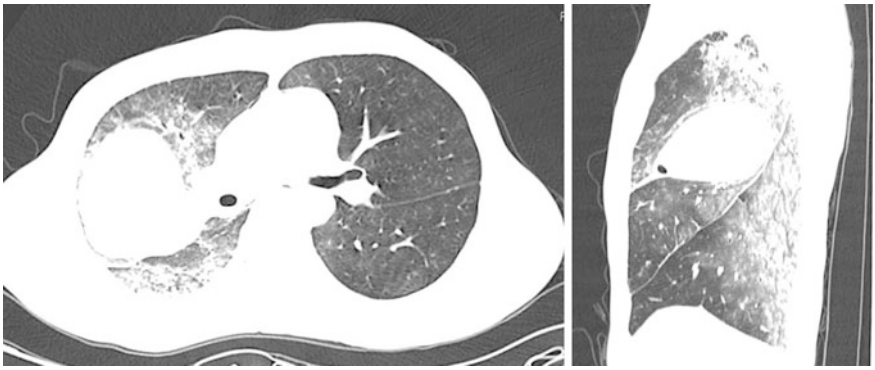

Fig. 2 A 41-years-old male patient with blunt chest trauma. Spiral CT scan showed a large intrapulmonary hemotoma (*black arrow*)

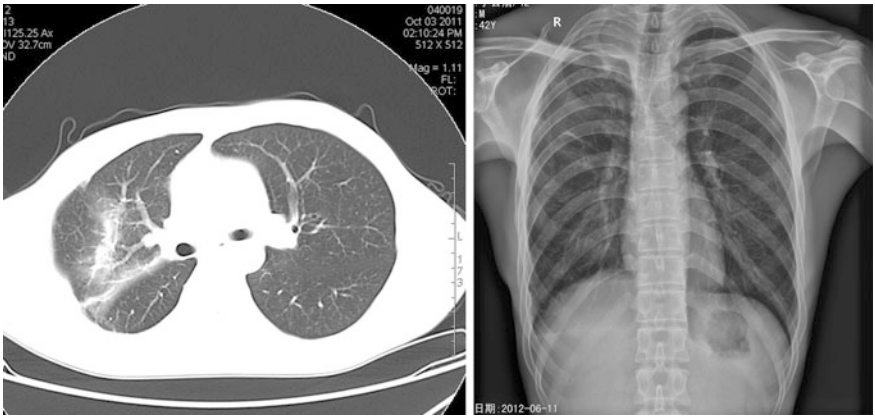

Fig. 3 CT or Chest X-ray followed up in 4 weeks and 9 months after removal of intrapulmonary hematoma and surgical pneumonorrhaphy

ligation, and non-anatomic resection [83]. These resection techniques are indicated for control of hemorrhage, control of small air leaks, to preserve pulmonary tissue, and/or when the pulmonary injury is amenable to reconstruction [84]. It is estimated that approximately 85 % of all penetrating pulmonary injuries can be managed with these procedures [82, 85–87]. Lung tractotomy allows for rapid exposure and selective ligation of injured pulmonary structures, and thus reduces the need for emergent lung resection [86]. Tractotomy has allowed achieving rapid hemostasis with preservation of lung tissue. Use of stapling instruments has simplified the procedure. Smaller lung resections involving peripheral portions of a lobe were performed as nonanatomic wedge resections using surgical staplers. Lobectomy and pneumonectomy was required for resection of devitalized or destroyed pulmonary tissue in severe lung injuries.

6 Traumatic Aortic Injury

Injury to the aorta and the arch vessels can occur following blunt and penetrating trauma. Traumatic aortic injury (TAI) is the second most common cause of death after blunt trauma [88]. As many as one-third of fatalities in motor vehicle collisions can be attributed to TAI [88, 89]. Burkhart and colleagues reported that 57 % of the deaths occurred at the scene or on arrival to the hospital, 37 % died within the first 4 h of admission, and 6 % died 4 h after admission [90]. In autopsy study involving traffic accidents, 33 % of the victims had associated TAI, 80 % patients with blunt TAI die prior to hospital arrival and only 20 % in hospital [88].

The most common anatomical site of aortic injury is the medial aspect of the lumen, distal to the left subclavian artery. Injury at this site is found in about 93 % of hospital admissions and in about 80 % of autopsy studies [91]. These resulting from a combination of high shear stress, heterogeneity in the wall architecture possibly contributing to focal wall weakness and acute transient intraluminal pressure loading [92, 93]. The most common type of injury is a false aneurysm (58 %), followed by dissection (25 %) and intimal tear (20 %) [94].

For those patients with TAI, timely diagnosis and prompt aggressive blood pressure control are essential in preventing free rupture of the contained aortic injury. Digital substraction angiography (DSA) was the gold standard for diagnosis of TAI traditionally. CT angiography (CTA) is now the new standard modality for screening and definitive diagnosis of TAI [91]. The diagnosis of TAI by DSA and CTA were showed in Fig. 4. The sensitivity and negative predictive value of the CT scan in the diagnosis of blunt TAI approaches 100 % [95]. Advances in CT technology have significantly improved the sensitivity of CT for the detection of TAI. The new-generation multi-slice CT scanners with 3-dimensional reformation have almost 100 % sensitivity and specificity, a 90 % positive and 100 % negative

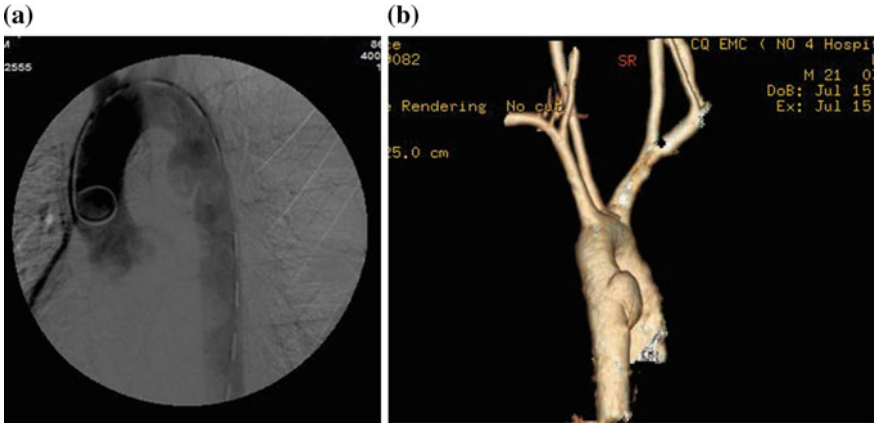

Fig. 4 A multiple trauma patient, male, 21 years-old, was impacted by traffic accident. The DSA (**a**) and CTA (**b**) examination showed traumatic aortic injury (TAI)

predictive value, an overall diagnostic accuracy of 99.7 %, and provide impressive anatomical details of the aortic arch and the injury site [91, 96]. Transesophageal echocardiography (TEE) as a diagnostic tool might be useful in critically ill patients in the intensive care unit who cannot be transferred safely to the radiology suite for CT scan [91]. Additionally, with regards to long-term surveillance and more specifically the detection of endoleaks, pseudoaneurysms and stent graft material-related complications, recent clinical practice guidelines by the Task Force for the Diagnosis and Treatment of Aortic Diseases of the ESC recommend the combination of a chest X-ray with either MRI or CT scan. Although CT is currently the preferred modality, they advise considering the dangers of radiation, especially in younger patients, and suggest the use of MRI except in cases of magnetic resonance-incompatible grafts [97].

Once the diagnosis is made, treatment must be properly timed. In general, minimal aortic injuries (intimal tear of less than 1 cm with no or minimal peri-aortic haematoma) receive conservative management [98]. Treatment of patients with TAI may be interventional surgical or conservative therapy dependent on clinical judgment on an individual basis [99]. The timing of repair according to the extent of injury on the thoracic aorta and the presence or absence of other injuries [100]. With regards to the best timing of intervention, the decision should be made based on the presence and severity of symptoms and related complications, comorbidities and the presence or absence of other injuries [100].

Prevention of free rupture by means of rigorous blood pressure control is the most urgent priority. The risk of free rupture is highest in the first few hours after the injury, with 90 % of ruptures occurring within the first 24 h [91]. Without rigorous blood pressure control, risk of rupture is about 12 %, and rigorous blood pressure control reduces the risk to about 1.5 % [101, 102]. Systolic blood pressure should be kept as low as tolerated, in most patients at about 90–110 mmHg. In elderly or head-injury patients, optimal systolic pressure might be slightly higher [91]. It is important to avoid excessive administration of intravenous crystalloid, as controlled hypotension is preferred to avoid blood pressure elevation and to decrease the likelihood of aortic rupture [103]. β-blockers and antihypertensive are the most commonly used modalities to modulate the systolic blood pressure [91, 103].

Interventional treatment for blunt TAIs can be either open surgical repair (OSR) or thoracic endovascular aortic repair (TEVAR). The TEVAR has been widely rapidly adopted as an alternative to the OSR for treatment of traumatic aortic injury (Fig. 5). TEVAR is minimally invasive compared to surgery and can be performed soon after the establishment of diagnosis prior to management of other concomitant severe injuries. TEVAR is an effective option for the treatment of blunt TAI, numerous reports have demonstrated that blunt TAI have benefits of lower blood loss, shorter hospital stay, and reduced mortality rate, which confirming the increased utilization of TEVAR as the primary approach in selected trauma patients. Unlike aneurysmal disease, TAI is usually a focal lesion in the setting of a relatively younger and healthier aorta. As a result, a properly sized, delivered, and deployed device may have a potentially lower rate of long-term complications compared with other aortic pathologies [104].

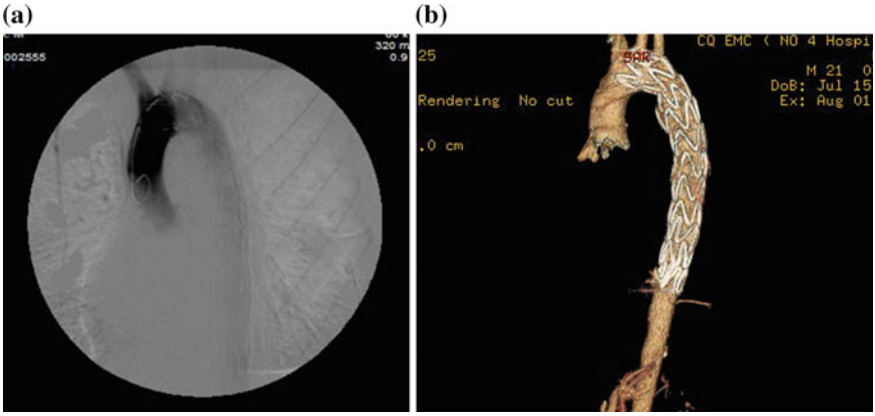

Fig. 5 The same trauma patient showed in Fig. 4. Repeated examination by DSA and CTA after thoracic endovascular aortic repair

The first case report of endovascular stent-graft treatment of a blunt TAI was published in 1997 [105]. Recently published observational studies and meta-analyses favor the use of the endovascular method as definitive treatment over open surgery in patients with TAI and support delaying repair of the injuries where possible. Azizzadeh and colleagues described an estimated odds ratio of 0.33 for complications including in-hospital mortality with TEVAR compared with open repair (OR), similar costs, and similar length of hospital stay [106]. In 2014, Branco et al. after a 9 year analysis of the same data bank, described favorable outcomes of the endovascular approach compared with OR in terms of in-hospital mortality (12.9 % *vs.* 22.4 %) and sepsis (5.4 vs. 7.5 %) [107]. Estrera et al. found that TEVAR was statistically superior to OR with cross-clamping but not to OR with distal aortic perfusion, in terms of survival (4, 31 and 14 % respectively). In the same study survival at 1 and 5 years post-intervention was 76 and 75 % respectively for OR, and 92 and 87 % for TEVAR [108]. According to Di Eusanio and colleagues, Delayed repair was used as first-line treatment for blunt TAIs and was associated with a very low mortality (3.9 %), mortality and paraplegia rates were not different comparing TEVAR and OR groups [109]. At midterm follow-up (median follow-up 2.3 years, range 0–7 years), TEVAR is an effective and durable option for the treatment of TAI in properly selected patients [104]. The incidence of in-hospital mortality, stroke, and paraplegia were 5.0, 2.4 and 0 %, respectively. The rates of device-related adverse events (2.4 %), secondary procedures (4.8 %), and open conversion are rare (2.4 %). Survival was 95 % at 30 days, 88 % at 1 year, 87 % at 2 years, and 82 % at 5 years. The late outcomes (mean, 103.9 months) following open and endovascular repair of TAI was reported by Patel shows that the overall crude mortality rate was 14.7 % and freedom from aortic reintervention at 4 years was higher after open repair (DTAR 100 % vs. TEVAR 94 %; $P = 0.03$) [110]. A recent study by Canaud et al. [111] described

data of follow-up of minimum 10 years post-TEVAR (mean 11.6 years) with very encouraging results. The authors showed that the favorable outcomes of TEVAR over OR in terms of mortality and complications last over time, follow-up computed tomography scans did not reveal any stent-graft migration or collapse, or secondary endoleaks [111]. The findings support the use of TEVAR over OR for patients with TAI. However, there are no RCTs conducted to determine whether use of TEVAR for the treatment of blunt TAI is associated with reduced mortality and morbidity when compared to conventional open repair. To perform a randomized controlled trial to clarify optimal management of blunt TAI would be very challenging to complete, mainly because of the natural history of the condition, usually seen in combination with other life-threatening injuries, the requirement for urgent intervention and the potential difficulties surrounding consent [112]. Despite lack of RCT evidence, clinicians are moving forward with endovascular treatment of blunt TAI on the basis of meta-analyses and large clinical series. Recent clinical practice guidelines of the Task Force for the Diagnosis and Treatment of Aortic Diseases of the ESC, that advise the use of TEVAR in suitable anatomies (Level of Evidence: C). There are still some unresolved issues and areas of concern.

There are currently some technical limitations to endografting. One of the most common problems in treating aortic arch pathologies is a short proximal landing zone. Injuries that occur adjacent to a sharp bend in the aorta may result in poor apposition of the covered stent to the aortic wall, which leads not only to failure in covering the injury but also to device collapse [113, 114]. Another technical issue relates to the management of the left subclavian artery. Lesions adjacent to the left subclavian artery may require covering this vessel in order to achieve adequate repair. Although usually well tolerated, coverage of the left subclavian artery can result in ischemia of the upper extremity or territory perfused by the left vertebral artery [98]. To further expand the applicability of endovascular repair of aortic arch and descending aortic pathology, alternatives have been proposed, such as the chimney graft technique, which involves placement of stents in side branches of the aorta alongside the main endovascular stent graft [115–117]. One advantage of the chimney technique is that readily available, on-the-shelf stents can be used. When these stents are placed in the side branches parallel to the aortic stent graft, a prolonged proximal landing zone can be created, and continued perfusion of the aortic side branches can be maintained [117]. Chimney graft use in the emergent setting is becoming widely accepted as a useful technique. Increasing amounts of data support the benefit of visceral and arch chimney graft techniques. In particular, the low early mortality and complication rates and high long-term patency seem advantageous in electively treated cases [118]. Elective TEVAR with the chimney technique is used more often and is a potential new technique to replace hybrid repair for thoracic aortic diseases.

7 Extracorporeal Lung Support in Trauma Patients

Acute respiratory distress syndrome (ARDS) is most frequently observed in the early course of intensive care in patients with severe trauma which has an associated mortality rate of 40–60 % despite many medical advances in its treatment [119–121]. The injury to the lungs in ARDS after trauma may be due to a direct pulmonary insult, such as pulmonary contusion or one that is indirect, as in severe sepsis, trauma, shock, and massive blood transfusion. Extracorporeal membrane oxygenation (ECMO) therapy presents a rescue therapy in severe trauma patients with concomitant chest injury suffering from refractory ARDS when conventional therapies have been exhausted [122, 123].

ECMO uses technology derived from cardiopulmonary bypass that allows gas exchange outside the body, circulatory support can also be provided. The first successful report use of ECMO as lifesaving treatment in respiratory failure was introduced by Robert Bartlett in 1972 and revolutionized the treatment of resistant hypoxemia in patients worldwide [124–126].

ECMO has been used for more than 40 years and its benefits in neonates with respiratory distress. The benefits of ECMO in adult patients with cardiac failure or refractory ARDS are still debated, as early studies demonstrated ECMO was initially associated with poor results [127]. However, with technological advances in the ECMO circuit including the use of polymethylpentene membrane oxygenators, centrifugal pumps, miniaturization of circuits, and heparin-bonded circuitry, which have led to a reduction in the rate of technical issues and complications. Moreover, improved understanding of the benefits of ECMO has emerged from its widespread use as a rescue therapy for patients with ARDS and refractory hypoxaemia associated with H1N1 (2009 influenza A). ECMO offers artificial temporary and respiratory support that should be maintained until the patient recovers from severe respiratory failure.

The management of severe ARDS in adult trauma patients presents a great challenge for physicians. In some ways, trauma patients are the ideal patients to undergo ECMO. Trauma patients often have recoverable injuries. Moreover, they are typically young and healthy, with good cardiopulmonary function at baseline [128]. ECMO more frequent considered as a viable treatment option for severe ARDS in the trauma patient. One potential contraindication to ECMO in the trauma population is the increased risk of hemorrhage during its use. However, this issue has recently been challenged, as advancements in ECMO circuits, specifically heparin-bonded systems and shorter circuit lengths, have allowed ECMO use with little or no anticoagulation. Several reports have shown that patients with traumatic intracranial hemorrhage and respiratory failure can be successfully managed on heparin-free ECMO without increasing the size of the lesion [129–131]. If the risk of bleeding is adequately reduced, trauma patients with severe pulmonary failure present an ideal population for treatment with ECMO. Cordell-Smith et al. reported a series showing a survival rate of 71 % in 28 trauma patients treated with ECMO [132]. In the report by Ried M showed that the overall survival rate in patients with

required pumpless extracorporeal lung assist and veno-venous ECMO for primary post-traumatic respiratory failure was 79 % compared with the proposed Injury Severity Score-related mortality (59 %) [133]. Extracorporeal lung support (ELS) devices are an excellent and life-saving treatment option in severe thoracic trauma patients with ALF. Thoracic trauma patients with concomitant refractory pulmonary failure have a remarkable potential to recover under ELS. The utilization of the ELS devices was safe and effective in these severe multiple trauma patients. In recent, retrospective analysis of ECMO patients in the Extracorporeal Life Support Organization database, Jacobs JV et al. showed that, outcomes after the use of ECMO in blunt thoracic trauma can be favorable, the rate of survival to discharge was 74.1 % [128].

There is no established standard of care regarding the use of ECMO in patients with blunt thoracic trauma. It would be difficult for any single institution with ECMO capabilities to collect a large number of trauma patients requiring the use of ECMO. Standardizing ECMO therapy and evaluating its efficacy in patients with multiple injuries is also problematic. Further study is needed, and multi-institutional collaboration will be paramount to make progress in this field.

In conclusion, new technology innovation and application significantly change the traditional path and method in treatment of chest trauma. Guidelines for trauma care seek to set achievable standards for trauma treatment services which should constantly update to propose important and evidence-based recommendations regarding chest trauma.

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The Normalization and Promotion of Large Craniotomy Treatment for Severe Traumatic Brain Injury

Baiyun Liu and Xiang Mao

Abstract The mortality rate of TBI currently remains as high as 30 %. To properly understand and apply minimally invasive TBI surgery is of great importance. Our present study encapsulates the foundation for decompressive craniectomy (DC) in diffuse, non-penetrating TBI, with the effects and complications of craniectomy in TBI. We introduced the information following: 1. why choose DC for TBI patients (including surgical technique); 2.outcomes after DC in TBI; 3. complications of DC. DC can save lives for patients with severe TBI, but many questions still remain for its application, and the outcome is highly related to the severity of the initial TBI. DC is a potential therapeutic option in a variety of situations, and neurosur-geons should be aware of all the complications of DC.

Keywords Traumatic brain injury · Large craniotomy · Surgical technique · Outcomes · Complications

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1 Introduction

The mortality rate of TBI currently remains as high as 30 %. Therefore, neurosurgeons and trauma physicians are devoted to reducing this mortality rate with its associated disabilities. To properly understand and apply minimally invasive TBI surgery is of great importance. The concept of minimal invasion refers to a set of medical behaviors (especially surgeries), performed to achieve the best therapeutic effect with minimal tissue damage. Since different tissues have different functions in the human body, physicians must focus on protecting the important tissues and perform a full assessment of their functions and injury levels before surgery. In neurosurgery, the importance of tissue protection should be from the inside to the outside (e.g. brain → dura → skull → scalp). Currently some intracranial lesions can be primarily treated using specific modern technologies, such as intraoperative navigation, ultrasound, intervention, and endoscopy, to achieve the best excision effect through minimally invasive approach to the scalp (i.e. small incision).

However, with regards to TBI surgery, simply applying the above-mentioned methods through a small bone window or small incision is clearly unfitting and cannot protect brain tissues due to the diversity and complication that come with TBI.

Our present study encapsulates the foundation for DC in diffuse, non-penetrating TBI, with the effects and complications of craniectomy in TBI.

1.1 Why Choose DC for TBI Patients

If TBI surgery were a battle, choosing an appropriate surgical approach would be the crucial strategy that determines its success or failure. The decisions regarding which surgical approach to choose, whether to use a large/small craniectomy or drilling alone, not only evaluates a physician's general understanding of the disease, it also tests his or her surgical skills. Inappropriate surgical approaches are bound to greatly reduce the surgical efficacy and can lead to serious adverse complications on the patient as well.

Large craniectomy was proposed and applied by American physicians in the 1970s. The incised bone flap area by this technique is nearly twice as large as that by conventional approach. It was introduced in China during the 1990s. After decades of practice, it is being widely used to treat severe TBI and has achieved acceptable effects.

Standard treatments, such as mannitol and hypertonic solution, can reduce the ICP; however, aggressive intracranial hypertension need further management procedures. We know that the concept of "widely opening the skull to decompress the brain in order to decrease the pressure" has been in existence for more than 100 years [1]. DC was used for the patients who presented with a progressive increase in intracranial pressure (ICP) after traumatic brain injury. DC has been

proven to decrease intracranial pressure where patients sometimes get good outcomes from this procedure, but there is a debatable effect of DC with its association on having a better clinical outcome.

Cranial cavity is fixed, if brain edema will cause intracranial hypertension and cerebral herniation. Thus, pieces of skull are under resection, so an increase in the cranial cavity will be the most effective treatment. DC, which can increase the cranial cavity, is a useful and invasive procedure which can reduce ICP immediately after TBI [2]. Severe TBI is usually a diffuse injury; DC can expand our operation horizons and avoid missing the original traumatic lesions.

1.2 Surgical Technique

DC was usually chosen to eliminate the contusion and evacuate the hematoma in order to reduce the ICP in TBI, stroke or other central diseases causing a mass effect [3–5]. The traditional process is that we confirm the place where DC has to be done and do operation a little maximal around that. However, for diffuse brain edema, we need to combine operations with neurocritical care, such as hypothermia [6].

For children, Mhann et al. [7] retrospectively analyzed the medical records of children with severe TBI who were treated with DC in one center reported that early DC improves functional outcomes in pediatric patients with severe TBI, but does not improve mortality rates which were similar to Taylor et al. and Prasad et al. [8, 9] reports. They used DC in a randomized trial of very early DC in children with TBI and sustained intracranial hypertension, reported that a benefit in mortality and improved functional outcomes.

Previously described procedures about DC in adults were unilateral craniectomy [10] and bilateral craniectomy [11]. Liu et al.'s [3] method comprised of the unilateral craniectomy or bilateral craniectomy opening to maximize the reduction in ICP. For bilateral craniectomy, the dura is then opened in a cutting that resembles an "X", (like that of a "fish mouth") with the sagittal sinus ligated and anteriorly cut. The size of dura which is being opened is close to the cranial window. The sort of craniectomy performed (unilateral/bilateral) is based on the computed chromatography (CT) scan results. Unilateral craniectomy is performed in patients with unilateral swelling; however, in cases with general swelling, bilateral craniectomy is chosen. The area covered in unilateral craniectomy is approximately 12 cm × 15 cm, and that in the bilateral craniectomy is about 10 cm × 25 cm. Larger craniectomies may reduce the risks of herniation that leads to contusions at the skull edge [12]. This procedure is similar to the description Kjellberg [11] and Polin et al. [13]. Other neurosurgeons avoid sectioning the sagittal sinus or parting a strip of skull over the sagittal sinus when choosing craniotomies [9, 10, 14–16]. Leaving a strip of skull over the sagittal sinus may be easy to do crainoplasty in the future. However, the first thing is to reduce the ICP maximal after TBI. It is lack of evidence that whether leaving a strip of skull can affect the outcome of patients with severe TBI. Although most of these patients require to remove bone flap, it should

be clear that we must know large craniotomy is not equal to decompressive craniotomy. Depending on the state of brain after the operation, we can choose whether we can remove the bone flap.

The illustration of large craniectomy:

The principle of surgical approach: "trapezia approach by layer" from skin to bone to dura to brain in turn.

The Surgical indications:

1. Severe widespread cerebral contusion or intracerebral hematoma
2. Cerebral hernia caused by acute subdural hematoma
3. Diffuse brain edema/swelling
4. Dilated double pupils caused by TBI.

Steps:

1. The unilateral frontotemporoparietal large craniectomy
   (a) Position: supine, head to the lateral about 45°, shoulder padded high 15° in surgery side.
   (b) Scalp incision: starting from the zygomatic arch up—1.5 cm beyond tragus—the auricle- parietal tuber—to the midpoint of the line along the midline sagittal forward-to former hair line—large "?" flap (Fig. 1).
   (c) Cranial window: the size is equivalent to two-thirds of the area of one side, and the average size is 12 × 15 cm^2 (Fig. 2a, b).

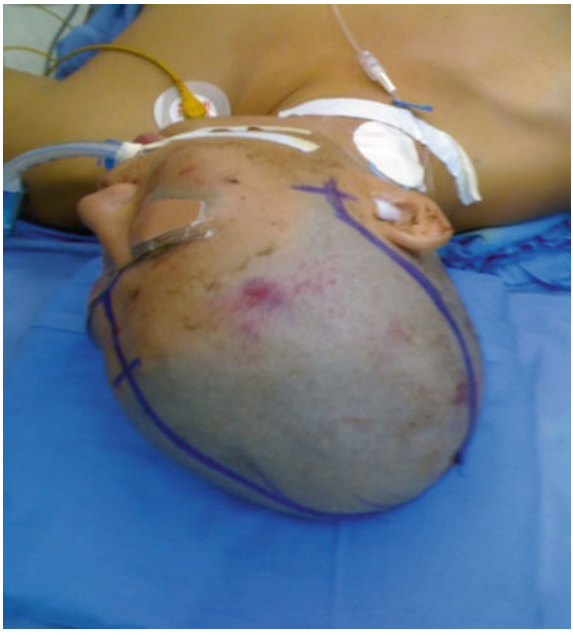

Fig. 1 Scalp incision: large "?" flap

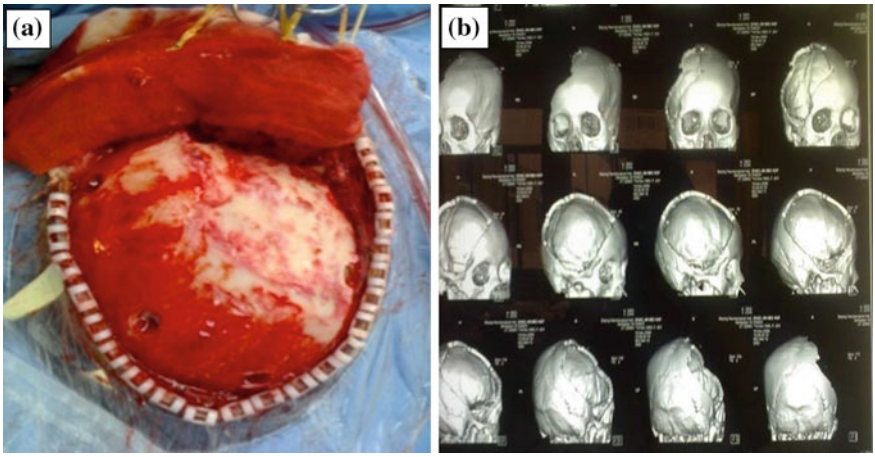

Fig. 2 Cranial window: the average size is 12 × 15 cm^2. **a** Patient; **b** CT

(d) Dura: It is easy to suturedura such as cutting like "H". The size is close to the cranial window (Fig. 3).
(e) Intracranial check: Check carefully and completely remove the hematoma/contusion.
(f) Acute encephalocele during operation.
(g) Check the airway carefully to confirm whether the airway is obstruction, using mannitol to reduce ICP at the same time.

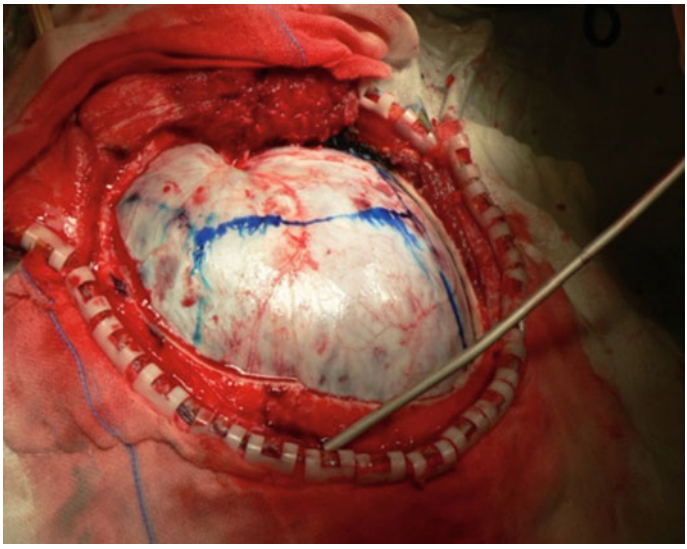

Fig. 3 Dura: suturedura such as cutting like "H"

(h) According to CT, it is to confirm the cause of acute intracranial hypertension, such as acute diffuse brain swelling (Fig. 4).
(i) Suture of dura: After the operation completed, the expanded suture of dura should be to expand the volume of the cranial cavity (Fig. 5).
(j) Superficial temporal fascia interrupted gashed (instead of the temporalis muscle resection). Height of the drainage bag is generally the same level with the head (Fig. 6).

2. Bifrontal larger decompressive craniectomy

(a) Position: supine, and shoulder padded 5 cm in both sides.
(b) Scalp incision: Coronal Suture—Pterional-Two sides Zygomatic Arch.

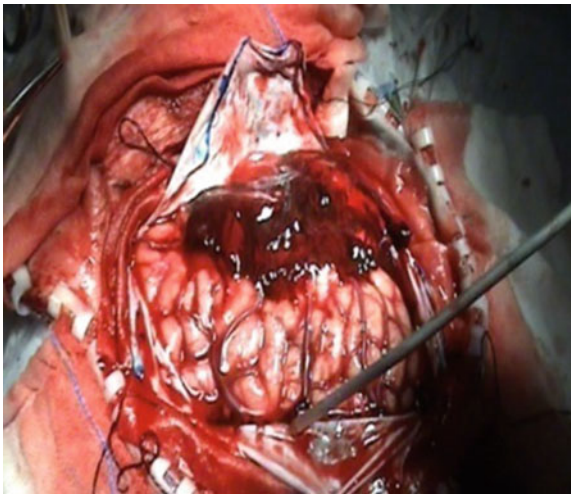

Fig. 4 Acute intracranial hypertension

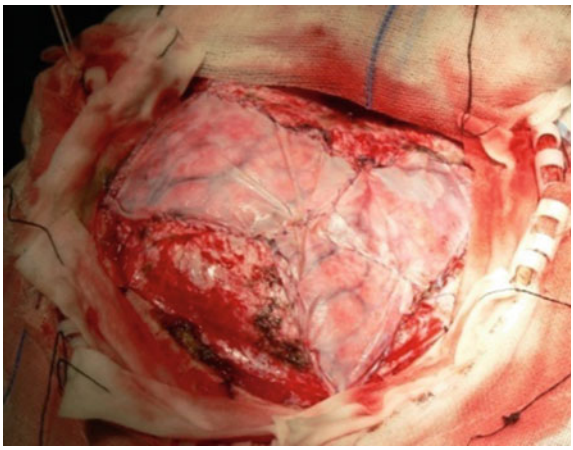

Fig. 5 Suture of dura

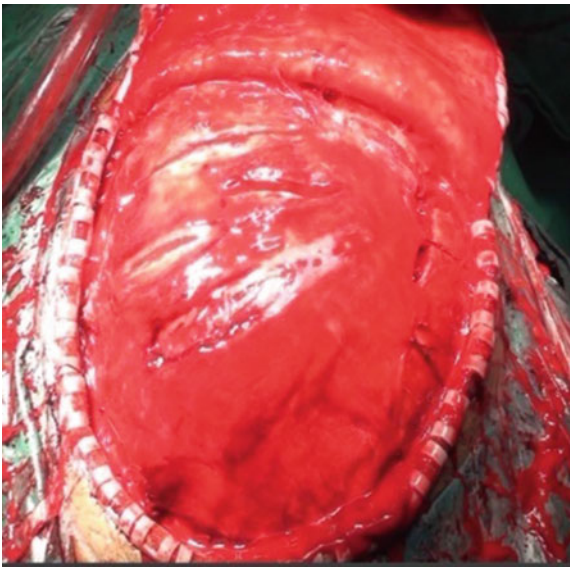

Fig. 6 Superficial temporal fascia interrupted gashed

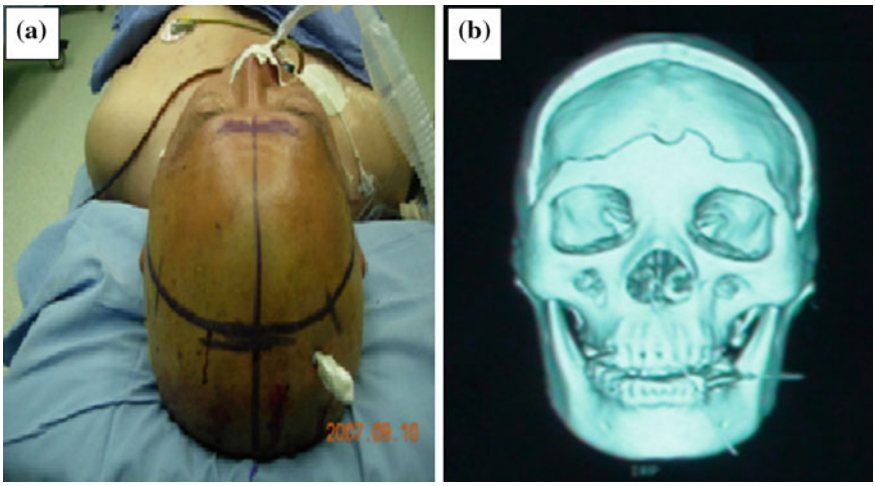

Fig. 7 Bifrontal larger decompressive craniectomy. **a** position; **b** cranial window

(c) Cranial window: Down to eyebrow, up to skin line, two sides to zygomatic arch by pterional avoiding opening frontal sinus (Fig. 7a, b).
(d) Dura: It is easy to suturedura such as cutting like "X". The sagittal sinus is ligated. The size is close to the cranial window (Fig. 8).

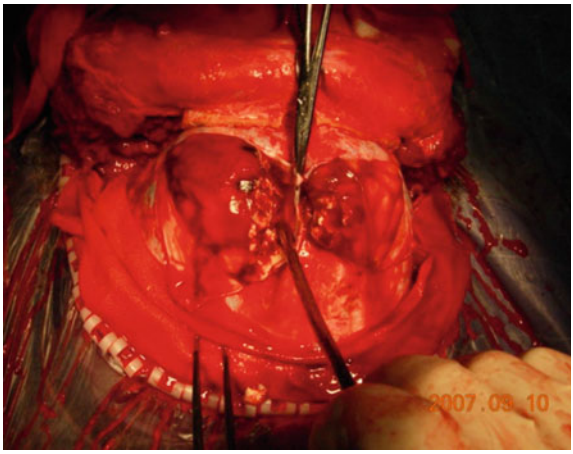

Fig. 8 Dura: suturedura such as cutting like "X"

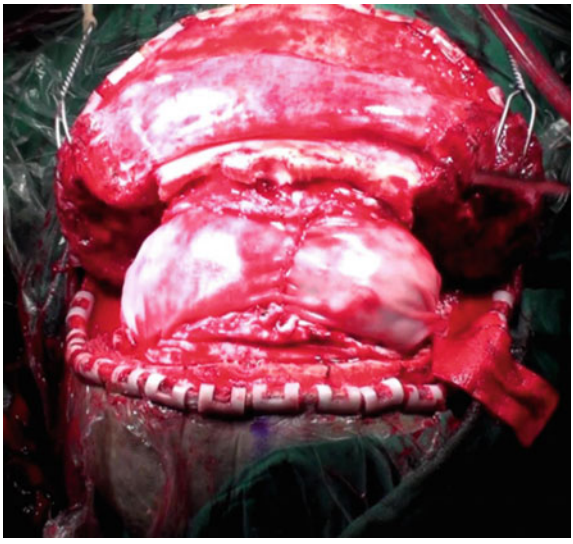

Fig. 9 Suture of dura

(e) Suture of dura: After the operation completed, the expanded suture of dura should be to expand the volume of the cranial cavity (Fig. 9).
(f) Superficial temporal fascia interrupted gashed (instead of the temporalis muscle resection). Height of the drainage bag is generally the same level with the head (Fig. 10).

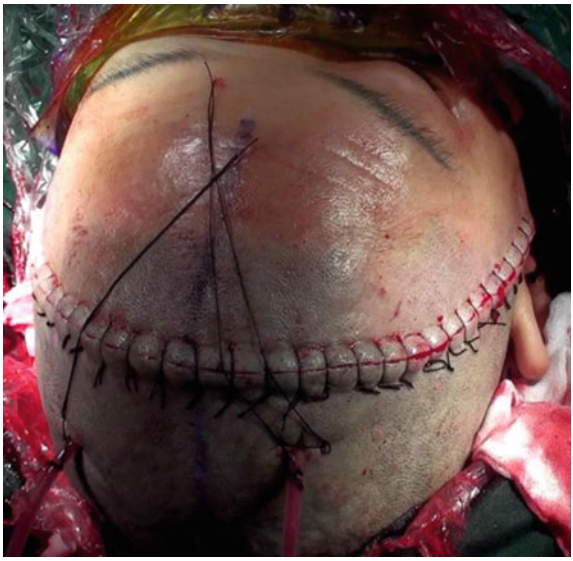

Fig. 10 Superficial temporal fascia interrupted

2 Outcomes After DC in TBI

TBI is known to be a highly varied disease and the factors that govern the outcomes are partly implicit. These concerns complicate clarification of all trials in TBI, and the case series and trials involving DC in TBI are no exception. Recognition of this knowledge gap has driven recent efforts to systematize data collection in the TBI literature.

A 2015 meta-analysis on the outcomes of early DC after severe TBI included 8 articles and 256 cases [17]. The results of this meta-analysis demonstrated that the benefits of DC in cases of TBI were not significant enough for DC to be recommended over conventional medical management. However, the limitation of results was the lack of the number of high-quality studies.

A 2012 meta-analysis on the relation between DC and ICP, CPP for TBI comprised of 20 articles and 479 cases [2]. The meta-analysis showed that ICP was decreased immediately after DC and CPP increased. In our study about the effect of DC for severe TBI patients with fixed dilated pupils [3], the 6 month follow up showed: 39.76 % of patients (DC group) and 87.80 % of patients (CC group) died, and 34.33 % of patients (DC group) and 0 % of patients (CC group) had mild or no disability.

Andrews et al. [6] showed that therapeutic hypothermia with standard care could reduce ICP but outcomes were not better than those with standard care alone. During this clinical trial, DC was not used more often, stage 3 treatments (barbiturates and decompressive craniectomy) were used if all stage 2 treatments failed to

control intracranial pressure. In that stage, the brain may be under irreversible edema. DC can reduce the ICP immediately, but the outcomes were related to lots of factors. In the future, in our experience, when ICP was more than 20 mm Hg, we need to take DC for patients with severe TBI.

Mhanna et al. [7] disclosed that there was no significant differences in survival between patients with DC and controls for children (71 % [12/17] vs. 82 % [14/17], respectively; $p = 0.34$). However, among survivors, at 4 years (IQR 1–6 years) after the TBI, 42 % (5/12) of the DC patients had mild disability or a Glasgow Outcome Scale score of 5 vs. none (0/14) of the controls ($p = 0.012$). In a study of 23 patients younger than 19 years of age, Jagannathan et al. [18.] reported that DC group had a favorable outcome, with a mean GOS score of 4.2 at follow-up. The outcomes was similar to the Pérez Suárez et al. [19] report.

3 Complications of DC

Complications happen during all surgical procedures, particularly in critical medicine; as such DC has been associated with a high rate of complications. The most common complications are hydrocephalus, subdural hygroma and infection.

A 2015 review on the complications associated with DC included a total of 142 eligible records. Kurland et al. [20] showed that complications were of three major types: hemorrhagic, infectious/inflammatory, and disturbances of the CSF compartment.

However, Mhann et al. [7], in comparison with their controls, patients who had a DC had a higher percentage of extradural hemorrhage, skull fractures, cerebral herniations, and cerebral edema underlying a more severe TBI on their admitting CT scans. However, the group of patients who had a DC had fewer CSF drainage devices in place in comparison with the control group. Although DC is known to be a simple surgical technique, complications commonly occur, sometimes with significant clinical impacts on patient outcome.

4 Conclusion

Decompressive craniectomy can save lives for patients with severe TBI, but many questions still remain for its application, and the outcome is highly related to the severity of the initial TBI. DC is a potential therapeutic option in a variety of situations, and neurosurgeons should be aware of all the complications of DC.

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Current Developments on Traffic Medicine

Jihong Zhou and Jun Qiu

Abstract Traffic medicine is a multidisciplinary and comprehensive subject pertaining to the development of traffic injury and is a subject of science and technology which studies the generation rules, preventions and treatments of traffic injuries. The disciplines contributing to traffic medicine include psychology, sociology, statistics, engineering, biomechanics, law, policing, policy analysis, pharmacology, emergency medicine, surgery, as well as rehabilitation medicine and much more. This chapter mainly shows the development of traffic medicine as below. (1) A good road safety strategy is the primary key for traffic safety. WHO calls countries to have their own road traffic safety strategies to control their traffic injuries and to make safer roads. To achieve safety strategies, countries need continuously to perfect road traffic safety laws and regulations, to provide safer roads and vehicles, to guide road user with safe behavior, and to provide efficient emergence system and medical care standards for the traffic injuries. (2) The study on epidemiology is a very important basis of looking for effective and targeted countermeasures on traffic injury control and prevention. The big data mining is an innovative and effective strategy for traffic safety. The cores of the big data are data integration, data link construct, data mining and service. (3) Traffic injury is the leading cause of death for children under the age of 19 in the world. Many projects which are driven by science studies, involving innovative behavior psychology—social theory and technologies, hierarchical school educations and multi-level implementation have effectively reduced child traffic injuries. (4) More and more studies focus on diseases and sickness, medicines and chemical reagents that may involve in traffic accidents, such as: attention deficit/hyperactivity disorder (ADHD), obstructive sleep apnoea, morbid obesity, dementia, after stroke, hearing impairment, epilepsy, hypoglycemia, posttraumatic stress disorder (PTSD), pregnancy, etc. (5) The performance and distinct biosocial profiles of high-risk drivers (HRDs) were studied by means of some new technologies, such as in vehicle driver monitoring system and medical-psychological assessment (MPA) system, and

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some positive results have achieved. (6) In recent years, forensic medicine of traffic injury has combined with more factors of clinic medicine, social psychology, vehicles, road and road users. (7) A high-efficiency medical rescue and care system could minimize potentially avoidable the deaths and the disabled caused by traffic injury. An effective medical rescue and care system should include an effective and efficient communication network, transport vehicles, pre-hospital care providers, emergency department, ICU, specialized department, and effective medical care standards. (8) There are more and more new developing traffic safety technologies appearing. In-vehicle information systems (IVIS), vehicular networking, internet of things (IoT), vehicular cloud computing (VCC), intelligent transportation systems(ITS), etc. make driving more safe. Intelligent safety roads will be more forgiving of humans' errors and could communicate with vehicles initiatively. Examples are presented in this paper.

Keywords Traffic medicine · Traffic injury · Traffic safety · Driving adaptability

1 Introduction

Data from World Health Organization (WHO) showed that there were more than 1.25 million people die on the world's roads in 2013, making road traffic injuries a leading cause of death globally [1]. Road traffic injuries have become a very important public health problem in our world, especially for low and middle-income countries in which road traffic injuries are a very severe development issue.

Traffic medicine is a multidisciplinary and comprehensive subject pertaining to the development of traffic injury. The first paper with insightful contents on car driving was published in the American Journal of Psychology in July 1938 [2]. Since that time an enormous technical literature devoted to traffic crash, traffic injury and traffic safety has emerged, resulting in a solid edifice of detailed knowledge about traffic medicine [3].

Traffic medicine is a subject of science and technology which studies the generation rules, preventions and treatments of traffic injuries. Traffic medicine is a subject that encompasses all those disciplines, techniques, methods, and policies aimed at reducing the harm traffic crashes inflict on human beings. It includes studies about characteristics and classification of traffic injury, epidemiology of traffic crash and injury, psychology and ability of road users, injury mechanism, death causes, emergency medical care and treatment of traffic injury, traffic injury prevention and traffic safety. For the traffic safety, it also includes studying the knowledge and ways to enable those with reduced capabilities due to aging, illness, drug usages, and policies to prohibit from driving which no longer able to drive with adequate safety. The disciplines related to traffic medicine include psychology, sociology, statistics, engineering, biomechanics, law, policing, policy analysis, pharmacology, emergency medicine, surgery, rehabilitation medicine and so on [4].

Some latest developments on traffic medicine will be list as follows in this paper.

2 Road Safety Strategy

Road traffic crash causes more than 1.25 million deaths per year and is a leading cause of death globally, and is the first cause of death among those aged 15–29 years. Road traffic injury is a heavy burden of national economies and households, and also a difficult burden for the sustainable development goals of the world and each countries. Facing the severe fact, it is very important that there must be definite road safety targets and goals for every region, every country and whole globe to effectively reduce and control road traffic injuries.

In September 2015, heads of state attending the United Nations General Assembly adopted the historic Sustainable Development Goals. One of the new Sustainable Development Goals targets is to reduce 50 % of the global road traffic deaths and injuries by 2020. Inclusion of such an ambitious road traffic fatality target is a significant advance for road traffic safety. It is also a significant advance that more and more people have accepted the knowledge based on strong scientific evidence that there is considerable evidence about interventions that are effective at making roads safer. There are many countries that have successfully implemented these interventions have seen corresponding reductions in road traffic deaths.

WHO has been promoting the Decade of Action for Road Safety (2011–2020) that calls on countries to implement the measures identified internationally to make their roads safer. The campaign has promoted countries and international community to do more for the road traffic safety and to galvanize greater and faster actions. Based on empirical and epidemiological researches, WHO calls countries to have their own road traffic safety strategies to control their traffic injuries and to make safer roads. WHO asks each country to focus their strategy more on road safety legislations, safer vehicles and roads.

2.1 Road Safety Legislations

According WHO's data, there are 17 countries, representing 409 million people, have amended their traffic laws on one or more key risk factors for road traffic injuries in the last 3 years and bring them in line with best practice. It is believed that the most positive changes to road user's behavior will occur when these road safety legislations were supported by strong and sustained enforcement, and public awareness. Road safety laws could improve road user's behavior and reduce the amount of road traffic crashes, deaths and injuries, especially laws about speed, drink driving, seat-belts, helmets and child restraints which relate to the five key risk factors of road traffic.

Research showed that the likelihood of traffic crash and severity of traffic injuries are rising when the average traffic speed rise, especially for motorcyclists, cyclists and pedestrians. The death rate of adult pedestrian has less than 20 % when impact speed of car is less than 50 km/h, but the death rate will be 60 % when impact

speed is 80 km/h. WHO suggests that maximum urban speed limits should be lower than or equal to 50 km/h, and should has sustained and visible speed limit legislation enforcement.

WHO indicates that drinking driving increases the likelihood of road traffic crash, traffic death and serious injury. Setting and enforcing legislation on blood alcohol concentration (BAC) limits of 0.5 g/L can significantly reduce alcohol-related crashes. For young and novice drivers, the legislation of lower BAC limits is suggested as BACs ≤ 0.2 g/L. There only are 34 countries, representing 2.1 billion people, have drinking driving laws in line with best practice. So it is necessary to extend the legislation and good practice globally.

Motorcycle helmet laws should apply to all riders and should combine with specify helmet quality standard. Research showed that motorcycle helmet can reduce almost 40 % of death and 70 % severe injury in motorcycle traffic crashes. But only 44 countries, representing 1.2 billion people (most in high-income countries), have laws that require the helmet to be weared and refer to a particular helmet standard. South-east Asia region and the western pacific region who are known to have a high proportion of motorcycle deaths have less laws related with motorcycle helmet wearing.

Seatbelt can prevent the fatality among drivers and front-seat passengers by 45–50 %, and the minor and serious injuries by 20–45 % respectively. Seatbelts also can reduce fatal and serious injuries by 25 % and minor injuries by up to 75 % for rear-seat passengers. Though 105 countries have comprehensive seatbelt laws, covering 67 % of the world's population, but only 52 countries are good in the enforcement of seatbelt laws. It suggests there are a lot should be done about the enforcement of seatbelt laws all around the world.

Child restraints could reduce the likelihood of crash fatalities by proximately 90 % among infants and between 54 and 80 % among young children. Only 53 countries have a child restraint laws based on age, height or weight, and apply an age or height restriction on children sitting in the front seat, representing just 17 % of the world's population. It still a big challenge for many countries to set the laws and to achieve compliance with child restraint laws.

2.2 Safer Vehicles and Roads

To provide safer vehicles and roads also are very important traffic safety strategies for each government.

Safe vehicles play a critical role in averting crashes and reducing the likelihood of serious injuries. The United Nation (UN) World Forum for Harmonization of Vehicle Regulations is the primary global body who is responsible for the development of passenger car safety standards. Its regulations provide a legal framework covering a range of vehicle standards which would potentially save many lives if it was applied to the manufacturing and production standards. To date, most countries

fail to apply minimum UN safety standards to new cars, only 40 countries which are high-income countries meet all seven vehicle standard regulations.

Road infrastructure has traditionally maximized mobility and economic efficiency at the expense of safety, especially to non-motorized road users. In many industrialized countries non-motorized road users have to face the mix traffic mode more and more severely and to involve in fast flowing vehicles. Ensuring safety measures are implemented in the design of road infrastructure projects can result in important safety gains for all road users. This is particularly true where road design and maintenance are strengthened by a safe system approach, which makes allowances for human error. The use of infrastructure interventions to help manage speed and reduce the likelihood of traffic crash (such as road widening and raised pedestrian crossings), and interventions to mitigate the severity of the traffic crash (such as using roadside barriers and roundabouts), all reduce road traffic deaths and injuries.

2.3 Implementation of Traffic Safety Strategy

To have the first level road traffic safety, it is important for every country not only to have a good road safety strategy, but also to have excellent practice and implementation. It is a common challenge for most countries in the world. To achieve safety strategies, countries need continuously to perfect road traffic safety laws and regulations, to provide safer roads and vehicles, to guide road user with safe behavior, and to provide efficient emergence system and medical care standards for the traffic injuries.

To achieve significant improvements, it is very important that there is a systematic approach in traffic safety management for each country [5]. (1) The first thing is to define the burden of road traffic accidents in the country. It includes the monetary valuation of the prevention of a fatality crashes. Generally, there is a negative correlation between the country's road safety situation and its monetary valuation of a statistical life. Namely, countries with poor road safety situation have low values of statistical life and vice versa. (2) The decision maker (government) must give commitment. Even the strategies and measures are the most efficient, these will not be realized without commitment from decision makers. For example, for the Vision Zero policy in Sweden, their parliament passed the Road Traffic Safety Bill on Vision Zero in 1997. (3) To create a responsible body for road safety on the national level. A National Road Safety Commission/Committee should include the relevant departments, and the most important is that it should be chaired by a committed person, respected by all departments. Before jumping to road safety actions and countermeasures, the problems related to road traffic safety should be identified in a systematic way. (4) Monitoring of performance. All performance of policies and countermeasures should be monitored and evaluated continuously. Indicator data on traffic safety performance should be collected accurately and their

status should be followed-up persistently and in the event of deviation from the target, relevant measures should be applied promptly.

2.4 Some Cases

In recent years, more and more countries and regions have made road safety strategies and made the traffic safety become a responsibility of the country and the society. For example:

Australian Transport and Infrastructure Council released a National Road Safety Strategy (NRSS) for Australia in 2011. The strategy is based on Safe System principles embracing the principle that no person should be seriously injured or killed on Australian roads in a longer term. NRSS asking for a reduction of at least 30 % of deaths and serious injuries on its roads by the year 2020. NRSS will focus on four key aspects—Safe Roads; Safe Speeds; Safe Vehicles; and Safe People. (1) Safe Roads: All road authorities at all government levels are asked to make sure that safe system principles are applied to every new road projects of the country. (2) Safe Speeds: The NRSS calls for a series of initiatives aimed to reduce speed-related risks. (3) Safe Vehicles: There should be a number of comprehensive regulatory and consumer tests to ensure that proven safety design features and technologies are mandated in all new vehicles of Australian. (4) Safe People: It includes to improve driver and rider behavior such as provisional licence periods, supervised learner driving hours, sanctions for speed and alcohol offences, passenger night time restrictions, and mobile phone bans [6].

Iran had passed a new traffic law in 2010 that replaced the previous one dating back to 1968. The new law have heavier penalties for major offences such as speeding, driving under the influence and dangerous driving; with stricter law enforcement, graduated driving license, and universal coverage of medical treatment costs for all traffic injuries. At the same time, Iran has noticed that there are some subjects need to be improved, for example: (1) Driving behavior either in public drivers and in private drivers; (2) Against driving under influence of drugs; (3) Improving the helmet usage in motorcycle riders; (4) Driving examinations about medical and psychological fitness for driving safety; (5) Safety vehicle manufacture within Iran; (6) More emphasis on expansion of safe highways in transportation engineering [7].

Netherlands has entered the aging society like many other developed countries. At the same time, Netherlands is a flat country with a moderate climate and generally small distances between destinations, bicycling is a very important mode of transport also for older persons. Older cyclist becomes a important factor in traffic safety in Netherlands. In the road safety strategy of Netherlands in recent years, they strengthened studies on traffic adaption of older people to help aging person and improve traffic safety. The main ways are strengthening traffic safety studies related with neurological disorders, dementia, impairment of sight and hearing of elders; studying on license issue according the physical condition, specific training

of safety riding adaption, and development of intelligent driving support/auxiliary system. These works have made good progress.

3 Epidemiology and Big Data

The study on epidemiology is a very important basis of looking for effective and targeted countermeasures for traffic injury control and prevention. It is a common view of experts all around the world that low-income and middle-income countries should strengthen the road traffic safety investigation to face the high traffic injuries and death caused by their fast increasing road traffic. The investigation of traffic safety not only should pay attention to road users, vehicles, roads and environment factors, but also should keep a watchful eye on policy, culture, education, engineering, medical care, and organization system, etc. at the same time.

WHO's data showed that road traffic caused 1.25 million deaths in 2013 all around the world and 84 % of the death came from low-income and middle-income countries. The fatality rates of low-income countries is more than double times of that in high-income countries. And it was disproportionate that the number of deaths relative to these countries' level of motorization. 90 % of road traffic deaths occurred in low-income and middle-income countries, but the number of vehicles in these countries was just accounted for 54 % of the world's. There are the highest road traffic death rates in the African region continues. The lowest death rates were in the high-income countries in European region which had been very successful at achieving and sustaining reductions in death rates despite rising motorization. Almost half of all deaths on the world's roads were motorcyclists (23 %), pedestrians (22 %) and cyclists (4 %) who had the least protection in 2013. There was the highest proportion of pedestrian and cyclist deaths in African region at 43 % of all road traffic deaths. The death rates were relatively lower in the south-east Asia region than that in African region.

This partly glasses the level of traffic safety measures appropriate to protect different kinds of road users and the predominant forms of mobility in different regions.

In recent years, more and more countries are promoting various projects for deep investigation on road traffic injury. For example, China has established traffic injury database and developed China In-Depth Accident Study (CIDAS) project. Kingdom of Saudi Arabia is carrying out a Injury Surveillance System project aimed to gather regular ongoing information for prevention and control of injuries and efficient use of resources.

The United States, Britain, Australian, Sweden, China, India and many other countries are process studies on epidemiology, prevention and control of traffic injuries based on their traffic accident and injury databases.

By means of epidemiologic studies, it shows that several factors are most notable causes in road traffic crashes. Some factors such as speed, road horizontal curvature, and congestion were found to have mixed effects on road traffic safety and need

particular examination. Future research directions on the traffic safety factors are improving most notably data quality, making use of advanced statistical models, and probing into the factors in rural areas and developing counties. It is also a need to have more deep investigations in the factors which are related with the effect of speed and congestion on road accidents, whether road traffic safety could be improved by the curvature improvement. The use of more sophisticated statistical models may be helpful to better understand the effect of factors on road accidents [8].

Today is the big data era. The traffic safety arena has great expectations of taking advantage of big data. It could make the traffic safety first by means of big data mining analysis. Processing the big data requires the integration and standardize of the database, different analytical and data mining techniques. More big data applications for the traffic safety improving may involve the combination of planning, census, safety, roadway and land data.

Big data can make the intelligent transportation system become more popular, the traffic management and traffic injury prevention and control become more predictable and targeted, traffic become safer and more effective. At present, the big data mining and analysis have been used in traffic safety analysis, "dark spot" screen and management, safety planning advice on the climate, real-time safety strategy and implementation, et al.

The cores of the big data innovation strategy for traffic safety are data integration, data link construct, data mining and service. Traffic safety data sources are involving multiple departments, including the police records and reports, first aid (EMS), emergency departments, hospitalizations, patient outcome, forensic identification, insurance, road construction and management, vehicle information, etc. Various data system has its own fields, tables, structure and relationships. So it is very important to integrate data according the same standards, to establish chains and links between each data, to establish high quality data sets, to constitute analyzing basis for big data of traffic safety. The analysis and mining traffic data by new analysis technology, methods and models can put forward effective decisions and countermeasures, and promote efficient traffic injury prevention and treatment. The Zero Death strategy will come true some day.

4 Child Traffic Injuries

Traffic injury is the leading cause of death for children under the age of 19 in the world. Most of child traffic injuries are pedestrians, cyclists and motor riders. It is much more severe in low-income countries. Even in the UK, 60 % of child deaths are from road traffic accidents. Risk of traffic injury in children under the age of 10 is 4–10 times higher than that in other pedestrians.

In China in 2013, for example, there were 14.6 died per million children under 18 because of road traffic injury. Middle school and high school children accorded for 42 % of total children traffic death with 18.2 death per million children. It even reached to 23.9 death per million children in high school students. In middle school

and high school students, motor vehicle violation (92.3 %, 1550 cases/1679 cases) was the first reason for these traffic deaths, but the reasons of traffic modes couldn't be ignored that the most was caused by motorcycle and bicycle. Motorcycle driver was the most part of traffic deaths and followed by passenger of motorcycle in high school students, and male was far more than female. Traffic death rate was the highest in summer vacation for boys, and the death peak was located in the summer vacation and the followed two months in girls. Brain injury was the main death cause with about 80 % of deaths and was much more in male than that in female.

Many studies have showed that around schools is a high traffic risk areas to children. It is effective for significantly reducing children's traffic injuries and deaths by means of increase in walking and cycling to reduce traffic congestion, setting standard traffic signs (e.g., 30 km/h speed limit and pedestrian passage ways), continuous educations and training on security behavior and cognition of children and parents, evidence-based safety assessment and improvement.

In recent years, Many countries have set up special child road safety projects to effectively reduce child traffic injuries. Many projects are driven by science studies, involving innovative behavior psychology—social theory and technologies, hierarchical school educations and multi-level implementation.

5 Health and Traffic Safety

In recent years, more and more studies focus on diseases and sickness, medicines and chemical reagents that may involve in traffic accidents, especially for some special groups of people, such as elder. Following are some aspects being concerned recently.

Attention deficit/hyperactivity disorder (ADHD) who are more common in children and adolescents. ADHD involved in road traffic accidents is more in male than in female, and had much higher ADHD scores than ADHD non-involved in road traffic accidents. More ADHD-related symptoms participated more likely in traffic crashes, both being responsible for and victim in accidents. It will be helpful for the traffic accidents prevention that ADHD symptoms is acknowledged in the curricula in driving schools [9].

Obstructive sleep apnoea is an increasing common disorder of repeated upper airway collapse during sleep, leading to oxygen desaturation and disrupted sleep. Features include snoring, witnessed apnoeas, and sleepiness. Research showed that obstructive sleep apnoea related with road traffic accidents. This may be related with fatigue, sleepiness, systemic hypertension, congestive heart failure, myocardial infarction, stroke, and diabetes mellitus which probably caused by obstructive sleep apnoea [10].

Morbid obesity could significantly increase risk of road traffic accident. A study of morbidly obese adult patients showed that the road traffic incidence in morbid obesity patients was three times higher than that in non-obese patients (7 per 1000 patient-years vs. 2 per 1000 patient-years). Compared to non-obese controls, obese

drivers had significantly higher risks for fatality ($1.10 \leq$ adjusted odds ratio (AOR) ≤ 1.47), seat-belt non-use ($1.00 \leq$ AOR ≤ 1.21), need for extrication ($1.01 \leq$ AOR ≤ 1.23), and ambulance transport time ≥ 30 min ($1.01 \leq$ AOR ≤ 1.28). At the same time, obese drivers were less likely to drinking driving ($0.41 \leq$ AOR ≤ 0.72) and speed ≥ 65 mph ($0.78 \leq$ AOR ≤ 0.93) compared to non-obese controls. Traffic crash risks were similar before and after surgery among those who were diagnosed with an obstructive sleep apnea. Facing the prevalence of obesity in the current world, it is necessary to improve seat-belt use, vehicle design, and post-crash care for this morbid obesity population who is vulnerable in road traffic crashes [11].

More and more countries have been entering the aging society, more and more studies focused on the driving adaptability of dementia, after stroke, hearing impairment, epilepsy and other aging diseases. Most studies focused on the physiological and cognitive ability evaluation and training, legal issues, assistive technology, etc.

For the hearing loss, drivers usually adopt more careful driving manner for the safety. Compensatory strategies for hearing loss include: reduce the speed, more visual search, less distracting activities. Research showed that tactile sense has compensative effect for hearing impaired drivers, so development of tactile driving assistant system can help hearing loss people to improve driving safety and mobility.

Hypoglycemia is one of major complications related to diabetes treatment and also is an unneglectable factor for traffic safety. Hypoglycemia has been associated with cardiac arrhythmia, decreased ability to drive and driving mishap. A meta-analysis of 15 studies showed that road traffic crashes in people with hypoglycemia were 12–19 % greater than that in general populations. The most significant subgroup of persons with diabetes is those on insulin therapy. So, some researchers suggested that more restriction regulations should be established for drivers who are using insulin, drive buses and heavy goods trucks. More frequent medical examination for drivers with diabetes treatment should be taken [12].

Pregnancy causes diverse physiologic and lifestyle changes that may contribute to increased driving and driver error. A study on serious motor vehicle crash with pregnant women in Canada showed that the risk of road traffic crashes increased 42 % during the middle of pregnancy. The increased traffic accident risk was largest in the early second trimester and compensated during the third trimester. The increased risk included varied obstetrical cases, diverse populations, and different crash characteristics. There were no significantly increase of traffic related injuries for pregnant women who were involved as passengers or pedestrians, inadvertent falls or intentional injury, or self-reported risky behaviors. The absolute risk in motor vehicle accidents amounted to an estimated 1 in 50 women at some point during an average pregnancy. All nine months and the full spectrum of severity were taken into account (injury, fatal, and vehicle damage combined). Therefore researchers suggested that pregnancy is associated with an increased risk of a serious motor vehicle crash during the second trimester. Pregnant women in this period should avoid to drive, or drive more carefully. The attentions should be taken in prenatal care guidelines [13].

Posttraumatic stress disorder (PTSD) is one of the more common psychiatric disorders after injury due to road traffic accidents and other forms of trauma injury. PTSD symptoms mainly represents intrusive thoughts, avoidance behavior and hyper-arousal symptoms. It may persist or even become worse if it was not diagnosed and treated within the first 12 months. These PTSD symptoms can be debilitating extremely and lead to relationship breakdown, social isolation and ongoing psychological dysfunction. Presence of substance abuse, head injury, depression and other psychiatric disorders may further entangle the diagnosis and treatment of PTSD and other psychiatric disorders. The incidence of PTSD has been reported at 10–25 % in a lot of well controlled studies, but it has been reported that was less than 10 % in some other studies. It was showed that 31 % of patients had developed a psychiatric diagnosis at 12 months and 22 % had a new psychiatric diagnosis in a large longitudinal study in which patients were followed up to 12 months after injury. It has also been shown that the functional impairments (psychological, physical, environmental and social impairment) at 3 months increase the risk of the development of a psychiatric disorder at 12 months. The most common new psychiatric disorders were generalised anxiety disorder, depression, PTSD and agoraphobia. Many trauma patients with psychiatric symptoms did not seek professional assistance. There were only a minority of patients (33 %) sought mental health treatment at 12 months. The promptly diagnosis and treatment of patients with PTSD after traffic injuries depends on appropriate presentation and available treatment options which includes cognitive behavioral therapy, desensitisation treatment and drug treatment [14].

More and more medicines which could influence road traffic safety are realized. For example, sedative-hypnotic drugs are proved that they could decrease driving ability and is concerned by researchers in recent years, such as zopiclone. A study showed that zopiclone 7.5 mg has significant and clinically relevant performance-impairing effects on driving in the morning, until 11 h after bed time ingestion. The effects of zopiclone 7.5 mg are comparable to the effects of a 0.5–0.8 mg/mL blood alcohol concentration, which has been associated with a 2–3 fold increase in the risk of involving in traffic accidents. It is suggested that patients using an evening dose of zopiclone 7.5 mg should avoid activity in skilled works and driving in the morning after intake [15]. Selective serotonin reuptake inhibitors (SSRIs) which are widely used medications to treat several psychiatric diseases and depression also are known that have some adverse effects on traffic safety. Its most reported undesirable effects referring to driving impairment were agitation, anxiety, headache, sleep disturbances, increased risk of suicidal behavior, and deliberate self-harm. There were inconsistencies between the outcomes of current experimental and epidemiologic studies. It suggested there should be more experimental and epidemiologic studies to elucidate the relationship between SSRI use and road traffic safety [16].

6 High-Risk Drive and Driving Adaptation

High-risk drivers (HRDs) are those are prone to repeat episodes of dangerous driving and over-represented in road traffic accidents. Traditional HRD research has focused on HRDs healthy, their self-reported personality features and driving behavior or intentions, but has not focused on HRD populations and direct observation of their risk-taking behaviors. So the traditional researches couldn't directly represent core characteristics of HRDs.

Recent researches showed that HRDs have different performance and distinct biosocial profiles. HRD with mixed profile closely resembles a 'cold' antisocial phenotype in which chronic under arousal interferes with avoidance learning which leads to a social risk seeking. Speeders/reckless drivers comprise a phenotype involving the most dangerous behaviour and externalizing features. The phonotype includes competitiveness, reward driven decision-making, impulsivity, and weak inhibitory control. The impaired drivers usually showed poor inhibitory control, safe driving in simulation and alcohol misuse. It means that HRDs' risk taking involves and interaction between alcohol misuse and poor inhibitory control. The dysregulation in HRDs related to the prefrontal cortex [17, 18], and the hypothalamic-pituitary-adrenal axis [19, 20].

The traditional interventions for HRDs are training and education based programs. But relatively few effective evaluation of these road safety education programs have been done. So we don't know whether these interventions are effective on reducing the subsequent crash risk of participants. There even are some negative reports that some interventions have been shown to increase the crash risk of drivers [21]. In recent years, some new technologies have been applied to HRDs training and have gotten good results. For example, driver monitoring system can identify key behaviors of high risk drivers and the system has demonstrated how it can be utilized to manage those HRDs behaviors and reduce crash risk [22]. This new technology enables intervention models that are more focused on the specific risky driving behaviors of individual drivers. A success intervention model for high-risk drivers, such as traffic offenders and young drivers, should involve a systematic long term monitoring and coaching/counseling of the individual driver. This type of intervention models has already been widely adopted in programs for drink driving offenders and has showed good results [23].

Medical-psychological assessment (MPA) system could significantly improve diving eligibility, prevent relapse and promote the driving safety for HRDs. The MPA is an integrated medical and psychological examination which usually contains elements Questionnaire (biography, knowledge of driving rules, driving history), Medical examination (physical examination, laboratory analysis, medical history), Psychological interview (change of behaviors and attitudes, alcohol or drug consumption style, perception of problems in future) and Psychophysical computer-based test of cognitive functions (concentration, visual perception, reaction capacity, vigilance). Research in Germany showed that the MPA-system is an effective measure in preventing recidivism and an important approach for

improving traffic safety with a significant decrease of the percentage DUI- and DWI-offenders with a relapse. But there are still problems with drivers with multiple traffic offences (e.g. tail gaiting, speeding) who have a relapse rate of more than 40 % [24].

Companied with the age growing older, the physiological and mental functions of elders are weakening. These make the elders be a important part of HRDs. The education and training for older drivers is a very important works in the future, because more and more older people need and want to be able to keep safe driving when the world has more and more older people. It is not necessary for older drivers to have more information concerning traffic rules, etc., but rather better helping older drivers to understand of themselves, their skills, their health restrictions, and their abilities to ensure their daily mobility safely. Hierarchical models of driving education and training are suitable in increasing understanding of older drivers' needs and abilities. In these models, goals for life, skills for living, social environment, driving ability, driving assistant system and skills affect everyday decision making in general, which all relate with elders' traffic safety. Giving up driving for older driver is very much a social decision. It is also very important that their closest companions take part to help them to make the discussion of traffic safety issues for older drivers [25].

7 Forensic Medicine in Traffic Medicine

Most forensic medicine reports of traffic injury were mainly focused on autopsy data analysis of traffic crashes, the reconstruction of the process of traffic crashes and injuries, classification of accident from suicide, et al. In recent years, more and more researches study the forensic problem of traffic injuries combined with factors of clinic medicine, social psychology, vehicles, road and road users.

The traffic medicine under the perspective of forensic medicine mainly focuses on the following areas: (1) Determination and prognosis of driving fitness with respect to illness, age and substance abuse/dependence. (2) Driving under the influence of alcohol, drugs and medicaments. (3) Chemical-toxicological analyses, breath alcohol analyses, drug recognition training and et al. (4) Demographic change, driver licence and elder medical condition. (5) Crash reconstruction, with special respect to the investigation of drivers. These also mean that traffic forensic medicine concerns drivers' fitness to drive, careful medical conditions (such as epilepsy and dementia) and psychological problems (including psychiatric illness and substance abuse of road users), toxicological screenings and investigations et al. [26, 27].

Crash reconstruction is a hot and fast progressing aspect of traffic forensic medicine. In the crash reconstruction, researchers concern the pattern of injury of vehicle occupants, vulnerability of children in vehicles, effect of seatbelts, injuries to motorcyclists, pedestrians and pedal cyclists, cause of death, railway injuries, homicide and suicide, special dynamics of other motorized transports and so on.

Special questions from the forensic medical point of view are associated with heavily mutilated and severely decomposed bodies, the pattern of injuries, toxicological findings, and trace evidence. For these situations, the vary considerably and the whole scale of forensic medical analyses is required. So it will be important to promote joint research uniting forensic medicine, clinical medicine, automotive engineering, and road engineering, etc. [28].

8 Medical Rescue and Care of Traffic Injury

A high-efficiency medical rescue and care system could minimize potentially avoidable deaths and disabled people caused by traffic injury. The medical rescue and care of traffic injury are generally divided into pre-hospital care and in-hospital treatment.

An effective medical rescue and care system should include an effective and efficient communication network, transport vehicles, pre-hospital care providers, emergency department, Intensive Care Units (ICU), specialized department, and effective medical care standards.

In recent years, China has made a great progress in study on standardization process of road traffic injury treatment and has significantly decreased the fatality and disability rate of severe traffic injuries [29]. In this study, hospitals located in different geographically and industrially cities in China were selected. A standard process was established as a general rule for staff training and patient treatment for medical care of severe trauma, especially for severe traffic injuries. A regional network were designed with efficiently integrating pre-hospital rescue, emergency room treatments, and hospital specialist treatments under the rule of information sharing and improving severe trauma treatments. As the results, the implementation of the standardization processes led to efficient co-operation and information sharing of different treatment services. The emergency response time, pre-hospital transit time, emergency rescue time, consultation call time, and mortality rate of patients were improved in different degree. All these results suggested that staff training and standardization processes can significantly improve the treatment efficiency of severe traffic injury based on current personnel and organizations of severe traffic injury treatments in China.

9 Safety Technology

It is a rapid developing era for traffic safety technology now. In addition to traditional active safety and passive safety technology which we are familiar with, there are more and more new developing traffic safety technologies appearing.

Development of in-vehicle technology now allows easier implementation of secondary and tertiary interventions aimed at young drivers, such as feedback

devices. Research showed that the in-vehicle feedback technology could effectively reduce some indices of young drivers' risk and strengths, risky behaviour (such as g-force events), limitations, and obstacles to implementation in primary, secondary, and tertiary prevention programs [30].

A new in-vehicle information systems (IVIS) was developed which includes a standardized task environment of information contents and applies, i.e. an advanced but relatively simple fixed-base driving simulator in a Human machine interface and the Safety of Traffic in Europe (HASTE) project which was organized by Europe to standardize the test regime for the task of driving a motor vehicle by standardizing its constituent parts. The new IVIS may be installed easily everywhere, following the HASTE guidelines, and it is a valid, reliable and efficient tool that may aid testing authorities in traffic safety evaluation. This system will formulate pass/fail criteria and safety guidelines that can be used in a cost-effective tool for safe driving training and test [31].

Internet and information technologies push traffic safety forward quickly by means of intelligent transportation. Vehicular networking has become a significant and hot research area because its specific features and applications such as standardization, road safety, efficient traffic management and infotainment. It has been used to maintain and promote Intelligent Transportation Systems (ITS). Vehicular Cloud Computing (VCC) is a new hybrid technology that has a extraordinary impact on traffic management and road traffic safety by instantly using vehicular resources, such as storage, computing and internet for decision making. The VCC is a technologically feasible and economically viable technological shifting paradigm. It converges intelligent vehicular networks towards autonomous road traffic, vehicle control and perception systems [32]. With the breakthrough of wireless technology, a plethora of its applications have shaped our modern traffic world. For the road traffic safety and crash preventions, Wireless technologies will play a pivotal role in saving lives by traffic accident prevention that it could assist drivers in detecting potential collisions from blind spots, in inclement weather conditions such as heavy rain, thick fog, etc. Wireless technologies include adaptive cruise control system for autonomous cars, automotive radars for collision detections, the 5G and Internet of Things (IoT), they provide greater level of driver assistance, decline in road accidents and a smoother traffic flow and control. Researchers have showed that wireless technologies for the automotive industry are key to the reduction of fatal road traffic accidents and could save millions lives [33].

Because bicycle-car accident usually is very dangerous and serious for cyclists. Some active safety devices to protect cyclists in bicycle-car accident are appearing. These devices that can detect cyclists were developed to be an effective countermeasure for reducing the severity of injuries and number of cyclist fatality. These devices would detect the detailed features of car–cyclist contact scenarios to estimate the collision time in the cases where bicycles emerged from behind unobstructed views and bicycle emerged from behind obstructions. These systems could be effective in preventing car-bicycle collisions and lethal accidents [34].

Modern high technologies not only will make vehicles safer, but also will provide more safe roads for road users. About safety road, most pioneer researchers

focus on more active intelligent safety road. The future Road Safety system will content more active safety factors and systems. These road system will be more forgiving of humans' errors, because more and more people realize that people using the road network will inevitably make mistakes. For example, active beside road facilities will interact with vehicles and take part in the traffic safety process forwardly by means of correcting deviated vehicle, helping vehicle back to the safety state or to stop by the roadside safety hardware. The intelligent roads who could communicate with vehicles initiatively are in researching now.

10 Others

Study showed that good behavior can influence other people to improve traffic safety. Even peer pressure can influence behavior in either way depending on what is promoted. The simple approach of good behavior can encourage a cultural shift promoting driving safety if it is spread. It can help communicating without opening windows by the side window stickers with clear messages. This can hence diminish the feeling of intimidation. Warm and good habits, such as open your window to amicably mention the issue, make a gentle hand wave in a welcome manner to the driver or passenger, upon resolution of the situation greet the person with a thumb up and a smile, have positive effects and will result in the return of a smile. It could readjust unsafe situation and even get many "Thank you" [35].

Pedestrian–vehicle conflicts are one of the most important safety concerns especially at intersections. Recent study showed that long crosswalks have significant higher risk of pedestrian–vehicle crashes which related with the higher percentage of pedestrian speed change occurrence. Pedestrians may rush or suddenly change their speed without paying attention to the surrounding conditions when they are at a long crosswalks, short green times, or other reasons. The sudden changes in pedestrian behavior at road usually cannot be predicted by the driver, which can cause severe conflicts. In general, the length of crosswalk have a significant impact on pedestrian speed change choices when pedestrian will enter to a crosswalk, and pedestrian will estimate the necessary speed to finish crossing before the onset of the pedestrian red signal indication. So, the application of two-stage pedestrian crossing will be helpful that is a potential solution to reduce crosswalk length and to make the pedestrian choice safer behavior [36].

Active and passive restraint systems are now required in all motor vehicles all around the world. Active restraints also include seat belts, either the manual lap belt that accompanies an automatic shoulder belt or the three-point seat belt (lap and shoulder belt). Passive restraints include air bags, automatic seat belts, and head restraints.

Many studies confirmed the findings of NHTSA about the seat belts that the seat belts have decreased fatalities by 43–50 % and serious injury has been reduced by 45–55 % comparing with no seat belts. Despite the injury and fatality reduction

remarkably, but the compliance with the seat belt laws is still not close to 100 %. Though seat belts reduction in fatal and serious injuries, there are reports on injuries caused by seat belts. Generally, seat belts decrease injuries in brain and facial injuries, intra abdominal solid organ injuries, and long bone fractures, but seat belts may increase injuries in hollow viscus injuries, abdominal wall hernias, thoracic injuries, and neck injuries [37, 38].

The death caused by motorcycle and bicycle accidents is very severe in developing countries. For example, up to one third of traffic deaths are due to two-wheelers in Asia. Observations showed that helmets can prevent deaths and severe head injuries effectively and can help reduce these fatalities by 25 % and more, but there is low utilization rate of helmet. Research showed that helmet had limited effectiveness in preventing facial injuries in this setting with poor helmet and poor helmet usage standards, and there were low helmet using in developing countries, especially in females and children. But many countries do not have laws about their quality assessment or how they should be worn yet. So more work is needed to do about the helmet quality and to understand the helmet wearing and rider behavior in helmet users.

Vehicle collisions with wildlife are a significant threat, especially accompanied with environmental conservation. General wildlife-vehicle collisions (WVCs) exhibit clustering on roads, which is related with specific landscape and road-related factors. WVCs usually occurred when foraging, roads bisect favorable cover, or breeding habitat for specific groups or species of species. Generally, WVCs were highest on road sections with low motorist visibility, high traffic volumes, and when roads cut through drainage and movement corridors. Birds, ungulates, carnivore collision locations and small-medium vertebrates were associated with road-side vegetation and other features such as salt pools. The WVCs were likely less to occur when a road bisected steep slopes. These characteristics are useful to facilitate application of this knowledge to planning, and design of mitigation strategies on roads. More and deeper researches are needed providing comprehensive guidelines for wildlife mitigation planning on roads [39].

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The Features of Explosive Fragments Induced Injury and Management

Jianmin Wang

Abstract Explosive weapons were mainly anti-personnel weapons in modern warfare, and fragments are the main killing factors of these weapons. Damage effect of the fragments is closely related to the shapes, speed, materials quality, and the environment in which people. We outline the epidemiology, characteristics, mechanisms and general principles in prevention and treatment of explosive fragment injury in this article. Especially, the injury characteristics of some fragments with special materials such as tungsten, depleted uranium, and some special environmental conditions such as high altitude, high heat, sea water immersion, were summarized. Our aim is to provide some references for the prevention and treatment of new high explosive fragment injury.

Keywords Explosive weapon · Fragments · Personnel injury · Prevention · Treatment

Fragment injury refers to injury from various types of firearm projectile fragments as well as bombs, missiles, mines and other explosive weapons where fragment impact ruptures, deforms or penetrates body tissue damaging its structure and function. Whether at peace or at war, fragment injury incidence rates are very high. Affected by factors such as arms control, whereas fragment injuries previously arose largely from slow-speed shotguns or homemade shotgun projectiles, in recent years because of an increase in intentional explosive actions there has been a clear rising trend in explosive injuries and concomitant fragment injuries [1, 2].

As several recent wars have demonstrated [3–5], explosive weapons are modern warfare's most commonly used deadly weapon, and the biggest cause of war casualties' deaths. During the 1991–1992 Gulf War, multinational force air strikes lasted 38 days, deployed more than 200,000 tons of bombs and launched 244

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Tomahawk cruise missiles, inflicting 70 % of overall conflict deaths (85–100,000) on the Iraqi army. In addition, 48 % of the multinational force's ground offensive casualties were also the result of Iraqi explosive weapons. According to incomplete statistics from the Kutina field hospital, during the Bosnian War injuries from mines, bombs, artillery shells and other explosive weapons accounted for 87 % of all wounds.

In 1991–1992, the Croatian War of Independence accounted for 1907 deaths from various kinds of explosive weapons. In 2003 NATO dropped over 80,000 tons of bombs and launched more than 3000 Tomahawk cruise missiles in the Kosovo War, resulting in the long-distance non-contact combat deaths of over 15,000 military and 10,000 civilian members of the Federal Republic of Yugoslavia. The application of high-technology in the military field has led to developments in explosive weaponry such as augmenting the speed of projectiles (fragments, steel balls etc.), boosting the amount of charge in warheads, increasing factors causing fatal injury, widening the area of fatal impact from the point of detonation, reinforcing projectiles' penetrative ability and broadening the use of precision-guided munitions. Thus within a short space of time, a large number of casualties can occur with injuries that can be complex, critical and difficult to treat.

1 Explosive Fragment Injury Epidemiology

1.1 Explosive Fragment Injury Incidence Rate

In modern warfare, explosive weaponry is the most commonly used weaponry causing fatal injury. Although damage factors also include the blast shock wave and thermal radiation, the main cause of fatal injury remains explosive fragments. In regards to the incidence rate of injuries from explosive weapons, previous war survey data illustrates that explosive injury and resulting fragment wounds remain a major part of modern warfare injury, showing a clear upward trend (Table 1) and, since World War 2, are the highest ranked causes of injury. During the Gulf War, the ratio of explosive weapon injury to other kinds of injury reached more than 80 % (Fig. 1) [4, 5].

Analyzing the relationship between different injury factors from explosive weaponry and biological causes of death, a combination of explosive fragments and blast shockwaves are the main cause of fatal injury, followed by incidents caused solely by exposure to explosive fragments. Different injury factors and their relation to biological death types are illustrated in Table 2 and Fig. 2.

Table 1 The ratio of different kinds of war injuries sustained in different wars (%)

Event	Explosive injury	Gunshot injury	Blade injury	Contusion injury	Burn injury	Frost bite	Concussion	Poisoning	Other
Anti-Japanese War	30.1	52.8	4.0	10.3	0.0	0.0	0.0	0.0	2.8
War of Liberation	58.9	36.9	0.0	0.0	0.1	0.0	0.5	0.0	3.6
Korean War	62.2	17.2	0.2	6.1	1.6	10.5	0.3	0.4	1.5
Sino-Vietnam War	57.7	37.1	0.2	2.8	0.9	0.0	0.0	0.0	1.3
Second World War	55.0	32.4	0.2	12.3	0.0	0.0	0.0	0.0	0.0
Battle of Vi Xuyen	53.7	6.7	2.2	22.7	4.2	0.0	1.1	0.0	9.4
Afghanistan War	60.0	38.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0
Vietnam War (American Casualties)	57.1	42.1	0.0	0.0	0.0	0.0	0.0	0.0	0.8
Vietnam War (Vietnamese Casualties)	45.0	19.0	0.0	0.0	9.0	0.0	0.0	0.0	27.0
First World War	35.3	64.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Falklands War	55.8	31.8	0.0	0.0	0.0	0.0	0.0	0.0	12.4
Gulf War	81.0	19.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

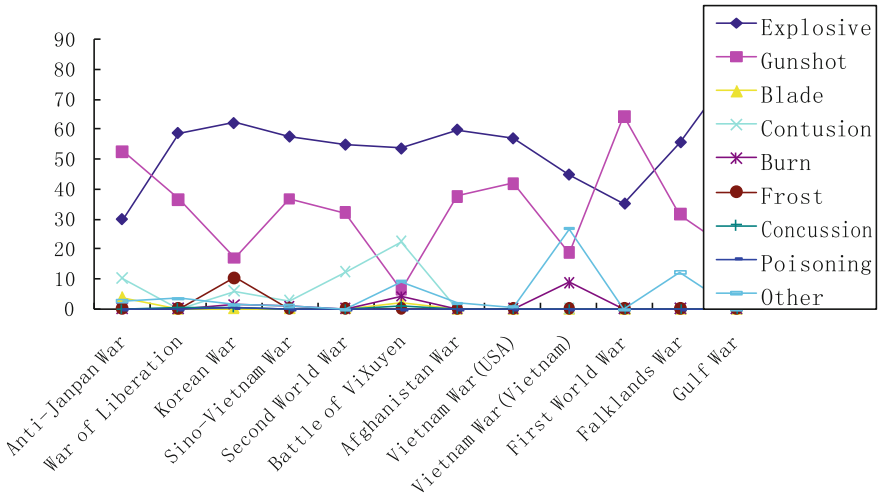

Fig. 1 Different factors causing personal injury in successive wars

Table 2 Different injury factors and related biological death rates

Cause of death	Explosive fragments + blast shockwaves	Explosive fragments	Blast shockwaves	Burn injuries	Total deaths
Number	31	30	5	2	68
Incidence rate (%)	45.6	44.1	7.4	2.9	

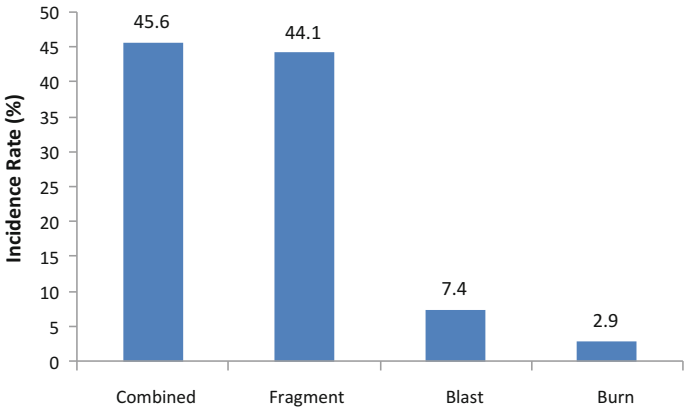

Fig. 2 Different injury factors and related biological death rates

1.2 Explosive Fragment Injury and Effects on Different Parts of the Body

Amongst modern warfare injuries, the majority are explosive fragment injuries. At the same time, because of differences in their exposed area and anatomical position, injury incidence rates for different parts of the body are also different.

Amongst war injuries, craniocerebral wounds have a high rate of incidence. In the Iraq and Afghanistan Wars, there were 1255 cases of brain injuries, of which 774 involved fragment penetration and 481 blunt trauma. An analysis of 10 years of recent American army injuries demonstrates that the majority of early deaths before arrival at military hospital are the result of craniocerebral wounds. Amongst injuries affecting different parts of the body, craniocerebral wounds account for 42.3 % of injuries causing immediate or early death (Table 3).

Maxillofacial fragment injury is a common injury in the firearm wound class. According to reports [4], head and facial injuries accounted for 13 % of injuries in the Soviet-Afghan war and 19 % of injuries in the Chechen conflict. During 1991s Operation Desert Storm, 17.3 % of injuries sustained by the US military were head and face injuries and 4.3 % neck injuries. Israeli war statistics demonstrate that 24 % of its military injuries were head and face injuries and 12 % neck injuries. From July 1998 to March 1999, during US military operations in Mogadishu, Somalia, head and facial wounds accounted for 36 % of all injuries.

Because of the increased use of explosive weaponry in modern warfare, eye wounds have also increased in incidence (Table 4). Artillery shell fragments and blast shockwaves can cause eye and adnexal lesions, with more than 70 % of eye injuries caused by penetration from explosive fragments. These injuries often result in the loss or damage of the eye and treatment is very difficult.

With regards to colorectal war wounds, data demonstrates 35 % of these were caused by explosive fragmentation, 5 % by vehicle accidents, 1 % by falls and 1 % by blunt trauma (Table 5).

Table 3 Proportion of immediate and early deaths for different injured parts of the body

Injury type	Immediate deaths (1619)	Early deaths (1624)	Total (3243)
Craniocerebral injury	38.3 % (620)	53.0 % (753)	42.3 % (1373)
High spinal cord injury	–	9.2 % (131)	4.0 % (131)
Limb severance	31.6 % (512)	–	15.8 % (512)
Chest injury	23.6 % (383)	21.8 % (310)	21.4 % (693)
Pelvic injury	–	6.5 % (93)	2.9 % (93)
Other	6.5 % (104)	9.5 % (134)	7.3 % (238)

The early period after injury can include anytime from several minutes to several hours before arrival at hospital

Table 4 Modern wars and eye injury causes

Event	Firearm injury	Explosive fragment injury	Other
World War 1	62.00	12.00	26.00
World War 2	65.80	15.00	19.20
Korean War (US military)	72.00	4.50	23.50
Egypt and Israel War	78.70	4.80	13.50
Sino-Vietnam Border War	74.00	13.00	13.00
Winter War (Russia-Finland)	72.00	12.00	16.00
Great Patriotic War (Russia-Germany)	85.00	11.00	4.00
Korean War (Russian military)	66.50	3.20	30.30

Table 5 Incidence rates for different areas of injury concurrent with colorectal injury

Injured area	Concurrence with colon injury (%)	Concurrence with rectal injury (%)	Difference in the incidence of colorectal injury *P* value
Head and neck	9.9	9.2	0.79
Face	0	6	<0.0001
Chest	22.1	10	<0.0001
Abdomen	68.2	57	0.003
Limbs	39.3	69.3	<0.0001
Skin and soft tissue	5.5	8	0.20

1.3 Recovery Rates and Mortality Rates for Fragmentation Injuries

In wars from the last decade, the two main causes of injury, accounting for nearly 75 % of wounds, have been penetrative explosive fragmentation injuries and penetrative gunshot wounds. During this period the survival rate from such wounds reached an historical record of 90 %. During the Vietnam War the survival rate from penetrative injuries was 84 % and in World War 2 80 %.

In the period from October 2001 to June 2011, 4596 cases of war injury deaths were retrospectively analyzed and in 73.7 % of cases the cause of death was identified as explosive fragmentation injury, in 21.1 % of cases the cause was gunshot wounds and in 4.2 % of cases the cause was other injuries including vehicle accident injuries. 87.3 % of deaths occurred before arriving at the medical treatment facility (MTF), of which 35.2 % (1619 cases) were instant deaths and 52.1 % (2397 cases) were deaths occurring several minutes to several hours subsequent to the incident but prior to MTF arrival. Only in 12.7 % of cases (580) did death occur after arriving at the medical treatment facility (Fig. 3). In addition,

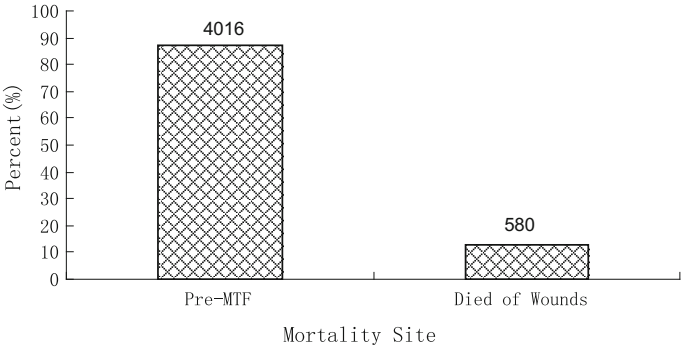

Fig. 3 Death rates from combat injuries before or after arrival at the medical treatment facility

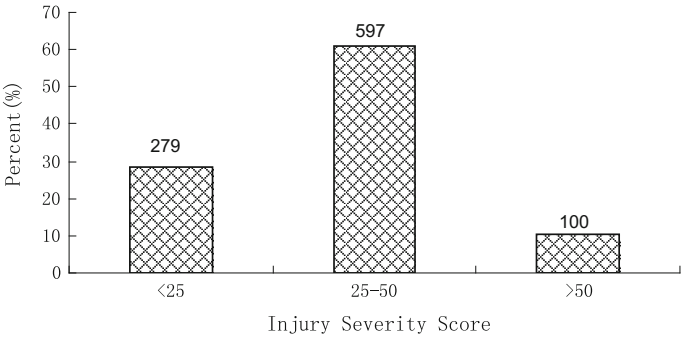

Fig. 4 Injury severity score (ISS) rates for fatal combat injuries

amongst fatal wounds, the injury severity score (ISS) was recorded as between 25–50 points in 61.2 % of cases (Fig. 4).

2 Types of Explosive Fragmentation

The main cause of fatal injury from fragmentation is explosive weaponry, namely the fragmentation warhead or shell. The fragmentation warhead is one of the main types of warheads, detonating high explosives to release a large quantity of high-speed fragments to destroy targets by way of such fragments' impact, and incendiary and explosive effects. These can be used to fatally injure biological creatures (humans, animals) and damage unarmored or light-armored vehicles, aircraft, radars, missiles and other armored equipment. According to their means of generating fragments, fragmentation warheads can be divided into three types: natural, controlled and pre-fragmentation. The grenade shell is a typical example of the pre-fragmentation type.

Natural fragmentation is caused by the shell expanding and breaking after detonation. Such shells are characterized by the casing not only acting as a container, but for the way the container itself also degrades into potentially deadly fragments. Such fragments are of an uneven, irregular shape but typically decelerate quickly in flight and have a limited range in causing fatal injury.

Controlled fragmentation employs a housing groove or lining for the explosive materials, or uses some other technical measures to partially weaken the shell and control its breaking points thereby producing fragments. Such warheads are characterized by broken pieces of uniform size and shape.

Pre-fragmentation warheads are molded to house a predetermined design and quantity of steel balls, steel arrowheads, tungsten balls, tungsten pellets or similar materials that can be fitted either on the inside or outside surface of the warhead or shell. These pre-made fragments and the natural fragments from the casing together create a field of deadly fragments (Figs. 5 and 6). Furthermore, because the

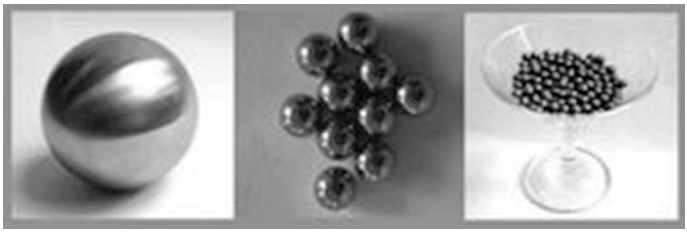

Fig. 5 Small tungsten alloy balls used as pre-made fragments

Fig. 6 Grenade shell capable of producing deadly fragments

pre-made fragments are designed to have uniform air resistance when flying they have a more efficient killing effect within a specified range, and the lethality of the whole warhead improves greatly.

3 Injury Characteristics from Explosive Fragmentation

3.1 General Characteristics of Fragment Wounds [6–8]

Projectile injuries to the body usually involve two forces. First, the forward momentum propelling the projectile along the projection axis directly causes penetrative or blind wound damage to body tissues and results in permanent wound tracts that are the main cause of fatal laceration injuries. In addition, the moment the high-speed projectiles strike the body's surface the projectile's forward momentum can produce a strong shockwave intensifying the damage to body tissue. Second, the lateral momentum of the projectile relative to the vertical wound means pressure waves can increase the size of the wound tract. Under a strong pressure wave, lateral momentum has a ripple effect producing transient cavities that can damage the surrounding soft tissue and bone tissue.

The ballistic characteristics of small fragments share some general characteristics with the gunshot ballistics, but also have some their own particular features. These main features are as below.

1. The transient cavity effect is not obvious. Transient cavity formation is one of the most important features of high-speed projectile injuries. When the high-speed projectile penetrates the body tissue, high pressure (10 MPa or more) extends the damage to the soft tissue surrounding the wound tract and can form cavities 10–30 times larger than the diameter of the projectile but only lasting a few milliseconds.
   When high-speed projectiles penetrate through body tissue, transient cavities are not simply fast-changing physical phenomena inside the tissue, but also the main cause of severe trauma of tissues and organs. Transient cavities may be much larger than the volume of the projectile itself and also have a sharply expanding and contracting pulsation cycle. When the cavities begin to form, there is maximum pressure inside the cavity then as soon as the cavity reaches its maximum volume the pressure suddenly reduces to a minimum and even reaches negative pressure. Transient cavities' cyclical expansion and contraction can produce strong waves of pressure in the body causing damage to near and distant tissues and organs. Besides this, negative pressure can produce a sucking action inside the cavity resulting in serious contamination of the wound tract. In contrast, due to their small size, fragments only strike a small surface area of biological tissue, therefore encounter little resistance from the body. They thus produce a big cutting force but small lateral momentum meaning the transient

cavity formed is small and that damage to distant parts of the body and wound contamination may be minor.

2. In most cases the entry wound for fragments is bigger than the exit wound. According to researchers such as Liu Yin Qiu, when a 0.44 g tungsten ball, cylinder, rectangle or triangle-shaped fragment causes damage to biological creatures, the wound tract caused by such small fragments features an entry wound that is typically bigger than the exit wound and that the faster the speed of the fragments, the bigger the size differential between these entry and exit wounds. When the speed reaches 1300 m/s, the entrance area is 10 or more times bigger than the exit area. Different impact speeds produce both different sizes and shapes of entry wounds. Thus when the fragment impacts at a low speed, the wound tract is cylindrical, at a medium speed it is gourd-shaped, and at high speed it is shallow and wide, resembling an inverted trumpet-shape. The latter is due to the fact that when the high-speed steel balls penetrate the body, they encounter big resistance from the tissue thus sharply reducing their speed. A large amount of energy is released in the area around the entry, thus causing the shallow and wide wound tract.
3. Fragments produce more blind wounds. A blind wound means that there is only an entry wound and no exit wound. Because the fragments are small, they quickly lose their energy, have weak penetration and are therefore more likely to remain inside the body resulting in blind wounds.
4. Fragments produce more complex wound tracts. The complexity of the wound is an important feature of projectile injuries. The complexity of high-speed projectile injuries is mainly reflected in two aspects. First, when the projectile penetrates the body it damages many tissues, resulting in more complex types of tissue injury. Second, the area surrounding the wound tract receives interlaced, jagged damage, resulting in a complex range of tissue damage. The wound tract caused by small fragments, however, demonstrates not only the complications mentioned above, but also its own complexities. The pathway of the fragment wound tract is typically more complex because the energy of small fragments quickly attenuates inside the tissue, meaning they are very likely to deviate in direction when penetrating tissues of different densities.

3.2 Characteristics of Wounds in Different Parts of the Body [9, 10]

The extent of the damage caused to the body by projectiles depends not only on the projectile's kinetic energy, structure, shape, flight stability and other physical factors of projection, but is also closely related to the anatomical, physiological and biomechanical properties of the tissues and organs affected.

In modern warfare, when an explosive weapon strikes roughly 75 % of its casualties suffer some kind of explosive fragment injury with a particularly high incidence of craniocerebral injury, which can inflict great physical harm. During the Gulf War, the incidence of craniocerebral injury caused by fragments constituted 20 % of all injuries amongst the multinational force. Craniocerebral fragment injuries can be divided into two categories, namely open and closed head fragment injuries. In earlier times, craniocerebral combat wounds were the main reason for war injuries and death, but the subsequent development of protective helmets significantly reduced the incidence of craniocerebral injury from fragments. Despite this, blunt injuries inflicted by fragments hitting the helmet can still be important causes of cerebral hemorrhage and cerebral edema.

Because the maxillofacial area is an exposed part of the body with a complex anatomy and special physiological functions, it is particularly vulnerable to trauma whether in peace or war. Clinical data shows that due to the widespread use of explosives, explosive damage to oral and maxillofacial areas has also followed an upward trend. Compared with general injuries, the wound tracts of explosive fragment injuries in the maxillofacial area are more complex, with greater likelihood of contamination and infection, more severe local damage within a wider affected area, and with higher incidences of shock, systemic contusion and significant concussion syndromes. It has long been a research focus of firearm wounds.

In regards to the effects of small fragments on the limbs, these mainly cause blind tract wounds in the local tissue, where the tracts' size and depth are closely linked to the weight and speed of the fragments. When steel balls of 0.15 and 0.25 g struck pigs' hind legs at a speed of 900–1000 m/s they were found to predominantly cause blind tract wounds to soft tissues (Figs. 7 and 8). The wound tract caused by 0.25 g steel balls was also found to be significantly greater than the wound tract caused by 0.15 g balls, but neither could fracture the pig's femur (Fig. 9).

When a small fragment hits the biological target in the chest area, the severity of the effect of the targets' thoracic trauma depends on the following two factors: first,

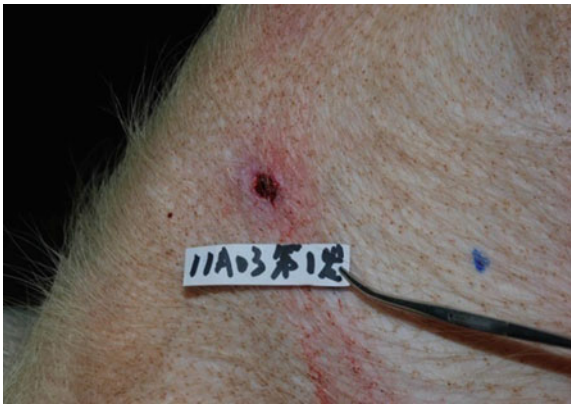

Fig. 7 Wound tract entrance from 0.15 g steel ball

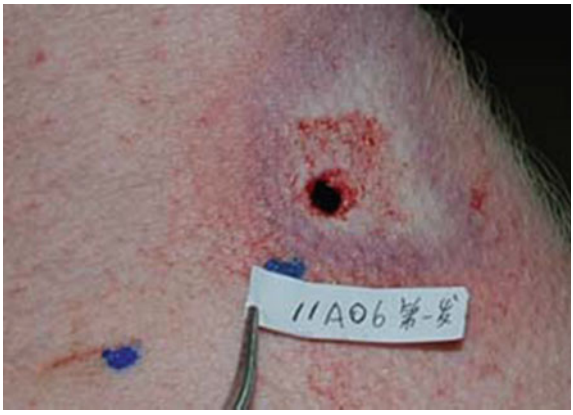

Fig. 8 Wound tract entrance from 0.25 g steel ball

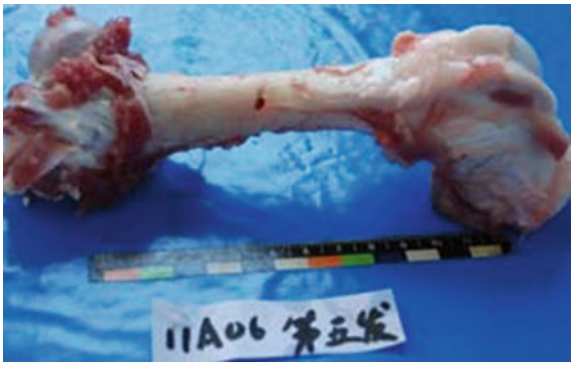

Fig. 9 Perforation of a pig's femur by a 0.25 g steel ball

whether the small fragment can penetrate through the target's chest; and second, whether the small fragment can hit vital tissues and organs. In regards to whether a small fragment can penetrate the chest, the most important factors are the fragment's degree of kinetic energy as well as whether it hits any bones when entering the body. In regards to the small fragment's ability to hit vital tissues and organs, this depends on the exact part of the body targeted and the area affected by the thoracic trauma. Regardless of the size of the fragment, as long as it can penetrate the chest, it can cause serious damage to the heart, lungs and other vital organs (Figs. 10 and 11). The first two pictures below demonstrates the damage to the lung caused by 0.15 g fragments penetrating the chest, the latter two pictures demonstrate heart and lung damage caused by 500 μm diameter tungsten balls penetrating the chest.

The abdominal area not only includes solid organs such as the liver, kidneys and spleen but also gastrointestinal and other hollow organs. At the same time, abdominal soft tissue is easily penetrated by fragments, meaning that the abdominal organs are easily damaged when the abdomen is targeted by explosive fragments. Trauma effect experiments of fragments striking biological creatures' abdomens

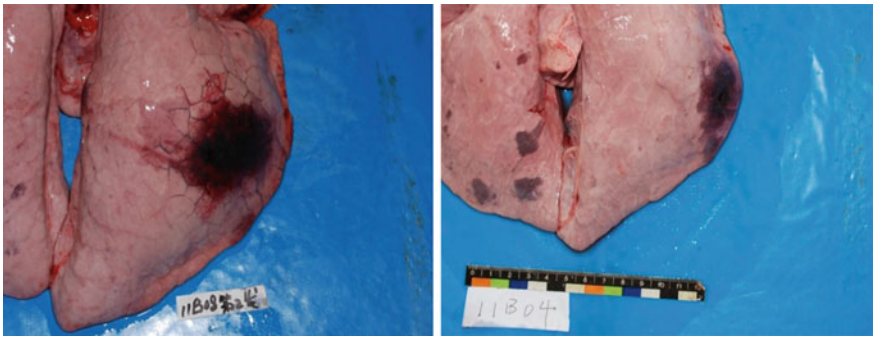

Fig. 10 Lung wounds caused by 0.15 g fragments penetrating the chest

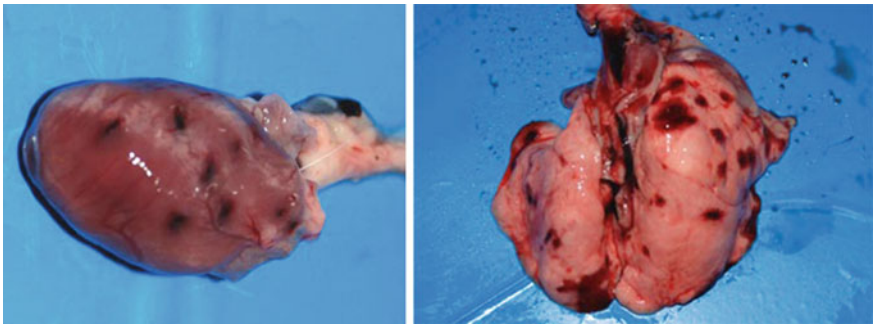

Fig. 11 Heart and lung damage caused by 500 μm diameter tungsten balls penetrating the chest

have demonstrated that the greater the kinetic energy of the fragment, the more severe the injury and trauma to the target. After penetrating the target's abdomen, the greater the speed and weight of the small fragments, the more damage is caused to the main organs and blood vessels, and the faster the target's blood loss. At the same time, the small fragments also impact nerve groups in the same way. The degree of the target's injury caused by small fragments hitting the abdomen can be judged as follows: A spleen ruptured by one third (>6 cm) or 80 % unilateral renal damage can be judged as severe to extremely severe injury (AIS score 3–5); splenic rupture of between 3 and 6 cm can be judged as moderate to severe injury (AIS score 6–8); whilst multiple perforations of the small intestine or colon can be judged as less than minor injury (AIS score 14–15).

3.3 *Characteristics of High-Speed Fragment Injuries (Remote Effects)*

The velocity and the damage caused by small fragments have significant correlation. Although earlier research was conducted on projectile speeds of less than 1000 m/s [11], with the continuous improvement of the speed of bullets and more widespread use of high explosives, the velocity of many explosive fragments now far exceeds 1000 m/s. Studies examining how these faster velocities can damage the body are, however, less common. Taking landrace pigs as experimental subjects, 0.72 g steel balls were used as projectiles at speeds of 2000, 3000 and 4000 m/s to target the hind legs of the pigs, with 1000 m/s projectiles also used as a control. The experiment aimed to understand the characteristics of ultra high speed projectiles in causing local damage to the body, and also to comprehend the systemic effects and injury mechanisms of these projectiles by analyzing the appropriate physiological and physical indicators. The studies show that the wound tract entrance area increased significantly with increasing velocities. When the velocity is 4000 m/s, the impact of the projectile produces a wound tract area approximately 344 times greater than at a speed of 1000 m/s. However, the depth of the wound tract decreases with the increasing striking speed.

Besides causing serious local tissue damage, injuries caused by ultra high speed projectiles have other particular features such as causing severe damage to distant tissues and organs [12]. When steel balls struck the hind legs of the pigs at 1000 m/s, local tissue damage was visible but there was no abnormality in near or distant organs noticeable to the naked eye; when the balls struck at 2000 m/s, a comminuted fracture of the femur was visible in the wound tract; when at 3000 m/s as well as comminuted fracture of the femur, there was also pulmonary hemorrhage and bleeding from the colon mucosa. Finally, when the balls struck at 4000 m/s there was extensive visible damage to various organs, principally comminuted fracture of the femur, bleeding adjacent to the lower intestine, bleeding from the bladder, pulmonary hemorrhage, pulmonary consolidation and subarachnoid hemorrhage (Figs. 12 and 13).

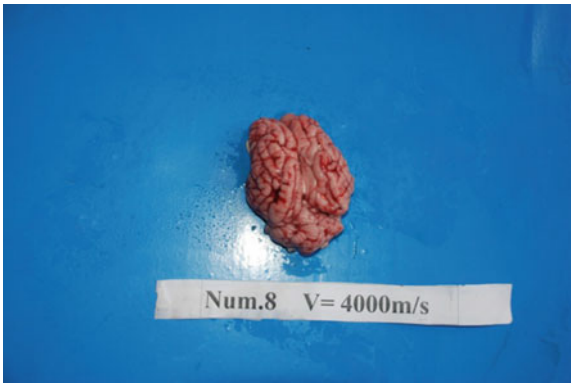

Fig. 12 4000 m/s Subarachnoid hemorrhage

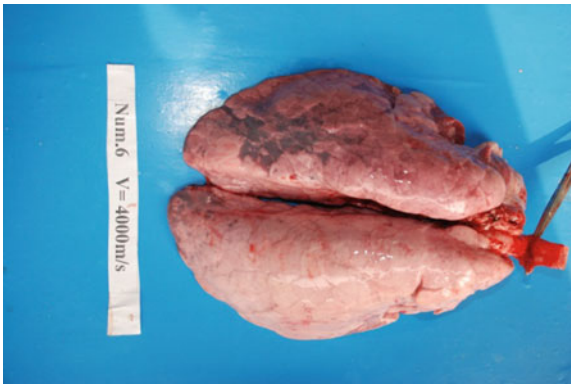

Fig. 13 4000 m/s Pulmonary hemorrhage

Remote effects of firearm wounds are one kind of firearm injury affecting the entire body, damaging distant tissues and organs with no direct anatomical connection to the original local wound tract. The notion was first proposed by the American scholar Callendar [13] in the 1930s, after which scholars such as Berlin [14], Suneson et al. [15] and Liu et al. [16, 17] have also found that when a high speed projectile strikes the hind legs of cats, dogs, pigs and other animals, high pressure changes were recorded in other body parts such as the head and abdomen. These pressure changes are regarded as the main cause of remote wounds. According to Chinese scholars such as Liu Yin Qiu, based on large amounts of animal experiment data, remote wounds are responsible for hyperemia, bleeding spots and other pathological changes and that the disturbance of blood flow is the main cause of such wounds. Some scholars, however, have claimed neurological dysfunction and other remote effects are caused by momentary shocks and pressure waves from transient cavities. Test results, however, are yet to confirm this, meaning that the existence of remote effects is still questionable.

When including both Chinese and foreign research results, scholars such as Wang Zhengguo believe: (1) Following a gunshot injury, areas both near and remote from the wound tract demonstrate injury; (2) Although some remote injuries (such as bleeding spots) are not always part of the systematic reaction after severe trauma, pressure waves causing injury in remote parts of the body definitely still exist; (3) Under most conditions, the damage manifest in remote parts of the body is very slight, but under certain conditions remote injuries could cause additional health complications meaning the whole body suffers further injury with clinical consequences.

3.4 Characteristics of Injuries from Particular Fragment Materials

The injury effects of explosive fragmentation on biological targets are not only related to the weight and speed of fragments, but also to the properties of the

fragments' materials. Studies have shown that in pre-fragmentation warheads using steel fragments, adding fragments made from combustible metals such as rare earth alloys, zirconium alloy and zirconium sponge can heighten the combustibility of the target's fuel system. Zirconium fragments are often used in the form of zirconium sponge, made up of tiny particles of zirconium pressed together. After an explosion from a warhead travelling at high speed, due to the impact of the explosion, and intense friction within the air, spontaneous combustion of the zirconium fragments can lead to temperatures as high as 3000 °C. Obviously, the use of such fragments on easily flammable targets produces greater fire damage, but they also have some specific fatal effects on biological targets.

Currently the American military widely uses depleted uranium as fragment shell material in combat, which has more severe side effects on biological organisms. Depleted uranium projectiles refer to missiles, bombs, shells or bullets where the main material used is depleted uranium [18]. Uranium is a rare element in nature composed of three isotopes, namely uranium-234, uranium-235 and uranium-238, which respectively account for 0.005, 0.720 and 99.275 % of naturally occurring uranium. Of these only uranium-235 has fissionable nuclides that under the effects of neutron bombardment can cause a chain reaction of nuclear fission used as "fuel" in atomic bombs and nuclear reactors. When using a core made from high density, high strength and high toughness depleted uranium alloys, upon detonation depleted uranium projectiles can produce high temperature chemical reactions used to destroy solid buildings and attack tanks. Radioactive depleted uranium can also cause greater harm to humans and the environment. Such harm comes from both depleted uranium radiation and chemical toxicity. Its health effects depend on the physical and chemical properties of the organism in contact with the depleted uranium and the extent and duration of exposure. After the depleted uranium projectiles hit the target, usually 10–35 % (maximum 70 %) of the depleted uranium forms toxic aerosols (oxides of uranium), whilst the majority of the depleted uranium spreads on or under the ground. The ways in which depleted uranium enters the body include inhalation, ingestion and contamination of wounds.

As evident from a large number of animal experiments and the results of human exposure, the kidneys are the organ most damaged by the chemical toxicity of depleted uranium. Research has shown that within a few days of exposure to uranium concentrations exceeding 50 μg per 1 g kidney, kidneys may suffer from renal failure. Furthermore in the short term, a sharp intake of uranium concentrations of 1 μg per 1 g kidney will also lead to minor renal dysfunction. The respiratory effects of depleted uranium depend on the size, solubility and quantity of depleted uranium dust inhaled. Because most of the depleted uranium particles can be inhaled, there is an obvious threat to health. In the case of acute exposure, lung tissue damage may occur, finally resulting in pulmonary fibrosis and emphysema. The impact of depleted uranium on the immune system is mainly in the effects of radiation on lymph nodes and red bone marrow. In recent years, studies of in vivo mice implanted with uranium demonstrate that the mice's lymph nodes contain a higher rate of uranium than other tissues. Other experiments also show that the intake of depleted uranium in the blood increases the quantity of eosinophils. This

means the body is more susceptible to allergic reactions and parasitic infections. In addition, depleted uranium has a significant effect on the nervous system. In 2000, results from the US Institute for Radiation Biology showed that uranium implanted in mice concentrated in and affected the hippocampus. Moreover, in comparison to these controlled situations, inhaled radiation leads to a significant increase of uranium concentration in the motor cortex, vestibular cortex, midbrain and cerbellar vermis. Other studies indicate that different concentrations of uranium implanted in in vivo mice can alter the biopotential of the hippocampus suggesting that long term exposure to uranium can lead to behavioral or nerve effects in wounded soldiers.

3.5 *Wound Characteristics Under Special Environmental Conditions*

Under specific environmental conditions such as high altitude plateaus, extremes of heat and aridity and immersion in seawater, explosive fragment wounds have their own particular characteristics. Amongst war wounds suffered on high altitude plateaus, explosive fragment wounds have an incidence rate of over 50 % [19, 20]. Current anti-personnel munitions widely use pre-fragmentation technology, and in cases where projectiles contain a large quantity of spherical, triangular or serrated fragments, an initial core fragment velocity of over 1000 m/s has been found to have a fragmentation radius in excess of 10–20 m. Experiments, however, have demonstrated that on high altitude plateaus fragments have faster terminal velocities than on low altitude plains. On plateaus a 1.03 g ball has a speed 112.79 % of that on the plain, triangular fragments 115.25 %. The reason for the differences in fragment velocities between the plateaus and the plains lies in the different atmospheric pressures. Under normal circumstances, each 100 m increase in altitude results in a decrease in atmospheric pressure of 7.45 mmHg. At an altitude of 3658 m, for instance, atmospheric pressure is only 64.35 % that at sea level, and closely related to atmospheric pressure, air density is also 37.35 % lower.

When in flight, the attenuation of a fragment's velocity depends upon resistance. A flying projectile's resistance can be expressed as $F = \frac{1}{2} C_x S \rho V^2$ where F is resistance (N), Cx is the drag coefficient of the projectile, S is the projectile's resistance area (m^2), p is the air density (kg/m^3) and V is the velocity of the projectile (m/s). As can be seen from the above equation, because air density is lower for fragments flying at high altitudes, resistance also decreases and velocity attenuates slowly. This explains why fragments fly faster on plateaus rather than on plains. The resistance area of flying triangular fragments is larger than that of a ball, explaining why the change in velocity for triangular fragments at high altitudes is more obvious.

Since the kinetic energy of a projectile has a square relationship to the projectile's velocity, when the terminal speed of a projectile at high altitude is high it produces a lot of kinetic energy entailing that all high speed fragments can penetrate

an average thickness of 15 cm in pigs' hind legs and 20 cm in soap bars. In lower altitude plains, however, only steel balls can cause penetrative wounds in pigs' hind legs, with 40 % of triangular fragments causing blind tract wounds and no recorded case of triangular fragments penetrating soap bars. It is generally agreed that a depth of penetration equal to or above 15 cm from the body's surface to the body's major blood vessels or organs can effectively be considered as fatally wounding. In regard to fragment penetration depths on plateaus and plains, the radius of fatal explosive fragments on plateaus is also greater than that on plains.

Muscle mitochondria's SDH is a key enzyme in oxidative phosphorylation and is sensitive to circulatory conditions. ATP is a substrate of the ATP enzyme, and a decrease in ATP can reduce enzyme activity. Typically SDH and ATP enzyme activity are taken as biochemical markers of tissue activity. Experiments show that in the case of fragment injuries sustained on plateaus, the amount of damaged tissue removed from wound tracts and the area of crushed muscle fiber were both greater than that from injuries sustained on plains. In addition, in the wounded area SDH and ATP enzyme activity decreased more significantly in plateau areas than on plains, which suggests that explosive fragmentation on plateaus not only has a greater killing radius but also causes more tissue damage.

Previous war wound statistics show that naval warfare had a higher mortality rate than land warfare. The fragment wound combined with sea water immersion produced injuries that had a key role in the build-up of casualties [21]. Due to the special nature of the maritime operations area, after injury the wounded often fell into the water making early rescue and treatment difficult and meaning the most crucial period for surgical treatment was missed. Even the most basic rescue and treatment required an average of at least 4–6 h, therefore improving the treatment of such injuries became an important focus in naval battles. The combination of fragment injury and immersion in seawater additionally meant that the wounded party's immune function was inhibited. Experimental studies have shown that when not immersed in water, injuries affecting experimental animals demonstrate no intestinal flora in blood cultures. This does not, however, exclude the possibility of immunosuppression and bacterial translocation as a result of seawater immersion. Because after fragment injuries there is a loss of local skin function, this, combined with seawater immersion, may further aggravate immunosuppression meaning the immersed local wound comes under attack both from specific seawater flora and also from translocated flora causing even earlier infection and an increase in the severity of infection. The earlier and more serious infections are due to the following: (1) Further disruption of the immune function. Low temperatures arising from immersion in seawater not only cause the dysfunction of immunocompetent cells (e.g. the deterioration of neutrophil's phagocytosis function and the reduced activity of macrohpages) but also a reduction in the immune activity of proteins (antibodies, complement functions). (2) Membrane phospholipid damage caused by tissue hypoperfusion also has a role in the pathogenesis of infection meaning the immersion process further increases local tissue necrosis.

4 The Mechanics of Injury in Explosive Fragmentation Wounds

Explosive fragment injuries to the body usually depend on two forces. First, the forward momentum propelling the fragments along their projection axis directly causing penetrative or non-penetrative damage to body tissues and resulting in permanent wound tracts that are the main cause of fatal laceration injuries. In addition, the moment a high-speed fragment strikes the body's surface the fragment's forward momentum can produce a strong shockwave intensifying the damage to body tissue. Second, the lateral momentum of the fragment vertical to the wound means pressure waves can increase the size of the wound tract. Under a strong pressure wave, lateral momentum has a ripple effect producing transient cavities that can damage the surrounding soft tissue and bone tissue [7, 11, 22].

1. Projectives directly causing laceration injuries. After the projectile strikes the body, when the striking force on the local tissues exceeds the tissue's tolerance levels it will cause injuries such as severance and laceration.
2. Hydrodynamic and accelerated particle effects. When projectile fragments act on body tissues, they can produce similar "explosive" effects. Some scholars believe that this effect is due to the diffusion of water particles, wherein the kinetic energy of the fragment transfers to the liquid particles surrounding the tissue making them accelerate and rapidly leave the wound tract like secondary projectiles, spreading to the surrounding areas like an "explosive" effect and causing extensive damage in the tissues around the wound tract.
3. The transient cavity effect. Transient cavity formation is one of the most important features of high-speed projectile injuries. When the high-speed projectile penetrates the body tissue, high pressure (10 MPa or more) extends the damage to the soft tissue surrounding the wound tract and can form cavities 10–30 times larger than the diameter of the projectile but only lasting a few milliseconds.

   When high-speed projectiles penetrate through body tissue, transient cavities are not only fast-changing physical phenomena inside the tissue (Fig. 14), but also the main cause of severe trauma of tissues and organs. Transient cavities are not only much larger than the volume of the projectile itself, but also have a sharply expanding and contracting pulsation cycle. When the cavities begin to form, there is maximum pressure inside the cavity then as soon as the cavity reaches its maximum volume the pressure suddenly reduces to a minimum and even reaches negative pressure. Transient cavities' cyclical expansion and contraction can produce strong waves of pressure in the body causing damage to near and distant tissues and organs. Besides this, negative pressure can produce a sucking action inside the cavity resulting in serious contamination of the wound tract.

   A high speed x-ray photograph taken 1.616 ms after a 5.56 mm bullet strikes both of a dog's hind legs at a speed of 950 m/s. The central light-colored area shows a huge transient cavity and the arrow represents the bullet's direction.

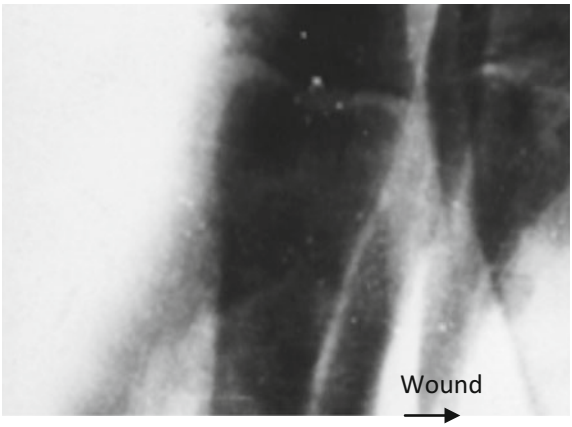

Fig. 14 Picture Transient cavity high-speed x-ray photograph

The size of the transient cavity depends on the energy impacting the tissues from the projectiles and the physico-mechanical properties of the tissue. When the projectile speed is less than 340 m/s, it rarely produces transient cavities. Due to the different structures and mechanical properties of various human tissues the transient cavities caused by projectiles are also different. The effects of transient cavities are more obvious in muscles, livers, brains and other tissues. In muscle tissue every joule of projectile energy produces a 0.7 ml volume of transient cavity, but in lower density tissues such as lungs, or higher density tissues such as bone, the effects of transient cavities are not so pronounced.

4. Shockwave effects. When a high speed fragment strikes the body surface it can produce a peak pressure of approximately 1.01 × 105 kPa (10 atm). Strong shockwaves have their own particular properties. Following the wavefront there is an exponential decrease in pressure, leading to an area of negative pressure lower than atmospheric pressure. When the propagation velocity inside the tissue is about 1450 m/s, the duration of the shockwave is very short, and within 30 s the negative pressure will reduce by half, so that in the past it was believed that shockwaves did not possess significant fatal effects. However, recent experiments demonstrate that overpressure, particle acceleration and displacement effects from the shockwave can, in fact, cause significant tissue damage. In particular, the overpressure effect of the shockwave, even when tissue displacement is prevented, can still cause significant and severe cell damage.

5 Protection from Explosive Wounds

On the battlefields of modern warfare, there has been a gradual increase in fragment wound casualties caused by artillery fire, RPGs, rockets, hand grenades and mines, which now constitute 67–75 % of total combat casualties and 82–87 % of fatal

head, chest and abdominal injuries, far exceeding fatal limb injuries. Statistical analysis of war wound surveys indicates that protection of the head, chest and abdomen, which account for 39 % of the surface area of the human body, can significantly reduce fatalities and that bulletproof vests and helmets are effective means of protecting these key parts of the body. The United States claims that during World War 2 protective equipment saved the lives of at least 70,000 American soldiers. According to British research reports, bulletproof vests can reduce incidents of injury by 27 %, and reduce fatal injuries by 40 %. As a result, after World War 2 protective helmets and armor for soldiers have developed rapidly [1, 23].

Development of protective equipment for individual soldiers is reflected in the development of protective materials. Progress in research and development in protective materials has played a decisive role in the development of protective technology, moving through carbon steel, manganese steel, titanium alloy, aluminum alloy, nylon fiber, glass fiber, Kevlar aramid fiber and thermosetting resin composite materials, in order to make continuous improvements to the defensive abilities of individual soldiers' protective equipment. In particular, the successful development of the US PASGT helmet in 1984 marks a modern milestone in the development of the modern military helmet and since then bulletproof helmets made of non-metallic composite materials have become the mainstream in modern military technology. With the constant improvement of aramid, an ultra high molecular weight polyethylene fiber with mechanical properties of high strength and high modulus fibers, the protective performance of non-metallic bulletproof helmets has continued to improve whilst their weight has nevertheless decreased. However, although the continuously improved bulletproof protective helmets can successfully block penetrative injury from high speed projectiles, at the same time, and in contrast to metallic materials, non-penetrative temporary and permanent deformations to the back surface of helmets have also correspondingly increased.

The reason for this is that helmets made of composite materials and helmets made of metals have completely different bulletproof mechanisms. Early metal bulletproof helmets blunt the high speed projectiles by way of the hardness of the metal, producing a larger compression zone and dispersing the impact energy to achieve their bulletproof function. Currently used non-metallic bulletproof helmets, on the other hand, absorb the kinetic energy of high speed projectiles through the expansion and deformation of bulletproof material fibers, thus achieving their bulletproof function. The deformation mechanism is thus a necessary part of their bulletproof ability.

A high speed projectile's impact energy, however, is proportional to the square of its velocity, meaning that when projectile velocity increases from 400 m/s to 600 m/s the degree of protection needs to increase by more than double. Extruded areas and deformed areas produced on bulletproof helmets may also still cause the inner surface of the helmet to strike the head thus causing blunt head injury.

Body armor is used to protect the viral organs of the chest and abdomen in order to resist the fatal effects of bullets and fragments. However, with the further development of ammunition technology and the continuous improvement of the

penetrative ability of bullets and fragments, since the mid-20th century high energy munitions have rendered bulletproof vests made of protective materials such as nylon unable to effectively resist small-caliber bullets and high speed projectiles. In this light, in 1972 the American DuPont company developed an aramid polyesteramide fiber material (named "Kevlar") to produce Kevlar body armor which is soft, light, heat- and cold-resistant, non-flammable and with strong bulletproof ability. Its bulletproof ability is twice that of alumina ceramic material, three times that of nylon fiber and other soft materials, and five times that of steel. It is also 45 % lighter than fiberglass, and with one fifth the density of steel. Modern body armor can be divided into soft and hard body armor vests. Soft body armor is made of high-strength fiber material for protection against knife wounds, fragmentation and low-speed projectiles, whilst hard body armor vests are used for protection against high-speed projectile weapons such as 5.8 and 7.62 mm rifles.

6 Treatment of Explosive Fragmentation Injuries

Explosive fragment wounds are one kind of firearm injury. Generally speaking, firearm injuries all suffer from infection and require debridement. Early and thorough debridement is the best way of preventing infection, but because of the asymmetry of the wound tract injury caused by fragments wounds "thorough" debridement can easily result in the excessive removal of muscle and other soft tissues often causing delayed healing. In recent years, both international and Chinese scholars emphasize that in the early surgical treatment of modern firearm injury more attention should be paid to the improvement of pressure reduction and drainage rather than to thorough debridement. Experimental studies have shown that there was no significant difference in incidence of infection between a sample group of injuries that underwent debridement, and a sample group of injuries that underwent surgical cutting and drainage, but the healing time the latter group was significantly shorter than that of the former group [24, 25].

Principles of early debridement: (1) Debridement should be performed sooner rather than later, and ideally within as short a time as possible prior to the occurrence of infection. Antibiotics should also be used as early as conceivable. (2) Aseptic surgical requirements should be adhered to as strictly as possible. Under wartime conditions there are often poor surgical conditions but nevertheless suitable surgical conditions need to be created and aseptic procedures employed. (3) The wound should be expanded to fully expose the wound tract so that the deep tissue injuries can be examined to avoid missed or inaccurate diagnoses. (4) Attempts should be made to remove any sediment, shrapnel, cloth fragments and other foreign matter together with damaged and necrotic tissue whilst bone fragments, nerves, tendons and blood vessels should all be properly treated. (5) Early debridement should involve delaying suture. Firearm injury effects can result in remote tissue damage so it is hard to decide in the early treatment period whether the damage to tissues is necrotic or not. Therefore in principle it is necessary to keep

the wound open rather than perform early suture. However for recently-inflicted, shallow and easily-cleaned injuries early suture can be taken into consideration after debridement. In addition, as much as is possible early suture should be given to craniofacial, genital and joint capsule injuries whilst wounds linking the pleural and peritoneal areas should also be closed as soon as possible.

Besides these principles of debridement, the timing of the debridement is also vital. The bacteria in firearm injury wound tracts can only cause infection after a certain period of reproduction (the incubation period). The shorter the time between injury and debridement, the lower the infection rates. In most cases the wound is contaminated within 6–8 h after injury but has still not reached the stage of infection meaning the generally accepted prime time for debridement is within this 6–8 h period. However, this time period is not absolute as there are also other factors affecting the formation time of wound infections. Generally, the length of the bacterial incubation period is related to environmental factors such as temperature and humidity. Under high temperature and humidity bacteria reproduce more rapidly causing earlier infection, with the opposite occurring in low temperatures and humidities. In addition, the length of the incubation period is also related to the nature of the wound, the type, quantity and toxicity of the bacteria, the soldier's level of local and systemic resistance and whether antibiotics are used or not.

In cases of serious wound contamination and poor overall physical health of the wounded soldier, especially where there is the existence of tissue hypoperfusion or local circulation dysfunction, the formation time of the wound infection can be reduced to 3–4 h. On the contrary, in cases of minor wound contamination, good overall physical health of the wounded soldier and good local blood circulation, the formation time of the wound infection can be delayed by over 12 h. In cold, dry areas at lower temperatures and with the use of antibiotics, within 12–24 h there may be no obvious sign of infection, so the wound is still suitable for debridement surgery. For already obviously infected wounds, or wounds untreated for 24 h or more, debridement surgery should be deemed unsuitable because debridement at this time can damage the granulation tissue barrier that has formed, further spreading the infection. The treatment requirement of such injuries is to remove obviously necrotic tissue and foreign bodies and to open the wound for changing dressings.

High-speed small fragment injuries have characteristics such as many wounded areas, numerous small wounds and many blind tract wounds. Besides following the general treatment principles of firearm injuries, treatment of small fragment injuries should also pay attention to these particular characteristics. For small fragment wounds, surgical debridement is still the first choice. However, the treatment may still vary from the principles of traditional debridement. Traditional debridement surgeries require complete opening of the wound tract in the early period, the rinsing and removal of all foreign bodies, the removal of inactive or necrotic muscle tissues due to injury, and a delay to suture of the wounds by 3–5 days after drainage. The treatment principles are based on the theory that transient cavity pulsation not only produces huge lacerations and bruising effects in the wound tract tissues resulting in serious contamination of the wound tract, but also serious

damage to the microcirculation of the surrounding tissue thus causing tissue necrosis from blood loss. This means that most of the wound tract tissues need to be removed.

The judgment of whether muscle tissues are necrotic is mainly based on the "4C" method: *color*: dark red; *consistency*: oozing; *contractility*: no shrinkage when it is pinched; *capillary*: no bleeding when it is cut open. Another important factor to support a wide range of tissue removal is the severity of the firearm wound tract tissue contamination. Fragments and noticeable remnants of clothing are not hard to remove, whilst mud, debris and other contaminants can also be removed through substantial rinsing. Some tiny, invisible foreign bodies, however, are radially dispersed into the depths of the tissues and become potential foci of infection. The removal of living but seriously contaminated tissues can indeed help control infection, but such procedures established a century ago when there were no antibiotics have increasingly been questioned. Removal of excess muscle or tissue edges will cause greater tissue damage and in certain circumstances requires the use of flap transfers or skin grafts that will also affect subsequent rehabilitation.

Bowyer has conducted more detailed research on the treatment of small fragment wounds. Using 0.2 g steel pellets striking pigs' hind legs at a rate of 500 m/s, it was found that at some time intervals the boundary area of the devitalized tissue was 1 mm, whilst at other time intervals there was no clinically significant boundary area. After surgery the type and quantity of skin surface bacterial flora showed no significant change compared to before surgery. The wound tract's bacteriological examination showed that out of 12 wound tract samples there were only two positive culture results, one of which was taken as a sample from the pig after one hour, in which both at the surface and depths of the wound there were mixed growths of small amounts of non-pathogenic Staphylococcus and Streptococcus faecalis. The other positive result was taken as a sample after six hours and in this case there was a small amount of mixed non-pathogenic Staphylococcus at the surface of the wound. All anaerobic cultures were negative. These results suggest that small fragment wounds have a limited devitalizing area in the surrounding tissue and in most cases require no surgical treatment. At the same time, wound infection incidents are relatively low thus most small fragment injuries do not require surgical treatment where there is no infection and can heal automatically. Therefore in modern warfare, conservative treatments are suitable for multiple small fragment wounds.

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Advances in Early Treatment of Combat and Traumatic Shock

Tao Li and Liangming Liu

Abstract Trauma has become a public hazard in modern society and its mortality has ranked the third place in all diseases. Traumatic-hemorrhagic shock is an important complication of trauma and accounts for nearly 50 % of the early death in trauma. In recent years, many new treatment measures and concepts have been put forward for the early treatment of war wound and trauma, including effective blood control, damage control resuscitation, early application of vasoactives and vascular hypo-reactivity control, etc. In this review, we will elaborate the new treatment concepts and measures to trauma shock and discuss the future trends.

Keywords War wound · Trauma · Shock · Emergency care · Resuscitation fluids · Anti-shock drugs

Shock, an old but still challenging issue, its incidence and mortality are still high not only in wartime but also in peacetime. Data showed that hemorrhage and hemorrhagic shock accounted for nearly 50 % of war deaths, and 40 % of trauma death at early stage. So the early treatment of combat casualty or trauma is very important. In recent years, many researchers have done a lot of work on this filed and made a quantity of progresses. This review discussed these progresses.

1 Bleeding Control and Early Resuscitation for Traumatic Shock

Data showed that, in conventional wars, 80 % of deaths occurred immediately within 30 min after injuries and most of them died from massive blood loss and shock. The same situation also occurred in disaster and road traffic injuries, in

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which 80 % of deaths occurred within the early 6 h [1–3]. The number of trauma deaths around the world is up to 3.5–5.8 million each year, which ranks the third place in the death profile of all diseases. Experts predict that the deaths from all kinds of trauma around the world may reach up to 8.4 million by 2020. Therefore, in order to get effective treatment of trauma at early stage, the concepts of "*Golden 1 hour*" and "*Platinum 10 minutes*" are put forward, which bring up the new requirements and directions to develop the new technologies and treatment measures for severe trauma. Based on these concepts, some developed countries have adopted theses measures to manage the combat casualties and trauma and have made a remarkable result.

Effective bleeding control Hemorrhage-induced deaths account for nearly 50 % of early deaths in combat casualty and trauma. An analysis from American military reported 25 % of deaths in Iraq war was avoidable by early and effective bleeding control, which suggests that bleeding control is important in combat and disaster spot [4]. In Iraq and Afghanistan Conflict, because large number of deaths were found from the extremity hemorrhage, so experts on military medicine recommended for the widespread application of tourniquets after that. By 2007, tourniquets were equipped in the majority of US military and became regular trained item [5]. Tourniquet application on the battlefield was found to be able to decrease the early deaths [6]. The death rate from extremity hemorrhage deceased by 85 % after full utilization of tourniquet protocols [4]. It is reported tourniquets have been widely equipped and used in both medical personnel and combatants in Israeli Defense Force (IDF) for the past 20 years. Based on these cognitions, many types of tourniquets and hemostatic dressings such as personal spinning and ratchet tourniquet, and HemCon and Celox hemostatic dressings have been used in combat casualty care and trauma care [7].

Rational fluid resuscitation The therapeutic principles to resuscitate combat shock in European and American military are: for controlled hemorrhagic shock, if the wounded is stable and has no shock presentation, fluid infusion is not suggested; if the wounded has shock presentation, the mean arterial pressure (MAP) is suggested to be resuscitated to 70 mmHg with *lactated ringer's solution* (*LR*) or 6 % *hydroxyethyl starch* (*HES*). For uncontrolled hemorrhagic shock, small volume of fluid resuscitation is suggested, of which, it is aimed to maintain the body's basic needs. 250 ml of 7.5 % *NaCl* and 6 % *dextran* (HSD) or Hextend was recommended as the initial resuscitation fluids in USA army. The total volume of HSD or Hextend is suggested no more than 500 ml. Subsequently, some isotonic solution can be given according to requirement. The goal of resuscitation is to that the radial artery pulse can be touched or the systolic blood pressure is at about 80–90 mmHg and the consciousness get recovery [3]. In Israel, since most of the war has occurred on their border and the distance to evacuate the wounded to the advanced medical center is less than 100 km, the evacuation time is less than 1 h. So in Israel, for uncontrolled hemorrhagic shock, fluid resuscitation is not suggested if the evacuation time is less than 1 h, if the evacuation time is more than 1 h, they suggested application of small volume of fluid until arriving the advanced medical center.

2 New Concept and Measures to Resuscitate Traumatic Shock

Traditional resuscitation for traumatic shock advocates rapid and aggressive resuscitation, means using inotropic and vasoactive drugs to rapidly raise blood pressure to normal level. But in recent years, with the research progress of the pathophysiology of shock and tissue fluid and oxygen metabolism, traditional fluid resuscitation measures experienced great challenge. Some new resuscitation concepts have been put forward, including permissively hypotensive fluid resuscitation, delayed fluid resuscitation and hypothermic resuscitation [8–11]. Some new measures for the early treatments of traumatic shock have been brought to clinic and get more and more accepts based on these new resuscitation concepts. The 2013 bleeding management guideline in European has collected these measures [12].

Permissive hypotensive resuscitation Traditional resuscitation concept that quickly restoring the blood volume and blood pressure is based on Wiggers controlled hemorrhagic shock model. But in clinic, especially for trauma shock in combat, most of the types are uncontrolled hemorrhagic shock, means with active bleeding. Recent studies suggested that substantially rapid fluid resuscitation for uncontrolled hemorrhagic shock patients before surgical bleeding control may increase the blood loss, result in dilutional coagulopathy and metabolic acidosis. Besides, large quantity of rapid fluid infusion may influence vasoconstriction, leading to thrombus translocation and re-bleeding. Studies of ours and other labs showed that the ideal resuscitation target pressure is controlling the systolic blood pressure at around 90 mmHg or mean arterial blood pressure at 50–60 mmHg. Using uncontrolled hemorrhagic shock model induced by transection of splenic parenchyma and one of the branches of the splenic artery, we compared the resuscitative effects of different target resuscitation pressure (MAP at 40, 50, 60, 70, 80 and 100 mmHg), the results showed that normotensive resuscitation (MAP at 80 and 100 mmHg) significantly decreased the animal survival and time, increased the blood loss and hemodilution, and resulted in severe organ function damage including mitochondrial function damage. In contrast, permissive hypotensive resuscitation (MAP at 50- and 60-mmHg) brought a longer survival time and higher survival rate for uncontrolled hemorrhagic shock rats. The hematocrit, hemodynamic parameters, and vital organ function of rats given appropriate hypotensive resuscitation got better improvement than normotensive resuscitation groups. Having too-low target MAP (40 mmHg) during uncontrolled hemorrhagic shock was not good; in which, the animal survival and survival time were significantly lower than those in the 50-, 60-, and 70-mmHg target MAP groups. The results suggests that 50–60 mmHg target resuscitation pressure is the more ideal resuscitation pressure for uncontrolled hemorrhagic shock. Further studies found that ninety minutes were the maximal tolerance limit of hypotensive resuscitation; over ninety minutes such as 120 min of hypotensive resuscitation could cause severe organ damage [13] (Fig. 1). With traumatic shock patients, we found that the target MAP controlled at 60 mmHg before arriving operation room could significantly

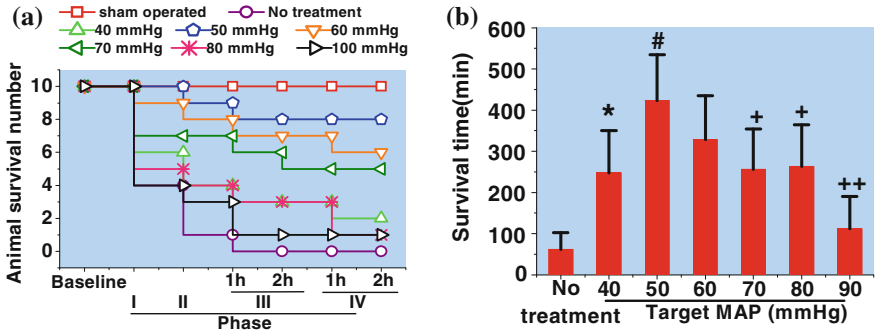

Fig. 1 Effect of different target **MAP** during uncontrolled hemorrhagic shock on survival in rats. **a**: survival number; **b**: survival time, Data are mean ± SD (n = 10). *: $P < 0.05$, vs no-treatment group; #: $P < 0.05$, vs 40-mmHg group; +: $P < 0.05$, ++: $P < 0.01$, vs 50-mmHg group

decrease the requirement of fluid and blood during prehospital and operation, and maintained a higher hemotocrit and hemodynamics as compared to normotensive resuscitation group (MAP maintained at 80 mmHg). Morrison et al. [14] compared the effects of 50, 65 mmHg and standard fluid resuscitation on traumatic shock using 90 trauma patients, and found 50 mmHg of target fluid resuscitation pressure could decrease the postoperative coagulopathy and lower the risk of early post-operative death. Although this new measure has achieved good results in trauma shock resuscitation in laboratory and clinic, but more clinical investigations are needed to further confirm the validity, security and application range of this measure [15, 16].

Delayed resuscitation The traditional viewpoints showed that fluid resuscitation and vasoactive drugs should be given immediately to rapidly increase the blood pressure to normal level after shock. But recent studies showed that for severe traumatic shock, especially for uncontrolled hemorrhage shock, early use of vasoactive drugs or large amounts of fluid to rapidly raise the blood pressure before completely controlling bleeding did not improve the survival rate of the patients. In contrary, it has big risk to increase the mortality and complication [17]. Based on the results of laboratory and clinical research, the new concept of "delayed resuscitation" is suggested for severe traumatic shock, especially for uncontrolled hemorrhagic shock. This concept advocated a small amount of balanced salt solution was given to this kind of patients to meet the basic need of body before operation, but not giving a large amount of fluid resuscitation. A very famous observation is from Bickell et al. They compared the effects of immediate versus delayed fluid resuscitation on severe trauma with 598 penetrating torso injured patients. The results showed that patients in delayed resuscitation group had better organ function, lower renal, respiratory, and infection complications and lower mortality than immediate resuscitation group [18]. Schreiber et al. investigated the effects of controlled resuscitation and standard resuscitation on trauma patients in out-of-hospital settings in nineteen emergency medical services (EMS) systems in the Resuscitation Outcome Consortium. Eligible patients had an out-of-hospital

systolic blood pressure (SBP) of 90 mm Hg or lower. Patients in controlled resuscitation group received 250 mL of fluid if they did not have radial pulse or an SBP lower than 70 mm Hg and another 250-mL boluses to maintain a radial pulse or an SBP of 70 mm Hg or greater. Patients in standard resuscitation group received 2 L initially and additional fluid to maintain an SBP of 110 mm Hg or greater if needed. The crystalloid protocol was maintained until hemorrhage control or 2 h after hospital arrival. The results found that 24 h after admission, there were 5 deaths (5 %) in the controlled resuscitation group and 14 (15 %) in the standard resuscitation group. Among patients with blunt trauma, 24-h mortality was 3 % in controlled resuscitation group and 18 % in standard resuscitation group. These results suggest that controlled resuscitation in out-of-hospital and hospital settings is proper and may offer an early survival advantage in blunt trauma [19]. Nevertheless, what type of and how much fluid are needed for different trauma patients during pre-hospital period needs further investigation. Large-scale, Phase III clinical trials to investigate the effects on survival and other clinical outcomes are needed.

Hypothermic resuscitation Hypothermic resuscitation has always been a controversial issue. Long-time profound hypothermia may influence the metabolism, coagulation and cardiovascular functions [20]. But in recent years, more and more studies suggested that, for severe traumatic hemorrhagic shock, short-term and mild hypothermia during the period from injury to operation may enhance the hypotensive resuscitation effects. Safer resuscitation center of America found that mild or moderate hypothermia (32–28 °C) implemented before bleeding control from injury could significantly increase the animal survival and vital organ function [21]. Our studies found that 1 h, 34 °C mild hypothermia significantly enhanced the resuscitation effect of hypothensive resuscitation, which include decreasing the cellular metabolism rate, reducing the oxygen demand, and extending the golden save time [22] (Figs. 2 and 3). Recently, Gu et al. [23] found pharmacologically induced hypothermia attenuates the traumatic brain injury in neonatal rats. These studies showed that hypothermic resuscitation has beneficial effect for traumatic shock before hemorrhage controlled. But how to implement hypothermic resuscitation at early stage of shock needs further investigation. In addition, it's worth pointing out that the controlled hypothermia at early stage of shock treatment is different from the spontaneous hypothermia in trauma patients. The former is beneficial for the trauma patients while the latter is harmful. Large amount of studies showed that severe hemorrhage, large amount of blood and fluid infusion after trauma would result in hypothermia, which severely interferes with the cellular metabolism and organ function [24].

Damage control resuscitation In patients with severe trauma, in addition to the limited fluid resuscitation, "damage control resuscitation" has also been put forward in recent years [4]. The damage control resuscitation is that, in the first 24–48 h treatments for the severe trauma patients, non-surgical treatment strategies are taken to prevent or reverse a series of hemorrhage induced damages, such as blood loss anemia, coagulopathy, hypothermia, acidosis (spontaneous), etc., which may improve the resuscitation effect. The specific measures include: application of

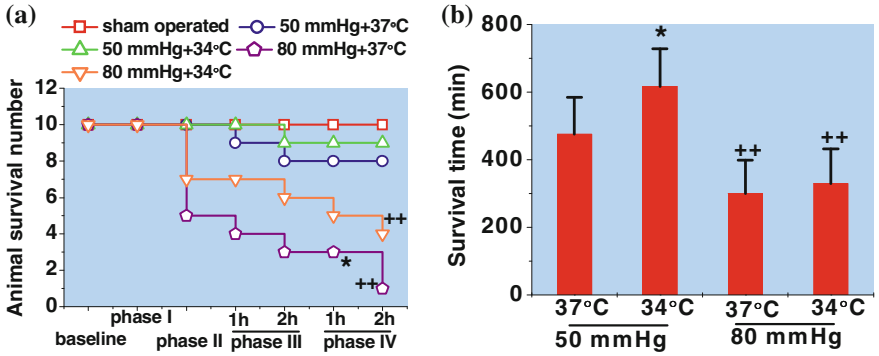

Fig. 2 Effect of normal and hypothermic resuscitation on survival time and survival rate following hemorrhagic shock in rats. Survival time and survival rate are represented by the median and Kaplan–Meier survival line and analyzed by the interquartile range (IQR) and Kaplan–Meier survival analyses, respectively. n=10/group. **a** survival rate; **b** survival time. * $P < 0.05$, ** $P < 0.01$, comparison of different temperatures at the same pressure, + $P < 0.05$, ++ $P < 0.01$, comparison of different pressures at the same temperature

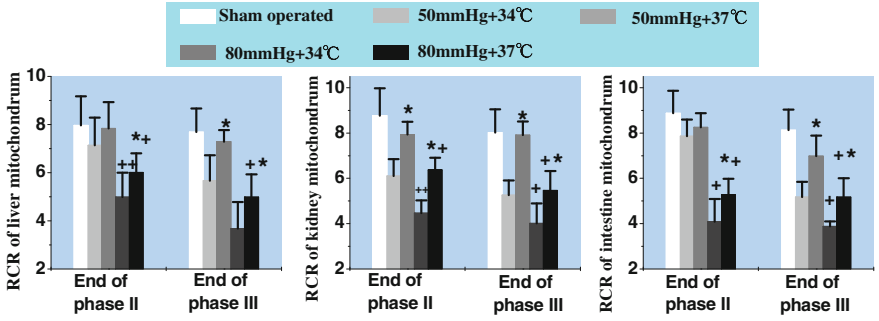

Fig. 3 Effects of normal and hypothermic resuscitation on mitochondrial function of liver, kidneys, and intestine following hemorrhagic shock in rats. Data represent the mean ±SD of 8 observations (n=8/group). **a** liver; **b** kidneys; **c** intestine. RCR: respiratory control rate. * $P < 0.05$, ** $P < 0.01$, comparison of different temperatures at the same pressure, + $P < 0.05$, ++ $P < 0.01$ comparison of different pressures at the same temperature

permissive hypotensive resuscitation, prevention and treatments for hypothermia including passive and positive warming measures, the use of exogenous buffer to correct acidosis, direct application of 1:1:1 fresh frozen plasma, packed red blood cell and platelet. Recombinant factor VII was recommended early use, etc. [25, 26]. The damage control resuscitation has been widely used in early treatment of severe trauma and shock, but some specific proposals and measures, especially for the treatment of coagulation function, still need further investigation to improve the efficacy [27, 28].

Adaptive hypotensive resuscitation The common view point of fluid resuscitation for traumatic sock is rapidly recovering the blood pressure via enough fluid resuscitation after surgical bleeding control. While the fact is that immediately implementing enough fluid infusion after surgical bleeding control would cause tissue edema and damage organ function. Our research team found that immediately recovering the blood pressure to normal level (MAP at 90 mmHg) for uncontrolled hemorrhagic shock rats after bleeding control further damaged the vital organ function, the main reason is the tissue edema and cellular function injury after immediately enough resuscitation. While transiently adaptive hypotensive resuscitation, maintaining MAP at 70 mmHg for 1 h enhanced the resuscitative effect of hypotensive resuscitation including significantly improved the tissue oxygen delivery and utilization, improved the vital organ function and animal survival [29] (Figs. 4 and 5).

The resuscitation end point of traumatic shock Restoration of blood volume, tissue perfusion and oxygen supply are the core issues of resuscitation for traumatic hemorrhagic shock. The traditional fluid resuscitation endpoints of traumatic hemorrhagic shock include blood pressure, heart rate and urine volume, etc. But these parameters cannot well reflect the tissue perfusion and oxygenation status, especially when the patients are in compensatory stage or after use of vasoconstrictor drugs. In recent years, many researchers have proposed many new resuscitation indices, including oxygen delivery (DO2), oxygen utilization (VO2), blood lactate, base deficit, and gastric mucosa pH value, etc. These indices provide reliable reference for ideal resuscitation and increase the success rate of resuscitation for trauma shock [30, 31].

DO2 and VO2 Studies have shown that tissue ischemia and hypoxia in patients with severe trauma and shock are not simply caused by insufficient blood supply, but are closely related to DO2 and VO2. Therefore, the recovery of shock in recent years, in addition to using blood pressure and cardiac index (CI) as resuscitation

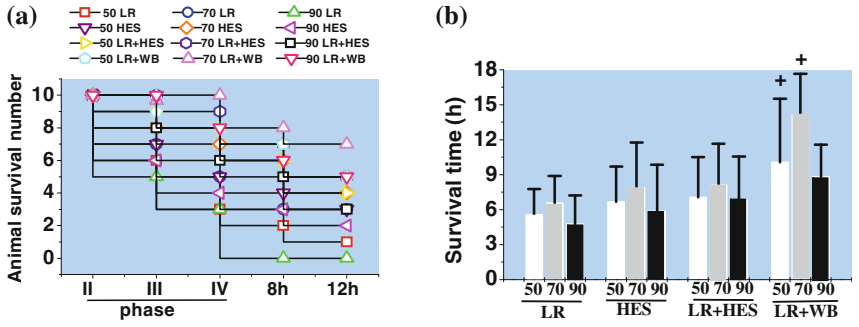

Fig. 4 Effects of different target resuscitation pressure after bleeding control on survival time and rate in hemorrhagic shock rats. a 12-h survival rate; **b** survival time; Survival time and survival rate were represented by median and Kaplan–Meier survival line and analyzed by interquartile range (IQR) and Kaplan–Meier survival analyses, respectively. LR: lactated Ringer's solution; HES: hydroxyethyl starch; WB: whole blood. 50, 70, 90 in X-axis means maintaining MAP at 50-, 70-, 90- mmHg with LR, HES, or LR+HES and LR+whole blood. + $P < 0.05$ vs the same MAP level of the LR group

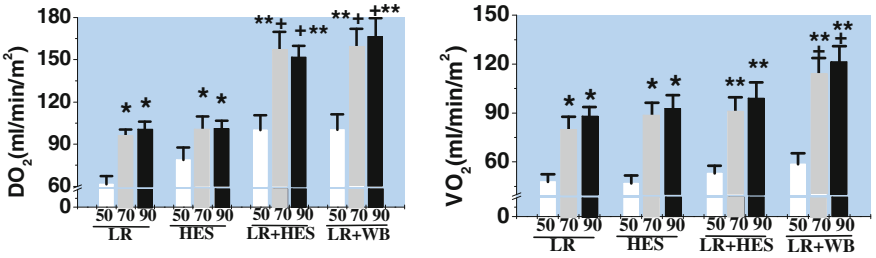

Fig. 5 Effects of different target resuscitation pressure after bleeding control on xygen delivery (DO_2,) and oxygen consumption (VO_2) in hemorrhagic shock rats. Data are expressed as mean+/-SD (n=10); LR: lactated Ringer's solution; HES: hydroxyethyl starch; WB: whole blood. 50, 70, 90 in X-axis means maintaining MAP at 50-, 70-, 90- mmHg with LR, HES, or LR +HES and LR+whole blood. * $P < 0.05$, ** $P < 0.01$ vs 50 mmHg group in the same fluid groups; + $P < 0.05$ vs the same MAP level of the LR group

endpoints, some new endpoints have been put forward such as DO2 and VO2. Many animal and clinical studies have shown that traumatized animals and patients who obtained super normal value of DO2 and VO2 in resuscitation can get higher resuscitation success rate and higher survival than those who did not obtain the super normal value of DO2 and VO2 in resuscitation [32]. Common measures to increase DO2, VO2 include: (1) expand capacity and improve the effective circulating blood volume; (2) use positive inotropic drugs, such as dopamine and dobutamine; (3) use vasoconstrictors (epinephrine, norepinephrine, and phenylephrine) when dopamine is invalid; (4) improve the ventilation and maintain arterial blood oxygen saturation.

Blood lactate, base deficit, the pH value of gastric mucosa Many studies have shown that blood lactate, base deficit and the pH value of gastric mucosa are good resuscitation end points and prognostic indicators, which are closely related to the prognosis and mortality of patients with severe shock. One study showed that the survival rate of patients whose blood lactate levels recovered to normal status within 24, 24–48 h and more than 48 h were 100, 78 and 14 %, respectively. All patients whose gastric mucosa pH < 7.32 and corrected into normal status within 24 h were saved, while for those whose pH was not corrected within 24 h, the mortality was 50 %. More organs had dysfunction in patients whose gastric mucosa pH was not corrected within 24 h than those whose gastric mucosa pH was corrected.

3 New Types of Resuscitation Fluids and Blood Substitutes

Fluid resuscitation is usually divided into crystalloid and colloid fluids. Crystalloid fluids include isotonic solution and hypertonic saline solution while colloidal fluids include albumin, dextran, gelatin and hydroxyethyl starch. However, they have their own advantages and disadvantages [33]. Isotonic crystalloid such as lactated

Ringer's solution (LR) or physiological saline, is inexpensive and easy to be stored, while their resuscitation effectiveness is low, it is only 25 % that whole blood has. It is difficult to be broadly applied in disaster or combat because of logistic issues. Hypertonic crystalloid such as 7.5 % hypertonic saline, has higher resuscitation effectiveness (4.5 times of whole blood), while because of its side effect (high concentration of chloride induced acidosis), 7.5 % hypertonic saline can only be temporarily used in disaster and combat at early stage [34, 35]. Colloids such as dextran, albumin, hydroxyl ethyl starch (HES), has good resuscitative effectiveness, which is often used to resuscitate traumatic hemorrhagic shock in disaster or at combat. While because of their side effects such as dextran affecting blood match test, HES affecting renal function, their application is limited [36, 37]. An ideal resuscitation fluid should meet with the following elements: (1) restore the plasma volume quickly and improve microcirculation perfusion and oxygen supply; (2) with oxygen-carrying function; (3) with no obvious side effects, such as immunoreactions; (4) with the function of cell protection; (5) the price is not expensive and easy for storage as well as transportation. Obviously, the resuscitation fluids commonly used in clinical practice cannot meet with these requirements.

Therefore, investigators have been studying to solve these issues and develop new types of resuscitation fluids: (1) modified hemoglobin. Investigators usually use human's waste blood or animal blood to develop modified hemoglobin via artificial modification or intermolecular cross-linking. The advantages of modified hemoglobin include no/less immunogenicity, without cross-matching and infection risk when used [38, 39]. In recent years, the United States, Japan, Canada and other countries have been spending a lot of money to develop this kind of product, part of products have completed phase III clinical trial and goes into market in some countries, such as South Africa and Mexico [40, 41]. However, such products have not large-scale listed yet because of some toxicities such as vasoconstriction action, renal toxicity and oxidative damage, thereby further investigations and newly types of products are still needed [42–44]. (2) Functional resuscitation fluids. Investigators have been trying to develop a kind of fluid that have cell protection, so as to prevent ischemia or hypoxia induced cell damage [45]. But till now, no this kind of product has been used in clinic. (3) In recent years, studies have shown that polymorphonuclears (PMN) can be activated after infusion of large amount of LR solution. It is demonstrated that D-lactic acid in LR solution is the main cause of PMN activation. The study of Lee et al. showed that LR solution contains 14 mmol/L L-lactic acid and D-lactic acid. The activation of PMN could be significantly decreased if D-lactic acid in LR solution was replaced by L-lactic acid. The same results could be obtained if lactic acid in LR solution was replaced by acetone. The result suggests that D-lactic acid is associated with the activation of PMN [46]. A kind of acetone Ringer's solution has been developed and has been proved with good beneficial to shock [47].

4 Early Application of Vasoactives

Usually, vasoactives are not applied at accident spot or at early stage of shock. It is only used when blood pressure dramatically decreases, which endangers life but without fluid infusion condition. In recent years, the application of vasoactives during hypotensive resuscitation period has been explored. It is aimed to reduce the fluid requirement and win the time for subsequent treatments. Stadlbauera et al. observed the maintaining effect of arginine vasopressein (AVP) on hemodynamics with swine uncontrolled hemorrhagic shock model. Pigs were cut a piece of mesentery (0.5 cm × 5 cm), when the MAP decreased to 20 mmHg, 0.4 U/kg of AVP or 25 ml/kg of LR + 25 ml/kg of 3 % gelatin were given. 30 min after administration, the surgical operation was taken to control the bleeding and gave the definitive management. The results showed that the MAP in AVP and fluid resuscitation group could increase to 55 mmHg from 15 mmHg 5 min after administration, AVP could better maintain the blood pressure, while the blood pressure in fluid resuscitation group decrease rapidly. All 7 pigs in AVP groups and 6/7 pigs in fluid resuscitation group survived to definitive treatment, none of 7 pig in saline group survived to definitive treatment. The results showed that AVP could increase the short-term survival of uncontrolled hemorrhagic shock animals. The application of vasoactives during uncontrolled hemorrhage period could win the time for the definitive treatment [48, 49]. Our research group observed beneficial effects of small dose of AVP (0.4 U/kg) in combination with norepinephrine (NE, 3 μg/kg) on uncontrolled hemorrhagic shock rats. We found small dose of AVP in combination with NE during the period of uncontrolled hemorrhage could maintain the hemodanamics, improve the cardiac function, prolong the survival time, and finally win the time for subsequent treatment [50, 51].

5 New Types of Anti-shock Drugs

With the development of pathophysiology of trauma shock, some targeted anti-shock drugs including new types adrenergic agonists (*dobutamine*), opioid receptor antagonists (*naloxone, naltrexone, nalbuphine*), calcium antagonists (*nicardipine, nimodipine*), arachidonic acid metabolite inhibitor (*ibuprofen, ketoprofen*), cAMP-PDE inhibitors (*pentoxifylline*), oxygen free-radical scavengers, shock cytokine antagonists and endotoxin antagonists, etc. were developed. These drugs have good therapeutic effects to endotoxic shock, hemorrhagic shock as well as cardiogenic shock, and exhibits a good prospect to shock therapy [52–59].

Nevertheless, there are two big problems unsolved for these anti-shock drugs: (1) the use of these drugs is based on sufficient volume resuscitation, which is not suitable for early emergency to traumatic hemorrhagic shock in disaster or combat [60–62]; (2) Some of these drugs can promote calcium influx and aggravate the calcium overload after shock. Thus, those anti-shock agents, which is not or less

dependent on fluid resuscitation, and can be used at early stage of shock, should be developed. Our research group found δ-opioid receptor antagonist, ICI-174864 has this potential [62].

6 In Summary

In recent years, early emergent care for war wound and traumatic shock have made great progresses. Some new concepts and measures to treat severe trauma or shock have been put forward as mentioned above. However, we should clearly realize that there are many problems which are unsolved. Thus following aspects should be strengthened in future to improve the entire ability to treat severe trauma and shock: (1) The construction of emergency system or first aid organization and their implementation mechanism should be strengthened, so as to make sure the severe trauma or shock victims can get effective management in platinum 10 min and golden 1 h. (2) Large scale multicenter clinical trials need to be organized to verify the effectiveness and safety of new fluid resuscitation regimes and new anti-shock agents. (3) The mechanisms of severe trauma and shock and its complications such as acute respiratory distress syndrome (ARDS) and multiple organ dysfunction syndrome (MODS) need further investigation.

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Calcium Desensitization Mechanism and Treatment for Vascular Hyporesponsiveness After Shock

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Abstract Shock, the common pathological process of many critical illnesses, is often accompanied by vascular hyporeactivity, which severely interferes with the development and treatment of shock and other critical conditions, especially interferes with the application of vasoactive agents. So, it is very important to shed light on the mechanisms and search for the effective measures. Lots of studies focused on the characteristics of vascular hyporeactivity and mechanisms following shock in recent years. Since the limitation of previous mechanisms for vascular hyporesponsiveness (receptor desensitization mechanism and membrane hyperpolarization mechanism), our research group raised calcium desensitization mechanism for vascular hyporesponsiveness after shock and proposed pertinent treatment measures.

Keywords Shock · Vascular hyporesponsiveness · Calcium sensitivity · Rho kinase · Rac · PKC · Therapy

1 Introduction

Shock,the common critical illness, is one of the main causes resulting in the mortality after severe trauma at early and late stage. Vascular hyporesponsiveness (hyporeactivity), the common complication of shock, is the main reason that results in poor tissue perfusion, refractory shock, multiple organ dysfunction syndrome (MODS)/multiple organ failure (MOF), and even death [1]. Meanwhile, vascular hyporesponsiveness is also the main reason that interferes with the application and effect of vasoactive during shock. Many factors may interfere with the vascular response following shock including acidosis, nitric oxide (NO), inflammatory

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cytokines such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β, etc. [2]. The present mechanism for vascular hyporeactivity includes receptor desensitization and membrane hyperpolarization mechanisms. Since the limitation of these two mechanisms for the occurrence of vascular hyporesponsiveness, our research group raised calcium desensitization mechanism of vascular hyporesponsiveness after shock. This review will discuss the characteristics of vascular hyporesponsiveness after shock, the calcium desensitization mechanism and pertinent treatment measures.

2 The Characteristics of Vascular Reactivity After Shock

Studies showed no matter in traumatic-hemorrhagic shock or septic shock, the vascular reactivity all appeared biphasic change and vasculature bed differences. Hemorrhagic shock also has gender and age differences.

2.1 In Hemorrhagic Shock

Biphasic change Studies showed vascular reactivity appeared biphasic change after hemorrhagic or traumatic shock [3–7]. The vascular response is significantly increased at early-stage shock (at 10 and 30 min after shock) and decreased at late-stage or prolonged shock (2 h after shock) [4]. Available data showed that the balance of RhoA/Rac and Ang1/Ang2 participated in the occurrence of the hemorrhagic shock-induced biphasic change of vascular reactivity. RhoA/Rac, the important member of small G protein family, was found participated in the regulation of biphasic change of vascular reactivity after hemorrhagic shock [5–7]. Li et al. [8] found that the activity of RhoA was increased and the activity of Rac1 was decreased at early-stage of shock, while at late-stage of shock, the activity of RhoA was decreased, and the activity of Rac1 was increased, the ratio of RhoA/Rac1 was positively correlated with the changes of vascular reactivity. Further studies showed that RhoA/Rac played important role in the regulation of vascular reactivity. Rho A may activate Rho kinase and inhibit p21 activated kinase (PAK) to increase the vascular reactivity, while Rac may activate PAK and inhibit Rho kinase to decrease the vascular reactivity [7–10].

Angiopoietin (Ang), the important factor promoting angiogenesis and repairing, includes Ang-1 and Ang-2. Our research group found that Ang1/Ang2 participated in the occurrence of biphasic change of vascular reactivity following hemorrhagic shock [11]. We found the expression of Ang-1 was increased at early shock, but it was decreased at late shock, which was positively correlated with the changes of vascular reactivity after hemorrhagic shock, whereas the expression of Ang-2 did not change at the early shock, while it was increased at late stage of shock, which was negatively correlated with the changes of vascular reactivity after hemorrhagic shock [11]. Exogenous application of Ang-1 could further increase the vascular

reactivity at early-stage shock and improved hypo-reactivity at late-stage shock while exogenous application of Ang-2 could suppress the vascular hyper-reactivity at early-stage shock and aggravated hypo-reactivity at late-stage shock.

Vasculature bed-difference Studies showed that hemorrhagic shock induced-vascular hyporesponsiveness presents vascular bed diversity, which means blood vessels in different places or organs have different change patterns in vascular response following shock. For example, Liu and Dubick et al. [12, 13] found that following hemorrhagic shock, the vascular responses in superior mesenteric artery (SMA), renal artery (RA), femoral artery (FA), celiac artery (CA), and middle cerebral artery(MCA) to norepinephrine (NE) were all significantly blunted but the loss of vascular reactivity in SMA, RA and FA was more severe and rapid than that in CA and MCA. The vascular response in SMA, RA and FA appeared more sensitive to hemorrhage insult than CA and MCA.

As for the reason for the different change pattern of vascular reactivity in different vasculatures, it may be related to Poiseuille Law and *"*Vascular Waterfall*"* in hemodynamics [14, 15]. For example, when systolic pressure decreases below 50 mm Hg, the auto-regulation of the blood flow in cerebral Willis artery circles would become weaken gradually, while the glomerular filtration pressure would drop directly to zero and the urine formation would completely stop at this situation, which indicates that the vasculature bed differences of vascular response following hemorrhagic shock are closely related to blood redistribution and the decrease of tissue perfusion. Besides, further studies showed that vasculature-difference in vascular reactivity was also correlated with different expression of cytokines and inflammatory mediators in various vasculatures. Our study showed that different expression pattern of cytokines and inflammatory mediators such as IL-1β, TNF-α and ET-1 in different organs was possibly the main reason for the difference of vascular hypo-reactivity in different vascular beds [16–20].

Sex and age-difference and the role of estrogen Large amount of studies demonstrated that different ages and genders of animals and human have different responses to trauma and hemorrhagic shock. Chaudry's research group found that female animals could tolerate more severe trauma stimuli than males [21]. Angele et al. found that male patient younger than 50 years old, had higher risks of death than the same ages of females when suffering from the severe blunt trauma, while the tolerate difference was not evident any more in those older than 50 years old when suffering from trauma insults [22, 23]. Further studies showed that sex- and age did not affect the tolerance and outcome of trauma, but also affected the vascular reactivity. Proctor and Newcomer [24] found that the vascular response in 7-week-old rats was higher than other ages of rats, as the increase of age, the vascular reactivity was gradually decreased. Our studies found that vascular reactivity in reproductive age of female rats was higher and had better tolerance to traumatic shock than the same age of male rats or female rats under and over the reproductive age. The same trends were observed in healthy human and trauma patients [25], young and middle aged of people and trauma patients had higher vascular response. Our further studies [25] found that administration of exogenous estrogen (17-βestradiol) played the protective effects in vascular reactivity in

normal and traumatic shock rats at reproductive age (8–24 weeks) but the protective effect of estrogen in older rats (1–1.5 years) was dis-appeared. These findings provided an important evidence for personalized treatment to estrogen-induced gender-/age-related diseases.

2.2 *In Endotoxic Shock*

Studies showed that after endotoxic or septic shock the vascular reactivity also appears biphasic change: increased at early stage and decreased at late stage [26, 27]. In endotoxic or septic shock, the vascular hyporesponsiveness occurred little bit late but more severe [28, 29] than traumatic and hemorrhagic shock. Chen and Li et al. found that the vascular reactivity was increased at 1 h after endotoxic shock, while rapidly decreased after 2 h of endotoxic shock. The loss rate of vascular reactivity reached as high as 80 % of normal level at 4 h after endotoxic shock [30, 31]. They also found that the difference of vascular reactivity existed vasculature difference in endotoxic or septic shock. They found that in early endotoxic shock, the increase of vascular reactivity in superior mesenteric artery was not obviously. The decrease rate of vascular reactivity in order was superior mesenteric artery at first (reduced by 34.8 %), next for renal artery (33.7 %) and celiac artery (16.7 %). They considered that these results may be correlated with the blood redistribution in various vasculatures after shock but its underlying mechanisms are not clear. Some studies showed that the seriously damage of vascular endothelial cells and smooth muscle cells may be the main reason for the hyporeactivity after endotoxic or septic shock [32].

Research showed that males and aged individuals are proved to have higher risk for the development of sepsis and multiple organ failure after severe trauma or shock [33, 34]. Bone [35] reported males with sepsis or septic shock had more morbidity and mortality as compared with females. Schroder et al. [36] found that women had a higher survival rate (74 %) as compared with men (31 %) following the onset of sepsis, which are consistent with the results in hemorrhagic shock. In addition, only few studies paid attention to vascular reactivity in burn injured shock. Available data did not clearly show burned shock had biphasic change and age- and gender-differences in vascular reactivity. Therefore, continuous investigations on vascular reactivity after burn shock are needed.

3 The Calcium Desensitization Mechanisms of Vascular Hyporesponsiveness

Previous studies showed that there are two mechanisms responsible for the occurrence of vascular hypo-reactivity after shock including receptor desensitization mechanism and membrane hyper-polarization mechanism. Based on the

problem of these two mechanisms, our research group raised calcium desensitization mechanism of vascular hyporesponsiveness after shock.

3.1 Receptor Desensitization and Membrane Hyper-Polarization Mechanisms

Receptor desensitization mechanism Studied showed that adrenergic receptors (ARs) are desensitized following shock [37]. Following hemorrhagic or septic shock, high concentration of catecholamine such as norepinephrine and adrenaline could cause receptors desensitization including the decrease of receptor amount or affinity. In addition, shock-induced ischemia and hypoxia and large amount of cytokines as well as endogenous opioid peptide (EOP) may also inhibit the functions of adrenergic receptors and result in the receptor desensitization [37].

Down-regulation of receptor number is one of the important mechanisms for receptor desensitization. Sandrini et al. [38] conducted an investigation on the changes of ARs in brain, heart and spleen in rats after hypovolemic shock and found that there were no obvious changes in Bmax and Kd of α1-,α2- and β-ARs, but the amount of α-, β-ARs in heart and α2-ARs in spleen were significantly reduced. Tait and Onuma et al. also found the β-ARs were down-regulated in liver and heart in traumatic-hemorrhagic shock rats [39, 40]. Down-regulation of the receptor number includes two types. One is receptor internalization, it is just The first step is the decrease of receptor number on the cell membrane surface but the receptor's total quantity in each cell has not changed. The second type is the decrease in receptor's total quantity, which means the real down-regulation of receptors. In the early stage of shock, receptor desensitization may mainly come from the decrease of receptor amount on the cell membrane surface. and this is possibly correlated to the internalization of surface receptors, which had been confirmed by Maisel et al. [41]. The real down-regulation of receptors is related to the degradation of internalized receptors and the decrease of receptor expression, which appears at 3-5 h even 1-2d after the first step (Fig. 1). High concentration of catecholamine and cytokines may down-regulate the adrenergic receptors. High concentration of catecholamine can result in the internalization of adrenergic receptor [37]. TNF-a and IL-1β may down-regulate the amount of adrenergic receptors via inhibition of the transcription of the adrenergic receptor [19].

Receptor affinity decrease is another important mechanism for receptor desensitization. It is often seen before the decrease of receptor number on the cell membrane surface. Previous studies showed that the receptor affinity of β-AR is generally declined during endotoxic and hemorrhagic shock but the affinity of α-AR remains constant [42, 43]. Decease of receptor affinity may also result in receptor uncoupling, and thereby decrease the activity of adenylyl cyclase (AC). Therefore, the coupling obstacles between adrenergic receptors and adenylyl cyclase may be the most important factor for receptor desensitization.

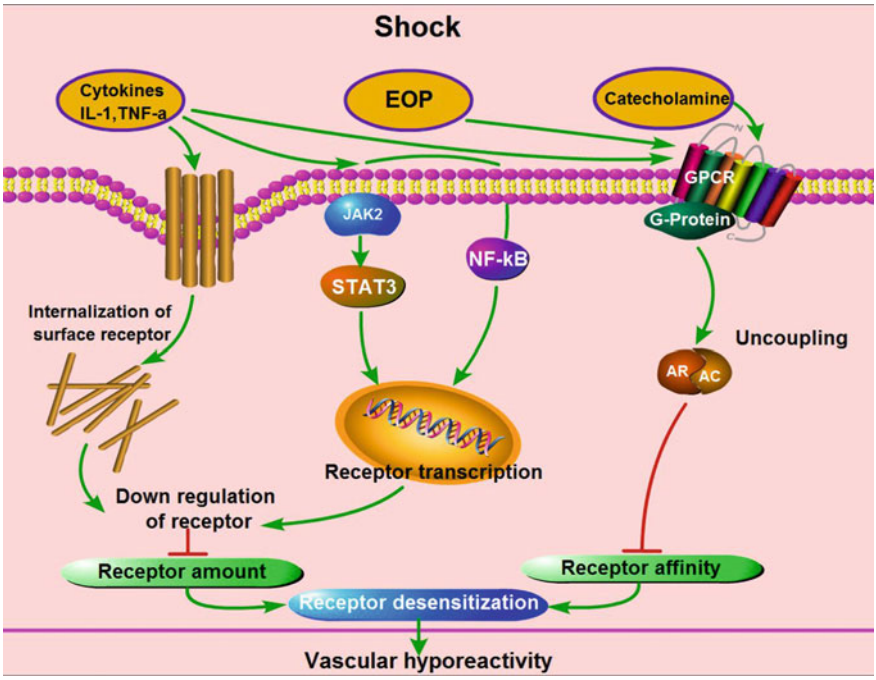

Fig. 1 Receptor desensitization mechanism of vascular hyporesponsiveness. The signal transductions for receptor desensitization include three pathways. One is the internalization of surface receptors, the second pathway is receptor transcription regulation through JAK2-STAT3 and NF-kB related pathways. Both of these two pathways might down-regulate the receptor amount. The third pathway is receptor uncoupling and receptor affinity regulation through G-proteins. *EOP* endogenous opioid peptide; *JAK2* janus activating kinase 2; *STAT3* signal transducer and activator of transcription 3; *GPCR* G protein coupled receptor; *AR* adrenergic receptor; *AC* adenylyl cyclase

Membrane hyperpolarization mechanism Studies showed after hemorrhagic and burn shock, vascular smooth muscle cell (VSMC) would appear membrane hyperpolarization. Membrane hyperpolarization is a crucial mechanism to vascular hyporesponsiveness after shock. VSMC membrane hyperpolarization mainly involves in two kinds of potassium channels including ATP-dependent K^+ channel (K_{ATP}) and large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel. In physiological conditions, cytoplasm ATPs are in mmol–magnitude, which is enough to completely close the K_{ATP} channels on cell membrane [44]. While in shock condition, the disorders of the cell oxidative metabolism or the huge reduction of ATP would cause the open of K_{ATP} channels on cell membrane [45, 46]. The over-opened K_{ATP} channels in VSMC would result in membrane hyperpolarization of VSMC. This process would inhibit the potential dependent calcium channel, decrease the Ca^{2+} inflow, and finally result in vascular hyporesponsiveness. The inducing factors for over opening of K_{ATP} channels include ATP decrease, nitric oxide (NO) production, and so on.

Although K_{ATP} channel plays an important effect in VSMC membrane hyperpolarization and vascular hypo-responsiveness following shock, the density of K_{ATP} channel in VSMC is only one channel per 10 μm^2. However, BK_{Ca} channel, not only broadly distributes on VSMCs (its density in VSMC is 1–4 channels/μm^2), but also plays an important regulatory role in vascular reactivity [47]. BK_{Ca} channel consists of α-subunit and accessory β-subunit, which co-affect the characteristics of the physiology and pathophysiology of BK_{Ca} channel [48]. Studies showed that Ca^{2+} sparks are the physiological activators of BKCa Channels. A single Ca^{2+} spark may cause the open of its surrounding BK_{Ca} channel and K^+ outflow, which forms spontaneous transient outward current (STOC) [47]. This process may induce membrane hyperpolarization. In turn, over-opened BK_{Ca} channels cause the decrease of external calcium influx and finally results in the vascular hyporesponsiveness (Fig. 2).

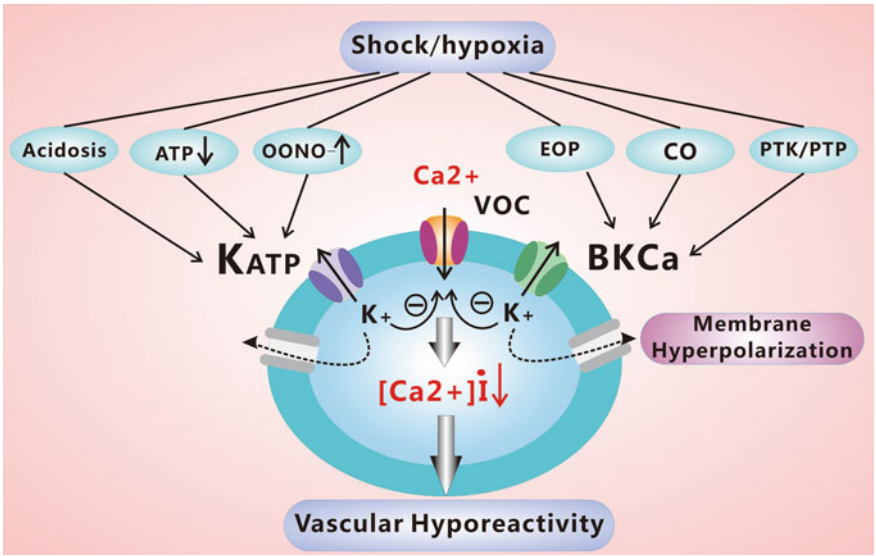

Fig. 2 Membrane hyperpolarization mechanism of vascular hyporesponsiveness. The membrane hyperpolarization of vascular smooth cell after shock is mainly involved in two channels- K_{ATP} channel and BKCa channel. The membrane hyperpolarization of vascular smooth cell may inhibit the open of VOC, and via which results in the decrease of intracellular [Ca^{2+}] and vascular hyporesponsiveness. *K_{ATP} channel* ATP-dependent K^+ channel; *BKCa channel* large conductance Ca^+- activated K^+ channel; *VOC* voltage-dependent calcium channel; *CO* carbon Oxide; *EOP* endogenous opioid peptide; *PTK* protein tyrosine kinase; *PTP* protein tyrosine phosphatase

3.2 Calcium Desensitization Mechanism

An interesting phenomenon should be concerned is that restoration of adrenergic receptor, K^+ and Ca^{2+} channels' functions cannot return the vascular reactivity to normal level, which suggests that there are other ways to regulate the vascular reactivity following shock. The key event of receptor desensitization and membrane hyperpolarization mechanism responsible for vascular hyporesponsiveness is the decrease of intracellular [Ca^{2+}]. While at late stage of shock or in severe shock, the intracellular calcium in VSMCs is over loaded, but the vascular hyporesponsiveness still exists [5]. This suggests there may be other mechanisms that participate in the occurrence of vascular hyporesponsiveness after shock. Based on the basic theory that the contractile force of VSMC is dependent on the ratio of force and calcium, our research group raised the calcium desensitization hypothesis for vascular hyporesponsiveness. With in vivo and in vitro, and animal and cell experiments, we found that following shock, VSMC indeed exists calcium desensitization. Calcium desensitization played very important effect in vascular hyporesponsiveness [49]. That is to say the occurrence of vascular hyporesponsiveness have calcium desensitization mechanism. Our further studies found that Rho kinase and PKC pathway are the key pathways in calcium sensibility regulation of vascular reactivity following shock (Fig. 3).

Role of Rho A-Rho kinase pathway in the regulation of calcium sensitivity Rho kinase, a Ser/Thr protein kinase, is a GTP-Rho binding protein. Previous studies showed that Rho kinase participates in the regulation of many biological process of cells, such as cell proliferation, cell differentiation and migration of tumor cells, the migration and invasion of trophoblast cells, etc. [50]. Our research group and Schmitz et al. found that Rho kinase plays critical role in the regulation of vascular reactivity and calcium sensitization following hemorrhagic shock [4, 50].

Basic research showed the calcium sensitivity regulation of VSMC depends on the phosphorylation and dephosphorylation of myosin light chain (MLC), which is regulated by myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP), respectively [51, 52]. Studies showed that there are three ways for Rho kinase regulating the calcium sensitivity of VSMC (Fig. 3): (1) Rho kinase phosphorylate MLC20 directly. The strength of Rho kinase phosphorylating MLC20 is about one third of MLCK. So this way is not the main way that Rho kinase regulates calcium sensitivity after hemorrhagic shock. (2) The main way of Rho kinase regulating calcium sensitivity is that Rho kinase phosphorylates myosin-binding subunits (MBS) of MLCP at Thr2695, Thr2850 and Ser2854, and via which inhibits the activity of MLCP and increases the phosphorylation level of MLC20 [53]. (3) Rho kinase can also activates CPI-17 via phosphorylation of the Thr238 site of CPI-17. The activated CPI-17 enhances the phosphorylation of MLC20 through inhibiting the MLCP.

Role of PKC pathway in the regulation of calcium sensibility regulation Protein kinase C (PKC), a Ser/Thr protein kinase, plays a critical role in cell

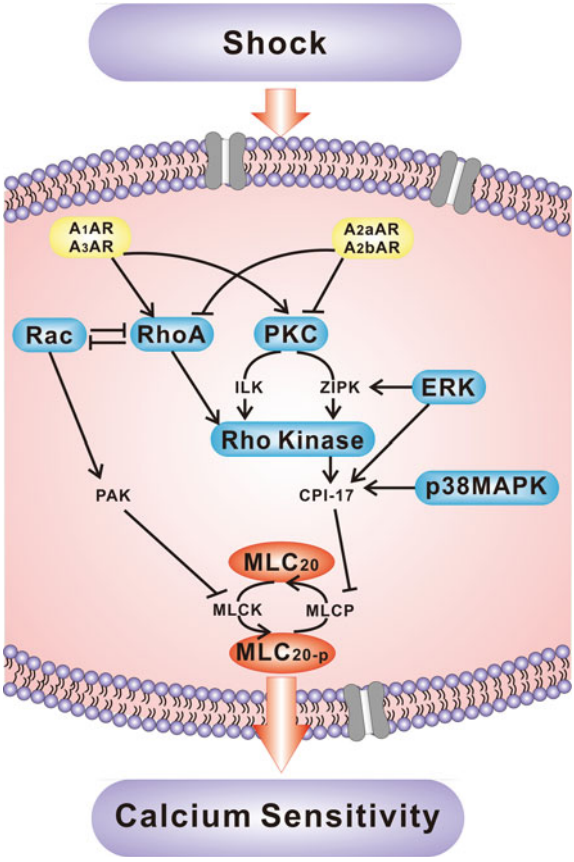

Fig. 3 Calcium desensitization mechanism of vascular hyporesponsiveness. RhoA regulates vascular reactivity mainly through activation of Rho kinase and inhibition of Rac1 while Rac1 regulates vascular reactivity mainly through inhibition of RhoA and activation of PAK. Rho kinase may be through three pathways to exert effect: directly activate MLC20, inhibit MLCP and via CPI-17. ZIPK and ILK are the important downstream molecules of PKC. ZIPK may directly bind with CPI-17. ERK and p38MAPK play effect mainly through ILK and ZIPK or CPI-17. A_1AR and A_3AR may enhance the activity of PKCε and RhoA while A_{2a}AR and A_{2b}AR may inhibit the activity of RhoA and PKCε. *RhoA* Ras homolog gene family member A; *Rac* Ras related C3 botulinum toxin substrate; *PAK* P21-activated kinase; *MLCP* myosin light chain phosphatase; *MLCK* Myosin light chain kinase; MLC_{20} 20-kDa myosin light chain; *PKC* protein kinase C; *MAPK* Mitogen-activated protein kinase; *ERK* extracellular signal-regulated kinase; *CPI-17* protein kinase C-dependent phosphatase inhibitor of 17 kDa; *ILK* integrin linked kinase; *ZIPK* Zipper- interacting Protein Kinase; *AR* adenosine receptor

adaptability to extracellular environment. PKC is also involved in varieties of physiologic functions including cell proliferation, differentiation and migration, cytoskeletal structure, and apoptosis [54, 55]. PKC is a big family consisting of at least 12 isoforms, and the main isoforms distributed in the vascular system are PKC

α, ε, δ and ξ. Basic research showed that the various isoforms of PKC, especially α and ε isoforms, may be activated through subcellular redistribution and transfer from cytoplasm to membrane, and then trigger a series of cascade reactions that ultimately interacts with the contractile myofilaments and leads to VSMC contraction [56].

Many studies showed that PKC participated in the regulation of vascular reactivity and calcium sensitivity following shock. Our previous study found that PKC agonist, phorbol-12- myristate-13-acetate (PMA), could improve and stabilize the hemodynamic parameters and play beneficial effect for hemorrhagic shock in rats through improving the vascular reactivity and calcium sensitivity [8, 18]. There are several mechanisms that PKC regulates the vascular reactivity and calcium sensitivity [57–59]: (1) The study of Woodsome et al. showed that PKC may phosphorylate CPI-17, and then inhibits the MLCP activity, via which increases the MLC20 phosphorylation and calcium sensitivity of VSMC [56]. (2) Our recent studies showed that the inhibitory effect of PKC on MLCP is not only related to CPI-17 but also related to integrin-linked kinase (ILK) [60, 61] and zipper-interacting protein kinase (ZIPK) [61]. Our results showed that ZIPK and ILK may be the direct downstream molecules of PKC α and ε, in which CPI-17 may play an indirect modulating role on MLCP. Our very recent study found that Rho kinase is the downstream molecule of ILK and ZIPK, and the upstream molecule of CPI-17 (Fig. 3) [25].

Role of MAPK pathway in the regulation of calcium sensibility Mitogen-activated protein kinases (MAPKs) belong to a family of serine/threonine protein kinases, which include extracellular-signal regulated kinase (ERK), jun NH2 -terminal kinase (JNK), and p38 MAPK in mammals. It was reported that MAPKs mediated the fundamental biological process to external signals, such as cytokines and inflammatory mediators [5]. Previous studies showed that MAPK had a critical role in regulating cell differentiation, proliferation and cell death [62, 63]. Our recent study [5] investigated the potential role of MAPK in the regulation of vascular reactivity and calcium sensitivity after hemorrhagic shock and interestingly found that the activity of ERK and p38MAPK were positively correlated with the change of vascular reactivity after hemorrhagic shock [4]. In SMAs, the activity of ERK and p38MAPK was significantly increased at early stage of shock (0.5 h) and decreased after prolonged shock (2 h), the inhibitors of ERK and p38MAPK decreased the vascular reactivity and calcium sensitivity following shock. This finding suggests that MAPK pathway participates in the regulation of vascular reactivity and calcium sensitivity following shock.

Role of adenosine and its receptor in the regulation of calcium sensibility Adenosine is one of the most important endogenous modulator released excessively after severe trauma and ischemia or hypoxia in tissue. It has been demonstrated that adenosine mainly produces the marked effect through adenosine receptor in VSMC. There are four types of adenosine receptor (AR) reported in VSMCs, including A_1AR, $A_{2a}AR$, $A_{2b}AR$ and A_3AR. Adenosines which combine with specific AR may cause vasoconstriction (A_1AR) or vasodilatation ($A_{2a}AR$, $A_{2b}AR$). The study of Srinivas et al. reported that exogenous adenosine may reduce the

phosphorylation of MLC in bovine cornea epithelial cells [64]. However, the report of Lai et al. demonstrated that exogenous adenosine may induce MLC phosphorylation on VSMCs and increase its calcium sensitivity [65]. The results suggest that adenosine is closely correlated with vascular reactivity and calcium sensitivity.

Huang et al. [53] demonstrated that A_1AR agonist (N6-cyclopentyladenosine, CPA, 10^{-5} mol/L) can induce renal artery constriction, which can be antagonized by Rho kinase inhibitor (Y-27632). This indicates that Rho kinase is correlated with A_1AR in the regulation of vascular tone. The study of Tawfik et al. [66] showed that PKC inhibitor U-73122 could abolish the vasoconstriction induced by A_1AR and A_1AR agonist may enhance the activity of PKCε. This indicates that A_1AR regulating vascular calcium sensitivity is also related to PKC pathway.

However, $A_{2a}AR$ and $A_{2b}AR$ can activate adenylate cyclase which causes the increase in cAMP concentration and PKA activation. The activated PKA may inhibit the activity of Rho kinase. This will induce MLC dephosphorylation and vascular smooth muscle dilation. Besides, the study of Gardner et al. showed that $A_{2a}AR$ may down-regulate the activity of PKC and $A_{2a}AR$ agonist CGS21680 may inactivate PKCε [67].

Our research group and Abbracchio et al. found that A_3AR is also involved in the modulation of vasoreactivity following shock and this regulation is closely related to Rho kinase pathway [68, 69]. Furthermore, Zhao et al. reported that the A_3AR agonist IB-MECA could up-regulate PKCδ activity and A_3AR antagonist MRS-1191 could counteract this function [52], which suggests that A_3AR regulating vascular reactivity and calcium sensitivity is related to PKCδ pathway.

4 The Approaches Towards Vascular Hyporesponsiveness Based on Calcium Desensitization Mechanisms

Based on the various vascular hypo-reactivity theories and its inducing factors, the therapeutic approaches may occur through blocking the related pathways.

4.1 Based on Receptor Desensitization and Membrane Hyperpolarization

Based on receptor desensitization Glucocorticoid (GC) may promote the catecholamine biosynthesis and potentiate the vasoconstriction effect of vasopressin (AVP), Angiotensin II and endothelin (ET) by increasing the sensitivity of their receptors [70–72]. In addition, studies showed cortisol has significant inhibitory effect on pro-inflammatory mediators, such as TNF-α and IL-1β, which are confirmed to be correlated with receptor desensitization [73]. A recent study showed that dexamethasone may rapidly reverse LPS induced hyporesponsiveness of

VSMC to NE and this effect of dexamethasone is related to increasing the phosphorylation of MLC_{20} via increasing activity of the RhoA-Rho kinase pathways. These results demonstrated that glucocorticoid increasing the vascular reactivity is not only related to increasing the sensitivity of related receptors, but also related to increasing the calcium sensitivity of VSMC [57, 58].

There still remains controversy about the application of small doses of GC in patients with trauma or shock. The Surviving Sepsis Campaign Guidelines (2012) [59] suggest if hemodynamic stability can be achieved via adequate fluid resuscitation and vasopressor therapy, intravenous hydrocortisone is not allowed to use in adult septic shock patients. If this is not achievable, 200 mg of intravenous hydrocortisone per day is recommended. In addition, the guidelines also suggest septic shock patients if they should receive hydrocortisone do not use the ACTH stimulation test. When vasopressors are no longer required corticosteroids should be reduced or stopped. If no shock exists, do not use corticosteroids for the treatment of sepsis [59].

Based on membrane hyperpolarization Glybenclamide is a kind of K_{ATP} channel antagonist. Zhao et al. [74] found that application of glybenclamide combined with $NaHCO_3$ could significantly increase the vascular reactivity in hemorrhagic rats. The blood pressures, arteriolar blood flow as well as the 24-h survival rate were also markedly increased after administration of glybenclamide, which indicates that glybenclamide combined with $NaHCO_3$ is an effective regimen in the treatment of severe hemorrhagic shock. Studies showed NO generated $OONO^-$ with superoxide anion can induce the membrane hyperpolarization of VSMC though activation of K_{ATP} channels. Superoxide anion scavenger Tiron may block the production of $OONO^-$ in hemorrhagic shock rats, and inhibit the membrane hyperpolarization of VSMC and improve shock-induced vascular hyporesponsiveness [75].

As a hotspot in recent years, mitochondrial function has important value in the genesis of many diseases, such as cardiovascular diseases, Alzheimer disease, and Parkinson disease, and so on. Mitochondrial dysfunction also takes part in the occurrence of vascular hypo-responsiveness after shock. Zhao et al. found that Polydatin, a mitochondrial protector, can protect the mitochondria of vascular smooth muscle and restore the vascular reactivity in hemorrhagic shock rats [76, 77]. Our recent study found that cyclosporine A (CsA), an inhibitor of mitochondrial permeability transition pore (MPTP), and 4-Phenylbutyrate (PBA), an anti-oxidation agent, could improve the vascular reactivity of traumatic hemorrhagic shock in rats via inhibition of oxidative stress and protecting the mitochondrial function [78, 79].

4.2 Based on Calcium Desensitization Mechanism

Based on RhoA-Rho kinase pathway As mentioned above, Rho kinase and PKC may be the important potential targets to the treatment of vascular

hypo-responsiveness. Studies showed arginine vasopressin (AVP) and its analog terlipressin (TP) have important role in vasodilatory shock animal and patients [80]. Our recent study found that small doses of AVP (0.03 μg/kg/h) and TP (2.6 μg/kg/h) significantly improved the decreased vascular reactivity in hemorrhagic shock and endotoxic shock in rats, rabbits, and septic shock patients. This effect of AVP and TP is closely related to activation of Rho A-Rho kinase pathway [27, 81, 82] (xiao ref 2016).

Further studies showed that ischemic preconditioning may activate Rho A-Rho kinase pathway and improve the vascular reactivity in hemorrhagic shock rats [83]. We observed the effects of ischemic preconditioning on vascular reactivity after hemorrhagic shock in rats and found that 5 % hemorrhage for 30 min before hemorrhagic shock may prevent the decrease in vascular reactivity and calcium sensitivity after hemorrhage. The study showed that hemorrhagic shock may attenuate Rho-kinase activity while ischemic preconditioning may increase the activity of Rho A and Rho kinase.

Based on PKC pathway Phorbol-12-myristate-13-acetate (PMA), a non-specific PKC isoform agonist, was found to have good protective effect on shock. We observed the beneficial effect of PMA in hemorrhagic shock rats. The result showed that 1 μg/kg PMA could significantly enhance the vascular reactivity and calcium sensitivity of hemorrhagic shock rats and improved the hemodynamic indexes as well as liver and renal function [18]. Although PMA has not been used in clinic yet, these findings provide a rational ground to develop this kind of drug or search for other approaches to induce or activate PKC to play protective effect on shock in clinic.

Pinacidil, an adenosine triphosphate–sensitive potassium channel (K_{ATP}) opener, is a common agent to be used to induce preconditioning protection against ischemia insult [84–87]. Our study found 25 μg/kg of pinacidil pretreated 30 min before hemorrhagic shock in rats could activate PKC α and ε to improve the vascular reactivity and calcium sensitivity in hemorrhagic shock [93]. This finding suggests that pinacidil pretreatment can improve the vascular reactivity and calcium sensitivity after hemorrhagic shock, the mechanism is related to the activation of PKC α and ε.

5 Summary

Vascular hypo-reactivity is the common complication of severe trauma, shock and multiple organ dysfunction syndrome (MODS). Many inducing factors have been proposed to be involved in the development of vascular hypo-reactivity. Previous studies showed that there were two mechanisms including receptor desensitization mechanism and membrane hyperpolarization mechanism responsible for shock induced vascular hyporeactivity. Based on the limitation of the present mechanisms, our research group raised calcium desensitization mechanism and pertinent treatment measures for shock induced vascular hyporeactivity. Nevertheless, there

are still some issues that need further investigation such as the more detailed mechanisms and target directed treatment approaches.

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Acute Coagulopathy of Trauma-Shock

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Abstract Acute coagulopathy of trauma-shock (ACoTS) occurs in 25 % of severe trauma patients, and the mortality is fourfold higher than the patients without coagulopathy. Pathophysiology of this complex phenomenon has been emphasized in recent years. Tissue injury, tissue hypoperfusion, activated protein C and the complements play important roles in the early phase after trauma. The use of blood products, hypothermia, acidosis and inflammation are the main mechanism in late stage. Supplementation of coagulation factors and platelets is not effective. Positive resuscitation and improvement of tissue perfusion may be beneficial.

Keywords Acute coagulopathy · Trauma · Shock

Trauma is a leading cause of death in modern society. Trauma mostly occurs in young adults and has a big influence on labor force and social stability for its high mortality and disability. Thus, trauma is called "disease of developed society" [1]. Despite great progress in trauma surgery and intensive care in recent years, mortality and disability of severe trauma remains high. Recent studies showed that 25 % of patients with severe trauma developed coagulopathy in the early phase after trauma, and the mortality in those patients was fourfold higher than patients without coagulopathy [2–4].

Acute coagulopathy after trauma is gradually becoming a hotspot in clinical and laboratory research [5]. It is usually called "acute traumatic coagulopathy" [6], "early coagulopathy of trauma" [7], or "trauma-induced coagulopathy" [8]. Hess and colleagues [9] named it acute coagulopathy of trauma-shock (ACoTS) in 2008. ACoTS is

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widely accepted because it reflects the nature of the responsible underlying processes and pathophysiology of this complicated phenomenon. Here, we focus on the progress of research on ACoTS in recent years, especially on its mechanism.

1 Mechanism in the Early Stage

1.1 Tissue Injury and Hypoperfusion

Loss of clotting factors caused by bleeding and consumption in thrombosis, dilution of clotting factors for massive transfusion, and effects of acidosis and hypothermia on coagulation function are considered the main mechanism for coagulopathy in trauma patients in the early phase. This coagulation disorder is described as systemic acquired coagulopathy (SAC) [10]. Recent studies found that the exact mechanism may be not like that. Brohi and MacLeod et al. found that 25 % of patients with severe trauma developed acute traumatic coagulopathy before arriving at emergency room. These patients always did not present with acidosis and hypothermia. Acute coagulopathy could only be detected in trauma patients with tissue hypoperfusion and those with inadequate fluid resuscitation [6, 7]. This coagulopathy after trauma in early stages is also called endogenous acute coagulopathy (EAC) or ACoTS. Tissue damage and post-traumatic hypoperfusion are the necessary prerequisites for early ACoTS.

1.2 ACoTS Mediated by Activated Protein C

Further studies showed that low level of activated protein C led by tissue damage and systemic hypoperfusion in patients with traumatic shock was the main pathogenesis of early ACoTS [11]. Thrombin combined thrombomodulin (TM) on the endothelial cell surface activates protein C (APC) anticoagulation pathway. APC plays a role of anticoagulation by inactivating factor Va and VIIIa irreversibly. In vivo, endothelial protein C receptor (EPCR) could activate protein C by thrombin-TM complex, which amplified its anticoagulant effect tenfold [12].

Hyperfibrinolysis is very common after trauma due to tissue injury and shock. Protein C could develop its anticoagulant ability by activating and consumption of plasminogen activator inhibitor (PAI)-1. Which is leading to low levels of PAI-1 and the increased release of tPA from the vessel wall, and finally resulting in hyperfibrinolysis [13]. Brohi and his colleagues examined 208 trauma patients and found that coagulation was activated and thrombin generation was related to injury severity, but acidosis did not affect Factor VII or prothrombin fragments 1 + 2 levels [14].

The initial purpose of fibrinolysis may be to limit the infinite expansion of blood clots in damaged vessels. However, early clot in patients with traumatic shock

always misses its physiological role. Instead, there happens the severe imbalance of coagulation and fibrinolysis.

1.3 Platelet Dysfunction

Platelet dysfunction in the early stage after trauma remains obscure. Although the complete blood count with difference provides a platelet count, Swallow pointed that this quantitative test does not provide an assessment of platelet function [15]. Wohlauer and his colleagues reported in a study with 51 trauma patients that there were significant differences in the platelet response between trauma patients and healthy volunteers. In trauma patients, the median ADP inhibition of platelet function was 86.1 % compared with 4.2 % in healthy volunteers. The impairment of platelet function in response to arachidonic acid was 44.9 % compared with 0.5 % in volunteers [16].

1.4 Complement

Complement plays a central role in the innate immune system and is always activated in the early phase of trauma [17]. It has been reviewed that there is a significant amount of crosstalk between the complement and coagulation systems [18]. Katharina and his colleagues found that mannose-binding lectin-associated serine protease-1 (MASP-1) interacted with plasma clot formation on different levels and influenced fibrin structure. Although MASP-1-induced fibrin formation was thrombin-dependent, MASP-1 directly activated prothrombin, FXIII and TAFI. And they suggested that MASP-1, in concerted action with other complement and coagulation proteins, may play a role in fibrin clot formation [19]. On the other hand, coagulation proteases can activate the complement system. For example, thrombin, FXa, FIXa, and FXIa, can cleave the central complement components C3 and C5 into their bioactive fragments [20]. Expression of TM and activation of protein C seems to be complement-dependent in ischemia-reperfusion injury, which indicates that there may be a certain link between complement activation and ACoTS in the early stage of severe trauma.

2 Mechanism in the Late Stage

In the late phase of trauma, consumption of clotting factors, clotting factors dilution as well as acidosis, hypothermia and inflammation play more important roles in coagulopathy. At this stage, the pathogenesis of ACoTS is more similar to traditional SAC.

2.1 Use of Blood Products

In the late phase of trauma and traumatic shock, coagulopathy is caused by excessive consumption and dilution of coagulation factors due to bleeding and massive fluid resuscitation. Lack of clotting factors and platelets secondary to transfusion of blood and its components contributes to the development of ACoTS as well. Appropriate use of blood products in trauma patients can improve the coagulation function.

Fluid resuscitation for trauma shock based on blood products went through three stages: whole blood recovery before the 1970s, the strategy of blood component transfusion later, and currently the fluid resuscitation strategy based on plasma. The strategy of blood transfusion has been a controversial topic for a long time. The core problem is the ratio of FFP and RBC. Borgman and colleagues [21] found that the mortality of trauma patients who receiving massive transfusion with FFP: RBC = 1:1.4 was significantly lower than that with FFP: RBC = 1:2.5 or 1:8 group. Some scholars also believe that FFP: RBC = 1:2 can replenish clotting factors and improve the coagulation function in trauma shock [22].

2.2 Hypothermia

Hypothermia worsens coagulopathy. The most significant effect is extending the coagulation cascade, which finally leads to bleeding. Hypothermia usually accompany with dissolved dysfunction of platelet and fibrin. Gregory and colleagues reported that 57 % of trauma patients got hypothermia after trauma, and body heat loss in emergency room is more severe. Large input of cryogenic liquids is the main cause of hypothermia [23]. Frank and colleagues [24] showed that body temperature less than 33 °C produced a significant coagulopathy that was functionally equivalent to factor deficiency states, which presented when clotting factor concentration was less than 50 %. Resuscitation with cold blood and fluids creates a vicious cycle of worsening haemodilution, acidosis, hypothermia and coagulopathy.

2.3 Acidosis

Acidosis directly reduces the activity of clotting factors in both endogenous and exogenous coagulation pathway and limits the function of platelets. Note that this effect can be seen only when pH is lower than 7.2.

2.4 Inflammation

Inflammation and coagulopathy are linked through several mechanisms. More clinical findings indicate that the complement and coagulation systems are interconnected at various levels in vivo. These interactions point to alternative ways in which complement or coagulation components can potentially become activated [18]. In the late phase of trauma, low level of serum activated protein C inhibits fibrinolysis through promoting a high level of serum PAI-1, which resulting in a increase in procoagulant activity and a decrease in complement activation by reducing the plasma mannose-binding lectin and deposition of complement-3b.

3 ACoTS and Disseminated Intravascular Coagulation (DIC)

In the early phase of trauma, coagulation disorder increased bleeding, followed by a hypercoagulating state and increased the risk of thrombosis. Gando called it transformation from fibrinolytic (hemorrhagic) DIC phenotype to antifibrinolytic (thrombus) DIC phenotype. He believed that ACoTS was equivalent to DIC consumptive coagulopathy and the secondary fibrinolysis, ACoTS and non-ACoTS might be the continuous states in the process of coagulopathy [25, 26]. While Hess believed that the mechanisms in tissue trauma, shock caused by acute coagulopathy of disseminated intravascular coagulation and, trauma, inflammation caused by coagulation disorders and trauma are not the same [8]. Johansson reported one study of 80 adult trauma patients from a level 1 trauma center and found that 15 % of the patients developed ACoTS, but DIC wasn't been observed significantly [27].

The essential difference between ACoTS and DIC is a controversial issue. Authors believe that when body suffers from severe trauma, tissue injury, they will release large amounts of tissue factors, start the extrinsic coagulation pathway, and show hypercoagulating state immediately. In this case, the body reacts to promote hemostasis, reduce blood loss and maintain stable circulation, which has a protective effect on the body. But if the damage is too strong and beyond body's compensatory limit, the number and/or function abnormalities in procoagulating factors, anticoagulating factors, platelet and fibrinolytic factors will cause coagulation and fibrinolysis imbalance, ultimately lead to coagulopathy. ACoTS and posttraumatic DIC have similar pathogenesis and pathophysiology, but they are not exactly the same. However, the strict distinction between the nature of these two cannot bring a positive role to guide clinical treatment. In contrast, improving the attention of ACoTS by early detection, early diagnosis and early treatment are more important to improve the prognosis of severe traumatic patients.

4 Prevention and Treatment

Conventional coagulation tests, e.g. PT, TT, APTT, play an important role for early detection of ACoTS. However, these detections are based on one time point. They cannot reflect the continuous changes of coagulation function and the function of platelet. Thus they have little diagnostic value in fibrinolysis and are more time-consuming. Nowadays, new small viscoelastic instruments to coagulation testing get rapid progress, which promptly evaluate the clotting process and may guide blood product therapy. The Sonoclot analyzer provides information on the entire hemostasis process both in a qualitative graph and quantitative results including the activated coagulation time, clot rate, and platelet function [28]. ROTEM measures and graphically displays the time until initial fibrin formation, the kinetics of fibrin formation, clot development, and the ultimate clot strength and stability.

Thromboelastogram (TEG) is introduced in clinical practice for its wide detecting contents and intuitive results. It can reflect the dynamic process of blood coagulation, platelet function, and the full picture of blood coagulation [29]. The risk of bleeding and thrombosis cause of bleeding can be evaluated and identified, and thus guide blood transfusion and clinical management. In this way, TEG lays a solid foundation for early and rapid diagnosis of ACoTS as well as individualized treatment and even promotes the generation of early goal-directed coagulation therapy (EGCT) [30, 31].

Treatments for ACoTS include the following aspects: (1) Damage control resuscitation: Early blood transfusion, blood component transfusion, permissive hypotension and the minimum dose crystal recovery. (2) Massive blood transfusion protocol: blood transfusion $\geq$ circulating blood volume or infusion of packed red blood cells > 10 U in 24 h, or packed red blood cells > 4 U within 1 h. (3) TEG guided transfusion: design a transfusion flowchart based on TEG monitoring results. (4) Using tranexamic acid, recombinant factor VIIa.

These treatments are facing controversy. Choi and Vogel [32] noted that massive transfusion protocols have improved outcomes in adults, but limited studies in pediatrics have not shown any difference in mortality. TEG guided transfusion is widely accepted nowadays. But Hagemo and his colleges [33] reported of 184 traumatic haemorrhage patients and made a conclusion that the inter-changeability between TEG and ROTEM is limited in the trauma setting. Besides, Da and coworkers recently published a descriptive systematic review article pointed out that limited evidence from observational data suggested that TEG/ROTEM tests diagnose early trauma coagulopathy and may predict blood-product transfusion and mortality in trauma [34]. Recombinant activated factor VII (rFVIIa) is increasingly being given to treat massive bleeding. Michael studied in vitro concluded that the efficacy of rFVIIa was largely dependent on the presence of high levels of fibrinogen in reversing this severe dilutional coagulopathy [35].

The pathogenesis of ACoTS and traditional SAC is different. Supplementation of coagulation factors and platelets is not efficient. Only positive resuscitation of

shock and improvement of tissue perfusion may be beneficial. For patients with traumatic shock, active resuscitation and surgical control of bleeding are important. At the same time, we should strengthen monitoring coagulation status and make adjustments to avoid surgical wound bleeding. Based on effective, accurate and dynamic coagulation monitoring, early goal-oriented recovery can help guiding fair use of blood products, saving medical resources, and improving the prognosis of trauma patients [36].

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Research Progress in Trauma Metabolism and Nutrition

Weiqin Li and Xiao Shen

Abstract Trauma causes a series of alterations in body metabolisms, which may further aggravate organ dysfunction and lead to multiple organ failure (MOF). The typical metabolic responses to trauma can be summarized in six points: increased energy expenditure, accelerated gluconeogenesis, increased lipolysis, increased water-sodium retention, increased nitrogen excretion as well as decreased muscle protein synthesis. Nutrition support is critical in the management of trauma patients. The major functions of nutrition support are to prevent acute protein malnutrition, to modulate the immune response as well as to promote gastrointestinal structure and function. Despite the widespread use of nutrition in trauma patients, there still remains controversies in the aspect of the route and timing of nutrition support. In reviewing the literature studies concerning the route and timing of nutrition support in the trauma condition, we concluded that: First, enteral nutrition is superior to parenteral nutrition in critically ill trauma patients; Second, early enteral nutrition (within 24–48 h after admission to ICU) was more preferred compared with delayed enteral nutrition.

Keywords Trauma · Metabolic response · Nutrition support

1 General

Trauma causes abnormal manifestations in the body. These manifestations can cause alterations in body metabolisms including hypercatabolism, hyperglycemia, hypoalbuminemia, et al. These metabolic abnormalities may aggravate organ dysfunction and lead to multiple organ failure (MOF). In addition, these metabolic abnormalities may also complicate nutritional management.

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2 Metabolic Response to Stress

Stress is a body's method of reacting to a challenge. According to the stressful event, the body's way to respond to stress is by sympathetic nervous system activation, which results in the fight-or-flight response [1]. Cannon first used the word "stress" to describe this fight-or-flight response. In the 1930s, Cuthbertson defined the ebb and flow of metabolic response after trauma [2]. A patient may undergo two phases during the time of stress: the ebb phase and the flow phase (Fig. 1).

The ebb phase, also named acute phase, usually lasts 1–3 days. The clinical presentation of ebb phase is hypometabolism, decreased temperature, decreased energy expenditure and normal glucose production, but with insulin resistance, mild protein catabolism, increased blood glucose, increased catecholamines, increased glucocorticoids, decreased cardiac output, lowered total oxygen consumption, and vasoconstriction. All these metabolic changes would lead to muscle catabolism.

The ebb phase ends after adequate resuscitation and replaced by the flow phase. The metabolic response of the flow phase is characterized by high oxygen consumption, hypermetabolism, increased resting energy expenditure (REE), increased cardiac output, increased glucose production, profound protein catabolism, increased catecholamines, increased glucocorticoids, increased glucagon, increased potassium, and increased nitrogen losses. During this phase, four types of mechanisms regulate metabolic changes: the release of tissue factors, the synthesis of cytokines, endocrine changes and central nervous system functions.

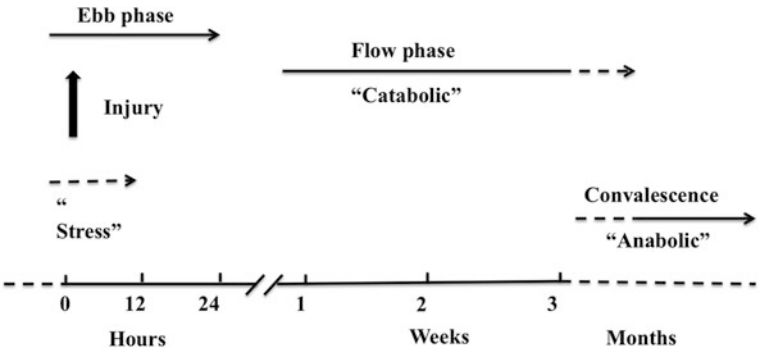

Fig. 1 Phases of the response to injury

3 Metabolic Response to Trauma

The typical metabolic response to trauma can be summarized in six points: increased energy expenditure, accelerated gluconeogenesis, increased lipolysis, increased water-sodium retention, increased nitrogen excretion as well as decreased muscle protein synthesis. The hypothalamus and the adrenergo-sympathetic system might play important roles in initiating these metabolic changes.

3.1 Increased Energy Expenditure

It has been shown that there is a moderate increase in the energy expenditure in patients after uncomplicated elective surgery. Only those patients with severe trauma, sepsis and burns would have a 50–100 % increase in energy expenditure. This increased energy demand can be met without problems by giving glucose and fat emulsions, but it may be necessary to measure the energy expenditure directly by indirect calorimetry in critically ill patients in order to prevent overloading of nutritional substrates, particularly in those with multiple organ failure (MOF).

3.2 Accelerated Gluconeogenesis

Hyperglycemia always follows injury. Hyperglycemia may be the result of rapid catecholamine-mediated mobilization of body carbohydrate stores in the early stage of trauma. The carbohydrate stores in the liver will last only 12–24 h without replenished. The body glucose requirement must be met with an increased hepatic production of glucose from protein precursors. While in the late stage, the hyperglycemia is always the result of an increased synthesis of glucose relative to an increased turnover rate. Injury and sepsis apparently do not impair the ability of the body to oxidize glucose, in turn, the glucose oxidation is actually increased in septic patients and gluconeogenesis cannot be suppressed even with intravenous glucose infusion. The study by Wilmore et al. [3] has demonstrated that wound is responsible for the increased glucose utilization instead of skeletal muscle.

3.3 Increased Lipolysis

The turnover rates of glycerol and free fatty acids are increased in postoperative patients, but the production of ketone bodies probably remains unchanged as skeletal muscle uses almost exclusively lipids as substrates.

3.4 Water-Sodium Retention

As is well known, trauma and sepsis would cause an increase in the muscle contents of water, sodium and chloride. Bergstrom et al. has revealed that, retained water is mainly distributed extracellularly in postoperative patients. Although potassium and magnesium are less affected, their concentrations in muscle are decreased after operation. And in the conditions of severe trauma and sepsis, these changes deteriorate more. For these reason, nutrition support may partly correct these water and electrolyte abnormalities.

3.5 Increased Nitrogen Excretion

Normally there is a balance between protein synthesis and breakdown, but the catabolic rate becomes much greater than synthetic rate in trauma patients. In most trauma patients, there is an increase in both synthesis and degradation. However, the enhancement of degradation is the more pronounced. As a result, the nitrogen balance becomes negative in the posttraumatic period. Skeletal muscle is the major source for the excreted nitrogen, leading to muscular fatigue and weakness. Therefore, it is very important to apply an appropriate nutrition formula to preserve the body proteins of the trauma patients. In addition to nutrition support, some adjuvant therapies such as insulin and growth hormone may also be necessary.

3.6 Decreased Muscle Protein Synthesis

O'Keefe et al. found that the total ribosome concentration per mg of deoxyribonucleic acid (DNA) and the proportion of polyribosomes decreased in postoperative patients, suggesting that the operative trauma would cause the decrease in ribosome utilization. The decrease in the concentration of polyribosomes is the same as that is observed during starvation, indicating that skeletal muscle is used as an important source of nutrients in trauma patients. Tissue analysis reveals a markedly decrease both in the activity of and the capacity for protein synthesis.

4 Nutrition Support in Trauma Patients

4.1 Rationale for Nutrition Support

Nutrition support is critical in the management of trauma patients. The rationale is 3-fold: First, to prevent acute protein malnutrition; Second, to modulate the immune response; Last, to promote gastrointestinal structure and function [4].

4.1.1 Prevent Acute Protein Malnutrition

Patients would always develop a systemic inflammatory response syndrome (SIRS), which resolves with recovery, after the initial traumatic insult. However, in the condition of patients with overwhelming SIRS, hypercatabolism would result in acute protein malnutrition and subsequent immune system impairment. This persistent hypercatabolism dominates the metabolic response to trauma. If exogenous amino acids were not supplied timely, the initial demand would be met by skeletal muscle proteolysis. Thereafter, there is depletion of visceral structural elements, as well as circulating proteins. The resultant acute protein malnutrition is associated with subclinical multiple organ dysfunction (MODS).

4.1.2 Immune Response Modulation

SIRS, characterized by the localized and systemic production and release of multiple pro-inflammatory cytokines, is an acute condition following trauma. However, the traumatic insult also stimulates a parallel release of anti-inflammatory cytokines, called the compensatory anti-inflammatory response syndrome (CARS). Overwhelming CARS seems to be responsible for post-traumatic immunosuppression, leading to increased susceptibility to infections, sepsis and MODS. Compared to parenteral nutrition, the use of enteral nutrition has been shown to improve clinical outcomes, decreased infective complications and reduced the incidence of MOF in patients with SIRS and CARS. Previous studies to explain these effects suggest immunomodulatory effects of enteral nutrition on both the systemic and intestinal mucosal immune systems [5]. Our previous studies have also shown that early enteral nutrition could moderate the excessive immune response during the early stage in severe acute pancreatitis (SAP) patients [6]. The integrity of the intestinal epithelial and immune cells of the gut-associated lymphoid tissue and the intestinal barrier plays an important role in maintaining the intestinal homeostasis and preventing bacterial translocation. The intestinal epithelial cells (IEC)-derived cytokines secretion plays a major role both in maintaining intestinal mucosal functions and in the maturation and optimum functions of lymphocytes. While enteral nutrients play a major role in maintaining the integrity of IEC. As a result, enteral nutrition could modulate the intestinal mucosal immune systems.

4.1.3 Gastrointestinal Structure and Function Promotion

Gut dysfunction occurs in the majority of the critically ill patients. In trauma patients, gut dysfunction occurs for multiple reasons. First of all, trauma itself would cause an ischemia/reperfusion injury to the intestine. Subsequent therapies such as anesthesia and bowel manipulation would cause further injury to the intestine. Consequently, the dysfunctional gut becomes a reservoir for pathogens and leads to infection, sepsis as well as multiple organ failure (MOF). A series of

animal studies have demonstrated that enteral nutrition could promote the protective effects of commensal bacteria, maintain the mass of gut-associated lymphoid tissue and preserve gastrointestinal mucosal structure and function [7–9]. Furthermore, clinical studies have shown these effects translate into better outcomes with respect to infection, organ failure and length of hospital stay [10, 11].

4.2 Route and Time of Nutrition Support

Despite the widespread use of nutrition in trauma patients, there still remains controversial in the aspect of the route and timing of nutrition support.

4.2.1 Parenteral Versus Enteral Nutrition

Early in the 1970s, enteral nutrition was preferred over parenteral nutrition as it was less expensive, safer and more convenient. However, the gut was regarded as dysfunctional after the traumatic insult in trauma patients at that time. Therefore, the implementation of enteral nutrition was always delayed until the gut function was certain. By the late 1970s, there was a better understanding of resuscitation and stress metabolism, which provided the rational for early nutrition. By then, parenteral nutrition developed by Dr. Stanley J. Dudrick was widely used in the surgical patients [12]. Therefore, parenteral nutrition gradually became the standard way for nutrition therapy in patients with severe trauma.

While, in the 1980s, a series of studies documented the physiologic advantages of enteral nutrition over parenteral nutrition. The reasons were as follows: First, as enterally delivered substrates pass first through the liver, they would be better used. Second, enteral nutrition does not produce the glucose intolerance associated with parenteral nutrition. Last, it was reported that enteral nutrition could prevent intestinal mucosal atrophy and bacterial translocation as well as maintain immune function. Taken together, enteral nutrition had regained the popularity by the late 1980s.

Later then, several clinical studies have pointed out the harmful effects in the patients receiving parenteral nutrition. Moore et al. [13] compared the effect of total enteral nutrition and total parenteral nutrition in the patients with major abdominal trauma. Results showed that the incidences of infection and major septic morbidity were significantly higher in the patients of parenteral nutrition group compared to those of enteral nutrition group. Soon after, a study conducted by Kudsk et al. [8] confirmed these findings in 98 patients with blunt and penetrating abdominal trauma. They found that although patients with enteral nutrition received significantly fewer calories/kg, there was no significant difference in nitrogen balance between the two study groups. In addition, patients in enteral nutrition group suffered significantly fewer pneumonias (11.8 % vs. 31.0 %, $p < 0.02$), intra-abdominal abscess (1.9 % vs. 13.3 %, $p < 0.04$), and line sepsis (1.9 % vs.

13.3 %, $p < 0.04$) as well as sustained significantly fewer infections per patient ($p < 0.03$) compared to those in parenteral nutrition group.

In 2003, the Canadian Critical Care Clinical Practice Guidelines evaluated 12 level 2 studies and 1 level 1 study that compared enteral nutrition to parenteral nutrition in critically ill patients with an intact GI tract [14]. The results showed that although there was no apparent difference in mortality rates across groups receiving enteral nutrition or parenteral nutrition (relative risk [RR], 1.08, $p = 0.7$, the incidences of infectious complications were markedly reduced in the group of enteral nutrition compared with that of parenteral nutrition (RR: 0.61, $p = 0.003$). According to the results, the committee strongly recommended the use of enteral nutrition over parenteral nutrition for critically ill patients.

More recently, the updated Guideline for the Provision and Assessment of Nutrition Support Therapy in the Adult Critically Ill Patient assessed six previous meta-analysis comparing EN with PN. The results revealed significant reductions in infectious morbidity with use of enteral nutrition. In conclusion, the committee also suggested the use of enteral nutrition over parenteral nutrition in critically ill patients including patients with trauma, burns, head injury and major surgery who require nutrition support therapy [15].

4.2.2 Early Versus Delayed Enteral Nutrition

As previously mentioned, a persistent hypercatabolic state dominates after trauma. This results in acute protein malnutrition and subsequent immune system impairment. If not timely supplied with exogenous nutrients, there would be a depletion of visceral and circulating proteins. Therefore, a series of clinical studies had been conducted to assess the implementation as well as the effect of early enteral nutrition in the trauma patients.

In 1986, Moore et al. first performed a prospective, randomized, controlled trial to evaluate the benefits of early enteral nutrition in 75 patients with major abdominal trauma [16]. Patients were randomly assigned to control group (no enteral nutrition within the first five postoperative days) or early enteral nutrition group (element enteral nutrition via a needle catheter jejunostomy initiated 18 h postoperatively). It turned out that the nitrogen balance was significantly improved ($P < 0.001$) and the infectious morbidity was significantly decreased ($p < 0.025$) in the early enteral nutrition group. According to these findings, the authors concluded that early enteral nutrition was feasible in the immediate postoperative period and that it decreased septic complications.

Later after that, Taylor et al. [10] performed a study to determine the effect of early enhanced enteral nutrition on clinical outcome in 82 patients suffering head injured. Patients were randomized to receive standard EN (gradually increased from 15 mL/h up to estimated energy and nitrogen requirements) or enhanced EN (started at a feeding rate that met estimated energy and nitrogen requirements) from day 1. The results showed that there was a tendency for more intervention patients to have a good neurologic outcome at 3 months than control patients (61 % vs.

39 %, $p = 0.08$). Fewer intervention patients had an infective complication (61 % vs. 85 %, $p = 0.02$) or more than one total complication (37 % vs. 61 %, $p = 0.046$) compared with control patients. These results indicated that early enhanced enteral nutrition appeared to accelerate neurologic recovery and reduced both the incidence of major complications and post-injury inflammatory responses. At the same time, Kompan et al. [17] conducted another study to determine the effect of early enteral nutrition on gut permeability and the development of MOF in multiply injured patients. They found that the patients started on enteral nutrition later than 24 h after admission to the ICU demonstrated increased intestinal permeability on the second day after sustaining multiple injury. In addition, those patients had a more severe form of MOF than the group with early enteral nutrition.

In 2003, the Canadian Critical Care Clinical Practice Guidelines Committee evaluated 8 randomized controlled trials (level-2 studies) comparing early with delayed enteral nutrition intake in critically ill patients with intact gastrointestinal tracts [14] and found that early enteral nutrition was associated with a trend toward a reduction in mortality (RR: 0.52, $p = 0.08$) when compared with delayed enteral nutrition. As a result, the committee recommended early enteral nutrition (within 24–48 h after admission to ICU) in critically ill patients. Later in 2011, Doig et al. [18] conducted a meta-analysis which included three randomized controlled trials (RCTs) with 126 participants and further confirmed the benefits of early enteral nutrition in the trauma condition. A more study by Chourdakis et al. [19] found a positive influence on endocrine function of early enteral nutrition. The results of their study showed lesser hormonal changes in the early enteral nutrition group compared with the delayed group, indicating that early enteral nutrition might exert beneficial effects on the hormonal profile of the patients with traumatic brain injury.

Lately, the updated Guideline for the Provision and Assessment of Nutrition Support Therapy in the Adult Critically Ill Patient retrospected three meta-analyses aggregated data from RCTs comparing early versus delayed enteral nutrition in critically ill patients including trauma [15]. All the studies revealed a better outcome for the patients when early enteral nutrition was established. As a result, the committee recommended that, nutrition support therapy in the form of early enteral nutrition should be initiated within 24–48 h in the critically ill patient who is unable to maintain volitional intake.

Whereas, the role of early enteral nutrition for burns patients was still undefined yet. Patients with large burns have significantly increased energy requirements. Basal metabolic rates can double when burns are >50 % total body surface area (TBSA) [20]. These patients, as well as those with inhalation injury who require mechanical ventilation, are not able to meet their requirements for macro- and micronutrients and fluids via the oral route. Under these circumstances, enteral nutrition is indicated.

Providing nutrition is clearly essential in the successful management of burn injured patient, there are several conflicting findings amongst research groups regarding the optimal method and timing of enteral nutritional support. Providing early nutritional support has a number of advantages including increased caloric intake [21] and improving bowl mucosa integrity [22]. However, it has remained

unclear whether early enteral nutritional support has any beneficial impact on a diverse field of nutritional, metabolic and biochemical outcomes and clinical indicators such as length of stay, infection rates and mortality [23]. In 2007, Wasiak et al. performed a systemic review to examine evidence for the effectiveness and safety of early versus late enteral nutrition support in adults with burn injury and suggested that early enteral nutrition support might blunt the hypermetabolic response to thermal injury but was insufficient to provide clear guidelines for practice.

5 Conclusion

In summary, the metabolic response to trauma involves a number of changes in the metabolism of the major energy sources as well as protein metabolism and adequate nutrition support is pivotal in the management of trauma patients. During the procedure of nutrition support, enteral nutrition is superior to parenteral nutrition in critically ill trauma patients and early enteral nutrition was more preferred.

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Metabolic Changes and Nutrition Therapy in Burn Patients

Xi Peng

Abstract The metabolism pattern changes obviously after severe burn injury, the primary pathological phenomena are energy consumption and catabolism increased significantly, and nutrients utilize barriers. Severe burned patients will lead to autophagy metabolism, continuous consumption, and progressive emaciation. If the pathological process can not effectively block, it will lead to organ damage, immune dysfunction, wound healing delay and other adverse outcomes. Therefore, hypermetabolism after burn is one of the leading cause of multiple organ dysfunction and even death. After many years research, although there is certain understanding of hypermetabolism mechanism, but it is difficult to fully explain the causes of the hypermetabolism after burn, up to now. At the same time, the therapeutic measures of regulating hypermetabolism are still not perfect, and obstacle to burn comprehensive treatment level continuous progress.

Keywords Metabolism pattern · Catabolism · Progressive emaciation · Hypermetabolism

This chapter will introduce the mechanism of metabolic changes and the nutrition therapy strategy on severe burned patients. Focus on discussing the indication, dose, course of treatment, and supplement pathway of macronutrients and micronutrients.

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1 Changes in Energy Metabolism After Burn Injury

Hypermetabolism after burn injury is characterized by increased metabolic rate, augmented protein metabolism, and energy consumption. Hypermetabolism reaction on severe burn patients could last several months even a few years. Previous studies have confirmed that the metabolic rates of burn patient maximum of up to 1.8 times the normal person [1]. With the development of the disease, the metabolic rate is gradually reduced, but the resting energy expenditure (REE) in the burn patient was still 10–20 % higher than that of the healthy people post burn 12 months [2].

There are two methods to detect the patient's energy consumption, the indirect calorimetry and the energy consumption estimation formula. Indirect calorimetry is accurate but requires expensive instruments, and it is difficult to be popularized [3]. Therefore, there are many energy consumption estimation formulas used in burn clinical, but their common problem is more energy estimates than patient confirmed consumption [4, 5]. Assessment of the energy requirements is an ongoing process and modified according to the progress of the disease. No single formula can accurately assesses the true energy consumptions. Take the third military medical university (TMMU) formula as an example, although the formula is convenient and practical, it overestimates energy consumptions in severe burned patients. Our further investigation demonstrated that estimated values produced from the TMMU formula is more about 15, 23, and 40 % than measured REE values in patients with total body surface areas (TBSA) burned of 31–50, 51–70, and 71–100 %, respectively [6]. Other formulas have similar problems, they usually overestimate the caloric needs of burn patients when compared to metabolic expenditure requirements. The overestimation may be related to the progress in burn treatment since the formula was developed. Early wound closure, higher ambient temperature, improvements in infection control, and pain management all reduce the hypermetabolic response to the burn injury. it is now recognized that the value of energy consumption estimation on 1 % burn area should be decreased, from 25 to 10–15 kcal. As a result, lead the daily total energy consumption in severe burn patients from 3000–3500 kcal drop to 2500–3000 kcal.

The changes of energy metabolism on burned patients are complex and are regulated by many factors. Therefore, no formula can be estimated completely consistent patient's energy consumption, and can only provide a rough range of energy consumption. Energy supply should be based on the specific conditions of the patient, in addition to considering the predicted energy consumption, the physician must also consider the patient's metabolic capacity. In the early stage of burn, the energy demand is often lower than the energy consumption in patients with extensive burns.

2 Changes in Substance Metabolism After Burn Injury

After severe burn injury, pathophysiological conditions is complicated and highly related to material metabolism change significantly [7]. As a whole, glucose utilization disorders, gluconeogenesis increase, protein catabolism enhanced, and the anabolism is relatively lower, induce protein net release. Moreover, the long chain fatty acid transport is blocked, and fatty acid beta oxidation is partially inhibited. The metabolism pattern changes obviously after severe burn injury, the primary pathological phenomena is energy consumption and catabolism increased significantly [8].

2.1 Changes in Protein Metabolism

Daily nitrogen intake can be calculated from food tables, and fecal and exudate losses can be estimated using information derived from previous studies. If a normal individual consumes 12 g nitrogen in 75 g protein, he will excrete a similar amount, thus maintaining nitrogen equilibrium. Following injury, however, several factors occur which lead to a depletion of nitrogen. Nitrogen intake decreases and lose increases, resulting in a negative nitrogen balance [9]. The period of negative balance is called the catabolic phase. Its duration and the total loss of nitrogen are related to the severity of the trauma. In the burned patient the severity of the catabolic phase as well as its duration are related to the quantity of tissue destroyed and the degree of sepsis.

Therefore, skeletal muscle is the main source of fuel for burn patients, resulting in apparent lean body mass (LBM) lose for a long period after burn injury. This muscle decomposition has been shown in systemic and cross legged in nitrogen balance studies in which pronounced negative nitrogen balances sustained for 6 and 9 months after burn injury. Since skeletal muscle has been shown to be responsible for 80 % of glucose uptake into the systemic insulin stimulation, muscle mass decline may contribute to persistent insulin resistance after burn injury [10]. The relationship between hyperglycemia and muscle protein catabolism has also been supported by many studies, these researches showed that there are remarkable increased in proteolysis rates occurring without any alteration in either leucine oxidation or nonoxidative disposal, suggesting that hyperglycemia induced increased protein decomposition. This loss of protein is directly related to increases in metabolic rate and may persist up to 9 months after critical burn injury, often resulting in significantly negative whole-body and cross-leg nitrogen balances. These protein loss is directly related to an increase in metabolic rate, which may be sustained for up to 9 months after severe burns, often resulting in remarkably negative whole-body nitrogen balances. Daily nitrogen loss on severe burned patients could reach 20–25 g/m [2] TBSA, and if no effective treatment, lethal cachexia is imminent in less than 30 days [11].

Persistent proteolysis may also account for the delay in allografted skin growth, pressing immune functions. Eventually leading to the occurrence of various complications, such as sepsis. Patients with increase metabolic rates and protein catabolism up to 40 % of the same burned size who do not develop sepsis. Ensuing vicious circle, because patients who are more susceptible to sepsis since changes in immunity and immunologic response. The emergence of multi-resistant bacteria, leading to infections and sepsis-related death. Immunological cells, in response to burn infections, metabolize glucose anaerobically to pyruvate and lactate. These compounds are returned to the liver for gluconeogenesis, which produces recycled energy for use by immunocyte and fibroblasts in the burn wound.

2.2 Changes in Glucose Metabolism

Some studies have found that hepatic glucose synthesis disorder after burn injury, result in increasing the level of gluconeogenic hormones, for instance, catecholamine, glucagon, and cortisol. Further data indicate that there are notable disorder in major ATP consumption pathways such as increased protein turnover and urea production and gluconeogenesis. Hypermetabolic response after burn could lead to Glycolytic-gluconeogenetic cycling increased 250 % and triglyceride-fatty acid cycling raised 450 % [12]. All of these reactions could cause severe hyperglycemia and impair insulin sensitivity.

After burn injury, there was remarkably increased insulin level and fasting glucose and glucose clearance was significantly decreased. Glucose levels were increased by threefold, although glucose oxidation was restricted to glucose delivery to the peripheral tissues. Increased glucose production is aimed at burn wounds, in order to support the anaerobic metabolism of vascular endothelial cells, fibroblasts, and inflammatory cells. Lactic acid, the end product of glucose anaerobic oxidation, the cycle to the liver of the sugar production of different ways to produce more glucose. Serum glucose and insulin level were still significantly increased through the whole acute inpatient treatment. Insulin resistance appeared in the first week after the burn and continued at least until discharge.

2.3 Changes in Fat Metabolism

In addition to these changes of glucose and protein, fat metabolism is also significantly changed after burn injury. The total free fatty acid turnover and plasma glycerol concentrations are increased. Compared with control group, the change of plasma free fatty acid concentration was not obvious, but the glycerol concentration was elevated above normal for the first 20 days after burn injury [13]. Each intracellular triglyceride molecule, the hormone sensitive lipase promotes the release of a glycerol and three free fatty acids, whereas in muscle cells free fatty

acids recovery may be responsible for the lack of increase in free fatty acids concentration. In spite of variable concentrations of free fatty acids, the free fatty acid flux is increased postburn [14]. The free fatty acids flux represents the futile cycle involving the breakdown of adipose and muscle triglyceride into free fatty acids, followed by reesterification into very low density lipoprotein and triglycerides in the liver, and eventually regenerating into adipocytes or muscle triglyceride. After burn injury, the rate of free fatty acids release far more than the required energy utilization, so that most of the free fatty acids is regenerating in liver and resecreted as very low density lipoprotein triglycerides. It appears that all parts of the regulator increase in trauma patients and may affect the effects of hormone sensitive lipase on the catecholamine.

3 Nutrition Therapy in Burn Patients

Nutrition therapy, also called nutrition supply therapy, is an important treatment method in the comprehensive management of severe burned patients. The aim of the nutrition therapy is to reduction in hypermetabolic response, maintenance cellular function, and improvement the prognosis.

3.1 Reasonable Energy Supplement in Burn Patients

The calorie of burn patient expenditure can be determined or estimated approximately by indirect calorimetry or energy expenditure equations. However, the energy expenditure is not entirely equal to energy requirement in the whole process of burn injury. The energy consumption is increased significantly, but the ability of nutrient absorption and anabolism is decreased remarkably in the early phase after burn injury, thus result in imbalance between energy demand and consumption. However, nutritional supplement as calculated according to energy consumption may lead to overfeeding. Supplement excessive nutrients can not be fully utilized, and it might aggravate metabolic disorder. Therefore, the nutrition administration should be lower than REE whether it is direct determination or by formula estimation. With the disease progresses, the internal environment gradually tends to be stable, ending in the balance between anabolism and catabolism. The amount of nutritional support should be increased gradually. During convalescence, anabolic metabolism outstrip catabolic metabolism, therefore the quantity of nutritional administration should be moderately higher than that of energy consumption. Therefore, in the whole treatment process, energy consumption and energy supplement may reach a balance point [15].

Assessment of the energy requirements is an ongoing process and modified according to the progress of the patient. There is no single formula accurately assesses the true energy needs and continued vigilance is required to prevent some

complications induced by overfeeding or underfeeding. Assessment of nutritional supplement is divide into two distinct categories: the initial demand and continuous demand. The assessment of caloric require within the first 24 h is an initial goal so that nutrition support can be initiated. However, it should be necessary to make adjustments for the continue needs during the whole course of disease. The assessment of the energy demand need comprehensive of the following factors: Basal metabolic rate, Hypermetabolism, Percent of total body surface area (TBSA) burned, Ventilatory support, Infections, Sepsis, Multiple organ failure, Level of physical activity, Thermic effect of food. Many mathematical equations have been developed to estimate the energy demand of the burn patients. Avoidance of overfeeding minimizes the risks of hyperglycemia, fat accretion, and infections. However, most of the formulas overestimate the amount of calories required in burn patients. The overestimation may be related to the changes in burn care since the formula was developed. Early wound closure, higher ambient temperature, improvements in infection control, and pain management all reduce the hypermetabolic response to the burn injury [16]. it is now recognized that the daily total energy required in severe burn patients is from 2500 to 3000 kcal.

3.2 Reasonable Nutrient Composition in Burn Nutrition Therapy

We recommended macronutrients and micronutrients in the nutritional formulas used for moderate to severe burn injury patients, including 1.5–2.0 grams protein/kg/day; 4–5 mg/kg/min of glucose per day representing approximately 50–65 % of total calories; Fewer than 30 % of nonprotein calories from fat sources; vitamins A, C and D in standard multivitamin formulations; trace minerals (e.g., selenium, zinc and copper) in standard formulations.

1. **Macronutrients**: In addition to establishing initial caloric energy requirements, determination of the appropriate composition of protein, carbohydrates, and fats for a nutrition support regimen is an early priority. Carbohydrates stimulate protein synthesis and limit the loss of lean body, thus making a high carbohydrate diet more desirable in patients who have sustained a moderate to severe burn.
2. **Protein**: Administration of nutrition support with protein of 1.5–2.0 g/kg/day, approximately 20–25 % of whole calories per day, will provide a balanced between synthesis and breakdown. Urea production, and therefore protein oxidation, in burned patients occurs at a rate approximately twice that of healthy individuals. Studies of protein intake in burns have examined intake levels of 1.5 g/kg/day and greater to determine optimal quantities. Delivery of greater than 1.5 g/kg/day in adult burns does not improve protein synthesis but does enhance maintenance of an isonitrogenous state [17].

Sufficient protein is a priority in the nutrition support of burn patients to minimize the impact of the catabolism to burn injury and to help the protein synthesis required for wound healing and immune function. It has been estimated that healthy individuals synthesize protein at a rate of approximately 4 g/kg/day, in contrast to the 7.6 g/kg/day rate of protein synthesis that has been estimated in burn patients.

3. **Glucose**: Glucose is the preferred substrate for tissue repair and should be the major source of energy in burn patients. Ideally, carbohydrates administered for burn nutrition support should consist of 4–5 mg/kg/min of glucose and represent approximately 50–65 % of total calories provided [18].
   Data from an observational study suggest that there is a maximal of glucose infusion over a physiologically significant increase in protein synthesis and the direct oxidation of glucose cannot be expected. There seems to be a physiological cost more than the optimal glucose infusion rate, such as in the infusion process in production of carbon dioxide the increase rate and large fat deposition in the liver injection of large amounts of glucose patients at autopsy.
4. **Lipids**: Lipids are an important dietary component as they contain the essential fatty acids, but should comprise no more than 30 percent of nonprotein calories. Lipids serve as transport lipid soluble vitamins. Following a moderate to severe burn injury, oxidation of free fatty acids occurs at a rate more than double that of healthy people. This enhanced process of lipolysis allows for excess exogenous fat to heighten recycling of free fatty acids and may result in increased fat storage [19].
5. **Micronutrients**: Maintaining appropriate levels of trace elements in moderate to severe burn patients is difficult due to exudative losses consequent to loss of the skin barrier. A comprehensive review of the impact of burns on micronutrients and the relevance of those micronutrients to recovery from burn injury is beyond the scope of this topic, but vitamins A, C, and D and three trace minerals copper, zinc, and selenium will be discussed.
6. **Vitamins**: Vitamin A plays an important role in immune function, wound re-epithelialization, and protection of free radical damage. Vitamin A toxicity can occur with high doses; vitamin C does not appear to have any known toxicities in high doses. Vitamin C, an antioxidant, is an essential component of collagen cross-linking and therefore also influences wound healing. Levels of vitamins A and C are decreased in patients following burn injury, but can be replenished with supplementation. Vitamin D deficiency is most likely due to a combination of acquired defects in vitamin D metabolism and immobility associated with significant burn injury. While vitamin D supplementation has been recommended in burn patients, an optimal dosing or method for delivery has not been identified.
7. **Trace minerals**: Copper, zinc and selenium serve as antioxidants. Zinc also plays a role in collagen cross-linking, wound healing, and immune function. The depletion of copper and zinc in burn patients is attributed to a combination of

urinary losses and exudative losses from wounds. The mechanism for the selenium deficiency is unclear and appears to be multifactorial.
ESPEN (European Society for Parenteral and Enteral Nutrition) recommends that the trace elements copper, zinc and selenium be supplemented in "higher than standard dose". Doses of 40.4 mmol copper, 2.9 mmol selenium and 406 mmol zinc are recommended for at least 30 days postburn [16].

8. **Electrolytes**: Burn injury involves loss of water, electrolytes, and protein. Resuscitation with a hypertonic lactated saline solution (e.g., lactated Ringer's solution) reduces the risk of electrolyte imbalances and tissue edema. Electrolyte balance, however, must be monitored and corrected throughout all phases of burn care. Hyponatremia and hyperkalemia occur during the initial resuscitation period. Hypernatremia, hypokalemia, hypomagnesemia and hypophosphatemia commonly occur during the early post-resuscitation period and the hypermetabolic phase. It is necessary to invite pharmacists driven electrolyte protocol at our institution to reduce the risk of electrolyte.

3.3 Parenteral Nutrition Support

Parenteral nutrition (PN) is an important nutritional treatment measures. But for burned patients, PN should be reserved for those with enteral nutrition (EN) intolerance.

1. **Indications and complications of burn PN support**

   (a) **Indications**

   ① Patients with burn surface area >20 %, or >30 %, and vigorous metabolism whose requirement can not be satisfied by EN.
   ② Patients with severe chemical burn on digestive tract.
   ③ Severe inspiration injury, tracheal cannula or respirator users.
   ④ Severe burn patients who can not chew and swallow.
   ⑤ Patients who can not take food or refuse to eat.
   ⑥ Digestive system complications such as stress ulcer, gastric retention, intestinal paralysis, intestinal obstruction, intestinal failure.

   (b) **Complications**

   ① Catheter related infection.
   ② Hypermetabolic response, hyperglycemia, and hyperketonemia.
   ③ Liver injury and Cholestasis.

2. **The principle of the implementation of PN**

   (a) The structure damage of gastrointestinal tract, and dysfunction are common complications after severe burns. Major pathogenic factors including stress,

pain, fever, trauma, inflammation, surgical excision, and anesthesia. Therefore gastrointestinal peristalsis function is usually suppressed in the early stage of burn injury, and gastrointestinal paralysis may occur in a few postburn hours. Series studies confirmed that the gastric emptying time delayed and intestinal peristalsis moved slowly in the early stage of burns. Moreover, even develop into gastric slight paralysis, enteroplegia, and stress ulcer. Accordingly, patients are hard to take food through mouth in the stage of infection or shock and need to be feed by parenteral nutrition support which is a complementation of enteral nutrition support. Therefore, patients in the infection or shock stage is difficult to take food, they need parenteral nutrition support, which is an alternative method of enteral nutrition.

Parenteral nutrition support is commonly used for large area burn patients with enteral nutrition as a complement to provides 50–75 % of total energy. Only little patients required total parenteral nutrition support. Parenteral nutrition support can be used in patients with different stages, shock recovery and hemodynamic stability should be supported by intravenous nutrition. Parenteral nutrition support treatment and metabolic process is basically the same, and 1 months after the recovery of gastrointestinal function in patients with parenteral nutrition support for 1.5 months, especially after the use should be stopped using enteral nutrition support.

(b) Medical and ethical principles should be followed in the use of parenteral nutrition. How to choose parenteral nutrition support is a serious problem, because of the complex operation, a large number of drugs and fluids, expensive costs, and potential complications. Therefore, the following principles should be followed: to bring benefits to the patient; no defects; independent decision of patients; individual treatment to each patient. The following points need attention: ① Parenteral nutrition administration should be practiced when burned patients refuse to eat or not get enough energy by oral administration. ② Parenteral nutrition administration should be used when the patients with malnutrition or malnutrition risk. ③ For severe burn patients, parenteral nutrition supplement is prohibited to be used until blood volume, hemodynamics, tissue oxygenation and internal environment are gradually stable and guaranteed [20].

3.4 Enteral Nutrition Support

Enteral nutrition (EN) is the first choice of nutrition support for burn patients, which is consistent with the gastrointestinal physiology and nutrients digestion and absorption. Series studies have confirmed that at the early stage of severe burns, early EN administration could increase intestinal blood flow, lessen intestinal damage, improve gastrointestinal energy metabolism, mitigate inhibition of mucosa

proliferation and reparation, and protect intestinal structure and function. Moreover, early EN could improve prognosis of burned patients.

The advantage of EN is the physiological nutrient absorption pathway. It can help to improve the portal system circulation and intestinal perfusion, to promote the intestinal peristalsis, gastrointestinal hormone and immune protein release, to maintain intestinal mucous membrane barrier function, to reduce the body hypermetabolic reactions, to improve nutrition status, and to decrease the incidence of multiple organ dysfunction syndrome. EN has less complications compared with PN. Nowadays, it has been widely used in burn patient nutrition support therapy.

When compared PN, severe burned patients supplement with EN receive less goal energy, and obtain better clinical efficacy. EN has been shown with fewer infections and lower mortality compared with PN. In early trials, the role of PN on burn patients was evaluated, results suggest that patients on the basis of EN added PN had a more incidence of infection and increased mortality compared to patients receiving EN only. Clinical trials compared early EN and PN effect in eighty two burn patients found greater infectious morbidity and higher mortality in those patients provided with PN, and these patients received significantly more energy than those patients supplement with EN. Early enteral feeding could improve gastrointestinal tract structure and function. It is proved the significantly greater gastrointestinal smooth muscle shrinkage, less ischemia/reperfusion damage, and reduce intestinal permeability on burn patients received EN compared with PN administration [21].

Based on evidence-based medicine and clinical expert consensus, we recommend early implementation EN as soon as possible on burn patients. A randomized trial of twenty cases of burn patients, sequentially assigned to EN within 5 h or after 48 h, showing in patients with early enteral nutrition group achieve positive nitrogen balance earlier, lower urinary catecholamines level, higher insulin and glucagon levels compared with patients on late EN group. But rates of bacteremia and hospitalization days were similar between two groups. Large research results showed that early EN has an advantage in burn stress, hypermetabolism, and inflammatory reaction compare to PN or delay EN [22].

1. **Indications, contraindications and complications of burn EN support** [23]

   (a) Indications

   ① Unable to tolerate oral intake.
   ② There is no serious damage of gastrointestinal function.
   ③ Disturbance of consciousness and coma.
   ④ Around the mouth and throat burn, mastication and deglutition are very difficut.
   ⑤ Chemical burn of upper digestive tract.

2. **Contraindications**

   ① Digestive tract obstruction.
   ② Alimentary tract ulcer, hemorrhage, perfor.

③ Jejunal fistula, persistent vomiting and diarrhea
④ Gastrointestinal function failure.
⑤ Serious metabolic disorder of sugar, water, and electrolyte.

3. **Complications**
The major complications of EN are pipeline, gastrointestine and metabolic complications.

① Pipeline complications including: Inflammation and injury of nasal, pharyngeal and esophageal; Feeding tube obstruction; Aspiration of nutrient solution.
② Gastrointestinal complications Including: diarrhea, nausea, vomiting, abdominal distension and abdominal pain.
③ Metabolic complications include: hyponatremia, hypernatremia, hypokalemia, hyperkalemia and hypomagnesemia etc.

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Early Prediction and Prevention of Trauma-Related Infection/Sepsis

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Abstract According to the World Health Organization, trauma still stands for one of the leading causes of death around the world. Although the incidence of post-traumatic sepsis in the hospital has decreased in the past two decades, the mortality (between 19.5 and 23 %) of septic trauma patients is still high. Early prediction of the sepsis development can help the subsequent intervention and treatment for the patients and contribute to improving the outcome. This chapter is mainly divided into two sections, early prediction and prevention of trauma-related infection/sepsis. The methods for predicting sepsis in trauma patients are primarily using biomarkers (e.g., PCT, IL-10, PSP/reg, IL-1, NT-proCNP, Lactate clearance, mHLA-DR), patient demographics (e.g., age, gender, race) and injury characteristics (e.g., Injury severity, mechanism of injury, number of injuries, hypotension on admission). According to the new definition of sepsis, the prevention of trauma-related infection/sepsis correspondingly includes infection prevention (e.g., surgical managements, prophylactic antibiotics, tetanus vaccination, immunomodulatory interventions) and organ dysfunction prevention (e.g., pharmaceuticals, temporary intravascular shunts, lung-protective strategies, enteral immunonutrition, acupuncture). As a single method of prediction and prevention may not have the desired level of sensitivity and specificity for diagnostic and apotropaic purposes, a new combination of measures can be generated to improve posttraumatic diagnosis and outcome. Overall, more efficient and accurate ways to predict and prevent the trauma-related infection/sepsis should be developed.

Keywords Prediction · Prevention · Trauma · Infection · Sepsis

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1 Early Prediction

Early prediction of the sepsis development can help the early intervention and treatment for the patients and contribute to improving the outcome. So far, the methods for predicting sepsis in trauma patients are mainly using biomarkers, patient demographics and injury characteristics. But the studies on verifying their predictive value are very few and the results are still controversial. More work should be done to explore more efficient and accurate ways to predict the post-traumatic sepsis.

1.1 Biomarkers

According to the Biomarkers Definitions Working Group, biomarker (biological marker) is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [1]. In another word, Biomarkers are tools to measure biologic homeostasis that give standard to what is normal, and providing a quantifiable method for predicting or detecting what is abnormal [2]. The ideal biomarker for sepsis should have a high sensitivity allowing for early diagnosis, and would be specific for pathogenic microorganism in order to allow appropriate therapy [3]. It has been reported that more than 80 molecules have been proposed as useful biomarkers of sepsis [4], and to date, the number increases to 178 or more [5]. The validation of a biomarker needs three aspects of its performance: "(1) proving that the test truly measures a particular molecular species, or its relevant biological activity; (2) proving that measurement of the biomarker discriminates patients with a disease from those who are without the disease; (3) proving that measurement of the biomarker can inform a clinical decision that can improve patient outcomes" [6]. Here we list some representable candidates among the potential biomarkers of posttraumatic sepsis.

Procalcitonin (PCT)

PCT is a precursor of the hormone calcitonin, which is codified by the CALC-I gene located on chromosome 11 and is produced and secreted by parafollicular C cells of the thyroid to sustain the calcium homeostasis [7]. PCT has been proved to be an marker of bacterial infection and sepsis [8, 9], while PCT is released systemically from various kinds of cells outside the thyroid as a response to bacterial infection [10]. On the condition of systemic bacterial infection or by stimulation with endotoxin or proinflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin-6 (IL-6) and interleukin-1 (IL-1), PCT levels increase 1000 times within a few hours [11, 12]. The half-life about 22 h of PCT is another characteristic that it can be used as a biomarker for bacterial infection because its level show a rapid decrease when infection is resolved whereas many other inflammatory biomarkers still keep high levels during the acute-phase response

[11]. For predicting posttraumatic sepsis, studies have shown the rapid kinetics of PCT, with levels peaking at 24–48 h after trauma and rapid decrease in non-complicated patients [13]. Continuous high levels or secondary increases of PCT are predictors of sepsis [10, 11, 14–19]. PCT as a biomarker is useful in predicting and early diagnosis of sepsis in trauma patients.

C-reactive Protein (CRP)

CRP belongs to acute phase protein family, and each one is made of five protomers of 206 amino acid residues, and belongs to the pentraxin family of calcium-dependent ligand-binding plasma proteins [20]. CRP is mainly synthesized in the hepatocytes and its transcription is reduced by the cytokine IL-6, which is predominantly released by macrophages in response to kinds of systemic inflammation, including infections or trauma [20–22]. So it is a sensitive marker of inflammation and tissue damage. The half-life of CRP is 19 h [23]. Serum CRP is as a biomarker because of the rapid concentrations increasing in response to inflammation, the shorter half-life, and the wildly available inexpensive test. Many researchers have explored the predictive value of CRP for posttraumatic sepsis, but the results are unsatisfactory. Both prospective studies and retrospective studies have been reported no predictive power of CRP for sepsis in trauma patients [11, 13, 19, 24–27].

Interleukin-6 (IL-6)

IL-6 is a glycoprotein synthetized by various kinds of cells including T- and B-cells and endothelial cells. Other cytokines (IL-1, TNF) and viruses and bacterial components such as LPS induce the production of IL-6. IL-6 induces hepatic producing acute-phase proteins such as CRP and complement factors, regulation of B- and T-lymphocytes, differentiation of cytotoxic T-cells and an enhanced activity of natural killer (NK) cells [28]. Its release is triggered by tissue damage or infection. It is a cytokine involving in both pro-inflammatory and anti-inflammatory response [29]. IL-6 has a rapid onset, peaking within 2 h after the infectious stimulus [30]. The results of studies on the predict value of IL-6 for posttraumatic sepsis are controversial. Some studies have found that IL-6 is able to discriminate trauma patients prone to sepsis [15, 16], while others have shown no correlation between the IL-6 levels and sepsis development [26, 27, 31–34].

Interleukin-10 (IL-10)

IL-10 is a protein produced by T-lymphocytes, B-lymphocytes, macrophages, and dendritic cells (DC) [35]. It is an anti-inflammatory cytokine playing a role in counter inflammatory and autoimmune pathologies [36]. IL-10 down-regulates MHC class II and costimulatory molecule B7-1/B7-2 expression on monocytes and macrophages, inhibiting their antigen-presenting function, and limits the synthesis of pro-inflammatory cytokines (IL-1, TNF-α) and decreases cytokines production of Th-1 cells [35]. IL-10 peaks quickly in a few hours (4 h) following trauma, and the levels decreasing rapidly in all patients (the first day after trauma) [37, 38]. IL-10 levels have been shown significant higher in patients who develop sepsis at the point of admission [13, 37–40].

Neopterin
Neopterin is a pteridine produced by monocytes or macrophages upon stimulation with interferon-γ (IFN-γ) then released into body circulation [41]. It is helpful in diagnosis of bacterial, viral infections and systemic inflammation. In addition, increased levels of neopterin are associated with endothelial damage, organ dysfunction and sepsis [42]. Among the studies performed on predicting posttraumatic sepsis, neopterin levels have shown no statistical difference between patients who developed and not developed sepsis [26, 42–44].

Pancreatic Stone Protein/Regenerating Protein (PSP/Reg)
PSP/reg is a lectin-binding acute phase protein and was initially found in patients with pancreatitis [45]. PSP/reg acts as an acute phase protein causing the activation of leukocytes and can also be observed in other cells outside pancreas [16]. Its release is reduced by IL-6 following tissue injury [46]. PSP/reg levels can predict and distinguish no infection, local infection and septic complications in posttraumatic patients [16].

Interleukin-1 (IL-1)
IL-1 is an important mediator of innate immunity and inflammation. It can significantly lengthen the lifespan and activate the function of neutrophils and macrophages in response to infections [47]. Its effects on central nervous system cause fever, then the elevated temperature leads to an increased migration of leukocyte. Few literatures give the evidence of predictive power of IL-6 to sepsis after trauma except Menges et al. [39] has reported the positive correlation between IL-1 and sepsis.

Amino-Terminal Pro-Peptide (NT-proCNP)
NT-proCNP is a part of the natriuretic peptide family and is first identified in 1990. CNP participates in physiological processes such as bone growth, reproduction, nerve growth, and re-endothelialisation [48]. ProCNP protein is a precursor of CNP. As a cleavage product of proCNP, Amino-terminal pro-C-type natriuretic peptide (NT-proCNP) is the N-terminal fragment of the C-type natriuretic peptide precursor [49]. The amounts of NT-proCNP are equal to CNP in human plasma and NT-proCNP is considered to be a more reliable indicator of the extent of CNP synthesis [49]. Results of a study show that the levels of circulating NT-proCNP can discriminate polytrauma patients without traumatic brain injury who develop sepsis from who do not [50].

Polymorphonuclear Elastase (PMNE)
In healthy adults, PMN is circulating in the resting state and it can be activated following major trauma [51]. PMN is the main effector cell of the inflammatory response posttrauma and it produces and releases toxic reactive oxygen species. PMN activation and inflammatory response post trauma may be reflected on serum elastase levels [51]. Some studies have proven the difference of PMN elastase between patients with and without infection or sepsis [31, 43], while some others have shown that it has no correlation with post-traumatic infective complications [26, 27].

Lactate Clearance

Persistent occult hypoperfusion is as a risk factor for infections following trauma [52]. And lactate clearance is proposed as a measure of early sepsis resuscitation effectiveness [53]. So lactate clearance can be a biomarker of sepsis. During the first 12–24 h, the lactate clearance is associated with the posttraumatic sepsis [15, 52].

IL-18

As a member of the IL-1 cytokine family, IL-18, which is produced by a variety of cells including Kuppfer cells, monocytes, dendritic cells (DC), macrophages and so on, induces the production of IFN-γ and other cytokines. It is found to be high levels in sepsis patients compared to healthy people [54]. Mommsen et al. [42] has proposed IL-18 concentrations as early markers for posttraumatic complications such as sepsis and MODS.

Monocyte Human Leukocyte Antigen DR (mHLA-DR)

Human leukocyte antigen-DR (HLA-DR) protein is a member of the MHC class II system. HLA-DR is expressed in antigen presenting cells (APC) including monocytes, macrophages, dendritic cells, B lymphocytes [55]. Low expression of Human Leukocyte Antigen DR on circulating monocytes (mHLA-DR) is reported as an indicator to post trauma immunosuppression [55, 56]. And studies show that the decreased level in mHLA-DR is a biomarker of sepsis development after major trauma [57–59].

Other Biomarkers for Sepsis Following Trauma

Some potential biomarkers such as Toll-like receptor-9 (TLR-9) [60], PMN CD11b [33], Soluble FAS (sFAS) [31], L- and I-FABPs (small fatty acid binding proteins) [61], Group-specific component globulin (Gc-globulin) [62], kynurenine values and kynurenine-tryptophan ratios [63], shock index (SI) [64], Protein phosphatase type 2A (PP2A) [65], NT-proBNP level [66], the soluble thrombomodulin (s-TM) level [67] and serum S100 beta [68] also have been reported to have predictive abilities to post traumatic sepsis. TNF-α as an important cytokine has been proved to have no sufficiently predictive value of sepsis development after trauma [34].

Up to now, a lot of biomarkers have been proposed in the field of sepsis. But to the posttraumatic sepsis, there are only a few biomarkers proved to be useful. Among the biomarkers for sepsis following trauma, PCT is the most extensively investigated biomarker, and the results show the good application to predicting this complication. But to others, like CRP, though many studies have been performed on it, the results show no predictive power for patients in trauma. Some biomarkers such as IL-6 and PMNE, the results are controversial. There are also some biomarkers such as IL-1 and IL-18, that show the predictive value of sepsis post trauma, but the studies are few and the results need further evidence to support. Traumatic injuries cause the great changes in the immunological and neurohormonal environments and then affect the physiological processes. After trauma, the innate immune system is activated,then a lot of pro-inflammatory cytokines such as TNF-α, IL-1,and IL-6 are released, leading to systemic inflammation. Activation of neutrophils and endothelial cells can cause the endothelium and tissue damage. To

counter these disadvantages, anti-inflammatory cytokines such as IL-10 are released,leading to the immune suppression and increased risk to secondary infections. The primary cytokines TNF-α and IL-1 can induce the release of following cytokines including IL-6 and IL-8. IL-6 again promotes the production of acute phase protein such as PCT and CRP [69].

Most of these biomarkers illustrated above participate in the reaction of systemic inflammation. But post trauma physiological processes may become more complicated due to the immune function disorders causing by multiple trauma. For example, under the condition of abdominal or brain trauma, the kinetics of biomarkers can be changed [13]. So more tests should be performed to verify the predict value of the present biomarkers and find more biomarkers suitable to the traumatic sepsis.

1.2 Patient Demographics

Patient demographics including age, gender, and race are as risk factors associated with posttraumatic sepsis.

Older age is an independent risk factor for sepsis following trauma [70–73]. This may because the elderly trauma patients have decreased cardiopulmonary function, poor nutritional status, and susceptible to increased bleeding after injuries, and these factors may contribute to the disorder of physiological processes and immunologic function. In addition, the elder traumatic patients may have more preexisting diseases than the young patients, while the preexisting diseases is also as an risk factor of posttraumatic sepsis [74].

Some studies have proposed the male gender as a predictor for sepsis post trauma [17, 71, 72, 74, 75]. After trauma, the continuous increased cytokines and the subsequent immunosuppression make the body prone to sepsis. Study performed on animals shows that proestrus females are not immunodepressed compared with male and ovariectomized mice after trauma [76]. There are also tests results that estrogen produces beneficial effects on immune and cardiovascular function after trauma [77] by reducing the release of cytokine production such as TNF-α and maintaining the immune response [78]. So estrogen plays an important role in the gender dimorphism of posttraumatic sepsis.

African American race is reported as a risk factor of sepsis following trauma [72]. But there are not extensive researches done to investigate the role of racial or ethnic factors in posttraumatic sepsis. More researches should be warranted to explore the association between ethnicity and this complication.

1.3 Injury Characteristics

Injury severity, mechanism of injury, number of injuries, hypotension on admission and other injury characteristics are factors associated with posttraumatic sepsis.

Trauma can cause the deficits in the immune system by depressing the humoral and cell-mediated systems. After major trauma, the function of lymphocytes is depressed. The neutrophil chemotaxis is decreased and monocyte antigen-presenting capacity is impaired. There are also changes in complement components [79]. Different degrees of trauma severity may lead to the different influences in immune function. The main measures of injury severity are trauma scoring systems.

Among the various scoring systems, Injury Severity Score (ISS) and New Injury Severity score (NISS), members of anatomical scoring systems, are most widely used. The ISS is based on the Abbreviated Injury Scale (AIS) severity values, and it is first developed in 1974 [80]. It is calculated as the sum of the squares of the highest AIS values from each of the three most severely impaired body regions. It has some limitations, for example, it does not represent multiple injuries in the same body region and it considers injuries with an equal AIS score to judge a same severity regardless of the injured body region [81]. The NISS is proposed by Osler et al. in 1997 to counter the limitations of ISS [82]. It is calculated as the sum of squares of the three most severe injuries regardless of the body region injured. The ISS or NISS ranges from 1 to 75. Increasing injury severity measured by ISS and NISS was associated with increased incidence of sepsis [70–74, 83].

Besides ISS and NISS, the Glasgow coma scale (GCS) which assess the level of clinical consciousness are also a predictor of sepsis [71, 74]. GCS is first described by Teasdale and Jennett in 1974 [84]. It is the sum of three components that describes a patient's best motor response, verbal response and eye opening to stimuli. It ranges from 3 to 15, and the lower scores patient gets, the worse condition patient is. GCS belongs to physiological scoring systems [85].

The anatomy scoring systems such as ISS and NISS represent the physical degeneration of the body, and the physiological scoring systems such as GCS stand for the physiological impair caused by trauma. Compared to biomarkers, getting the indexes of these scoring systems are easier, earlier and cheaper.

Liang et al. [86] reported two novel formulae based on LD_{50} values of ISS and NISS were superior to PCT for prediction of sepsis in trauma patients. The performance of ISS/LD_{50ISS} + SIRS score and $NISS/LD_{50NISS}$ + SIRS score were equivalent (area under the ROC curve (AUC) = 0.816 vs. 0.819, $P > 0.05$) and both performed better than PCT (AUC = 0.592, $P < 0.05$) in predicting posttraumatic sepsis. Overall, the two novel formulae ISS/LD50ISS + SIRS score and NISS/LD50NISS + SIRS performed well and both better than PCT in predicting sepsis following trauma. The value of the two formulae can be easily calculated in real-time and can identify the high-risk patients susceptible to sepsis. This method may become an effective way to guide the early assessment and treatment in trauma patients.

There are also several injury characteristics reported as risk factors such as number of red blood cell units transfused [70], number of injuries [71] and hypotension on emergency department presentation [72].

2 Prevention

The greatest danger after haemorrhage in trauma patients is sepsis. Sepsis 3.0 was put forward by Society of Critical Care Medicine, the chairman of the United States, Professor Craig Coopersmith on Chinese Medical Association (CMA) ninth intensive medical conference in 2015. The experts suggested the new definition of sepsis should take organ dysfunction (OD) as a core. Thus Sepsis 3.0 is composed of two parts (1) Infection; (2) Sequential Organ Failure Assessment (SOFA) ≥2, regardless earlier or later, as long as the two coexist and then diagnosed. According to the new definition of sepsis, the prevention of trauma-related infection/sepsis correspondingly includes infection (wound infection, nosocomial infection primarily) prevention and OD prevention.

2.1 Infection Prevention

Preventing infection following trauma basically involves preventing wound infection and nosocomial infection. Wound care methods commonly include surgical managements (disinfect, debridement, profuse irrigation and wound cleansing, negative-pressure wound therapy, wound drainage, appropriate wound closure etc.) and pharmaceuticals (prophylactic antibiotics, tetanus vaccination, immunomodulatory interventions, etc.) The prevention of nosocomial infection is another aspect. Immune dysregulation is a well described consequence of trauma, increasing the risk of nosocomial infection. Regional proper clinical protocols and hygiene are the correct methods in the accepted prevention principles. Here, we mention the following measures: chlorhexidine, hydrocortisone, detrusor botulinum toxin A injection, enteral nutrition and management of tube system etc. to prevent ventilator associated pneumonia (VAP), central line-associated bloodstream infection and urinary tract infection (UTI).

2.1.1 Surgical Managements

Hair Removal and Skin Disinfection

Hair is autologous source of wound contamination, and removing hair from the wound can avoid entangling during suture and closure [87]. The type and time of shaving has been revealed necessary in reducing the chance of infection. The

infection rate of surgical wounds after electric clippers preparation of the skin is markedly lower than razor [88]. Moreover, shaving hair before wound repair is proved to be higher risk of surgical site infection than clipping hair immediately [89]. Although the antiseptic agents containing iodophor or chlorhexidine can suppress a broad spectrum of organisms and bacterial proliferation, they may damage the wound defenses and promote the development of infection [90]. Consequently, reasonable application of antiseptic agents into the wound should be considered.

Debridement

Wound debridement is the most common surgery used in conflict and civilian cases. First surgical treatment in war surgery at the first echelon hospital is debridement, without primary closure [91]. USA military recommends that repeat debridement and irrigation every 24–48 h before wound clean should be insist on [92]. Debridement can remove devitalized and severely contaminated tissues for preventing infection, and the basic principles of wound debridement are well accepted in field of surgical managements [93, 94]. However, Edlich et al. suggested that the less tissue debrided had been associated with lower wound infection. Thus, it is important to identify the definite limits of dead tissue, for instance, the "4C" guidelines (color, consistency, contraction, circulation) of muscle viability [87]. In the case of complex traumatic hand injuries, meticulous initial debridement of nonviable tissue and skeletal stabilization are paramount in preventing hand infection [95]. Multiple debridements will be necessary if significant contamination is present.

Mechanical Cleansing

Early and thorough irrigation following wound debridement is one of the important steps in basic principles of management of war wounds [91, 92]. Gentle irrigation with low pressure and normal saline will wash out any residual debris and clot and dilute any bacterial load, while high-pressure irrigation (7 psi, pounds per square inch) is applied to dirty or heavily contaminated wounds [91, 96]. Additionally, mechanical cleaning with high-pressure may effectively decrease the level of bacterial contamination and reduce the incidence of wound infection [87, 97].

Negative-Pressure Wound Therapy (NPWT)

NPWT systems (also referred as vacuum-assisted wound closure), composed of open-pore sponge, semi-occlusive dressing, negative pressure source, are commonly available in USA [94, 98]. And negative pressure ranging from −50 to −200 mmHg may effective in higher risk of infective wounds [99]. NPWT has the frequently cited advantage of bacteria clearance from wound environment. It can reduce the wounds bacterial bioburden in animal open fracture model which was contaminated with gram-negative bacilli. But the colonization of gram-positive cocci (e.g., *Staphylococcus aureus*) also exist [100]. In addition, NPWT has more benefits than dressings in the setting of wound infection [99–101]. Patients with persistent drainage at least 5 days treated with NPWT have lower rate of wound infection and shorter period of drainage than compressive dressing group [99].

Several studies have showed that the wound infection rate in the patients who adopted NPWT is significantly lower than WTD group [100, 101]. In military fields, NPWT used during intercontinental aeromedical evacuation of combat casualties also provide many benefits such as earlier wound closure, lower infection rates, and better pain management [92, 102].

Wound Drainage

Thorough wound drainage following debridement and irrigation is one of the steps in basic principles of management of combat-related injuries [91]. Traditional drains within 24 h is commonly used in wounds with deep cavities and dead space. Stannard et al. [99] had evaluated the efficiency of NPWT for management of persistent wound drainage. In addition, Rispoli et al. [103] reported a new technique, combining NPWT with traditional drainage, which allowed deep cavitary defects conversing to superficial defects for facilitating drainage. At the same time, wound deep infecton was better controlled and there were no complications, such as abscess formation, tube-associated skin necrosis, sepsis detected.

Wound Dressings

Abulky absorbent dressing or cotton wool is necessary for adequately excised wound. Bandage wounds with sterile dressings are commonly used in the battle field initial care. Silver nitrate solution applied to dressings is routinely done following burns [91, 92]. WTD dressings were suggested to be the standard of method for soft-tissue defects and open wounds in the past. Since WTD has increased patient pain, healthcare cost and risk of nosocomial infection, the safe and effective wound dressings are required [94]. Then, Guthrie et al. compared 3 dressings, Inadine® (USA), Acticoat® (Hull, UK) and Activon Tulle (Nottingham, UK) in a rabbit model of contaminated forelimb muscle injury. They found Inadine and Acticoat groups had significantly lower bacterial counts [104].

Wound Closure

It is important that the wound is closed as soon as safe, not before and not long after [91, 105]. Traumatic laceration wounds (≤5 cm) without signs of infection can be closed immediately, and disinfected wounds may be closed up to 24 h afterwards (based on the Friedrich dogma), while the wounds with active signs of infection should allow for secondary closure after 3–5 days [106]. Because there is no powered evidence to prove the efficiency of dogma which traumatic wounds should not be sutured after 6 h. Then, van den Baar et al. [107] conducted a prospective cohort study, showing the wounds older or younger than 6 h was not a critical factor in the decision of wound closure. Contaminated wounds should never be primarily closed. It seems necessary for delayed primary closure (DPC) to treat severely contaminated or macerated wounds after multiple debridement and irrigation procedures [93, 105].

2.1.2 Pharmaceuticals

The most common effective intervention is pharmaceuticals besides surgical managements after trauma. Antibiotics are now generally recommended for wounds and nosocomial infection prevention. In addition, tetanus vaccination, chlorhexidine, hydrocortisone, detrusor botulinum toxin A (BoNTA) injection, immunoglobulin, IFN-γ, and glucan are mentioned in several researches and take an active role in preventing trauma-related infection.

Prophylactic Antibiotics

According to the International Committee of the Red Cross (ICRC) Antibiotic protocol, proper use of antibiotics is based on different types of injury [91]. Additionally, USA guidelines for antibiotics of combat-related injuries suggest that post-traumatic antimicrobial agent selection and duration should base upon different combat-related injuries pattern. For instance, extremity wounds (cefazolin, 2 gm IV q6-8h, 1–3 days), thoracic wounds (cefazolin, 2 gm IV q6-8h, 1 day after washout; if penetrating chest injury with esophageal disruption, metronidazole 500 mg IV q8-12 h is added), abdominal wounds (cefazolin 2gm IV q6-8h with metronidazole 500 mg IV q8-12h, 1 day after washout), maxillofacial and neck wounds (cefazolin 2gm IV q6-8 h 1 day), central nervous system wounds (cefazolin 2gm IV q6-8h, 5 days or until CSF; if contamination and abdominal cavity are involved, metronidazole 500 mg IV q8-12h is added), penetrating eye wounds (levofloxacin 500 mg IV/PO once a day, 7 days) [92].

Afterwards, many exploratory studies are drawing wide concern on prophylactic antibiotic after trauma. Here, we list the basic principles.

- **The Time of Administration**

As the bacteria increases exponentially with the time from trauma, six hours seem to be a vital period after wound contamination. It is necessary to extend the time of antibiotics if an unavoidable delay in administering during which the wound is open [87]. The ICRC recommends that penicillin if not begun within 6 h in prehospital uncomplicated soft-tissue Grade 1 wounds, great risk of infection may be unavoidable [91].

- **The Choice of Antibiotics**

Immediate broad-spectrum intravenous antibiotics based on the Gustilo and Anderson classification should be given in open fractures or extensive soft tissue loss patients [95, 108]. High dose intravenous 3rd generation cephalosporins rather than oral 1st generation drugs may effective in open fractures patients [109]. The incidence of wound infection in fresh traumatic wounds/laceration is low after co-amoxiclav application [106, 110]. Cefazolin, or vancomycin if penicillin allergic, cefoxitin/clindamycin and gentamicin, clindamycin and gentamicin are commonly applied in trauma intensive care unit (TICU) [111].

- **The Course/Dose of Antibiotics**

Both civilian and military studies suggest that short course and single dose of cephalosporins are important to prevent wound infection in open fractures, either three days from injury or until 24 h after wound closure [112]. Patients with penetrating abdominal trauma and concomitant TLS fracture receiving prophylactic antibiotic for ≤48 h are not develop spinal infections [113]. Prophylactic antibiotics researches apply not merely in the site of trauma wound infection, but also in nosocomial infection (early VAP and clostridium difficile infection) [114, 115].

- **The route of antibiotics administration**

There are several routes of antibiotics administration depend on the different types of trauma. Administration of oral antibiotics is often applied to prevent wound infection for simple traumatic wounds [116, 117]. In addition, the antibiotic ointments containing bacitracin, polymyxin, neomycin or cetrimide are often used in minor uncomplicated soft tissue wounds and have lower skin infections rates [118].

Overall, recent studies concentrated on the prophylactic antibiotics after trauma are mostly retrospective and integration of experts' opinions, which lack of considerable random, double-blind, prospective studies.

Tetanus-Diphtheria/Tetanus Vaccination

Tetanus, the incubation period is 3–21 days, has a great risk of any penetrating wounds infection, especially in deep, small, punctate ones. It is crucial for all trauma patients with deep wounds to receive appropriate immunization against tetanus. The present tetanus vaccination condition, a booster or revaccination, should also be considered for clinicians according to local protocols [106]. As emergency clinicians are frequently facing to patients who are sensitive to tetanus infection in emergency department, attitude should be changed on tetanus prophylaxis among emergency physicians [119].

Chlorhexidine (CHX)

For incontinence care, wiping the involved skin should use as many chlorhexidine cloths as necessary after routine cleaning with soap water. Chlorhexidine is proved to be useful in reducing Acinetobacter skin colonization of ICU patients [92]. However, the effect of CHX for preventing nosocomial infection in trauma patients is controversial. Critically injured patients receiving daily bathing with 2 % CHX are associated with a lower rates of catheter-related bloodstream infection and MRSA (methicillin-resistant Staphylococcus aureus) VAP [120]. Receiving CHX both from admission to 48 and 72 h are also effective [121]. But oral chlorhexidine over the first 48 h could not minimize the risk of VAP for intubated trauma patients [122].

Hydrocortisone

Adrenal insufficiency that alters organism immunity often occur in severe trauma patients. Intravenous stress-dose of hydrocortisone have been associated with lower incidence of hospital-acquired pneumonia (HAP) in ventilated patients with trauma [123, 124]. Subsequently, researches have detected the mechanism of hydrocortisone in a posttraumatic pneumonia mouse model, which can decrease the

trauma-induced immunosuppression by modulating the DC/NK cell cross talk [125].

Other Interventions

The most common clinical complication in patients with indwelling catheter after spinal cord injury (SCI) is urinary tract infection (UTI). And neurogenic detrusor overactivity (NDO) is frequently detected in SCI population, increasing the risk of UTI. BoNTA injection may significantly reduce UTI in SCI patients with NDO which appears to decrease detrusor pressure [126]. Besides, Immunomodulatory interventions such as immunoglobulin, IFN-γ, or glucan have the most effective significance to improve infection and MOF in trauma patients [127].

2.1.3 Enteral Nutrition (EN)

Receiving EN within 24 h of severe injury and/or ICU admission can significantly reduce pneumonia rate [128]. Some studies have indicated that both the nutritional quality and type of EN are also critical for reducing hospital-required infections following trauma. High quality EN formulas containing omega-3 fats, extra levels of vitamins, minerals, and amino acids (such as glutamine) are proved to reduce the rates of nosocomial pneumonia, bacteremia, abdominal abscesses, urinary tract infections than standard EN [129]. And EN containing fish oil but no arginine has lower risk of secondary nosocomial infection [130]. EN added probiotics is associated with lower incidence of VAP [131]. In addition, the type of transpyloric feeding (TPF) has a lower rates of VAP in severe TBI patients than gastric feeding (GF) [132].

2.1.4 Management of the Tube System

Severe trauma patients placing central venous catheter should strictly observe sterile techniques to reduce central line-associated blood stream infections (CLBSIs) [133]. Choosing proper tube type is as critical as indwelling time on intubation trauma patients. Traumatic hemothorax placing central venous catheter (CVC) has associated with lower infection rate of surgical wounds than conventional large-bore chest tube [134]. And reducing the time of indwelling urinary catheter could reduce the rate of UTI [135–137].

2.2 Organ Dysfunction Prevention

Organ Dysfunction (OD) prevention has been received increasing attention in patients with serious infection. Calcium channel sensitizer levosimendan has been

shown to have potentially advantages on organ function in severe sepsis, specially on myocardial function [138–142]. Cardiopulmonary Bypass (CPB), an essential technique of cardiac surgery, seems to alleviate inflammation and prevent organ dysfunction [143]. Critical ill patients who have an increased risk of extensive endothelial damage receiving autologous transplantation of endothelial progenitor cells (EPCs) may restore blood flow, which could improve the function of important organs, and thus, MODS can be prevented [144]. In addition, Chinese medicine therapy of clearing-heat and detoxifying has also demonstrated beneficial effects on MODS prevention [145]. The effect of Remote ischaemic preconditioning (RIPC), a strategy to reduce ischaemia in remote organs, has been controversial in renal injury however [146]. Though above pharmaceuticals and medical strategies have shown an potentially prevention effect on OD, the evaluation of these measures used on trauma patients is unknown.

Some studies have reported several major risk factors in trauma patients leading to MODS, e.g. older age, chronic diseases, hypoperfusion, infection, immunodepression [147–150]. So far, few effective interventions apply in the organ dysfunction (OD) prevention following trauma instead of some exploratory researches.

2.2.1 Pharmaceuticals

Immunoglobulin, IFN-γ, or glucan have effective significance to improve MOF in trauma patients [129]. Obese trauma patients (BMI >30 kg/m^2) with an increasing risk of multiple organ failure (MOF) receiving preinjury ACE/ARB (angiotensin-converting enzyme inhibitor/angiotensin receptor blocker) therapy have markedly higher Marshall and Denver-2 scores than that of not receiving these medications [151, 152]. Patients receiving either 7.5 % hypertonic saline (HS) or 7.5 % HS with 6 % dextran-70 (HSD) have lower incidence of MODS than that of 0.9 % normal saline (NS) [153].

2.2.2 Healthcare Strategy

Patients with vascular injuries (arterial/venous) at a Civilian Level I Trauma Center applying to temporary intravascular shunts (TIVS) treatment have lower rates of MOF and sepsis [154]. MOF seems to be prevented in critically multiple trauma patient with mechanical ventilation by using lung-protective strategies, avoiding high volumes and inspiratory pressure and improving the proportion of aerated lung during expiration. It has been turned out that the occurring rates of single organ failure, two organ failure and MOF are low [155]. High quality of EN is important in trauma patients to prevent nosocomial infection, such as VAP, UTI [128–132]. The immunonutrition of EN and immune-enhancing diet also have demonstrated a vital role in reducing MOF following severe trauma. A combination of arginine, *n*-3-fattyacids and nucleotides also have lower septic complications and MOF score [156]. In addition, Chinese traditional treatment also has potential validity in

prevention OD. Acupuncture, using special thin needles which are pushed into the skin in particular parts of the body, can activate vagal activity and cholinergic anti-inflammatory pathway, then improve the outcome of multiple traumatic patients. Liang and Qu reported that acupuncture at ST-36 and PC-6 acupoints was associated with lower incidence of systemic inflammatory response syndrome (SIRS), acute respiratory distress syndrome (ARDS), sepsis and MOF [157].

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Genetic Polymorphisms and Trauma Precision Medicine

Wei Gu and Jianxin Jiang

Abstract Major trauma is the leading cause of death in young adults. Post traumatic complications, especially sepsis and MODS, are the main causes of death of trauma patients in hospital. Recent advances in researches have showed a close relationship between genetic background and outcomes of trauma patients. The emerging field of precision medicine is expected to provide the best available care for every patient based on accurate clinical information and evidences at an individual level or at a community level. The further studies on genetics of trauma patients will certainly lead to a better understanding of post-trauma complications and personality treatment of trauma patients in the future.

Keywords Trauma · Precision medicine · Polymorphisms · Sepsis · MODS

1 Background

1.1 Precision Medicine

The core concept of precision medicine is the consideration of individual variability during the prevention and treatment of patients. It is believed to be an innovative approach to disease treatment, disease prevention and health promotion. The main purpose of precision medicine is to integrate basic science, diagnostic tests and the best evidence-based knowledge to conduct personalized health education, counseling, and prevention. This advanced approach will take the man's genetic profile and predisposition, environment, emotional and psychological state, and lifestyle choices into account when make a medicine care plan. In addition, it is the time for us health care providers to embrace and increase our knowledge to develop and

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manage the best health care system that provides safe, patient-centered, transparent, and quality health care based on those big data sets of population health and genomics.

There are many favorable condition for the propose of precise medical. The cost of sequencing a genome has dramatically dropped since 2001, when the first draft of the human genome sequence was published. Besides of that, the prospect of applying this concept broadly has been dramatically improved by the recent development of large-scale biologic databases (such as the human genome sequence), powerful methods for characterizing patients (such as proteomics, metabolomics, genomics, diverse cellular assays, and even mobile health technology), and computational tools for analyzing large sets of data. It could image that precision medicine's more individualized, molecular approach to disease will certainly encourage and support the next generation of scientists to develop creative new approaches for detecting, measuring, and analyzing a wide range of biomedical information, including molecular, genomic, cellular, clinical, behavioral, physiological, and environmental parameters.

1.2 The Application of Precision Medicine in Trauma

Trauma is a major public health problem worldwide, ranking as the fourth leading cause of death. In 2010, there were 5.1 million deaths from injuries, and the total number of deaths from injuries was greater than the number of deaths from HIV/AIDS, tuberculosis, and malaria combined (3.8 million) [1, 2]. With the promotion of emergency technology and the advent of new medical treatment, the early mortality of trauma was effectively reduced [3]. However, the late complications, especially infectious complications, such as sepsis and multiple organ dysfunction syndromes (MODS) are serious threats to the trauma patients, and dramatically increase the burden of cost to society. The cause of sepsis and MODS are numerous, including age, sex and injury. However, it is still not well known why some patients may develop sepsis, while others consulting similar injury not. Growing evidences show that the interaction between gene and host or gene and environment may play an important role in it.

The results from an early animal experiment supporting the role of genetic background in trauma outcomes. Radojicic et al. [4] observed a difference of survival rates among four inbred strains of mice (AKR, CBA, BALB/c and C57BL/6) after suffering mechanical, radiation and thermal injuries. Our results also demonstrated a significant differences of mortality between C57BL/6 and BALB/c mice after blast wave injury [5]. Another strong evidence comes from an early epidemiological study. Sorensen et al. [6] reported a close relationship between death from infection in adoptee and his/her biological, instead of adoptive parents, indicating a role of genetic background in risk of infectious outcomes. Besides of these initial findings, more and more studies indicated that genetic factors may influence the reaction and susceptibility of complications in trauma patients.

Genetic polymorphism refers to the occurrence of two or more alleles at one locus in the same population, each with a distinct frequency, where the minimum frequency is typically 1 %. This word is now used to describe the differences among individual DNA sequence that make each human genome unique. There are generally two types of genetic polymorphisms, DNA site polymorphisms and length polymorphisms. DNA site polymorphisms refer to those alleles at specific sites on the DNA sequence differences, also different genome scattered in the base, including point mutation (transition and transversion) and single base substitution, deletion and insertion. DNA length polymorphisms refer to the polymorphisms of DNA fragment length difference between the alleles of the same gene locus, including VNTR (variable number of tandem repeats), STR (short tandem repeats) and MVR (minisatellite variant repeat mapping). Among them, single nucleotide polymorphisms (SNPs), known as DNA sequence polymorphism caused by variation of a single base pair of nucleotide at the genome level, have been extensively studied.

In association studies, SNP markers have more advantages than microsatellite markers. (1) For SNP markers, there are always two alleles or three alleles in the population. Therefore, the allele frequencies can be easily estimated. (2) SNPs' distribution in the genome, with minor allele frequency >0.1 occur once every 600 kb, is much wider than that of microsatellite markers. (3) SNPs are highly stable compared with tandem repeat microsatellite loci, especially in the coding region of SNP (cSNP). (4) Researches have found that some SNPs, especially those exist in the coding region of a gene, may directly affect the structure or expression level of encoded protein. (5) It is easy to carry out automatic and high-throughput genotyping analysis for SNPs, which shortens the research periods. In addition, compared with protein biomarkers that are always transiently expressed in the disease course, SNPs do not alter in response to underlying disease, and may be the most suitable predictors for disease susceptibility research.

Since genotyping can be easily carried out using a small amount of peripheral blood, it is attractive to assess individual reaction after major trauma in a genetic approach. Identifying patients at risk of developing complications may improve their outcome by precise and targeted treatments such as antibiotic prophylaxis, substitution therapy, or plasma transfusions.

2 Relationship Between Genetic Polymorphisms and the Outcomes of Trauma Patients

Sepsis and MODS are among the most severe traumatic complications burdened with high mortality. Although a number of investigations have been conducted, the underlying pathogenesis is still not very clear, mainly due to its complex and multiple influencing factors. In the middle of 1980s, sepsis was considered as an excessive inflammatory response to infection, characterized by large number of

pro-inflammatory cytokines released by immune cells. However, subsequent so-called "anti-inflammatory therapies" were all failed. Later, Bone et al. [7] proposed the "compensatory anti-inflammatory response syndrome" (CARS) Hypothesis in 1996. It points out that the inflammatory response induced by early stage of sepsis can induce immune suppression in the later stage. Nowadays, it has become more clear that infection can trigger a much more complex, variable and prolonged host response, in which both pro-inflammatory and anti-inflammatory mechanisms are existing at the same time, contributing to clearance of infection and tissue repair on the one hand and organ damage and secondary infections on the other [8]. In order to determine the patients' risk of developing infectious complications after major trauma, a number of genes have been studied, including pattern recognition receptors (PPRs), signal transducing adaptors, inflammatory cytokines, complement system and coagulation system related genes.

2.1 Pattern Recognition Receptors and Complexes

Pattern recognition receptors are proteins mainly expressed on the surface of innate immune cells, with the role of identify two classes of molecules. The first is pathogen-associated molecular patterns (PAMPs), derived from microorganisms. The other one is damage-associated molecular patterns (DAMPs), which are cell-derived and released during cell damage or death. Among them, Toll-like receptors (TLRs), homologues of the Drosophila Toll gene, plays a critical role in early innate immunity to recognize invading pathogens by sensing microorganisms [9, 10]. TLRs are considered as bridges that connect both innate immunity and adaptive immunity, so they are widely studied. There are totally ten human and nine murine TLRs have been characterized so far, which can be divided into two groups. One group of TLRs are all expressed on the surface of immune cells, in charge of the recognition of microbial cell walls components or microbial proteins. TLR1, TLR2, TLR4, TLR5, TLR6 and TLR11 all belongs to this group. Another group of TLRs, including TLR3, TLR7, TLR8 and TLR9, are expressed inside the cell and mainly in charge of recognize nucleic acids, such as single-stranded or double-stranded RNA, or CpG-rich DNA in specific cellular compartments. The relationship between polymorphisms in TLR1, TLR2, TLR4, TLR9 gene and patients' outcomes has been well studied in trauma cohort (Table 1). Three SNPs in TLR1 were studied in trauma patients. Among them, rs5743551 and rs4833095 were associated with increased risk of mortality in sepsis and gram-positive sepsis, respectively [11]. A SNP in TLR2, Arg753Thr, although has been reported to be associated with Gram-positive infections [12, 13], does not exist in Chinese population. While another tagging SNP (TLR2 19216T/C) has been shown to be associated with LPS induced cytokine production and an increased risk of sepsis and MODS after severe trauma [14]. Besides, two functional SNPs in TLR4 gene (−2242T/C and 11367G/C) were found to be related to sepsis morbidity [15–17].

Table 1 Effects of pattern-recognition receptors gene polymorphisms on the outcomes of trauma patients

Gene	Chrome location	Variation	Population	Study size	Fuctional effects	Associated clinical phenotype	References
TLR1	4p14	−7202A/G (rs5743551)	Whites	1498		Higher mortality with sepsis after traumatic injury	[11]
		742A/G (Asn248Ser) (rs4833095),	Whites	1498		Higher mortality in Gram positive sepsis	[11]
TLR2	4q32	−16934 T/A (rs4696480)	Mixed Ethnic	219		Higher risk of a gram-positive infection and SIRS	[71]
		R753Q (rs5743708)	Mixed Ethnic	68		Higher sepsis morbidity rate	[35]
		19216T/C (rs3804099)	Han Chinese	410	Cytokine production	Higher sepsis morbidity rate and MOD scores	[14]
TLR4	9q33.1	−2242T/C	Han Chinese	303	Cytokine produciton and promoter activity	Higher sepsis morbidity rate and MOD scores	[15]
		896 A/G	Whites	598		Decreased risk of complicated sepsis	[72]
			Mixed Ethnic	159		Increased risk for severe sepsis following burn trauma	[31]
			Mixed Ethnic	228		Increased risk for severe sepsis.	[40]
		11367G/C	Han Chinese	132	mRNA stability and TLR4 expression	Decreased sepsis morbidity rate and MOD scores	[16, 17]
TLR9	3p21.3	−1486T/C (rs187084)	Han Chinese	557	Cytokine production	Higher sepsis morbidity rate	[18]
		6577T/C (rs352162)	Han Chinese	557	Cytokine production	Higher sepsis morbidity rate and MOD scores	[18]

(continued)

Table 1 (continued)

Gene	Chrome location	Variation	Population	Study size	Fuctional effects	Associated clinical phenotype	References
MD-2	8q21.11	−1625C/G (rs11465996)	Han Chinese	105/726	MD-2 promoter activity,MD-2 expression	Higher sepsis morbidity rate and MOD scores	[19, 73]
CD14	5q31.1	−159C/T (rs2569190)	Han Chinese	105	CD14 promoter activity	Increased sepsis morbidity rate and MOD scores	[20]
			Han Chinese	106		Increased MOD scores	[74]
			Mixed Ethnic	228/149/233		Decreased risk for severe sepsis and mortality	[40, 48, 75]
		−1145G/A (rs2569191)	Han Chinese	105	CD14 promoter activity	Decreased sepsis morbidity rate and MOD scores	[20]
			Han Chinese	106		Increased MOD scores	[74]
LBP	20q11.23	Pro436Leu (rs2232618)	Han Chinese	454/1215	Higher median basal serum LBP levels	Higher susceptibility to sepsis and MOD	[23]
RAGE	6p21.3	−429T/C (rs1800625)	Han Chinese	728	Decreased production of TNFα and promoter activities	Decreased sepsis morbidity rate and MOD scores	[24]
NLRP3	1q44	−1017G/A (rs2027432)	Han Chinese	718	Increased production of IL-1βand transcription activity	Increased MOD scores	[25]
		5134A/G (rs12048215)	Han Chinese	718	Decreased production of IL-1β	Decreased sepsis morbidity rate	[25]
hGR/NR3C1	5q31	BclI C/G (rs41423247)	Han Chinese	95			[76]

We also found that TLR9 6577T/C (rs352162) were associated with sepsis morbidity and MOD scores [18].

MD-2, CD14 and LPS-binding protein (LBP) are the co-molecules involving in TLR4 sensing. A polymorphism in MD-2 promoter (MD2 −1625C/G) was reported to increase the promoter activity and expression level of MD2 in vitro. Patients carrying −1625G allele are more likely to develop sepsis and MODS after major trauma [19]. Although the results of CD14 researches are not consistent, our study found a synergistic effect of −159C/T and −1145G/A on the development of post traumatic complications [20]. The researches regarding LBP SNPs and sepsis also got conflicting results [21, 22]. However, we identified that people carrying LBP 436Leu had an increased risk of infection in Chinese population [23].

Besides of classical receptors involved in TLR4 pathways, several other PPRs have been also investigated. RAGE −429T/C polymorphism (rs1800625) was shown to be related to sepsis and MODS in severe trauma patients [24]. Compared with those carrying T allele, patients carrying C allele had a significantly lower sepsis morbidity rate and MOD scores. Rs2027432 in NLRP3, a member of NOD-like receptor family, was found to be significantly associated with higher risk of MODS. In addition, the NLRP3 5134A > G (rs12048215) polymorphism was found to be significantly associated with a lower sepsis morbidity rate. Moreover, the rs2027432 polymorphism was significantly associated with higher IL-1β levels [25].

2.2 Signal Transducing Adaptor Proteins

Interleukin-1 receptor-associated kinases (IRAKs) are a family of molecules, which play an important role in the regulation of natural immune system, as mediators of TLR/IL1R superfamily signaling. There are four IRAK genes found in the human genome (IRAK1, IRAK2, IRAK3 or IRAKM, and IRAK4). All of them have the similar domain structures, including a praline/serine/threonine-rich (PEST) kinase domain (KD) and an conserved N-terminal death domain (DD), which is important for dimerization and interaction with MyD88. Except for IRAK4, the other three mebers in IRAK famil all contain a C-terminal domain, which is required for TRAF6 binding and activation [26].

The relationship between IRAK1, IRAK3 and outcomes of major trauma patients were investigated (Table 2). One MODS-related polymorphism in IRAK1 gene was found out. IRAK1 encodes the interleukin-1 receptor-associated kinase 1, a serine/threonine kinases belongs to the Toll/IL-1 receptor (TIR) signaling family and a key regulator of NF-kappa B pathway. Sperry et al. [27] studied a cohort of 321 patients with a median ISS of 16 for the 1595T/C substitution (rs1059703) in exon 12 of IRAK1 which results in a non-synonymous mutation (p.L532S). They found patients carrying this polymorphism have an eightfold and 11-fold risk of MOF and death, respectively. Specially,this phenomenon is most prominent in males, whereas females carrying heterozygous are more likely to have a worse

Table 2 Effects of signal transduction gene polymorphisms on the outcomes of trauma patients

Gene	Chrome location	Variation	Population	Study size	Fuctional effects	Associated clinical phenotype	References
IRAK-1	Xq28	1595 T/C (rs1059703)	Mixed Ethnic	321		Greater risk of MOF and mortality	[27]
IRAK-3	12q14	15SNPs	African ancestry and European ancestry	474		Greater risk of ALI in African descent	[28]
REL	2p13-p12	rs842647 G/A	Chinese	753	Lower TNF-α production	Lower sepsis morbidity rate and MOD scores	[30]

outcome. Meyer et al. [28] genotyped 25 candidate genes for 474 critically ill trauma patients with acute lung injury (ALI) in a prospective cohort study using the IBC chip. IRAK3 was found to be associated with ALI in patients from African descent but not in European ancestry trauma subjects.

Nuclear factor-κB (NF-κB) family contains five members, p50, p52, p65 (RelA), RelB and c-Rel. The complexity of NF-κB can be activated by either canonical or non-canonical pathways and plays an essential role in inflammation [29]. More and more evidence indicates that polymorphisms in the NF-κB family genes may affect the magnitude of proinflammatory response. Our research investigated the relationship between Tag SNPs selected from NF-κB family genes, including NFKB1, NFKB2, RELA, RELB and REL, and outcomes in a Chinese trauma cohort [30]. One SNP, rs842647 in REL gene, was found to be associated with lower sepsis morbidity and MOD scores. Patients carrying rs842647 A allele had lower plasma TNF-α levels.

2.3 Inflammatory Cytokines

In the course of sepsis, there is a comprehensive and systemic activation of immune responses. The markedly imbalanced cytokine response accompanying with sepsis forms a kind of 'cytokine storm', which converts normally beneficial responses of anti-inflammation into excessive, and finally causes damage to normal tissues. Various cytokines released from immune cells work as effectors and play an important role in the inflammatory response to infection. Thus, a number of polymorphisms in cytokine genes have been investigated using association studies (Table 3).

Table 3 Effects of cytokine gene polymorphisms on the outcomes of trauma patients

Gene	Chrome location	Variation	Population	Study size	Fuctional effects	Associated clinical phenotype	References
IL-1α	2q13	−889C/T (rs1800587)	Han Chinese	308	The lower serum levels of Il-1α	Higher sepsis morbidity rate	[77]
IL-1β	2q14	−1470G/C.	Han Chinese	308/238	Cytokine production	Lower sepsis morbidity rate	[77, 78]
		−511T/C (rs16944)	Han Chinese	308/238	Cytokine production	Higher sepsis morbidity rate	[77, 78]
			Caucasian	100			[79]
			Greek	183			[80]
		−31C/T (rs1143627)	Mixed Ethnic	159/228/149			[31, 40, 48]
			Han Chinese	308/238	Cytokine production	Higher sepsis morbidity rate	[77, 78]
		3953C/T (rs1143634)	Caucasian	100			[79]
			Unknown	97			[41]
IL-1RN	2q14.2	intron 2, VNTR	Greek	183			[80]
		rs315952	European	1002		Decreased risk of ARDS	[81]
IL-4	5q31	−589T/C (rs2243250)	Han Chinese	308	Higher plasma IL-4 and lower interferon-gamma production	Increased susceptibility of sepsis	[77, 82]
IL-6	7p21	−174G/C	Mixed Ethnic	68			[35]
			Mixed Ethnic	159/228/149			[31, 40, 48]
			African ancestry and European ancestry	474			[28]
			Caucasian	100			[79]
			Unknown	71			[83]
			Caucasian	57			[84]

(continued)

Table 3 (continued)

Gene	Chrome location	Variation	Population	Study size	Fuctional effects	Associated clinical phenotype	References
			Unknown	47			[85]
			Unknown	77		Increased mortality after acute severe TBI	[86]
		−572C/G (rs1800796)	Han Chinese	105/308	Reduced transcriptional activity of the IL-6 promoter, IL-6 production from leukocytes	Lower risk of sepsis	[42, 77]
			Unknown	47			[85]
		−597G/A (rs1800797)	Han Chinese	105			[42]
IL-8	4q13	−251A/T (rs4073)	Unknown	47			[85]
IL-10	1q31-32	−1082G/A (rs1800896)	Mixed Ethnic	68	Lower interleukin-10 production	Lower risk of sepsis	[35]
			Unknown	71	Lower interleukin-10 production		[83]
			Han Chinese	308	Lower lipopolysaccharide-induced IL-10 production	Higher sepsis morbidity rate and MOD score	[87]
			Caucasian	211	Lower interleukin-10 production	Higher severity of illness on admission, daily organ dysfunction scores and 60-day mortality	[88]
			Chinese	314		Higher morbidity rate of ARDS and 30-day mortality	[89]
			Unknown	119		Higher relative risk of MODS	[90]
		−819C/T (rs1800871)	Han Chinese	308	Lower serum levels of IL-10	Lower sepsis morbidity rate	[77]
			Mixed Ethnic	265	A trend for decreased levels of IL-10	A decreased risk for death	[91]
		−592C/A (rs1800872)	Unknown	119		Lower relative risk of MODS	[90]
			Mixed Ethnic	265		A decreased risk for death	[91]

(continued)

Table 3 (continued)

Gene	Chrome location	Variation	Population	Study size	Fuctional effects	Associated clinical phenotype	References
					A trend for decreased levels of IL-10		
TNFα	6p21.3	−308G/A (rs1800629)	Mixed Ethnic	159		Increased risk for severe sepsis	[31]
			Mixed Ethnic	228		Lower risk for severe sepsis	[40]
			Mixed Ethnic	69		Increased risk of mortality	[32]
			Han Chinese	308	Cytokine production	Increased sepsis morbidity rate and MOD scores	[77]
			Han Chinese	306	Increased TNFαproduction	Increased sepsis morbidity	[92]
			Unknown	159	Higher TNF-alpha serum concentrations	Increased sepsis morbidity and mortality rate	[33]
			Unknown	152		Increased sepsis morbidity and mortality rate	[34]
TNFβ	6p21.3	252T/C (rs909253)	Unknown	70	Higher cytokine-producing capacity	Increased severe sepsis morbidity	[36]
IFN-γ	12q14	874A/T	Mixed Ethnic	68	Lower IFN-γproducing	Lower sepsis morbidity in African American	[35]
			Han Chinese	308	Lower IFN-γproducing		[77]
		CA repeat	Unknown	61		Increased sepsis morbidity	[93]
HMGB1	13q12	2179C/G (rs2249825)	Chinese	556	Higher HMGB1 production	Increased sepsis morbidity and MOD score	[94]

Tumor necrosis factor alpha (TNFα) is a typical pro-inflammatory cytokine which has been widely studied. The relationship between TNFα/−308 variation and sepsis has also been reported extensively. It was found that TNFα/−308 was association with sepsis severities and outcomes of patients repeatedly. Those A allele carriers have a tendency towards increasing TNFα plasma levels and a stronger inflammatory response [31–34]. However, conflicting results were reported in other studies [35, 36]. Interleukin (IL)-1 is another kind of important pro-inflammatory cytokine, including isoforms α and β. A polymorphism (46 bp VNTR) in the intron 6 of IL-1α gene was described as having no association with sepsis [37]. However, we found that genetic variations in the IL-1β gene had a close relationship with worse outcomes in major trauma patients [38, 39]. In addition, researches focused on polymorphisms in other cytokine genes, including IL-6, IL-10, TNFα/β, MIF and IFN-γ (Table 1). IL-6 −174G/C variation was studied in six cohorts of trauma patients, three cohorts of burns patients, and a cohort of traumatic brain injury (TBI) patients. Only two out of these articles described an increased risk of sepsis with presence of the −174C allele [40, 41]. However, only −572C/G, instead of −174G/C was identified in the promoter of IL-6 gene in Chinese Han population. Patients carrying the IL-6 −572 CC genotype had significantly more sepsis morbidity than with a CG or GG genotype [42]. Three SNPs (rs1800896, rs1800871 and rs1800872) in IL-10 promoter have also been widely studied in trauma cohorts. However, conflicting results were reported. A meta analysis from our lab [43] didn't find a strong association between those three SNPs and sepsis morbidity. Subgroup analysis by ethnicity indicated −592C/A was association with sepsis susceptibility in Caucasians, while −1082A/G in Asians, indicating there is a racial difference.

During the process of sepsis, cytokine genes tend to form crosstalk, interact with each other. Therefore, we further investigated the synergetic effects of 13 SNPs in 9 cytokines Among them, eight SNPs, including IL-1β/−31, IL-1β/−511, IL-1β/−1470, IL-4/−589, IL-6/−572, IL-8/−251, IL-10/−819, and TNFα/−308 were found to be susceptibility loci for sepsis morbidity and organ dysfunction in severe trauma patients. Additionally, patients carrying more than four risk alleles of these eight SNPs had more than 50 % risk to develop sepsis and multiple organ dysfunction [44].

2.4 Vascular Endothelial Cells Function

Endothelial cells, a truly pervasive organ in human body, are highly active and alterative during the progress of sepsis [45]. Pathogens may directly infect intact endothelial cells in some cases. Endothelial cells can also be activated by components of the bacterial wall (e.g. LPS), as well as various host-derived factors, including cytokines, chemokines, complement body, serine protease, protein fiber, platelets activation, leukocytes, hyperglycemia, oxidation and blood flow changes [46]. Activated endothelial cells may undergo structural changes and functional

changes, involving a great number of genes. Therefore, the relationship between gene variations and outcomes of trauma patients was studied.

The interaction between inflammatory mediators and endothelial cells induces a net procoagulant phenotype, such as an increasing level of plasminogen-activator-inhibitor-1 (PAI-1). The insertion/deletion polymorphism in the promoter of PAI-1 gene(4G/5G) is investigated in a small severe trauma cohort by Menges et al. [47]. The PAI-1 4G allele was found to be associated with high plasma concentrations of PAI-1 and poor outcomes after severe trauma. Barber et al. [48] reported the similar results. Vascular endothelial growth factor A (VEGFA) encodes VEGF, a protein that is the most important member of the platelet-derived growth factor (PDGF)/vascular endothelial growth factor (VEGF) family. It acts on endothelial cells and has various effects, including inducing angiogenesis, vasculogenesis mediating increased vascular permeability and endothelial cell growth, inhibiting apoptosis and promoting cell migration. Meyer et al. [28] found that a set of SNPs in VEGFA gene (VEGFA block 1) was significantly associated with the morbidity of ALI in both African and European Ancestry trauma subjects. Angiopoietin-2, encoded by ANGPT2 gene, is expressed only in vascular remodeling. Two ANGPT2 polymorphisms, rs2442598 and rs1868554, were found to be strongly related to the plasma Angiopoietin-2 isoforms, as well as the morbidity of ALI in major trauma patients [49].

2.5 Acute-Phase Protein

Acute-phase proteins include two classes of proteins. One class is positive acute-phase protein whose plasma concentrations increase in response to inflammation. While the other class is negative acute-phase protein whose plasma concentrations decrease in response to inflammation. In response to injury, the liver produces a large number of acute-phase reactants. Their genetic variations have also been studied in trauma patients. Hildebrand et al. didn't find an association between SNPs in the calcitonin (CALCA) gene and systemic PCT levels or clinical outcomes of polytraumatized patients. Heat shock proteins (HSP) are released by cells when exposure to stressful conditions. High levels of heat shock proteins can be introduced by various kinds of environmental stress conditions, including trauma and inflammation. An association study in eighty major multiple trauma patients showed that HSPA1B AG and HSPA1L CT genotypes were significantly associated with increased plasma production levels of TNF-α and IL-6. HSPA1L CT genotype was also a significant risk factor of the development of liver failure [50]. However, Bowers et al. reported polymorphisms of HSP-70 (HSPA1B and HSPA1L loci) have no effect on infection morbidity or outcomes in critically ill patients after surgery [51]. The −144C/A loci in the promoter of HSP90beta gene was reported to be associated with higher expression of HSP90beta and low expression of TNF-alpha, as well as decreased MOD scores in a Chinese severe trauma cohort [52].

2.6 Other Genes

The mitochondrial genome (mtDNA) is the main source of oxygen-derived free radicals, also called as reactive oxygen species (ROS). ROS is an indispensable active substance for human beings. It can increase the activity of some enzymes, involve in the synthesis of some active substances such as prostaglandin. During inflammatory process, ROS can promote inflammatory cells phagocytosis and kill bacteria. However, the overabundance of ROS caused by oxidative stress reaction cause cell damage and result in patterns of secondary injury [53]. There are three association studies focusing on 4216T/C polymorphism of the NADH dehydrogenase 1 (ND1) gene conducted in burn and trauma cohort. However, conflicting results were reported. Canter et al. [54] showed that 4216T allele increased the in-hospital mortality after major injury. Trauma patients who carried 4216 T allele have 2.1 times more risk of death than C allele carriers. While Huebinger et al. [55] and Gomez et al. [56] both found that sepsis-related organ dysfunction and shock was significantly increased in burn and traumatic injury patients carrying 4216 C allele.

Micro RNA (miRNA) is a class of short single stranded endogenous non-coding RNA molecule (about 22 nucleotides) which participated in post-transcriptional regulation of gene expression function as RNA silencing [57]. It has been known that miRNAs can recognize their target mRNAs by seed sequence, 2–8 nucleotides at the 5′ end of the miRNA. Thus, a miRNA may have hundreds of mRNA targets. Meanwhile, a given target may be regulated by multiple miRNAs [58]. In recent years, growing evidence indicates that miRNA may play a major role in the pathogenesis of sepsis [58–60]. It has been reported that the miRNA expression profiles in both plasma and leukocytes are significantly different between sepsis patients and healthy controls [61]. There is also quite different between sepsis and nonsepsis systemic inflammatory response syndrome (SIRS) patients [62]. Recently, we conducted a systematic research of polymorphisms in pre-miRNA and their clinical relevance in major blunt trauma patients [63]. Nine SNPs were selected out from a total of 1048 human miRNAs and genotyped in three independent cohorts of severe trauma patients. Only one single SNP (miR-608 rs4919510) were identified to significantly increase the expression level of mature miR-608, as well as proinflammatory cytokines, such as TNF-α, IL-6 and IL-1β. Furthermore, patients carrying rs4919510 had a higher risk of developing sepsis and MODS in three independent study cohorts.

3 The Effect of Precision Medicine on Trauma Therapy

As the concept of precision medicine, it is encouraged to use accurate clinical information and evidence to appropriately manage a patient at an individual level or at a community level. Thus, clarifying the relationship between genetic background

and trauma can not only provide early warning diagnostic methods for traumatic complications, but also directly affect the clinical treatment of trauma patients.

An excessive immune inflammatory response and the imbalance of pro-inflammatory and anti-inflammatory factors are believed to be, at least partly, the underlying pathogenesis of complications after trauma. A group of cytokines, such as TNFα, TNFβ, IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10 and IFN-γ, were involved. The plasma levels of several cytokines have been reported to be associated with the course of disease and clinical outcome in trauma patients [64] and were thought to have potential therapeutic role in trauma patients. Injection of recombinant TNFα into human or animal can induce various symptoms of sepsis, suggesting that TNFα is a key factor in the pathogenesis of sepsis. Therefore, researches attempted to reduce the inflammatory response of sepsis patients using TNFα inhibitors [65]. Although the method has a certain effect on animal model, results from large-scale clinical trial showed that anti-cytokine therapy can not reduce, but even increase the mortality rate of sepsis patients [66]. However, the anti-TNFα therapy has been considered as an effective treatment in patients with arthritis [67] and inflammatory bowel disease [68]. The major challenges for anti-cytokine therapy maybe just how to choose the appropriate subjects.

Recent studies found that the individual TNFα expression levels induced by LPS stimulation among different healthy people may vary as much as 10 times. It is mainly determined by the genotype of two polymorphisms (−308 and −376) in the promoter region of TNFα gene. We can selected out the patients with potential high expression levels of TNFα on admission to hospital by just a simple genotyping. Thus, the anti-TNFα treatment may achieve success in clinical.

The traditional surgical procedures, including access, exposure, bleeding, resection, reconstruction, and drainage etc. Surgeons should strictly abide by the principles to achieve a perfect operation. However, in clinical practice, it is common that patient died due to the neglect of physiological state although underwent a successful surgery. It is well recognized now that multiple trauma patients have more possibility to die from their intra-operative metabolic failure rather than a failure operation. For example, patients with major injuries and shock will not survive if had a complex operation such as pancreaticoduodenectomy or formal hepatic resection. The surgeons should undergo a shift in their mind and aim at save patients first rather than complete a perfect operation. Therefore, Rotondo et al. [69] proposed the damage control operation (DCO) in 1993. Taking patients with massive hemorrhage as an example, coagulation dysfunction is the main cause of poor prognosis. Therefore, the surgeon should end the surgery as soon as possible, and transfer the patient to a critical care facility to restore his coagulation dysfunction. The deterministic surgery should be performed only when the patient achieve a stable physiological state. Pape et al. [70] conducted a multi-center clinical study in 2007. They observed 165 cases of major blunt trauma patients, and compared the morbidity rate of acute lung injury (ALI) between patients with an external fixator first, conversion to an intramedullary nail later and initial definitive stabilization of the femur shaft with an intramedullary nail. The results showed that in stable patients, primary femoral nailing could significantly decrease the

ventilation time. However, in borderline patients, those who underwent definitive stabilization at the first time had a higher incidence of lung dysfunctions. Based on that, the authors suggested that it is crucial to take the patient's preoperative condition into account when deciding on what type of initial fixation to perform. Thus, it is essential to find an early and accurate way to determine the response of trauma patients to certain treatment.

Researches on the relationship between gene polymorphisms and post traumatic response have made a great breakthrough in the recent years. It is possible to use genetic polymorphisms to evaluate the patients' response and predict the morbidity of complications. Selection an appropriate treatment or operation according to the patient's genetic background may completely change the current treatment of trauma patients, and bring the clinical treatment of trauma into the era of personalized medicine.

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Novel Inflammatory and Immunomodulatory Mediators in Sepsis

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Abstract Sepsis is a global problem with substantial morbidity, mortality, and health care expenditures in the U.S. and worldwide. Although we have improved understanding of the pathophysiology related to sepsis, rapid progress of research in this growing field requires a more nuanced approach to matching pathophysiology to therapeutic options against sepsis in a timely manner. Identification of novel pathophysiological events and the development of drugs by targeting novel inflammatory and immunomodulatory molecules have opened up different channels for attacking sepsis. Our current chapter encompasses a comprehensive, though by no means complete, summary of novel inflammatory and immunomodulatory mediators in sepsis via screening of current literature resources.

Keywords Sepsis · Inflammation · Cytokine · Chemokine · Macrophage · Neutrophil · Lymphocyte

Abbreviations

ICU	Intensive care unit
CARS	Compensatory anti-inflammatory response syndrome
HLA	Human leukocyte antigen
TNF	Tumor necrosis factor
LPS	Lipopolysaccharide
IL	Interleukin
MD2-TLR4	Myeloid differentiation factor 2-toll-like receptor 4
TGF	Transforming growth factor
SCID	Severe combined immunodeficiency
BCL-2	B cell lymphoma-2

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Bim	Bcl-2 interacting mediator of cell death
Puma	P53 upregulated modulator of apoptosis
IFN	Interferon
LFA	Lymphocyte function associated antigen
VLA	Very late antigen
NK	Natural killer
PD-L1	Programmed cell death receptor ligand-1
CTLA	Cytotoxic T lymphocyte associated protein
Th	T helper
CLP	Cecal ligation and puncture
MFG-E8	Milk fat globule-EGF-factor VIII
DCs	Dendritic cells
IL-22BP	IL-22 binding protein
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
MAPK	Mitogen-activated protein kinases
VCAM	Vascular endothelial cell adhesion molecule
AP-1	Activator protein-1
IL-1RAcP	IL-1 receptor accessory protein
IL-1Rrp2	IL-1 receptor related protein-2
GM-CSF	Granulocyte-macrophage-colony-stimulating factor
sTREM-1	Soluble triggering receptor expressed on myeloid cells-1
I/R	Ischemia reperfusion
OPN	Osteopontin
BSP-I	Bone sialoprotein-I
ETA-1	Early T lymphocyte activation-1
SPP-1	Secreted phosphoprotein-1
ECM	Extracellular matrix
ALI	Acute lung injury
PD-1	Programmed death-1
APCs	Antigen presenting cells
BTLA	B and T lymphocyte attenuator
GRAIL	Gene related to anergy in lymphocytes
DAMP	Damage-associated molecular patterns
HMGB1	High mobility group box 1
RAGE	Receptor for advanced glycation end-products
CIRP	Cold-inducible RNA-binding protein
S1P	Sphingosine-1-phosphate
LXs	Lipoxins
ICAM-1	Intercellular adhesion molecule-1
PBEF	Pre-B cell colony-enhancing factor
GHSR	Growth hormone secretagogue receptor
AM	Adrenomedullin
AMBP-1	AM binding protein-1
ET-1	Endothelin-1

PS	Phosphatidylserine
MPO	Myeloperoxidase
MSP68	MFG-E8-derived short peptides 68

1 Introduction

Sepsis is a pervasive medical syndrome that continues to be a leading cause of death. It is a syndrome characterized by systemic inflammatory response to invading pathogens leading to derangements in vital signs and leukocyte count [1]. The term "sepsis" is accredited to Hippocrates (ca. 460–370 BC), who claimed that sepsis was the process by which flesh rots [2, 3]. With the formation of germ theory in the 1800s, sepsis was understood as "blood poisoning" by the invasion of pathogenic organisms into the bloodstream [3, 4]. However, patients with sepsis continued to die despite treatment with antibiotics, suggesting that sepsis could not fully be explained by microbial invasion [3]. This finally led to the understanding that the pathogenesis of sepsis involved the host response to the pathogens. We now understand that the response to sepsis is mediated by the release of various inflammatory mediators, which can lead to severe sepsis with the presence of organ dysfunction, shock with the presence of hypotension despite resuscitation, and eventually death [5].

The heterogenous nature of sepsis has led to myriad definitions and clinical criteria that complicate epidemiological and clinical trials. Most recently, a task force was convened by the Society of Critical Care Medicine and the European Society of Intensive Care Medicine to revise the definition of sepsis. They concluded that sepsis should be defined as "life-threatening organ dysfunction caused by a dysregulated host response to infection" [6]. A Sequential (Sepsis-related) Organ Failure Assessment (SOFA) identifies organ dysfunction and a score of ≥2 points is associated with in-hospital mortality greater than 10 % [6]. Furthermore, sepsis progresses to septic shock when profound circulatory, cellular, and metabolic abnormalities develop [6]. These updated definitions can facilitate earlier recognition and management of patients with sepsis.

Despite varied definitions of sepsis as mentioned, the incidence of sepsis is approximately 19 million cases globally [7]. In the United States alone, there are approximately 1 million cases annually, with mortality generally quoted between 20–40 % [8, 9]. Treatment for sepsis, unlike other epidemic illnesses, is largely supportive [10]. In the United States, over $24 billion are spent on managing sepsis, an amount that has increased over time, but is only reflected in a modest improvement in mortality [11]. A large multicenter, multinational audit of critical illness in ICUs showed a stepwise increase in the adjusted risk of in-hospital death with decreasing global national income in sepsis patients [8]. Yet the amount of money that is spent on treating sick patients still leads to over 200,000 deaths in the

US every year [8]. Those who survive often suffer from significant morbidity, and continue to be at risk for early death [10].

The natural evolution of sepsis contains different stages. It was first believed that the initial hyper-inflammatory, cytokine storm phase of sepsis is followed by a compensatory anti-inflammatory response syndrome (CARS) [3, 12, 13]. These were believed to be distinct, with experimental therapies mostly targeting pro-inflammatory mediators in the former phase, in which patients exhibit clear signs like fever or an elevated heart rate [14]. The anti-inflammatory stage, however, is more difficult to detect, and can occur in patients that appear immunocompetent [15]. Hematological parameters like a complete blood count or white blood cell differential do not necessarily indicate immunosuppression; instead, the degree of anti-inflammatory response can be quantified through tests that measure monocyte human leukocyte antigen (HLA)-DR expression, or measure levels of tumor necrosis factor (TNF)-α after ex vivo stimulation of whole blood with lipopolysaccharide (LPS) [15]. Critically ill patients who have low levels of HLA-DR monocytes or TNF-α are at higher risk for the development of nosocomial infection and death [15].

More recently, the concept of sepsis has evolved to a more nuanced view [16]. Studies have shown that both pro-inflammatory and anti-inflammatory responses occur early and simultaneously, and it is the net effect of the hyperinflammatory phase that is seen first in most patients [17–19]. The length of this initial phase varies with factors like patient co-morbidities, nutritional status, and extent of injury [16]. Mortality can occur early from the overwhelming hyperinflammation, or later, when immunosuppression dominates [16].

Attempts to find a treatment for sepsis focused mainly on targeting proinflammatory mediators. Anti-TNF antibodies were shown in baboons infected with live *E. coli* prevented the development of acutely lethal septic shock despite bacteremia, showing that TNF-mediated acute shock and tissue injury [20]. Unfortunately, clinical trials using antibodies and other agents to block TNF in patients with severe sepsis failed to improve mortality [10]. Blockade of another pro-inflammatory cytokine, interleukin (IL)-1 using a receptor antagonist also failed to demonstrate a significant reduction in mortality compared with standard therapy [21, 22]. A randomized, double-blinded, multinational study with a synthetic lipid A antagonist that blocks LPS from binding toll-like receptor 4-myeloid differentiation factor 2 (TLR4-MD2) was unable to show any difference in 28-day mortality among patients with severe sepsis, compared to placebo [23].

Finally, drotrecogin alfa (activated), or recombinant activated protein C, was one drug that was able to pass clinical trials to obtain FDA approval [24]. In the PROWESS trial, patients with severe sepsis had a significant reduction in 28-day mortality [24]. However, to assess efficacy in different populations of patients, additional studies were conducted. Several named follow-up studies were performed that showed conflicting data in patients that were less critically ill, or in pediatric populations [25–27]. This led to the PROWESS-SHOCK trial, which randomized over 1600 patients to treatment or placebo, and the study found no

difference in survival. Drotrecogin alfa was voluntarily withdrawn from the market, and the FDA withdrew its approval in the United States.

Overall, there have been more than hundreds of millions of dollars spent on potential sepsis treatments, and over 100 Phase III clinical trials performed in septic humans, but none of them have proven to be effective [28]. While our understanding of sepsis is evolving as more research is conducted, it remains an area of great challenge. Considerable progress has been reported delineating the pathophysiological role of the most commonly encountered pro- and anti-inflammatory cytokines in sepsis, which include TNF-α, IL-1β, IL-6, IL-8, IL-10, and transforming growth factor (TGF)-β. Beyond these factors, studies have revealed many novel mediators of inflammation and immunomodulation which have an immense role in influencing sepsis pathophysiology, but are comparatively less well-known. Our current chapter provides an inclusive overview of these novel mediators which not only shed more light on the complex pathophysiology of sepsis, but also provide information to develop effective targeting strategies for the treatment of sepsis.

2 Cytokines, Soluble Membrane Markers, Extracellular Phospho-Proteins

IL-3 IL-3 is a pleiotropic cytokine which plays an essential role for the differentiation of pluripotent hematopeotic stem cells into myeloid progenitor cells [29]. In an animal model of sepsis, IL-3 knockout mice showed lower mortality compared to WT mice, and had overall improved clinical scores, body temperatures, and blood pressure [29]. The WT mice had an accumulation of monocytes and neutrophils in the lungs and livers indicating higher organ injury [29]. In human studies, detectable levels of IL-3 correlated with the level of circulating monocytes in septic patients, and higher levels of IL-3 conferred poorer prognosis [29]. Inhibition of this cytokine is therefore able to indirectly decrease the amount of pro-inflammatory cytokines and reduce septic injury.

IL-7 IL-7 is an immunostimulatory cytokine required for T lymphocyte development, homeostasis, and maintenance [14]. Humans who have mutations in the IL-7 gene lack T cells and have severe combined immunodeficiency (SCID) [14, 30]. In patients with sepsis, there is apoptosis-induced cell loss which contributes to immunosuppression [31]. IL-7 counteracts this by modulating the expression of pro- and anti-apoptotic members of the B cell lymphoma 2 (BCL-2) family, such as Bcl-2, Bcl-2 interacting mediator of cell death (Bim), and p53 upregulated modulator of apoptosis (Puma) [32]. IL-7 also reverses sepsis-induced depression of T cell cytokines like interferon (IFN)-γ, which activates macrophages [14]. Additionally, IL-7 aids lymphocyte trafficking by increasing cell adhesion molecule expression via upregulation of lymphocyte function associated antigen (LFA)-1 and very late antigen (VLA)-4 [33]. Finally, IL-7 increases T cell receptor diversity that

leads to a broadened response against foreign pathogens [34]. As such, IL-7 is being studied for use particularly for viral infections such as HIV and hepatitis C, and for cancer [35].

IL-15 Closely related to IL-7 is IL-15, another pleuripotent cytokine that supports homeostasis, activation, and proliferation of B and T cells [14]. In contrast to IL-7, IL-15 is also essential for natural killer (NK) cell development, survival, and cell function [14]. The broad role that IL-15 has on different parts of the immune system makes IL-15 very promising as an immunotherapy agent in sepsis. Like IL-7, IL-15 treatment of septic mice decreased apoptosis of immune cells by increasing Bcl-2 expression, showing improved overall survival [36]. Against cancer, IL-15 has also shown antitumor activity in preclinical mouse tumor models [37]. In combination with blockade of other immune system checkpoints like anti-programmed cell death receptor ligand-1 (PD-L1) or anti-cytotoxic T-lymphocyte associated protein (CTLA)-4 antibodies, to be discussed later, antitumor activity was enhanced in a murine model of colon carcinoma [38].

IL-17 IL-17, a pro-inflammatory cytokine, whose members includes IL-17A, -B, -C, -D, -E, and -F [39]. IL-17 is mainly produced from T helper (Th) 17 cells, yet it is also produced by other innate and adaptive immune cells which include neutrophils, lymphocytes, inducible natural killer T cells (iNK T cells), γδ T cells, and Paneth cells [39–41]. IL-17A promotes inflammation and injuries in tissues, and is known to interact predominantly with endothelial cells, epithelial cells, fibroblasts, and macrophages through binding its receptor, IL-17R thereby producing pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 [39]. IL-17 is involved in a wide range of cellular events that include bacterial defense, rheumatoid arthritis, graft rejection, tumor modulation, and asthma and allergic reactions [42]. Although it is in these latter autoimmune processes that IL-17 has been more widely studied, IL-17 has recently attracted attention as a regulator of innate immunity in host defense. In a murine model of sepsis using cecal ligation and puncture (CLP), IL-17 was shown to promote high levels of pro-inflammatory mediators and bacteremia [42]. Levels of IL-17 increased in a time-dependent manner after CLP, and in vitro incubation of macrophages with LPS and IL-17 increased production of TNF-α, IL-1β, and IL-6 [42]. Furthermore, targeting of IL-17 with neutralizing antibodies showed a protective outcome, with reduced bacteremia and increased survival [42]. Additionally, neutralization of peritoneal IL-17 after CLP-induced sepsis resulted in markedly improved neutrophil infiltration and decreased levels of pro-inflammatory cytokine production [43]. Thus, the regulation of IL-17 expression could be beneficial in controlling inflammatory diseases. It has recently been reported that the administration of a homeostatic growth factor, milk fat globule-EGF-factor VIII (MFG-E8) during the time of CLP-mediated sepsis significantly improved the disease phenotypes [41]. Collectively, these finding reveals IL-17 to be an outstanding therapeutic target in sepsis.

IL-22 The cytokine IL-22 is a member of the IL-10 superfamily (IL-19, IL-20, IL-24, and IL-26) which functions in intracellular signaling [44]. IL-22 is produced

by activated dendritic cells (DCs) and T cells, and it plays a significant role in responding against bacterial pathogens, particularly in epithelial cells located in the pulmonary and intestinal mucosa [45]. In patients with sepsis, IL-22 levels were modestly elevated in the serum, likely contributing to host defense by stabilizing the mucosal barrier during infection [46]. Adverse effects, however, have also been reported in a model of polymicrobial peritonitis [47]. There is an IL-22 binding protein (IL-22BP) that modulates IL-22 activity. Treatment of mice with IL-22BP prior to sepsis led to higher infiltration of neutrophils and mononuclear phagocytes resulting in a reduced bacterial load at the site of infection [47]. Like the pathogenesis of sepsis, the role of IL-22 is complex with both pro- and anti-inflammatory components, and its duality may make it a good candidate for sepsis treatment.

IL-27 IL-27 is a cytokine produced by antigen-presenting cells upon exposure to pathogenic molecules and inflammatory mediators [48, 49]. It is believed to play a role in immunosuppression, which can lead to late deaths in sepsis due to secondary infections. In one recent study, IL-27 was shown to be upregulated after an animal model of sepsis, and IL-27R KO and IL-27-neutralized mice showed improved survival, with enhanced pulmonary neutrophil recruitment [50]. They were more able to clear bacteria from the lungs of septic mice from *P. aeruginosa* infection, and addition of recombinant IL-27 led to increased susceptibility to infection [50]. Studies of parasitic infections in IL-27-deficient mice all exhibited enhanced pro-inflammatory responses to control parasitic replication [51]. Mechanistically, IL-27 has been shown to induce T cells production of anti-inflammatory cytokine IL-10 [52]. In human studies, IL-27 may serve as a biomarker for risk of bacterial infection in critically ill pediatric patients with systemic infections [49]. IL-27 may be an important target to reduce the immunosuppressive phase of sepsis in which people are susceptible to secondary infections.

IL-33 IL-33 is the newest member of the IL-1 cytokine family. It is expressed in structural and lining cells, including fibroblastic reticular cells of lymphoid tissues, and epithelial cells [53]. At baseline, IL-33 localizes to the nucleus, but after exposure to LPS, IL-33 is released to the extracellular space [54]. IL-33 binds to its receptors, ST2 and IL-1R accessory protein, which are expressed on the surface of Th2 cells and mast cells, and this drives production of IL-5 and IL-13 via activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinases (MAPK) pathways [55]. On mast cells, IL-33 triggers the production and release of pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6, promotes maturation, and induces degranulation [56]. One of the receptors, ST2, can be spliced to either a localized form bound to the cellular membrane or a soluble form. This soluble variant, sST2, can act as a decoy receptor by binding IL-33, but does not induce signaling [57]. High levels of sST2 are associated with poor prognosis in sepsis [58]. Thus, the pro-inflammatory role of IL-33 seems to be beneficial in the sepsis response, and function therapeutically to prevent mortality during the immunosuppressive phase of sepsis.

IL-35 IL-35 is a newly described cytokine in the IL-12 family, which includes IL-27, an immunosuppressive cytokine discussed earlier [59]. IL-35 has been shown to be induced in the plasma of mice after LPS injection, and in the plasma of sepsis patients [59]. In addition, IL-35 decreased LPS-induced pro-inflammatory cytokines and chemokines in the plasma of mice. Levels of IL-35 in serum from septic adult or child patients were significantly higher than in healthy control, and its levels increased with severity of sepsis [60]. Mechanistically, IL-35 can inhibit LPS-induced upregulation of vascular endothelial cell adhesion molecule (VCAM)-1 via inhibition of the MAPK-mediated activator protein-1 (AP-1) signaling pathway [59]. Although IL-35 is known to be anti-inflammatory, administration of anti-IL-35 antibodies in murine sepsis significantly diminished bacterial dissemination, which was accompanied by enhanced local neutrophil recruitment and early increased release of inflammatory cytokines and chemokines [60]. Therefore, IL-35 facilitates bacterial dissemination in polymicrobial sepsis. These studies show that IL-35 plays a major role in the pathogenesis of sepsis by compromising innate immune function, though further studies of its role in early versus late phase sepsis may be valuable to delineate effects of IL-35 neutralization.

IL-36 IL-36 is a cytokine that predominantly acts on naive CD4 T cells via its receptor which is a heterodimer of IL-1 receptor accessory protein (IL-1RAcP) and IL-1 receptor related protein-2 (IL-1Rrp2) [61, 62]. After binding its receptor, IL-36 activates NF-κB and MAP kinases to play a role in inflammation [61, 62]. IL-36 has also been found to activate T cell proliferation and release IL-2 [63]; thus, it may directly be involved in maintaining the functions of the innate and adaptive immune system. Although the direct role of IL-36 in sepsis is still under investigation, a recent study reports that an IL-36 receptor antagonist can ameliorate inflammation [64]. Furthermore, a new cytokine, IL-1F10 (IL-38) has been shown to be capable of inhibiting IL-36 by analogous action [65]. Thus, the growing body of evidence in this new innate immune field is promising in understanding sepsis pathophysiology and developing potential therapeutic interventions.

IL-37 IL-37, formerly IL-1 family member 7, is a protein that is encoded in humans by the IL1F7 gene, and functions to downregulate inflammation [66]. Expression of IL-37 in macrophages or epithelial cells almost completely suppresses production of pro-inflammatory cytokines, while silencing of endogenous IL-37 leads to increased cytokine production in human blood cells [67]. IL-37 transgenic mice were protected from endotoxin-induced shock, and showed remarkable improvement of lung and kidney function, and reduced liver damage after treatment with endotoxin [67]. Transgenic mice containing the human IL-37 gene had significantly reduced levels of systemic and tissue cytokines compared to wild-type mice by reducing dendritic cell activation [67]. IL-37 interacts with the transcription factor, Smad3, and under Smad3-deficient conditions, neither IL-37-expressing cells nor transgenic mice were able to show suppression of cytokine expression [67]; thus IL-37 emerges as a natural suppressor of innate inflammatory and immune responses.

GM-CSF Granulocyte-macrophage-colony-stimulating factor (GM-CSF), a 23-kD growth factor, exhibits potent immunostimulatory effects on a variety of innate immune cells [68]. It promotes host defense against invading pathogens by improving survival, proliferation, differentiation, phagocytosis, and bacterial killing of neutrophils and monocytes/macrophages [68]. GM-CSF has been shown to increase HLA-DR expression and endotoxin-induced pro-inflammatory cytokine production in whole blood cultures of patients with severe sepsis under an ex vivo condition [69]. In order to define its role in sepsis, it has been demonstrated that GM-CSF secreted from a distinct type of B lymphocyte protects mice against polymicrobial sepsis [70]. Additionally, a recent clinical trial reported that administration of GM-CSF successfully reversed long-lasting monocyte deactivation (anergy) in sepsis [71]. In a multicenter, prospective, randomized, double-blind, placebo-controlled trial examining patients with severe sepsis or septic shock and sepsis-associated immunosuppression, GM-CSF has been shown to be protective by shortening the time of mechanical ventilation and hospital stay [72]. Thus, GM-CSF serves as a safe and effective therapeutic potential for restoring monocytic functions in sepsis.

sTREM Soluble triggering receptor expressed on myeloid cells 1 (sTREM-1) is a member of the Ig superfamily, originally described as a receptor expressed on the surface of mononuclear cells such as, macrophages and neutrophils [5]. Recent studies reported that sTREM-1 is increased in the plasma of septic patients and in the bronchial aspirates of patients diagnosed with chronic obstructive pulmonary disorder (COPD) [73]. High levels of sTREM-1 were also noted in the plasma of patients after surgical and ischemia reperfusion (I/R) stress [74]. After LPS injection in healthy subjects, plasma sTREM-1 levels increase, such that sTREM-1 may be a useful marker or predictor for bacteremia [75]. In a murine model of endotoxin-induced septic shock [76], and in *Pseudomonas aeruginosa*-induced sepsis [77], blockade of sTREM-1 signaling protects from exaggerated inflammation and improves survival. sTREM-1 can function as a diagnostic marker, as well as effective therapeutic target in attenuating sepsis.

OPN Osteopontin (OPN), a secreted glycophosphoprotein also known as bone sialoprotein-I (BSP-I), early T lymphocyte activation-1 (ETA-1), or secreted phosphoprotein-1 (SPP-1), is found in the extracellular matrix (ECM) and recognizes integrins to promote its physiological function [78]. OPN is mainly produced by immune cells, osteoblasts, and tumor cells [78]. OPN has a highly conserved N-terminal Arg-Gly-Asp (RGD) which binds to $\alpha_v\beta_3$-integrin [79]. OPN is a component of the ECM and can be secreted as a soluble cytokine into bodily fluids, like blood, urine, and breastmilk [78]. Pro-inflammatory cytokines such as TNF-α, IL-1β, IFN-γ, and IL-6 induce OPN production in macrophages [78]. OPN is known to promote macrophage function by enhancing their migration, survival, phagocytosis, and pro-inflammatory cytokine production [80]. Additionally, OPN knockout mouse neutrophils display reduced chemotaxis toward chemokines, suggesting OPN is important for the infiltration of neutrophils to the site of inflammation [81]. OPN expression in T cells has been shown to be controlled by

Tbet, a transcription factor that promotes Th1 cell lineage commitment, which promotes cell-mediated immunity and phagocyte-dependent inflammation [82]. The expression of OPN has been demonstrated to become induced in a wide range of chronic inflammatory diseases like cancer, Crohn's disease, autoimmune diseases, and atherosclerosis and may act as a pro-inflammatory cytokine to exaggerate inflammation and tissue damage [78, 83, 84]. Aside from chronic inflammatory diseases, OPN has also been found to be upregulated in acute inflammation. Vaschetto et al. [85] reported increased levels of plasma OPN in patients with sepsis. In an animal model of sepsis, mice deficient in OPN have been shown to have reduced levels of plasma cytokines and chemokines [86]. Furthermore, migration of neutrophils into the lungs to cause acute lung injury (ALI) was diminished by neutralization of OPN with an anti-OPN antibody [87]. These findings implicate OPN as a novel mediator whose blockade show potential in sepsis treatment.

3 Co-inhibitory Cell Surface Molecules

PD-1 Programmed death-1 (PD-1) is a co-inhibitory receptor molecule that is expressed on T and B lymphocytes [14]. Its ligand, PD-L1 is expressed by epithelial, endothelial, and antigen presenting cells (APCs) such as monocytes, macrophages, and dendritic cells [88]. PD-1/PD-L1 binding results in an inhibitory signal for T cells and negatively regulates their activation [14]. Inhibition of PD-1 and PD-L1 has been successful as immunotherapy in clinical trials against cancer, and this has led to a similar effort to combat sepsis-related immune suppression [89].

In a model of sepsis, PD-1 deficient animals showed reduced mortality as compared to wild-type mice, with less bacterial burden and a lower inflammatory cytokine response [90]. Post-treatment with an anti PD-1 neutralizing antibody led to similar results [91]. In a model of a secondary fungal infection after primary sub-lethal sepsis, anti-PD-1 and anti-PD-L1 therapy in animals also led to a survival benefit [92]. In human studies of septic shock and trauma patients, increased levels of PD-1 and PD-L1 have been observed in monocyte and T lymphocyte populations [93], which suggests that PD-1 may be an indicator of immune suppression, and be a target for therapeutic intervention.

BTLA Like PD-1, the co-inhibitory protein B and T lymphocyte attenuator (BTLA) has been studied in infection and sepsis. Knockout studies have shown that infected BTLA-deficient mice were depleted of $CD4^+$ cells, and were moderately protected from parasitemia in the early stages of infection [94]. They also found that in *Listeria monocytogenes* infection, BTLA contributes to increased infection leading to higher pro-inflammatory cytokine release [95]. In a model of sepsis, BTLA-deficient animals had improved mortality, while WT animals had higher levels of inflammatory cells [96]. Blockade with anti-BTLA antibody in animals

with LPS-induced endotoxin shock have improved survival [97]. Studies of septic humans showed that increased BTLA expression on $CD4^+$ T cells correlated with higher levels of nosocomial infections and longer length of hospital stays [96]. The immune suppression mediated by BTLA is therefore associated with poorer outcomes in septic patients, and its inhibition may be a useful treatment for sepsis.

CTLA-4 T lymphocytes express CTLA-4, also known as CD152 on their surface which competes for binding to co-stimulatory molecules on antigen presenting cells [14]. CTLA-4 binding prevents T cell proliferation, expansion, and activation [14]. Activation of CTLA-4 has been used in clinical trials to help regulate hyper activation of T cell functions minimizing transplant rejection and ameliorating symptoms of rheumatoid arthritis [98]. On the other hand, inhibition of CTLA-4 has been successfully used to reduce immune tolerance of cancer in patients with metastatic melanoma who failed previous therapies [99]. In sepsis, inhibition of CTLA-4 led to reduced apoptosis and improved survival of secondary fungal infections in septic mice [98, 100].

GRAIL The reduction in cell population and functionality of CD4 T cells is one of the characteristic features of developing immunosuppression during sepsis. Gene related to anergy in lymphocytes (GRAIL), also known as ring finger protein-128, is a novel E3 ubiquitin-protein ligase that induces and maintains anergy in CD4 T cells [101]. It has been reported from a recent study that the expression of this ring finger anergy-inducing protein was dramatically upregulated in lymphoid organs in a murine model of sepsis, which may lead to an antigenic unresponsiveness to CD4 T cells in terms of proliferation during sepsis [102]. Thus, inhibition of GRAIL may reduce impairment of CD4 T cell proliferation, and attenuate sepsis immunosuppression.

4 Damage-Associated Molecular Patterns (DAMP)

HMGB1 High mobility group box 1 (HMGB1), a highly conserved DNA binding protein that is necessary to maintain nucleosome structure and regulate gene transcription, is also a potent pro-inflammatory mediators in sepsis [103]. It is constitutively expressed in cells and localized to the nucleus, but secreted extracellularly from activated macrophages, NK cells, dendritic cells, endothelial cells, neurons, smooth-muscle cells, osteoclasts, and intestinal epithelial cells during inflammation [104, 105]. Apart from active release into the extracellular milieu, HMGB1 can also be passively secreted from cells undergoing necrosis [103]. HMGB1 interacts with different cell surface receptors, such as receptor for advanced glycation end-products (RAGE), TLR-2, TLR-4, and syndecan, to initiate cellular responses [105]. In macrophages and neutrophils, HMGB1 can dose–dependently upregulate TNF-α, IL-1β, and IL-8 [106]. Endothelial cells treated with HMGB1 can induce the release of chemokines and cytokines and up-regulate the expression of cell

adhesion molecules which promote leukocyte adhesion and migration in the inflammatory response [104, 105]. HMGB1 increases permeability of epithelial cell monolayers in a time- and dose–dependent manner, implicating HMGB1 to serve as a mediator of epithelial barrier dysfunction [107]. HMGB1 antagonists are protective against lethal sepsis. Nicotine and acetylcholine can inhibit LPS- or TNF-α-induced HMGB1 release by inhibiting the NF-κB pathway [108]. A survival benefit in septic mice was shown with administration of ethyl pyruvate which significantly decreased serum HMGB1 levels [109]. Additionally, other compounds such as oleanolic acid, edaravone, epigallocatechin-3-gallate have been found to be effective in reducing HMGB1 and prolonging survival in septic mice [110–112]. These results suggest that antagonism of HMGB1 may be a strong therapeutic approach to sepsis.

CIRP Cold-inducible RNA-binding protein (CIRP) is a damage-associated molecular pattern discovered to be upregulated in various organs in animal models of hemorrhage and sepsis [113]. CIRP belongs to the family of cold shock proteins that respond to hypothermic stresses, and it functions as an RNA chaperone to facilitate translation [114]. Under homeostatic conditions, CIRP is constitutively expressed at low levels, but is rapidly upregulated during hypothermia, as well as exposure to UV irradiation and hypoxia [113]. During hemorrhagic and septic shock CIRP is found to be actively released into the extracellular space, where it functions as an endogenous pro-inflammatory mediator causing further deleterious effects [113]. Studies have shown that the activity of extracellular CIRP is mediated through the TLR4-MD2 complex [113]. Administering recombinant CIRP stimulates TNF-α and HMGB1 release in macrophages, and it induces inflammation and tissue injury. Blockade of CIRP with neutralizing antibodies reduced levels of inflammatory cytokines and tissue injury, and also conferred a survival benefit [113]. In human studies, an elevated plasma level of CIRP was strongly associated with poor outcome among patients with sepsis [115]. Inhibition of CIRP may be useful as a target to reduce inflammation and tissue injury in sepsis.

5 Lipid Mediators

S1P/Sphk1 Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid metabolite that regulates several cellular events, such as cell growth, survival, differentiation, lymphocyte trafficking, vascular integrity, and pro-inflammatory cytokine production [116]. S1P is formed by phosphorylation of sphingosine, a backbone component of all sphingolipids, by Sphk1, an enzyme activated by inflammatory signals including LPS and cytokines [116]. Deletion or inhibition of Sphk1 demonstrated protection against CLP-induced sepsis in mice [117]. Puneet et al. [117] showed that Sphk1 expression is up-regulated in peritoneal macrophages from severe sepsis patients, and that administration of a Sphk1 inhibitor suppressed inflammatory

cytokine production. Inhibition of Sphk1 with 5c, a selective inhibitor of Sphk1, exhibited a protective role against lethality in a murine model of polymicrobial sepsis by enhancing bacterial clearance [117]. Addition of broad spectrum antibiotics to the administration of 5c greatly improved its efficacy in protecting infection [117]. S1P, via inhibition of its activating enzyme, Sphk1, may be an important target against the inflammatory injury in sepsis.

Resolvin/Lipoxin Resolvins are a group of endogenous lipid mediators generated during the resolution phase of acute inflammation from ω–3 polyunsaturated fatty acids to resolve hyperinflammation [118]. Resolvins RvD1 and RvE1, derived from DHA and eicosapentaenoic acid, respectively, are known to reduce inflammatory and postoperative pain when administered peripherally or centrally [119]. In sepsis, RvD2, a novel resolvin, acts as a potent regulator of leukocytes by modulation of endothelial-dependent NO production and by direct downregulation of leukocyte adhesion receptor expression in sepsis [120]. In CLP mice, RvD2 decreased excessive bacterial load, pro-inflammatory cytokine production, and neutrophil recruitment, and increased phagocytic functions of peritoneal macrophages [120]. Lipoxins (LXs) are an analogous class of endogenously produced eicosanoids that are derived enzymatically from arachidonic acid, an ω-6 fatty acid [121]. LXA is a lipoxin that inhibits chemotaxis, transmigration, superoxide generation, and NF-κB activation [121]. Based on the inflammatory resolution properties of LXA, Walker et al. [122] showed that LXA treatment in sepsis animals reduced systemic inflammation and NF-κB activation without compromising host defense. Additionally, LXA has been found to reduce blood bacterial load by enhancing macrophage recruitment [122]. Both resolvins and lipoxins have been shown to improve survival in septic animals and may be useful anti-inflammatory agents against sepsis.

Resistin Resistin is an adipose tissue–derived secretory protein initially known to play a role in insulin resistance, but has been identified as a pro-inflammatory cytokine [123]. In mammals, resistin is also secreted from macrophages and epithelial cells [123]. In a hospital–based study, Sundén-Cullberg et al. [124]. reported increased resistin in the serum of severe sepsis and septic shock patients, which was correlated to serum levels of TNF-α, IL-6, IL-8, IL-10, and procalcitonin, and associated with decreased overall survival. In vitro stimulation of macrophages with LPS or HMGB1 caused significant upregulation of the expression of resistin [124]. Administration of recombinant resistin up-regulated intercellular adhesion molecule (ICAM)-1 on monocytes and promoted monocyte trafficking [124]. Resistin may thus be an effective target in resisting sepsis.

6 Adipocytokines

Adiponectin Adiponectin is a novel adipocytokine that can exert several anti-inflammatory responses in macrophages and endothelial cells [125]. Treatment of cultured macrophages with adiponectin significantly inhibits LPS-induced production of TNF-α [126]. In murine polymicrobial sepsis, exogenous administration of adiponectin reduces mortality and plays an anti-inflammatory role through inhibiting HMGB1 [127]. In another study, administration of exogenous adiponectin to the peritoneum in abdominal sepsis increased survival and decreased intra-abdominal adhesions by decreasing TNF-α and IL-6 production [128]. Additionally, recent studies have demonstrated that adiponectin also induces various anti-inflammatory cytokines, such as IL-10R and IL-1R antagonists [129]. These anti-inflammatory properties of endogenous adiponectin make it a therapeutic option in sepsis.

Visfatin Visfatin, also known as pre-B cell colony-enhancing factor (PBEF), is a new adipocytokine, with a role in modulating the innate-immune system [5]. It has been shown that recombinant visfatin activates human leukocytes to release IL-1β, TNF-α, and IL-6, and increases the surface expression of costimulatory molecules CD-54, CD-40, and CD-80 via MAPK pathways [130, 131]. Visfatin expression is upregulated in sepsis, and facilitates prolonged inflammation by inhibiting neutrophil apoptosis [132]. Elevated levels of visfatin have also been shown to be a potential diagnostic marker for neonatal sepsis [133].

7 Vasoactive Peptides

Ghrelin Ghrelin is a vasoactive peptide secreted from the stomach that plays an essential role in stimulating growth-hormone release [134]. During sepsis, serum levels of ghrelin were shown to be reduced, while exogenously administered ghrelin improved survival in sepsis [135]. Ghrelin suppresses inflammation in sepsis by modulating peripheral and central sympathetic nerve activity through its receptor, growth hormone secretagogue receptor (GHSR) in the brain [136]. This requires that ghrelin cross the blood-brain barrier, which is normally difficult, but the systemic inflammation of sepsis disrupts the blood-brain barrier [136]. Studies have also demonstrated its functions to be mediated by vagus nerve stimulation [135, 136]. Through sympatho-inhibition and suppression of inflammation, ghrelin rescued animals from sepsis–associated multi-organ dysfunction by attenuating lung injury and intestinal barrier disruption [135, 136]. In an animal model of radiation and sepsis, ghrelin decreased levels of pro-inflammatory cytokines and improved survival [137, 138].

AM/AMBP1 Adrenomedullin (AM) is a member of the calcitonin gene-related peptide family of proteins [139]. It is an evolutionarily conserved neuropeptide that

was first isolated from human pheochromocytoma cells and later found to be widely distributed throughout the mammalian tissues [139]. Elevated plasma AM levels were reported in patients with sepsis [140] and following major surgery [141]. The small intestine is a major source of AM during CLP-induced polymicrobial sepsis in rats, which correlates with the increased portal blood flow observed in the early stage of sepsis [142]. Inflammatory cytokines, particularly IFN-γ, have been recognized as positive regulators of the AM binding protein (AMBP-1) expression, while oxidative stress and NF-κB–sensitive microRNA-146a have been shown to down-regulate AMBP-1 [143, 144]. In an in vitro study, administration of AM or AMBP-1 alone only moderately reduced LPS-induced TNF-α production in Kupffer cells, though AM and AMBP-1 given in combination, dramatically down-regulated TNF-α production [145]. AM also has the potential to inhibit neutrophil activation and migration to sites of inflammation by inhibiting up-regulation of the adhesion molecule CD11 on neutrophils [146]. These beneficial effects of both AM and AMBP-1 may be combined into a therapeutic regimen for sepsis treatment.

ET-1 Endothelin 1 (ET-1) is a vasoactive peptide ubiquitously expressed in many cell types, and it functions in maintaining vascular homeostasis. Under normal conditions, endothelial cells, cardiac myocytes, and fibroblasts synthesize ET-1 at a low level [147]. Under stress conditions such as vasospasm, vascular damage, cardiovascular remodeling, renal ischemia, and inflammation, expression of ET-1 is increased [147, 148]. In severe sepsis patients, plasma ET-1 is markedly increased and its levels correlate significantly with the degree of sepsis severity, and specifically, the amount of renal dysfunction [149, 150]. Recent studies demonstrated that during the early phase of sepsis, the initial up-regulation of ET-1 seems to be beneficial for maintaining blood pressure and organ perfusion [151]. However, high levels of ET-1 for longer periods evoke profound vasoconstriction and tissue hypoperfusion, which is harmful [152]. Blockade of ET-1 via antagonism at its receptor ETR has been shown to be protective in reducing organ injury, splanchnic hypoperfusion, and bacterial translocation in septic shock [153].

8 Growth Factor/Hormone

MFG-E8/MSP68 MFG-E8 is a secretory glycoprotein that functions in the engulfment of apoptotic cells by professional phagocytes [154]. The MFG-E8 peptide contains an arginine–glycine–aspartate (RGD) motif which recognizes $\alpha_v\beta_3/\alpha_v\beta_5$-integrin on phagocytic cells, while its C-terminus binds to apoptotic cells via phosphatidylserine (PS) [154]. This is how MFG-E8 scavenges dying cells from the tissue micro-environment. It is ubiquitously expressed in various tissues such as mammary cells, splenocytes, monocytes, peritoneal macrophages, dendritic cells, glial cells, APCs, stromal cells, fibroblasts, osteoblasts [154, 155]. During CLP-induced sepsis, levels of endogenous MFG–E8 are reduced dramatically,

which leads to accumulation of apoptotic cells in the spleen and thymus [154]. This leads to exaggerated inflammation accompanied by elevated levels of TNF-α, IL-6, IL-8, and myeloperoxidase (MPO) [154]. Exogenous administration of rMFG-E8 can restore immune homeostasis by reducing these pro-inflammatory mediators and accelerating tissue regeneration [156, 157]. In ALI caused by gut I/R and endotoxemia, MFG-E8 has been found to inhibit neutrophil migration in lungs [158, 159]. Although the protective roles of MFG-E8 are being mediated through the modulation of hyperactive innate-immune functions, its roles in the adaptive immune system need to be defined.

More recently, a related protein, MFG-E8-derived short peptides (MSP68), has also shown promise in reducing organ injury in sepsis. MSP68 contains the RGD motif involved in cell-cell and cell-matrix interactions that enhances neutrophil migration, systemic inflammation, and organ damage [160]. In an animal model of sepsis, MSP68 was found to effectively inhibit excessive neutrophil infiltration to organs, attenuating organ injury and significantly improving survival rates in septic mice [161]. These peptides may serve as a more efficient way to treat sepsis by administering the active portion of MFG-E8 responsible for its useful functions.

Growth Hormone and Ghrelin Treatment in Combination It has been reported earlier that ghrelin can inhibit sympathetic nerve activity and inflammation in young septic animals by binding to its receptor in the brain [136]. However, in a recent article, hyporesponsiveness was noted after treatment of ghrelin administration in sepsis in elderly animals, likely due to decreased expression of ghrelin receptors in the brain [162]. Treatment with a cocktail of human growth hormone (GH) and human ghrelin was shown to lead to enhanced ghrelin receptor expression in the brain, allowing ghrelin to recognize its receptor to exert beneficial out comes in elderly sepsis [162]. Treated rats showed increased expression of ghrelin receptor in the brainstem combined with an improvement in cardiovascular function [162]. Ten-day survival of aged septic rats was increased after combined treatment and was associated with less body weight loss [162]. Combined human ghrelin and GH for sepsis treatment may be important for reducing morbidity and mortality in the elderly population.

9 Conclusion and Future Directions

In summary, the mediators discussed above represent new advances in understanding the very complicated pathophysiology of sepsis. The list of these potential inflammatory and immunomodulatory agents will continue to grow and change as research progresses in this field. As we have a better understanding of the relationship of pro- and anti-inflammatory phases in sepsis, we may be able to develop better targets for treatment. It is clear that best treatment should be a combination of mediators, and timing may be the key to providing the correct immunomodulatory agent at the right phase. It is hopeful that these promising animal studies will lead to

human clinical trials, and reverse the trend of prior failures to combat the problem of sepsis and its related morbidities.

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Research Advances in Biomarker for Sepsis

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Abstract Sepsis is one of the most common causes of death in severely injured patients worldwide. The early detection of sepsis still has to be solved in clinical practice. The delayed diagnosis often contributes to inappropriate antimicrobial treatment and subsequent high mortality. Sepsis biomarkers are produced during the host response to infection. Traditional biomarkers are polypeptides and/or proteins derived from this response. Omics-based biomarkers are screening out from all kinds of molecules of host response while high-throughout omics technologies are emerging. This review describes traditional and potential omics-based sepsis biomarkers from currently available literatures. The combination of these biomarkers would refine the identification of sepsis for further clinical and experimental sepsis studies.

Keywords Trauma · Sepsis · Diagnosis · Biomarker · Omics technology

1 Introduction

Sepsis is one of the leading fatal causes of critical injured and ill patients all over the world. Despite recent advances in comprehensive management of trauma patients, sepsis is still a life-threatening condition with poor outcome. The risk factors of patients for the development of sepsis usually refer to their physiological characteristics, underlying illnesses and clinical treatment backgrounds. The patients with one or more of these factors are susceptible to sepsis. The most vulnerable populations for sepsis are the elderly and infants, patients with chronic diseases, patients with severe trauma and burns, those who are immunocompromised or receiving immunosuppressive therapy, and malnourished and debilitated

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patients. Early diagnosis of sepsis plays a significant role for each hour of delay of appropriate antibiotic therapy increases mortality by 7.6 % [1]. However, the accurate and timely detection of sepsis remains a great challenge nowadays, because of the various, insidious and nonspecific clinical manifestations as well as the complex and indeterminate pathophysiologic process. Therefore, neither the clinical microbiological detection, which is known as the golden standard of infection, nor the most traditional biomarkers can fulfill all the existing needs in the early diagnosis and management of sepsis [2].

Originally, sepsis has been defined as infection with at least 2 of the 4 SIRS criteria, which mainly focus on inflammation that is not covered the full pathobiology. Sepsis is now recognized to involve early activation of both pro- and anti-inflammatory responses, along with major modifications in nonimmunologic pathways such as cardiovascular, neuronal, autonomic, hormonal, bioenergetic, metabolic, and coagulation, all of which have prognostic significance [3]. Sepsis is refined as "life-threatening organ dysfunction caused by a dysregulated host response to infection" [3]. There is an extensive and complicated pathogen-host interaction during the process of infection. Sepsis develops when the initial, appropriate host response to pathogens becomes amplified and then dysregulated [4]. It is the ability of host immunologic defense that determines the fate of infecting organisms: whether be localized, phagocytized and erased by immunocytes which may cause the release of their components from the invading pathogens; or multiply in the local tissues, successfully leak into the bloodstream and eventually become bacteremia and sepsis. Pathogen itself and its structural components not only cause extensive changes of both innate and acquired immunities, but also exert a profound influence on the systems of nerve, endocrine, respiration, circulation, and metabolism etc. [4]. Traditional biomarkers of sepsis are mainly derived from this host immuno-inflammatory response. The emergence and development of a variety of high throughput omics technologies will contribute to a more comprehensive screening for sepsis-specific biomarkers. Based on the discussion of current traditional sepsis biomarkers, we address new insights into sepsis biomarkers in the field of genomics, transcriptomics, proteomics, and metabolomics, those may show the hope for more comprehensive understanding of sepsis and help to overcome the present diagnostic uncertainty.

2 Traditional Biomarkers

Biomarkers are molecular indicators that help doctors diagnose illnesses, predict the outcome or identify which people are susceptible. Biomarkers, should be some quantifiable measurements of biological homeostasis and by defining the normal status can provide a frame of reference for predicting abnormal or pathogenic processes [2], and make an impact on clinical decision making in time. Most commonly proposed sepsis and infection biomarkers including C-reactive protein (CRP), procalcitonin (PCT) [5, 6], cytokines (TNF-α, IL-1, IL-6, IL-10, osteopontin) [7, 8],

chemokines [macrophage migration inhibitory factor (MIF), high-mobility-group box 1 (HMGB1)] [9, 10], soluble receptor [soluble triggering receptor expressed on myeloid cells 1 (sTREM-1), soluble urokinase-type plasminogen activator receptor (suPAR)] [11, 12] etc.

Given the complicated pathophysiology in sepsis which involves hundreds of mediators or single molecules, it is unlikely to identify one single biomarker which is able to satisfy all the existing needs and expectations in sepsis research and management. CRP, for example, is frequently used to assess the presence of infection and sepsis [2], and there is a positive correlation between its plasma level and the risk of organ dysfunction and death [13, 14]. However, plasma concentrations of CRP may increase during minor infection and do not adequately reflect the severity of infection, or remain at a high level for several days, even overstay the infection [2]. Besides, CRP may experience an increase during the inflammation caused by noninfectious etiologies, such as tumor, tissue necrosis or operation, which explains its nonspecificity as an early stage sepsis biomarker. Meanwhile, PCT, perceived as the most potential biomarker, is listed as one of the diagnostic criteria for sepsis [15]. A recent meta-analysis of PCT that included 30 studies found mean sensitivity and specificity of 0.77 and 0.79 respectively, with the area under the receiver operating characteristic curve (AUC) was 0.85 [16]. Although PCT was thought as a helpful biomarker for early diagnosis of sepsis in critically ill patients, it was not suitable to be recommended as the single definitive diagnostic test [16].

Therefore, some researchers have put forward that combinations of biomarkers may overcome the limitations mentioned above. In a prospective cohort study including 151 patients with systemic inflammatory response syndrome (SIRS), Kofoed et al. [17] found that the AUCs of six biomarkers—suPAR (0.5), sTREM-1 (0.61), MIF (0.63), PCT (0.72), neutrophil count (0.74), CRP (0.81)—for detection of a bacterial cause of inflammation had ranged from 0.5 to 0.81. With method reported by Xiong et al. [18], which discussed the statistical estimation of the optimum linear combination test and the associated maximum area under the ROC curve, Kofoed got the combined AUC of the six-marker test at 0.88. Consequently, the six-marker test had a better diagnostic accuracy in detecting bacterial versus nonbacterial causes of inflammation, and significantly greater than that of each single marker. Similarly, in another prospective research among the critically ill patients [19], a 'bioscore' combining the polymorphonuclear neutrophil (PMN) CD64 index together with PCT and sTREM-1 serum levels was put forward to diagnose sepsis and had a better performance with an AUC of 0.97 than that of each individual biomarker, and was externally confirmed in the validation cohort with 90.9 % of patients being correctly classified by the very model. Although the combination of biomarkers do improve diagnostic sensitivity and specificity, due to the factors of time-consuming, economic cost, the amount of sample, the feasibility of biomarker detection method and so on, it has a limited application in clinical practice and still needs further prospective studies conducted in multicenter on cost-effectiveness.

Circulating DNA, including nuclear DNA (nDNA) and mitochondrial DNA (mtDNA), can either actively release or be released passively into the blood stream after rupture or necrosis of host cells [20, 21]. These nucleic acids released in the plasma during sepsis could serve as danger associated molecular patterns (DAMPs) [22], which make them potential biomarkers for the very condition [23, 24]. A clinical study [22] found that plasma cytokine concentrations, as well as nDNA and mtDNA levels of septic shock patients were increased at the onset of septic shock and remained elevated. And during the first 5 days of septic shock, nDNA levels consistently correlated with plasma cytokine concentrations as well as with the shock-related parameter norepinephrine infusion rate and markers of organ damage (total bilirubin and creatinine). These findings not only indicate a relationship between plasma nDNA levels and the inflammatory response, but also demonstrate that nDNA levels are associated with markers of shock and organ damage in septic shock patients.

3 Genomics-Based Biomarkers

Genomics, a discipline in genetics and an emerging field, explains physiological or pathophysiological events from the point of view of complete set of DNA, including recombinant DNA, DNA sequencing methods, and bioinformatics to sequence, assemble, and analyze the function and structure of genomes. Sepsis can be regarded as a polygenic syndrome initiated by infection. Genetics plays a crucial role in both susceptibility and response to infection [25], and genetic predisposition influences clinical outcomes of infectious diseases [26, 27].

3.1 Polymorphisms and Single Nucleotide Polymorphism (SNP)

A gene polymorphism is defined as regular occurrence (>1 %) of two or more alleles at a particular chromosome location. Several polymorphisms of genes broadly involved in inflammation, immunity, and coagulation have been linked with susceptibility to sepsis, or outcome of sepsis [25], and have become the focus of most gene association studies of sepsis as well. SNP, the most common type of polymorphisms, is a substitution, deletion, or insertion of a single nucleotide occurring in approximately 1 per 1000 base pairs of human DNA. SNP can lead to an altered protein, a change in the amount of normal protein expression, or no discernible change in protein function [28]. Study of SNP genotypes in sepsis helps identify potential markers of susceptibility, severity, and clinical outcome.

Extensive researches on SNP genotyping of main genes CD14 [29–32], Toll like receptors (TLRs) [27, 33], lipopolysaccharide-binding protein (LBP) [34],

cytokines [26, 33, 35, 36] and coagulation factors [37, 38]—have provided valuable information for sepsis. For example, burn patients with TLR4 and TNF-α polymorphisms were 1.8 times more likely to develop severe sepsis, but none of them were significantly associated with mortality [33]. TLR1 SNPs are associated with increased mortality in patients with gram-positive sepsis after traumatic injury, which may represent a novel marker of risk for death in critically injured patients [27]. And most recently, it was found that there was a new association between vascular endothelial growth factor (VEGF) +936 CC genotype and the risk to develop acute kidney injury (AKI) in severe sepsis patients [35]. Genome-wide SNP genotyping assays allow to detect hundreds of thousands of SNPs accurately in a single experiment [39, 40] and are expected to be of great applicative prospect in finding novel sepsis susceptibility-associated SNP genotypes.

In order to evaluate the validity of these studies and translate this concept to the bedside, several important factors have to be kept in mind: potential confounding variables should be recognized and matched; positive association studies and replicate studies should be validated and analyzed on the basis of the primary hypothesis other than multiple comparisons; large scale collaborations and studies on sepsis susceptibility-associated SNP genotypes need to be performed for the sake of possible new risk factors at the genetic backgrounds of sepsis development [25, 41].

3.2 Epigenetics-Based Biomarkers

Genes concerning immunity and inflammation are subject to epigenetic regulation [25], which refers to heritable changes in gene expression that are not related to direct DNA sequence changes [42]. DNA methylation and histone post-translational modifications play vital roles in the epigenetic control [43] and gene expression, and strongly impact on the host defense responses.

Sepsis induces epigenetic changes in dendritic cells and lymphocytes rendering the host immune deficiency for a long period after the initial sepsis challenge [44–46]. Late-phase immunosuppression of sepsis is strengthened by a postmortem study [47]. By suppression of proinflammatory gene products and subsequent immune cell activation and proliferation, epigenetic mechanisms are put forward to have an influence on the very stage of sepsis, which may not only provide a better understanding of septic mechanism but also yield important biomarkers [48].

DNA methylation, the addition of methyl group to cytosine or adenine nucleotide, is now replacing the biological markers with their high specificity, sensitivity and prognostic efficacy. A retrospective investigation has showed that calcitonin-related polypeptide α (CALCA) gene promoter methylation varied with different types of preterm bacterial sepsis [49]. Based on this finding, another study demonstrated that global DNA methylation varied significantly among newborns with sepsis and those without sepsis [50]. More specifically, a recent study analyzed the CpG methylation status in the epigenome of septic and non-septic babies [51] or

ICU patients [52]. Given biological and clinical significance, they found 81 differentially methylated CpGs located in 64 genes, and a panel of differentially methylated protocadherin β (PCDHB) genes that play vital role in leukocyte cell adhesion and Wnt signaling pathway. These genes play vital role in calcium dependent cell to cell adhesion and other immunological processes like antigen processing and presentation. In sepsis, suppression of leukocyte cell adhesion and migration may exaggerate disease severity and poor outcome due to multiple organ dysfunctions. Therefore, this study provides some novel insights into the role of DNA methylation in neonatal sepsis. However, further studies are called for exploring the clinical relevance as well as related therapeutic approaches of the observed findings.

All the above mentioned sepsis biomarkers are derived from the development of genomics technology and summarized in Table 1.

Table 1 Potential genomic biomarkers for sepsis

Genomics	Biomarkers (reference)	Patients/animals (reference)	Changes	Clinical relevance (reference)
SNP	TLR1 [28]	1961 trauma patients	−7202G +742A/G (Asn248Ser)	Association with increased mortality after traumatic injury and sepsis
	CD14 [30–33]	14 septic patients and 30 healthy controls [30] 514 critically ill patients [31] 58 severely injured blunt trauma patients [32] 228 burn patients [33]	−260C → T	1. No association with an increased risk of severe sepsis in trauma patients [30] [32] 2. Association with increased susceptibility to sepsis [33] 3. Higher −260TT genotype frequency in ICU survivor patients [31]
	IL-6、TLR4 and TNF-α [33]	228 burn patients	IL-6 -174 G → C TLR4 +896A → G TNF-α -308G → A	Association with increased risk for severe sepsis after burn injury
CpG	81 differentially methylated CpGs located in 64 genes [51]	3 septic and 3 non-septic babies	Protocadherin beta genes (PCDHB11/12/16/5/6/7/9) hypermethylated in newborns with sepsis. CCS-hypermethylated, DEGS2-hypomethylated	Provide some novel insights into the role of DNA methylation in neonatal sepsis

4 Transcriptomics-Based Biomarkers

4.1 Gene Expression

The immune responses involved in sepsis are so complicated that the exact molecular mechanism remains to be fully elucidated [53]. The balance between pro-inflammatory responses and anti-inflammatory responses is closely related to the expression and regulation of relevant genes [54]. Hence, evaluating the key gene expression profiles by high throughput DNA chip may reveal the immune status of septic patients. Researchers have found specific changes of gene expression with microarray in certain organs and tissues of septic mice model, which including heart [55], liver, spleen [56], leucocytes [57] and so on.

Accordingly, Lukaszewsk recruited 92 ICU patients who had the risk of developing into sepsis [58]. The mRNA expression levels of IL-1β, IL-6, IL-8, IL-10, TNF-a, FasL and CCL2 in their blood leukocytes were measured on a daily basis by means of real-time reverse transcription PCR (RT-PCR), and analyzed with a nonlinear technique (neural network analysis). The data correctly predict the onset of sepsis in an average of 83.09 % of patient cases between 1 and 4 days before clinical diagnosis with high sensitivity and selectivity (91.43 and 80.20 %, respectively). Sutherland et al. [59] evaluated transcriptional profiles in circulating white blood cells of ICU sepsis patients, post-surgical patients and healthy controls with a microarray and multiplex tandem (MT)-PCR. A panel of 42-gene expression markers was identified, by which the prediction of sepsis within a mixed inflammatory population had an AUC between 86 and 92 %. Sepsis has a unique gene expression profile that is different from uninfected inflammation and becomes apparent prior to the clinical manifestations of sepsis for 0–48 h [60]. In that case, the specific gene expression profile, which may involve the function of innate immunity, cytokine receptors, T cell differentiation as well as the protein synthesis, may make a reference for early diagnosis of sepsis.

However, an important limitation of transcriptomics is that it only partially reflects the steady-state mRNA abundance, and the degree of mRNA abundance is influenced by multiple factors, and does not provide any direct information about gene end products (proteins), nor post-translational modifiers of protein function [61]. When it comes to the sample used as RNA source, there is a contradiction. For the whole blood approach, it may be difficult to interpret the confounded data of RNA for the reason of the heterogeneity among blood cell populations. As for the cell-specific approach, there is a possibility to miss relevant expression information from other cells due to the complexity of clinical sepsis [61]. It highlights the necessity of linking theory to clinical practice.

4.2 miRNAs

MicroRNAs (miRNAs) is a class of short RNAs with 18–25 nucleotides in length which regulate gene expression in a post-transcriptional manner via sequence-specific interaction with target sites in mRNA [62], associated with various physiological and pathological processes. The levels of miRNA in serum and plasma are consistent among individuals of the same species, resistant to RNase A digestion, and stable even after the freeze-and-thaw and a long term of storage [63, 64]. The stability of miRNA makes it a potentially useful candidate for diagnostic and other clinical applications. Although the source of circulating miRNA is still unclear, it has been proved that there is a link among a range of diseases, such as circulating miRNA and cancer [65, 66], trauma [67, 68], acute pancreatitis [69], and hepatitis [70].

When it comes to sepsis, by using genome-wide miRNA profiling with microarray in peripheral blood leukocytes and quantitative RT-PCR, Vasilescu [71] found that miR-150 levels were significantly reduced in both leukocytes and plasma of sepsis patients and had a negative correlation with the level of disease severity measured by the Sequential Organ Failure Assessment (SOFA) score, which made it a biomarker of early sepsis. Similarly, Zeng [72, 73] investigated the levels of miR-150 and miR-143 in peripheral blood leukocytes in sepsis patients with RT-PCR, and found that the expression levels of miR-150 and miR-143 were significantly decreased in sepsis patients and could reflect the severity of sepsis in certain degree, which not only made it a marker to reflect the situation of inflammatory response, but also made it a prognostic marker in sepsis. Recently, higher serum miR-133a levels were found among sepsis patients in ICU [74]. As they were significantly correlated with disease severity, classical markers of inflammation and bacterial infection, as well as organ failure, high miR-133a levels were considered as independent biomarkers for unfavorable prognosis of critically ill patients.

However, given that the pathophysiological process of sepsis involves a variety of tissues and organs, a simple screen for miRNA differentially expressed in leukocytes may omit those secreted by other cells. Wang et al. [75] used genome-wide microarray to identify differential serum miRNAs in survival and non-survival sepsis patients, and then further validated the differential expressions of miR-297 and miR-574-5p by RT-PCR in a larger group. The serum miR-574-5p together with sepsis stage and Sepsis-Related Organ Failure Assessment scores has a better predictive capability for the death of sepsis patients. In addition, serum miR-146a and miR-223 were found significantly reduced in septic patients compared with SIRS patients and healthy controls which might serve as new biomarkers for sepsis with high specificity and sensitivity [76]. Due to our knowledge on serum miRNAs is still at a primary stage, the expression level of circulating miRNAs at different stages of sepsis and their potential correlation with injured organs need further investigation.

To sum up, from the point of view of gene transcription, miRNA may undertake the task of diagnosing sepsis in an early stage and evaluating the prognosis, as well as becoming the new target for sepsis therapy.

4.3 Long Non-coding RNAs (LncRNAs)

As discussed above, epigenetic factors not only include histone modifications and DNA methylation, but also contain non-coding RNAs(ncRNAs), which have diverse size and can be generated from intergenic regions, introns, or enhancers [45]. LncRNAs are transcripts longer than 200 nucleotides and lack protein-coding capacity. Peng et al. [77] first discovered the widespread differential expression of lncRNAs in response to severe acute respiratory syndrome coronavirus (SARS-CoV) virus infection. Accordingly, there is a possible link between lncRNAs and the host defense response against infection. LncRNA has the potential to become new class of biomarkers and new therapeutic target for infectious diseases. However, as the functions of lncRNAs remain largely unexplored, there is a need for future studies on their regulatory role in infection.

All the transcriptomics-based biomarkers mentioned above are outlined in Table 2.

5 Proteomics-Based Biomarkers

Proteome is the complete set of proteins that can be expressed by the genetic material of an organism. Proteomics is the analysis of the expression, localizations, functions, and interactions of proteomes. Compared to other immunologic tests, proteomics is a novel method with advantages of high throughput, high sensitivity and specificity. The development of proteomics has allowed for a better understanding of the molecular bases concerning the identification of cell signaling, modifying protein, post-translation modification pathway, as well as the characterization of specific biological markers [78].

Proteomics has irreplaceable clinical significance and an expansive application prospect in studies of sepsis biomarkers. In a rabbit sepsis model by intravenous injection of *Pseudomonas aeruginosa* at 24 h after scald, 11 discrepant expression proteins from lymphocyte were found by matrix-assisted laser desorption/ionization time of flight mass spectrometry(MALDI-TOF MS). They are related with the folding, assembling, transportation and degradation of proteins, signal transmission, inflammation, immunization, energy metabolism, the proliferation, differentiation and apoptosis of cells [79]. In a recent research, 41 differential expressed proteins in the neutrophils from *Acinetobacter baumannii* sepsis rats were identified using two-dimensional electrophoresis and mass spectrometry [80]. They included antioxidant proteins, signaling proteins, cytoskeleton and regulatory proteins,

Table 2 Potential transcriptomic biomarkers for sepsis

Transcriptomic	Biomarkers (reference)	Patients/animals (reference)	Changes	Clinical relevance (reference)
Gene expression	A panel of 42 sepsis gene expression markers [60]	Mixed inflammation group (28 sepsis and 38 post-surgical patients in ICU), and 20 healthy controls	NA	A novel molecular biomarker test has the capacity for early detection of sepsis via the monitoring of patients
	IL-1β, IL-6, IL-8, IL-10, TNF-a, FasL and CCL2 mRNA expression [59]	92 ICU patients	NA	Provide a generic indicator of sepsis and help its early diagnosis
miRNAs	miR-150 [71, 72]	17 sepsis patients and 32 healthy controls [72] 40 sepsis patients,20 SIRS patients, and 20 health controls [73]	↓	The miR-150 levels in both leukocytes and plasma correlate with the aggressiveness and prognosis of sepsis and can be used as a marker of early diagnosis
	miR-133a [74]	223 critically ill patients(138 with sepsis and 85 without sepsis) and 76 healthy controls	↑	High miR-133a levels were associated with the severity of disease and predicted an unfavorable outcome of critically ill patients
	miR-143 [73]	40 sepsis patients, 20 SIRS patients and 20 healthy controls	↓	The expression level of miR-143 may be a marker for judging the severity of sepsis and its prognosis
	miR-146a [76]; miR-223 [76]	50 sepsis patients, 30 SIRS patients and 20 healthy controls	↓ ↓	Serum microRNAs might be used as biomarkers for early diagnosis and reflecting severity of sepsis
	miR-574-5p [75]	12 surviving and 12 nonsurviving sepsis patients for	↓	The miR-574-5p combined with SOFA scores and

(continued)

Table 2 (continued)

Transcriptomic	Biomarkers (reference)	Patients/animals (reference)	Changes	Clinical relevance (reference)
		microarray scan; 118 sepsis patients for validated by qRT-PCR		sepsis stage provides a prognostic predictor of sepsis patients

NA Not available

energy metabolism and protease protein, which may play a key role in such kind of sepsis and provide potential clues in early diagnosis and treatment of sepsis.

In clinic, YKL-40 was identified with proteomics analysis on a significantly higher expression level in serum samples from sepsis patients and considered as a possible biomarker of sepsis [81]. Paugam-Burtz et al. [82] used plasma profiling coupling proteinchip array with surface-enhanced laser desorption ionization time-of-fly mass spectrometry (SELDI-TOF MS) to analyze the plasma of post-operative patients, and found that a combination of five plasma protein peaks may have potential as diagnostic biomarkers of postoperative sepsis in patients undergoing liver transplantation. Even so, these proteins remain to be identified and validated in more clinical trials.

6 Metabolomics-Based Biomarkers

Although many potential sepsis biomarkers have been revealed by genomics, transcriptomics, and proteomics, the changes of cellular metabolism in sepsis should be paid attention. Metabolomics is an emerging omics technology following genomics and proteomics and focuses on the metabolic products with a molecular weight less than 1000 kD under the physiological or pathological status. It can analyze the biochemical events of cells, tissues or organs and evaluate the disease and its severity. The research methods of metabolomics mainly include nuclear magnetic resonance (NMR), gas chromatography/mass spectra (GC/MS), high performance liquid chromatography/mass spectra (HPLC/MS).

The development of sepsis involves the reactions of multiple systems on various levels, which has a significant influence on the expression levels and activities of metabolic enzymes. And by detecting the concentration and ratio changes of those metabolites involved, a better understanding of condition and prognosis of sepsis may be achieved at an early stage [83]. Metabolic profile of the serum from septic rats with cecal ligation and puncture was achieved with the help of NMR and LC/MS [83]. In the septic rats, especially the non-survivors, many free fatty acids

showed a lower level which may be consumed greatly for energy supply in sepsis and may be related with the prognosis of sepsis. Moreover, there was a rise of some polyunsaturated fatty acids in the very group, which may have a relationship with the increased anti-inflammatory effect. Based on the metabolic profile analysis, a model for outcome predication was built with high sensitivity and specificity, which provided a novel method for sepsis prognosis judgment. NMR-based metabolic profiling revealed the difference of metabolites of energy metabolism and inflammation in lung tissue, bronchoalveolar lavage (BAL) fluid, and serum samples between the septic rat and the control rat [84]. In septic rats, creatine concentration increased in all the three types of samples, whereas alanine and phosphoethanolamine concentrations increased only both in lung tissue and in serum. Myoinositol increased in lung tissue but decreased in BAL fluid. In addition, acetoacetate increased whereas formate decreased in serum. And with the construction of a predictive model for diagnosis of sepsis using partial least-squares discriminant analysis, the preliminary goal of sepsis diagnosis was achieved.

The possible sepsis biomarkers screened out from the application of proteomics and metabolomics technologies are summarized in Table 3.

Table 3 Potential proteomic and metabolomic biomarkers for sepsis

	Biomarkers (reference)	Clinical relevance (reference)	Changes	Patients/animals (reference)
Proteomic	YKL-40 [82]	YKL-40 may involve in the pathophysiology of sepsis and be a biomarker of sepsis	↑	45 sepsis or septic shock patients, 22 healthy controls and 23 patients who received off-pump coronary artery bypass grafting
Metabolomic	Acetoacetate, Alanine, Creatine, Phosphoethanolamine, formate [84]	NMR metabolomics analysis is a potentially useful technique for sepsis diagnosis	↑ (serum) ↑ (serum) ↑ (serum) ↑ (serum) ↓ (serum)	14 rats underwent cecal ligation and puncture as septic group;14 rats with sham procedure as control group
	Linoleic acid, Oleic acid, Atearic acid, Docosahexaenoic acid, Docosapentaenoic acid Linolenic acid [83]	A model for outcome predication was built with high sensitivity and specificity	↓ (serum) ↓ (serum) ↓ (serum) ↑ (serum) ↑ (serum) ↑ (serum)	23 surviving and 22 nonsurviving septic rats; 25 sham-operated rats

7 Prospects

Sepsis involves sophisticated pathophysiologic changes among various organs and different systems, so that comprehensively identifying sepsis biomarkers and understanding sepsis molecular mechanism from the perspectives of omics may provide valuable information about a more macroscopical and authentic state following infection.

The most important issue involved in the researches of sepsis biomarkers is the criteria of septic cases. The golden standard of infection, currently, still depends on the results of clinical microbiology laboratory, which, however, due to the severity of the disease, the load and type or growth capacity of pathogens, and the use of antibiotic treatment may show a negative result while patients has clinical manifestation of infection [85]. Or false positive results may present for the reason of contamination. Therefore, whether this gold standard is appropriate in sepsis needs further reflection and investigation.

There is a lack of effective and united assessment methods for sepsis biomarkers, especially in the multi-centered or multi-index researches. Valid evaluation needs to be performed to pick out the ideal diagnostic indicators, those both of high sensitivity and specificity in the field of data statistics and of great practical use in clinical practice.

Majority studies of sepsis only focus on a single set of omics technology (Table 1) rather than apply the combination of multiple omics approaches. Since different omics may display sepsis mechanism at various levels of a specific molecule or a particular group of sepsis-associated molecules, the comprehensive application of two or more omics may provide integrated information of potential sepsis biomarkers. MAPIT algorithm (Multi Analyte Pathway Inference Tool), for example, enables principled integration of epigenomics, transcriptomics, and proteomics data for cancer diagnosis, prognosis, and biomarker discovery [86].

Last but not the least, as mentioned above, the lack of a clear insight of the pathophysiology of sepsis process will, to some extent, put off the sepsis biomarker researches [2]. But with the application of state-of-art technology and exploration in a novel view, the progress in sepsis biomarkers will promote awareness and understanding of sepsis.

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Trauma, Regulated Cell Death, and Inflammation

Jie Fan and Liyan Fan

Abstract Trauma is a significant regulator of cell death, which, in turn, plays an important role in the regulation of inflammation. The efficacy of tissue homeostasis includes several factors such as the removal of foreign microbial pathogens and the removal and identification of dead and dying cells. Further research has led to an enhanced knowledge on the connection between cell death and inflammation, expanding past understanding of the signaling pathways that regulate and affect different forms of cell death and inflammatory responses. This chapter presents an overview of the major types of cell death related to inflammation and the mechanisms underlying trauma regulation of cell death. The impact of these cell death pathways allows for the identification of a therapeutic target for inflammatory diseases.

Keywords Alveolar macrophages · Apoptosis · Autophagy · Caspases · Cell death · Cold-inducible RNA binding proteins (CIRP) · Damage-associated molecular patterns (DAMPs) · Inflammasome · Necrosis · Necroptosis · Netosis · Pyronecrosis · Pyroptosis

1 Introduction

Cell death is an important factor in the development and maintenance of an organism. The early 1960s saw the classification of apoptosis as the only form of cell death [1, 2], while necrosis was seen as a form of 'accidental' cell death that would only occur in response to harmful chemical or physical stimuli. Further

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development in cell death research allowed for the observation of the relationship between cell death and inflammation that is that, in host defense, cell death can be used defensively, reducing infections by separating unaffected cells from infected cells. However, cell death can also increase inflammation. Trauma regulates cell death through the damage and destruction of tissue and cells, but also through the release of signals that induce cell death and thus affect inflammation and organ dysfunction following trauma.

Two criteria were proposed by the Nomenclature Committee on Cell Death (NCCD) in 2015 for the identification of dead cells. These criteria include: (1) the permanent loss of the barrier function of the plasma membrane; and (2) the destruction of cells into discrete, separate pieces, called apoptotic bodies [3, 4].

There are two categories that instances of cell death and by classified into: "accidental" and "regulated". Accidental cell death (ACD) and regulated cell death (RCD) are contrasted by the factors that initiate these types of cell death. ACD is causes by severe physical (e.g. high temperatures and pressures), chemical (e.g. variations in pH and detergents), and mechanical (e.g. shearing) insults. These cells die in an uncontrolled and unpreventable manner which does not allow for therapeutic intervention or the use of specific molecular machinery. By contrast, RCD can occur as part of physiologic programs or can be activated once adaptive responses to perturbations of the extracellular or intracellular microenvironment fail. The biochemical phenomena that accompany RCD may be classified into several subtypes, which usually exhibit stereotypical morphologic features.

This chapter describes the link between trauma, cell death, and inflammation, focusing on the proteins in each mechanistic module that executes the process of cell death and inflammation.

2 Necrosis, Necroptosis, and Inflammation

Historically, necrosis was viewed as a type of ACD, resulting from extreme physiochemical insult and thus is morphologically characterized by swelling of organelles which leads to increased cell volume and weakening or breaking of the plasma membrane and thus resulting in the release of intracellular content. These intracellular materials, damage-associated molecular patterns (DAMP), can cause an inflammatory response; therefore, necrosis is generally viewed as a cause of inflammation. DAMPs are the critical factors to the pathogenesis of sterile inflammation, including ischemia-reperfusion, atherosclerosis, gout, and Alzheimer's disease. For example, the release of high-mobility group box 1 (HMGB1), a DAMP molecule, from necrotic cells can cause neighboring cells to express chemokines, cytokines, and adhesion molecules though the activation of the receptor for advance-glycation end-products (RAGE), inducing inflammation [5]. Recent studies explored the existence of multiple pathways of regulated necrosis [6–11].

A pathway of regulated necrosis, also named as necroptosis, has been heavily studied. Necroptosis can be defined as cell death that is regulated by a pathway that depends on the receptor-interacting protein kinase (RIPK)1-RIPK3 complex and that can be inhibited by Necrostatin-1 (Nec-1) [10] (Fig. 1). Necroptosis is induced by a class of death receptors that includes tumor necrosis factor receptor (TNFR)1, TNFR2, and Fas. Of these, the TNF-α/TNFR-induced pathway is the most widely studied. Binding of TNF-α to the extracellular portion of TNFR1 causes allosteric changes in the intracellular portion of TNFR1 followed by the release of the silencer of death domains (SODD) from the intracellular domain of TNFR1 [11]. TNFR1 and TNFR2 form complex I containing a death domain [e.g., TNF-α receptor-associated death domain (TRADD)], RIPK1, Fas-associated death domain (FADD), and several E3 ubiquitin ligases, such as TNF-α receptor associated factor 2/5 (TRAF2/5) and inhibitor of apoptosis proteins (IAPs) cIAP1 and cIAP2 [12]. RIPK1 is initially recruited to complex I and is polyubiquitinated by TRAF2/5, cIAP1, and cIAP2 [13, 14]. Because RIPK1 exhibits a biphasic effect based on its ubiquitination state, complex I is situated at the crossroads of cell survival and death. Deubiquitination of RIPK1 can inhibit the NF-κB pathway, which promotes cell death pathways. Whether TRADD is required for necroptosis depends upon the type of stimulus. TNFR1 activation together with the absence of c-IAPs (IAP antagonist treatment), translation inhibition (cyclohexamide treatment), or RIPK1 deubiquitination by the deubiquitinating enzyme (DUB) CYLD may promote the translocation of RIPK1 to a secondary cytoplasmatic complex, Complex II [15–17]. Complex II is formed by the death domain containing protein FADD, caspase-8 and cellular FLICE (FADD-like IL-1β-converting enzyme)-inhibitory protein (cFLIP). Complex II may activate either apoptotic or necroptotic downstream signaling pathways. Activation of caspase-8 drives complex II into a pro-apoptosis state by cleaving RIPK1 and RIPK3. However, when the apoptosis pathway is inhibited, a complex named the "necrosome" is formed (Fig. 1). The necrosome is primarily composed of RIPK1 and RIPK3 and distinctly enhances necroptosis [18].

The pseudokinase mixed lineage kinase domain-like (MLKL) protein is a substrate of RIPK3 and required for necroptosis [6, 19]. Unlike its previously discovered function in regulating mitochondrial fission, MLKL recruitment and phosphorylation caused by RIP homotypic interaction motif (RHIM)-dependent oligomerization and intramolecular RIPK3 autophosphorylation [20, 21] results in an activated state able to induce necroptosis [22]. Furthermore, several studies have deciphered a role for MLKL in necroptosis. MLKL oligomerization induced by RIPK3 and plasma membrane localization is associated with its cytotoxicity [23–26]. MLKL binds to phosphatidylinositol phosphates (PIPs) [23, 25] and subsequently modifies sodium or calcium influx through ion channels, thereby increasing osmotic pressure and promoting plasma membrane rupture [24, 26, 27].

It is unclear what the mechanism in which the necrosome causes cell death is. Necroptosis and necrosis shares several identical sub-cellular events, including: mitochondrial membrane hyperpolarization, oxidative burst, and lysosomal and plasma membrane permeabilization. However, the underlying mechanisms for these processes may differ [28]. Reactive oxygen species (ROS) potentially lead to cell

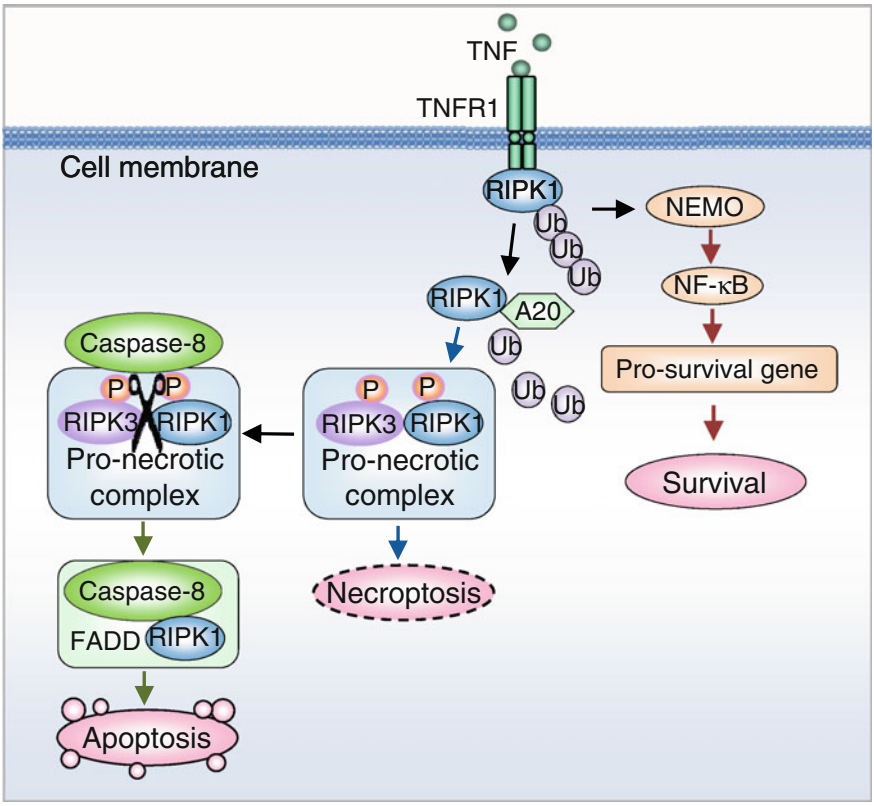

Fig. 1 TNF receptor signaling regulation of cell fate. Upon the binding of TNF to its receptor TNFR1, RIPK1 is recruited to TNFR1 and is subsequently ubiquitinated. The polyubiquitinated RIPK1, in turn, binds to NEMO, the regulatory subunit of NF-κB, to promote NF-κB activation, which leads to the induction of pro-survival genes to counter the death signals. Cell survival is a result of this pathway. The polyubiquitinated RIPK1 can also migrate to the cytoplasm, where RIP1 is de-ubiquitinated by A20, the de-ubiquitylating enzyme. RIPK1 and RIPK3 can then form a pro-necrotic complex followed by phosphorylation on both kinases and induction of necroptosis. In circumstances in which caspase-8 is activated, RIPK1 and RIPK3 can be cleaved by caspase-8, and the pro-necrotic complex is blunted, which stimulates the cell to undergo apoptosis

death by directly oxidizing or triggering various downstream pathways in the mitochondria [29–31]. RIPK3 accelerates mitochondrial ROS production and mitochondrial metabolism through the activation of a series of metabolism-related enzymes, including nicotinamide adenine dinucleotide phosphate (NADPH) and c-Jun N-terminal kinases (JNK) [32, 33]. Through an ADP/ATP-related pathway in addition to ROS production, mitochondria affect necrotic cell death. Adenine nucleotide translocase (ANT), an ADP/ATP carrier located in the inner mitochondrial membrane, is required for the synthesis of ATP in the mitochondria. RIPK1-dependent inhibition of ANT is reportedly involved in the programmed

necrosis induced by TNF-α and zVAD-fmk, whereas the latter potentially blocks the ability of ANT to transport cytoplasmic ADP and thereby induces massive ATP depletion in mitochondria. The activity of ANT is potentially affected by interactions with VDAC and cyclophilin D (CYPD). Two other potential executional proteins are calcium-dependent phospholipase A2 (cPLA2) and lipoxygenase (LOXs). cPLA2 plays an important role in TNF-α-induced necrotic cell death in L929 cells and MEFs [34]. LOXs act as downstream effectors of cPLA2 and lead to the disruption of organelle and plasma membranes [35]. LOXs is reportedly involved in both apoptosis and necrosis induced by TNF-α, although the exact mechanism has yet to be defined [36, 37].

Necroptosis can initiate inflammation. The triggering of inflammation by necroptosis has been seen in a study using mice with deletion of FADD [38] or Casp8 [39] in intestinal epithelial cells (IECs). In this study, it was observed that RIPK3-dependent cell death caused intestinal inflammation. RIPK3-mediated necroptosis may play a role in the pathogenesis of Crohn's disease, as evidenced by the high RIPK3 expression in Paneth cells of these patients [39]. Necroptosis has been found to stimulate the immune system to elicit inflammatory responses and has also been characterized in animal models of acute pancreatitis, ischemic injury, and neurodegeneration [40–43]. RIPK3$^{-/-}$ mice are protected from systemic inflammation caused by TNF stimulation and experimental sepsis induced by cecal ligation and puncture (CLP) [44, 45]. RIPK1 and RIPK3 also play crucial roles in the pathogenesis of *Salmonella enterica serovar* and *S. typhimurium* infection [46]. Necrotic macrophages have been observed in atherosclerosis lesions from both animals and human patients [47]. RIPK3-dependent necroptosis is a key driver of inflammation in atherosclerosis; RIP3 deficiency alleviates macrophage necrosis in advanced atherosclerosis lesions in atherosclerosis-prone LDL-R$^{-/-}$ or ApoE$^{-/-}$ mice [48]. The contribution of RIPK1-dependent necroptosis to multiple organ failure has also been observed in models of ischemia reperfusion (IR) and can be rescued by Nec-1 inhibitor [49–51]. In addition, necroptosis has been shown to contribute to neuronal damage in neonatal brain injury [52].

Necrosis and necroptosis both influence host disease outcomes through triggering inflammation. Determining the relative contribution of necroptosis-dependent and -independent pathways in inflammation may lead to new and more specific therapeutic targets.

3 Apoptosis and Inflammation

Apoptosis is a major type of cell death. Two separate signaling cascades for apoptosis have been identified: intrinsic and extrinsic pathways [53]. The binding of Fas plasma membrane death receptor to Fas ligand (Fas-L) or other like receptors triggers the extrinsic pathway [54]. Fas-L combines with Fas to form a death complex. The Fas/Fas-L composite binds with pro-caspase-8 and a death domain containing protein (FADD) to form the death-inducing signaling complex (DISC).

The protein complex then activates pro-caspase-8 which then activates pro-caspase-3 [55]. Mitochondrial pro-enzymes control the intrinsic pathway. Stimuli affecting the cell causes outer mitochondrial membranes to become permeable and release cytochrome c into the cytosol In the cytosol, cytochrome c binds with Apaf-1, an adaptor protein, and forms the apoptosome, triggering downstream caspase-9 [56]. Caspases-3 and -7, processed by caspases-8, -9, and -10 are executioner caspases that cleave many substrates, resulting in apoptosis. The biochemical and morphological changes caused by these caspases include membrane blebbing, nuclear condensation, phosphatidylserine exposure, and genomic DNA fragmentation.

Inflammation and apoptosis are heavily related as the onset of inflammation activates a number of signaling pathways that are critical in the regulation of apoptosis. Absent in melanoma 2 (AIM2), a member of the pattern recognition receptors (PRRs) in the cytoplasm, has been found to activate caspase-3 in parallel with caspase-1 [57]. AIM2 can recognize DNA released by the cytosolic bacteria [58], whereas NLRP3, another member of the cytoplasmic PRRs, responds to the bacterial pore-forming toxin nigericin [59], both of which elicit apoptotic caspase activation [60, 61]. Apoptotic responses can be observed in wild type cells responding to AIM2 or NLRP3 stimuli [59]. AIM2 and NLRP3 inflammasome-dependent apoptosis requires caspase-8, which is recruited to the inflammasome through interaction between its DED domains and the pyrin domain (PYD) of apoptosis-associated speck-like protein containing a caspase activation and recruitment domains (CARD), an adaptor molecule of the inflammasome [58, 59, 62]. In contrast, BCL-2 can negatively regulate NLRP3 inflammasome activation by preventing the cytosolic release of mitochondrial DNA [63].

In order to initiate phagocytosis of apoptotic cells, these cells release signals that are composed of either newly expressed molecules or modified existing molecules [64]. Phagocytosis of apoptotic cells is an anti-inflammatory mechanism. Phosphatidyl serine (PS) localized to the outer leaflet of the plasma membrane is the predominant “eat me” molecule upon apoptosis [64, 65]. Specific molecules such as milk fat globule epidermal growth factor 8 (MFG-E8) links PS to phagocyte a_vb_3 integrin [64], whereas growth-arrest-specific 6 (GAS6) links PS to the receptor tyrosine kinase MER [64]. PS acts as a ligand for the T-cell immunoglobulin domain and mucin domain (TIM)-4 molecule on macrophages and dendritic cells (DC) [66], and TIM-4 helps promote the uptake of apoptotic cells [67]. Two other molecules, brain-specific angiogenesis inhibitor 1 (BAI1) and stabilin-2, have also been shown to mediate uptake of apoptotic cells via recognition of PS [68, 69].

Although apoptotic cells are rarely seen under normal physiological conditions, the build-up of uncleared apoptotic cells is an indicator of many distinct diseases and the expression of inflammation and infection Tissue-resident cells, as a response to infection or tissue injury, detect PAMPs and DAMPs. Leukocytes then collect at the site of inflammation. Here, innate immune cells, such as neutrophils, are usually first to appear then macrophages and mononuclear cells appear afterwards [70]. This initial robust immune response is designed to destroy invading pathogens and enhance tissue repair [71, 72]. After the initial threat is eliminated,

leukocytes are cleared. Leukocytes are primarily cleared through neutrophil apoptosis and phagocytosis [73, 74]; however, another route of clearance is transepithelial migration into the airway lumen in regards to lung inflammation [75] or via lymphatic vessels [76]. The phagocytosis of pathogens, such as *Escherichia coli* or *Staphylococcus aureus*, promotes neutrophil apoptosis following neutrophil recruitment, which is termed phagocytosis-induced cell death (PICD) [77]. This response is believed to be primarily protective for the host, and incidentally, pharmacological acceleration of neutrophil apoptosis is protective in pneumococcal meningitis by reducing the incidence of brain hemorrhage [78]. The failed clearance of apoptotic neutrophils can lead to a prolonged inflammatory response, and this phenomenon has been observed in disease, including chronic obstructive pulmonary disease (COPD) [79], pulmonary fibrosis [80] and cystic fibrosis [81]. The production of ROS by neutrophils involves this impaired phagocytosis process, in which ROS activate the GTPase ras homolog gene family member A (RHOA) in surrounding phagocytes and reduces apoptotic cell engulfment by neighboring cells [82–85]. Alveolar macrophages from patients with severe asthma and children with poorly controlled asthma are defective in clearing apoptotic cells [86, 87]. As the mainstay of treatment for asthma, corticosteroids not only induce eosinophil apoptosis [88] but also enhance monocyte-derived macrophage engulfment [89]. The mechanism underlying the enhanced clearance seems dependent on the binding of protein S to apoptotic cells and the upregulation of tyrosine-protein kinase MER on the surface of macrophages [90]. Recently, airway epithelial cells have been found to be capable of engulfing neighboring apoptotic cells, and deficiency of this engulfing function increases pro-inflammatory mediator production and exacerbates airway inflammation [91]. Apoptotic cells are well established to induce the synthesis of anti-inflammatory mediators such as TGF-β, prostaglandin E2, and platelet activating factor by macrophages [92, 93].

In summary, apoptotic signaling pathways may be activated by specific PRRs which contrasts with the traditional model. Furthermore, inflammation is affected by neutrophil apoptosis and the clearance of apoptotic cells. Therapeutic induction of neutrophil apoptosis at the inflammatory site may be a powerful pro-resolution intervention and could fulfill the clinical need to prevent the harmful consequences of inflammation.

4 Pyroptosis and Inflammation

Pyroptosis, a form of cell death, is dependent on the activation of caspase-1. Pyroptosis is characterized by the rupture of the plasma-membrane, releasing proinflmmatory intracellular content. Cell lysis during pyroptosis results from caspase-1-mediated processes [94–102]. Plasma membrane pores dependent on caspase-1 dissipate cellular ionic gradients, producing a net increase in osmotic pressure, water influx, cell swelling, and eventual osmotic lysis, followed by release of inflammatory intracellular content [103]. Cell death due to pyroptosis results in a

measurable cellular size increase and cleavage of chromosomal DNA [96, 98, 103–106].

The inflammasome, a caspase-1-containing complex that activates the proinflammatory cytokines IL-1β and IL-18 and results in proinflammatory cell death, is one of the drivers of pyroptosis. The inflammasome activates caspase-1 through a Nod-like receptor (NLRP1, 3, 6, 7, 12, NLRC4), AIM2, or Pyrin, all of which contain a CARD or PYD [107, 108]. Many inflammasomes recruit the ASC adaptor via homotypic interactions. Additional ASC molecules are incorporated via CARD-CARD and PYD-PYD interactions, until all ASC molecules are collected into a single focus. The recruitment of procaspase-1 into the ASC focus via CARD-CARD interactions results in its dimerization and proximity-induced autoproteolytic processing into the p10 and p20 subunits. This processed and catalytically active caspase-1 cleaves pro-IL-1β and pro-IL-18.

Studies have shown that ASC specks collect in extracellular space and promote maturation of IL-1β after pyroptosis [109]. In addition, phagocytosis of ASC specks by macrophages induces lysosomal damage and nucleation of soluble ASC as well as activation of IL-1β in recipient cells [109]. These findings indicate that pyroptotic cell-released inflammasomes serve as danger signals promoting enhanced activation of macrophages.

IL-1β and IL-18 are inflammatory cytokines secreted after caspase-1 activation by pyroptotic cells. IL-1β is a potent endogenous pyrogen that stimulates fever, leukocyte tissue migration, and expression of diverse cytokines and chemokines [110]. IL-18 induces IFNγ production and is important for the activation of T-cells, macrophages, and other cell types [111]. Cytokine secretion occurs through caspase-1-dependent pores in the plasma membrane. Pharmacological inhibition of cell lysis does not prevent caspase-1-dependent pore formation and cytokine secretion, suggesting that lysis is not required for the release of active IL-1β and IL-18 [103]. Thus, cytokine secretion and cell lysis are both downstream consequences of caspase-1-dependent pore formation. Notably, caspase-1 activation cannot trigger pyroptosis in all cell types; specifically, epithelial cells use caspase-1 activation to prevent cell death—i.e. caspase-1 activation stimulates lipid production and membrane repair in response to the pore-forming toxins aerolysin and α-toxin [112].

In addition to caspase-1, caspase-11 has also been found to be involved in pyroptosis [113–115]. A recent study revealed that caspase-11 participates in the process of non-canonical inflammasome activation downstream of a cytosolic ligand released from bacteria [116, 117].

Pyroptosis can induce pathological inflammation as a defense against infection. However, exuberant or inappropriate caspase-1 activation and pyroptosis can be detrimental. During infection, caspase-1 activation helps to clear pathogens, such as *Salmonella* [118, 119], *Francisella* [120], *Legionella* [102, 121], *Shigella* [122], *Anaplasma phagocytophilum* [123], *Burkholderia thailandensis* [124], *Burkholderia pseudomallei* [125] and *Listeria* [126]. Mutations in nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) proteins can lead to improper caspase-1 activation and can cause hereditary

autoinflammatory syndromes [127]. Moreover, caspase-1 is involved in the pathogenesis of several diseases characterized by inflammation and cell death, including myocardial infarction [128], cerebral ischemia [129], neurodegenerative diseases [130], inflammatory bowel disease [131], and endotoxic shock [132].

As one of the most recently recognized types of cell death, pyroptosis exhibits a particular relationship with common pathogens, and clinic inflammatory disease for caspase-1 connects to both cell death and pro-inflammation directly. Pyroptosis and other caspase 1-dependent processes are therefore relevant to our understanding of the pathophysiology of inflammatory disease.

5 Pyronecrosis and Inflammation

Similar to necrosis, pyronecrosis is a cell death process that is dependent on ASC and lysosomal protein cathepsin B but is independent of caspase-1 and -11. HMGB1, a pro-inflammatory mediator, is secreted as a result of pyroptosis [133]. Recent studies have demonstrated that pyronecrosis can be induced by several pathogens, including *Neisseria gonorrhoeae* [134], *Toxoplasma gondii parasitophorous* [135], *Bacillus anthracis lethal toxin* [136] and *Staphylococcus aureus* [137]. The mechanism underlying pyronecrosis remains unclear at present and requires further investigation.

6 NETosis and Inflammation

A type of polymorphonuclear neutrophil (PMN) death, NETosis releases neutrophil extracellular traps (NETs) [138]. NETs are composed of decondensed chromatin and different neutrophil proteins to form a web-like structure. The purpose of NETs is the capture, neutralization, and clearance of microbes. These large extracellular structures provide a physical barrier to prevent microbial dissemination and increase the local concentration of antimicrobial effectors [139, 140]. NETosis can be categorized by occurrence time, early or late. Late NETosis is more often observed as cell death induced NET release, defined as suicidal NETosis, is a relatively slow process (120–240 min). Suicidal NETosis is NADPH oxidase-dependent and requires chromatin decondensation, followed by nuclear envelope disintegration and mixing of nucleic acids and granule proteins within a large intracellular vacuole [141]. However, it remains unclear how oxidants participate in the dismantling of the nuclear envelope and mixing of the NET components. Classically, suicidal NETosis occurs following stimulation by phorbol myristate acetate (PMA) through activation of protein kinase C and the Raf–mitogen-activated protein kinase (MEK)–extracellular signal-regulated kinase (ERK) pathway. NADPH assists in the translocation of neutrophil elastase from cytosolic granules into the nucleus, where it aids in chromatin breakdown via

histone cleavage. Myeloperoxidase (MPO) is required for chromatin and nuclear envelope breakdown and granular mixing within the NET vacuole. One hundred twenty minutes after intracellular NET formation, the neutrophil outer membrane ruptures, and the mature NET is extruded.

The early form of NETosis occurs rapidly in response to a pathogen, e.g., after in vitro *Staphylococcus aureus* stimulation for 5–60 min. Early NETosis has also been termed vital NETosis in some studies [142]. In general, NETosis begins when the nucleus loses its characteristic lobulated architecture. Subsequently, nuclear membranes disassemble, and the chromatin decondenses into the cytoplasm while the plasma membrane remains intact. Finally, the plasma membrane bursts, leading to NET released [138]. This process is mainly dependent on ROS, such as superoxides generated by the NADPH oxidase Nox2. This mechanism spares the PMN outer membrane, thereby allowing the PMN to continue to function, even to the point of becoming anuclear. There are three major differences between suicidal NETosis and vital NETosis, including the nature of the inciting stimuli and the timing, the functional capacity of the PMNs during NET release, and the mechanisms employed to make and release NETs. In addition to PMN, NETosis has also been observed in eosinophils and mast cells [143]. Therefore, the more generalized term 'ETosis' may be more accurate [144].

Apart from immobilization and capture, NETS are able to directly kill a number of pathogenic bacteria [145–148]. Studies show that bacterial virulence factors can be inactivated by NETs [138]. NETs may also serve to opsonize certain fungi, such as *A. fumigatus* via long pentraxin 3 [149]. NETs generated from PMNs can inhibit the growth of *Aspergillus* [145] and kill *C. albicanscan*, even the opportunistic pathogen *P. aeruginosa* [150]. The gram-negative bacterium *K. pneumoniae* is not sufficient to induce NETosis in isolated neutrophils ex vivo but is a good inducer in a mouse lung infection model [151]. Human immunodeficiency virus (HIV)-1 has been shown to induce NETosis through a cell death pathway [152]. Feline leukemia virus (FeLV) was able to inhibit neutrophil activation by inhibiting the activation of PKC to reduce ROS production [153].

NETs and NETosis are associated with many types of inflammation. NETs are observed in both infection- and sterile- acute lung injury (ALI) models related to influenza virus [154, 155], bacteria or bacterial component LPS [156–158], fungi [148, 159, 160], and transfusion [161, 162]. Among them, human neutrophil antigen (HNA)-3a causes the most severe transfusion-related ALI and has been shown to promote NETosis in human neutrophils in vitro [161]. Extracellular neutrophil elastase release via NETosis may be an important cause of lung tissue damage and cystic fibrosis progression [163]. NETs have been shown to form scaffolds in circulation that promote thrombus formation by interacting with the endothelium, platelets, coagulation factors and red blood cells, which cause deep vein thrombosis. IL-8 and ROS release from endothelial cells can recruit and trigger neutrophils to form NETs, which subsequently promote damage to the endothelium through the binding of histones [164].

NETosis is a type of cell death specific to neutrophils that gives neutrophils the capacity to capture numerous viruses and pathogenic bacteria. A deeper

understanding of the relationship between NETs and invaders would increase comprehension of inflammation and the processes behind it. Furthermore, NETotic products could be treated as prognostic biomarkers for inflammatory disorders, and whether the products correlate with clinical outcome in a variety of diseases requires further translational investigation.

7 Autophagy and Inflammation

Autophagy is a pathway for the degradation and clearance of subcellular component; this pathway is evolutionarily conserved and genetically regulated [165, 166]. Autophagy has previously been classified as a form of programmed cell death to describe a form of caspase-independent necrosis-like cell death associated with the accumulation of autophagosomes in cells [167]. This classification is now controversial, and the causal relationship between autophagy and cell death remains uncertain [168, 169].

When an autophagic isolation membrane, a phagophore, engulfs a portion of cytoplasm, autophagy formation begins [170]. Beclin 1, the serine/threonine protein kinase ULK1, autophagy-related LC3 proteins, and γ-aminobutyric acid receptor-associated proteins are key regulators of phagophore formation [170]. A phagophore sequesters captured cytoplasmic cargo, and a double-membraned autophagosome is formed following elongation and closure. Autophagosome formation is largely controlled by mammalian target of rapamycin (mTOR). Inhibition of mTOR leads to the interaction between ULK1 and AMPK [171, 172], which in turn recruits the type III PI3 kinase VPS34 to promote the development of autophagosome [173, 174]. The degradation of the captured cargo begins when the double-membraned autophagosome matures into a single membrane-delimited autolysosome [175, 176]. Following this step, lysosomes can be recycled from autolysosomes, thereby permitting the cell to reuse a critical component required for further autophagy.

Autophagy can be activated by PRR signaling induced by DAMPs and PAMPs. For instance, TLRs can cooperate with autophagy in response to PAMPs [177, 178], and NLRs can interact with ATGs to localize autophagy [179, 180]. Inflammatory cytokines such as IL-1 family members [181, 182] and IFNγ [183–185] are also involved in the activation of autophagy, whereas T_H2 cell-associated cytokines, IL-4, and IL-13, inhibit autophagy [184].

Multiple studies have confirmed the important role of autophagy during the infection process. Autophagy protects organisms from infectious disease by degrading intracellular bacteria, viruses, and protozoan pathogens [186–188].

Autophagy plays a key role in regulating inflammation; this has been observed in Crohn's disease, a type of chronic inflammation, and sepsis. Polymorphisms in the genes encoding the autophagy-related proteins Atg2a, Atg4a, Atg4d, death-associated protein, immunity-related GTPase family M protein (IRGM), and ULK-1 have been found to be associated with susceptibility to Crohn's disease

[189–191]. NOD2 mutations cause impairment in autophagosome induction and bacterial clearance [179]. Autophagy formation downstream of NOD2 activation controls IL-1β and IL-6 release [192, 193] and results in the tolerogenic presentation of commensal bacterial components on MHC class II complexes in dendritic cells [180]. Inhibition of autophagy in septic mice boosts inflammatory cytokine levels and increases mortality. This effect may be due to the failure to clear damaged or dysfunctional mitochondria, which activate the NLRP3 inflammasome [194].

Although the relationship between autophagy and cell death remains uncertain, several members of the inflammation process are involved in autophagy. The function of autophagy in related inflammatory diseases requires further investigation. A better understanding of the relevance of the contribution of autophagy to inflammatory diseases has great clinical potential.

8 Trauma Regulation of Cell Death

Several experimental and clinical studies have shown that surgical and trauma injury markedly affects the immune system, including both the specific and the nonspecific immune responses [195–198]. The protective immunity of the hosts may critically depend on an appropriate cytokine balance, proper activation and recruitment of PMNs and monocytes/macrophages, an intact macrophage–T-cell interaction, and an adequate T-helper (Th)1/Th2 conception of T-helper cell activation. The surgical and trauma injury potentially disintegrates these complex regulatory systems and induces the deterioration of immune function [195–198]. Trauma is an important regulator of cell death, not only through damaging tissue and cells, but, more importantly, also through releasing endogenous danger signals that induce regulated cell death, and thereby, influence the development of post-trauma inflammation and subsequent organ dysfunction. Trauma regulation of cell death exhibits complicated mechanisms and multiple facets associated with different post-trauma situations, including temporal and infectious factors.

8.1 Trauma Regulation of Macrophage Autophagy

Patients are especially susceptible to a secondary inflammatory stimulus as a response to multiple organ dysfunction syndrome (MODS) and systemic inflammatory response syndrome (SIRS) after major trauma and surgery that results in hemorrhagic shock (HS). This increased susceptibility is due to a cell priming mechanism [199]. ALI, a main cause of patient death following HS, is a major component of MODS. Inflammation is self-regulated through the balance of interaction between pro- and counter-inflammatory factors. Alveolar macrophages (AM) are critical to the development of inflammation. Macrophages are activated

via families of related PRRs, including TLRs and NLRs [200–203]. NOD2, the product of *CARD15*, is a member of a growing family of NLRs that have been implicated in the regulation of immune responses and cell death in animals and plants [204, 205]. NOD2 acts as a cytosolic recognition molecule of bacterial peptidoglycan (PGN), which is found on both Gram-positive and Gram-negative bacteria, through specific detection of the conserved muramyl dipeptide (MDP) structure [206]. NOD2 have been shown to associate with RIPK2/RICK, via CARD-CARD interactions, which allow RIPK2 to associate with TRAF6/TAK1 [207]. Subsequent signaling leads to activation of NF-κB and upregulation of inflammatory mediators, such as IL-6 [207]. Studies have also shown that NLRs, including NOD2, regulate autophagic processes during bacterial infection, which are now recognized to influence pro- and anti-inflammatory responses in cells [179, 180].

We demonstrated that HS upregulates NOD2 expression in AM through HMGB1/TLR4 signaling. Upregulated NOD2 subsequently sensitizes AM to respond to NOD2 ligand MDP, which initially leads to augmented inflammation in the lung. NOD2 signaling also induces autophagy in AM, which in turn exhibits a potent anti-inflammatory effect on lung inflammation at later time points, thereby negatively regulating inflammation. However, this anti-inflammatory effect was concealed by HS-activated PMN that migrated into alveoli and counteracted the effects of autophagy in AM (Fig. 2) [208]. This study identifies a previously unrecognized HMGB1/TLR4-NOD2-autophagy axis that serves as a macrophage self-regulatory mechanism governing post-HS inflammatory responses to bacterial products. The findings also explored a novel function of PMN NAD(P)H oxidase-derived oxidant signaling in enhancing HS-primed lung injury. PMN NAD(P)H oxidase activates transcellular oxidant signaling through its ability to counteract the autophagy-induced anti-inflammatory mechanisms, and therefore enhances post-HS lung inflammation and injury. In the broadest sense, these findings may also be valid in other human diseases in which macrophages play a role, including diseases associated with acute and chronic inflammation.

8.2 DAMP Molecule HMGB1 Triggers Pyroptosis

DAMP molecule HMGB1 is a ubiquitous protein present in almost all cell types in the cytoplasm and nucleus that can regulate and activate macrophages [209]. During infection and sterile tissue injury, HMGB1 is released from cells and serves as a necessary and sufficient mediator of inflammation to induce a variety of cellular responses including cell chemotaxis and release of pro-inflammatory cytokines [210, 211]. Inflammatory functions of HMGB1 are mediated by binding to the cell surface receptors, including the receptor for advanced glycation end products (RAGE), TLR2, TLR4, and TLR9 [212, 213]. RAGE is a type I transmembrane protein and a member of the immunoglobulin superfamily expressed in many cell populations including endothelial cells, vascular smooth muscle cells, neurons,

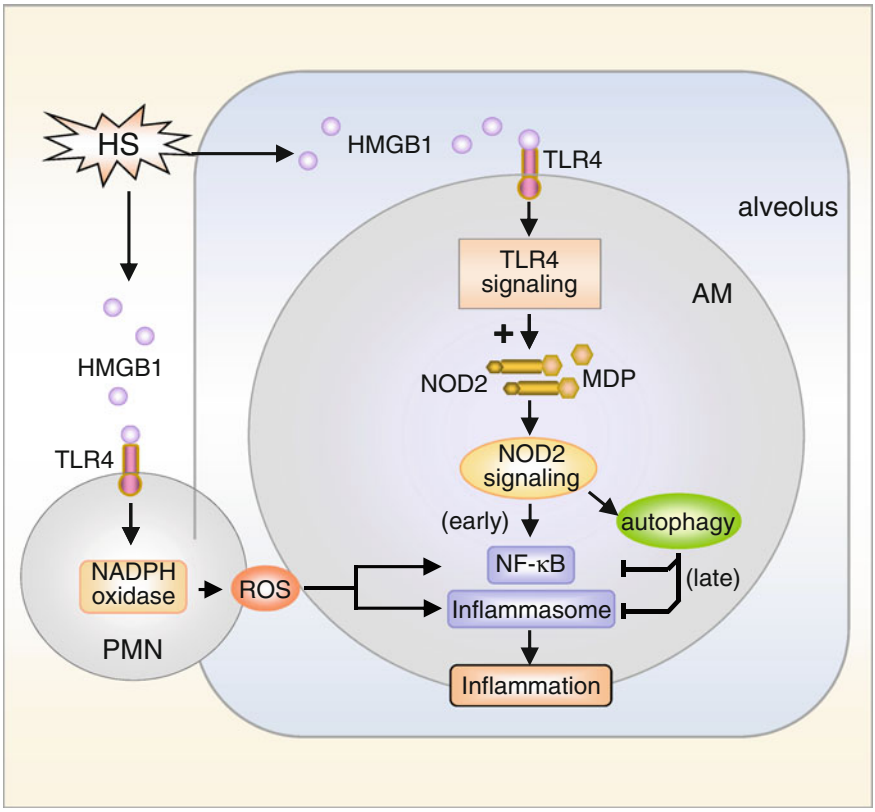

Fig. 2 PMN counteraction of autophagic anti-inflammatory mechanisms to augment ALI following hemorrhagic shock (HS). HS increases HMGB1/TLR4 signaling upregulates NOD2 expression in alveolar macrophages (AM), with a subsequent sensitization of AM to NOD2 ligand MDP, which leads to augmented inflammation in the lung. Additionally, upregulated NOD2 signaling induces autophagy in AM, which in turn negatively regulates lung inflammation by suppressing NOD2-RIP2 signaling and inflammasome activation. PMN counteract the anti-inflammatory effect of autophagy, possibly via NAD(P)H oxidase-derived ROS, and therefore enhance post-HS lung inflammation

neutrophils, and macrophages/monocytes [214]. RAGE has been implicated as a receptor mediating the chemotaxis and cytokine activity of HMGB1 in macrophages and tumor cells [213, 215, 216]. RAGE engagement by multiple ligands is linked to a range of signaling pathways including activation of NF-κB [217, 218], PI3K/Akt [219], MAPKs [220, 221], Jak/STAT [222], and Rho GTPases [223], although how RAGE transduces the signaling is not fully addressed.

A novel pathway of HMGB1-induced pyroptosis has been recently identified. We demonstrated that HMGB1 acting through RAGE on macrophages triggers dynamin-dependent endocytosis of HMGB1, which in turn induces cell pyroptosis. The endocytosis of HMGB1 initiates a cascade of molecular events, including

cathepsin B (CatB) activation and release from ruptured lysosomes, followed by pyroptosome formation and caspase-1 activation (Fig. 3) [224].

Endocytosis plays important roles in many different areas of cell biology, ranging from the uptake of nutrients to regulation of intercellular signaling [225]. Endocytic pathways have been mainly classified as clathrin-dependent or clathrin-independent [225], the later can be further classified as dynamin-dependent or dynamin-independent pathway. Dynamin is a large GTPase directly involved in pinching off endocytic vesicles from the plasma membrane [226–228]. For many years, endocytosis of ligand molecules has been considered as a mechanism of signal attenuation via receptor and ligand clearance from the cell surface [229, 230]. The finding that the dynamin-dependent endocytosis of HMGB1 activates a cascade of intracellular events leading to cell pyroptosis, rather than diminishes the effect of

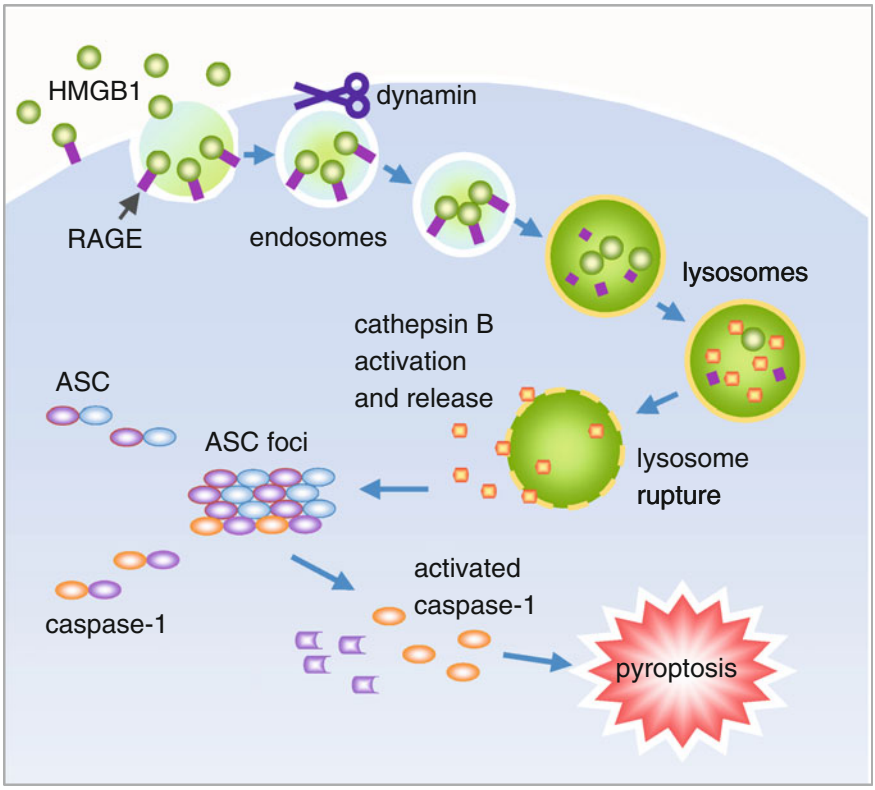

Fig. 3 Macrophage endocytosis of HMGB1 induces pyroptosis. HMGB1 acting through RAGE on macrophages triggers dynamin-dependent endocytosis of HMGB1, which in turn initiates a cascade of cellular and molecular events. These include CatB activation and release from ruptured lysosomes followed by pyroptosome formation and caspase-1 activation, which promotes HMGB1-induced pyroptosis

HMGB1, represents a shift in our understanding of the significance of ligand endocytosis.

8.3 *Tissue Damage Negatively Regulates LPS-Induced Macrophage Necroptosis*

After severe trauma or surgery, infection is a common complication affecting patients [231]. The immune system responds to infection by releasing proinflammatory mediators. This response can have serious consequences. Research has established that antecedent trauma and tissue damage caused cell pre-activation heavily affects innate immune cell response to a secondary infectious stimulus. This affected response is typically expressed through enhanced inflammation [224, 232, 233].

It has been observed through previous studies that DAMPs released after trauma serves as a priming factor, which increases the inflammatory response to infection [234–239]. Those results, therefore, suggested that blocking DAMP-signaling may attenuate inflammatory responses to a secondary infection. In the current study, however, we demonstrate a novel finding that tissue damage suppresses subsequent LPS-induced macrophage necroptosis through DAMP-signaling, thereby exhibits a negative regulatory effect on the inflammatory response to a secondary LPS stimulation. We show, as others have, that LPS acting through TLR4 promotes macrophage necroptosis. However, in the setting of trauma, release of HMGB1 by damaged tissue upregulates caveolin-1 expression in macrophage via HMGB1/RAGE signaling, which in turn induces caveolae-mediated TLR4 internalization to reduce LPS-TLR4-induced macrophage necroptosis. Part of the mechanism for upregulation of caveolin-1 is through RAGE-MyD88 signaling and downstream activation of Cdc42 leading to nuclear translocation of transcription factor Sp1 and alteration of caveolin-1 expression (Fig. 4). It seems clear that transcriptional upregulation of caveolin-1 is important in inducing TLR4 internalization; although posttranscriptional modification of caveolin-1, i.e. tyr14 phosphorylation may also needed [240]. Data from this study therefore suggest that DAMP molecules, in a defined period following tissue damage, are not just pro-inflammatory but can also negatively regulate host inflammatory responses to LPS, as shown by our in vivo findings. This suggests that targeting DAMP molecules as a therapeutic strategy for post-trauma inflammation may need to take timing or potential treatments into consideration to avoid bad outcomes.

In summary, trauma is a significant regulator of cell death. Trauma regulation of cell death exhibits complicated mechanisms and multiple facets associated with different post-trauma situations, including temporal and infectious factors. As shown in Fig. 5, trauma causes tissue damage and induces release of DAMP molecules, i.e. HMGB1 and cold-inducible RNA binding proteins (CIRP). HMGB1 acting through RAGE and dynamin-dependent signaling induces macrophage

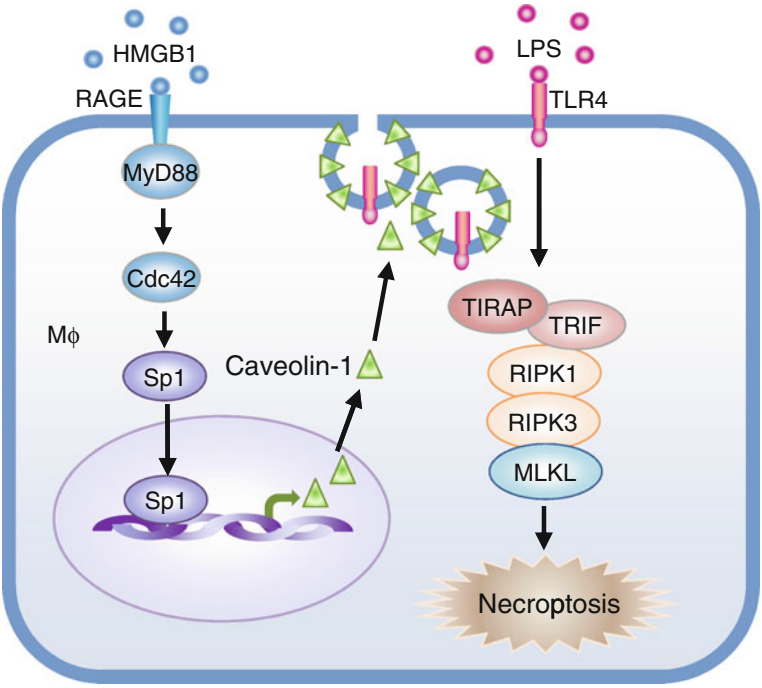

Fig. 4 Mechanism underlying tissue damage regulation of LPS-induced macrophage necroptosis. LPS acting through TLR4 promotes macrophage necroptosis. However, damaged tissue through HMGB1/RAGE signaling upregulates caveolin-1 expression in macrophage, which, in turn, induces caveolae-mediated TLR4 internalization and desensitization, thereby, ameliorates LPS-TLR4-induced macrophage necroptosis. RAGE-MyD88 signal activation of Cdc42 and the consequent nuclear translocation of Sp1 serve the mechanism of upregulation of caveolin-1

pyroptosis. On the other aspect, HMGB1 suppresses LPS-induced necroptosis. HMGB1 also up-regulates NOD2 expression, which, in turn, sensitizes the macrophage to NOD2 ligand MDP and results in autophagy in the macrophage. DAMP molecule CIRP acting through TLR4 causes mitochondria DNA fragmentation, and subsequent autophagy and necroptosis of the macrophages. The autophagy serves as a negative regulator suppresses necroptosis. However, hemorrhagic shock, a systemic ischemia/reperfusion process inhibits autophagy and therefore, enhances macrophage necroptosis.

9 Conclusion and Prospective

The strong connection between inflammation and cell death has been observed through recent research. A better appreciation of the cross-regulatory relationships between different forms of cell death and pathways will be crucial for understanding

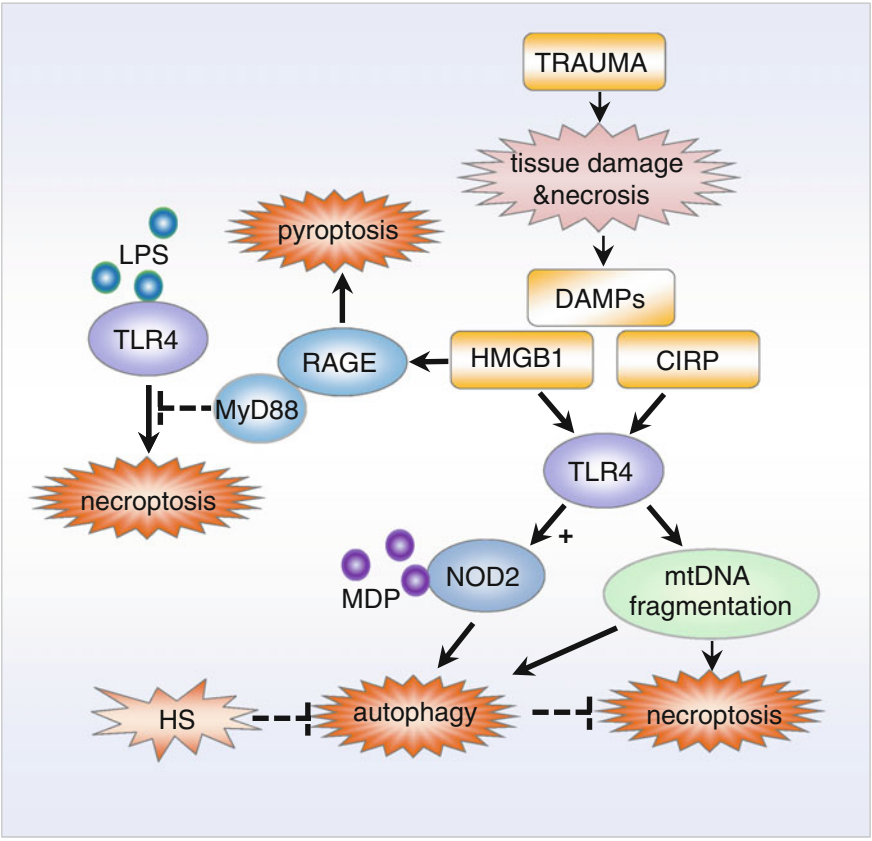

Fig. 5 Trauma regulation of macrophage death. Trauma is a significant regulator of cell death. Trauma causes tissue damage and induces release of damage-associated molecular pattern (DAMP) molecules. DAMP molecule HMGB1 acting through RAGE and dynamin-dependent signaling induces macrophage pyroptosis. On the other aspect, HMGB1 suppresses LPS-induced necroptosis. HMGB1 also up-regulates NOD2 expression, which, in turn, sensitizes the macrophage to NOD2 ligand MDP and results in autophagy in the macrophage. DAMP molecule cold-inducible RNA binding proteins (CIRP) acting through TLR4 causes mitochondria DNA fragmentation, and subsequent autophagy and necroptosis of the macrophages. The autophagy serves as a negative regulator suppresses necroptosis. However, hemorrhagic shock, a systemic ischemia/reperfusion process inhibits autophagy and therefore, enhances macrophage necroptosis

their roles in the inflammation process. It is crucial that we comprehend the therapeutic possibility of targeting programmed cell death in patients as an increased understanding of the pathways controlling programmed cell death will allow the development of reagents that can regulate cell death, thereby serving as a novel strategy for interventions in inflammatory diseases. Some types of cell death that do not seem to be related to inflammation may also be considered in future studies in

light of their possible interaction with inflammation; these approaches will help us better understand the entire inflammatory process network.

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To Explore Sepsis, We Need New Thought

Lei Li, Guie Liu and Qing Ouyang

Abstract Sepsis is a life-threatening condition initiated by invasion of pathogenic microorganism. In essence, it refers to the excessive inflammatory response induced by various harmful stimulating factors after infection. Numerous sepsis-related factors, like triggers, involved cells and receptors, signaling pathways and secreted products by immune or inflammatory cells, form a complex network, confusing and frustrating current researchers. For this reason, we need a new way of thinking and the term-*inflammationomics* might be an idea.

Keywords Inflammatory response · Inflammatory cells · Inflammationomics

Sepsis, in essence, refers to the inflammatory damage caused by excessive stimulation of various harmful stimulating factors on the body. Since 1991 when the modern concept of sepsis was proposed, its definition has been constantly revised, with both the connotation and denotation. The treatment strategies and techniques are improved as well. However, it is a pity that up to now sepsis-induced mortality rate has not been reduced significantly [1, 2]. The development law of medical science tells us that the establishment of an ideal treatment depends on the full understanding of the pathogenesis of a disease. It is obvious that for sepsis, there are still many unknowns that need to be explored.

1 Diversity of the Triggers for Sepsis

The main stimuli to sepsis response on body are harmful pathogenic bacteria. In addition, fungi, viruses and parasites also can invoke sepsis, and even some normally harmless bacteria in some cases can induce sepsis as well. In fact, the stimulus for the

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occurrence and development of sepsis is not limited to pathogenic microorganisms [3]. The broken and necrotic cells, cell metabolites such as high uric acid, and even the disorder of electrolyte balance can induce or aggravate the inflammatory response of the body and eventually lead to severe inflammatory damage [4]. The stimuli to the occurrence and development of sepsis are complex and varied. Therefore, it is hard to improve the therapeutic effects of sepsis by only blocking one or two stimuli [5].

2 Variety of Cells Involved in Sepsis

For a long time, there has been a misunderstanding that the inflammatory response is only an immune consequence induced by sepsis and those participating in the response are immune cells [6]. In fact, cells involved in the sepsis response are diverse, mainly in two aspects. First, the cell types are varied. Not only the traditional immune cells, such as mononuclear macrophages, lymphocytes and granulocytes directly take part in the inflammatory response, but also some somatic cells such as endothelial cells, liver cells, etc., are involved in the synthesis and release of inflammatory mediators. Second, the immune cells in the inflammatory response themselves have subsets or varied polarity. Usually, they participate in the inflammatory response in the form of subsets or with different polarity, either accelerating, or maintaining, or restraining the inflammatory response [7, 8]. The most typical ones are ratio of CD4/CD8 and the shifts of Th1/Th2 subsets [9]. Recently, even the macrophages have been found to have obvious polarity and subsets, which takes major role to balance the inflammatory response stimulated by harmful factors. Therefore, it is not enough to achieve effective treatment for sepsis only by regulating a certain cell, which is no doubt like a drop in the bucket. In recent years, some articles report relatively ideal therapeutic results of severe sepsis from animal experiments by stem cells injection. However, whether the stem cells are effective for human body is still unclear and needs to be tested and verified [10].

3 Intricacy of Receptor Signal Pathways in Sepsis-Related Inflammation

Various harmful factors are likely to be the activating factors of sepsis [11, 12]. The recognition of harmful factors by pattern recognition receptor is the key link in the initiation of sepsis. Besides, the most important event that induces the inflammation shifting toward to sepsis is out of control of the dynamic network balance of the protein signaling pathways of inflammation-related receptors [13]. The intricacy is expressed in at least three aspects: First is the intricacy of receptor locations. That

is, the receptor can be at the cell membrane, in the cytoplasm or in the nucleus, and even outside the cell, there are soluble receptors with negative regulatory role. Second is the diversity of receptor functions: accelerating or inhibiting the inflammatory response. Third is the diversity of reaction ways: direct activation or direct inhibition of inflammatory response; acceleration effect of positive feedback, or speed limit effect of negative feedback. This vast network balance is to maintain the balanced development of the inflammatory response. Otherwise, if it is out of control, the whole body will face a catastrophe [14].

4 Complexity of the Metabolites of Sepsis-Related Inflammation

Once the sepsis has been initiated, the outbreak release of various inflammatory mediators claims major responsibility for the development of sepsis, which eventually induces multiple organ failure and leads to death [15]. Inflammatory mediators, according to their functions, can be divided into pro-inflammatory factors which promote the development of inflammation and anti-inflammatory factors which suppress inflammatory response (immunoparalysis) [16]. They can be also divided into vasoconstriction promotion factors and capillaries dilation inducing factors which cause the outleakage of blood constituent; or blood coagulation promotion factors which accelerate thrombotic formation and overactive fibrinolysis inducing factors. According to their own physicochemical properties, inflammatory factors can be divided into proteins, lipids, polysaccharides, and even electrolytes which may involve in the development of sepsis [17]. Various mediators combine as a huge network and interact with each other as both cause and effect. Through this way, they together regulate the occurrence, development and final results of sepsis. Therefore, it is meaningless to control septic inflammatory by blocking the release of inflammatory mediators [18, 19]. As mentioned above, the pathophysiologic process of sepsis is very complex, so single and linear thinking mode is no longer suitable for sepsis study. The implementation of human genome project is prominent not only because it is a landmark for the human to fully understand themselves, but also because it provides a new mode of thinking. Especially with the development of chip technology and the arrival of the big data era, the concept of "-omics" provides us with a new thinking way in the face of complex pathophysiologic status of diseases. We used DNA chip technology to carry out a large scale of detection, analysis and comparison of gene expression, which brought the birth of genomics. In the same thinking way we proposed proteomics, which means we analyze and compare the expressions, functions and interactions among a family of proteins for one type of cells or tissues.

Correspondingly the concepts of enzymeomics and metabonomics are put forwarded. Therefore, it is necessary to set up a thinking mode of "-omics" in inflammation study, namely inflammationomics, which carries out systematic research on the occurrence, development and regulation of inflammatory diseases. As for sepsis response, when the body is exposed to a variety of harmful stimuli, inflammatory reaction cell receptors on the membrane and plasma are rapidly activated, and all the related inflammatory signal protein networks are aroused sequentially, which leads to a cascade amplification and activation of a series of inflammation mediators. Centralized release of abundant inflammation mediators is completed in a very short time, just like waterfall. Corresponding negative regulation pathways and proteins are also rapidly activated. A large amount of anti-inflammatory mediators, especially those inhibiting body's immune defense functions, are synthesized and released to achieve a new dynamic balance. When the dynamic response is in control and reduces normally, the patient's condition is improved. On the contrary, if the dynamic balance gets out of the control and goes to the extreme, the disease tends to deteriorate and eventually leads to death.

5 Puzzle in the Treatment of Sepsis

There are so many reports declaring that they could effectively prevent the occurrence and development of sepsis, even significantly reduce the mortality rate of severe sepsis recently. However, the common sense suggested that the more the treatment methods, the less the therapeutic effects. As the four aspects mentioned above, our limited understanding of the mechanism of sepsis seriously restrict us to produce accurately assessment about the pathophysiological state of sepsis patients. In addition, the current deficient detection techniques also limit the accurate estimation of immune status on sepsis patients.

Though the mechanism of the occurrence and development of sepsis remains unclear, it may be an ideal strategy for sepsis treatment that we speculate sepsis patient's immune function condition based on some existing immunological or inflammatory indicators and then carry out targeted therapy. Unfortunately, there are at least three shortcomings we have to face right now, real time, specificity and dynamics.

For real time, it means that these techniques can immediately reflect the patient's condition. Regrettably, some significant data reach physician' hand after 12 or even 24 h, even more due to the obligatory process of collecting samples, sending for clinical laboratory, machine testing and analyzing, etc. It is well known that the condition of sepsis patient changes rapidly within 3–5 days after trauma, and therefore, the delayed parameters can hardly reflect the real state of the patient.

For specificity, actually every detected indicator has its own limitation. TNF-alpha, whose original biological significance is in the killing effect of tumor cells and promotion of cell apoptosis, has a raised expression early and reach to its peak at around 2 h after severe trauma. Normally speaking, the more severe the trauma is, the higher level TNF-alpha will reach. But for some very severe trauma, TNF-alpha may do not show any increase respond and go to profoundly decrease directly. Theoretically, the Gram-negative bacteria may stimulate the expression of TLR4. In fact, there are a variety of negative feedback signal pathways which could inhibit the elevated expression of TLR4, therefore, TLR4 often are present in an abnormal low level. Actually, the number of cytokines involved in trauma or sepsis is large. That is how the term "cytokines storm" derived. To determine the patient's status simply through the level of several cytokines is far from enough and may have the adverse effect of overgeneralization, which is no doubt like the Chinese proverbial the blind men and the elephant. Apparently, the more specific parameters related to the pathophysiological status of sepsis patient are emergent needed, in spite of there are long way to go.

For dynamics, immunological parameters change with human biological circadian in one day even under normal physiological state. Definitely, this change becomes even intense after trauma, especially at the early stage. Hence, the routine daily monitoring can not reflect the true condition of the patient. Therefore, instantaneous, dynamic and sequential monitoring, especially at the early period of trauma sepsis, should be paid particular attention.

In fact, pathogenic microorganisms are the key stimuli triggering sepsis inflammatory reaction. Broken tissues and necrotic cells, electrolyte balance disorder, and some noninfectious harmful stimuli promote the development of sepsis. Receptors recognizing various noxious stimuli exist not only on the cell membrane, but also in the cytoplasmor even outside the cell. Inflammation factors are usually proteins, but some nonproteins like lipid, reactive oxygen species (ROS) also directly participate in the development of inflammation. Therefore, it is necessary to study thewhole process of inflammatory systematically and comprehensively. The authors think that the so-called inflammatoryomics means that inflammation study, based on functional proteomics, should focus on inflammation-related genome and proteome, meanwhile combine the nonproteins involved in the inflammatory reaction to reveal the dynamic changes and interactions among the abovementioned substances and systematically explain the pathophysiologic process of the occurrence and development of inflammation. Inflammatoryomics provides a foundation for human intervention and reasonable regulation of various inflammatory diseases. All in all, we would like to summarize Inflammatoryomics as the following diagram (Fig. 1).

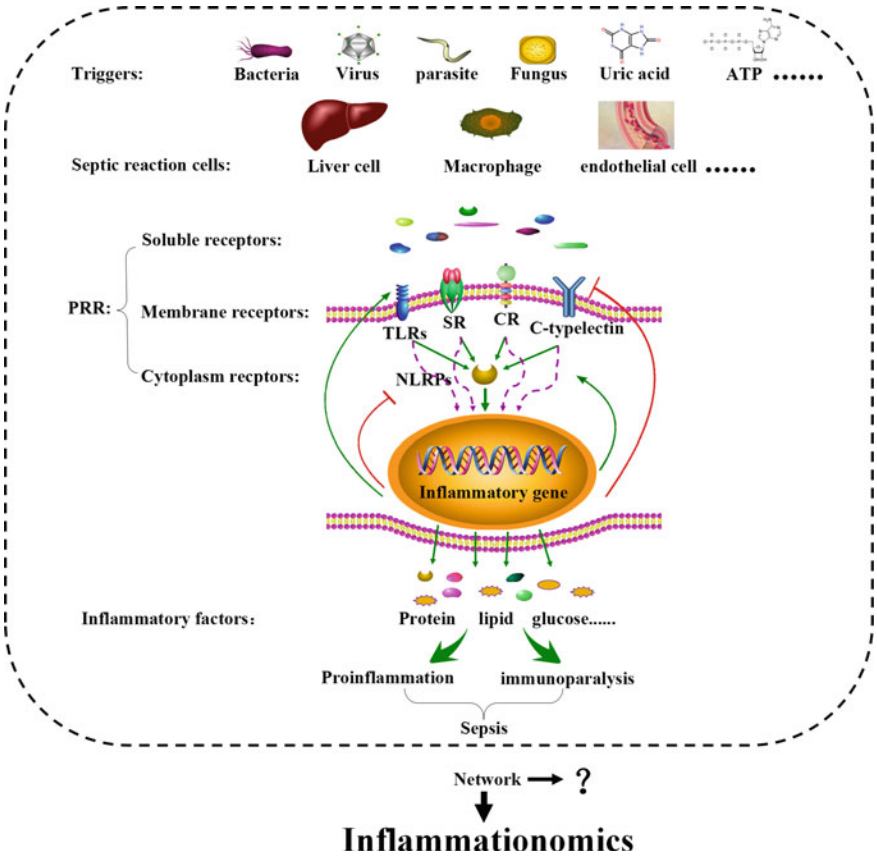

Fig. 1 Normally, sepsis is triggered by infection, however besides the microorganism, many harmful factors, such as necrotic cells, adenosine triphosphate, etc. also take part in the process of the excessive inflammatory response after infection and tissue injury. Not only the immune cells, such as macrophages, DC cells, but some other cells like liver cells, endothelial cells, etc., play important roles during sepsis as well. The pattern recognition receptors involved in sepsis cover extracellular proteins, transmembrane proteins, cytoplasma proteins, even nuclear proteins. These proteins constitute a complex signaling pathway network to regulate or balance the septic response. After being stimulated, the septic cells deliver overwhelmed inflammatory cytokines or factors that cause organ dysfunction and other severe inflammatory damage. Faced with such a crisis, a simple ordinary way of thinking may need to be replaced by "**omics**" idea

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Regenerative Medicine in China: The Capacity, Capability and Reliability

Biao Cheng, Shuliang Lu, Xiaosong Gu and Xiaobing Fu

Abstract Regenerative medicine (RM) is an emerging interdisciplinary field of research. Its clinical application focuses on the repair, replacement and regeneration of cells, tissues, or organs by approaches including stem cell transplantation, tissue engineering, and colonel treatment. RM has become a hot point of research in China and other countries. China has developed the research quickly and impressed the world with numerous research findings in stem cells, tissue engineering, active molecules and gene therapy. China's main and local governments have attached great importance to RM and given strong support in relevant policies and funding. About 3.5 billion RMB has been invested in this field. Since 1999, China has established more than 30 RM centers. Important directions are induced differentiation of induced pluripotent stem and embryo stem cells as well as somatic stem cell differentiation potential and their application in trauma, burns, diseases of aging and nerve regeneration. The products ActivSkin and bone repair scaffolds have been approved and are applied in the clinic, and similar products are being studied. About 10 engineered growth-factor drugs for repair and regeneration have been approved and are used in the clinic. Gene therapy, therapeutic clone and xeno-

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transplantation are widely of the strategies being studied. China cooperates with many advanced countries in RM research and benefits from their cooperation. However, China needs to develop standards, regulations and management practices suitable for the healthy development of RM. Aspects that should be strengthened include sound administrative systems, laws, and technical specifications and guidelines; conservation of stem cell resources; emphasis on training and retention of talented stem cell researchers; and reasonable allocation of resources, diversification of investment and breakthroughs in genetic and tissue engineering, etc areas. Finally, broad and deep international cooperation is necessary.

Keywords Regenerative medicine · Stem cell · Tissue engineering · China

1 Background

Annually, about a hundred million Chinese patients will receive treatment by tissue repair and regeneration technologies because of the sharp increase in various injuries, accidents and diseases of aging. However, the current paradigm of "healing by scar tissue replacement", regardless of superficial tissues or visceral organs, is stagnating and is far away from the ultimate goal of "regenerating the impaired organ". Regenerative medicine (RM) is gradually being used to restore the intrinsic repair ability with stem cell transplantation, tissue engineering, activating factors, cell reprogramming and genetic treatments. RM holds sound promise of restoring organ function that is impaired because of congenital disorder, acquired disease, trauma and aging by replacing or regenerating cells, tissues and even organs. RM is expected to transcend traditional organ transplantation and replacement. Stem cell technology and tissue engineering have an outstanding role in RM. RM will become one of the most promising areas of life science in the 21st century [1].

In the past 20 years, the RM market has continued to grow in China and other countries such as the United States of America, Europe, Japan, and Singapore. As the largest developing country, China has impressed the world with its findings in stem cells, tissue engineering, active molecules and gene therapy as well as its national strategies and regulation of RM. These achievements may benefit China in both disease treatment and society development [2].

2 Strategies, Guidance, Funding and Industrialization of RM in China

2.1 National Strategies

The central government of China supports the development of RM. In the 2006 *National Plan for Long-and Medium-Term Scientific and Technological Development* (2006–2020), stem cells and RM technologies were the important fields among the five biotechnologies [3]. Also, local governments have adopted stem cell research as one of the priorities of technological development and provided active support. Relevant government departments and academia have paid close attention to and encouraged the development of RM. In the *Science & Technology on Public Health in China: A Roadmap to 2050*, issued by the Chinese Academy of Sciences (CAS) [4], and the Study on the Long- and Medium-Term Development Strategy for China Engineering Science and Technology, issued by the Chinese Academy of Engineering (CAE) [5], RM was considered a major research field. Industrialization of RM is a part of the "12th Five-Year Planning" and will be nurtured as a source of economic growth.

The strategic science and technology projects from CAS can be divided into "Forward-Looking Strategic Priority Research Program of Science and Technology" and "Construction of Research Centers for Basic and Forefront Scientific Research". Academia held three times Xiangshan Science Conferences on RM, in 2005, 2010 and 2015, to discuss the philosophy, scope and major breakthroughs needed for the development of RM in China and the key scientific issues to be addressed. In addition, the Xiangshan Science Conference organized seminars on stem cell biology and cloning, strategies for research and development of gene therapy and biomaterials, and tissue engineering.

2.2 Regulatory and Scientific Guidance

Policies and regulations reflect that China is gradually strengthening the management of RM research and clinical application. Since 1999, when the Ministry of Health (MH) promulgated the first "*Umbilical cord blood stem cell bank management approach (Trial)*", about 30 rules and regulations have been issued by the Ministry of Science and Technology (MST), the MH and the State Food and Drug Agency (SFDA) (Table 1). In 2011, The First National Stem Cell Research Guidance and Coordination Committee was established for the overall design and scientific planning of stem cell research in China. In December 2011, the "Notice on carrying out self-inspection and self-rectification campaign regarding stem cell clinical research and application" was issued. In 2013, the stem cell clinical research and application rectification lead group of the MH and SFDA formulated the regulations "*Management Specification of Stem Cell Clinical Trials (Trial)",*

"Management Specification of Stem Cell Clinical Trial Research Base (Trial)" and "Stem cell Preparation Quality Control and Pre-clinical Research Guidelines (Trial)". These regulations will be implemented soon and help in the development of RM in China. In 2015, MH and SFDA approved to open the clinical trial of stem cells in qualities hospitals, which will accelerate the stem cell translational application in the future.

2.3 Funding Support and Resources

Multiple sources are funding RM studies and translational application. After 1999, the MST approved the National Program on Key Basic Research Project (973 Program) related to tissue engineering, and the stem cell field had the largest number of "973 Program" projects. Research into the clinical transformation and application of stem cell therapy was established in the biotechnology and medical technology field of the "863 Program". The National Natural Science Foundation of China (NSFC) funded about 2000 and 2 hundred million RMB Yuan for this study, including 5627 items (Fig. 1a, b). Up to now, about 3 billion RMB from the MST, CAS and NSFC has been invested in this field. Both the amount of funding and number of projects is increasing annually. Other funding for RM from companies is about 5 hundred million RMB. By the end of 2015, China MST approved its first key project in the fields of science and technology was stem cells and their translational application.

2.4 Translating Outcomes into Industrialization

The establishment of centers and technological translational application are important for RM development. Since 1999, China has established more than 30 RM centers. These centers are involved in stem cell research and its translational application (e.g., national stem cell east centre and national stem cell centers in Tianjin, Qingdao, Wuxi, Taizhou etc.) [6], national stem cell and RM technology innovation strategic alliance (sponsors and governing members include 27

Table 1 Management specification of stem cell transplantation techniques

Time	
2006	Unrelated hematopoietic stem cell transplantation technique
	Unrelated hematopoietic stem cell transplantation collection technique
2009	Cord blood stem cell therapy technology (trial)
	Tissue-engineering tissue transplantation therapy technique (trial)

(Reprint from Biao Cheng, Shuliang Lu and Xiaobing Fu, Regenerative medicine in China: demands, capacity, and regulation. Burns & Trauma 2016. 4:24)

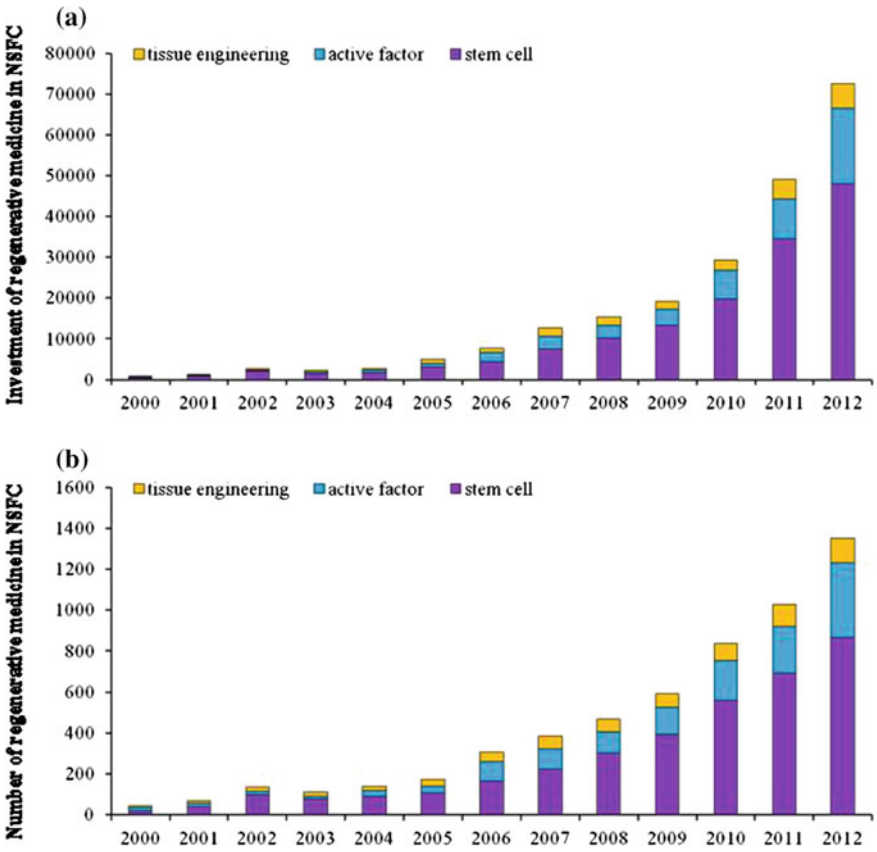


Fig. 1 National Natural Science Foundation of China investment in regenerative medicine from 2000 to 2012. **a** Amount of funding; **b** Number of funds (Reprint from Biao Cheng, Shuliang Lu and Xiaobing Fu, Regenerative medicine in China: demands, capacity, and regulation. Burns & Trauma 2016. 4:24)

first-class research institutes, well-known three-A hospitals, several "211 Project" key universities and industry leaders) and a tissue-engineering innovation center in Shanghai. In 2011, the first academic workstation for the industrialization of stem cell technology was launched in the Inner Mongolia Autonomous Region. Companies such as Cyagen Biosciences (Guangzhou) and Hangzhou Biowish Technology (Biowish) are specialized in the development and sales of stem cell products. In 2009, NeoStem announced that it had reached an exclusive agreement for strengthening biomedical cooperation with Shanghai enterprises. This agreement aims to establish a network of stem-cell collection and treatment centers in Shanghai, Jiangsu, Zhejiang, Fujian, Anhui and Jiangxi provinces. In 2010, the Beike Stem Cell Bank and Stem Cell Preparation Laboratory successfully passed the ISO9001 quality management system certification and obtained the qualification

certification issued by China Quality Certification Center, becoming the first comprehensive stem cell bank to pass ISO9001 quality management system certification in China [7].

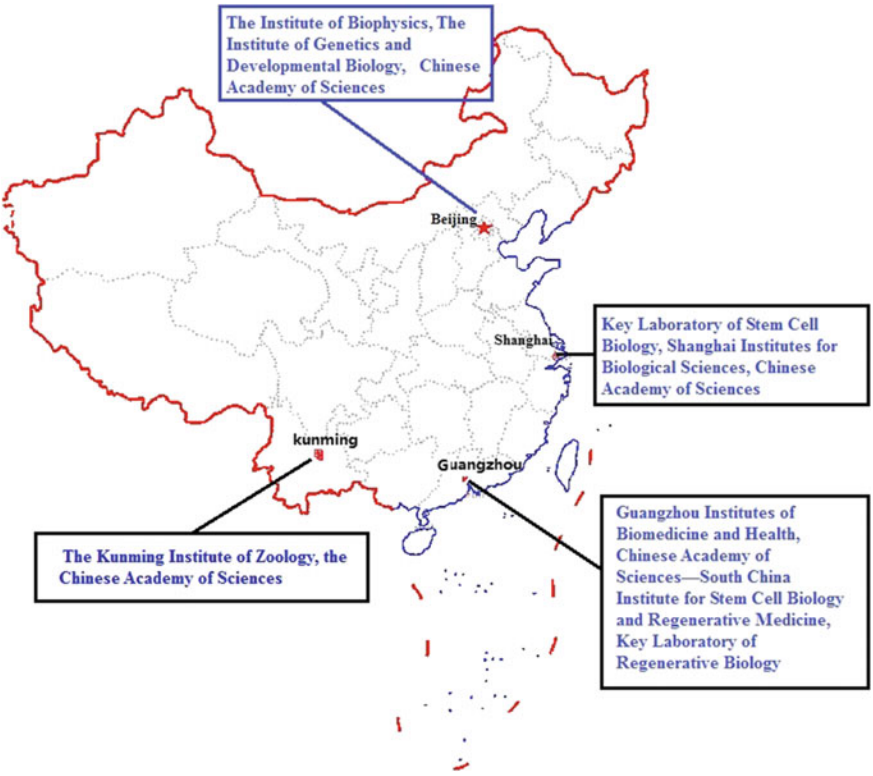
3 Major Progress in RM in China

Since 2002, major foreign media paying attention to the progress of RM in China included the *Wall Street Journal*, *New Scientist* and *Times*, and *Journal of Applied Behavioral Analysis* [8, 9]. In 2009, more articles were published in *Nature* and the subjournal *Nature Reviews Molecular Cell Biology* [10, 11], *Cell* and subjournal *Cell Stem Cell* [12], New *England Journal of Medicine* [13], and *Regenerative Medicine* and *Science* [14–16]. The media has noted the significant progress in China and interpreted China's policies on stem cells, tissue engineering and related areas. It noted that the Chinese government has invested a large amount of funding in RM represented by stem cell study, tissue engineering and clinical translational studies and the country has made great progress. The reports indicated that the Chinese government will enhance investment in RM and has been active in recruiting well-trained researchers in the 21st century and that China is leading the area in some aspects. However, China still faces challenges in regulation, governance and management.

3.1 Stem Cells

3.1.1 Overview

Chinese researchers started to pay attention to stem cells during the 1980s. In China, it is prohibited to conduct reproductive cloning, utilize a human embryo beyond day 14, fuse human and non-human gametes and implant research embryos into a human or animal uterus. The government has greatly invested in stem cell research concentrated in several key labs in Beijing and Shanghai. In 2001, the CAS established a key lab of stem cell biology, which was followed by the establishment of a stem cell research network composed of the Shanghai Life Science Institute of CAS, Guangzhou Institutes of Biomedicine and Health, Biology Physics Institute, Zoology Institute, Genetics and Development Institute and Kunming Zoology Institute (Fig. 2). Other famous universities and institutes have established stem cell institutes or centers with or without international cooperation (Table 2). Many papers and monographs on stem cells and RM have been published (Tables 3 and 4). Thus, China is close to the global advanced-research level of embryo stem cells and other stem cells.



3.1.2 Stem Cell Banks

In 2007, the MST established 4 stem cell banks covering the north, south and east of China. The banks support each other with their own technological advantages and are expected to create a platform for 3 or 4 key technologies of stem cells (Fig. 3) [17]. In 2002, the information network for hematopoietic stem cell donators was released formally online. In 2010, the MH planned to establish 10 hematopoietic stem cell banks.

3.1.3 Basic Stem Cell Studies

Before 2007, basic stem cell studies in China concentrated on bone-marrow and embryo stem cells [18]. Then, China was gradually moving toward the top position in basic stem cell research. In 2001, Fu reported that epidemial cells could be induced into eipdemial stem cells with growth factors in wound healing [19]. Hongkui Deng et al. reported the first inducible pluripotent stem (iPS) cell line from rhesus monkey in *Cell Stem Cell* [20]. In 2009, Wu et al. first isolated reproductive stem cells and cultured reproductive stem cell strains capable of long-term

◀ **Fig. 2** Major Research Institutions for Stem Cell Research in China. (1) Key Laboratory of Stem Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. Partners are Shanghai Jiaotong University School of Medicine, Shanghai Xinhua Hospital, Changzhou City First People's Hospital, The Third Hospital Affiliated to Suzhou University, established a biomedical translational research base. The research focuses on establishing embryonic stem cell lines, isolating tissue stem cells, studying their stemness and differentiation induction, and stem cell immunology. The main focus is on regulating the differentiation of stem cells, aiming to solve several major problems in the clinical application of stem cells and developing protocols to derive iPS to further understand their roles in disease development. (2) Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences—South China Institute for Stem Cell Biology and Regenerative Medicine, Key Laboratory of Regenerative Biology. This is first international demonstrative base of technology collaboration. Guangdong International Technology Collaboration Demonstrative Base of Stem Cell and Regenerative Medicine. Cooperation institutions are Korea Stem Cell Research Center/YonSei University College of Medicine, Faculty of Medicine, and the Chinese University of Hong Kong. Research Areas are chemical biology for stem cell, stem cell physiology, therapeutic, differentiation, molecular diagnoses and self renew the mechanism of induced pluripotent stem cell and its clinical application. (3) The Institute of Biophysics, Chinese Academy of Sciences. Research areas are biology of embryonic stem cell, pluripotent stem cells (PSCs), the function and regulation of neural stem cells in the mammalian brains; Institute of Zoology, Chinese Academy of Sciences—The Research Center of Stem Cells and Regenerative Medicine. The Chinese-French Laboratory of Biology of Embryonic Cells of Mammalians (LABIOCEM) combined with the French National Institute for Agricultural Research focusing on stem cells and iPS cells of domestic animals, the mechanisms of cloning and therapeutic cloning, which markedly improved the efficiency of animal cloning; The Institute of Genetics and Developmental Biology of the Chinese Academy of Sciences and Nanjing Drum Tower Hospital the affiliated hospital of Nanjing University Medical School established the Nanjing Stem Cells and Biomaterials Research Center, focused on stem cell 3D culture and self-renewal regulatory network, stem cell and biomedical materials, tissue regeneration and wound healing and stem cell translational medicine. (4) The Kunming Institute of Zoology, the Chinese Academy of Sciences, cooperation with Yunnan provincial government established the Key Laboratory of Animal Reproductive Biology focused on rhesus monkey embryonic stem cell self-renewal mechanism with primate animal disease models, research stem cell pharmacology, and promoting Chinese clinical stem cell therapy. "Springer publications remain neutral with regard to contested jurisdictional claims in published maps"

Table 2 Main Stem Cell Research Institutes in China

Time	Name	Composition
2001.01	Peking University Stem Cell Research Center	Peking University
2013.07	Peking University Center for Craniofacial Stem Cell Research and Regeneration	
2001.02	Union Stem Cell and Gene Engineering	Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College
2002.01	Institute of Reproductive and Stem Cell Engineering, Central South University	Central South University
2004	National Engineering Research Center of Human Stem Cells	
2005	Key Laboratory of Human Stem Cells and Reproductive Engineering	

(continued)

Table 2 (continued)

Time	Name	Composition
2003.01	Center for Stem Cell Biology and Tissue Engineering Sun Yat-Sen University	Sun Yat-Sen University
2008.10	Med-X-Renji Hospital Clinic Stem Cell Research Center	Med-X Research Institute of Shanghai Jiao Tong University, Renji Hospital
2011.03	Center of Stem Cells and Regenerative Medicine, Tsinghua University	Tsinghua University
2012.01	Research Center of Stem Cell and Developmental Biology, Zhejiang University	Zhejiang University
2012.04	Sino-US Research Center of Regenerative and Translational Medicine	Institute for Regenerative Medicine, Wake Forest University, Key Laboratory of Neuroregeneration, Nantong University
2012.06	Sino-US Research Center of Stem Cell	Tongji University, California Institute for Regenerative Medicine
2012.12	Southern China Center for Stem Cell and Regenerative Medicine	Academy of Military Medical Sciences, Guangdong Provincial Department of science and technology

Table 3 Main monographs on regenerative medicine published in China

Time	Name	Author	Publishing company
2008.03	Regenerative Medicine: From Basic to Clinic Research	Fu Xiaobing, Wang Zhengguo, Wu Zuze	Shanghai Scientific and Technical Publishers
2010.05	Regenerative Medicine: Theory and Technology	Pei Xuetao	Science Press
2012.03	Regenerative Medicine	Ding Fei, Liu Wei, Gu Xiaosong	People's Medical Publishing House
2013.08	Regenerative Medicine: Basic and Clinical Research	Fu Xiaobing, Wang Zhengguo, Wu Zuze	People's Medical Publishing House

(Reprint from Biao Cheng, Shuliang Lu and Xiaobing Fu, Regenerative medicine in China: demands, capacity, and regulation. Burns & Trauma 2016. 4:24)

Table 4 Main monographs on stem cells published in China

Time	Name	Author	Publishing company
1988.01	Basic Hematopoietic Stem Cell Transplant	Wu Zuze	People's Medical Publishing House
2000.04	Stem Cells and Developmental Biology	Ye Xinsheng, Xu Tian, Tang Xifang, Pei Xuetao	Military Medical Science Press
2000.09	Peripheral Blood Stem Cell Transplantation	Da Wanming, Pei Xuetao	People's Medical Publishing House
2000.11	Hematopoietic Stem Cells Theory and Transplant Technique	Han Zhonchao	Henan Science and Technology Press

(continued)

Table 4 (continued)

Time	Name	Author	Publishing company
2003.07	Stem Cell Biology	Pei Xuetao	Science Press
2005.03	Stem Cells Theory and Technique	Wang Tinghua, Li Liyan	Science Press
2005.05	Stem Cells Biology	Hu Huozhen	Profile of Sichuan University Press
2006.05	Principles, Technology and Clinic of Stem Cell	Zhan Chunhua	Chemical Industry Press
2006.07	Neural Stem Cell Foundation and Application	Zhu Xiaofeng	Science Press
2006.12	Neural Stem Cell	Xu Ruxiang	Military Medical Science Press
2007.03	Hematopoietic Stem Cell Biology and Research Methods	Wang Yaping	Science Press
2007.07	Stem Cell Aging and Disease	Wang Yaping	Science Press
2008.02	Fundamental and Clinic Research of Stem Cells	Yu Yue	Press of University of Science And Technology of China
2010.07	New Technologies of Stem Cell Application	Yang Xiaofeng, Zhang Sufen, Guo Zikuan	Military Medical Science Press
2010.01	Clinical Research of Mesenchymal Stem Cells	Wang Tong	People's Medical Publishing House Co., Ltd
2011.08	The Basis, Ethics and Principles of Clinical Applications of Stem Cells	Jin Kunlin	Science Press
2011.12	Research Legal Regulation of Human Embryonic Stem Cell	Zhao Xu	Shanghai People's Publishing House
2012.04	Mesenchymal Stem Cells: Basic Research and Clinical Application	Han Zhongchao	Science Press
2012.10	Clinical Research and Application of Stem Cells	Gu Yongquan, Han Zhongcao, Fu Xiaobing	People's Medical Publishing House
2012.10	Application of Stem Cell Technology for Cardiovascular Diseases	Ma Yitong, Ge Junbo	People's Medical Publishing House
2012.06	Development Report on Technology and Industry of Stem Cell	Dai Tao, Chi Hui, Fu Xiaobing, Pei Xuetao, Zhou Qi, Li Defu, Lan Baoshi	Science Press

(Reprint from Biao Cheng, Shuliang Lu and Xiaobing Fu, Regenerative medicine in China: demands, capacity, and regulation. Burns & Trauma 2016. 4:24)

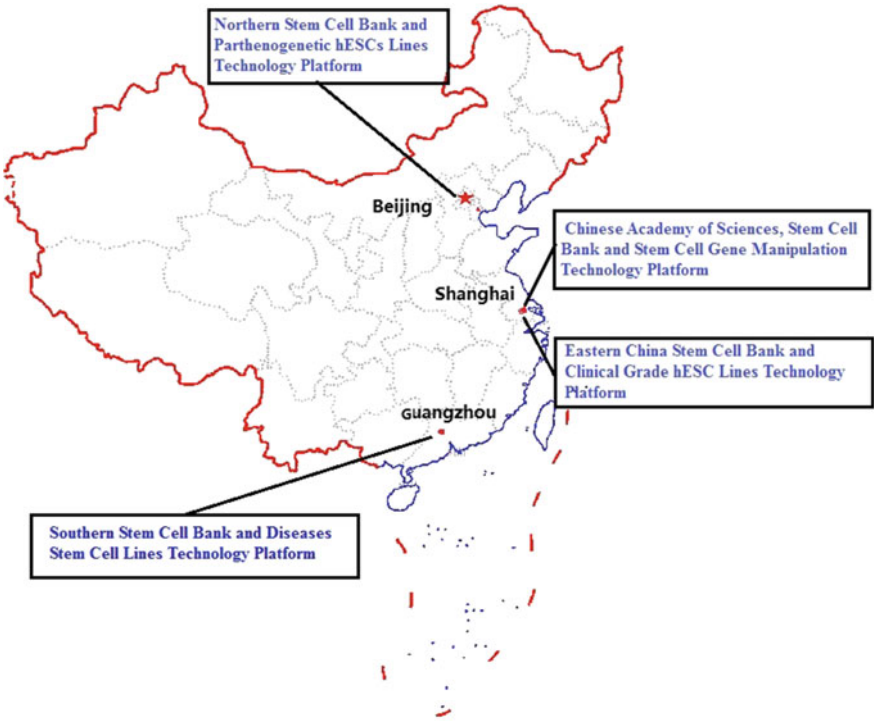


Fig. 3 Stem Cell Banks in China. (1) **Northern Stem Cell Bank and Parthenogenetic hESCs Lines Technology Platform**. To establish key technology of clinical-grade human embryonic stem cells and human parthenogenetic embryonic stem cell bank; to create their own stem cells and collect a variety of resources; supply stem cell materials, information, knowledge and technology services; and support for research institutions. (2) **Southern Stem Cell Bank and Diseases Stem Cell Lines Technology Platform**. To establish disease stem cell lines and hpESC lines, and parthenogenetic technology platform in hESC lines, complete common stem cell culture technique and operating instruction. (3) **Chinese Academy of Sciences, Stem Cell Bank and Stem Cell Gene Manipulation Technology Platform**. To establish, collect, identify, store and provide stem cells and relevant technique and materials, and improve China stem cell resources (especially human embryonic stem cells), and promote China stem cell research and international academic exchange. (4) **Eastern China Stem Cell Bank and Clinical Grade hESC Lines Technology Platform**. To take charge of the National Stem Cell bank websites and databases and stem cell bank management and coordination. To establish clinical-grade stem cell lines and non-animal ingredients hESC lines, offer a variety of standardized stem cells, and provide stem cell technical consultation and training. "Springer publications remain neutral with regard to contested jurisdictional claims in published maps"

self-renewal [21]. Zhou and colleagues first cultivated a mouse by using iPS cells [22]. This was the first proof of the totipotency of iPS cells. This finding was elected by *Times* as one of the Global Top 10 Biomedicine Advances. The journal believed that "this study is a symbol of a major step forward of stem cell research". In early 2010, Pei and associates increased the iPS induction efficiency by 10-fold by adding vitamin C [23]. The *Proceedings of the National Academy of Science*

USA published the finding of the new function of the stem cell factor receptor C-KIT and application in translational medicine [24]. Chinese researchers published their success in creating cell lines from androgenetic haploid embryonic stem cells, a breakthrough in embryo stem cell research [25]. In 2013, Deng and colleagues used a small molecular compound to induce the reprogramming of somatic cells into multipotent stem cells [26]. In 2013, *Cell* published a special "*Spotlight on China*" edition that highlighted the rapid development of immunology study in China and in particular, positively commented on the immunology aspects of applying stem cells for clinical treatment [27, 28].

3.1.4 Therapeutic Applications

The practice of stem cell clinical trials or treatment in China dates back to bone-marrow transplantation in the 1960s, really the transplantation of stem cells in bone marrow. China declared the legality of stem cell treatment as a medical technique following the United Kingdom and the United States.

For therapeutic applications, Zhu and colleagues treated a woman who had chopsticks inserted into her brain from the eyes, which resulted in frontal cerebral-cortex injury. The authors cultivated the brain tissue attached to the chopsticks and were interested in stem cell–motivated self-repair [29]. In 2009, Fu and colleagues regenerated sweat glands by using bone-marrow mesenchymal stem cells (MSCs). This technology has been applied in more than 30 cases with follow-up for more than 4 years [30]. In 2013, umbilical-cord MSC transplantation was performed in patients with post-traumatic brain syndrome. A total of 40 patients with post-traumatic brain syndrome were randomized to receive stem cell or control treatment. Umbilical-cord MSC transplantation improved the neurological function of the patients. However, these results need to be confirmed by prospective, randomized, multi-center, large clinical studies [31]. At the website of the SFDA (date of search: 2013-08-01), a few stem cell–related products have been approved for clinical trials (Table 5).

Before 2005, China had no stem cell therapies and activity factor clinical trials registered at ClinicalTrials.gov (http://www.clinicaltrials.gov/, Fig. 4). After then, Chinese scholars have been paying increasing attention to this issue and the projects registered have been increasing annually, reaching 138 in 2012. In 2013, at the time of our search (August 20, 2013), the number of projects registered reached 28.

3.1.5 Comparison with Research-Advanced Countries on Stem Cells

The United States maintains a leading position in stem cell research, for a significant difference between the United States and China (Fig. 5). The United States has implemented strict management since 1998. The CAS, MST and MH in China started to make laws and policies associated with stem cells in 2003. However, China has improved a variety of policies so far and hopes for real enforcement. In

Table 5 Stem cells products approved for clinic pre-clinic trial by the SFDA

Accept no.	Generic name	Usage	Date	Unit	Status
X0400586	Bone marrow mesenchymal stem cells	Injection	2004.02	The Foundation of the Chinese Academy of Sciences Institute of Medicine	Approved
CSL20020071	Human recombinant stem cell factor for injection	Injection	2003.05	The Second Military Medical University	Approved
X0408234	Mesenchymal stem cells in myocardial infarction for injection	Injection	2005.01	Beijing Yuanhefa Biotechnology	Approved
X0407487	Autologous bone marrow mesenchymal stem cells for injection	Injection	2004.12	Institute of Transfusion Medicine, Academy of Military Medical Science of PLA	Approved
CSL01037	Recombinant stem cell factor for injection	Injection	2001.09	Institute of Transfusion Medicine, Academy of Military Medical Science of PLA	Approved
X0404120	Umbilical cord blood nuclear progenitor cell for injection	Injection	2004.07	Institute of Transfusion Medicine, Academy of Military Medical Science of PLA	Approved
X0404119	Umbilical cord blood red blood cell precursors for injection	Injection	2004.08	Institute of Transfusion Medicine, Academy of Military Medical Science of PLA	Approved

countries such as the United Kingdom and Sweden, collecting human embryonic stem cells for application is relatively free but is more stringent in other countries. In all countries, human cloning and destruction of human embryos are prohibited.

In the field of stem cell drug development and clinical trials, before December 31, 2009, the US Food and Drug Administration approved 2980 projects on stem cell therapy. In 2009, it allowed Geron, a biotech company in California, to perform the world's first clinical trial on human embryonic stem cells, which was a milestone in

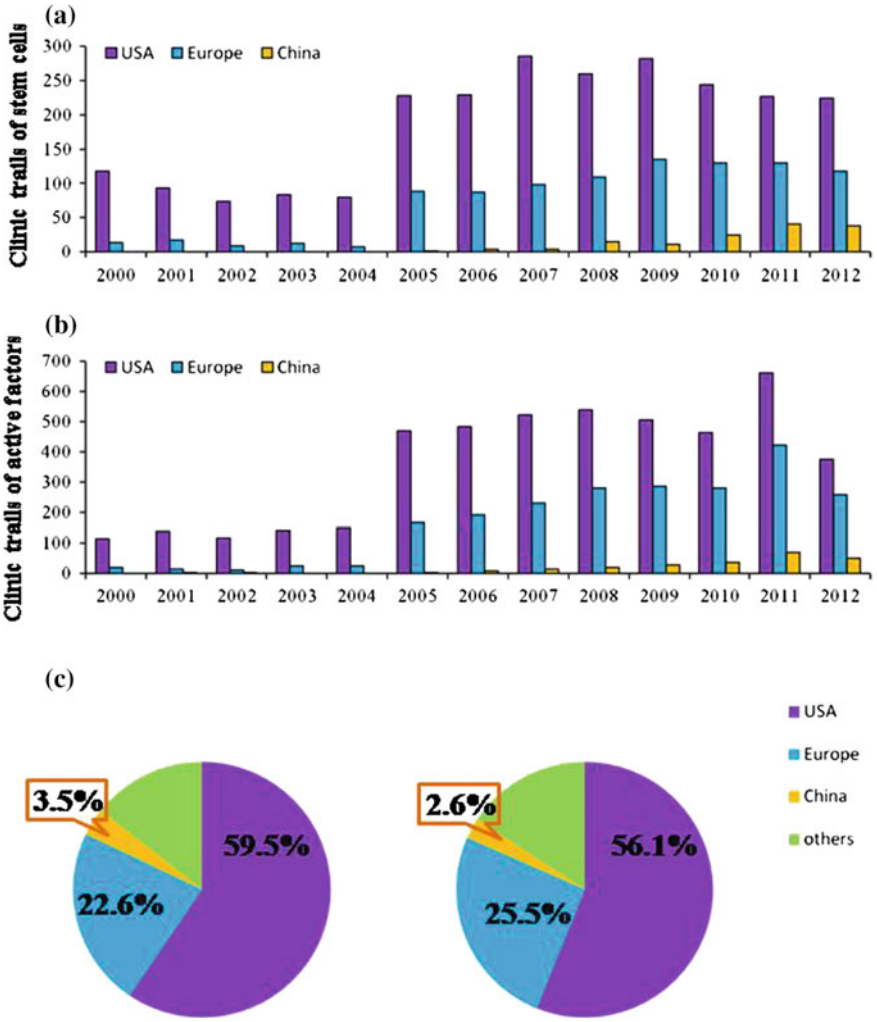


Fig. 4 Clinical trials about stem cells and active factors in China, United States and European countries (www.clinicaltrials.gov/). **a** Stem cells; **b** active factors; **c** stem cells and active factors

the history of medicine [32]. Subsequently, another clinical trial of human embryonic stem cells, performed by Advanced Cell Technology, was approved by the US Food and Drug Administration (FDA). In 2010, the United Kingdom approved its first stem cell trial in humans. In recent years, 3 stem cell therapeutic agents have been approved in South Korea. A stem cell product, Prochymal (manufactured by Osiris), was approved by Health Canada in May 2012. This product was the first non-prescribed MSC agent approved by a developed country for treating acute graft-versus-host disease worldwide. In addition, the Australian Therapeutic Goods

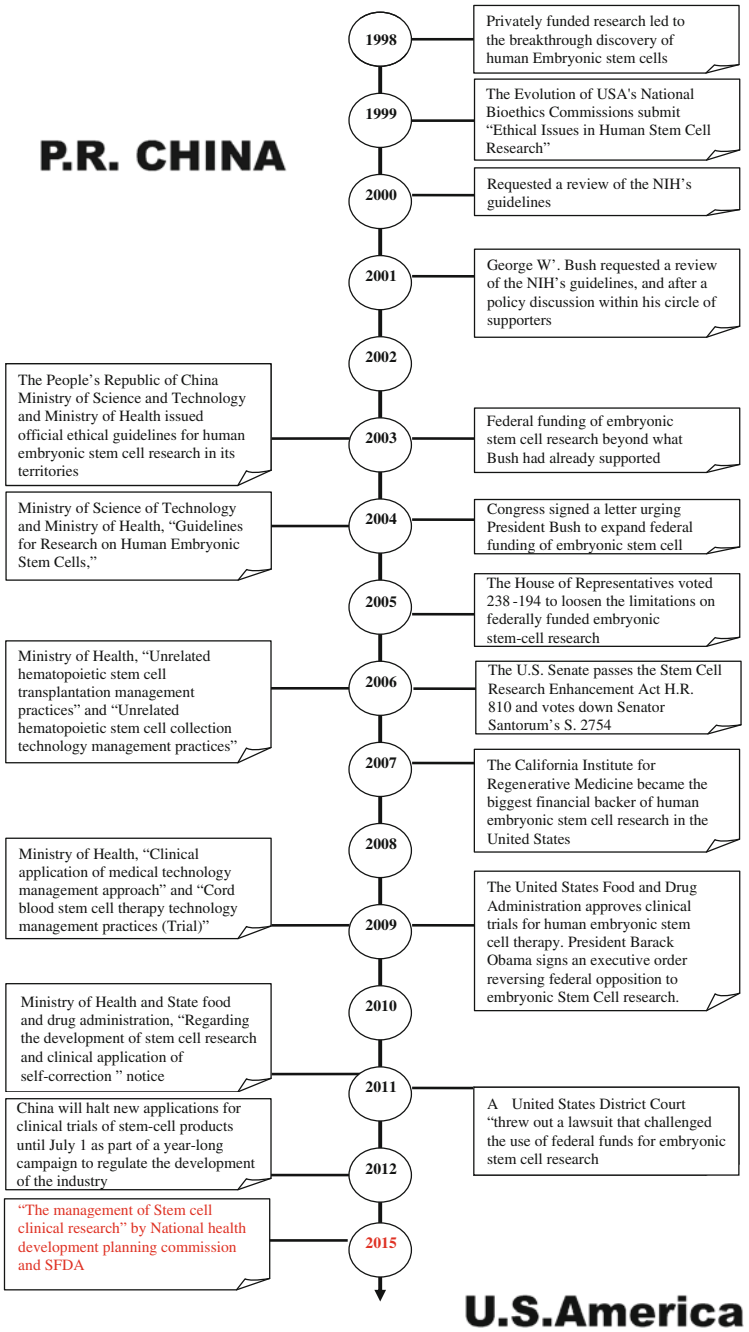


Fig. 5 Comparison of stem cell research policies between China and the United States and related organizations

Administration approved the production and supply of an autologous mesenchymal precursor cell product, Mesoblast. About 3500 clinical trials involving somatic stem cells are registered by the US National Institutes of Health. More than 2000 clinical trials of stem cell therapy are ongoing. Most of these clinical trials are phase I to II. These clinical trials were performed mainly in the United States and Europe. In China, stem cell therapy is restricted, and the hospitals need to file with the regulatory authorities to perform studies. In 2012, the project "Mesenchymal stem cells in myocardial infarction injection", involving one of the 3 stem cell drugs improved for clinical trials in China 6 years ago, completed its phase I clinical trials. Before this, the SFDA approved 2 stem cell drugs for clinical trials: "primitive bone-marrow MSCs" and "autologous bone-marrow MSC injection".

For stem cell drugs, China has lagged behind other countries in strong stem cell research capability. Because of lack of detailed rules in laws, the stem cell market is in chaos driven by financial interests. The chaos in the area of stem cell therapy in China was reported in the *New England Journal of Medicine* and *Nature* in 2009 and 2010 [13, 33, 34]. The articles indicated concern about the safety of stem cell therapy in China. The MH has implemented regulations on the clinical application of cutting-edge therapies such as stem-cell injections. In December 2011, the MH report of quality control in the implementation of stem cell clinical study and application indicated that stem cell clinical study and application activities not approved by the MH or SFDA should be discontinued (Table 5).

A total of 35 stem cell clinical trials have been performed in mainland China, 3 in Hong Kong and 22 in Taipei. As compared with the leading countries in the area of stem cell research, in China, the number of stem cell clinical trials is significantly smaller. The application of stem cells has mainly focused on hematopoietic stem cells and MSCs because of the type of stem cells used in clinical trials of hematological diseases, vascular diseases and diabetes mellitus.

The total number of scientific papers dealing with RM have increased quickly in China, as have the number published in leading international scientific journals in China and internationally (Fig. 6). Since the 1960s, the United States has published 599 articles about stem cells in *Cell* and its subjournals [35], Germany published 45, Japan 36 and China only 17. Since 2000, the number of annual patent applications for stem cells has increased quickly and amounted to 1333 in 2009. In 2011, the Chinese literature related to stem cells outnumbered that published by German, Japanese and United Kingdom researchers and ranked second. In 2012, it had increased. In terms of citations, the United States ranked first, with a mean of 32.2 citations per item. However, citations for Chinese publications is increasing annually and the mean is currently 10.19. For patent applications, as of March 2010, the number of China stem cell–related patent applications and patent applications as a patent priority country were ranked 6th and 3rd in the world. The United States, Japan and China have applied for more patents as patent priority countries [36].

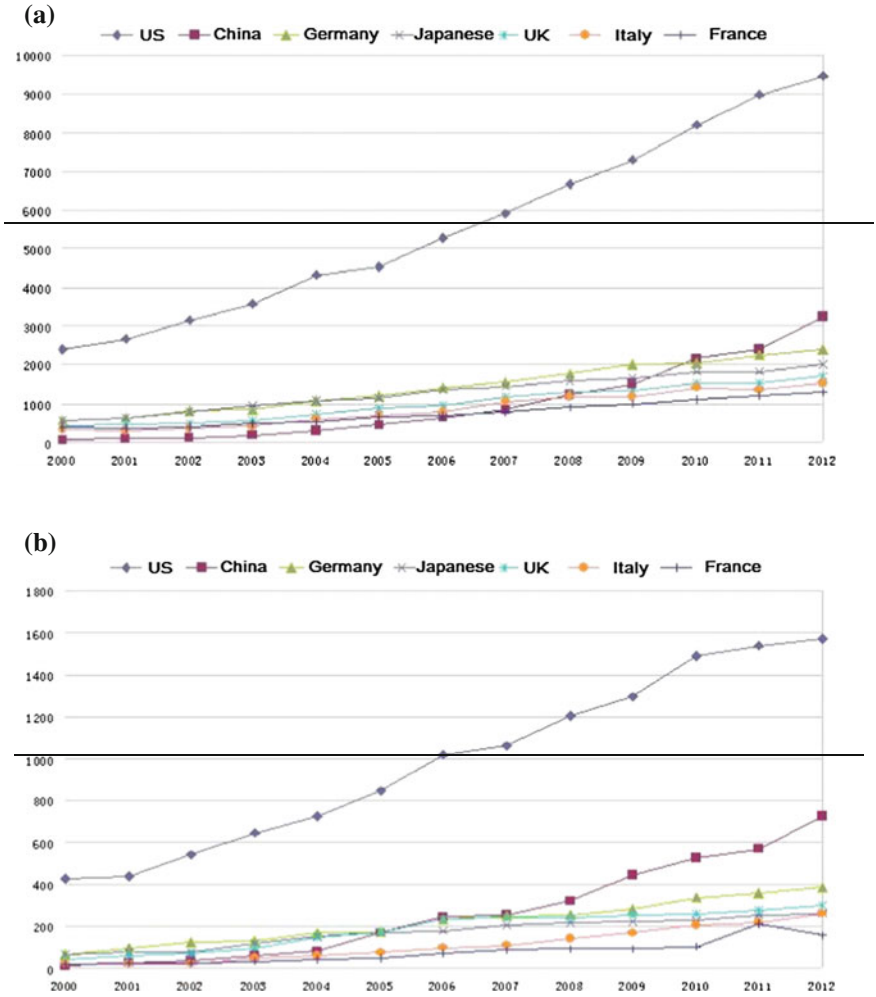


Fig. 6 **a**, **b** Outputs of regenerative medicine research papers compared with major countries (ISI Web of Knowledge). **a** Stem cells; **b** Tissue engineering (Reprint from Biao Cheng, Shuliang Lu and Xiaobing Fu, Regenerative medicine in China: demands, capacity, and regulation. Burns & Trauma 2016. 4:24)

Therefore, the gap between China and the United States as well as other leading countries in stem cell research is mainly characterized by innovation, regulation, and quality control. Also, the quality person system has not been established, and thus cannot be used extensively. The review procedure and laws on stem cell clinical trials have not been established and the long-term mechanism needs to be improved.

3.2 Tissue Engineering

3.2.1 Construction of Tissue-Engineering Research System in China

Tissue-engineering research in China started in the 1990s. In 1994, the Shanghai Science and Technology Committee established tissue-engineering research as a priority. In 1997, the topic of tissue engineering was officially recognized by the NNSF of China. In the same year, the first tissue-engineering laboratory, Shanghai Key Laboratory of Tissue Engineering Research, was established. In 1998, the national "973 Program" for basic research officially established tissue engineering as one of the research topics. In 2001 and 2002, the national "863 High-tech Research and Development Program" consecutively funded the application research and product development of tissue engineering. In 2001, the Shanghai Research and Development Center of Tissue Engineering, also known as the research and development center of tissue engineering in the biological field of the national "863 Program", was established. In June 2012, 39 representative colleges and universities in the field of tissue engineering and RM cooperatively founded the collaborative innovation center for tissue engineering and RM [37, 38].

In 2007, the SFDA announced the requirements for research and submission of tissue-engineered medical products (No. [2007]762 for medical devices). Requirements for the production environment of tissue-engineered technology in technical management specification for tissue-engineered tissue transplantation therapy (trial) was released by the MH in November 2009. At the same time, the MH issued the Management Specification for Class III Medical Device, New Technology for Cell Transplantation and Tissue-Engineered Tissue Transplantation. In 2013, China completed the registration and was granted the right to vote for International Standards Organization (ISO)/TC150/SC7 standardization activity as an active member country (P member) of the ISO/Technical Committee [39] and Dr. Xiaobing Fu was appointed the chair of this committee in China. China was the 13th member and the first member in developing countries in Asia. Thus, the standardization task in the field of tissue engineering in China would officially go to the international stage for involvement in standardization activity of international relevant fields (Tables 6 and 7).

3.2.2 Tissue-Engineering Studies

In 2007, the SFDA approved the first tissue-engineering product, ActivSkin, developed by the Fourth Military Medical University, Xi'an, which made China the second country in the world with the technology of artificial skin after the United States. Moreover, the country continued to develop tissue-engineered de-cellular dermal matrix, skin containing adipose layers, skin containing pigmentation, skin containing capillary-like network, skin containing hair follicles and dermal equivalents. In 2010, the SFDA approved bone-repair scaffolds developed by Fuzhai Cui,

Table 6 Main Monographs of Tissue Engineering Published in China

Time	Name	Author	Publishing Company
2002.09	Tissue Engineering	Yang Zhiming	Chemical Industry Press
2004.06	Principles and Protocol of Tissue Engineering	Jin Yan	Fourth Military Medical University Press
2004.12	The Theory and Practice of Tissue Engineering	Cao Yilin	Shanghai Scientific and Technical Publishers
2005.06	Basic and Clinic Research on Tissue Engineering	Yang Zhiming	Sichuan Scientific and Technological Press
2006.05	Tissue Engineering: A Laboratory Manual	Pei Guoxian, Wei Kuanhai, Jin Dan	Military Medical Science Press
2008.01	Tissue Engineering	Cao Yilin	Science Press
2009.05	Tissue Engineering of Skin	Wu Jinjin, ZhuYoutang	Military Medical Science Press
2011.03	Stem Cell Tissue Engineering: Basic Theory and Clinical Application	Wang Dianliang	Science Press

(Reprint from Biao Cheng, Shuliang Lu and Xiaobing Fu, Regenerative medicine in China: demands, capacity, and regulation. Burns & Trauma 2016. 4:24)

Table 7 Centers/key laboratories for stem cell biology and tissue engineering

Time	Unit
2001	Key Laboratory for Tissue Engineering Research, Institute of Chemistry, Chinese Academy of Sciences
2003	Center for Stem Cell Biology and Tissue Engineering, Sun Yet-Sun University
2003	Center of Tissue Engineering, Chinese Academy of Medical Sciences
2003	Center of Tissue Engineering, the Fourth Military Medical College
2005	Key Laboratory for Tissue Engineering Research, West China Medical University and Tsinghua University
2007	Center for Biomedical Materials and Tissue Engineering, Peking University
2008	Key Laboratory for Tissue Engineering Research, Polymer Research Institute of Tianjin University
2009	Key Laboratory for Tissue Engineering Research, Institute of Basic Medical Sciences Academy of Military Medical Sciences
2011	Zhejiang Key Laboratory for Tissue Engineering and Regenerative Medicine Founded by Zhejiang University

in Tsinghua University. This material has been used in 30,000 patients and was promoted to other parts of the world. Other products include tissue-engineered tendons, cartilage/bone, and neural tubes (Table 8). Fundamental research or investigational use has been conducted for tissue-engineered oral mucosa, bladder,

Table 8 Tissue engineering products approved for marketing by the SFDA

Company	Time	Name	Constitute	Application	Registration No.
Beijing Jieya Laifu Biotechnology	2006	J-1 allogeneic acellular dermal matrix	The donation of human body's skin, removed the host immune rejection of all cells, while keeping intact the original structure with the same extracellular matrix	To repair oral mucosal defects, soft tissue defects	SFDA (quasi-) word No. 2000 No. 346027, and No. 2006 3460430
	2010	AlloDerm	The donation of human body's skin, removed the host immune rejection of all cells, while keeping intact the original structure with the same extracellular matrix	To repair defect of human dermal	SFDA (quasi-) word No. 2010 No. 3461247
Beijing Qingyuan Albert Tissue Engineering Biological Technology	2007	Rhino (acellular dermal matrix medical tissue patch)	Acellular dermal matrix derived from donated human skin that undergoes a multi-step proprietary process that removes both the epidermis and the cells that can lead to tissue rejection	To repair various causes of oral mucosa and soft tissue defect. Closing the wound, dental implantation, hernia repair, urethral	SFDA (quasi-) word 2004 No. 3460736
Chongqing Zongshen Junhui Biotechnology	2007	Artificial skin—gene transfection pigskin	The product performance and composition with fresh skin tissue taken from Bama miniature pig as basic material, and through the introduction of technology and gene transfection of CTLA4Ig gene research	To repair burns and other trauma wound coverage, to promote wound healing, prevention of microbial infection	The Food Drug Armed (quasi) Word 2007 No. 3461287
Qidong Oriental Medicine Research Institute	2010	Acellular dermal matrix dressings	Pigskin as raw material, such as viral inactivation process with acellular prepared from a pig dermal extracellular matrix, is a porous	To repair superficial II degree burn wounds, donor site wounds, deep cut (cut) scab wound granulation wounds and other wounds	SFDA (quasi-) word No. 2010 No. 3641111

(continued)

Table 8 (continued)

Company	Time	Name	Constitute	Application	Registration No.
			three-dimensional network structure; mainly composed of collagen		
Shaanxi Eyre skin Biological Engineering	2007	Tissue engineering skin	A bilayer artificial skin substitute: epidermal layer is composed of human epidermal cells, dermal fibroblasts from human and bovine collagen	To repair deep II degree burn wound, not more than III degree burn wound 20 cm^2 (diameter less than 5 cm)	The Food Drug Armed (quasi) Word 2007 No. 3461110
BiotechnologyYantai Zhenghai	2009	Skin repair film	Cattle skin tissue after treatment prepared by a series of acellular dermal matrix is composed primarily of collagen	To repair various causes dermal wound repair defects	SFDA (quasi-) No. 2009, No. 3460425
	2009	Biofilm	Cattle skin tissue processed through a series of prepared acellular dermal matrix is composed primarily of collagen, collagen retains the unique three-dimensional structure	To repair a variety of causes dura (spinal) membrane defects	SFDA (quasi-) No. 2009, No. 3460602
	2009	Dental film	The leather prepared after a series of acellular dermal matrix, whose main ingredient is collagen, sterilized by radiation, one-time use	To repair various causes shallow intraoral soft tissue defect repair	SFDA (quasi-) No. 2009, No. 3460404
Biological Technology of Guangdong Grandhope	2007	General thoracic surgical repair film	Pig tissue membranes crosslinked epoxy chemical reagents and biochemical transformation of materials made of surgical repair	To repair chest wall, bronchial stump, diaphragmatic, visceral capsular defect, etc.	SFDA (quasi-) word No. 2007, No. 3461317
	2009	Sterile biological care record film	The pig offal film, by the addition of antigen and a series of biochemical treatment and viral inactivation and made	skin burn, burn and trauma, skin defects	SFDA (quasi-) No. 2009, No. 3640426

(continued)

Table 8 (continued)

Company	Time	Name	Constitute	Application	Registration No.
	2011	Biotypes dura (spinal) membrane patch	Pig membranous tissue as raw material, processed into biotechnology, should be finished smooth side, the other side is allowed villous or striped or grid-like native structure	To repair hard brain (spinal) membrane defect	SFDA (quasi-) word 2008 No. 3460637
Beijing Datsing Bio-Tech	2011	Allogeneic bone grafts	Cadaveric bone usually obtained from a bone bank	Dental implants, to repair broken bones that have bone loss, and repair broken bone that has not yet healed	Fresh Armed State Drug Administration (prospective) No. 3460627(2011)

artificial liver and kidney, heart valve prostheses, cardiac patch, blood vessel, nerves, urethra, testes and thyroid glands. Thus, scientists in China have an interest in different fields and are involved in tissue-engineered grafts to repair and regenerate tissues and organ. In 2012, *Science* reviewed the development of tissue engineering in China; work from Xiaosong Gu at Nantong University, Yilin Cao at Shanghai Jiaotong University and Fuzhai Cui at Tsinghua University were highlighted for their outstanding contribution in this area. In 2016, Fu and his team reported in Acta Biomaterialia that new tissue engineering skin with functional sweat glands have been made with 3D biological printing technology, which offer new hope for new generation of tissue engineering skin in the future.

3.2.3 Comparison with Other Research-Advanced Countries

The concept of tissue engineering was formally proposed by the US National Science Foundation in 1987. Moreover, the United States subsidized research projects for tissue engineering as early as 1988. Institutions involved are research institutes (National Aeronautics and Space Administration, the US Department of Energy, NIH), universities (Massachusetts Institute of Technology, Harvard Medical School, Georgia Institute of Technology, and University of California at San Diego), and enterprises (Sandoz, Organogenesis, Advanced Tissue).

The US FDA granted marketing approval to about 10 products including tissue-engineered skin and cartilage. In China, tissue-engineered skin has been approved, which marked the beginning of the industry for tissue-engineering products. The technical level of China in tissue engineering is basically geared to international standards, but the delay in tissue-engineered research and translational application in China stems mainly from regulations and product standards. With respect to tissue-engineering publications, China exceeded Germany, Japan and the United Kingdom as early as in 2000 and the gap with these countries has widened since 2008 (Fig. 6a, b) [40].

3.3 Growth Factors

3.3.1 Overview

The supplement of exogenous bioactive factors is important for accelerating wound healing and tissue regeneration. These bioactive factors include basic or acidic fibroblast growth factor (bFGF or aFGF), epidermal growth factor, nerve growth factor and bone natriuretic protein. Some have been approved for clinical application as new drugs and approved with good clinical effects.

3.3.2 New Growth Factors

As early as in 1991, Chinese scientists published the first academic monograph that systematically addressed "growth factor and wound healing" (Table 9). Since 1998, they have published results of a series of multicenter, randomized clinical trial about growth factors accelerating wound healing in *The Lancet* and other international journals [41]. The results indicated that bFGF and other active molecules play a key role in regulating tissue and organ repair and regeneration. About 10 growth-factor products have been approved by the SFDA and used in the clinic (Table 10).

Compared with China, the United States is more cautious about the clinical application of growth factors. Only recombinant human platelet-derived growth factor by Chiron was approved by the FDA in 1998 and is used for débridement healing and repair of advanced diabetic foot ulcers, severe burns, skin diseases, and bone and teeth defects. The recombinant human keratinocyte growth factor palifermin, by Amgen, was approved in 2004 and is used for treatment of severe oral mucositis caused by chemoradiotherapy. Therefore, the number is far less than that of growth factors approved in China.

Cell differentiation and dedifferentiation induced by growth factors are a "hot" topic in RM. In 2001, Chinese scientists published results of a series of in vivo and vitro studies dealing with epidermal cells dedifferentiating into epidermal stem cells in *The Lancet* and other international journals, which addressed dedifferentiation as a new approach to make stem cells from skin [19, 42, 43]. Particularly, the authors found that growth factors have a significant dedifferentiation effect on cells in in vivo settings. Although the innovative theory immediately caused controversy between Chinese and foreign scholars, this groundbreaking thinking has great significance for the later practice of using active factors to reprogram adult cells into adult stem cells. These results offer direct evidence that the potential of plasticity and transdifferentiation of some adult tissue stem cells could lead to differentiation into other series of cell types without developmental correlation [44].

Table 9 Main monographs on growth factors in China

Time	Name	Author	Publishing Company
1991	Growth Factor and Wound Healing	Fu Xiaobing	Military Medical Science Press
1992	Basic and Clinical of Polypeptide Growth Factors	Zhou Tingchong	Science Press
1994	Basic and Clinical Cell Growth Factor	Qi Yanchao	Henan Science and Technology Press
2003	Polypeptide Growth Factors and Spinal Cord Injury	Wang Tinghua, Feng Zhongtang	Xinjiang Science and Technology Publishing House
2007	Basic and Applied Research on Fibroblast Growth Factor	LI Xiaokun, Gong Shouliang	Jilin University Publishing House

Table 10 Growth factors for external using products approved for marketing by the SFDA

Company	Time	Products	Effect	Status
Zhuhai Essex Bio-Pharmaceutical	1998	Recombinant bovine basic fibroblast growth factor	Burn and scald wounds: including shallow II degree and deep II degree wounds, granulation wounds and inhalation injuries * Acute wounds: bruises, contusions, combined injuries and cuts * Surgical incisions: incisions of surgery, orthopedics, gynaecology (such as lateral episiotomy and cesarean incision), otolaryngology, urology and proctology * Chronic wounds: diabetic ulcers, vascular ulcers, radiochemotherapy ulcers, bedsores, fistulas, residual wounds and cervical erosions * Skin grafting: skin donor site, skin grafting site, skin flap handling * Other applications: after plastic surgery, skin resurfacing, dermabrasion, nevus removal and laser therapy wounds	SFDA Approval No. S10980077
	1999	Recombinant bovine basic fibroblast growth factor eye drops	* Various corneal defects and punctate keratopathy * Recurrent punctate keratopathy in the shallow layer * Mild or moderate dry eye * Corneal operation and poor corneal healing after operation * Geographic (or nutritional) herpes simplex keratitis * Ballous keratitis * Corneal abrasion, mild and moderate chemical burns	SFDA Approval No. S19991022
	2005	Recombinant bovine basic fibroblast growth factor Gel	Treatment of various keratopathy, ocular traumas and foreign body removal * Corneal transplantation and eye surface reconstruction * Dry eye: especially dry eye due to corneal injury * Cataract surgery (ECCE. Phaco etc.): restoration of endothelium and reduction of edema * Corneal refractive surgery: repair damaged corneas before surgery, repair damaged nerves after surgery and treat postoperative dry eye	SFDA Approval No. S20050100

(continued)

Table 10 (continued)

Company	Time	Products	Effect	Status
Beijing SL Pharmaceutical	2002	Lyophilized recombinant human basic fibroblast growth factor (rh-bFGF)	Chronic cutaneous ulcer and burn wounds	SFDA Approval No. S20020025
Nanhai Longtime Pharmaceutical	2004	Recombinant human basic fibroblast growth factor for external use	Chronic cutaneous ulcer and burn wounds	SFDA Approval No. S20040052
Shanghai Wanxing Bio-Pharmaceutical	2006	Lyophilized recombinant human acidic fibroblast growth factor for external use	Chronic cutaneous ulcer and burn wounds	SFDA Approval No. S20060102
Guilin Pavay Gene Pharmaceutical	2002	Recombinant human epidermal growth factor hydro gel	Chronic cutaneous ulcer and burn wounds	SFDA Approval No. S20020111
Wuhan HITECK Biological Pharmaceutical	2006	Mouse nerve growth factor for injection	Peripheral nerve injury and peripheral neuropathy; Brain and spinal cord injury; Acute cerebrovascular disease, cerebral atrophy, Parkinson's and Alzheimer's disease	SFDA Approval No. S20060051

3.4 Other: Gene Therapy, Therapeutic Clone and Xenotransplantation

Gene therapy has an important place in neuron, cardiovascular and islet-cell regeneration. Self-developed recombinant hepatic growth factor is the first gene therapy product for cardiovascular disease and is now in a phase II clinical trial. In addition, pcD2/hVEGF121 gene therapy for peripheral vascular diseases has been approved by the SFDA for a special clinical trial, so China is the second country conducting such research after the United States.

"Therapeutic cloning" has always been listed as a national basic research program [45]. It is divided into upper, middle, and lower research parts according to national strategic planning. Drs. Guoxiang Chen (Transgenic Research Center in Shanghai), Huizhen Sheng and Yilin Cao (Shanghai Second Medical University) are responsible for the upper, middle and lower research parts, respectively. With cloning, cells may be induced to differentiate into specific tissue and organs in vitro, such as skin, cartilage, heart, liver, kidney and bladder, then these tissues and organs are implanted into patients [46].

Xenotransplantation is being accepted gradually in addition to tissue engineering and stem cell technology. The first batch of inbred Wuzhishan mini-pigs with GT knockout (homozygous with 2 copies of GGTA1 gene deletion) were established by Deng and colleagues at the institute of animal husbandry. Pigskin excipients with reduced immunogenicity treated by several different means have been approved by the SFDA. In addition, patents have been applied for α-galactosidase–treated porcine heart valve, pericardium and ligament. Functional cells and various humanized organs for human transplantation may be obtained if the same method is applied in large animals. The immunological rejection of xenotransplantation would be overcome and result in functional organs.

4 International Collaboration and Opportunities for RM in China

Open and cooperative regulations are basic in China. Since 2005, China has cooperated with many countries that are advanced in RM at different levels. Six world-renowned comprehensive RM research institutions from Germany, the United States, Canada, Spain and The Netherlands established a Regenerative Medicine Coalition (RMC) to jointly promote the research and innovation of RM therapy at cellular levels (Table 11). Even some large foreign pharmaceutical companies, such as General Electric and Sanofi-Aventis, have invested in China for stem cell–related research and achieved relevant results [47, 48].

Table 11 Collaborations between China and other countries in regenerative medicine research

Country of collaboration	Time	Objects
France	2005	Institute of Zoology (IOZ), Chinese Academy of Sciences, and the French National Institute for Agricultural Research
	2007	The Chinese-French Joint Laboratory of Biology of Embryonic Cells of Mammals
Australia	2007	Sino-Australia Center of Excellence for Stem Cell Science
Canada	2007	Monash University's Immunology and Stem Cell Laboratories (MISCL) was awarded a federal government grant to establish a joint Australia-China Centre for Excellence in Stem Cell Science with Peking University
	2009	The Ministry of Science and Technology and the Canadian Institutes of Health Research signed a memorandum of understanding
United Kingdom	2005	The Committee of the National Natural Science Foundation of China and the UK Medical Research Council signed a memorandum of cooperation
	2009	Scottish Centre for Regenerative Medicine (SCRM) and Peking University Stem Cell Research Center (PKUSCRC) established a National International Joint Research Center
	2012	UK Medical Research Council and the National Natural Science Fund Committee cooperated to jointly fund a stem cell research project
United States	2009	The California Institute for Regenerative Medicine (CIRM) and the Chinese Ministry of Science and Technology (MOST) signed an agreement to collaborate on stem cell research
Germany	2009	The National Natural Science Fund Committee and the German Science Foundation cooperated to jointly fund a stem cell research project

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5 Summary

In 2012, a special edition of *Science* (sponsored supplement for Regenerative Medicine in China) gave a comprehensive snapshot of the development of RM in China, with particular emphasis on stem cells, tissue engineering and trauma. As the core scientific journal, *Science* echoed with positive comments for the achievements in China. For scientists in China, change and challenge in RM exists. Although its achievements are remarkable and impressed, China needs to strengthen the following aspects: **(1) Sound administrative system, laws, technical specifications and guidelines**. China has not yet established a truly effective management system for clinical application of somatic stem cells, and detailed regulatory rules are not clear. The standards for stem cell conservation, research, clinical trials, eligibility criteria for hospital and medical staff performing stem cell therapy, relevant instruments and

devices remain to be determined. The implementation should be in accordance with the guidelines for stem cell clinical translation released by the International Society of Stem Cell Research. Ethical norms and legal provisions should be strictly followed to constrain the application and clinical trials of stem cells. Emphasis should be paid on the protection of intellectual property, allowing healthy and orderly development of the study of RM. **(2) Emphasis on training and retention of talented stem cell researchers**. China has become an important member in RM competition. However, some prominent problems should be solved. Compared with international advanced levels, in China, the scale and overall level of stem cell and RM research still has a long way to go. There is not much originality, especially not many innovative ideas and research directions that can lead the trend. China lacks worldwide influential scientists in the area of tissue regeneration (including stem cell, tissue engineering) and coordination among multi-disciplinary experts. **(3) Reasonable allocation of resources and breakthroughs**. China will use the limited funds to concentrate on RM research in inducing stem cell differentiation, synchronous repair and regeneration of multiple impaired tissues, construction of tissue-engineered major organs and driving tissue-engineered products from bench to bedside. Substantial progresses and breakthroughs have been made in further establishing and improving the rules and laws involved in RM and construction of a RM translational base. **(4) Broad and deep international cooperation**. China should continue to emphasize in-depth and practical cooperation with foreign well-known universities, scientific centers and key laboratories. The construction of programmed, systemic and open research teams at the national level can avoid the waste of resources and concentrate on advantages to overcome difficulties. **(5) Diversification of investment and other new technologies**. Stem cell technology is attracting scientists and entrepreneurs from various countries with its huge market potential and has tremendous business opportunities for stem cell industry. Some new technologies may bring breakthrough developments for RM, such as the effect of 3D biological printing technology on RM. Dr. Mingyan Xu, at Hangzhou University of Electronic Science and Technology, also the director of the research and development team for the Chinese biomaterials 3D printer, independently developed the first domestic biomaterial 3D printer. This printer has successfully printed small proportions of cartilage tissue of human ears and liver. This biomaterial 3D printer is characterized by a great variety of printed biomaterials, low incidence of cell damage, high printing precision and convenient operation. The development of nano-intellectual materials for RM is important. The intellectual biomaterials may induce molecular modifications of degradable materials, resulting in the interactions between cell integrins, induction of cell proliferation and differentiation and synthesis and assembly of extracellular matrix, thereby initiating the body's regenerative system. For example, the new intellectual biomaterial can have the function of signal transduction and control the release of growth factors or drugs intelligently, thus inducing the regenerative repair of tissues and organs directly.

Finally, RM in China in the next 10 years is expected to achieve substantial progress in systemic and effective regulation as well as a management system for RM research, application and synchronous repair by inducing stem cells and regenerating a variety of impaired tissues. The construction of major organs by

tissue engineering and large-scale application of tissue-engineered products will be completed. These achievements will bring hope to China improve healthcare and build a healthy society [49, 50].

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The Differences of Cell Biology in the Repair Process of Wound and Refractory Wound Surface

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Abstract In the following paragraph we will discuss the differences of the cell biology in the repair process of wound and refractory wound surface. In the repair process of wound surface the cell biology in hemostasis phase, in inflammation phase, in proliferation, angiogenesis, fibroplasia and epithelialization phase and in contraction, maturation and remodeling phase in the normal organ or tissue such as skin after injury will be shown. The cell biology in the repair process of refractory wound surface, we mainly discuss the cell biology in refractory wound surface of the diabetes such as the effect of diabetes on the biological function of fibroblasts, M1/M2 macrophage imbalance in the repair process of refractory wound surface of diabetic, the effect of glycosylated extracellular matrix on fibroblasts and so on.

Keywords The cell biology · Repair process · Wound surface · Refractory wound surface · Diabetes

Skin repair after injury such as scald includes a complex programmed sequence of cellular and molecular progresses that involves hemostasis, inflammation, proliferation, and maturation, which include multiple cell populations, the extracellular matrix (ECM) and the action of soluble mediators such as cytokines (including growth factors). In this paragraph we mainly talk about the differences of cell biology in the repair process of wound and refractory wound surface.

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1 The Cell Biology in Hemostasis Phase

The platelets that can release cytokines (including growth factors), chemokines, and hormones play a crucial role in clot formation during hemostasis after aggregation and attachment to exposed collagen surfaces and activated in the initial stage of injury. Cytokines (including growth factors) have emerged as important mediators in repair process. As we know, cytokine is released from various cells, which can bind to target cell surface receptors to stimulate a cell response by endocrine, paracrine, autocrine, or intracrine routes. Platelets elaborate a number of proinflammatory substances and growth factors such as platelet-derived growth factors (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF) and so on. PDGF is released from the alpha granules of platelets and is responsible for the stimulation of neutrophils and macrophages, and also a mitogen and chemotactic agent for fibroblasts and smooth muscle cells, which can stimulate angiogenesis, collagen synthesis, and collagenase. VEGF contributes to angiogenesis by stimulating the mitosis of endothelial cells. TGF-β promotes proliferation of fibroblasts, regulates its own production in an autocrine manner and produces proteoglycans, collagen, and fibrin. It also promotes accumulation of ECM and fibrosis. All these cytokines (including growth factors) act on surrounding cells and stimulate chemotaxis of neutrophils, monocytes, and fibroblasts to the area of injury. So chemokines released by platelet activation attract inflammatory cells to the area, leading to the next phase i.e. inflammatory phase in the repairing process in the adult body.

1.1 The Cell Biology in Inflammation Phase

Neutrophils, monocytes/macrophages and lymphocytes are the main cells in the inflammatory phase in the wound surfaces area.

Neutrophils cleanse the wound site of bacteria and necrotic matter and release the chemotaxis such as interleukin 8 (IL-8) that chemotactic the macrophages and other cells involved into the wound site. Chemokines (or chemotactic cytokines) are small heparin-binding proteins that direct the movement of circulating leukocytes to sites of inflammation or injury via interaction with specific membrane-bound receptors and, as such, contribute to the pathogenesis of a variety of diseases [1]. Depending on the spacing or presence of four conserved cysteine residues, chemokines are classified into CC, CXC, CX3C, and XC families. CXC chemokines primarily attract neutrophils and lymphocytes and are believed to orchestrate the early phases of wound healing [2].

On the other hand, neutrophils produce reactive oxygen species (ROS) and proteases and also function to debride devitalized tissue. These functions are required in a timely manner. Neutrophils are produced in the bone marrow from stem cells that proliferate and differentiate to mature neutrophils fully equipped with

an armory of granules. Neutrophils are dormant in the blood circulation. Once trauma and infection occurred the neutrophils are activated and the first to arrive the wound site. Neutrophils can release the particles of granules not only to fight microorganisms but also to cause great tissue damage. In the burn wounds, neutrophil infiltrates in the skin tissue in 4 h after the injury and reached the peak level 24 h later. The number of neutrophils decreased after 48 h of injury. At sites of infection and trauma, endothelial cells capture bypassing neutrophils and guide them through the endothelial cell lining whereby the neutrophils are activated and tuned for the subsequent interaction with microbes.

Neutrophils are the predominant cell type in the first inflammation phase (48 h after injury) and begin to wane after 24–36 h by apoptosis in the time of circulating monocytes enter the wound and mature into tissue macrophages that play the very important role in the wound site. In the adult body, no macrophages, no wound repair.

Following the neutrophils the monocytes involve in the wound site and become the macrophages. Macrophages play a central role not only in the inflammatory phase but also in all stages of repairing. Their functional phenotype is dependent on the wound microenvironment. During the early and short inflammatory phase macrophages phagocytose debris and bacteria and produce and orchestrate inflammatory cytokines (including growth factors) such as Tumor Necrosis Factor (TNF), Interleukin-6 (IL-6), Interleukin-1 (IL-1) and basic fibroblast growth factor (bFGF) and so on. IL-1 stimulates inflammatory cell proliferation and promotes angiogenesis. TNF-α is secreted from macrophages and as a mitogen for fibroblasts. bFGF is a chemotactic and mitogenic factor for fibroblasts and endothelial cells and other mesenchymal cells and also is an important stimulus for angiogenesis, that facilitate the repairing process.

Then let's talk about macrophages. Macrophages are known to produce collagenases and elastases, which remove the damage tissue by phagocytosis and make the wound clean.

Depending on the stimulus in vitro, activation of macrophages has been classified into two populations. The classical (M1) activation results in a highly pro-inflammatory macrophage phenotype, with microbicidal activity and pro-inflammatory cytokine production, and is mediated by like Toll-like receptor (TLR)-4 ligands and interferon-γ (IFN-γ). The alternative (M2) activation can reduce inflammatory reaction, promote tissue repair and humoral immunity, and is mediated by IL-4 and/or IL-13.

The phenotype of wound macrophages in this phase is probably the classically activated or the so-called M1 phenotype. During the proliferative phase, macrophages stimulate proliferation of connective, endothelial and epithelial tissue directly and indirectly. M2-type macrophages release some growth factors such as PDGF, acid fibroblast growth factor (αFGF) and bFGF, transforming growth factor α (TGFα), macrophage-derived growth factors. Especially fibroblasts, keratinocytes and endothelial cells are stimulated by macrophages during this phase to induce and complete ECM formation, reepithelialization and neovascularization. Subsequently, macrophages can change the composition of the ECM both during

angiogenesis and in the remodeling phase by release of degrading enzymes and by synthesizing ECM molecules [3, 4].

Besides, M1 and M2 promote T help 1 cells (Th1) that play the main role in cellular immunity and T help 2 cells (Th2) that play the main role in humoral immunity responses, respectively. Products of Th1 such as interleukin-2 (lL-2), interferon (IFN) and Th2 such as interleukin-10 (IL-10), interleukin-4 (IL-4) responses also down regulate M1 and M2 activity, respectively. The balance of the products of Th1 and Th2, is the balance of cellular immunity and humoral immunity. Thus, M1/M2 also demonstrated the importance of Innate Immunity [5, 6]. This suggests an important role for alternatively (M2) activated macrophages in this phase of wound healing.

Recent studies have been showed another factor, autophage, may play role of the cell biology in the repair process, because autophage has a lot of functions that influence infection, inflammation and immunity. Autophage is induced by pattern recognition receptors and, through autophage adaptors, provides a mechanism for the elimination of intracellular microorganisms. Autophage regulates inflammation through controlling interactions with innate immune signaling pathways, by removing endogenous inflammasome agonists and through effects on the secretion of immune mediators. At the same time, autophage participate in antigen presentation and to T cell homeostasis, and it can affect T cell polarization and repertoires [7].

During the inflammatory phase, lymphocytes migrate into the wound area approximately 72 h post injury. Lymphocytes produce lymphokines such as bFGF, heparin-binding epidermal growth factor (EGF) and so on. T lymphocytes arrive to wound through IL-1 induced, which also contributes to the regulation of collagenase. Therefore, lymphocytes also play an important role in antibody production and cellular immunity. As mononuclear cells, T lymphocytes continue to replace macrophages and other inflammatory cells, their proliferation phase begins. They take wound repair into the end of inflammatory phase, the evolving milieu of eicosanoids in the wound interact with the cell types present, resulting in fibroblast synthesis of collagen and other substance. Additionally, the macrophage-derived growth factors are now at optimal levels, strongly influencing the influx of fibroblasts and then endothelial cells and keratinocytes into the wound.

2 The Cell Biology in Proliferation, Angiogenesis, Fibroplasia and Epithelialization Phase

Angiogenesis, fibroplasia and epithelialization occur during the proliferation phase. Formation of granulation tissue is a central event during the proliferation phase. Its formation occurs 3–5 days following injury and overlaps with the preceding inflammatory phase. A rich blood supply is vital to sustain newly formed granulation tissue. The macrophage is essential to the stimulation of angiogenesis and

produces macrophage-derived angiogenic factor in response to low tissue oxygenation. This factor functions as a chemoattractant for endothelial cells. Besides, the macrophages secrete bFGF and vascular endothelial growth factor (VEGF), which are also important to angiogenesis. Endothelial expansion contributes to angiogenesis, as intact vessels generate buds in granulation tissue. Neovascularization facilitates growth of the advancing line of fibroblasts into the wound, providing them with necessary nutrients and cytokines. The fibroblasts is a critical component of granulation tissue, which grow in the wound as the number of inflammation cells decrease. Two to three days after injury, the fibroblasts migrate inward from wound margins over the fibrinous matrix, which has been established during the inflammatory phase. During the first week, fibroblasts begin to migrate, proliferate and produce glycosaminoglycans and proteoglycans, the ground substance for granulation tissue, as well as collagen, in response to macrophage-synthesized growth factors such as PDGF, FGF, VEGF, TGF-α, TGF-β and etc. Type III collagen is the primary component of early granulation tissue. Fibroblasts soon become the dominant cell type, peaking at 1–2 weeks. The synthesis and deposition of collagen is a critical event in the proliferation phase and to wound healing in general. They generate not only collagen molecules but also growth factors such as PDGF, TGF-ß, bFGF, insulinlike growth factor-1(IGF-1), keratinocyte growth factor (KGF) and so on. Angiogenesis results in greater blood flow to the wound and, consequently, increased perfusion of repairing factors. Degradation of the fibrin clot and provisional matrix is accompanied by the deposition of granulation tissue (ground substance, collagen, capillaries), which continues until the wound is covered. Angiogenesis ceases as the demand for new blood vessels ceases. New blood vessels that become unnecessary disappear by apoptosis.

Fibroplasia starts on 3–5 days following injury and may sustain as long as 14 days. Fibroblasts produce the collagen, fibronectin, glycosaminoglycans, and other components of ECM. Fibroblasts are able to assemble cross-linked and fascicular fibers using collagen molecules. This synthesis work would last about 2–4 weeks. In normal skin there is approximately 80 % of the collagen identified type I collagen; the remaining is mostly type III. Collagen is the major component of acute wound connective tissue, it will continue to produce in the next 6 weeks. The accumulation of wound collagen is related to the increase of tensile strength. Collagen is rich in hydroxyproline and hydroxylysine moieties, which promote to form a strong cross-link structure. The hydroxylation of lysine and proline residues depends on the presence of oxygen, vitamin C, ferrous iron, and α-ketoglutarate. Particularly, deficiencies of vitamin C and oxygen result in under-hydroxylated collagen that is less capable of forming strong cross-links and is easier to breakdown. The formation of collagen is carried out on extracellular. First cells secret procollagen. Then procollagen is cleaved of its terminal segments and called tropocollagen. Collagen filaments can be formed through aggregate of tropocollagen molecules. Moreover, the cross-linked structure of intermolecular makes collagen fiber stabilize and resistant to destruction. Collagen fibers are deposited in a framework of fibronectin, which is closely connect with fibronectin In addition,

fibronectin can paly a role of an anchor to make the myofibroblast migrate into the wound. At the moment, granulation tissue is gradually formed, and the wound begins to contract.

Epithelialization is the formation of epithelium that involves cell migration and covering the wound area. Firstly, epidermal cells at the wound edges, under their structural changes, detach from their basement membrane. Cellular movement relies on the establishment of physical forces by means of protrusive forces that lead to membrane extensions and traction forces allowing the cell to contract and slide forward [8]. The polar change of actin cytoskeleton intracellular causes the cell generate these deformations. Protrusions rely on polymerization and depolymerization of actin filaments while the traction is generated by myosin-based motors which pull actin filaments past one another. In a word, cell movement is based on the direction of polarity of the cells.

The initial step of cell polarization is that intracellular actin polymerizes to form ruffles or leading pseudopodia. The Rho family small guanosine triphosphate (GTP)-binding proteins (GTPases) are pivotal regulators of actin organization and control the formation of lamellipodia and filopodia. At the sites where contact with the extra cellular matrix (ECM) occurs, big protein complexes are assembled through the recruitment and the clustering of receptors of the integrin families. These large protein molecule structures are known as focal adhesions or focal contacts. There are known two types of migration mode: “Integrin/MMP dependent mode” and “Integrin/MMP-independent mode”.

“The dependent mode of cell migration” is called “mesenchymal”. Surface proteases, such as MT1-MMP, break down pericellular matrix molecules locally to provide sufficient space of cell expanding. Shortly after integrin binding with ECM, contractile proteins connect with cytoplasmic actin filaments, such as myosin II, which can stabilize and shorten the membrane-tethered actin filaments. This results in local cell contraction, generally at the opposite pole respect to the leading edge. Another mode is called “ameboid”. Cells also can migrate across connective tissue within pre-existing ECM pores by simply squeezing.

The formation of Intracellular actin microfilaments makes the epidermal cells crawl across the wound surface. Epidermal cells can secrete collagenases and plasminogen activator, collagenases break down collagen, plasminogen activator stimulates the production of plasmin, which promotes clot dissolution along the pathway of epithelial cell migration. Migrating epithelial cells interact with a provisional matrix of fibrin cross-linked to fibronectin and collagen. In particular, fibronectin seems to promote keratinocyte adhesion to guide these cells across the wound base. This epithelial layer provides a seal between the underlying wound and the environment. Besides, as the cells migrate, they dissect the wound and separate the overlying eschar from the underlying viable tissue. The stem cells are found in the deep rete ridges, leading them to propose that this site may provide protection for the long-lived stem cell population from harmful environmental mutagens. The sebaceous glands and hair follicles contribute to reepithelialization.

When epithelialization is complete, the epidermal cell restores its original morphology, and forms new desmosomal linkaging to other epidermal cells, and

hemidesmosomal linkages to the basement membrane are restored. At the same time, epithelial cells continue to migrate inward from the wound edge until the defect is covered. The transformation of fibroblasts into myofibroblasts which contain contractile actin fibers, is contact inhibition induced. Then the new tissue replaces injured tissue volume.

2.1 The Cell Biology in Contraction, Maturation and Remodeling Phase

Contraction, defined as the centripetal movement of wound edges that facilitates closure of a wound defect, is maximal 5–15 days after injury. The result of contraction is decreased wound size which depends on the degree of tissue laxity and shape of the wound. The process of wound contraction is usually accompanied by collagen synthesis. During this phase, collagen remodeling depends on continued collagen synthesis in the presence of collagen destruction. For the first 6 weeks, new collagen production dominates the wound healing process, deposited randomly in acute wound granulation tissue. As the wound matures, collagen is remodeled into a more organized structure with increased tensile strength. With collagen synthesis, matrix metalloproteinase collagenolysis achieves a steady state.

Collagen forms tight cross-links to other collagen and with protein molecules, increasing the tensile strength of the healing wound. Stress, age, pressure and tension affect the rate of collagen synthesis. Loose tissues contract more than tissues with poor laxity, and square wounds tend to contract more than circular wounds. Wound contraction does not seem to depend on collagen synthesis but depends on the myofibroblast located at the edge of the wound, its connection to myofibroblast proliferation and components of the ECM.

In remodeling phase, collagen becomes organized increasingly. During this phase, a balance exists between formation of new collagen and removal of old collagen depending on collagenases and matrix metalloproteinases in the wound to assist removal of excess collagen while synthesis of new collagen persists. Fibronectin gradually disappears, and proteoglycans instead of hyaluronic acid and glycosaminoglycans. Gradually, type I collagen replaces type III until the normal skin ratio of 4:1 is achieved. The cross-links of Intramolecular and intermolecular collagen result in increased wound bursting strength. Remodeling begins approximately 21 days after injury, when the net collagen content of the wound is stable. Remodeling may continue indefinitely. Bursting strength varies with skin thickness. The tensile strength of the wound reached its peak at about 60 days after injury.

2.2 The Biology of Stem Cell in the Repair Process of Wound

Stem cell research has become one of the hot points in the repair process of wound because the stem cells have the characteristic of self-renewing, differentiated into multiple types of total specialized cells of the body [9]. But it still exist many problems because we still don't know how many stem cells which include many types still existing in our organism when we leave the uterus. Such as what kind of damage and microenvironment can "home" the stem cells and induce them to differentiate into the appropriate cells to remodel damaged tissue. Nevertheless, the stem cell research has helped our mankind to understand how single cell can grow and develop into tissue and organ, and how the damaged cells can be replaced by healthy cells in adult body, which guides us to explore the cytological pathway to treat disease.

Kucia et al. have found and identified a population of stem cells in the BM, they are small (about 2–4 μm), but have large nuclei surrounded by a narrow rim of cytoplasm, and contain open-type chromatin (euchromatin), express several markers such as SSEA-1, Oct-4, Nanog and Rex-1, they are typical embryonic stem cells (ES). These cells can differentiate into all three germ-layer lineages in vitro. So they are also called very small embryonic-like (VSEL) stem cells. These cells have the characteristics of age-dependence. With the increase of age, the number of them is gradually reduced. They are barely detectable in 1 year old mice which correspond to a 50 year old human. This feature may be one of the reasons why the regeneration of young individuals is more effective than aged. It has been provided that as the organ is damaged, non-hematopoietic stem cells (including VSELs) are enter the peripheral blood circulation from the BM to "home" to the damaged tissues and participate in tissue repair. These cells may efficiently differentiate and regenerate into special tissue cells to replace the damaged cells in injuries. During this time damaged tissues up-regulate the expression of several chemotactic factors, which may participate in the homing and inducing differentiation of VSELs. But, if these cells migrate to the wrong place or/and migrate at the wrong time, they may lead to the formation of pathological diseases, such as tumor formation.

Adult stem cells are present in tissues and organs of the body, which have the potential to self-renew and differentiate into various types of cells. The process of differentiation is regulated by multiple genes. So that they can develop into specific structures and perform special biological functions. Some local adult stem cells are differentiated to supply new cells that effectively replace senescent ones or those undergoing apoptosis such as epidermal stem cells differentiated and developed into several layers of epidermis in maintaining normal metabolism condition of our skin [10]. In some injuries such as second-degree burns, some adult stem cells that are located in the wound or wound edge can be rapidly differentiated, proliferated and migrated with still healthy terminated cells to replace the damaged tissue cells, even in the microenvironment of chemoattractants that may express and secrete by damaged tissue cells or/and healthy cells, and finally restore the damaged tissue to

the normal condition. Because there are no healthy cells, structures and even adult stem cells in site used to wound repair, such as the local area of severe burns or three-degree burns, bone marrow stem cells as precursor cells of other stem cells, play an important role in the reconstruction of various kinds of trauma. For example: many fibroblasts are derived from the blood delivered cells harvested from BM in the early stage of granulation formation of the repairing process in the local severe wound surfaces area.

Now the hypothesis of "Stem cell Niche" has become the hot point to stem cell research. Some scholars believe that "Niche" may be a habitat, such as the limbal SC niche, in which SCs could remain stable in this environment or microenvironment that not to differentiation. Adult SCs are regulated by microenvironment of their "niche", i.e., the adult-specific SCs in "niche" are maintained in undifferentiated state, and their biological functions, consisting of other cellular and extracellular components have been adjusted accordingly in the vicinity of the area. So, the differentiation of stem cells may be synergistically regulated by various factors of micro-environmental, such as gene expression, cell-cell contacts, cell-matrix interactions and etc. The existence of niche and surrounding cells may guard stem cells to have a stable reserve force, by avoiding stimulation of differentiation and apoptosis. Stem cells, in the niche, not only have the ability of self-renewal, but also when the niche or the surrounding environment is stimulated from outside, they are able to differentiate. At that time the niche would modify to ensure that SC activity parallels the organism's needs for particular differentiated cell types. However, if the adult stem cells are differentiated or/and differentiated cells migrate and proliferate into the inappropriate region, such as defection or without of "dermal template" or niche, it may result in "abnormal repair", e.g. scar formation. But there are still a lot of issues that need to be explored, such as the multiple signal-pathways relative to stem cell differentiation, and the corresponding microenvironment of niche and surrounding that is suit for stem cell biological changes.

In view of the characteristics of bone marrow stromal cells (BMSCs), which are easily harvested from bone marrow, easy to culture in vitro and could be re-introduced into patients as autografts without serious ethical and technical problems, many researchers have used them as the ideal seed cells to transplant onto various medias such as denuded human amniotic membrane (AM). It has been used in many surgical procedures, such as skin equivalent and vaginal reconstructive surgery, and also for human embryonic SC differentiation into neural cells as well as for supporting chondrocyte proliferation and phenotype maintenance in vitro and the regeneration of osteochondral defect in rabbits [9]. Some researchers have confirmed that the amnion-derived cellular cytokine solution can promote the migration of macrophages during wound repair [11]. So the bio-scaffold with appropriate three-dimensional structure e.g. niche or "dermal template" and their components such as collagen of ECM may have a special influence on SC differentiation or "homing" SC and then assisting them to differentiate to form a functional tissue or organ. And the problem we are facing now is that due to the structure and content of bio-scaffold is always suffered a certain degree of damage

in the process of production, we can't create an ideal bio-artificial scaffold that is completely consisted with natural structure and environment.

Above all shows the cell biology in normal organ or tissue such as skin after injury. But many common chronic wounds such as diabetic foot ulcer, pressure ulcer, venous stasis ulcer and etc. Considers specific type of nonhealing wounds such as pressure ulcer, leg ulcers, diabetic foot wounds, surgical and malignant wounds as well as lymphoedema and dermatological conditions associated with skin breakdown [12]. Here we mainly discuses the diabetes and cell biology in refractory wound surface.

3 Diabetes Has Multiple Effects on Cell Biology in Refractory Wound Surface

Chronic wounds include vasculitis, non healing ulcer, pyoderma gangrenosum, and disease that cause ischemia. There are so many physiologic factors which contribute to wound healing deficiencies in individuals, such as decreased, hyperglycemia or impaired growth factor production, cytokine receptor, angiogenic response, macrophage function, collagen accumulation, epidermal barrier function, quantity of granulation tissue, keratinocyte and fibroblast migration and proliferation, number of epidermal nerves and balance between the accumulation of ECM components and their remodeling by MMPs [13–18].

Diabetic patients with spontaneous rupture of skin, such as diabetic lower extremity ulcers, wound or trauma is difficult to heal, all is a hotspot and difficulty in clinical research.

In recent years, the research on the mechanism of diabetic wound healing focus on harmful substances deposited, such as advanced glycation end products (AGE), high glucose deposition, cell signal transduction, cell apoptosis and cell function, blood vessels and extracellular matrix etc. Histological observation also showed that the thickness of the epidermis and the dermis of skin tissue of diabetic rats was significantly thinner, the epidermal cell layer is not clear, partial epidermis lack of multiple layer arrangement, significant reduction in the number of heckle cells, dermal collagen arrangement disordered, partial collagen degeneration, fracture, focal infiltration of chronic inflammatory cells was seen in the area of collagen degeneration. This shows that the skin tissue of diabetic patients in the absence of injury has been the existence of the changes in histology and cell biology behavior; this is a kind of "**Underling Disorder**", that does not result in the integrity and continuity damage of the skin. This damage is endogenous, although it does not cause skin defects or damage to the visibility of the damage, but because of the change of histology and cell function, can make the skin tissue to increase the vulnerability of exogenous damage [19].

3.1 Neutrophils

The normal healing process can be defined by a number of overlapping events: clot formation, inflammation, reepithelialization, angiogenesis, granulation tissue formation, wound contracture, scar formation, and tissue remodeling. Diabetic wounds are characterized by functional defects in the majority of these events, leading to impaired wound healing, in addition to local ischemia caused by well-recognized macro- and microvascular occlusive disease. Usually, impaired wound healing in diabetic patients is accompanied by decreased early inflammatory cell infiltration but persistence of neutrophils and macrophages in the chronic, impaired wounds [20].

3.1.1 The Biological Characteristics of Neutrophils in the Diabetic Impaired Wound Healing

A series of function changes of neutrophil will occur in diabetic state. Insulin levels in patients with diabetes have a certain effect on the function of neutrophils. In the research [21] of 8 healthy volunteers showed that after treated with insulin, neutrophil chemotaxis, phagocytic ability and sterilizing ability were improved, and the control group showed significant differences. At the same time, Okonchi's [22] research found, high concentrations of insulin can promote neutrophil transmembrane swimming and the expression of the platelet endothelial cell adhesion molecule-1 increased. When used with Gliclazide drug, can inhibit the abnormal function of neutrophils. From the above research, it can be speculated that the level of insulin in the body can directly affect the function of neutrophils. Then, the secretion of insulin in patients with type 1 diabetes is insufficient. This is one of the reasons that diabetes patients are susceptible to infection and impaired wound healing. Although there is no reduction in insulin secretion in patients with type 2 diabetes, neutrophil receptor glycosylation also affects the binding of neutrophils to insulin, which affect neutrophil function.

Tennenberg et al. [23] found that neutrophils from patients with diabetes are prone to apoptosis, the authors believe that this may be related with hyperglycemia. This would cause decreased functional longevity of neutrophils and increased neutrophil clearance from infectious sites, possibly contributing to the increased susceptibility and severity of infections in diabetic patients.

Long term hyperglycemia may lead to the production of a large number of advanced glycation end products (AGEs) in the body. Study of Collison et al. [24] found that AGEs could be high affinity with the human neutrophil AGER (AGE Receptor) and lead to increase in intracellular calcium and actin polymerization, which will depress the transendothelial cell migration and sterilization ability of neutrophil. Tian et al.'s research found [25, 26] that neutrophils couldn't reach the basal part of the wound in time and form a dense inflammatory zone. A large number of neutrophils were scattered around the wound. Immunohistochemistry

showed that AGE was distributed in the skin tissue of diabetic rats. Neutrophil migration test is shown in vitro that AGE can inhibit the migration of the neutrophil by binding its receptor on the surface of the neutrophil. At the same time, neutrophil and AGE combined outside the vascular tissue leads to a large number of inflammatory cytokines are released and oxidative stress burst by neutrophils. This release and burst are delayed and lasts longer than the normal wound.

Some studies have also similar views, Osar et al. [27] confirmed that the neutrophil oxidative burst index decreased significantly compared with the control group ($p < 0.05$), coenzyme I (NADPH) activity decreased by studying 30 type 2 diabetes patients. Gustke et al. [28] found that the average neutrophil chemotaxis index was significantly lower than that of the control group ($p < 0.02$) in type 1 diabetic patients. This changed cell function of neutrophil were dependent on HLA-DR3, DR4, and DR5 genes.

Wound healing involves many complex, interrelated processes that involve multiple cell types. Neutrophil plays an important role in the normal healing process, but abnormal neutrophil function may contribute to the pathogenesis of nonhealing wounds present in diabetic patients. A better understanding of the molecular mechanisms and cellular interactions of neutrophil in diabetic patients, is critical for the development of novel therapeutic strategies to promote diabetic wound healing.

3.2 Macrophage

Abnormal macrophage function in process of wound healing may not be conducive to the normal development of wound and lead to adverse results, such as the formation of ulcers, chronic wounds, hypertrophic scars and keloids. During the process of impaired wound healing, macrophage activation phase and degree were abnormal, wound repair process cannot be in accordance with the conversion from severe inflammation to mild inflammation state. Compared with the normal repair of acute wound, impaired wound is usually stuck in inflammatory phase and it was found that there was an in situ retention of macrophages.

3.2.1 M1/M2 Macrophage Imbalance in the Repair Process of Refractory Wound Surface of Diabetic

Impaired wounds such as diabetic wounds and chronic venous ulcer were found abnormal inflammatory retention and reduced granulation tissue state [29]. The first evidence is the number of aaM in wound area more than caM in diabetic wound healing model using db/db mice. Miao et al. [30] found a decrease in iNOS level, which is marker of caM(M1), on days 1 and 3 after wounding in STZ-induced diabetic rat lesion, especially on day 3, compared with the normal rats. The expression of Arg-1, which is marker of aaM(M2), in the diabetic group was lower

than in normal group on day 7, but increased sharply and significantly higher on day 13. The study showed that the M1 in the non diabetic SD rats mainly appeared in the inflammatory phase and gradually replaced by the M2 in the repair of the proliferative phase. The infiltration of macrophages ($CD68^+$) in the scald wound of STZ-induced diabetic rats was "slow in and slow out" which insufficient at early stage, and detained at late stage. Compared with normal rats, the expression of iNOS in the early stage of diabetic rats was decreased, Arg-1 was increased in expression, IL-4, IL-10 and other anti-inflammatory factors were relatively higher, indicating that Th1/Th2-M1/M2-iNOS/Arg-1 adjustment mechanism of normal healing was inclined to the side of Th2-M2-Arg-1 in diabetic wound, that is, changes in performance for insufficiently pro-inflammatory at early stage, at late stage the pro-inflammatory and anti-inflammation disordered, and with anti-inflammatory as the main.

So the balance of caM/aaM is very important in the process of wound healing. It is found Th2-aaM-Arg-1 increased in streptozotocin-induced diabetic wounds. But, abnormal caM may also lead to bad results. Unrestrained proinflammatory caM induced by iron and too many TNF-a positive macrophages, which are considered as caM cells, impairs wound healing in humans and mice [31]. An imbalance of caM/aaM in wound healing may delay and even hinder skin defect restoration. It appears that successful healing requires the activation of macrophages at an appropriate phase and a suitable extent.

There is large number of accumulated AGEs founded in diabetes mellitus, and AGEs might induce macrophages to product TNF-α to influence wound healing. Goren et al. [32] found that in the dorsum of ob/ob mice full thickness concise was observed in the number of abnormal TNF-a positive caM, and at the late stages of inflammation (post injury 7, 9, 11 days) in removal wound caM secreting TNF-a, thus launched a fast impaired wound epithelialization process. Dong et al. [33] also found that activating α7 nicotinic acetylcholine receptor (α7nAChR) can promote diabetic wound healing by suppressing AGE-induced TNF-α production, which may be closely associated with the blockage of NF-κB activation in macrophages. Suggesting that, there are inflammatory disorders during the process of some impaired wound healing such as diabetes, in this environment due to anomaly time phase of activated macrophages, macrophages cannot successfully complete from the early inflammation M1 based active state to late inflammatory M2 based activation state transition. Trem2 is a cell surface receptor that is specifically induced in macrophages by IL-4/IL-13 and is important in injury responses. Wound healing in Trem2−/− mice showed an increased expression of caM markers, decline aaM markers. This wound also demonstrated diminished burst of epithelial proliferation and wound closure rate [34].

One of the key factors of effective wound healing is proper phenotypes transformation of macrophages from the phase of pro-inflammatory to healing. The switch in macrophage phenotypes during skin wound healing was associated with up-regulation of the peroxisome proliferator-activated receptor (PPAR)γ and its downstream targets, along with increased mitochondrial content. Miraz et al. [35] reported that in the setting of diabetes, up-regulation of PPARγ activity

was impaired by sustained expression of IL-1β in both mouse and human wounds. In addition, experiments with myeloid-specific PPARγ knockout mice indicated that loss of PPARγ in macrophages is sufficient to prolong wound inflammation and delay healing. Furthermore, PPARγ agonists promoted a healing-associated macrophage phenotype both in vitro and in vivo, even in the diabetic wound environment.

In short, many studies confirm the importance of macrophage activation in wound healing. The imbalance of CaM and aaM may cause impaired wound. Moreover, regulation of the balance between caM and aaM may be regarded as a therapeutic strategy to promote wound healing.

3.3 Endothelial Cells

Wound healing process can be split to 3 distinct phases [36], endothelial cells participate in all the phases, and play the deferent roles in each one. That can be summarized as the excessive activation of ECs in inflammation phase (lost or declined vascular barrier function), but the weakened or obstacled ability of angiogenesis in late phase.

It has been reported that ECs were suffered from increased apoptosis, up-regulation of secreting of adhesion molecule (such as ICAM-1, VCAM-1), increased ROS, MDA, decreased SOD level and activated cell signal pathway of MAPK, NF-kb under the high glucose or AGEs in vitro [37, 38]. All that could help leukocytes to gather on vascular wall, and cells more easily migrate from vessel to the injured area. It may be the one of the reasons for the presence of sub-inflammatory phenomena, being susceptible to infection and the sustained inflammatory response after trauma in diabetic skin.

As we all known, diabetic skin inflammatory cells infiltration excess normal skin. The disordered ability of re-establish blood supply in injury area can ultimately lead to the occurring of chronic wounds. Many researchers have confirmed that some of the biological functions of ECs, such as, migration, angiogenesis, and secretion of angiogenic-relative cytokine, have been down-regulated in high glucose or AGEs environment. Dr. Qiao. investigated the degree of neo-vascularization in STZ-induced diabetic rats suffered partial thickness scalding of 20 % total body surface area (20 % TBSA) on back, and the quantity of vascular ECs by double-labeling immunofluorescence. He found that vascular ECs can proliferate actively in the diabetic wound with burns, but it is still poor in blood supply due to lack of functional capillaries. The mechanism may be related to sustained abnormal high expression of Ang-2 and down-regulated VEGF [39].

New evidence-based medical research shows that: if the patient in hyperglycemic state for a certain time, even after the blood glucose levels returned to normal, still prone to vascular complications, this phenomenon called “hyperglycemia metabolic memory” (metabolic memory) [40]. Dr. Li et al. established endothelial cells memory model of high glucose to evaluate cell proliferation,

apoptosis, ROS, SOD, MDA, eNOS mRNA, and NO levels in supernatant of endothelial cell culture media. These results suggest that in hyperglycemia memory cell model, transient hyperglycemia may lead to persistent imbalance in oxidative stress and reduce endothelium—derived relaxing factor NO level, indicating that hyperglycemia memory may play an important role in persistent vascular endothelial cell injury.

3.3.1 Endothelial-Mesenchymal Transition, EnMT

Recently, the concept of EnMT has attracted more and more attention of researchers. Cell differentiation is a process which is characterized by the loss of the intrinsic and special phenotype of a cell and the transformation of a new phenotype into another, which is characterized by a change in phenotype, morphology and function [41]. Once ECs are subjected to mechanical tension, oxidative stress, abnormal lipid metabolism, inflammation, hypoxia and other external stimuli, will change their composition, structure to adapt to these changes. EnMT could be occurred by TGF-ß signal pathway, and also by Notch signal pathway which inhibits the expression of endothelial cell adhesion molecule VE-cadherin [42–44]. Endothelial cells which were occurred EnMT, the adhesion junctions were disrupted and the destruction of this continuity makes the vascular wall surface to become rough, exposed sub-endothelial collagen, platelet adhesion enhancement, coupled with the increased expression of endothelial cell adhesion molecule, promoting the adhesion of leukocyte-endothelial and the adhered leukocyte can also injury endothelial cell through the release of elastase, forming a vicious circle. High-glucose environment can activate oxidative stress in ECs by AGEs, the classical polyol pathway, PKC, the sorbitol pathway, the acetylglucosamine pathway, and et al. The activated oxidative stress can increase the active oxygen species in blood, reduce vascular diastole factor such as endothelial cell NO and prostacyclin, raise vascular contraction factor such as ET-1, thromboxin A2. ET-1 can active inflammatory reaction to promote the formation of leukocyte adhesion, TNF-α and so on. This may also be one of risk factors of wound healing.

3.3.2 Endothelial Progenitor Cells

Another important factor for revascularization in wound area is successful mobilization of endothelial progenitor cells (EPCs) from the bone marrow to injury area and participate in vasculogenesis. EPCs proliferate, migrate, differentiate, and produce proangiogenic cytokines during the process of angiogenesis. They are also charge of maintenance and repair of endothelial cells. EPCs express markers of both hematopoietic stem cells (CD34 and CD133) and endothelial cells (CD146, vWF, and VEGFR2) [45–47]. Dysfunction of EPC may promote to vascular pathological disease. Also, the decrease in number of EPCs from peripheral blood and dysfunction EPCs were found in patients with diabetes and cardiovascular disease.

In the normal process of wound healing, EPCs are effectively recruited to participate the remodeling microcirculation, and leading to wound repair in time. But in diabetic ulceration, this situation could be partial inhibited. Such as, it is recently reported that EPCs from normal but not diabetic patients contribute to postischemic revascularization. The number and function of diabetic EPCs were 5 times lower than that of normal EPCs, and show a tendency to pro-inflammatory phenotype. The bone marrow derived EPCs in diabetes mellitus are dysfunctional, due to oxidative stress, they produce fewer endothelial cells with reduced proliferative, and migratory potential. The number and function of EPCs act as a surrogate marker of vascular health and indicate cardiovascular risk in healthy persons [48–50]. Feng et al. [51] showed that oxidized low-density lipoprotein (OxLDL) can inhibit survival of EPCs that derived from umbilical cord, and impair their function, thus inhibiting the production of eNOS.

As we know there are increased oxidative stress levels in the body of diabetes, ROS can promote EPCs to secret many pathologic cytokines to increase iNOS level and decrease eNOS level. The reduced functional activity of EPCs during hyperglycemia involves the Akt/eNOS pathway, where signaling is downregulated under diabetic conditions [52, 53]. Ii et al. [54] have attributed the phenotypic differences of EPCs during diabetes to decreased thrombospondin-1 expression. There is an indication that upregulation of cyclin-dependent Kinase (CDK) inhibitors p16 and p21 leads to a reduction in proliferating EPCs under hyperglycemic conditions [55]. Although we have got a lot of evidence as above, but we still don't known clearly what molecular mechanisms affect the number and function of EPC in diabetes mellitus.

3.4 Fibroblast

The role of fibroblast (FB) in wound healing process is extremely important. They are involved in establishing the emerging basement membrane and subsequent reepithelialization. So any dysfunction of FBs will affect wound healing and ultimately lead to chronic, nonhealing wounds. FBs in diabetic skin and mellitus have been threaten by high glucose environment, and their biological function also occurred corresponding change, therefore we frequently find the cases of impaired wound healing with diabetes in clinical.

3.5 *The Effect of Diabetes on the Biological Function of Fibroblasts*

3.5.1 Fibroblast Proliferation

FB is a higher proportion in the number of cells in the dermis and a kind of dominant repair cells, the proliferation of normal metabolism and repair of dermal tissue after trauma depends mainly on the FB, cell cycle analysis is to understand the direct index of cell proliferation. Cell proliferation is a multi stage, multi factor involved in the orderly regulation process, which is achieved through the cell cycle. In this process, the cells were followed by phase G1, S, G2 and M to complete the proliferation. The most critical stage is the S phase because DNA multiplication and chromosome replication in this stage. The study of Wang et al. [56] found that FB were increased significantly in S phase but decreased obviously in phase G0/G1 and in G2/M phase in diabetic skin of rats compared with normal skin of rats (showing in Table 1). The results indicated that FB have the ability to self renew but can't effectively complete the proliferation in the diabetic state. This phenomenon of "DNA replication but can't effectively enter the G2/M phase and finish chromosome replication and cell division and proliferation" may be one of the important reasons of having complication such as diabetic foot. This phenomenon of "DNA replication but not division or proliferation" may be one of the important reasons of delayed or impaired wound healing in diabetes. But further biological effects in this phenomenon should be explored. Other results showed [57] that the number of FB in phase S were all significantly lower than those in normal rats in diabetic skin with deep II degree burns, on the 3, 7, 14 and 21 days of post injury. This indicated FB proliferation was inhibition completely during the repair process of diabetic wound. In the diabetic skin, FB due to the long-term in a composite environment of high glucose, high AGEs or other substances, its proliferation state has been affected (percent of S phase was increased) and entered self-renew stage. When the diabetic skin tissue was injured, the original should enter the repair process (the percent of S phase of FB in normal group was observably increased in 3 days post injury, and gradually decline in the late) has not been started, FB has unexpectedly been in state of inhibited proliferation, which affected the process of wound healing. It suggests that the changes of diabetic wound microenvironment may be one of the causes of impaired diabetic wound healing.

3.5.2 Skeleton Structure of Fibroblast

Many functions of the cell such as chemotaxis, movement and proliferation are mediated through the cytoskeleton, cell migration, differentiation and proliferation during the process of wound healing are accompanied by significant changes in the cytoskeleton system. Chen et al. [58] observed FB with transmission electron microscope and found that there is the expansive endoplasmic reticulum, the less

Table 1 Percentage of cell cycle of dermal FB in wounds after burns

Group	Before injury	Time after injury (day)			
		3	7	14	21
Normal rats (n = 6)					
G0/G1 phase (%)	82 ± 10	42 ± 13	56 ± 13	70 ± 7	77 ± 4
S phase (%)	17 ± 10	55 ± 16	44 ± 16	29 ± 6	23 ± 4
Diabetic rats (n = 6)					
G0/G1 phase (%)	66 ± 5**	70 ± 4**	88 ± 5**	82 ± 6*	84 ± 5*
S phase (%)	33 ± 5**	30 ± 4*	11 ± 5**	17 ± 6*	12 ± 4*

There is significant difference compared with normal group, $^{*}p < 0.05$, $^{**}p < 0.01$

developed Golgi complex, the lack of actin filaments and microtubules, and obviously increased lysosome in the cytoplasm in the dermal fibroblast of diabetic group But FB on normal control group within the dermis can be seen in different forms and maturity of FB, mostly fusiform, cytoplasm with abundant rough endoplasmic reticulum and well-developed Golgi complex, the nucleus was oval, nuclear chromatin pale staining, uniform distribution, and can be seen with FB and smooth muscle cells with characteristics of myofibrlblast (MFB), the cytoplasm appeared cells arranged parallel to the long axis of the microfilament bundle visible electron dense plaques (Fig. 1).

α-SMA can be connected to FB through “fibronexus”, a kind of transmembrane complex, to regulate cell contraction. It has been confirmed that there is a positive correlation between tissue contraction and the expression of a-SMA. MFB not only has strong synthesis function as FB, but also because of its expression of a-SMA

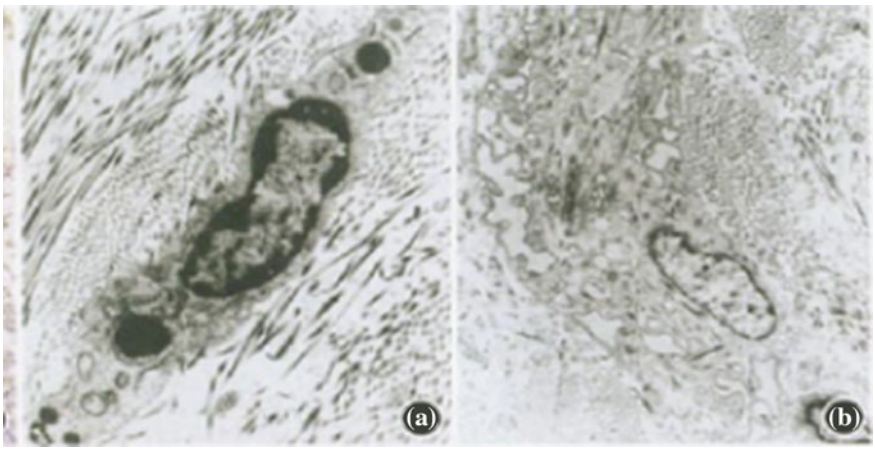

Fig. 1 Ultrastructural changes of FB in the 14 days after the burn injury of the rat model of diabetes mellitus and normal rats. **a** In diabetic rats, the cytoplasm of dermal fibroblasts lysosomes was significantly increased. TEM × 15000. **b** The normal rat dermis were observed in mature FB with spindle shaped. TEM × 800

and has stronger contraction ability than FB, is the main power of wound contraction. The lack of MFB and/or a-SMA inadequate expression in diabetic wound, which suggested that delayed or impaired diabetic wound healing may be related to the deficiency of wound matrix synthesis and contraction disorder. More important is that in the FB cytoplasm of diabetic wounds, in addition to the endoplasmic reticulum expansion and the lack of actin filaments, the lysosomal increased obviously, this might suggest the FB synthetic function of diabetic wounds was impaired, but the degradation function was enhanced.

3.5.3 The Effect of Glycosylated Extracellular Matrix on FB

AGE mainly accumulated in a long half-life, large molecular weight of protein, skin tissue is rich in collagen protein, AGE is easy to accumulate those parts, and more in the form of glycosylated collagen. Niu et al. mode the glycosylated ECM model in vitro, they confirmed that glycosylated matrix induced cell-cycle arrest and apoptosis of dermal fibroblast, while application of RAGE blocking antibody redressed these changes [59]. 3-deoxyglucosone (3DG) is one of the AGEs precursors. The migration ability of FBs can be efficiently reduced when cultured on 3DG-treated collagen [60]. Two years later this group provided that the inhibition in fibroblast migration, proliferation, and collagen expression by exposure to 3DG-collagen was mediated via extracellular regulated kinase 1/2 (ERK1/2) and Akt downregulation through activation of p38 MAPK (Mitogen-Activated Protein Kinase). They also indicated that 3DG-modified collagen can lead to oxidative stress, endoplasmic reticulum stress, and induce apoptosis via caspase-3 activation [61]. Therefore, Glycosylated ECM has an critical influence on the function of FBs in diabetic skin.

3.5.4 Secretion and Synthesis Disorder of FB

The synthesis of collagen in the matrix is the basis of tissue repair after skin burns. Lin et al. indicated the declined ability of collage synthesis and secretion of FB in diabetic wound tissue by measuring the contents of hydroxyproline and the ratio of collagen types I and III [58]. As the main cells of collagen synthesis, the proliferation disorder of FB obviously had a negative effect on collagen synthesis and wound repair.

It's reported that diabetic mice fibroblasts were detected with highly expression of matrix metalloprotease type 9 (MMP-9), and a severe impairment in VEGF production under normoxic and hypoxic conditions, showed an increased prodegradative activity [62]. The ability to synthesize NO was failure with the increase of MMP-8 and MMP-9 in human diabetic fibroblasts [63]. Also in human fibroblasts, they were confirmed the prodegradative phenotype by the increased MMP-2 and MMP-3 production and reduced collagens gene expression [64, 65]. In the process of wound healing dysfunction of producing NO is detrimental to wound

repair. NO donors' administration is considered to be a controller to restore the balance of MMPs and has the ability of stimulating cell proliferation [66].

3.5.5 The Effect of AGEs on Fibroblast

Any endogenous or exogenous factors that may lead to changes in the biological function of dermal fibroblasts may affect the biological process of wound healing. Wang et al. [56] confirmed that in vitro AGEs can inhibit the proliferation of FB, and induce cell apoptosis with dose dependent. And compared with diabetic rats, the sensitivity of FB to AGEs was higher in normal rats. Many scholars reported that primary dermal FB cultured in high glucose or AGEs medium, that was found inhibited the proliferation, decreased collagen synthesis, decreased synthesis of hyaluronic acid, abnormal expression or activity of proinflammatory cytokines or growth factors (such as IL-1, IL-6, TNF-a, PDGF and CTGF) and matrix metalloproteinase (MMP-2,3,9,13). It has confirmed that the molecules that can bind to AGEs are P60, P90, galectin-3, macrophage scavenger receptors and AGEs specific receptors (such as RAGE, AGER1, AGER2 and AGER3). AGEs receptor (RAGE) was found on the membrane of fibroblasts,and persistent high expression of RAGE on FB in diabetic patients.

3.5.6 Effect of Oxidation Stress on FB in Diabetic Condition

AGEs was not conducive to wound repair, and it is found that there was an increase in oxidative stress in the site of AGEs deposition. Extended exposure to reactive oxygen species (ROS) is thought to lead to cellular dysfunction and organismal death via the destructive oxidation of intracellular proteins, lipids, and nucleic acids.

Diabetic wound is a special wound, diabetes itself can cause oxidative stress through a variety of ways. Ge et al. [67] detected out the significantly increased plasma H_2O_2 in STZ-induced diabetic rat model on the 14th day after deep II degree scald, and decreased GSH (with antioxidant capacity), that may also one of the reason to cause impaired wound healing. In vitro, H2O2 could decrease FB vitality and induce cell apoptosis, these damage could be partly reversed by treatment with antioxidant aminoguanidine [68]. Extracellular superoxide dismutase (ecSOD/SOD3) is a prime antioxidant enzyme in the extracellular space that eliminates ROS. Fujiwara et al. [69] confirmed that reduced SOD3 levels contribute to healing impairments in aged mice. SOD3 deficiency and aged fibroblasts both display reduced production of TGF-β1, leading to decreased differentiation of fibroblasts into myofibroblasts. These impairments include delayed wound closure, reduced neovascularization, impaired fibroblast proliferation, and increased neutrophil recruitment.

3.6 Keratinocytes

Diabetes is an important health issue because of its increasing prevalence and association with the development of serious complications include impaired wound healing [70].

In the process of wound healing, epidermal keratinocytes is a new final epidermal layer to cover the wound formed by migration, proliferation and differentiation, as an important symbol of wound healing. The biological behavior of normal play a very important role on wound healing process and quality plays. However, in diabetic skin tissue, due to the influence of the microenvironment of the skin tissue, the biological behavior of epidermal keratinocytes will have a series of changes and will affect wound re epithelization.

3.6.1 The Changes of Epidermal Keratinocytes in Cell Proliferation Capacity

The proliferation of epidermal keratinocytes is one of the most important repair processes in the wound healing process. There are many factors regulating epidermal keratinocyte cell proliferation and apoptosis. The NF-κB can regulate a variety of epidermal keratinocytes and genes related to cell proliferation and signal transduction pathways, such as apoptosis pathway and TNF receptor promoter Fas.

The normal operation of the cell cycle is largely dependent on the precise regulation of the signal transduction pathway. Takao et al. [71] study confirmed that the PMA (BU quinoline alcohol myristate acetate) can significantly activate the NF-κB activity, with the increase of the dose of PMA, will have more epidermal keratinocyte cells to enter the G0 phase. Takao speculated that PMA can inhibit the growth of epidermal cells by launching the cell cycle of the epidermal keratinocyte. Studies [71] also showed that NF-κB activity in the epidermal keratinocytes of diabetic rats was significantly higher than that in normal rats. In vitro studies showed that AGEs could activate the activity of NF-κB in normal epidermal keratinocytes, while the activity was decreased, and the activity was correlated with the concentration of AGEs. S phase and G2/M phase epidermal keratinocytes percentage were significantly lower than normal group rats epidermal cell in AGEs intervention in vitro 48 h. However, inhibiting the activity of NF-κB can partly enhance the activity of epidermal keratinocytes. The classical theory believes that there are two decisive phases in the cell cycle, the transition from G1 phase to S phase when DNA replication; another decisive stage is G2 phase transition to M phase, at chromosome condensation and mitotic stage.

This experiment shows that AGEs hamper two key stages of cell cycle and inhibit the keratinocyte proliferation function of keratinocytes. When the NF-κB activity is inhibited, the proportion of cells cycle in G2/M phase can be increased obviously, but the changes in the percentage of cells cycle in S phase is not

obvious. This suggests that the AGEs can inhibit the cells transition from S to G2/M phase by activating NF-κB pathway.

At the same time, the proportion of cells in G2/M phase increases and cell proliferation ability enhance when inhibited the activity of NF-κB pathway. however, the inhibitory NF-κB pathway does not promote G0 cells into S phase. It showed that AGEs can inhibit the S phase transition from the G1 phase to the S phase through other pathways.

Similar results have been obtained in the study of the apoptosis of epidermal keratinocytes. AGEs can promote the apoptosis proportion of epidermal keratinocytes by regulating the NF-κB signal.

3.6.2 The Changes of Epidermal Keratinocytes in Cell Migration Ability

The normal play of epidermal keratinocyte migration function is an important repair behavior in the process of re-epithelial and wound healing. It plays an important role in the process and quality of wound healing. The formation of skin wound can stimulate the migration of epidermal keratinocytes to the wound surface, and participate in wound healing.

A study found [72], in impaired diabetic wound the migration ability of epidermal keratinocytes is enhanced, at the same time. In vitro research, AGEs intervention can significantly promote the migration, the ability of cell migration returned to normal when inhibiting the activity of NF-κB.

3.6.3 The Changes of Epidermal Keratinocytes in Other Biological Functions

The adherent ability of epidermal keratinocytes in culture medium reflects the activity of cells. Cell proliferation and division can only be carried out after the cell is adherence to the culture flask bottom. Tian et al. found that [73], the epidermal keratinocytes come from diabetic skin, were cultured in normal medium in 48 h, its adherence rate was significantly lower than the epidermal keratinocytes come from normal rat. AGEs in vitro can significantly reduce the adherent rate of epidermal keratinocytes, but the inhibition of NF-κB activity could not increase the rate of adherence of epidermal keratinocytes.

EGF (epidermal growth factor) can promote the proliferation of epithelial cells, fibroblasts, enhance the vitality and delay the aging of the skin cells, so that the composition of the skin to maintain the best physiological state. After the trauma, a large number of EGF expression is helpful to the early epithelium of the wound. Studies have found that [72] AGE can hinder the use of epidermal keratinocytes in EGF in the culture medium. Epidermal keratinocytes derived from diabetic rats reduced the use of EGF in culture medium. In vitro experiment showed that AGE

intervention of epidermal keratinocytes will reduce its use of EGF, inhibit the activity of NF-κB, the utilization ability of EGF to improve.

3.7 Neuropathy and Diabetic Wound Healing

Some scholars believe that the function changes of neuroendocrine system may be related with impaired wound healing. The relationship between the nerves and immune or cutaneous cells is closely, nerves can effect wound healing through inflammatory pathway, even in early and complication of diabetes [74]

Diabetic neuropathy (DNP) is one of the most common chronic complications of diabetes, and is the leading cause of diabetic foot ulcer impaired healing. As a part of peripheral nerve system, cutaneous nerve plays an important role in the protection, defense, metabolism, temperature regulation and sensation of the skin. But because of the anatomical and structural characteristics, make it become the most easily involved tissue in the course of diabetes. Long-term complication of diabetes is characterized by the progressive loss of somatic and autonomic nerve fibers, so DNP is often easy to be unnoticed [75]. In recent years, the study found that DNP could damage intercellular information exchange transfer between skin tissue and central nervous system, and cause abnormal structure and function of skin, also may be an important cause of wound healing [76].

3.7.1 The Pathological Changes of Skin Nerve in Diabetic State

Skin is rich in peripheral nerve fibers, according to the function, that can be divided into two categories: sensory nerve fibers and autonomic nerve fibers. In diabetic state, the pathological change of skin nerve is mainly including two aspects: structural change and function change. Structural change is mainly manifested as a reduction in the number of nerve fibers, myelinated nerve myelin edema, degeneration, dissolution, axon squeezed, degeneration of Schwann cell, exposed fibers; unmedullary nerve edema, vacuoles, actin filaments and microtubules are not arranged neatly. Function changes mainly include somatosensory and autonomic dysfunction, mainly manifested as pain, feeling of allergy and loss of one or several senses, as well as blood vessels, vertical hair muscle contraction dysfunction and abnormal sweat gland secretion. The risk of foot amputation was greatly increased in patients with peripheral sensory neuropathy [77].

3.7.2 The Pathogenesis of DPN

The pathogenesis of diabetic neuropathy is complex, and many factors may be involved in its occurrence and development. The metabolic factors, oxidative stress,

vascular factors, neurotrophic factors and immune factors were discussed in this paper.

3.7.3 Metabolic Factors

Diabetes mellitus is a metabolic disease characterized by persistent hyperglycaemia. There are many abnormal pathways and the accumulation of metabolites in the course of disease, diabetes cause a series of metabolic disorders, which interfere with the nervous material and energy metabolism, resulting in damage to its structure and function.

3.8 Excessive Activation of Polyol Metabolic Pathway

Aldose reductase and sorbitol dehydrogenase is a key enzyme in the polyol metabolic pathway. The polyol metabolic pathway is excessive activation in diabetic state, fructose and sorbitol accumulation in nerve cells, leading to the cell degeneration, edema, and segmental demyelination and axonal necrosis. The polyol pathway can also induce the depletion of glutathione by overexpression of aldose reductase, and then activate NF-κB, leading to Schwann cells secrete neurotrophic factors decreased, resulting in repair disorders after nerve injury [78]. Treated with aldose reductase inhibitors (ARIs) to animal models can effectively reduce the levels of sorbitol in nerve cells, Na^+-K^+-ATP enzyme activity recovery and improve of nerve conduction velocity and abnormality morphology [79].

3.9 Inhibition of Inositol Metabolism Pathway

In diabetic state, the over-activation of the polyol pathway makes the synthesis of inositol decrease, at the same time glucose competitive inhibition of inositol transport, which further increases the intracellular inositol decreased state. Inositol is necessary for the Na^+-K^+-ATP enzyme, the decrease of the inositol can lead to the decline of Na^+-K^+-ATP enzyme activity, which in turn can affect the carrier transport of inositol, and form a vicious circle. Inositol is also a component of the myelin sheath, its metabolic disorder, which will certainly affect the energy metabolism and structural integrity of the nervous tissue.

3.10 Accumulation of Advanced Glycation End Products

Enhanced AGEs deposition in the nerve tissue can lead to the increase of the cell skeleton protein, which can damage the axial plasma transport, and affect the intracellular signal transduction and protein phosphorylation or phosphorylation, so that axonal degeneration. At the same time, AGEs deposition in nutrient vessels of the nerve intima, is not only make the lumen stenosis, occlusion, but also combine with RAGE on the endothelial cells, reduce the formation of NO, affect the nerve blood supply and lead to neuropathy. Recently Duran-Jimenez and et al. found glycosylated extracellular matrix can also be affecting the nutrient supply of nerve fibers and bud proliferation and impact regeneration ability after nerve injury [80, 81]. As the support cells of peripheral nerve cells, Schwann cells play a crucial role in the process of injury and repair and regeneration of nerve. Chen etc. observed the effects of high glucose and AGE-HSA on Schwann cells in vitro, results showed that, high glucose, AGE-HSA could obviously inhibit the proliferation of Schwann cells, promote the apoptosis, and the effect of AGE-HSA is closely related to its concentration. This view is further validated theory of "diabetes of Schwann cell disease". It also showed that repair and regeneration barriers after nerve injury in diabetic patients and diabetic neuropathy had a common cause in the pathogenesis [82].

3.11 Others

Currently, it is found that the essential fatty acid metabolic disorders, vitamin deficiency, accumulation of homocysteine, abnormal cytokine and growth factors secretion and et al. have a certain relationship with occurrence and development of DPN [83].

4 Oxidative Stress Enhancement

High glucose environment in diabetic patients can damage the mitochondrial electron transport chain, so that the oxygen free radical (ROS) such as hydrogen peroxide, ultra hydrogen peroxide and so on production increased. ROS can cause lipid, nucleic acid, protein oxidation, reduce the nerve blood vessels and increase the apoptosis of nerve cells. At the same time, oxidative stress was also enable to reduce neurotrophic factor level by 64 %, and produces 8 hydroxy-2′-deoxy guanine, damage the DNA and Na^+-K^+-ATP enzyme activity decreased, resulting in nerve function and abnormal structure. Thermal pain sensitivity of motor nerve was significantly improved in the animal model of treatment with anti oxidation.

5 Abnormal Neurotrophic Factor

Neurotrophic factor is a kind of protein molecules secreted by the body, maintaining the survival, growth and differentiation of nerve cells. It plays an important role in the maintenance of nerve morphology, regeneration and the release of the transmitter. It was found that the nerve growth factor (NGF), brain derived neurotrophic factor, IGF-I, IGF-II and other neurotrophic factors decreased in diabetic state. Chu et al. [84] found in animal models, To improve systemic IGF-I levels can recovery hypoalgesia. But there are also contrary reports, Kim HC and other found that skin tissue of diabetic patients NGF mRNA expression increased, but the level of serum NGF was elevated [85].

6 Vascular Factors

Diabetic nerve biopsy showed nerve ischemia, infarction, endothelial cell proliferation, capillary basement membrane thickening. Microvascular structure and function abnormalities, abnormal blood rheology, anticoagulation and fibrinolysis imbalance, and induced nerve ischemia, hypoxia, is an important cause of neuropathy. Moreover microvascular disease can cause nerve aregeneratory after damage. Recently, Doupis et al. [86] also found in diabetic patients with peripheral neuropathy that the function of endothelium-related vasodilatation and C fiber-mediated vasoconstriction were impaired.

7 Immune Factors

The research confirmed that the existing autoantibodies to nerve tissue in patients with type I and type II diabetes, such as ß-microglobulin antibodies, anti microglobulin antibody, can cause nerve damage. Peripheral nerve myelin protein combined IgG and IgM in diabetic patients was respectively 4 times and 14 times of in non diabetic patients [87].

7.1 The Relationship Between DPN and Impaired Wound Healing

Wound healing is a complex network process with inflammatory cells, repair cells, extracellular matrix and multiple factors, which is highly coordinated and controlled. Post trauma, skin nerve endings can be involved in wound repair process through the release of a variety of neuropeptide. Diabetic skin sensory neuropathy

patients are easy to suffer from injury, delayed wound healing and even nonunion. Diabetic skin neuropathy through the secretion of neuropeptides may play an important role in wound healing in diabetes mellitus.

7.2 The Effect of DNP on Inflammatory Cells

A variety of noxious stimulus can induce skin nerve endings release neuropeptides and cause neurogenic inflammation in the local. CGRP and Substance P are the most common neuropeptide released from the cutaneous nerve endings, they are often released at the same time, all belongs to a strong vasodilator. Different neuropeptides play different roles in the inflammatory process. Now that, the substance P, bradykinin and vasoactive intestinal peptide are as proinflammatory mediators, CGRP is considered as a potent anti inflammatory mediator. CGRP in skin tissue in early stage of STZ induced diabetic rats, vasoactive intestinal peptide positive fibers was increased. In diabetic mice model, the early inflammatory response of the wound was delayed, and the total inflammatory reaction time was prolonged, which may be related to the abnormal release of the neuropeptide in the skin nerve endings [88].

7.3 The Effect of DNP on Cytokines

Cytokines play an important role in regulating the proliferation, migration and differentiation of various repair cells. Richards AM found that substance P can promote the synthesis of keratinocyte interleukin, TGF-α, and increased INF-γ mediated expression of keratinocyte adhesion molecules, their expression and synthesis can promote wound healing. In addition to the direct effect, but also the neuropeptide synthesis and expression of indirect effects of various cytokines related to wound repair [89]. Some neuropeptides are downregulated (SP, NPY, CGRP) in diabetes and others upregulated (CRF, α-MSH and NT) with the net effect being that downstream cytokines in the skin are dysregulated. This disruption in the balance of cytokines may cause impaired wound healing.

7.4 The Effect of DNP on Repair Cells

Although there is no direct evidence that neuropathy can lead to impaired wound healing, but the neuropeptides do play an important role in this connection. Neuropeptides exert their biological effects by binding to specific receptors on the cutaneous cells, that such as immune cells, Langerhans cells, ECs, mast cells, fibroblasts and keratinocytes, or through direct activation of intracellular G-protein

signaling cascades [90]. Known neuropeptides that related to wound repair are SP, NPY, CGRP, corticotropin releasing factor (CRF), α-melanocorticotropin releasing hormone (α-MSH) and neurotensin (NT). The roles for NT and α-MSH in diabetic wound need in further verification [91, 92].

8 Summary and Outlook

There is no doubt that DNP is one of the important causes of diabetes impaired wound healing. The neuropeptide is an important material in the process of wound healing. Peripheral vascular disease with neuropathy may result in impaired wound healing. The homeostasis maintained by the nerve-immune system is disrupted in diabetic skin. Because of the limited cognition and condition, we have a lot of unknown function in the process of wound healing of diabetic skin, so much knowledge is worthy of depth study. Such as, we know that the skin nerve endings can secrete a variety of neuropeptide and expression of related receptors, but we don't know what changes in their expression, binding, and subsequent reactions in diabetes. We know that many kinds of neuropeptides may be involved in wound healing, but it is still not clear what kinds of neuropeptides and through which signal transduction pathways are involved in wound healing, as well as in diabetic state, what changes have occurred. Believe that with the solution of these problems will help to deepen our understanding for the role of cutaneous neuropathy in diabetic wound healing, provide new ideas and methods for the treatment of diabetic wound healing.

9 Summary of All

The causes of refractory diabetic wound is so complex, it involves many links and cross-linking. Before the trauma diabetic skin has been different from the normal skin of some changes, including the decline in the sense of nerve,sub inflammatory state, some diseases of micro blood vessels and so on, making it more susceptible to infection and injury. And once the wound is formed, it is difficult to heal. Treatment of diabetic refractory wound is a difficult clinical problem. The summary of the relevant mechanism in this paper is just "the tip of the iceberg", the more clear mechanism is to be further explored by researchers.

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Stem Cell Based Biotherapy for Radiation Related Injury

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Abstract Radiation injury occurs after nuclear explosives, radiological or nuclear terrorism, nuclear accidents, and radiation therapy in combination with surgery, and it is more complicated and difficult to heal than single radiation or wound injuries. The stem cell-based therapy holds promise for radiation related injuries treatment. In this review, we summarized some representative biological properties of MSCs pre- and post-irradiation, discussed the feasibility of MSCs to apply to the treatment of radiation injuries on animal models and clinical patients, and elucidated the mechanisms of MSCs-based therapy to radiation injuries.

Keywords Radiation · Stem cells · Skin · Radiation related injuries · Biotherapy

1 Introduction

Radiation injury is a medically challenging and complicated disorder that involved in nuclear explosives on the battlefield, a domestic terrorist attack and cancer treatment [1–3]. Cells suffered to IR fail to repair the lesion will result in cell cycle arrest, mutagenesis or apoptosis [4]. Total body irradiation (TBI) is even associated with bone marrow failure, organ dysfunctions and acute radiation syndrome (ARS) [5]. Unfortunately, efficient treatment for radiation injury is limited to conventional medical care. Therefore, it is urgent to seek new approaches to treat radiation injury.

Resident stem cells with properties of self-renewal and differentiation are responsible for the recovery of tissues and organs from IR [6]. Studies have described adult stem cell efficacy in a variety of disease and injury models, and this guided a countermeasure for the radiation injury treatment. So researchers began to

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focus on MSCs, which have a paracrine effect and participate in the regeneration of damaged tissue repair, and the stem cell based therapy were proved to be an efficient approach to repair radiated tissues.

2 Biological Characteristics of MSCs

Over the course of the past decades, the sources of MSCs have been expanded to almost all tissues, including adipose tissue [7], skin dermis [8], umbilical cord [9], liver [10], et al.

Previous studies have proved that MSCs presented easily attachment to the surface of culture dishes and generally exhibited elongated spindle morphology, fibroblast-like shape [11]. These cells can proliferate rapidly and be stably passaged for several generations [12, 13]. In addition, MSCs are characterized by two of the most primitive capacity. One is to form colonies (CFU-F) in vitro which reflects the self-renewal capacity [14], the other is differentiation potential into multiple lineages [15–17].

Over the years, extensive experimental studies have enhanced our knowledge of MSCs. MSCs were reported to express variable levels of CD105, CD73, CD44, CD90 (THY1), CD106/VCAM-1, CD71, CD166, CD146, STRO-1 [15, 18, 19]. And the CD markers such as the haematopoietic markers CD45, CD14 and CD34 or the co-stimulatory molecules CD86, CD80 and CD40 were established as negative MSC markers [20–22]. However, to date, there were no generally accepted specific and unique surface markers for MSCs. Furthermore, the expression of these surface markers varies from different species, tissues and conditions [23].

As the rapid proliferation and differentiation capability of stem cells attracted a lot of attention, many following researches have been carried out on the topic of the function of MSCs. Studies have demonstrated that MSCs can significantly suppress the proliferation of T-lymphocytes and up-regulate regulatory T (Treg) cells proportions and regulate the immune system, which suggested that MSCs may have therapeutic potential for immune diseases treatment [24, 25]. In addition, MSCs can produce and secrete various cytokines, growth factors, and chemokines that may be essential for medical effect [26]. Studies showed that MSCs can locate to a wide variety of tissues following intravenous administration and may be capable of participating in ongoing cellular replacement and turnover within an engrafted organ [27, 28].

3 The Effects of Radiation Upon Biological Characteristics of MSCs

3.1 The Disparity of Sensitivities to Irradiation Between Various Tissues

Radiation injury is characterized as a whole body ranged damage, but the sensitivity to IR varies in individual cells and tissues. It is well known that cells with rapid renewal such as haemopoietic stem cells (HSCs) and lymphocytes are sensitive to radiation [29], and were suggested as acutely responding cells. While muscle cells, cartilage cells, osteocytes were not affected that much. Furthermore, MSCs is one of the survived cell populations with the capacity of resistance to irradiation. The retaining radio-resistance of MSCs is seemed to be one of the most important features for their potential to support tissue repair following radiation injury.

As MSCs consisting of multiple populations of cells, the influence of IR on them is largely depending on the dosage of irradiation. Reports demonstrated that the cells increased in size, exhibited a flat and large cellular phenotype, with reduction in the number of mitoses after radiation exposure [30]. And capacities of MSCs such as proliferation, colony-forming and differentiation altered in a dose-dependent manner [31, 32]. Interestingly, though irradiated cells showed a significant increase in the percentage of apoptotic cells, the degree of cycling cells reduction did not grow progressively as the doses were increased [33].

3.2 Stable Characteristics that Does not Affect Apparently by Irradiation

As a population with the capacity of resistance to radiation, MSCs have been reported to retain the stem cell related characteristics after radiation both in vitro and in vivo. Irradiation with high dosage resulted in little reduction in relative and absolute velocities for cells, and a hallmark of stress-induced premature senescence up to 20 Gy irradiation [31]. However, the adhesion and migration abilities of MSCs were not markedly altered by IR. In addition, data have suggested that the radiation exposure did not significant reduce the apoptosis level of MSCs and the numbers of viable cells remained relatively stable [34]. As a hallmark of MSCs, the oriented-differentiation ability was also not abrogated even following sublethal dose or high radiation dose radiation exposure [30, 35].

Analysis of cell surface markers has showed that radiation has no significant effect on gene expression pattern of MSCs. The osteogenic-specific markers BMP-6 and RUNX2 showed stable expression following IR. Similarly, PPARγ, the marker

for adipogenic differentiation, was stably expressed following IR [30]. Furthermore, the gene expression of IR-induced DNA damage markers ATM, ATR, BRCA1, CHEK2, MDC1, CHEK1, and TP53BP1 mostly showed the same as high-dose γ-irradiation [36]. Furthermore, the T-cell suppressive function of MSCs was also preserved after exposure to ionizing radiation [37]. And researchers also indicated that irradiation does not impair the immunosuppressive capacity of bone marrow-derived MSCs in vitro and thus might promise the safety of MSC-based cell products in clinical applications [38].

3.3 *Irradiation Affects the Cell Cycle, Secretion, Autophagy of MSCs*

Many studies showed that there was an accumulation in the G2/M phase, which suggested that MSCs have an arrest in cell cycle [31, 39]. As a result of gene analysis, the expression p21 and p16 were upregulated after irradiation, indicating cell cycle arrest, and expression of MYC, TP53 (p53), and KLF4 were downregulated [36].

Considering the secretion ability of MSCs, high dose of radiation treatment (30-60 Gy) to MSCs significantly influenced the cytokine profile in culture media, including less consumption of platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF), and increased of interleukin (IL)-6, compared to the culture medium of non-irradiated cells [36]. Besides, MSCs preserved the antioxidant capacity for scavenging reactive oxygen species (ROS) and active double-strand break (DSB) repair to increase their radioresistance [40]. The expression of oxidative stress genes as oxidative stress-specific dismutase-1 (SOD1) increased in radiated MSCs [41].

Moreover, previous studies have demonstrated that exposure to IR can induce a reduction of autophagic flux, suggesting damage to the autophagy process, which was a protection to prevent radiation deteriorative processes, and its decline closely contributed to senescence [33, 42].

3.4 *DNA Repair and DNA Damage*

Ionizing radiation (IR) can induce DNA damage depending on the type and dose. Irradiation can induce DNA double-strand break, and these breaks are repaired by two processes known as nonhomologous end joining (NHEJ) and homologous recombination (HR) DNA repair systems. Any defect in these pathways may

increase cellular susceptibility to irradiation-induced mitotic death or apoptosis [40]. Experiments suggested that the reduced activity of NHEJ system rather than HR system is the main cause to the impaired DNA repair capacity of MSC following IR. And the NHEJ is the only double-strand break repair system that is active in non-cycling cells [33]. Taken together, these important cell characteristics may facilitate resistance to irradiation and the less-sensitivity of MSCs hold promise for the clinical application for treatment.

4 Therapeutic Potential of MSCs for Radiation Induced Injuries

4.1 Various Sources of MSCs Became a Therapeutic Approach to Irradiation Injury

Currently, there is no effective medical countermeasure for the management of radiation induced injuries. Years ago, various synthetic agents were investigated for the efficacy in protecting against IR induced tissue damage. However, these drugs have the potential to cause serious side-effects including decreased cellular function, hypotension, nausea, and even death [43]. Effective treatment should be developed to mitigate IR-induced injury.

Emerging adult stem cell-based therapy has aroused great hopes for the treatments of diseases and injuries, including radiation induced injury. Increasing evidence has shown that transplantation of MSCs was a potent therapeutic strategy for radiation induced damages in various organs and tissues, such as salivary gland [44], lung [45], liver [46], skin [47], bone marrow [48] and intestine [49]. In recent years, autologous MSCs were also reported to apply on clinical patients with system or local radiation exposure, and the efficiency of stem cell based therapy has been proved [50, 51]. The transplantation of MSCs was chosen as a new approach to treat the radiation related injuries (Table 1).

Furthermore, MSCs were confirmed to play an important role in tissue regeneration after IR. Experimental data demonstrated that human mesenchymal stem cells (hMSCs) migrated into various tissues including bone marrow, liver, lung et al. following transplanting into adult unconditioned mice. They suggested that the property of hMSC to home to various organs in response to tissue injuries might be a strategy to repair the IR induced damages [64].

Table 1 MSCs therapy for radiation related injuries

Amount and source of MSCs	Recipient	Dosage of radiation	Infusion approach	Target	Article
5 × 105 bone marrow-derived mesenchymal stem cells from C57BL/6 mice	C3H/HeN mice C57BL/6-Tg mice	35 Gy right hind leg	Intravenous injection six weeks post-ionizing radiation	Cutaneo	[47]
1 × 106/50 μl, from C57Bl/6-Tg mice	C3Hf/Kam female and C57Bl/6 male mice	4, 6, 8, 15, 18, 21 Gy WBI/skin-only irradiation	Fibrin microbead implantation less than a 15-min interval	Cutaneo	[52]
50 × 106 MSCs × 3 autologous MSCs	Female minipigs	≥50 Gy local irradiation (at the right lumbar)	Intradermal day 27, 48, 69, 81, 96 after irradiation	Cutaneo	[53]
6 × 106 BMSC from rat	Rat	140 Gy locally to skin	Subcutaneous injection early, day 8 after exposure, during the early desquamation	Cutaneo	[51]
1 × 107, bone marrow-derived mesenchymal stem cells	SD rats	6 Gy whole body	subcutaneously around the wound time not mentioned	Wound	[54]
1.2 × 105 cm^2 (area 12.56 cm^2 × 2) BMSC from porcine	Minipig	20 Gy local irradiation dorsal regions beside the spine	Construct cultured cutaneous substitutes/human amniotic membrane cover	Wound	[55]
1 × 106/100 μl murine BMSC line	Female C57/Bl6 mice	7 Gy	Intravenous from 16 to 24 h after radiation	Hematopoiesis	[48]
5 × 106 UCB-MSCs from human	Female BALB/c	7 Gy TBI	Intravenous 4 h after irradiation	Hematopoiesis	[35]
6 × 106 Trx-1-overexpressing ucMSC from human	NOD/SCID Mice	4.5 Gy	Intravenous	Hematopoiesis	[56]
	SD rats			Intestine	[49]

(continued)

Table 1 (continued)

Amount and source of MSCs	Recipient	Dosage of radiation	Infusion approach	Target	Article
1 × 106, adipose-derived mesenchymal stem cells from male human		15 Gy whole-abdominal irradiation (from the xiphoid process to the pubic symphysis)	Intraperitoneally injection within 2 h		
1 × 106/200 μl umbilical cord-derived mesenchymal stem cells-medium	BALB/C male mice	10 Gy local abdominal irradiation	Intravenous injection immediately	Intestine	[57]
5 × 106 BMSC from human	NOD/SCID mice	3.5 Gy TBI 5.7 Gy abdomen	Intravenous 24 h after irradiation	Small intestinal	[58]
BMSC from rat	SD rats	14 Gy whole abdominal irradiation	Peritoneal cavity conditioned medium intravenous concentrated	Intestine	[59]
5 × 106/100 μl BMSC from human	NOD-SCID mice	3.2 Gy TBI	Intravenous 24 h after TBI	Liver	[46]
5 × 105/15 μl, BMSC from C57/BL6 mice	C57BL/6 mice	15 Gy local irradiation (the head and the neck fields)	Intraglandular 24 h after irradiation	Salivary gland	[44]
1 × 106/100 μl, Ad-HGF-modified MSCs from adult human	Female B6 mice	20 Gy of γ rays locally to the lung	Intravenously 6 h after radiation	Lung	[45]
5 × 106/0.5 ml Ad-MSC from female rat	Rat	15 Gy local irradiation to the right thorax	Intravenous 2 h after irradiation	Lung	[60]
0.1, 1, 5, or 10 × 106, autologous MSCs	Male SD rats	27 Gy local irradiation (the colorectal region)	Intravenous injection immediately after radiation	Colon	[61]
2 × 106 per kilogram autologous MSCs	Male pigs	Between 21 and 29 Gy delivered to the rectum	Ear vein on days 27, 34, and 41 postirradiation	Rectum	[62]
6 × 106 BMSCs from mice	Mdx mice	Lethal radiation TBI	Intravenous	Central nervous system	[63]

4.2 Application of MSCs to Cutaneous Wound and Inflammation

Skin is the largest organ in the body, and dermis has been proved to contain various stem cells populations [65]. Researchers have established the importance of skin dermis as an easily accessible source of stem cell populations and their promising significance in wound repair and other diseases [66, 67]. Cutaneous reactions are major actors in radiation accidents and a limitation for long term radiotherapy, and despit the radiation insensitivity of dermis derived stem cell, wound healing time would prolong following combined radiation injury.

Results of experimental applications of MSCs on the radiation induced wound were given to show that MSCs can significantly improve the rate of cell growth and wound recovery [67]. Lymphocytes accumulated locally at the dermis/subcutis border and vascularization was improved [53]. Moreover, cytokine significantly architected dermal reconstitution, improved angiogenesis, and faster epidermal recovery [68].

Cutaneous fibrosis has long been considered as a significant adverse issue after IR exposure. Previous studies have shown that systemic BMSC administration can inhibit drug induced pulmonary fibrosis [69, 70] and that local application can improve cutaneous wound healing [71, 72]. In addition, systemic infusion of MSCs was also discovered to reduce radiation-induced fibrosis by altering macrophage phenotype and suppressing local inflammation [47]. Furthermore, with the application of autologous MSCs around and in the lesion in clinic case, the healing of the lesion proceeded smoothly without side effect [73].

4.3 Application of MSCs to Haematopoietic System

As we already known, bone marrow is sensitive to radiation damage, and MSCs were wildly used in reconstruction of haematopoiesis and anti-GVHD after HSCs transplantation. Much work has been reported recently in these filed. Experimental data demonstrated that a lower apoptotic ratio, a lower ratio of G0/G1 cell cycle phases and a higher ratio of G2/M and S phases were observed in MSCs treated bone marrow [74]. Moreover, another report documented that the hUCB-MSCs treatment had regenerative effects on monocytes, lymphocytes, and white blood cells, and could significantly downregulate plasma levels of Flt-3L and upregulate transforming growth factor (TGF-β1) [35].

4.4 Application of MSCs to Intestine

The rapid regeneration and short growth period of the gastrointestinal epithelial cells give gastrointestinal tract, especially intestine, the characteristic of high sensitivity to radiation. Radiation-induced gastrointestinal tract (GIT) damage often occurs after radiotherapy for digestive neoplasms, and a considerable amount of research has been done about the stem cell based therapy for radiation-induced intestinal injury (RIII) during the last decade.

Experimental data indicated that the microvascular endothelial apoptosis was the primary radiation damage lesion leading to stem cell dysfunction [75]. Furthermore, MSCs were reported to improve intestinal integrity through regulation of endogenous epithelial cell homeostasis by both increasing proliferation and inhibiting apoptosis of intestinal epithelial cells [58, 76]. After MSCs administration, the moderated (RIII)-healing effects, including neovascularisation, anti-inflammation, and maintenance of epithelium homeostasis were observed [49]. And the efficient anti-inflammatory effects of MSCs based therapy were again confirmed in clinical case [77]. The injection of MSCs could also significantly accelerated recovery of the gut by stimulating proliferation of the crypt cell pool [78]. With different animal models, many studies proved similar positive effect of MSC to RIII [76, 79, 80]. The components of MSCs conditioned medium could also increase the survival rate, and improved the small intestinal structural integrity of irradiated mice, which supported the paracrine effect of MSCs [57].

4.5 Application of MSCs to Other Organs

To evaluate the potential therapeutic effect of MSCs, a total body radiation exposure was performed on NOD/SCID mice. The results indicated that the plasmatic transaminases (AST and ALT) levels, oxidative stress and apoptosis process in the liver vascular system were all significantly decreased, and cell proliferation in the liver increased that might improve liver dysfunction [46]. In fact, MSCs can exert their therapeutic functions without a grafting on damaged region. There were reports that the infused MSCs were not found in liver, and they corrected liver dysfunction in an indirect manner [81].

Besides, the bone marrow-derived clonal MSCs could ameliorate salivary damage following irradiation by decreasing the apoptotic cells and increasing microvessel density in salivary glands [44, 82]. Earlier studies found that MSCs resident in lung mostly at early stages. A different retention peak with the model of engrafting MSCs to SD rats of 15 Gy local irradiation were reported, which was probably due to the number of engrafted cells or the dose of radiation, and affect chemokine release [60]. Researchers found that MSC-based therapy could effectively reduce secretion and expression of pro-inflammatory cytokines, decrease

expression levels of profibrosis factors, and protect lung epithelial cells, which might also inhibit lung fibrosis [45].

4.6 Application of MSCs to Radiation Combined Injury

Combined injuries are defined as the combination of radiation exposure and tissue injuries from blast and thermal energy, which is much more complicated and difficult to heal than single injuries. Adult stem cell-based therapy has aroused great hopes for the treatments of diseases and injuries, including combined radiation and wound injury.

Experimental data demonstrated that BMSCs over-expressing hPDGF-A and hBD2 could significantly accelerate wound healing process of combined radiation wound injury [83]. Furthermore, the therapeutic effects of MSCs were reported to exist in various aspects of wound healing, including collagen deposition, re-epithelialisation, and granulation formation [55]. Very recently, the granulation tissue derived cells (GTCs) were further characterized as an abundant cell source for their important therapeutic efficacy in wound healing and tissue repair [84]. For skin is relatively insensitive to radiation, in our previous study, we demonstrated that the GTCs from combined radiation and wound injury may represent an alternative source of autologous adult stem cells for transplantation (not published).

5 Therapeutic Implications of Dermal Multipotent Cells for Wound Repair

It has been increasingly established that stem cells play an important role in wound healing. Several studies in recent years also suggest that adult stem cell-based therapy has aroused great hopes for regenerative medicine and the treatments of diseases and injuries [85]. Ideal stem cell sources are thought to be easily accessible, capable of rapid expansion in culture, immunologically compatible, and amenable to stable differentiation or transdifferentiation. Skin, the largest organ of the body, is considered as a natural resource of adult stem cells [65]. However, it is surprising that the dermis, which represents a larger adult stem cell reservoir than the hair follicle and epidermis together, has largely escaped the focus of the majority of the stem cell community.

Until in 2001, our and other groups first described the existence of dermal multipotent cell populations that were unidentified previously in the skin of adult mammals, such as mice, rat, and human, and these cells were considered similar to bone marrow mesenchymal stem cells [86]. By transplanting dermal cells from green fluorescent protein (GFP) transgenic mice intravenously, we provided evidence that these dermal cell populations could differentiated into different cells in

many organs and tissues, such as bone marrow, lung, liver and kidney. Moreover, we established a unique clonal population of dermal multipotent cells (DMCs) from neonatal rat skin [87]. Based on the adhesion to tissue-culture plastic and multi-lineage differentiation potential in vitro, DMCs were easily isolated and incubated on standard culture condition. The surface antigenic profile of DMCs has been shown to be positive for CD44 and CD90 expression, but negative for CD34, similar to marrow mesenchymal stem cells. However, the former showed a much higher proliferation potential. The number of stem cells in the adult tissue is very low, and seeking method to expand the supply of undifferentiated cells remains a challenging issue. We have established that beta-microglobulin, a MHC class I subunit, could act as a novel growth factor to simulate the ex vivo expansion of undifferentiated multipotent stem cells (such as dermal multipotent cells) to reduce the use of fetal bovine serum, which can elicit possible contamination or immunological reaction for clinical application [88].

To investigate the therapeutic effects of the multipotent cell populations within dermal tissues for the repair of wounded tissues, we applied exogenous DMCs to skin wounds and both topical and systemic transplantation of DMCs accelerated the healing of simple wounds in rats. We also noted that topical transplantation exhibited an earlier healing effect than systemic transplantation [89]. The engrafted DMCs were observed to differentiate into multiple cell types that would promote tissue repair, which indicated that they may be a potential source of wound-healing fibroblasts [87]. DMCs also produce many growth factors—such as hepatocyte growth factor (HGF) to improve wound healing [89]. HGF not only induces mitogenic and antiapoptotic activity, but also has an immunomodulatory action to create an appropriate inflammatory response for DMCs at the wound. Furthermore, we also observed that DMCs have the capacity to induce the formation of hair follicle-like structure at the subcutaneous sites in nude mice when implanted along with follicle epithelial cells, suggesting that these multipotent dermal cells are also involved in skin regeneration [90].

In addition to skin wound healing, DMCs are also capable of promoting the hematopoietic recovery in sublethally irradiated rats. These cells can represent an alternative source of adult stem cells for transplantation, restore microenvironment and promote hematopoietic recovery [91]. The implanted DMCs were detected to recruit preferentially to wounded tissues, which was mediated by an elevated expression of stromal-derived factor (SDF-1) after an injury. SDF-1 is a ligand for the CXC chemokine receptor 4 (CXCR-4) on DMCs and SDF-1/CXCR4 signaling is critical for the recruitment of stem cells to the site of injury. DMCs were further applied in rats that received combined radiation and wound injury through tail vein. And we have reported for the first time that systemic transplantation of neonatal dermal multipotent cells significantly promoted survival and accelerated both hematopoietic recovery and wound healing in rats with combined radiation and

wound injury, suggesting that stem cell therapy can achieve multiple therapeutic effects and provides a potential new strategy for the treatment of severe traumatic injuries with multiple tissue/organ damage, such as radiation combined injuries [92].

Given that DMCs play an important role in wound repair and are now emerging as potential therapeutic agents [90], we propose that the dermis may represent one of the best autologous sources of stem/progenitor cells for therapeutic applications in skin replacement and repair of other organs. However, the lack of specific markers for histological localization of stem/progenitor cells within the dermis still remains a problem. Moreover, although stem cell based biotherapy has aroused great hopes for the treatments of diseases and injuries, we and others highlighted that there is a risk that cell therapies will produce malignancies [93]. A spontaneous transformation were observed in the dermal multipotent cells after long-term in vitro culture and that Ras/Raf/MEK signaling pathway, protein phosphatase 2A (PP2A), and transcriptional coactivator PC4 may play a role in the malignant transformation of these cells [93, 94]. Furthermore, we have identified a class of lipophilic heptamethine cyanine cells via an energy-dependent pathway. These dyes have superior optical, biocompatible, and pharmacokinetic properties for tumor targeting and for imaging with a superb contrast index value that make them attractive for detecting tumorigenic cells [95]. Considering this safety issue, the long-term risk for tumor occurrences that results from the use of adult multipotent stem/progenitor cell in different pathological conditions must be further investigated. Moreover, there is an urgent need to develop more sensitive methods to detect tumorigenic cells at early stages.

It has long been a goal in biomedical science to harness endogenous repair mechanisms to promote tissue repair and regeneration. However the recent focus is mainly upon stem cell-based transplantation to treatment of various diseases. It would be another interesting approach to recruit resident dermal stem cells for further chronic wound treatment and skin regeneration. As the evolving body of work shows that stem cell populations resided in many adult tissues, another therapeutic approach would be promising. Recently, the skin granulation tissue has been proposed as a promising source of dermal stem cells, and the newly formed granulation tissue is enriched in cells that expressed stem cell surface markers after wounding [96]. However, the origin and biological characteristics of these cells have not been well investigated. Our team explored that, in normal conditions, dermal stem/progenitor cells keep quiescent, and could be activated to proliferate upon injury and enriched in granulation tissues (Fig. 1). The activated dermal stem/progenitor cells [here we termed granulation tissue derived cells (GTCs)] exhibited with enhanced proliferation, self-renewing, and multi-differentiation abilities. And topical transplantation of GTCs into the combined radiation and wound injury mice accelerated wound healing and reduced tissue fibrosis. We also proved that miR-21 mediated ROS generation negatively regulates the

stemness-related properties of granulation tissue derived cells [97]. Considering the complex factors involving in the wound microenvironment, we further study the response of dermal cells to a single mechanical injury. The wound scratch model in vitro is administrated to mimic the wound trauma. Following the mechanical injury, dermal cells could be completely activated to proliferation at about 36 h, which is consistent with the in vivo data. Furthermore, dermal cells were changed into a stemness phenotype with increase expression stem cells marker Sox2 and vimentin by mechanical injury, which also supplies evidence for in vivo data that GTCs showed enhanced stemness related properties (Fig. 2).

Combined injury involving radiation and a wound is considered a clinical challenge, because previously studies demonstrated that radiation exposure combined with wound trauma results in a negative synergistic effect that is much more harmful than either insult would produce alone. However, our latest experimental result demonstrated that the isolated GTCs from combined radiation and wound injury mice (C-GTCs) possessed comparable stem cell-owned properties with neonatal dermal mesenchymal stem cells and GTCs from normal wounds without irradiation (Fig. 3). Further, we discovered that the order of occurrence of radiation and mechanical injury affected the outcome of combined effects such that survivals of dermal cells improved when mechanical injury occurred prior to IR exposure. Upon mechanical injury, the cell-cell cadherins interactions and focal adhesion complexes were interrupted, and then the inner cellular PI3K/Akt and β-catenin pathway were activated. Following, the stemness, antioxidant ability, and DNA repair ability were increased, which contributed to the radio-resistance of dermal cells (Fig. 4). Considering the easy accessibility, we propose that the granulation tissue may represent one of the best autologous sources of stem/progenitor cells for therapeutic applications in combined radiation and wound injury, not only in the replacement of skin, but also for the tissue repair of other organs.

6 Potential Mechanisms of MSCs Based Therapy

6.1 Anti-inflammation

Transplantation of mesenchymal stem cell (MSC) has been explored as a new clinical approach for tissue repair, and the mechanism of the stem cell based therapy attracted a lot of attention. MSCs can secrete various anti-inflammatory factors, including IL-10, basic fibroblast growth factor (bFGF), Bcl-2, TGF-β1 and so on. Among them, IL-10 is the most concerned cytokine after MSCs engraftment, however, the possible mechanism of IL-10 is a controversial problem which has attracted considerable attention.

Many research studies have suggested that MSCs can inhibit inflammation via autocrine IL-10 signalling, and the BMSC-induced production of IL-10 in

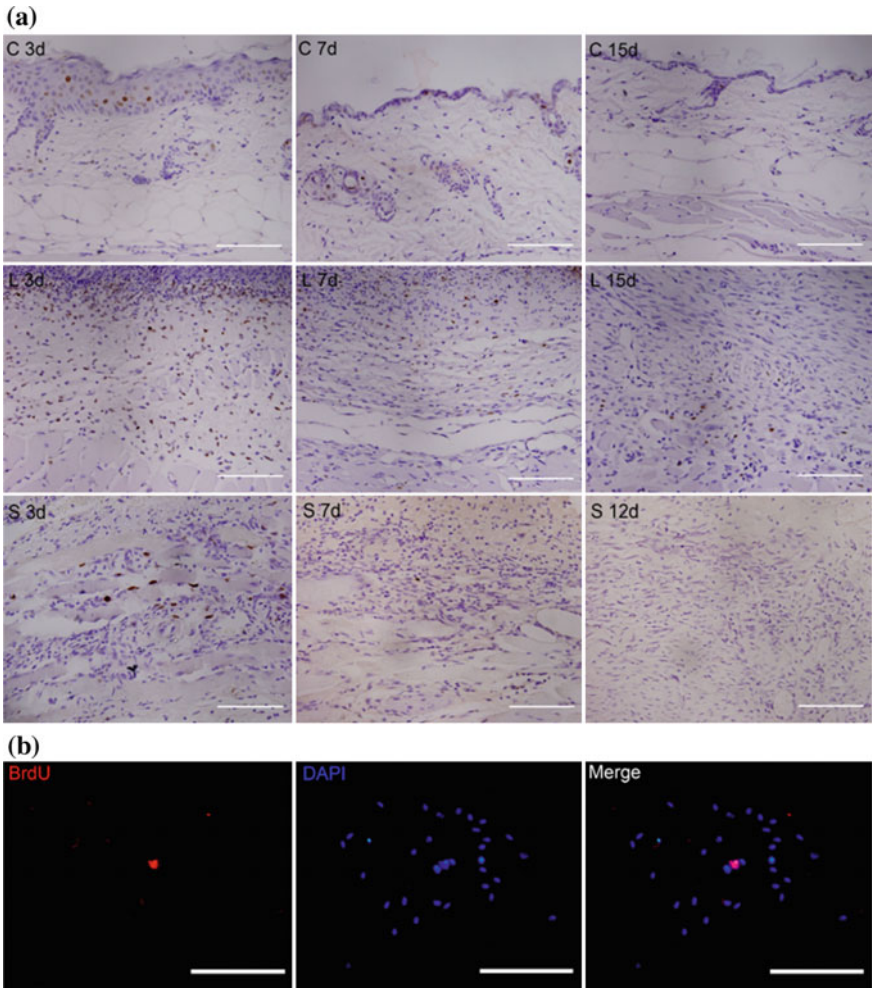

Fig. 1 Activation of dermal stem cell proliferation after wounding. **a** BrdU in normal skin or wound sites was measured by immunohistochemistry. *C*, control group, BrdU was injected consecutively three days in normal mice; *L*, long term BrdU labeling, BrdU was injected consecutively three days after wounding; *S*, short term BrdU labeling, BrdU was injected a single time at the 60 h after wounding; *d*, days after wounding. Scale bar = 100 μm. **b** BrdU in cultured clones of newly isolated in vivo labeled GTCs was measured by immunofluorescence. Scale bar = 500 μm

macrophages is TNF-α dependent, requiring TNF-R1, but not TNF-R2 [98]. While others proposed the novel mechanism that IL-10-secreting Treg was partially involved in the therapeutic effect of human adipose-derived mesenchymal stem cells (hASCs) in colitis mice [99]. Interestingly, researchers also pointed out that although IL-10 associated with an increased number of Treg is involved in the

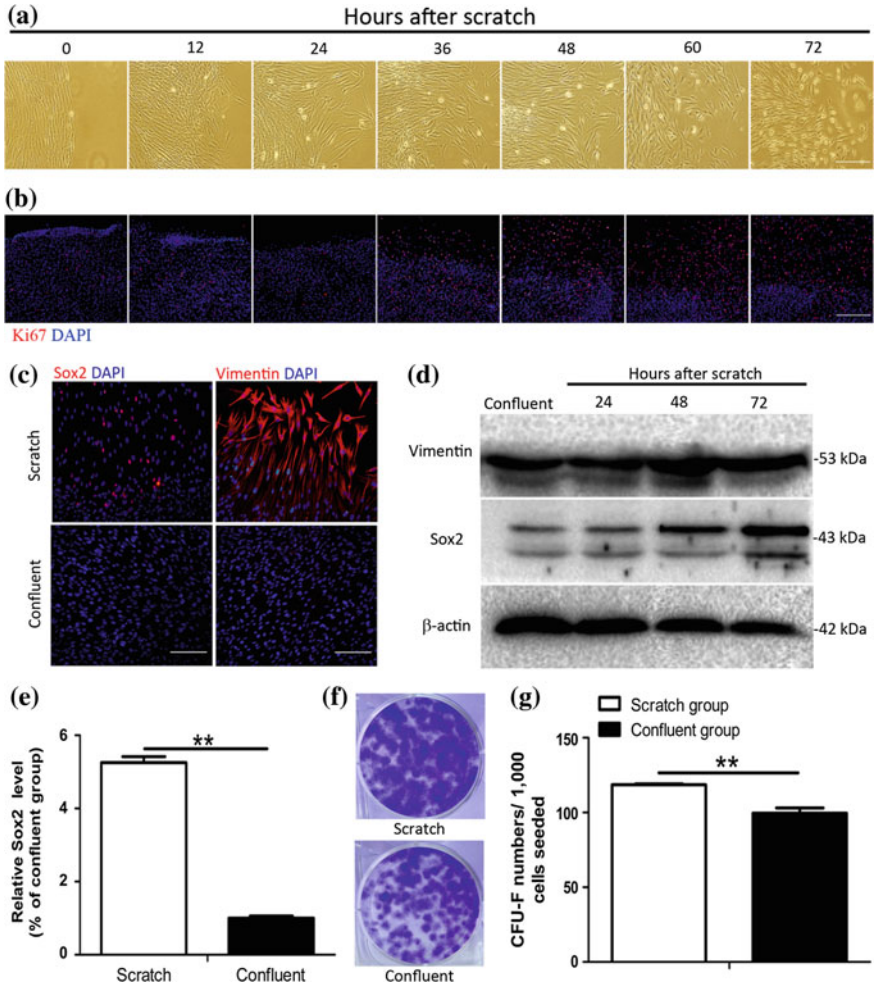

Fig. 2 Characterization of changes in dermal cells following mechanical injury. **a** Representative pictures of dermal cells in wound margins following mechanical scratch. Scales are 500 μm. **b** Immunofluorescence staining for Ki67 demonstrating activation of dermal cells following mechanical scratch. Cell nuclei are counterstained with DAPI. Scales bars are 500 μm. **c** Immunofluorescence staining for Sox2 and Vimentin in dermal cells of scratch edge 72 h following mechanical scratch. Cell nuclei are counterstained with DAPI. Scales bars are 200 μm. **d** Western blot analysis of Sox2 and Vimentin expression at indicated time points in dermal cells following mechanical scratch. **e** Real time PCR analysis of Sox2 expression in dermal cells 72 h following mechanical scratch. **f** Colony formation assay of dermal cells 72 h following mechanical scratch. **g** Quantification of colonies in **f**. **, $p < 0.01$. p values were calculated using the independent-samples t test in **e** and **g**

therapeutic effect of MSCs, the immunosuppressive effect of MSCs may be Treg independent [61]. Though these studies shared different mechanisms, all of them reported a high expression of IL-10 which inhibits the inflammatory process,

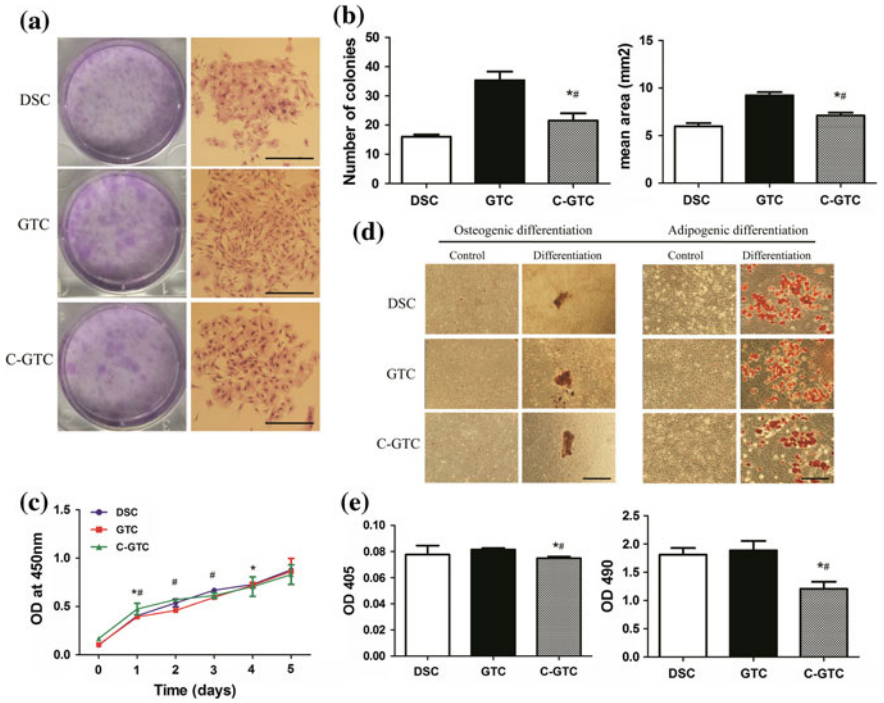

Fig. 3 Isolated GTCs from combined radiation and wound injury mice (C-GTCs) possessed stem cell-owned properties. **a** Colony-formation ability assay (*left* visual observation; *right* images of 200 × magnification). Scale bar = 500 μm. **b** Quantitative analysis of colony-forming ability. **c** Proliferation analysis with cell counting kit-8. **d** Osteogenic and adipogenic differentiation in DSCs, GTCs and C-GTCs (*left side*: osteogenic differentiation and stained with Alizarin Red; *right side* adipogenic differentiation and stained with Oil Red O). Scale bar = 500 μm. **e** Quantitative analysis (*left side* mineralized nodule; *right side* Oil Red O positive staining). $^{*}p < 0.05$ as compared with DSC, and $^{\#}p < 0.05$ as compared with GTCs

including adhesion, rolling and transepithelial migration of neutrophils in the inflammatory host.

Furthermore, after being modified with HGF, MSCs reduced secretion of many pro-inflammatory cytokines, such as TNF-α, interferon (IFN)-γ, IL-6, and decreased expression levels of Col1α1 (collagen type 1, α1), transforming growth factor-β, and Col3α1 [45]. The skin of BMSC-treated mice displayed significant down-regulation of IL-1β, which is a major pro-inflammatory cytokine induced by a number of proinflammatory stimuli and secreted by activated macrophages, and also suppressed macrophage infiltration [47].

6.2 Cell Contact Signaling

The systemic infusion of BMSCs in mice were reported to induce transient T cell apoptosis via the CD95/FAS ligand (FASL)-dependent FAS pathway, and the apoptotic T cells subsequently induced macrophages to produce high levels of TGFβ, which in turn led to the upregulation of Treg and, ultimately, immune tolerance [100].

In addition, the human MSCs induced adult CD34+ hemopoietic progenitor cells differentiating into regulatory dendritic cells via Notch pathway [101]. Furthermore, when co-cultured with hMSCs, the expression levels of Notch-1 were significantly increased in human neural stem cells (hNSCs), and co-culture increased immunoreactivity of CD15, a neural stemness marker but decreases

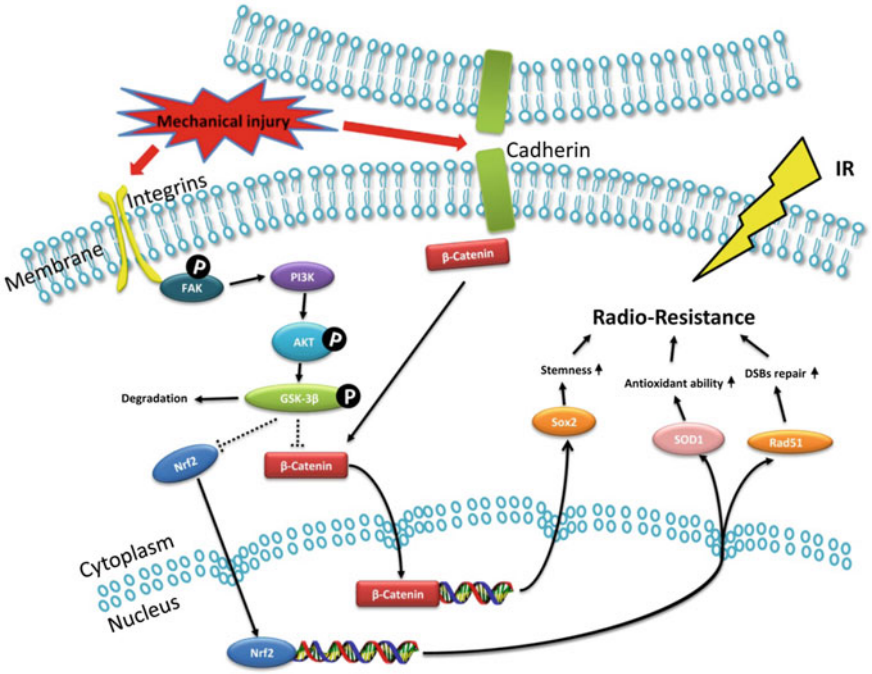

Fig. 4 A schematic illustration of mechanical injury induced radio-resistance enhancement of dermal cells. Flowing mechanical injury, cadherin–cadherin interactions are broken and dissociation of β-catenin from membrane complexes. On the other hand, focal adhesion complexes mediates mechanotransduction of mechanical injury signals into intracellular chemical signals by activating focal adhesion kinase (*FAK*), and the down-stream PI3K/Akt pathway is activated, following, the downstream protein GSK-3β is phosphorylated at Serine-9 by Akt to degradation. Thus, the GSK-3β targeted proteins Nrf2 and β-catenin are accumulated and then translocate into the nucleus, where they promotes stemness, antioxidant ability, and DSBs repair of dermal cells through activation o f related genes such as Sox2, SOD1 and Rad51, which finically enhances radio-resistance

CD24, a marker of neural commitment in hNSCs, markedly augments hNSCs proliferation rate [102, 103].

6.3 Immunoloregulation

It is well known that BMSCs with low levels of MHC-II molecules on the cell surface could evade rejection by the host immune system. Researchers showed that MHC-II expression on BMSCs may be inducible by interferon (IFN)-γ, resulting in in vivo elimination of the transplantated donor cells [104]. In addition to immunosurveillance evasion, MSCs have their therapeutic effects with a function of immunoloregulation.

Various in vitro studies have demonstrated that IFN-γ, and TNF-α stimulated MSCs to upregulate several soluble factors such as TGF-β, HGF, IL-10 and PGE2, which would modulated the cytokine secretion from MSCs and the inhibition of T-cell proliferation and DC maturation [103]. Additionally, studies suggested that MSCs may induce a cytokine profile shift from the T helper Th1/Th2 balance toward the anti-inflammatory phenotype Th2 due to increase of regulatory T (Treg) cells [77]. MSC inhibit the proliferation of B lymphocytes that are activated with soluble CD40 ligand, cytokines, or anti-immunoglobulin antibodies [105]. It is likely that both cell-to-cell contact and soluble factors of MSCs are involved in immunosuppressive mechanisms of MSCs.

MSCs were proved to be able to induce macrophages to become immunosuppressive [98]. Studies showed that CD11b+ was the mediator of this process. There were reports indicated that the number and proliferation ability of both CD4 and CD8 T cells return to base levels and the apoptosis of proliferating CD8 T cells increases following MSCs treatment [61]. Moreover, MSCs transplantation were able to down-regulate CCR5 and CXCR3 expression and up-regulate CCR7 expression on CD3+ T in irradiated mice, which would reduce the migration of activated T cells to inflammation sites, thus attenuating the immune injury [106].

6.4 Secretion of Growth Factor, Cytokine

Current evidences suggested that MSCs can stimulate tissue repair via paracrine actions rather than through direct transdifferentiation [107]. Recent studies have documented hMSC synthesis and release of several cytokines and growth factors, for instance, IL-6, IL-11, HGF, FGF-2, PDGF, EGF, KGF, VEGF, erythropoietin, G-CSF, insulin-like growth factor 1 (IGF-1), NFκB, extracellular signal-regulated kinase (ERK) and so on, to mediate recovery/regeneration of hematopoietic or

non-hematopoietic tissues [57, 108, 109]. All these factors has been described earlier as ameliorating intestinal mucosa injury, either through increasing cell proliferation and/or inhibition of epithelial cell apoptosis [58].

In vitro studies have demonstrated that the various factors secreted by MSCs could influence the migration, extracellular matrix (ECM) invasion, proliferation and survival of endothelial cells [110, 111]. Furthermore, the healing effects of MSCs were confirmed through serum IL-10 elevation, up-regulation of VEGF, bFGF and EGF in irradiated intestine, mobilisation of CD31-positive HSCs or haematopoietic progenitor cells, and the prolonged presence of Bmi1 (stem cell marker)-positive cells within crypts [49].

6.5 Homing and Regeneration

A growing corpus of studies highlighted that systemically administered MSCs home to sites of ischemia or injury. After MSC administration, radiation-induced dysfunctions could recover fast, that would prevent or reduce the potentially chronic inflammatory response caused by bacteria [76]. Transplantation of MSCs has the ability to accelerate structural restoration, like enhance or maintain the re-epithelization process.

After transplantation, MSCs upregulated expression of monocyte chemotactic protein (MCP)-5, macrophage inflammatory protein (MIP)-1 and MIP-2, as major chemoattractants to accelerate the recruitment of macrophages into injured sites [109]. Following, macrophages secreted pro-angiogenic factors, such as HGF, VEGF and angiopoietin-1 [112]. Homing of HSCs to the ischaemic area is mediated by the interaction between stromal cell-derived factors 1 (SDF-1) and CXCR4 [113]. SDF-1 and CXCR4 have been shown to be upregulated during hypoxia and tissue injury. Researchers showed that the upregulation of CXCR4 can increase recruitment of MSCs to injured tissues, which express a high amount of SDF-1 than normal ones [114]. Furthermore, the engraftment of hMSCs can downregulate the level of mir-27b in liver which can suppress the directional migration of MSC by binding indirectly to the SDF-1α 3′-untranslated region (3′UTR) to downregulate SDF-1α expression [46, 115].

MSCs were induced by VEGF to differentiate into endothelial-cell-like cells and form capillary-like structures [54]. Besides, PDGF play a role in each stage of wound healing, and the events that it is involved in are chemotaxis of inflammatory cells and repair cells, regeneration of fibroblasts and endothelial cells, debridement and granulation tissue formation, maturation of blood vessels and ECM synthesis [116, 117]. It's also reported that integrin α4/β1-fibronectin interaction plays a major role in transmigration of MSCs into ECM [103].

6.6 *Reduce Apoptosis*

Researchers observed that apoptotic epithelial cells in the small intestine were increased in early stage after irradiation [58]. These cells were preferentially located in positions corresponding to stem/progenitor cells. Significantly decreased apoptosis was observed in injured tissue following MSCs administration. They hypothesized that the re-establishment of the self-renewal ability of small intestine after irradiation requires a decrease in apoptosis.

After MSCs treatment, anti-apoptotic gene B-cell leukemia 2 (Bcl-2) and Mcl-1 expression was robustly induced [118]. While pro-apoptosis gene Bcl-2-associated X (Bax) expression reduced to protect the respiratory chain [119]. Experimental data have indicated that apoptosis, not necrosis, was the main mechanism of the radiation induced damage [120]. Besides, the protective effect on the epithelial cells might be either by the activation of antiapoptotic gene expression such as bc1-2, or the inhibition of the apoptotic pathways through downregulation of the expression of proapoptotic genes such as p53 [121].

7 Conclusions and Future Perspectives

To date, MSCs were focused for academic- and industry-based purposes to expand their therapeutic use, for example, regenerative medicine, inflammatory and immune-mediated diseases, gene vehicle, drug screening, and tissue/organ substitution. Parts of the studies have come to clinical trial, commercial product, even successful clinical application. But it's still an absence in clinical therapy for radiation injury. Fortunately, the activity of MSCs studies provide a tool for immunological tolerance following systemic injection and seem to depend on the capacity to anti-inflammatory, regeneration through the modulation of secretion, apoptosis, autophagy, differentiation, oxidative stress and so on. The final outcome of the administration of MSCs is likely to regenerate damaged tissues, recover organ functions, prolong the survival time and raise survival rate. Indeed, the microenvironment (niche) dictates the final effect of MSCs on target tissues, moderated by the outcomes of anti-inflammatory effect, section of trophic factors, paracrine cytokines and immunomoderation.

Bone marrow transplantation has exemplified the power of stem cell therapy in the field of hematopoiesis recovery and leukemia treatment. Functional improvements of MSCs treatment has been observed in many irradiated tissues in different animal models. The effectiveness of MSC-based therapies were confirmed in various studies to accelerate cutaneous healing, preserve gland functions, correct liver dysfunction, improve small intestinal integrity and inhibit lung fibrosis as a

systemic therapeutic approach. However, there is a long road ahead before MSC-based therapies to radiation injury become common clinical practice. One of the reasons is the lack of a sufficient number of survived stem cells in radiated patients and the lack of differentiation of MSCs into functional cell types after transplantation. Allogeneic MSCs transplantation faced the same matter even after in vitro expand culture. In some preclinical studies and animal models, successes have been obtained in the treatment of radiation damage and other disorders with MSCs, but still a great amount of uncertainty exist about their location, phenotype, tumorigenic risk, effective dose, safety profile and interaction with host. Furthermore, as sufficient numerical MSCs need to be cultured for several passages in vitro, the phenotype of which drift a lot that researchers can not ensure the influence of in vitro cultivation to hosts/patients.

As a result of those restrictions, future studies have to be focused on cell safety and protocols of expand cultivation to promote clinical application. In our previous study, we have reported for the first time that systemic transplantation of neonatal dermal multipotent cells significantly promoted survival and accelerated both hematopoietic recovery and wound healing in rats with combined radiation and wound injury, suggesting that stem cell therapy can achieve multiple therapeutic effects and provides a potential new strategy for the treatment of severe traumatic injuries with multiple tissue/organ damage, such as radiation combined injuries [92]. In addition, issues dealing with immune function and tumorigenic risk should be extensively studied before clinical trials are initiated. Finally, it is necessary to study the capacity of MSCs to exert their therapeutic properties through paracrine mechanisms which demonstrates that persistent engraftment at the damage site is not a mandatory prerequisite for having an effect on injured cells.

As the functions of released stress-induced therapeutic molecules from MSCs were accepted wildly, scientists focused on “artificial cells” named microencapsulated MSCs that induced minor inflammation and reserve the secretion of important soluble growth factors, cytokones [122]. The microencapsulated MSCs showed anti-fibrotic and anti-inflammatory effects that may have effects on radiated injury.

Unmodified MSCs have certain but not absolute therapeutic effect in various radiation injuries, especially in severe acute radiation syndrome (sARS), fibrosis. Administration of beneficial gene therapy can improve dysfunction of radiation injury. However, a high level of expression of beneficial gene in the injured areas is unachievable. Studies serve MSCs as cellular vehicles for gene delivery to achieve better effects or specified purpose.

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Spinal Cord Injury and Regenerative Repair

Chaozhi Liu and Yamin Wu

Abstract Spinal cord injury is a disease difficult to restore that function more than 2.5 million people worldwide. How to improve the prognosis of spinal cord injury is a matter concerned by clinicians and scientists. Complex pathophysiological processes after spinal cord injury are the important reason to hinder restore. To clarify its mechanism can provide ideas for clinical treatment. Neurotrophic factors, biological engineering and Chinese medicine have been applied to adjust the microenvironment after spinal cord injury. Transplanted cell can replace cells which were damaged and apoptosis in a certain extent, while secretion of a variety of nutritional factors improve the extracellular environment. Combined different therapy method can produce a synergic effect and improve the prognosis of spinal cord injury.

Keywords Spinal cord injury · Regenerative repair · Neurotrophic factors · Cellular transplantation · Traditional Chinese medicine · Biological engineering

1 Introduction

People have realized for a long time that some symptoms, such as the partial or complete loss of sensory and motor function, chronic pain, spasticity and incontinence of defecation and so on, which can be caused by the spinal cord injury (SCI) [1]. Scientists all over the world are keen to find the solution to the problem of SCI patients which is still a worldwide challenge at present, because SCI may bring serious threat to the patient's health and life, and cause heavy burden on the society and family. Spinal cord injuries can be subdivided into traumatic injuries (including traffic accidents, being struck by falling objects, crushing injuries, vio-

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lence and sports-related injuries) and non-traumatic injuries (including inflammation, tumors, ossification, degenerative damage and vascular injury). About 73.7 % of the SCI was traumatic injury [2]. In order to increase the recovery degree of SCI, many treatment methods are developed. The defects of various methods have been constantly fixed. This article mainly discusses the research progress of the traumatic SCI and its regeneration repair.

2 Pathophysiological Changes of SCI

A series of pathophysiological changes after SCI is complex cascade reactions that can be divided into primary injury and secondary injury. At the instant of the injury, mechanical power of the external force or fracture causes the direct ruin of neurons and endothelial cells. The damage of cells and tissue necrosis produced in a very short time. The damaged cells and tissues will activate some complex molecular and cellular mechanisms and evolved into secondary injury and brought greater harm [3].

2.1 Primary Injury

In the primary injury, mechanical power direct shear membrane of nerve cells and endothelial cells. Due to the gray region is soft and rich in blood vessels, hemorrhage and necrosis will appear in these areas for the first time. In the central area of the spinal cord, tissue dislocation will lead to hemorrhage and the ruin of the neuron membrane and connective tissue after injury. The peripheral area of the spinal cord, tissue dislocation is minimal; axons near the spinal dura mater usually can survive. On the contrary, axon near the gray matter will be severely damaged after SCI [3, 4]. Therefore, as the damage continued, some special molecular and cellular mechanisms will be activated, and further evolved into the secondary injury.

2.2 Secondary Injury

In the secondary injury stage, damaged cells dysfunction of the reuptake of glutamate. A large number of glutamates will enter into the extracellular by exocytosis and cell lysis to over stimulate ionic glutamate receptors. Excitatory neurotoxicity will be required to lead to cell death [5]. Ischemia-reperfusion injury is another reason to cause cell death. The reactive oxygen species (ROS) and calcium overload produced by ischemia-reperfusion is directly related to the neuron apoptosis [6]. Wallerian degeneration is an important feature of the nervous system injury. It occurs in the axon stump distal to the site of injury and usually begins within 24–36 h after injury. The axonal skeleton of stump distal disintegrates, and the axonal

membrane breaks apart. In the central nervous system (CNS), myelin sheath is constituted by oligodendrocytes. Unlike Schwann cells (SCs), oligodendrocytes are unable to clean up the myelin sheaths and their debris after Wallerian degeneration. Oligodendrocytes also can't recruit macrophages for debris removal. Microglia plays a critical role in the central nervous system for debris removal but their recruitment and clearance is far slower than macrophage [7]. During this period, inflammatory cells begin to enter the ruin area and inflammation expands the extent of damage. So Wallerian degeneration can't provide enough help for repair of SCI. The glial scar can limit inflammation but it is another important factor to further hinder chances for axonal regeneration and neuronal reinnervation. Early scar is comprised of oligodendrocytes fragments and myelin sheath which allow the axonal regeneration. Then meningeal cells from the surface of the CNS and precursor cells with multi-differentiating potential from the central tube migrate into the glial scar. Eventually astrocytes are seeping into the cavity cause by the primary injury to form a large amount of the glial scar. The glial scar and the surrounding environment are met with a variety of molecules called neurite growth inhibitor which can interrupt the growth cone of damaged neurons to disintegrate. To prevent the final scar formation is one of the essential parts to repair SCI [8].

Large number of experimental studies have shown that promoted regeneration after spinal cord injury SCI treatment and functional rehabilitation is the key. Factors limiting the central axonal growth or regeneration after spinal cord injury can be divided into two categories: First, the lack of neurotrophic factors and matrix components that guide and support nerve growth; Second, cysts and scar tissue resulting spinal cord stump and some inhibitory growth factor hinder and inhibit axonal regeneration. In recent years, research carried out in the treatment of spinal cord injury is mainly around these two aspects.

3 Using Neurotrophic Factor Treatment for SCI

Neurotrophic factors (NTFs) are a family of proteins that are responsible for the growth and survival of developing neurons and the maintenance of mature neurons. It includes Nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophins including neurotrophin-3 (NT-3) and neurotrophin-4/5 (NTF-4/5), ciliary neurotrophic factor (CNTF), glial cell line-derived neurotrophic factor (GDNF) and so on. The receptor of NTFs including Tyrosine receptor kinase (Trk) family and P75 receptor family. Trk receptor family has high affinity and high selectivity to the NTFs when P75 just opposite. Trk receptor mediated NTFs positive signal pathway of neurons. When P75 receptors coexpression with Trk, they will improve the affinity and selectivity of Trk to NTFs and promote the function of Trk receptor at the same time. When the Trk inactivation or low energy, p75 will mediate apoptosis [9].

3.1 Endogenous Neurotrophic Factors

NTFs and its receptors exist widely in the spinal cord. NTFs levels are low in the spinal cord except NT-3 is higher in the early development of the spinal cord [10]. Nerve growth factor is highly expressed in dorsal root ganglia after SCI [11]. Geng et al. [12] use enzymes linked immunosorbent assay (ELISA) to measure BDNF at 24 h after SCI which appeared incremental expression, returned to normal level after 28 days, has the time correlation. TrkC protein appears a downward trend within 7 days after spinal cord transection in the rat, began to rise after 7 days, rising speed at 14days is the most obvious which is consistent with the TrkC mRNA expression curve [13]. Unlike Trk, the expression change of P75 receptor mRNA is fluctuant after SCI, and positively correlated with neurons apoptosis. This fluctuation changes associated with the neurons apoptosis have significant relationship with regulation and repair after SCI [14]. So that NTFs and its receptor increases with time correlation after SCI, and it has different degree function to promote repair of the spinal cord. But due to its increase is limited, and cannot maintain the effective concentration for a long time, they limited to promoting the neural repair and functional recovery. Therefore, many scholars tried to utilize exogenous NTFs to further enhance the repair of SCI.

3.2 Exogenous Neurotrophic Factors

Exogenous neurotrophic factors can reach the spinal cord by injecting into the vein, abdominal cavity, and muscle and subcutaneous tissue. But as NTFs concentrations decrease quickly, and difficult to enter into the blood-spinal cord and brain barrier by this administration route, NTFs eventually reached the injured area are extremely rare and can't display the neural protective effect. NTFs can also be directly injected into the injured area, subarachnoid etc. Damaged area local drug delivery is advantageous to the formation of high concentration in the location, be helpful for neuronal survival. But the half-life of NTFs is short [15], so can't maintain adequate concentration at a long time. Demand for NTFs after SCI has time correlation. The biological characteristics of the short half-life of NTFs lead to that single NTFs have been impossible to meet the needs of the repair of SCI. Although exogenous neurotrophic factor has a very good effect in inhibiting apoptosis of neurons, but each kind of NTF effect has a limit, and single neurotrophic factor is not sufficient to help neuron axons completing reconstruction.

3.3 Multiple Factors Combination Therapy

To meet the needs of the repair of SCI, the combined use of different NTFs or with other small molecules to repair SCI is a research hotspot in recent years.

Methylprednisolone was believed to have nerve protective effect, but recently the research of Aomar et al. [16] think it has no improvement for neurological symptoms. However, Kim and Jahng [17] used BDNF and methylprednisolone for combined treatment of SCI which detected myelin regeneration, accorded with the results of immunohistochemical, and confirmed the combination therapy is possible and effective. Arvanian et al. [18] used NTF-3 and lysergic acid diethylamide (LSD) to combine treatment rats with hemisected SCI and found that compared to control group with the simple use of NTF-3 or LSD, combination group had earlier behavioral recovery time and better effect. Lee et al. [19]. reported that NTF-3 and thermo stabilized chondroitinase ABC (ChABC) combined use to treat the rats with SCI, the effect to restrain glycosaminoglycan's side chain, the important component of glial scar is 3 times as monotherapy. Sharma [20] found after application of BDNF with high concentration in combination with GDNF after SCI, the damage of blood spinal cord barrier has been inhibited and edema also be alleviated. Thus the recovery of motor function has obviously improved. Nevertheless, some of the combined treatment effect can't play the corresponding function even appear antagonism. Lang et al. [21] found that BDNF and CNTF combined treatment in the nerve root avulsion model showed no synergy. Donnelly et al. [22] also found small interference RNA and NTF-3 combination therapies with SCI did not show a better therapeutic effect. The synergy of NTFs to enhance the repair of SCI, but how to improve NTFs and additional small molecule combination plan needs further research.

3.4 Cooperate with Stem Cells

In addition to promoting the growth of axons, NTF can promote stem cell proliferation and differentiation. Therefore, NTFs can be followed in the stem cell transplants for the treatment of SCI. Tang et al. [23] developed NT-3-immobilized scaffolds which can sustain release of bioactive NT-3 to help with neural stem cell transplantation. And they observed the rate of survival and differentiation of stem cells had been strengthened considerably. Besides, for the half-life of NTFs is too short to maintain function for a long time, we can transfer NTFs gene to neural stem cells. So these neural stems cells will keep sustained secretion of NTFs to maintain the survival and the growth. I'll consider this method later in the article.

3.5 Load in Biological Materials

To add NTFs into the biological materials and use the characteristics of biological materials to control the release of NTFs is a great method to control and guide the

growth and differentiation of neuron, and help axon directional growth, reconstruct the original structure, and realize the functional recovery. See below for biological scaffold.

3.6 Neurotrophic Factor Receptor

Wang et al. [24] constructed a kind of immunoglobulin called p75NTR-ED-Fc for human p75 neurotrophic factor receptor (NTR), which can suppress the effect of p75NTR for improving the axonal regeneration and functional recovery after spinal cord injury. Research showed inhibition of p75 receptor, the spinal cord function has a certain degree of recovery while the axon length is actually increased. Figure 1 showed the function recovery after the hp75NTR-ED-Fc fusion protein therapy (Fig. 1).

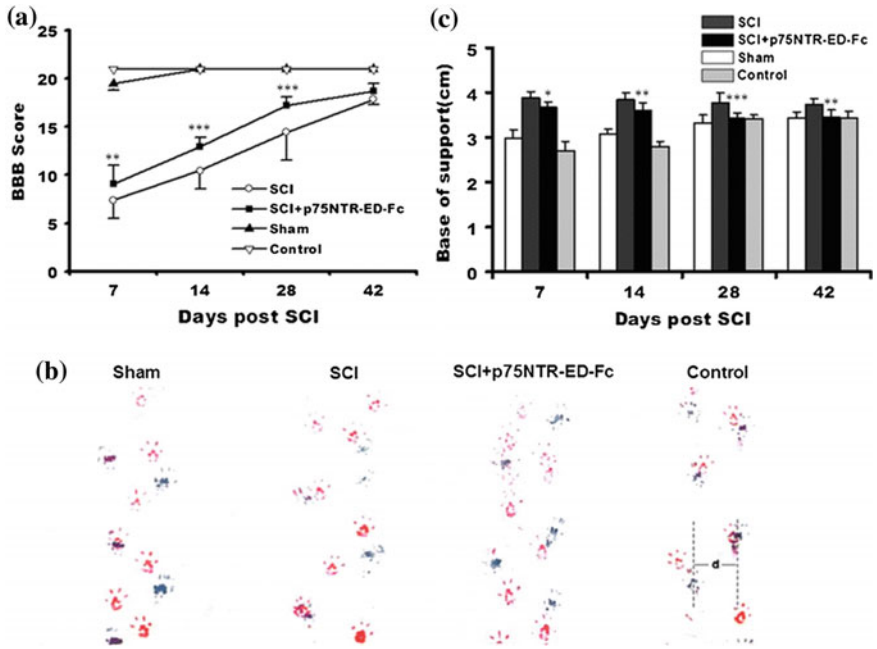

Fig. 1 Treated with hp75NTR-ED-Fc fusion protein promoted functional recovery after SCI. **a** The BBB scores in hp75NTR-ED-Fc-treated rats were consistently higher than those in SCI group. **b** Footprint analysis showed that the prints of all hind toes in hp75NTR-ED-Fc-treated rats were very visible, while in SCI rats, they were not clearly separated which indicated the signs of toe dragging. Fore paw footprints (*red*) and hind paw footprints (*blue*) from rats tested. "*d*" shows the vertical distance between the hind paws. **c** The distance of the base of support between the hind paws in hp75NTR-ED-Fc-treated rats was significantly reduced compared with SCI rats. $*p < 0.05$, $**p < 0.01$, $***p < 0.0001$, compared with SCI group. (Reprint with permission from the article of Wang et al. [24])

4 Tissue Engineering

Although above-mentioned methods have made some important progress. However, there are still cannot achieve the structure and function of the damaged spinal cord repair completely. The main problems are: (1) the number of neuron rescues by various means is limited and no extensive growth potential of axonal fiber will give full play to. (2) It is very awkward that renewable fiber long distance search for the "target" and establish the functional synaptic. (3) Functional remodeling is needed for the regenerative repair of nervous structure to show the effective function. With the deepening of the research, people gradually realize that increase the number of regenerated fiber and promote synaptic connections and structure and function remodeling between renewable fiber and the "target" is essential to repair spinal cord injury. Nonetheless, in recent years, the rise of tissue engineering research offers hope for it.

4.1 Biological Scaffolds

A large number of cells necrosis and apoptosis after SCI lead to the formation of a spinal cord defect [25]. Survival neurons can't meet the needs of rebuilding of neural pathways. And application of biological engineering technology to build the bridge can be attached on both ends of the injured spinal cord, lead to rebuilding orientation structure to targets axon and guiding axon directional growth. So as to realize the spinal cord regeneration and reconstruction of almost normal physiological structure [26]. Biological scaffold can be divided into natural biological material scaffold and synthetic biological material scaffold according to the source, and can be divided into the Hydrogel biological scaffold, spongy scaffold, tubular biological scaffold and membrane biological scaffold according to the form. The advantages of natural biological scaffold whose materials usually are saccharide and protein are good biocompatibility, biodegradable, degradation products can be absorbed and usually can't produce inflammation. The main disadvantages of them are that the mechanical properties are poor and degradation process is not easy to be controlled. Agarose is linear polysaccharide which extracted from seaweed and curdlan. It is widely used drugs and macromolecular carrier. Above mentioned Lee et al. [19] used agarose contained NT-3 as a scaffold, to improve the thermostable ChABC local sustained delivery, and found the axonal regeneration and the motor function recovery in rats with SCI. The mechanism may be sufficient NT-3 and ChABC can promote new axons across the glial scar and reach its remote control targets [27]. Alginate is a linear polysaccharide produced by brown algae, are commonly used in spinal cord defect. Shahriari et al. [28] implants calcium alginate

hydrogel in a rat model of spinal cord hemisection. And they use Fourier-transform infrared (FT-IR) spectroscopy to measure the stability of the implants and the effects on morphology and biochemistry of the injured tissue. The result shows that non-functionalized low-gelation soft Ca^{2+}-alginate hydrogel has an enhanced long-term stability in vivo and help spinal cord repair by limiting demyelination and reducing scar. Grulova et al. [29] used the affinity-binding alginate scaffold with sustained delivery of basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) to treat rats with spinal cord crushing and observed the recovery of function of rats. But commercial alginate products contain a large number of toxic materials so it should be super purified before use. And alginate degradation products also have slight cytotoxicity. Chitosan which also is called soluble chitin is obtained by deacetylation of chitin, the structure of which is similar to the plant fiber. Chitosan has excellent film-forming property and permeability, no or low immunogenicity. Yang et al. [30] found chitosan loaded with NT-3 can slow release of this neurotrophic factor to make an optimal microenvironment for regeneration. And they confirmed sensory and motor functional restorations in rats with SCI treated by this way. The drawback of chitosan scaffold is that the mechanical strength is negatively related to the biodegradability. So chitosan with superior mechanical strength will be difficult to degrade and can't adapt the speed of tissue rebuilding. Self-assembling peptide (sapeptide) is a kind of emerging material consists of repeated sequences of short chains of amino acids, belongs to nano-scale hydrogel biological scaffold materials, with similar characteristics of the extracellular matrix. The advantage of self-assembling peptide scaffold is that can be injected in the damage area, to prevent iatrogenic injury. Its disadvantage is too sensitive to change of temperature and PH, so it is necessary for practical application to closely monitor and adjust the local microenvironment [31]. In China, Hou et al. [32] seeded the neural stem cells or motor neurons in the sapeptides scaffolds and found the sapeptides with motor neurons have a better effect for functional recovery after SCI in rats.

Sun et al. [33] tried to use the functional motifs containing cell adhesion peptide RGD and neurite outgrowth peptide IKVAV to change this weakness and had a good effect. Compared to the natural biological scaffolds, synthetic biological scaffold both have superior performance of biology, mechanics and material science, also has a unique degradation controllability at the same time. Its main drawback is that the concentration release of monomer produced from degradation often cause the immune reaction. Polycaprolactone which is a kind of aliphatic polyester with good biocompatibility and degradation performance, is widely used in many medical products, including wound dressings. Silva et al. [34] use a blend of starch with polycaprolactone to product a 3D scaffold. And they found that the scaffold can quickly restore spinal stability of rats with a T8-T9 spinal hemisection. Poly (lactic-co-glycolic acid) (PLGA) is synthesized by means of ring-opening co-polymerization of two different monomers, the cyclic dimers (1,4-dioxane-2,5-diones) of glycolic acid and lactic acid. Depending on the ratio of lactide to glycoside used for the polymerization, different forms of PLGA can be obtained. It can be bending, expansion, deformation and changed of permeability by this way.

Wen et al. [35] adopt scaffold which is modified by PLGA binding with an anti-Nogo receptor antibody containing BDNF and vascular endothelial growth factor (VEGF) to implant into the injured area created by a dorsal hemisection at T9-10 of the spinal cord in rats. And they observed the inhibition of inflammation and gliosis, and large numbers of new blood vessels and regenerated nerve fibers around the implants. There are some other synthetic biological scaffolds have also made very good progress, but to date the United States Food and Drug Administration (FDA) has not yet approved its wide application in the clinical.

5 Genetic Engineering and Protein Engineering

Genetic engineering and protein engineering both is an important branch of biological engineering. Genetic engineering which is also called gene splicing technology and recombinant DNA technology is a complex technology to manipulate gene at the molecular level and import the restructured exogenous gene to recipient cells for make the gene can be expressed, replication, transcription, translation in recipient cells. Protein engineering is the process of promoting useful or valuable proteins. In protein engineering, scientist uses detailed knowledge of the structure and function of the protein to ensure that desired changes. Genetic engineering is used to transfect the neural stem cells (NSCs) by neurotrophic factor gene, and make the cells can continue secrete NTFs to promote the repair of spinal cord. Protein expression carrier construction also is required to use genetic engineering. After SCI, apply recombinant proteins and monoclonal antibody target to inflammatory mediators maybe significantly reduce inflammation, improve the possibility of recovery of SCI [36]. Yune et al. [37] produced a fusion protein PEP-1–SOD1 by fusing a human SOD1 gene with PEP-1 in a bacterial expression vector. They confirmed this fusion protein has antioxidant agents that can protect neurons from ischemia reperfusion injury. With the progress of protein engineering, human knowledge of protein structure is more and more. A growing number of recombinant proteins will be utilized in SCI, help to get better curative effect in the treatment of SCI. The use of genetic engineering and protein engineering to construct a vaccine is also a hot spot of research. Mentioned above the Wang et al. [24] using genetic engineering to construct the p75 receptor immunoglobulin for promoting axonal regeneration and functional recovery by inhibiting p75 receptor. Vaccination also can be constructed for further inhibit factor, example Nogo-66 receptor (NgR) which a common receptor for three myelin associated inhibitors mediates their inhibitory activities on neurite outgrowth in the adult mammalian CNS. Wang et al. [38] uses 15 nm gold nanoparticles (GNPs) which can boost the immunogenicity of human NgR-Fc (hNgR-Fc) protein vaccine effectively, to improve the therapeutic efficacy of hNgR-Fc protein immunization in spinal cord-injured rats. We can also transfer some gene which is helpful for protecting the injured spinal cord into cells that can be transplanted. Zhang et al. [39] used rubrospinal neurons transferred by adenoviral cardiotrophin-1 (CT-1) gene to

transplant into spinal cord with a shallow incision which was made on the left dorsal. They have demonstrated that adenoviral CT-1 gene transfer promoted the survival and regeneration of rubrospinal neurons and enhanced the partial functional recovery of forelimb usage after cervical spinal cord injury in adult rats.

In conclusion, the main mechanisms of tissue engineering used to repair spinal cord injury and paraplegia recovery include: (1) Filling defect of the organizational structure: after spinal cord injury, due to the primary and secondary injury caused local tissue necrosis at the injury site. If not removed the necrotic tissue, it is easy to form scar tissue to impact new tissue ingrowth, is not conducive to the functional recovery. If cut it, the local tissue will form defects and adjacent tissues will collapse, that destroy the original anatomy. Therefore, researchers fill cells, nerve tissue and a variety of materials at the defect. (2) Create a suitable microenvironment: primary and secondary damage caused by a large number of inflammatory cells infiltration around the injury site and release large amounts of inflammatory factors after SCI, which caused the change of microenvironment is not conducive to the cells survival and axons growth or extend. Also, because a large number of cell necrosis, trophic factors secreted by the remaining cells cannot meet the needs of tissue regeneration. Therefore, transplant exogenous cells transfered by genetically engineered can produce large amounts of cytokines, in favor of the axon extension and regeneration. Scaffold material itself can also carry nutrition factor and blocking inflammatory cells infiltration, further create a suitable microenvironment. Material also can separate transplant cell from residual myelin and surrounding glial cells. In the process of material degradation, it provides a time window for the extension of new axons reduce the inhibition of surrounding cells or factors (3) a greater degree of repair organizational structure after SCI: mainly through the orderly proliferation, growth, migration and differentiation of seed cells on a biological scaffold with certain rules and the internal arrangement of the network structure, and try to restore the connection of the damaged spinal cord fiber and the corresponding neuronal network links. (4) Remodeling of damaged spinal cord functional linkages: the damage of spinal cord leads to tissue disintegration and apoptosis at injury site. The functions of the corresponding structure also will be destroyed or lost. Tissue engineering can fill the defect that formed by spinal cord injury and also can create a suitable microenvironment for the regeneration of their own or transplant tissue or cell. However, it is still not clear whether the functional regenerating organization ultimately can achieve partial or complete replacement for the original function. Remodeling functional structure mainly to establish a complete anatomical structures, remain intact nerve fibers communicating and ensure synaptic activity. Restoration of function mainly includes the improvement of incoming and outgoing signals of damaged spinal cord and the function of preliminary integration information. In addition to axoplasmic transport function of neurons and axons, etc. Spinal cord tissue engineering is still considered one of the most challenging therapeutic strategies to repair injured spinal structure.

6 Cellular Transplantation

SCI in addition to resulting in a large number of axons damaged, also will lead to a large number of cells death. The research of Liu et al. [40] showed that endogenous neural stem cells increased significantly at the injury site after mild spinal cord injury, and the endogenous neural stem cells in central canal of spinal cord can differentiated into different type of neural cells of adult rats. Proliferative activity of endogenous NSCs of spinal cord after injury was examined via DAPI staining as shown in Fig. 2. But they differentiate into astrocytes more than neurons, which may be associated with high expression of inhibitory Notch1 and Hes1 genes after injury (Fig. 2).

Because of the endogenous neural stem cells proliferation and differentiation slowly, we can transplant exogenous cells to help repair damaged parts. Mechanisms of cellular transplantation to repair SCI are not completely understood, but it can be summarized as the following aspects. (1) Through secreting NTFs to protect the remaining neurons. (2) To differentiate into neurons, oligodendrocytes and astrocytes, promote axonal regeneration and myelination under the specific environment of the spinal cord. (3) Improve the local microenvironment after SCI

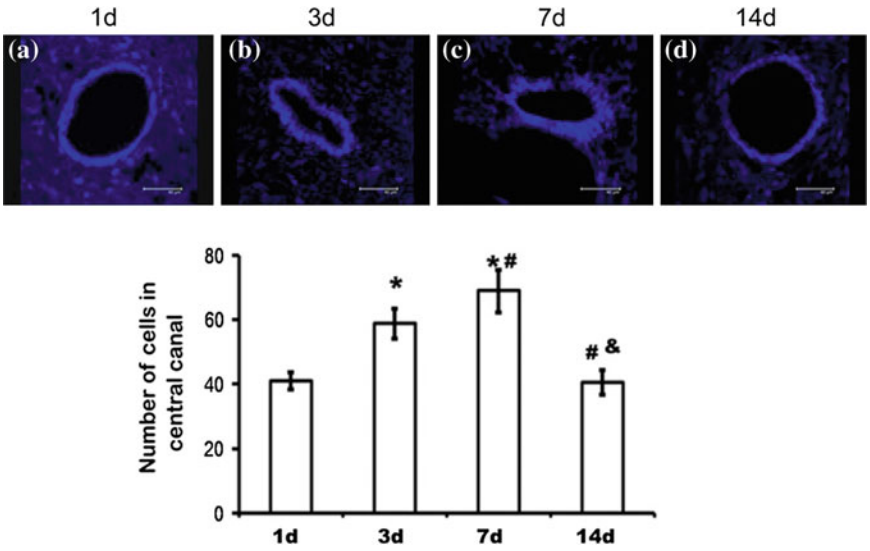

Fig. 2 Proliferation of endogenous neural stem cells in central canal of T10 level after injury. **a** DAPI labeling endogenous neural stem cells of spinal cord 1 day after injury. **b** DAPI labeling endogenous neural stem cells of spinal cord 3 days after injury. **c** DAPI labeling endogenous neural stem cells of spinal cord 7 days after injury. **d** DAPI labeling endogenous neural stem cells of spinal cord 14 days after injury. $^{*}p < 0.05$ indicated statistical significance compared with 1 day post-injury. $^{\#}p < 0.05$ indicated statistical significance compared with 3 days post-injury. $^{\&}p < 0.05$ indicated statistical significance compared with 7 days post-injury. Data represent mean ± SD of five independent samples in each group. [Reprint from the article of Liu et al. [40]

to reduce inflammatory reactions. (4) Promote cells to produce a variety of extracellular matrix, filled with gap in the spinal cord, is compatible with the axon regeneration [41, 42].

6.1 Transplantation Method

There are many ways to stem cell transplantation and basically has the following four. (1) Orthotropic transplantation on the injury site: Transplant stem cells directly into the damaged area around through surgery, can promote the improvement of the nerve cell function and recovery. However, to grasp the operation time is difficult, especially in emergency surgery. (2) Transplantation by lumbar puncture: this method which can choose the appropriate time for stem cell transplantation is injected stem cells into the cerebrospinal fluid through vertebral puncture, and stem cells will migrate to the injury site and repair the damaged nerve cells. This method is simple, easy to follow and has good repeatability. (3) Intravenous transplantation: inject stem cell which via proliferated and differentiated in vitro into the vein. And stem cell will be along with the circulation of the blood through the blood spinal cord barrier to reach the lesion site. (4) Intra-arterial transplantation: inject stem cell through arterial. The study of Amemori et al. [43] found that compared with implanted into the subarachnoid space, implanted stem cell into the lesion center can positive effect on the expression of endogenous neurotrophic and better to increase gray and white matter survival, axonal sprouting and reduced astrogliosis.

6.2 Transplantation Time

After SCI, gray and white matter immediately occur necrosis, spinal cord is in congestion and/or ischemia and edema are also found. It will reach its peak within 2–4 h, and extensive necrosis will appear in a few days in the injured spinal cord. However, after 12–14 days, injured spinal cord will be under a cyst. Glial cells will proliferate to form scars. That will severely harm the integrity of the spinal cord and blocks nerve cells regeneration and crossing. So stem cells need to be transplanted before that happens. But in the acute phase of SCI, a large number neurotoxic inflammatory factors produced in acute inflammation reaction, often cause the transplanted stem cells by death and/or differentiate into glial cells. This phase will last approximately 7–9 days [44]. After the acute phase, the nerve tissue become into the repair phase, which is conducive to the survival and differentiation of transplanted cells. So it advocates the delayed transplantation, namely transplantation after the acute phase. Research indicates that 1–2 weeks after SCI is the window phase for cell transplantation, and transplantation can achieve the best effect in this stage [41].

6.3 Types of Transplant Cells

There are a lot of cells can be used for cell transplantation. Different cells have unique effects. The following are the main types of the source of the transplanted cells.

Embryonic stem cells (ESCs) are derived from a highly undifferentiated cells in the blastocyst. ESCs are highly totipotent that can be differentiation for a variety of cells. ESCs can adjust the external environment for nerve cell growth and increase the survival rate of nerve cells. The main mechanism is inducing neurons and glial cells regeneration, and repairing the demyelinated axons. Shroff and Gupta [45] for treatment of SCI, inject ESCs into patient's body through a variety of methods. At first to make the body produce immune tolerance to embryonic stem cells by intramuscular injection. Then gradually adopt intramuscular, intravenous, brachial plexus block, intrathecal, caudal, epidural, popliteal block and/or deep spinal muscle and epidural catheter, in order to inject the ESCs as near the injured site as possible. Following the treatment, all patients showed improvement in different aspects including their power and movement of limbs, sitting balance, control and sensation of bowel and bladder. Vadivelu et al. [46] transplanted embryonic stem cell-derived neural lineage cells (ESNLCs) directly into the cavity of a contused spinal cord 9 days after injury. They observed axons had grown through long distances after transplanted ESNLCs. And they found ESNLCs prominent expression nerve glial antigen 2 (NG2) which can promote the expression of matrix metalloproteinase 9 (MMP-9) to make an increasingly inhibitory gradient of chondroitin sulfate proteoglycan (CSPG). CSPG is an essential part of the glial scar. That may be the mechanism of ESNLCs to help axons growth. However, the strong ability of ESCs for proliferation has shown tumorigenic potential. Salewski et al. [47] used an alternative method of clonal neurosphere generation to ensure the safety of the transplanted cells. They generated clonally derived definitive NSCs (dNSCs) from ESCs and proved that transplantation of these dNSCs can promote motor recovery in SCI mice without tumor formation.

Neural stem cells (NSCs) are pluripotent cells within the CNS which has the ability of self-renewal and multi-directional potential differentiation. The discovery of NSCs that broke the traditional concept of no regeneration of the CNS. Both embryonic and adult CNS can find the neural stem cells and they exist in specific niches where can provide a relatively stable environment for the survival, self-renewal and differentiation of the stem cell [48]. For the application of NSCs come from fetal brain or adult CNS the ethics are debatable. In order to solve the problems, the researchers used some methods including cell reprogramming or chemical induction indirectly obtained NSCs from other cells. Common sources include embryonic stem cells [47], human induced pluripotent stem cells [49] and immortalized cell lines [50]. NSCs under certain conditions can only be differentiated into neurons and glial cells, so it is easier to get the desired cell types. Relative to other cells transplantation, NSCs transplantation has unique advantages that NSCs can differentiate into all kinds of nerve cells have corresponding

phenotype at the particular area in the spinal cord after transplantation, and may rebuild connection. NSCs also can make through passage, separation of self-renewal constantly and produce enough amount of transplanted cells. NSCs will generate into neurons and glial cells in terminal differentiation after transplanted into the body, which makes NSCs no longer have the ability of rapid proliferation of stem cells. No report of these cells to become cancerous, as a result, the NSCs is considered to be safe and reliable clinical cell transplantation.

Mesenchymal stem cells (MSCs) that originally obtained from the bone marrow are a kind of self-renewal and multi-directional differentiation potential of pluripotent stem cells. MSCs are not difficult to be amplified, preserved, donated and be accepted in ethic, so it is studied for the treatment of SCI. And among them, the bone marrow mesenchymal stem cells (BMSCs) have been widely concerned because it is easy to obtain, can be autologous transplantation, and minimize invasive operation. MSCs can play its role through diverse mechanisms after SCI. In the initial SCI, MSCs can secrete various NTFs to protect neurons from the effects of excitatory neurotoxin and promote axon growth. Zhou et al. [51] found that adipose tissue-derived MSCs (AT-MSCs) transplantation for SCI can improve the recovery of the spinal cord function in mice, and its mechanism is increasing the expression of BDNF and enhanced regrowth of serotonergic fibers. They also think the improvement is better than BMSCs transplantation. At the intermediate phase of SCI, MSCs can adjust immune by molecule secretions and cell-cell contact, and powerful alleviate inflammation to damage cells in the spinal cord. The previous view believes that MSCs can differentiate into neurons and oligodendrocytes directly participated in the reconstruction of the spinal cord and myelin formation. Matrix metalloproteinase also can be secreted to degrade extracellular matrix which have the benefit of axon growth. But the new research thinks that MSCs mediate functional recovery through a paracrine effect, rather than by transforming into and replacing damaged glia in the spinal cord [52]. MSCs are often used by transfection NTFs gene for more NTFs secreted and better neurotrophic effect. Zhang et al. [53] significantly improve functional recovery and nerve regeneration of rats with SCI by precise transplanting NT3 gene-transfected BMSCs. Compared with other cells, BMSCs is easy to extract and separate from the bone marrow, and it transplants back also have no risk of immune rejection. Therefore, BMSCs autologous transplantation clinical trials have developed for the treatment of SCI in many countries in recent years, and significant results have been achieved.

Schwann cells (SCs) are a kind of glial cell distributed in the peripheral nervous system (PNS) to form the myelin sheath surrounding the axons. SCs can secrete NTFs and extracellular matrix to support neurons. SCs also play an important role in the axon regeneration and the process of myelination after peripheral nerve injury. The CNS without SCs and this is one of the reasons for the CNS damage is difficult to restore. SCs is easy to obtain and separation, and can be auto-transplantation without immunological rejection. So SCs is considered to be an ideal and feasible transplanted cells. SCs secrete a number of NTFs and improve the extracellular microenvironment for neuronal survival and axonal regeneration [54]. But SCs grafted into the CNS is unable to long-term survival or unable to help

complete the functional recovery of the spinal cord. So we need to combine a variety of method to realize the maximum potential of Schwann cells [55].

Olfactory ensheathing cells are a special type of glial cell that presents in the olfactory system, and distributed in the olfactory nerve, the olfactory bulb layer 1, 2 and olfactory epithelium. Olfactory ensheathing cells exist in both the central nervous system and peripheral nervous system at the same time. Many studies have shown that olfactory ensheathing cell transplantation can promote axon regeneration at a distance, make the demyelinated axons remyelination, and play a role in neuroprotection [56]. Many clinical trials also proved that OECs transplantation is safe and effective. Rao et al. injected OECs into the area surrounding the SCI under magnetic resonance imaging guidance in patients with cervical SCI, twice a week for four weeks. The recovery of patients has noticeable improvement after three months, but there is no further increase in a year. And no serious complications postoperatively were discovered during the follow-up period [57].

6.4 Combination Strategy

Biological engineering technology provides a great help for cell transplantation. Biological scaffold provides mechanical support for transplanted cell growth, proliferation. Biological scaffolds that load NTFs and chondroitin enzymes can create a perfect external environment, so as to promote the transplanted cells long-term survival, growth, proliferation and differentiation. And with the rapid progress of molecular biology and molecular mechanism of SCI, genetically modified technology in cell transplantation research has made significant progress. Through gene transfection technique, modified cells specifically expressed the required material, aimed at improving the local environment, which can greatly improve the curative effect of the transplanted cells. The combined use of different cells also can mutually optimize and create synergistic effects. Hu et al. [58] used co-transplantation oligodendrocyte progenitor cells (OPCs) and SCs to treat SCI. They believe the extracellular matrix produced by SCs can promote OPCs survival, proliferation and differentiation, and OPCs differentiation to oligodendrocytes can form the myelin sheath, which is beneficial to the spinal cord function recovery. And the experimental results accord with their ideal.

7 Electroacupuncture and Chinese Herbology

Traditional Chinese Medicine (TCM) is the national cultural treasures and the fruits of the Chinese people's prevention and treating disease for thousands years. In recent years, many studies have shown that the TCM have surprising effects in many diseases. In the cognition of TCM, SCI is associated with mechanical injury of Du meridian, which leads to the disturbance of qi and blood, the stasis of

meridian, and the instability in body warm-reinforcing and nourishing of qi and blood.

7.1 Electroacupuncture

The electroacupuncture therapy is an improved traditional Chinese acupuncture. It is a type of acupuncture where a small electric current is passed between pairs of acupuncture needles. Electroacupuncture has a good curative effect on the treatment in pain, neurosis, nerve palsy, gastrointestinal disease, high blood pressure and so on [59]. In the treatment of SCI, electroacupuncture is usually used to stimulate the Du meridian and Jiaji point. The position and function of the Du meridian describe in the traditional Chinese medicine is similar as the spinal cord. TCM holds that stimulate the Du meridian can restore the function of the Du meridian, namely the recovery of the spinal cord function. The Jiaji point locates at the T1 to the L5 spinous process adjacent to open 0.5-inch. Jiaji point near the Bladder Meridian of Foot-Taiyang and the Du meridian, so stimulation of the Jiaji point can help to restore the SCI.

In recent years, the study concluded that electroacupuncture can inhibit the formation of oxygen free radical and lipid peroxidation, inhibit apoptosis gene expression in neurons and increase the synthesis of NTFs and receptors expression [60]. Electroacupuncture can suppress the Notch signaling pathway to decrease the protein expression levels of Nogo-A and Nogo-66 receptor-1, so it can promote neural stem cell proliferation to help the recovery of the SCI [61, 62]. Changed microcirculation and neuronal morphology is another mechanism of electroacupuncture to improve the function of the spinal cord [63]. Jiang et al. [63] found to use different modalities of acupuncture to treat SCI in rats and found that electroacupuncture have better anti-inflammatory, antioxidant effect than other method of acupuncture which can protect neurons to improve recovery. In addition, electroacupuncture are effective to relieve pain by activating a variety of bioactive chemicals including opioids, serotonin and norepinephrine [64]. Combination of electroacupuncture treatment after cellular transplantation is a good strategy. Electroacupuncture can prolong the survival time of the transplanted cells, and promote the proliferation and differentiation of transplanted cells. Ding et al. [65] combinedly used electroacupuncture and grafted mesenchymal stem cells for SCI and detected that the expression of NTFs receptor TrkC is increased and remyelination and function in demyelinated spinal cord are improved.

7.2 Chinese Herbology

Chinese herbs have been used for centuries and formed a set of unique theory. According to the theory of Chinese herbology, the treatment of SCI mainly involves

activating blood circulation, removing blood stasis, smoothing the meridian, nourishing kidney and benefitting qi (This is a traditional Chinese medicine theory, distinct from the theory of modern medicine). The commonly used prescription include the single prescriptions such as *Salvia miltiorrhiza* (Dan Sen), *Panax notoginseng* (San Qi), *Panax ginseng* (Ren Shen), *Ligusticum wallichii* (Chuan Qiong) or their active ingredients and the compound prescriptions such as Buyanghuanwu Decoction, Xuefuzhuyu Decoction, Fangjihuangqi Decoction and so on. Panax notoginsenoside is a compound isolated from *Panax notoginseng*. The study found that Panax notoginsenoside can inhibit the inflammatory cytokines release and signaling pathways in cell apoptosis for the effect of neuroprotection [66]. Panax notoginsenoside also caused an upregulation of NGF and BDNF for improving the hind limb motor function [67]. Ligustilide is one of the main active components of *Angelica sinensis*, which can reduce the generation of reactive oxygen species to reduce the neuron damage and promote restoration of the injury [68]. Compatibility of medicines is an important component of Chinese traditional medicine theory. Buyanghuanwu Decoction is composed of astragalus, *Angelica sinensis*, radix paeoniae rubra, lumbricus, *Ligusticum wallichii*, safflower, peach kernel. The study found Buyanghuanwu decoction can promote the proliferation and differentiation of neural stem cells [69], and also can decrease in expression of caspase-3 and Bax and increase in Bcl-2 expression to exert anti-apoptosis effect [70]. Buyanghuanwu Decoction also can be used to combine with embryonic neural stem cell transplantation, and that will be under a synergistic effect on the recovery of neurological function [71].

8 Conclusion

SCI brings a series of functional impairment and pain to the patient caused devastating shock and heavy burden. Owing to the unique nature of the central nervous system and complex pathophysiological mechanisms after SCI, it is difficult to form a suitable environment conducive to recovery of SCI. On the contrary, scar formation and low levels of NTFs hinder the repair of the spinal structure. The treatment of SCI is creating a suitable microenvironment on the damaged area and supplement the new cells to instead the damaged and apoptotic cells to support the reconstruction of the original structure of the spinal cord. Supplementary exogenous NTFs is the principal part of creating suitable microenvironment. Nonetheless, management strategies of different NTFs at different times and different site need to be more sophisticated regulation. Biological scaffold that can cope with the delivery of NTFs, while cell transplantation can also offer support. The presence of a large number of cells destroyed or apoptosis after SCI, and the insufficient endogenous stem cells in the central nervous system makes it is necessary to transplant exogenous cells. Exogenous cells can supplement the lack of cell, while the secretion of extracellular matrix can play a role in improving the microenvironment. And genetic engineering can help the transplanted cells be best to play this role.

Chinese medicine is one kind of magical drugs and treatment methods. Continue to clarify the biological mechanisms of Chinese medicine also can provide excellent ideas for the treatment of SCI. At present the main problems or challenges existing in the research are: first, the cells transplanted into the defect is not able to completely differentiate to form the cell suiting the functional and the structure. Such as how to regulate the biology behavior including proliferation and differentiation of NSCs as seed cells, so that it can provide important cell structure basis and neurotrophic support for the reconstruction of spinal cord tissue engineering, and avoid unfavorable side for SCI repair, such as the transplanted neural stem cells in a quiescent state or mostly differentiate into glial cells and so on. Secondly, the number of new tissue growth in both ends of the fiber is limited and it is difficult to form synaptic connections. Thirdly, how to exclude or counteract the cytokines or negative components that inhibit axonal regeneration and the formation of synapses in the environment. Fourthly, the transplantation related issues such as immune rejection, revascularization and so on are also very important issues. Finally, more important is to repair the injured spinal structure but not functional rehabilitation. After various conditions stimulus in vivo and vitro environmental reached the remodeling of structural and functional, and ultimately to achieve important goals that the function of patients with paraplegia rehabilitation and quality of life improved obviously. We still have a hard and slow way to get that.

After comprehensive analysis of large number of domestic and foreign basis and clinical research about SCI, the author put forward the treatment of spinal cord injury with paraplegia rehabilitation treatment, which should be taken to a new comprehensive treatment strategies and methods called “5R-Combined Strategies”. The concrete content includes: (1) Rescue: including spinal surgical fixation, decompression, neural protection and stress protection and so on; (2) Regeneration: a various kinds of treatment for promoting damaged spinal cord regeneration. Main idea is to promote spinal cord neurons axon regrowth, such as use NTFs, transgenic therapy; (3) Replacement: It is all kinds of neurons, stem cells or other cells for the transplant therapy; (4) Remyelination: It is mainly to promote form new myelin of the demyelinated spinal cord fibers and restore conduction function of damaged nerve fiber, including the appropriate drugs, factors and myelin forming cells therapy; (5) Rehabilitation: including almost all of the neurological rehabilitation physiotherapy methods and measures. We think we should establish the following concepts: (1) Rehabilitation is not an adjuvant therapy. Breaking the idea in traditional sense that the rehabilitation dispensable; (2) Adhere to the principle of individual rehabilitation. According to each the actual injury patients and the development of the situation, make a scientific and reasonable rehabilitation methods and embodiments; (3) early intervention and perseverance principle. Breaking the traditional concept that the rehabilitation is the late treatment and establishing the concept of lifelong rehabilitation; (4) Rehabilitation techniques adhere to integration of Chinese and western, and value both physical and chemical. Breaking the traditional concept that the rehabilitation just includes physiotherapy and physical exercise therapy. Rehabilitation equipment should adjust local conditions and personalized; (5) Rehabilitation theory need innovation and absorbs

new knowledge. Such as the present, in particular, should be focused on the psychological rehabilitation of paraplegics, truly improve physical and mental health of paraplegics. 5R-Combined Strategies also should adjust the local conditions and personalized. In summary, the combination of a variety of methods for clinical therapy of SCI is the development direction in the future, and basic research continuously investigation of the downstream mechanism of each method can provide fresh ideas for clinical combined strategy.

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Cells Transplantation for the Repair of Peripheral Nerve Injuries

Bingcang Li

Abstract Peripheral nerve injury, resulted from different trauma and diseases, is quite popular in clinic and frequently leading to life-long disability. Although the peripheral nerve system has a more suitable environment for axon regeneration than the central nerve system, the treatment for the nerve injury is still a practical problem in clinic. Particularly, it is more difficult to repair the nerve defects with a greater gap, which needs to be bridged with different grafts. Autografts are considered as "gold standard" for surgical repair, but have many limitations. Therefore, the cells transplantation for the repair of peripheral nerve injury is a current interesting field in order to replace autografts. Generally, the donor cells derived from different tissue and ages, such as stem cells, mesenchymal stem cells, olfactory ensheathing cells and Schwann cells, are filled into the lumen of the synthetic and natural conduits to form artificial nerve for repairing the peripheral nerve defect, but the outcomes are various in the current literatures due to different donor cells, nerve conduits and animal injury model. The firearm nerve injuries are more special for surgical treatment as their wound features, so the research about it should be enhanced in the future.

Keywords Peripheral nerve injury · Peripheral nervous system · Central nerve system · Treatment · Transplantation · Graft · Nerve conduit · Acetylcholine receptors · Adipose derived stem cell · Amniotic fluid derived stem cell · Basic fibroblast growth factor · Brain-derived neurotrophic factor · Bone marrow derived stem cell · Ciliary neurotrophic factor · Chondroitin sulphate proteoglycan · Dental pulp stem cell · Dorsal root ganglia · Extracellular matrix · Endothelial growth factor · Embryonic stem cell · Fibroblast growth factor · Fetal derived stem cell · Firearm wound · Glial cell derived neurotrophic factor · Hepatocyte growth factor · Interferon-γ · Interleukin · Mesenchymal stem cell · Nerve growth factor · Nerve stem cell · Neurotrophin · Olfactory-ensheathing cell · Polyester poly

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(E-caprolactone) · Polyglycolic acid · Poly(lactic-co-glycolic acid) · Schwann cell · Silk fibroin scaffold · Sciatic function index · Skin derived precursors · Tumor necrotic factor-α · Vascular endothelial growth factor

Abbreviations

AchR	Acetylcholine receptors
ADSCs	Adipose derived stem cells
AFDSCs	Amniotic fluid derived stem cells
CAP1	Cyclase-associated protein 1
BDNF	Brain-derived neurotrophic factor
bFGF	Basic fibroblast growth factor
BMSCs	Bone marrow derived stem cells
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
CSPGs	Chondroitin sulphate proteoglycan
DPSCs	Dental pulp stem cells
DRG	Dorsal root ganglia
ECM	Extracellular matrix
EGF	Endothelial growth factor
ERK1/2	Extracellular regulated protein kinases
ESCs	Embryonic stem cells
FGF	Fibroblast growth factor
FOX	Forkhead box
FSCs	Fetal derived stem cells
GDNF	Glial cell derived neurotrophic factor
GFP	Green fluorescent protein
HGF	Hepatocyte growth factor
IFN-γ	Interferon-γ
IL	Interleukin
MSCs	Mesenchymal stem cells
NGF	Nerve growth factor
NSCs	Nerve stem cells
NT-3	Neurotrophin-3
OB-OECs	Olfactory-ensheathing cells from the olfactory bulb
OECs	Olfactory-ensheathing cells
OM-OECs	Olfactory-ensheathing cells from the olfactory mucosa
PCL	Polyester poly(E-caprolactone)
PFTBA	Perfluorotributylamine
PGA	Polyglycolic acid
PLLA	Poly(L-lactide)
PLGA	Poly(lactic-co-glycolic acid)
PNI	Peripheral nerve injury
PNS	Peripheral nervous system
SCs	Schwann cells

SFS	Silk fibroin scaffolds
SFI	Sciatic function index
SKPs	Skin derived precursors
SKPSCs	Schwann cells derived from skin-derived Schwann cell precursors
TNF-α	Tumor necrotic factor-α
VEGF	Vascular endothelial growth factor

1 Introduction

1.1 Peripheral Nerve Injury

Peripheral nerve injury (PNI) can be resulted from trauma, firearm wound, tumors section, inflammatory diseases, congenital deformities, and surgical interventions, frequently leading to life-long disability. At least 2 million people worldwide suffer annually from peripheral nerve injuries [55] or 13.9 per 100,000 per year in Europe [2]. In US over 200,000 peripheral nerve repair procedures are performed annually. Around 5 % of wounds in the extremities can be associated with peripheral nerve injuries [10]. Although the peripheral nerve system (PNS) has a more suitable environment for axon regeneration than the central nerve system (CNS) due to the trophic influence and guiding effects of Schwann cells (SCs) and lack of growth inhibitory molecules such as Nogo in the CNS, the repair of PNI is still a practical problem in clinic, especially for the repair of nerve defect. PNS has the intrinsic capacity to regenerate at a rate of 1–3 mm/day after injury, but it is influenced by the injury severity, such as the nerve trunk completely interrupted and the gap larger than 2 cm, in which the spontaneous regeneration cannot be achieved.

1.2 Peripheral Nerve Regeneration

The peripheral nerve regeneration starts immediately after nerve injury, which covers the three different phases [10]. (i) The early phase (1–5 days): this phase is characterized by axon and myelin degeneration occurred mostly distal to injury sites (Wallerian degeneration). Simultaneously, the nucleus of influenced neurons adopts an eccentric position within the cell body and the nucleolus becomes more prominent. During this period, neuronal biochemistry and function are altered to increase protein synthesis required for axonal sprouting and growth. (ii) The intermediate phase (from 5 days to weeks): during this phase macrophages infiltrate at the injured site for removing cellular and tissue debris. At the same time, SCs start a robust proliferation due to the lost contact with axons and proximal stumps. The newly proliferated SCs, together with pre-existed SCs that survived from the

nerve injury, form the bands of Büngner, which are highly aligned tubes formed by basal lamina secreted by SCs in the distal nerve segment and crucial for the directional guidance of axon growth, as the growth cones of regenerating axons use these bands as a regenerative substrate. (iii) The late phase (from weeks to months): the growth cone of regenerating axons extend within bands of Büngner and result in complete axon regeneration and functional recovery. The regeneration process can be compromised in neurotmesis when endoneurial tubes are damaged. Under this case, SCs and fibroblasts proliferate and re-organize for the attempt to re-establish a connective bridge across the lesion, while distal stumps release chemotactic cues to attract axon sprouts.

1.3 Treatment of PNI

The treatment procedure depends mainly on the etiology, type of injury, and the anatomic region. The aim of treatment is to improve function due to motor and sensory nerve loss at the distal part of the injury. Regardless of the cause of the injury, optimal treatment of PNI should provide adequate coaptation of proximal and distal stumps without tension, which increases connective tissue proliferation, then leads to scar formation [19]. Neurorrhaphy, also known as direct nerve repair, end-to-end suturing, end–end repair or end-to-end coaptation, is the preferred method for the repair of nerve defects shorter than 5 mm, and required tension-free suturing. For optimal regeneration, the nerve stumps must be correctly aligned and sutured with minimal tissue damage. This repair method is limited to nerve gaps shorter than 5 mm. Beyond this nerve defect, alternative tissue engineering nerve grafts are adopted to repair peripheral nerve defect, but it is still a special challenge to repair long-distance nerve defect greater than 4 cm.

Although 50 years have passed, autologous nerve grafts are still considered to be a gold standard for bridging the greater nerve defect. These grafts are primarily taken from the sural nerve which is primarily sensory, so are limited for the repair of pure motor nerve deficits such as tibial nerve injury and mixed nerve defects like sciatic nerve injury. The use of sensory nerves for repairing motor nerve defects can causes mismatch in axonal size, distribution and alignment. The SCs resulted from sensory and motor nerve have different properties, if placed in the incorrect microenvironment, may limit their regenerative ability [13]. Secondary to this limitation, the autograft has a number of disadvantages, including denervation of the donor site, limited amount of available donor nerve, scarring, and neuroma formation. In addition, the survival rate of the autograts is of only 50 %, even much lower when being placed in a poorly vascularized recipient bed, such as a heavily irradiated or scarred bed. Also the autografts longer than 4–6 cm more often show poor regeneration and functional recovery [51].

Despite allograft and xenograft transplantations can be an alternative to autologous nerve grafts [3], but a major problem is the necessity of immunosuppression up to 18 months post transplantation, so makes patients become susceptible to

opportunistic infections and occasionally results in tumour formation. For removing antigenic cellular components and reducing the immunological response, acellular nerve grafts prepared using freeze thaw cycles, cold preservation and detergent treatment are recommended to repair PNI.

The nerve conduits are a promise alternative to autograft. These conduits have a number of advantages for the repair of PNI including limited fibroblast infiltration, reduced neuroma and scar formation, and no associated donor site morbidity. They facilitate the accumulation of a high concentration of neurotrophic factors, guide regenerating axons to their distal targets, and create favorable microenvironment for the nerve fibers regeneration. However, the nerve conduits are currently used to bridge critical nerve gap of approximately 4 cm, fail to match the regenerative levels of autograft and show poor functional recovery. Generally, the ideal nerve conduit should fulfill following criteria: (i) limiting scar infiltration, while allowing diffusion of nutrients into the conduit and wastes to exit the conduit, which can be achieved by the permeable material with a molecular weight limit of approximately 50 kDa; (ii) providing sufficient mechanical properties for structural support; (iii) exhibiting a low immune response; and (iv) biodegradability for avoiding the secondary surgery and chronic inflammation and pain caused by nerve compression due to the eventual collapse of the conduit [13].

The nerve conduit is composed of various synthetic and natural materials, and often combined with extracellular matrix (ECM) and different donor cells to form artificial nerve as guiding channels for regenerating axons [36, 46]. Therefore most of the present studies about cells transplantation for the repair of PNI focus on the design of artificial nerve.

2 Nerve Conduits

2.1 Structures

The basic structure of a nerve conduit consists of a tubular device with a single lumen for providing the general functions of a bridging device, isolating the regenerating axons from scar tissue, protecting the regenerating nerve against compression by the surrounding tissue, guiding longitudinally the regenerating nerve and concentrating the growth factors secreted by SCs in the end stump. More complex nerve conduit designs (Fig. 1) include (i) single hollow lumen porous or not porous conduit with longitudinally oriented grooves in their lumen surface or functionalized with bioactive molecules, such as adhesion proteins, bioactive peptides. This design is benefit to enhancing SCs attachment, proliferation and migration, but only recommended for the gaps less than 30 mm in the sensory nerves, due to poly-innervation of different targets by the axons of the same motoneuron; (ii) single lumen conduit with longitudinal aligned fibers, porous sponges or gels. Such a design is for simulating the endoneurial-like structure of the

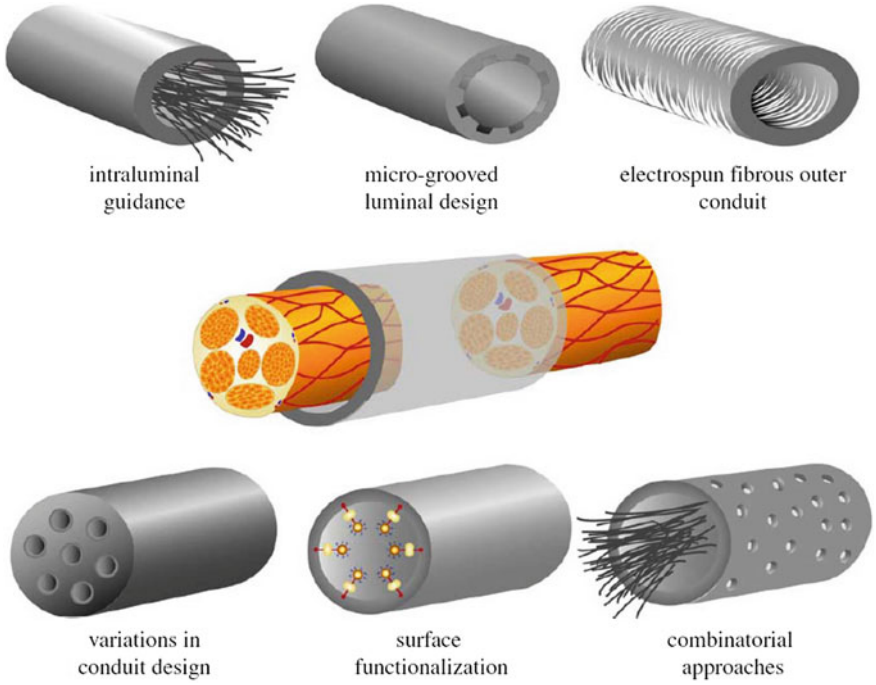

Fig. 1 Conduit design. Reproduced from Daly et al. [13]

nerve. Fillers can be functionalized with specific peptides/proteins or neurotrophic factors; (iii) multichannel conduit, which mimicks the natural compartment structure of the nerves, reduces axon dispersion, and offers superior surface area for cell adhesion and migration. However, multichannel conduit design reduces permeability and mechanical flexibility [10].

An ideal nerve conduit should have characteristics of biocompatibility, biodegradability, semi-permeability, ability for axon regeneration, and easy handling. There are three types of the nerve conduits. The first is synthetic scaffolds including silicone, polyglycolic acid, poly L-lactic acid, poly-3-hydroxybutyrate and polytetrafluoroethylene. The second is natural polymers such as chitosan, silk fibroin, collagen, chitin and gelatin. The third is natural conduits such as vein and artery grafts which are better for maintaining cell viability, supporting proliferation and permitting intercellular communication and growth factor elution [15, 27, 43, 44, 63]. For the present, several nerve tubes have been approved by US Food and Drug Administration for human uses, based on type I collagen (Neuragen®, Neuroflex™, NeuroMatrix™, NeuraWrap™, NeuroMend™), porcine small intestinal submucosa (Surgis® Nerve Cuff), poly(glycolic acid) (Neurotube®) and poly(D,L-lactide-co-e-caprolactone) (Neurolac®) [10, 51], but they are not recommended for gaps larger than 3 cm. In addition to their supportive role, the nerve conduits also help prevent unwanted cell dissipation from the injured site.

By realizing the importance of basal lamina and ECM framework for axonal guidance, the nerve conduits with different internal structure composed of multiple fibers have become more popular than hollow tubes. Recent advances in the design of internal structure of the nerve conduit focus on topographical features of guiding axonal regeneration and cell migration, based on the concepts of large interior channels to separate individual fiber fascicles, scaffolds with many longitudinally orientated pores, and parallel aligned polymer fibers produced by melt extrusion or electrospinning. Recently, the biodegradable polyester poly(E-caprolactone) (PCL) was electrospun into microfibers, then embedded in collagen gels and incorporated parallel in PCL tubes. This composite conduit could guide the direction of SCs migration and axonal growth of embryonic chicken dorsal root ganglia [35]. It is suggested that packing density and distribution of intraluminal structures can influence nerve regeneration, for example, higher densities (approx. 15–30 % of the cross-sectional area) of poly(L-lactide) (PLLA) microfilaments inhibit nerve regeneration, while lower densities (approx. 3.75–7.5 % of the cross-sectional area) enhance nerve regeneration [13].

The morphology and behavior of the cell are different according to the different fiber thickness. The poly(lactic-co-glycolic acid) (PLGA) fibers with 700 nm diameter increase olfactory-ensheathing cells (OECs) attachment with rounded cells in random orientation, whereas culture on 250 nm fibers enhance a unidirectional alignment with a characteristic bipolar shape. After seeding on silk fibroin nanofibers, migration, morphology, adhesion, spread, gene and protein expression of OECs could be influenced and modulated by the content and structure of these nanofibers. OECs with polygonal morphology could be observed on short nanofibers, on longer and parallel alignment nanofibers, OECs presented characteristic bipolar shape with more extensive migration properties [17]. 1800 nm silk fibroin scaffolds made OECs randomly disperse, however 300 nm scaffolds with the native fibrils of the ECM induced OECs to display a superior alignment and a better migration [54].

The natural silk from silkworm consists primarily of two major proteins: sericin and fibroin. Sericin is responsible for the cell adherence and has an important role for regarding nerve regeneration, but sericin is well known for inducing immunoreactivity. Up to the present, there are no study for comparing silk from silkworm (fibroin) and spiders (spidroins) or artificially prepared silk proteins. The different silk may has its own advantages and disadvantages for the spatial structures, cell seeding and release of bioactive molecules, which may have an important influence on the three-dimensional cell assembly, cell migration and axon regeneration.

Previously, the nerve conduits are mainly used for bridging the nerve defects of 2 cm or less, but it is a challenge to bridge larger nerve defects. Recently, Radtke et al. [50] adopted acellularized veins filled with spider silk to bridge a 6-cm peripheral nerve defect in adult sheep and showed that regenerated axons within the graft were found 8 months after the lesion and myelinated by endogenous invading SCs, followed by an improved electrophysiological and locomotor function that was comparable to that of autologous nerve transplantation.

2.2 Delivery Methods

Delivery method involves how to give the contents like the donor cell and the muscle into the lumen of the nerve conduit (Fig. 2). Generally, the donor cells can be directly injected within the conduit lumen or can be seeded onto the conduit matrix. If the autograft or allograft nerve is going to be used for peripheral nerve repair, the donor cells suspended in culture medium can be microinjected into ends of the grafts. However this delivery method can be traumatic to the delicate intra-neural architecture and can result in unpredictable cell distribution. The vein and artery grafts being rich in ECM proteins like collagen and laminin can provide a useful substrate for cell adhesion. Vessel grafts can remain empty or can be pre-filled with tissue such as muscle to support axonal guidance. Usually commercially available natural conduits are composed of ECM components such as collagen and fibrin. The degradation profile of the conduits should be carefully considered when used for cell transplantation. Natural conduits can degrade in a predictable, non-toxic fashion, but their degradation rate may not be sufficiently slow to allow for adequate regeneration time. Some synthetic polymers can acidify the microenvironment during degradation, so can result in a detrimental impact on cellular activity [16].

Recently, it has also been evidenced that systemic administration of the donor cells can be effective for peripheral nerve repair. With further refinement, this delivery method may become possible to sustain regenerative support through

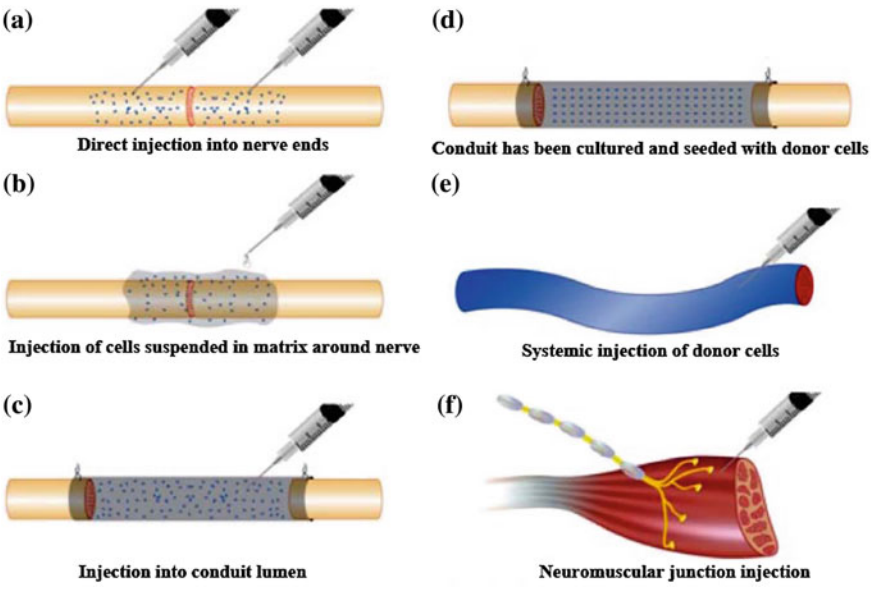

Fig. 2 Delivery methods. Reproduced from Fairbairn et al. [16]

regular systemic dosing. It has also been confirmed that the donor cells can be effective when administered at the site of neuromuscular junction or at denervated muscle [12]. A comparative study of the delivery way has recently been performed by intramuscular and intravenous injection of BMSCs after rat PNI with small gap neurorrhaphy, the results shown that the targeted muscular injection resulted in much superior outcomes compared to the intravenous injection, evidenced by increases in the sciatic function index, nerve conduction velocity, myelin sheath thickness and restoration rate of gastrocnemius muscle wet weight [61].

2.3 Oxygen Supply

For long nerve conduit a major problem is the oxygen supply within the tube, which can affect the survival and function of the filled cells and be a limiting factor for the repair of long nerve defect. To increase the oxygen supply, perfluorotributylamine (PFTBA) is used as a synthetic oxygen carrier and combined into a collagen-chitosan conduit filled with OECs for repairing 15-mm-long rat sciatic nerve defect. The survived OECs are more in number in the PFTBA-enriched tubes than in the tubes with OECs alone, suggesting the beneficial effect of PFTBA on the OECs survival in vivo [67].

2.4 Clinical Trials

The synthetic nerve conduits have been used for the repair of human PNI in China [18, 23] and USA [29]. A chitosan/polyglycolic acid (PGA) nerve conduit is adopted for repairing a 35-mm-long median nerve defect at elbow of a human patient. During the 3-year follow-up period, functional recovery of the injured median nerve is assessed by pinch gauge test, hydraulic hand dynamometry, static two-point discrimination and touch test and electrophysiological examinations. This implantation improves the recovery of the motor and sensory function at M4 and S3 + levels [18]. In another case, 30-mm-long median nerve defect in the right distal forearm of a 55 year-old male patient is repaired with same synthetic nerve conduit, and results in the recovery of the palm abduction of the thumb and the thumb-index digital opposition. In addition, compound muscle action potentials is recorded on the right abductor pollicis and perspiration function of the injured thumb, index and middle fingers is partially recovered, indicated by the ninhydrin test which is a classical method for assessing sympathetic nerve function. These repair cases show that the synthetic nerve conduits are beneficial to the repair of larger defect of human peripheral nerve trunk in clinic.

3 ECM

ECM is an acellular component composed of proteoglycans (collagen and elastin) and fibrous proteins (fibronectin and laminin). It provides an environment for the survival, development and differentiation of the cells and the tissues. In PNS, ECM has profound influences on SCs behavior such as adhesion, differentiation, survival, growth, and migration. During the peripheral nerve regeneration, SCs migration from both proximal and distal nerve stumps into the site of the lesion will be limited without the formation of ECM cable.

Among ECM proteins, fibronectins and laminins are the most widely studied in peripheral nerve regeneration. Fibronectin is mainly synthesized and secreted by SCs and forms a fibrillar network in association with type IV collagens and laminins. Although it is expressed at relatively low levels in the SCs basement membrane of adult peripheral nerves, fibronectin is rapidly upregulated following nerve injury, either deposited from plasma or synthesized by fibroblasts and endothelial cells. During the development of the nervous system, fibronectin is mainly involved in the migration and differentiation of the neural crest cells and responsible for SCs adhesion, proliferation, and neurite outgrowth. Fibronectin knockout mice die early in gestation, and nervous system abnormalities are apparent, including incomplete closure of the neural tube. Laminin, a complex trimeric glycoprotein composed of one *a*, one *B*, and one *y* chain and ranged in size from 400 to 900 kDa, is a main protein for the maturation of the PNS and appears to be crucial for the SCs to successfully myelinate axons [21]. During the repair of PNI, this glycoprotein has positive effects on SCs adhesion, proliferation, migration, and the ability to improve nerve regeneration. It has been confirmed that the lack of laminin is manifested as reduced myelination, discontinuous basal lamina, and atypical SCs ensheathment.

In vitro cell culture laminin and fibronectin are usually added to the culture disk for coating. Although both have a ability for SCs proliferation and neurites sprout of dorsal root ganglia (DRG) neurons, the best outcome result from the synergistic action of fibronectin and laminin [20]. In vivo, laminin and fibronectin not only benefit to the behavior of glial and neuronal cells, but also promote stem cells differentiate into SC-phenotype. After adding ECM to the nerve conduits, ECM modified-scaffold can promote survival and migration of endogenous cells and transplant exogenous cells in the PNI repair.

Also ECM plays a role for selectively attaching and releasing growth factors such as brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), fibroblast growth factor (FGF), glial cell derived neurotrophic factor (GDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3). For example, NT-3 and BDNF have shown good affinity to fibrinogen, whereas CNTF is easy to combine with laminin. This selective binding property of ECM can be benefit to the drug design and release.

4 Donor Cells

The ideal donor cell for peripheral nerve repair should be easily accessible, rapidly expandable in culture, capable of in vivo survival and integration into host tissue [16]. In addition the donor cell should be amenable to stable transfection and expression of exogenous genes.

4.1 Stem Cells

It has been widely demonstrated in vitro and in vivo that stem cells can replace lost neurons, increase the number of glial cells, rescue axotomized neurons, and manipulate the microenvironment of the neurons during regeneration. During regeneration of peripheral nerve, increasing number and activity of SCs is especially emphasized. Exogenous stem cells can differentiate into SC-like phenotype, form Büngner Bands, guide axon regeneration, and re-myelinate nerve fibers. After stem cells being transplanted, growth factor secretion and ECM production can be enhanced by direct releasing and paracrine signaling. Then, secreted factors stimulate endogenous SCs to upregulate secretory activity. ECM proteins such as collagen I, collagen IV, fibronectin and laminin have regenerative effects, but ECM components of chondroitin sulphate proteoglycan (CSPGs) is a potent inhibitor of axonal regeneration. However CSPGs can be cleaved from the basement membrane by activated matrix metalloproteinases [21], thus still providing a permissive ECM environment for axonal regeneration.

Both transplanted stem cells and endogenous SCs can produce NGF, BDNF, GDNF, CNTF and NT-3. Some angiogenic factors like vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF) and angiopoietin-1 may also be released by the stem cells. In addition, the stem cells express leukemia inhibitory factor and insulin-like growth factor, which are potential to improve neuronal survival, promote corticospinal tract growth, increase astrogliosis and potentiate the inflammatory response. The immunomodulatory effects of the stem cells are believed to be due to the secretion of granulocyte and macrophage colony stimulating factor (G-CSF, M-CSF), interleukin-6, 7, 8, and 11 (IL-6, IL-7, IL-8, IL-11) and tumor necrotic factor-α (TNF-α). If the host immune response is properly suppressed, the detrimental impact of inflammation and fibrosis following injury can be reduced. The levels of ECM protein and growth factor production may vary not only with stem cell type but also with the differentiation status of stem cells [16].

The stem cells can be used in their undifferentiated state or SC-like cells differentiated in vitro by exposure to β-mercaptoethanol, all-trans retinoic acid, fetal bovine serum, forskolin, recombinant human bFGF, recombinant human platelet derived growth factor-AA, and recombinant human heregulin β-1. The benefits of differentiation include superior in vivo viability and an enhancement of

neurotrophic factor secretion and myelinating ability. However, differentiation in vitro can incurs an unnecessary delay for clinical applicability, reduces the secretion of neurotrophic factors, maintains in vivo neuronal differentiation difficult. The undifferentiated cells may undergo in vivo differentiation in response to local stimuli, but have a risk of differentiation along unwanted non-neuronal lines [16].

4.1.1 Embryonic Stem Cells (ESCs)

ESCs isolated from blastocysts can form derivatives of all three embryonic germ layers (Fig. 3). These cells are homogenous, and can provide an unlimited source of cells with superior differentiation potential and long-term proliferation capacity. Their disadvantages include immunogenicity and tumourigenicity. Additionally, ethical controversy exists potentially due to the fact that cells are harvested during

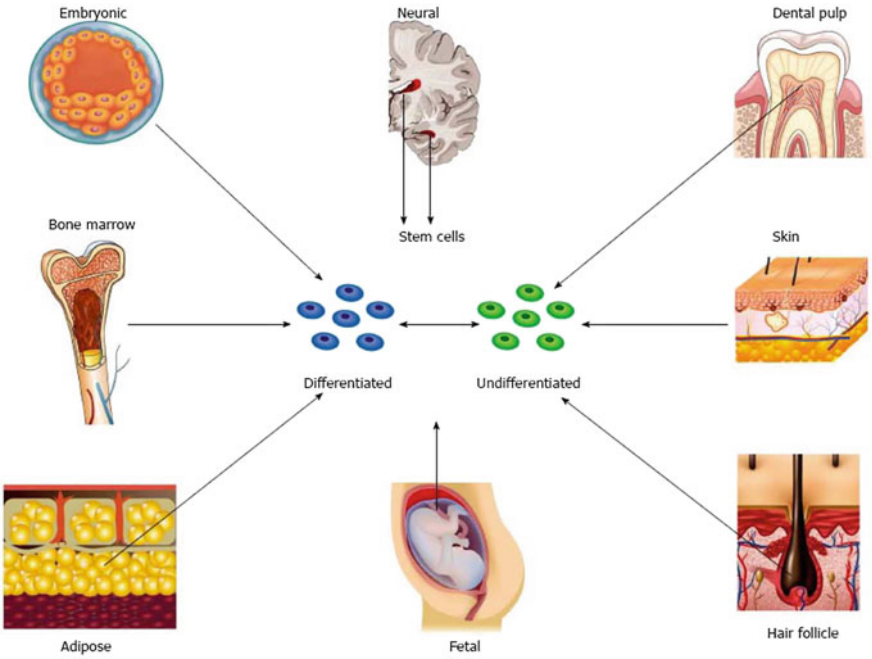

Fig. 3 Different stem cell sources. ESCs are obtained from the inner cell mass of the blastocyst, therefore require destruction of the embryo. NSCs are harvested from the subventricular layer of the lateral ventricle and the subgranular layer of the hippocampus. BMSCs are harvested from the marrow cavity of long bones. ADSCs are derived from subcutaneous fat, which is abundantly available following liposuction. SKPs are harvested from the dermis and represent a related populations of cells harvested from hair follicles. FSCs can be obtained from amniotic membrane, amniotic fluid, umbilical cord blood, umbilical cord tissue and Wharton's jelly. DPSCs can be harvested from deciduous teeth. Reproduced from Fairbairn et al. [16]

the blastocyst stage of development, which consequently result in the destruction of embryos [16].

It has been shown that ESCs can differentiate into neurons and glial cells of the central and peripheral nervous system. The capacity for in vivo myelination also has been confirmed [6, 66]. Previous studies about application of ESCs to peripheral nerve repair are limited, perhaps reflecting the lack of facile clinical translatability [11], but few studies reported that after ESCs transplanted directly into muscle of small animal with sciatic and tibial nerve transection can preserve short-term muscle mass and myocyte cross-sectional area [37]. Realizing this limitation, investigators have derived mesenchymal precursors from ESCs. It has been reported that transplanting spheres of mesenchymal stem cells (MSCs) derived from ESCs can improve the peripheral nerve regeneration [39].

4.1.2 Nerve Stem Cells (NSCs)

NSCs are first isolated from adult murine brain in the early 1990s, followed by similar discoveries in humans and non-human primates [16]. The brain subventricular zone and subgranular zone represent the primary sites of NSCs differentiation in adult mammalians (Fig. 3). Populations of NSCs and neurogenesis have been found in adjacent areas of the adult human striatum [14]. Many studies have reported positive results following NSCs implantation into injured peripheral nerve (reviewed by Fairbairn et al. [16]). NSCs transplantation is not only beneficial to the acute PNI, but also beneficial to the chronically denervated nerve for its recovery of structure and function [28]. Unfortunately, transplantation of NSCs into rat models of crush and transection, neuroblastoma formation has been encountered [32]. At present, immortalized murine C17.2 NCSs are commercially available and are commonly used for in vivo animal studies, but there are not uniform positive outcomes experienced with C17.2 NSCs in the repair of PNI.

4.2 Mesenchymal Stem Cells (MSCs)

MSCs are multipotent stromal cells originating from the bone marrow and different non-marrow tissue such as adipose tissue, skin, hair follicle and dental pulp (Fig. 3). Originally the potency of these cells is considered to be limited to tissues of mesodermal origin. Now it is generally accepted that they can differentiate into non-mesodermal lineage. MSCs can differentiate in vitro into osteoblasts, chondrocytes, adipocytes, cardiomyocytes, hepatocytes and neural lineages, including neurones, astrocytes, oligodendrocytes, microglia and SCs. Here the main sub-types of MSCs and their application to peripheral nerve regeneration will be briefly discussed as following.

4.2.1 Bone Marrow Derived Stem Cells (BMSCs)

BMSCs are easily accessible from the marrow cavity of long bones without potential ethical concerns (Fig. 3). Therefore these cells are more clinically applicable than ESCs, NSCs and SCs. Under appropriate conditions BMSCs can differentiate into neurons, astrocytes and SC-like cells of non-mesodermal lineages. It has been shown that adding BMSCs to the nerve conduits and acellular grafts results in superior results when compared with empty or cell depleted channels. Although few studies are failed to show that BMSCs can match outcomes achieved with cultured SCs, the majority of reports demonstrate that performance is at least equivalent [4], even a dearth of studies have shown superiority over gold standard autograft, which may be dose dependent [52]. BMSCs generally have inferior proliferation capacity and differentiation potential with low stem cell fraction. Recently, BMSCs with Platelet-rich plasma filled into Neurolac is used to repair 1 cm-defect of the sheep radial nerve (sensory) and the tibial nerve (motor). After 6 months increased myelinated nervous fibers and conduction velocity can be observed from both repaired radial and tibial nerves [9]. As BMSCs harvest is invasive and painful, less invasive MSCs sources such as adipose tissue, skin, hair follicle and fetal tissue are adopted.

4.2.2 Adipose Derived Stem Cells (ADSCs)

ADSCs result from the adipose tissue, and is abundant and easily harvested from abundant subcutaneous fat tissue via small biopsies or conventional liposuction procedures (Fig. 3). Especially, ADSCs can enhance neovascularization in ischemic conditions by secreting endothelial growth factor (EGF). In comparison to BMSCs, ADSCs have superior proliferation and differentiation potential [58]. Unlike BMSCs, donor age and anatomical site of origin do not seem to significantly influence therapeutic effect of ADSCs [56]. Based on the expression of myelin protein zero, peripheral myelin protein and myelin basic protein by ADSCs, capability of ADSCs myelinating regeneration axons is kept. Although some studies report that ADSC-filled conduits have no difference in outcome compared to cell deplete products, several studies have shown that ADSCs are at least as effective as autologous SCs, even have equivalent and superior outcomes in comparison to autograft. By comparing the performance of ADSCs and BMSCs, no significant differences have been found [45]. Low harvest morbidity, wide availability and superior stem cell characteristics have made this stem cell to be preferred one for pre-clinical studies.

4.2.3 Skin Derived Precursors (SKPs)

SKPs from the dermis (Fig. 3) are easily expandable in culture, and can give rise to different cell types such as melanocytes, craniofacial cartilage, bone and connective

tissue, smooth muscle of vasculature, endocrine cells, and neurons and glial cells of the autonomic and peripheral nervous system, which are similarity to embryonic neural crest cells. When cultured with neuregulin-1β, known to promote proliferation and differentiation of SCs from embryonic neural crest precursors and to mediate SCs proliferation during Wallerian degeneration, SKPs express markers consistent with functioning SCs. Both undifferentiated and differentiated SKPs can maintain differentiation and viability, myelinate axons, and exhibit superior outcomes following de-myelination and crush injury, acute and chronic transection injury [16]. SCs differentiated from skin-derived SC precursors (SKPSCs) are highly proliferative glial cells, can enhance functional recovery after acute and chronic PNI [34] by modulation of the immune response and enhancement of macrophage recruitment to injury sites for more efficient debris clearance through the expression of interferon-γ (IFN-γ), interleukin (IL)-1β, and IL-6 [57].

4.2.4 Fetal Derived Stem Cells (FSCs) [16]

Fetal tissue represents a promising alternative source of stem cells. FSCs can be harvested from fetal tissues such as amniotic membrane, amniotic fluid, umbilical cord cells, umbilical cord blood and Wharton's jelly (Fig. 3). FSCs are readily expandable in culture and possess the ability to differentiate into neural phenotype. It has been confirmed that amniotic fluid derived stem cells (AFDSCs) possess characteristics of both MSCs and NSCs and can differentiate into neural tissue. Following transplantation into rat sciatic nerve gap models, AFDSCs have been shown to promote peripheral nerve regeneration, but its survival following transplantation is limited. Umbilical cord-derived stem cells have also been shown to improve outcomes following crush and transection injuries in rodent models. Wharton's jelly derived stem cells can differentiate into functional SC-like cells, produce NGF, BDNF, NT-3 and stimulate neurite growth in vitro.

4.2.5 Dental Pulp Stem Cells (DPSCs)

Dental pulp, originating from neural crest, can be harvested from exfoliated deciduous teeth (Fig. 3). It represents the most convenient source of multipotent stem cells. DPSCs can successfully differentiate into SCs in vitro and is able to support DRG neurite outgrowth. Within a collagen gel matrix, DPSCs form aligned columns and had the ability to guide and myelinate neuritis [16]. After transplantation, this type of stem cell can support peripheral nerve regeneration.

4.3 Olfactory Ensheathing Cells (OECs)

OECs are specialized glial cells within the olfactory system. They ensheath bundles of nonmyelinated olfactory nerve axons and provide a channel for axons to grow from the PNS into CNS. There are two sub-population of OECs including these derived from the olfactory mucosa (OM-OECs) and olfactory bulb (OB-OECs). When comparing function of these sub-populations, different outcomes are resulted from a vagus nerve lesion model, indicating that OM-OECs influence primarily inflammation processes and extracellular matrix formation with a minimal effect on regeneration, whereas OB-OECs enhance axonal regeneration, thus leading to functional improvements [47]. These outcomes are consistent with examination of gene profiling in both OECs groups, showing that OM-OECs overexpress genes characteristic of wound healing and regulation of the extracellular matrix, however OB-OECs overexpress genes responsible for axonal guidance. In vitro, OB-OECs display faster migratory properties and more effective neuritic outgrowth than OM-OECs. The OECs in the nasal epithelium are more immature and change to a more mature state in the olfactory bulb, they allow growth of olfactory nerve axons and regulate the extracellular matrix. Whereas OB-OECs play a role in guiding axons to specific targets in the olfactory bulb glomeruli. These differences are important for the selection of OECs as a cell therapy candidate.

In a vagus nerve lesion model the functional differences of OB-OECs and OM-OECs are carried out with a comparative transplantation analysis. Transplantation of OM-OECs to the microsutured vagus nerve results in improved electrical activity and tissue healing, but induced aberrant movements of the laryngeal muscles and poor functional recovery. However, OB-OECs induce some functional recovery, increase number of myelinated axons and improve conduction velocity. Again, these results indicate that OM-OECs primarily influence inflammation processes and extracellular matrix formation, but had a minimal effect on axonal regeneration, whereas OB-OECs enhance axonal regeneration and targeting to laryngeal muscles, thus leading to functional improvement [47, 51]. Co-transplantation of both OEC subpopulations into a recurrent laryngeal nerve injury model is also carried out and result in enhance functional recovery of injured nerve [24].

4.3.1 OEC for Sciatic Nerve Repair

As the sciatic nerve is easily accessible and its axonal regeneration rates, remyelination patterns and hind limb motor performance are well characterized after the injury, this peripheral nerve is used as a common model for the study of transplantation and nerve repair. Within transected and crushed sciatic nerve, OB-OECs can survive and remyelinate regenerated axons, which reinforce the idea that OECs share many properties with SCs and indicate that although OECs normally do not form myelin in the olfactory system, they can do so when transplanted

into an appropriate pathological environment. OB-OECs are also microinjected immediately distal and caudal to the microsuture site of the transected sciatic nerve as an adjunct therapeutic approach. This transplantation can increase the scores of sciatic functional index and the numbers of remyelinated axons distal to the lesion site. Furthermore immunostaining for the sodium channel displays a high density of channel subtype 1.6 (NaV1.6) at the newly formed Ranvier nodes. After sciatic nerve transection, axons typically die-back 1–2 mm over the course of a day or two, and then regenerate toward peripheral targets. OECs transplantation may reduce the normal dieback of axons, thus allow regeneration across the injury site prior to scar formation which can impede axonal regeneration [48].

Likewise, OECs have been used to repair the severe sciatic nerve injury with 10 or 20-mm defect [24]. Usually the nerve conduits filled with the donor cells are adopted for repairing the nerve defect. Recently, PLGA scaffold filled with EMC and OB-OECs is used to bridge 10-mm defect of the sciatic nerve by a comparative observation with silicon filled with ECM and OB-OECs [40]; (Fig. 4). PLGA possesses complete compatibility with OB-OECs. After implantation, OB-OECs migrate along the axis of the nerve and survived longer in the PLGA + OEC bridged animals than in the silicon + OEC bridged animals. Gross recovery of the animal, like ulcerious and autophagical rate as well as relative diameter recovery rate of the nervier, is more successful in the PLGA + OEC group than in the silicon + OEC group. Although the number of the fiber in the middle and distal segments of bridged sites and neurons in anterior horn of the spinal cord is increased in both groups, the diameter and the myeline thickness of the fiber are increased only in the PLGA + OEC group. Thus the nerve conduction velocity and the amplitude of compound muscle active potential are improved much successfully in the PLGA + OEC transplanted animals. These results suggest that PLGA filled with OECs is a significant alternative to conventional autograft in repairing sciatic nerve defects, and OECs are potential seed cells for peripheral nerve tissue engineering. PLGA is much superior for repairing the nerve defect than silicon due to small pores within the wall of PLGA which allow the nutrient to pass through. Recently, Lokanathan et al. [42] and Tan et al. [59] use OEC-seeded muscle-stuffed vein and OEC-seeded PLGA to repair 15-mm sciatic nerve defect, the nerve conduction velocity is promoted. Especially the outcome from the biological nerve conduit is superior to the autologous graft [42].

4.3.2 OECs for Facial Nerve Repair

Both OB-OECs and OM-OECs have been transplanted for the repair of facial nerve lesions in rats. After the facial nerve is either anastomosed or resected out 5-mm, OECs filled within silicone tube or alone are transplanted and result in enhanced axonal sprouting, promoted recovery of vibrissae motor performance and increased rate of eye closure. However, endogenous return of vibrissae motor performance and increases in the rate of eye closure can also be demonstrated without cell

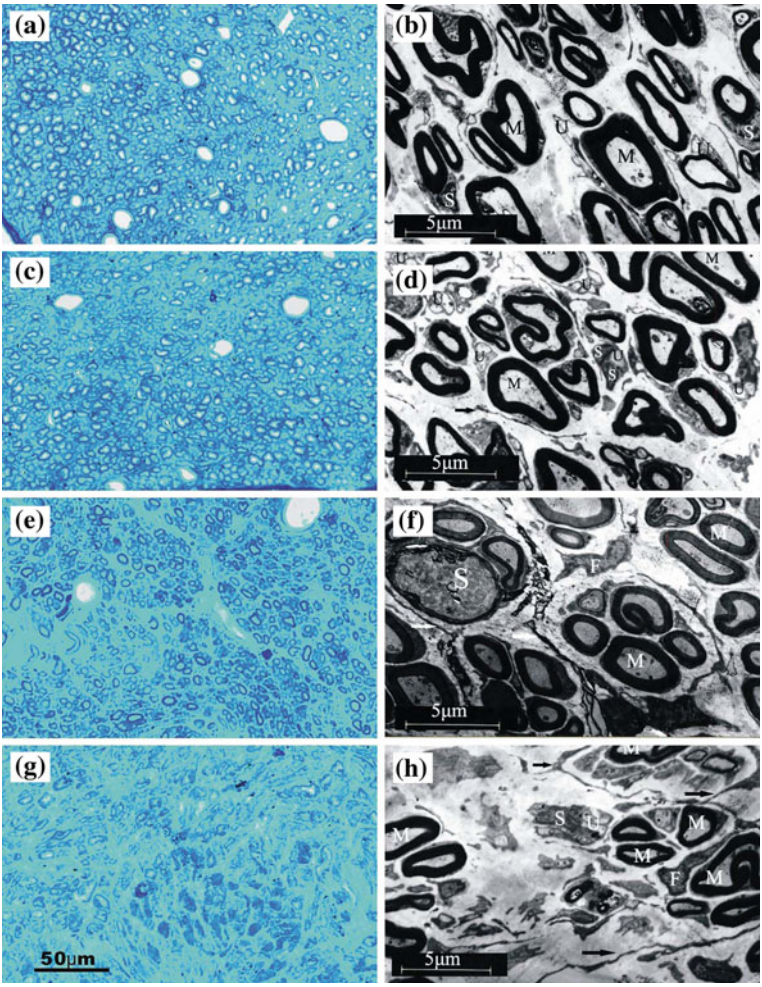

Fig. 4 Rat sciatic nerve with 10 mm gap was bridged by PLGA-OECs-ECM (**a**, **b**), Silicon-OECs-ECM (**c**, **d**), PLGA-ECM (**e**, **f**), and Silicon-ECM (**g**, **h**). Pictures in *left column* are distal segment of each group and nerve fibers were stained by methylene blue/Azure II on semi-thin sections. Pictures in *right column* are also distal segment, but observed under electromicroscope. *M* myelinated axons; *U* unmyelinated axons; *S* Schwann cells; *F* fibroblasts. *Arrow* indicates endoneurium-like structure formed by extending of fibroblast cytoplasm. Scale bars = 50μm in **a**, **c**, **e**, **g**; 5μm in **b**, **d**, **f**, **h**

transplantation [25, 26], indicating that more detailed experiments in this system with OEC transplantation are required [51].

4.3.3 OECs for Sensory Nerve Repair

If peripheral sensory axons are transected distal to the DRG, they can regenerate to peripheral targets in muscle or skin, but is very difficult to regenerate into the CNS after the nerves are injured proximal to the DRG. For reconnecting sensory pathway between the CNS and PNS, OB-OECs are transplanted into the spinal cord after dorsal root axons being transected, this performance promote dorsal root axons to regenerate into the spinal cord and improve sensory reflex responses of the spinal cord, but these do not occur after that OM-OECs are transplanted [53].

Again the repair outcomes of OB-OECs and OM-OECs are compared in rat rhizotomy model, OB-OECs promote axonal regeneration and restore electrophysiological transmission and forepaw grasping during the climbing test, however no axonal regeneration and forepaw grasping recovery can be observed after OM-OECs transplantation [30]. The comparative results for promoting axonal regeneration to transverse CNS-PNS transitional zone confirms once more that OB-OECs are more effective for the sensory nerve repair than OM-OECs.

The loss of spiral ganglion neurons can result in sensorineural hearing loss. Strategies are being explored to preserve and protect auditory neurons with neurotrophic factors released by transplanted cells. In in vitro studies, BDNF secreted from OECs promote the survival of spiral ganglia neuron [41]. OEC-conditioned medium derived from neonatal rats reduces apoptosis of spiral ganglia neurons with an increase of cell survival [64]. However in vivo studies that transplanting OECs into the inner ear have not yet been performed, but are being considered [51].

4.4 SCs

SCs, originating from the neural crest, are the most abundant glial cell type in the PNS. They can be subdivided into myelinating SCs, non-myelinating SCs, perisynaptic SCs and satellite cells. The major function of SCs is the establishment of compact myelin around large diameter axons, which is essential for fast saltatory nerve conduction. In contrast to the CNS in which most axons are myelinated, many axons including small diameter axons, terminal branches of motor neurons and sympathetic nerve fibers in the PNS are surrounded by SCs cytoplasm, which do not form myelin. Under normal conditions, engulfing SCs and bidirectional signaling of axons maintain axonal integrity. Loss of signaling molecules on SCs or on the axons may result in axonal loss. In addition, SCs express neurotrophins such as NGF, BDNF and GDNF for providing trophic support to axons. These neurotrophins are essential to promote axonal outgrowth and prevent neurons from initiating programmed cell death [38].

In the injured PNS, SCs remained after Wallerian degeneration migrate and proliferate to form aligned glial bands of Büngner for guiding axons regeneration toward their distal targets. It is previously suggested that SCs exhibit a specific motor or sensory phenotype based on their source, which can influence axonal

regeneration toward their correct target. However, a recent study about the effects of SCs source on the sensory or motor-specific regeneration is carried out, showing that SCs derived from the motor and sensory branches of the femoral nerve have same ability for repairing 14 mm rat sciatic nerve defect [31].

The migration and proliferation of SCs response to the peripheral nerve injury are related with adenylate cyclase-associated protein 1 (CAP1) and Forkhead box 1 (Foxj1). CAP1 regulates actin filaments and mediate processes establishing cell polarity, motility, morphogenesis, receptor-mediated endocytosis and mRNA location. Foxj1, a major regulator of cilia development, can be expressed in multiple tissues during development. It plays an important role in the injury and repair of CNS. After sciatic nerve crushed, both CAP1 and Foxj1 express a high level, which is related to the biological behavior of SCs both in vitro and in vivo [8, 68]. Recently, a study of the molecular signals for triggering SCs migration in vitro has been published and indicates that elevated extracellular regulated protein kinases (ERK)1/2 is coupled with the migration of SCs, however, inhibition of ERK1/2 activity has no significant negative effect on SCs migration, only combined inhibition of ERK1/2 and AKT activity result in a significant decrease in SCs motility. These molecular characteristics suggest that both ERK1/2 and AKT signals are involved in the migratory potential of SCs [65].

The use of autologous or allogenic SCs is taken as the gold standard for peripheral nerve repair due to their inherent advantages, such as producing a number of neurotrophic factors, building their own basal lamina, expressing cell adhesion molecules and remyelinating nerve fibers at a later stage. SCs can be added into the nerve conduit via a number of methods including injection, suspension within an intraluminal hydrogel, and release from intraluminal guidance structures or the luminal wall. At optimum concentration, the implanted SCs can be successfully incorporated into the host regenerative process and furthermore promote the nerve regeneration. For example, SCs seeded within the hollow fibrin conduit increase nerve regeneration and functional recovery compared to the control conduit in a 10 mm defect sciatic nerve model. It should be noted that canine SCs is a more clinically relevant seed cells as they display characteristics similar to primate SCs. Unlike those of rodent cells, canine SCs can stably express low-affinity binding receptor (p75NTR) and grow for long periods in the absence of mitogens [13].

The viability of the exogenous SCs in the host tissue is an important issue for their supporting role in the peripheral nerve regeneration. Transplantation of green fluorescent protein (GFP)-expressing SCs from male rat into an acute female rat sciatic nerve axotomy confirm survival and integrated into the injured nerve by GFP expression and fluorescence in situ hybridization for the Y chromosome (male donor cells) [49]. Furthermore these transplanted SCs can survive in the injured sciatic nerve for 56 days [22] or 112 days [5]. Survived SCs increase the number of myelinated fibers, promote the regeneration and improve motor recovery after sciatic nerve defect, also increase number of neurons in L4 segments and dorsal root ganglion sensory neurons. At the same time, the expression of neurotrophic factors such as BDNF, NGF, NT-3 and NT-4 is increased in the transected nerve, dorsal

root ganglion and spinal cord, which contribute to the better nerve regeneration both functionally and morphologically. The above positive results are enhanced by the treadmill training, indicate that combination of SCs transplantation and treadmill training can significantly improve functional and morphological recovery after sciatic injury [22].

The injured axons can regenerate into the motor endplate and reform synapse under direction and guidance of SCs. It has been confirmed by vital imaging that SCs at neuromuscular junctions play active roles in synaptic homeostasis and repair. Motor axons most commonly regenerate to the original synaptic site guided by SCs-filled endoneurial tubes. During the period of denervation, terminal SCs extend processes from the junction, but they also retract some processes from the territory occupied by them previously within the endplate. The degree of this retraction depends on last time of the denervation. The topology of SCs processes influences the branching pattern of regenerating axon terminals and the redistribution of acetylcholine receptors (AchR). Upon arriving at the junction, regenerating axons follow SCs processes within the original synaptic site. New AChR is also induced by axon terminals that follow SC processes extended during denervation [33].

4.5 Co-transplantation of SCs and OECs for Sciatic Nerve Repair

SCs and OECs share common growth factor responses, antigenic and morphological characteristics, and transcriptional regulation, both of them have been widely advocated for transplant-mediated repair of PNI and central nerve injury in animals and human. However they differ at inducing reactive astrocyte response. In vitro assays, both SCs and OECs migrate across astrocyte monolayers, but only OECs will migrate into an area containing astrocytes. Astrocytes undergo hypertrophy when in contact with SCs but not with OECs. It is further demonstrated in vivo that astrocytes mingle with OECs but not SCs after injection into normal spinal cord. Besides, OECs secret NT-4/5, which not expressed by SCs. Therefore, a hypothesis is suggested that if OECs and SCs are co-transplanted, can they play a synergic effect for the repair of PNI.

Recently we test above premise by comparing axon regeneration, gastrocnemius muscle restoration, functional recovery, and electrophysiological changes after a 10 mm sciatic nerve gap is bridged by PLGA conduits filled with SCs, OECs, or SCs/OECs and by autologous nerve grafts [62]. The results show that compared with SCs transplants, axon regeneration is 25 % less with OECs transplants but 28 % more with SCs/OECs transplants. Gastrocnemius muscle restoration is similar with SCs or OECs transplant and 35 % better with SCs/OECs transplant. With SCs transplants, motor and sensory function recovery and electrophysiological outcomes are similar as with OECs transplants and 33 % better with SCs/OECs

transplants. Compared with the mixed SCs/OECs transplants, axon regeneration is 21 % better and gastrocnemius muscle restoration is 18 % better with autologous peripheral nerve transplants. These findings support our hypothesis that the combination of SCs and OECs is more effective in repairing the sciatic nerve than the individual cell type alone. OECs synergistically improve SCs mediated anatomical and functional repair after sciatic nerve injury.

More myelinated axons with larger diameters and thicker myelin sheaths are found in SCs transplants than in OECs transplants, suggesting that SCs establish a more conducive terrain for axon myelination and maturation in the injured sciatic nerve than OECs, which can be expected considering the natural role of SCs as repair-promoting cell in injured peripheral nerves and myelinating cell in uninjured functioning peripheral nerves. The co-transplantation of SCs and OECs promoting axon myelination better than the individual cell types alone may be due to that OECs increase the levels of trophic factors which further facilitating the myelination process. An alternative explanation for the increased myelination in SCs/OECs transplants is that OECs encourage SCs migration from the nerve stumps, so as to much more effectively form myelin. Another reason against the better myelination after co-transplantation is that OECs are a type of effective myelin forming cells as described above.

Besides myelination, it is crucial for the regenerating axons to grow beyond the transplant into the distal sciatic nerve stump, as this would subsequently allow innervation of the denervated muscles. The number of axons that have regenerated beyond the transplant is larger in rats with SCs/OECs transplant than with OECs and SCs only transplant. It is likely that OECs contribute to this response by further eliciting axon growth across scar tissue that usually forms at the interface between the transplant and the distal nerve stump. After PNI, besides re-innervation of an affected muscle, it is also important that motor endplates are formed, which is necessary for muscle function. This has been demonstrated by the co-transplantation of SCs and OECs, showing that SCs and OECs transplants are similarly effective in increasing the number of motor endplates and their surface area but less effective than SCs/OECs transplants. It is likely that the increase in axon growth into the distal stump of bridged sciatic nerve is directly involved in the increase in motor endplate number and surface area. Subsequently, this may have resulted in a stronger and heavier gastrocnemius muscle.

The sensory function recovery as measured with the withdrawal test in the SCs/OECs transplant is better than a SCs-only or OECs-only transplant. It can be reasonably deduced that improved sensory function is associated with higher numbers of myelinated axons with larger diameters and thicker myelin sheaths in the transplant, axon growth beyond the transplant, higher numbers of motor endplates on the gastrocnemius muscle, and improved electrophysiological parameters. Motor function, as measured by sciatic function index (SFI), is also better in rats with a SCs/OECs transplant compared with a SCs- or OECs-only transplant. However, recovery of motor function is much slower than that of sensory function. SFI is based on analysis of footprints of the affected and unaffected limb, and depends on body weight and postural muscle tone. Recovery of muscle tone after

PNI is typically slow, which may at least in part explain the delayed restoration of motor function.

5 Conclusions and Future Perspectives

Despite some synthetic nerve conduits have been used in a few clinic studies, the donor cells for peripheral nerve repair have yet to make an impact in the clinical arena. Translation from basic research to clinical application is currently limited by some concerns about risks of tumorigenesis, genetic manipulation, and cell instability. Additionally, although the great efforts have been made to promote the peripheral nerve regeneration and some reported results are satisfactory, even much better than autografts, the outcomes of the donor cells for repairing PNI have not led to those which significantly and consistently surpass that achieved with conventional techniques. The peripheral nerve repair with different stem cells must consider their type, differentiation, as well as scaffold and method of cell delivery in order to offer novel and effective treatment methods. Unfortunately, these factors vary widely in the current literature. In addition, animal and injury models are very different in the current studies. All of these discrepancies make it very difficult to draw definitive conclusions.

Optimal peripheral nerve repair will not be achieved by single factor strategy. An appropriate combinatorial approach should be considered. Although some researchers have sought different methods of combination, the best possible combination of factors still need to be realized, which may need to considerate the natural sequence of the nerve regeneration. Particularly, the events occurred during regeneration in nerve trunk and nerve defect with larger gap should be completely understood. Also, a thorough understanding should be learned for why larger gap is, more poor recovery of structure and function is, even with nerve regeneration promoted by the cells transplantation. If the mechanisms of these limitations are clearly understood, the complete functional nerve regeneration will be realized. Overcoming these limitations is a key step towards the optimal peripheral nerve repair. Finally, how to use the cells to repair firearm-induced PNI should be emphasized. Although the model making, experiment procedures and technologies for the study of repairing this type of nerve injury are more difficult, as more serious injury such as more extensive damage range, more irregular defect, more length gap and more bleeding can be caused by firearms, it is well worth researching in order to save young adults labor and improve their life qualities.

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Sweat Gland Regeneration: Basic Scientific Problems and Possible Technical Approaches

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Abstract The term 'sweat gland regeneration' refers to a new and expanding field in regenerative medicine research that focuses on the development of innovative therapies allowing the body to replace, restore and regenerate damaged or diseased sweat gland cells and tissues. It combines basic scientific theory and technological approaches including dedifferentiation, biomaterials, tissue engineering, stem cell transplantation and the reprogramming of cell and tissue types. Because of its importance for skin reconstitution in patients suffering from chronic wounds and extensive burns, sweat gland regeneration is becoming an rapidly developing field in regenerative medicine.

Keywords Sweat gland · Regenerative medicine · Stem cells · Tissue engineering

With the usage of measures to prevent conflagration and improved treatment of burns, the occurrence rate of fire decreased and survival rate for serve burn patients in China is almost 100 %. However, these survivors face functional loss of skin because healing involves scar formation and the skin lacks skin appendages such as hair follicles, sweat glands and sebaceous glands. In humans, about 25 % of body heat is expended by perspiration through skin. Because of loss of sweat glands, burn survivors cannot sweat, which harms body temperature regulation. Thus, the functional recovery of wounded skin is a great challenge for physicians and medical scientists.

Skin is one of the largest organs in human body and different kinds of tissue are involved in its development and repair. If we focus on the sweat gland, tissues of the different germ layers were involved, such as sweat gland cells come from the

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ectodermal origin and their actions regulated with neuroendocrine. The repair and regeneration of sweat gland are very complicated biological progress.

In recent years, we have been investigating the theory and technology of sweat-gland regeneration to help meet this need for sweat gland regeneration in burn patients. We have developed an innovative method to recover sweat function in transplanted skin. In addition, the innovative methods can be used to establish a new generation of tissue-engineered skin with sweat glands and treat some heritable skin diseases that lack the sweat function.

1 Dedifferentiation and Its Role in Stem Cells and Tissue Regeneration

Firstly, what are the theories of sweat gland regeneration? As we know that stem cells are seed cells for tissue repair and regeneration, however, all adult stem cells can not became the target cells directly and should go back to a "chick point", in which all of these stem cells have characteristics of "multiple functions", it is the dedifferentiation.

Dedifferentiation is an important biological phenomenon whereby cells regress from a specialized function to a simpler state reminiscent of stem cells. It occurs during wound repair and regeneration, determining the regeneration capacity of a specific species or a specific organ. It was confirmed that about 70 % of regenerative ability in damaged tissues in mammalian come from the dedifferentiation of cells in wounded sites [1–3]. For skin, epidermal stem cells (ESCs) belong to a regenerative cell type and play an important role in wound repair and tissue engineering of replacement skin [4]. After skin injury, dedifferentiation may be a prerequisite for epidermal cells to process regenerative proliferation and redifferentiation to repair the damage. About ten years ago, we compiled the advances we have acquired in the research on dedifferentiation of mature epidermal cells into stem cells or progenitor cells and its contribution to the regeneration of injured skin.

In 2001, we found that groups of cells positive for β1 integrin and keratin 19, the ESC markers, appeared in the spinous and granular layers between the basal layer and the stratum corneum in the biopsy samples from the healed chronic skin wounds in patients with traumatic leg ulcers treated with topical recombinant human epidermal growth factor (rhEGF). These stem-cell islands are a specific occurrence in wounds treated with rhEGF and not found in normal skin. After excluding other interventions, such as methodology error, we concluded that rhEGF serves as a dedifferentiation signal to induce cell reversion under the wound microenvironment and the formation of stem-cell islands involved in accelerating skin wound healing process. The questions which should be answered include that whether dedifferentiation could be observed in different conditions and different tissues? Are there is a direct relationship between dedifferentiation processes and sweat gland regeneration?

In successive researches, we collected more evidence to demonstrate the dedifferentiation of differentiating epidermal cells. First, we established stable and efficient dedifferentiation-inducing models in vivo and in vitro. In the animal model, human epidermal sheets eliminated of basal ESCs and transit amplifying (TA) cells were transplanted onto the skin wounds in mice with or without the topical application of rhEGF and basic fibroblast growth factor (bFGF). Examination of these transplanted skin grafts showed a significant number of cells positive for CK14, CK19 and β1 integrin, the ESC and TA cell markers, in the suprabasal layer, indicating that some of the differentiating cells in grafted epidermal sheets might have dedifferentiated into stem cells or stem cell–like cells. After isolated and cultured in vitro, these dedifferentiation-derived cells showed characteristics of ESCs, as evidenced by the morphology of small round shape with large nuclear-cytoplasmic ratio, the phenotype of positive expression for CK19, β1-integrin, Oct4 and Nanog, the functions of rapid adhesion to type IV collagen, high colony-forming efficiency, long-term proliferative potential and the redifferentiation capacity to regenerate a skin equivalent. Based on in vivo studies, it is concluded that dedifferentiation process could be achieved by introducing or remodeling a microenvironment composed of intrinsic and extrinsic cellular molecules. Given that growth factors, including FGF, EFG, and their receptors may play important parts in wound healing in impaired and unimpaired wounds, we further established efficient dedifferentiation induction model of epidermal cells in vitro by introducing injury stimulation or growth factors [5–7]. These attempts include such treatments as ultraviolet (UV), heat, oxidant injury and various growth factors. Based on results acquired, we found that the treatment of UV or bFGF was relatively stable for epidermal cells to dedifferentiate and the induction procedure was easily controllable [8]. The occurrence of dedifferentiation was confirmed by cell changes from five levels: (1) morphology (showing smaller cell size, less number of organelles and higher nuclear-cytoplasmic ratio), (2) phenotype (reexpression of ESC and TA cell markers and redistribution of α6-integrin and CD71), (3) proliferation ability (regaining the high colony-forming efficiency, marked cell-cycle progression, and enhanced telomerase activity), (4) redifferentiation capacity (reconstructing a well-formed epidermis with regular stratification), (5) gene expression profile (genes controlling cell adhesion and mitotic cell cycle were upregulated, but those controlling epidermal cell development and differentiation, as well as keratinization, were downregulated).

Second, we also reported the signaling pathways involved in the epidermal reversion and dedifferentiation process. One is Wnt/β-catenin signaling pathway. In animal model, we observed a significant increase in nuclear β-catenin accumulation and an elevated nuclear expression of β-catenin target genes, cyclin D1 and c-myc in the grafted epidermal sheets. When an inhibitor of Wnt/β-catenin pathway was applied to the wounds, the phenomenon of dedifferentiation disappeared, demonstrating that the β-catenin-dependent canonical pathway, but not the β-catenin-independent pathway, are responsible for epidermal cell dedifferentiation in vivo. In ex vivo model, activation of the Wnt pathway inhibits GSK-3β activity and induces stabilization of β-catenin, causing translocation of β-catenin to the

nuclei, where its association with T-cell factor/lymphocyte enhancer factor (Tcf/Lef) causes transcriptional activation of target genes. Accumulation of β-catenin and subsequent stimulation of Tcf/Lef transcriptional activity causes dedifferentiation of epidermal cells, as indicated by their changes in morphology, phenotype, and function. Furthermore, the study of *Smad4* knockout mice also showed that the indirect activation of β-catenin by disruption of *smad4* increased the number of CK14- and Ki67-positive TA cells, which were distributed widely in both basal and suprabasal layers, as compared with their distribution in the basal layer of normal epidermis. Another signaling pathway is extracellular signal-regulated kinase (ERK). In UV-induced dedifferentiation model, we determined all three subfamilies of mitogen-activated protein kinases (MAPKs), including ERK, p38, and c-Jun N-terminal kinase (JNK). During the process, dedifferentiation is strongly activated with the expression of phospho-ERK. Inhibition of ERK activities substantially abrogates dedifferentiation. In contrast, a p38 kinase inhibitor and the dominant negative mutant JNK1 have little effect on the UV induction of cells. These results suggest that the ERK-dependent signaling pathway is involved in the proliferation and dedifferentiation of differentiated epidermal cells in a stage-specific manner. Although several signal mechanisms have been found to play important roles in dedifferentiation, they are members of a regulatory network with multiple feedback loops [9, 10]. We believe that dedifferentiation and regeneration are more complicated than previously thought, and that numerous inducing factors and signal pathways coordinate mutually to promote these processes.

Finally, insights into mechanisms of epidemic dedifferentiation might arise some concerns of safety due to the involvement of several common molecular factors associated with carcinogenesis. Therefore, before the clinical application of dedifferentiation-derived ESCs, it is critical to fully evaluate the risk of carcinogenesis in the process of dedifferentiation. We translated ESCs induced by in vivo and in vitro dedifferentiation models into mice and found no xenograft during the whole observation period. In contrast, human melanoma cell bowes (HMCBs) as the positive control formed obvious xenograft at the sites of injection in short time. This result suggested that dedifferentiation-derived ESCs were not of tumorigenicity in vivo. It is widely accepted that chromosomal damage is one of the most important properties of tumor cells. Genetic instability can predispose to cancer by increasing the rate of potentially oncogenic mutations and chromosomal alterations. Remaining genetic intactness is very crucial during dedifferentiation. We, thereby, evaluate the genetic stability of all dedifferentiation-derived ESCs. Those induced by UV showed light double strands DNA damage, appearance of γH2AX foci, the activation of cell cycle checkpoint factors including p53 and p21, as well as apoptosis. It should be cautious when UV is used as an external factor to induce dedifferentiation, although the dose we adopted to induce dedifferentiation of epidermal cell is not enough to form tumors in vivo. In comparison, ESCs induced by bFGF showed no obvious DNA damage and any activation of related responding factors, which make it the most promising candidate method for dedifferentiation induction.

Taken together, the dedifferentiation process of epidermal cells have been systematically studied during the past decade. We first observed the phenomenon of dedifferentiation in repaired skins in wound patients. Then we introduced novel dedifferentiation-induced models with non-invasive methods in vivo and in vitro. We also explored the main signaling pathways which are incited to initiate the dedifferentiation process and subsequently terminated so that redifferentiation can take place. We further evaluate the tumorigenic risks of dedifferentiation-derived ESC-like cells and feel comfortable that they are safe to use. By doing this, abundant ESCs can be generated with an efficient dedifferentiation process involving manipulating the related pathways. When it comes to regeneration, however, the situation may not be so simple. To induce endogenous regeneration in the wound microenvironment, it may be necessary that all the genes and molecules controlling dedifferentiation, redifferentiation, and patterning be expressed at the appropriate levels in the correct temporal and spatial context. This is why mammals are not able to fulfill complete regeneration as their lower counterparts do. Stimulating and regulating regeneration-competent cells in situ is the most desirable way to restore damaged and diseased tissues. Therefore, future work is required to make clear more unidentified signaling molecules and pathways involved in the dedifferentiation, which and how the genes altered during the process, how to ensure benign changes, how to enhance the dedifferentiation efficiency, and whether dedifferentiated cells attain the competence to respond to the extrinsic factors to complete regeneration. If the above challenges can be resolved, humans may acquire the ability to replace lost, diseased or senile structures as newts do.

2 Dedifferentiation and Sweat Gland Regeneration

How to realize the sweat gland regeneration with key innovative techniques? We have established ten steps to realize the regeneration of sweat glands following our discovery in dedifferentiation study.

The first step was to understand the developmental characteristics of sweat glands in skin in the embryo and adult. Sweat gland development is a complicated biological process of 3 stages in the embryo beginning from 12 weeks to about 36 weeks. In the early stage, the basal cells gradually enlarge, and some aggregate to form cell clusters at about 12 weeks and then continue to form cell buds and epidermal ridges. Then, the buds migrate downward as cords into the dermis to form the anlagen of skin appendages. In the middle stage, sweat glands develop a coil-like structure and primitive intracutaneous ducts. In the last stage, they continue to mature and become vascularized.

Sweat-gland development is a synergetic development of stem cells, growth factors, and matrix metalloproteinases. Epidermal stem cells are the source of sweat glands in human fetal skin. Growth factors, cytokines, and components of the extracellular matrix are required for proliferation and differentiation of some stem and progenitor cells, including mature epidermal cells. Epidermal growth factors

may serve as autocrine or paracrine modulators of epidermal cells to form clusters of sweat gland cells. Matrix metalloproteinases help "pave the way" for sweat-gland bud-like formation from the epidermis to dermis.

Because the numbers of sweat glands are limited and adult skin lacks a natural and post-damage regeneration process, sweat-gland regeneration must be stimulated by exogenous stem cell therapy. In 2001, this capability was made possible by our discovery of cell dedifferentiation from epidermal cells to epidermal stem-like cells. We found and reported in *The Lancet* that epidermal cells could be dedifferentiated into epidermal stem cells in vivo and in vitro with growth-factor stimulation. Since then, we have evaluated the possibility of using epidermal stem cells, mesenchymal stem cells (MSCs) and adipose stem cells as seed cells for sweat-gland regeneration after severe burn and found MSCs the best choice. Then, we selected biomarkers to identify sweat-gland cells in vivo and in vitro. We found that carcinoembryonic antigen (CEA), β1-integrin, cytokeratin 19 (CK19), CK14, CK10 and CK7 can be used as markers to identify sweat glands in different development stages and different parts of the body. For example, we found CK7 expressed in the early and middle stages of sweat-gland development, but after gland maturation, it is expressed only in the secretory and not duct portion. CEA is expressed in the secretory and duct portions of sweat glands in the final stage of sweat-gland development [11–15].

From these works, we then successfully induced human bone-marrow MSCs (hMSCs) into sweat-gland–like cells (SGCs) with co-culture methods in vitro. The key steps in phenotype change from hMSCs into SGCs are heat-shock stimulation and temperature control. SGCs treated with 47 °C for about 40 min then cooled for 1–2 h at 37 °C was best for disrupting the intercellular junction with retraction of cytoplasm. The co-culture method produces about 37 % of MSCs with the SGC phenotype, a normal phenotype.

In our subsequent animal experiments, we labeled induced SGCs with BrdU for implantation onto paws of BALB/c nude mice with full-thickness scald injury. Iodine-starch perspiration test 2 weeks later confirmed a positive reaction in SGC-implanted wounds, and histological examination confirmed that perspiration cells came from the labeled SGCs (Fig. 1).

To follow up the successful cell and animal experiments, we began a clinical trial of burn patients. We obtained autologus bone-marrow MSCs from patients and co-cultured them as previously described for transplantation onto skin wounds; repaired skin showed regeneration of a sweat-gland-like structure. We then confirmed sweat-gland-like structures in biopsies of treated wounds, and these sweat-gland-like structures showed normal sweating function (Fig. 2). The pH and levels of biochemical components, such as Na^+, K^+, Cl^- and iCa^{2+}, in sweat obtained from the transplantation area were similar to that in normal sweat. To date, 23 patients with severe burn have undergone this innovative sweat-gland regeneration process, with satisfactory sweating results. The follow-up in some typical cases over 2 years has confirmed continuous sweating function (Fig. 2). Another interesting finding is that the scar formation in induced MSC-treated wounds is less than that in controls (Figs. 2 and 3) [16].

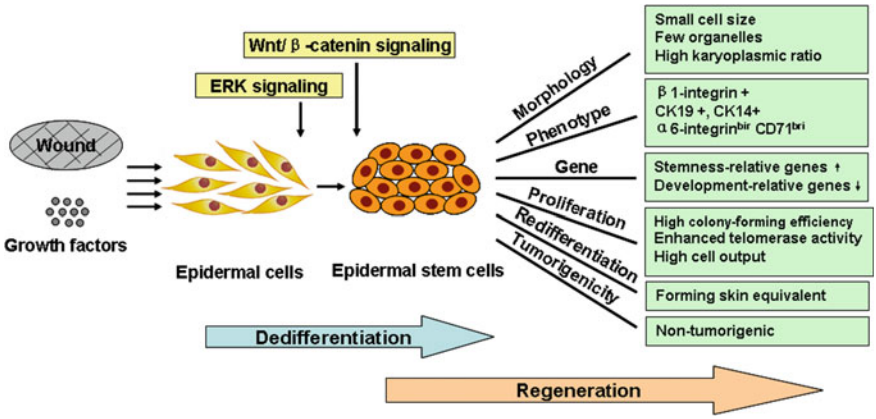

Fig. 1 Schematic illustration of dedifferentiation of epidermal cells. Differentiated or differentiating epidermal cells respond to internal or external signals by undergoing dedifferentiation. In this context, stimulation of wound or growth factors induce reentry into cell-cycle, proliferation, and redifferentiation. We postulate that Wnt/β-catenin pathway and ERK pathway underlie epithelial cell dedifferentiation and that the reversion is a crucial or even necessary step in the endogenous regeneration when endogenous stem cells are lost or exhausted

From these results, we are further studying whether these induced SGCs could be used as seed cells to establish a new generation of tissue-engineered skin with sweat glands. Because of the different pH and cell culture conditions needed for SGCs and tissue-engineered skin, a medium suitable for both is a key issue. Our preliminary studies confirmed that a new generation of tissue-engineered skin containing sweat glands could be established with this method.

Our studies are preliminary successes in sweat-gland regeneration and bring new hope for small-organ repair and regeneration through stem-cell dedifferentiation or transdifferentiation, but we still have a long way to go. Questions of mechanisms and clinical application still need to be answered. The first issue is whether the co-culture media and condition we used is the best niche for transdifferentiation of MSCs into SGCs. On histology, we found sweat-gland-like structures in biopsies from induced MSC-treated wounds, but the structures differed from the normal sweat-gland structures. Thus, whether these structures have the same perspiration mechanisms and function as normal sweat glands still needs to be studied. Another issue is whether our method can solve all perspiration anomalies in massive and deep skin burns. We observed perspiration results only in some small areas. As well, we wonder whether our innovative methods can be used for a new generation of tissue-engineered skin containing sweat glands. However, our preliminary work investigating engineering skin constructs with sweat glands provides new hope for burn patients.

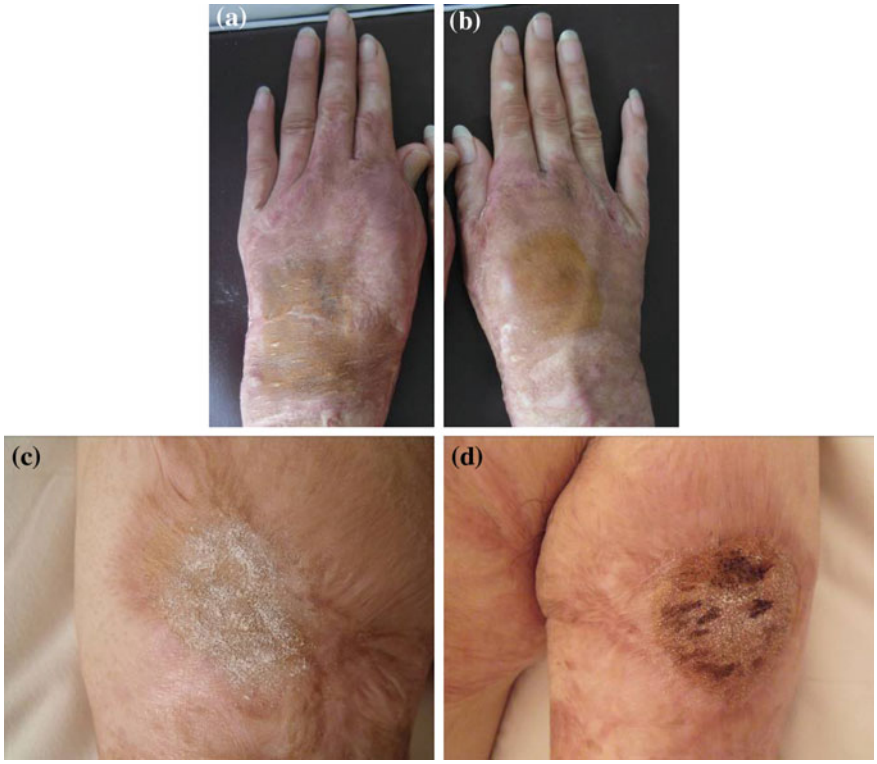

Fig. 2 One-year follow-up of a burn case treated with induced mesenchymal stem cells (MSCs) and its control. Iodine-starch perspiration test confirmed a positive reaction in wounds implanted with sweat-gland-like cells (**a**) and a negative reaction in the control (**b**). Two-year follow-up in a burn case treated with induced MSCs and its control. Iodine-starch perspiration test confirmed a negative reaction in the control (**c**) but a positive reaction in wounds implanted with sweat-gland-like cells (**d**)

3 Sweat Gland Regeneration and Its Translation Application: New Generation of Artificial Skins with Sweat Glands

Current available artificial skin products has offered great promise in the treatment of burns, donor sites, chronic skin ulcers (e.g., venous, pressure and diabetic foot ulcers), and various other dermatological conditions. Despite therapeutic efficacy of these skin substitutes shown in vivo, they have not yet replaced the current "gold standard" of an autologous skin graft and have even not achieved wide use in clinical treatment. This situation may be explained, at least in part, by the fact that no artificial skin product can completely replicate the anatomy, physiology or biological stability of nature skin. One of the main issues in structure and function

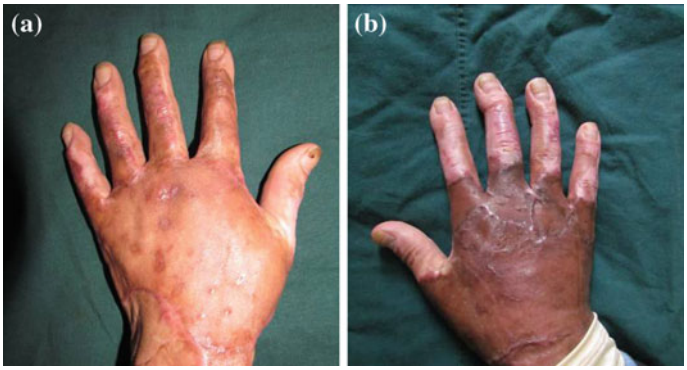

Fig. 3 Three-month follow-up in a burn case treated with induced MSCs. Scar formation in wounds treated with induced MSCs (**a**) is less than that in the control (**b**)

loss is the shortage of skin appendages. From a therapeutic standpoint, the presence of skin appendages is of major clinical importance for the maintenance of skin homeostasis and physiological function. For example, sweat glands is likely to play a key role in regulating body temperature, which may offer the hope to improve comfort level and living quality of survivors from severe skin injury.

The regenerative role of stem cells residing in skin appendages is multiple; such cells can participate in the repair and regeneration of injured skin. However, with extensive exploration on the road of regenerating defective skin tissues, hardly have we seen the structural and functional formation of sweat gland, which derives from epithelial compartment during development. Recent work of Reichmann proposed that human sweat glands were an alternative source of keratinocytes to generate a stratified epidermis. However, sweat gland-derived epithelial cells switched their phenotype to keratinocytes and there were no sweat gland in the ultimately formed epidermis. These observations may confer us with the clue that proper epithelial-mesenchymal interaction which exists throughout sweat gland development is essential for its morphogenesis and maintenance of epithelial homeostasis in the engineered models. Actually, from a tissue engineering point of view, this limitation can be overcome by the development of bioengineered 3D models that mimic nature skin microenvironment, in which epidermal cells are grown at an air–liquid interface on a connective tissue substrate harboring viable fibroblasts.

Although a number of tissue engineering-based therapies for research purposes and for clinical applications have been vigorously investigated, this strategy for restoration of functional sweat glands in renewal skin remains unmentioned. By multiple steps of tissue engineering operation, we propose that an artificial skin incorporating sweat glands can be constructed in vitro for improving the quality of skin repair and sweat gland regeneration during wound healing process (Fig. 4). To ensure sweat glands can be integrated, 3D human skin reconstruct is engineered to recapitulate natural sweat gland growth matrix. The reconstruct consists of a "dermis" with fibroblasts embedded in a collagen-based matrix, which provides

scaffolding, nutrient delivery, and potential for cell-to-cell interaction; an "epidermis", which is comprised of sweat gland cells and epidermal growth factor (EGF)-loaded microspheres, which represent as both a slow-release depot for growth factors and a delivery vehicle for sweat gland cells [17]. Naturally derived materials-based microsphere-technology has attracted growing interest as promising tools in cell transplantation and tissue engineering applications. By this method, trypsinization could be avoided and the cells could be transported to grow in a more natural state. Moreover, more potent and constant effect is demonstrated to come from the growth factors incorporated into microspheres, which would otherwise rapidly diffuse or be readily enzymatically digested or deactivated. Based on our previous founding, EGF exhibited the potential of stimulating sweat gland regeneration. This is in consistency with the report that postnatal application of EGF could induce the formation of functional sweat glands on the paws of the tabby mouse, a model for X-linked anhydrotic ectodermal dysplasia characterized by lack of sweat glands and other tissues of ectodermal origin [18–26].

Encouragingly, we demonstrated that engineered skin constructs integrated with bud-like sweat gland structures can be generated in vitro. As well, we found clear evidence that cells expressed markers of sweat gland cells in artificial skin models. We also found that the novel engineered skin construct enhanced the degree of wound healing. This supports the hypothesis that existence of sweat glands may help in the formation of a niche-like microenvironment for cell differentiation and stratification. Although the wounds treated with this animal model showed improved outcome, extrapolating these findings to the clinic is premature. Further

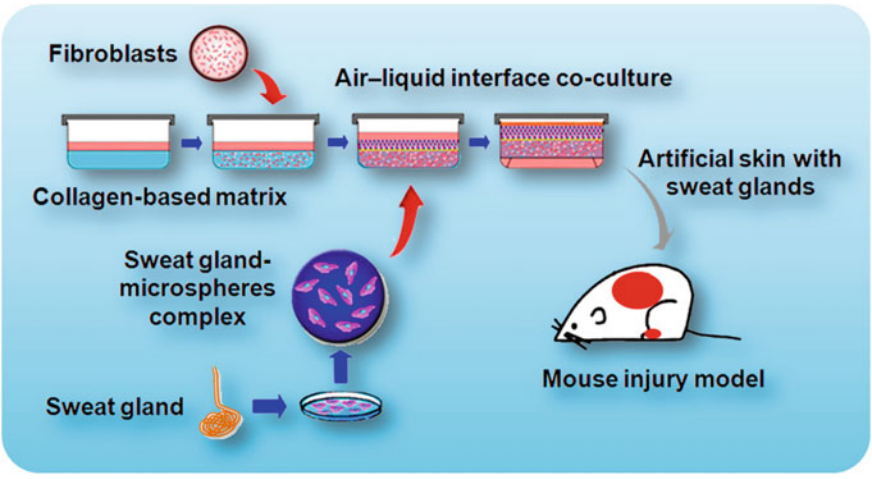

Fig. 4 Construction of artificial skin incorporating sweat glands. The reconstruct consists of a "dermis" with fibroblasts embedded in a collagen-based matrix and an "epidermis" with sweat gland cells and epidermal growth factor (EGF)-loaded microspheres complex. The efficacy of wound healing and sweat gland regeneration was examined by implanting the artificial skin into excisional wounds on both back and paws of hind legs in a murine model

work needs to be carried out to explore the mechanism underlying the beneficial effect of sweat glands in this model. Moreover, the interaction between sweat gland cells and other skin cells needs to be further ascertained.

Besides these unsolved issues, there are still many challenges remaining before this artificial skin can be translated to the clinic, such as a useful cell source. Actually, the remaining skin tissue was extremely limited after severe injury. Therefore, another strategy is to explore how stem cells can be candidate cells for artificial skin constitution that lead to regeneration of both skin tissue and sweat glands during skin repair. An example of this approach is our interest in bone-marrow–derived mesenchymal stem cells (BM-MSCs) application. Considerable evidence suggests that BM-MSCs have a strong propensity to ameliorate cutaneous damage in response to injury and are the most preferred cell types in clinical applications currently. Evidence from our laboratory and others also suggests that MSCs, particularly those derived from BM, may contribute significantly to promoting the repair and regeneration of skin by altering the tissue microenvironment and differentiating within injured tissue. However, stem cells also need to be delivered safely and effectively to a wound. Within an engineered skin model, they can remain in contact with the wound bed and be kept viable in the often-hostile wound microenvironment. To further investigate whether BM-MSCs can contribute to sweat gland repair via this novel tissue-engineering strategy, we engineered the same artificial skin model except for the cells application in vitro. In coupled with the advantages of artificial skin, engrafted BM-MSCs, or paracrine factors released by engrafted BM-MSCs in the wound may contribute to regenerating sweat gland-like structures in healed wounds. As well, this novel skin model could potentially serve as a feasible and effective delivery template for stem cells in regenerative skin applications. Ongoing study indicates that BM-MSCs delivered via an instructive microsphere-based engineered skin model has significant effects on enhancement of healing quality and sweat gland repair during the whole healing process. Furthermore, this model underscores the need to identify the mechanisms that govern cell fate in vivo as well as practical and relevant biomarkers that can be used to monitor the activity of MSCs after administration. From these observations, it is supposed that BM-MSCs and its related functions during wound healing are more complex than initially envisioned. Although the detailed mechanisms of specific cell-type differentiation from BM-MSCs still remain to be identified, this strategy has already shown prospective future in functional recovery of sweat gland in vivo. To better handle this potentially useful cell and provide promising, novel techniques of regenerative cell therapies and deeper understanding of BM-MSCs' complex functions is required.

While the ultimate goal is to build a functional artificial skin as healthy environment for sweat gland regeneration, these explorations are also indicative for the regeneration of many more spatially organized organ which can be reconstituted by means of tissue engineering methods [27–29]. Although the challenge of building a completely structural and functional tissue model is ostensibly insurmountable, researchers of multiple disciplines, including biologists, clinicians and engineers need to work together to deal with this challenge.

To date, various attempts have been made to improve the feasibility and applicability of regenerating sweat glands for its clinical importance. Notably, the emergence of bioprinting technology seems to provide a favorable tool of meeting the demands of tissue engineering and regenerative applications. In contrast to common cell culture and then transplantation, the designed 3D bioprinted extracellular matrix (ECM) for sweat gland regeneration upon enhancing specific differentiation of epidermal lineages offers a direct and effective strategy that may serve as a therapeutic tool for translational application. Theoretically, regeneration by cell differentiation is not unique for 3D printing technology. However, maintenance of high cell density and feasibility of in situ organized printing are its overwhelming advantages over other cell delivery approaches. Our latest study demonstrated that a high level of cell viability and proliferation during the printing process, which is further corroborated by the tissue-formation capabilities in the subsequent transplantation. This can be explained through the cushion and promoting-growth function of our printing structure. Further, transplantation of epidermal progenitors, which is typical of all multipotential cells, must rely on ECM for differentiation into a specific tissue. Our in vitro and in vivo data are encouraging confirmed the previous speculation of a gland lineage-inductive power from adult dermal components as embryonic tissue for regenerating sweat glands.

Using iodine/starch sweat test, we observed a near-complete loss of sweat function in the burned paws of mice. As expect, our studies show that the designed bioprinted 3D ECM could achieve functional regeneration or restoration of sweat glands [30–32]. No previous reports to our knowledge have shown such a fast functional regeneration of sweat glands, although some studies have shown somewhat healing by gene-correction during pregnancy or long-term repair. Combined with strong evidence of in vitro culture, these findings supporting that this instruction conveyed through bioprinted 3D ECM facilitates the directed differentiation of epithelial progenitors to sweat gland lineages. Although further work is required to dissect the molecular mechanisms, it seems likely that bioprinted 3D ECM is an attractive option that opens up new avenues for tissue reconstruction. Furthermore, this reproducible 3D ECM may offer the insights into the broad translational implications in the regeneration of other tissues by recreating the representative microenvironmental characteristics that provide convincingly and accurately inductive niche.

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