Current Osteoporosis Reports (2018) 16:155–168
https://doi.org/10.1007/s11914-018-0432-1

ORTHOPEDIC MANAGEMENT OF FRACTURES (S BUKATA AND L GERSTENFELD, SECTION EDITORS)

Clinical and Research Approaches to Treat Non-union Fracture

Claudia Schlundt[1,2] · Christian H. Bucher[1,2] · Serafeim Tsitsilonis[1,2] · Hanna Schell[1] · Georg N. Duda[1,2] · Katharina Schmidt-Bleek[1,2]

Published online: 13 March 2018

Abstract

Purpose of Review Impaired healing outcomes or even non-unions after bone injury are still a highly relevant problem in the daily clinical life. Especially within an aging population, the occurrence of bone fractures increases and thus novel treatment approaches to overcome compromised bone regeneration are needed.

Recent Findings The gold standard to treat delayed or non-healing bone injuries is still the use of autologous bone grafts to foster regeneration. Besides its successful treatment outcome, it also has disadvantages: a second surgery is needed in order to harvest the bone material and the material is highly limited. Looking into the recent literature, a multitude of different research approaches were already conducted to identify new possible strategies to treat impaired bone regeneration: application of mesenchymal stromal cells, platelet lysates, growth factors, interference in the immune system, or bone formation stimulation by ultrasound.

Summary This review gives an overview of the treatment approaches actually performed in the clinic as well as at the bench in the context of compromised bone healing. It clearly highlights the complexity of the nature of non-healing bone fractures as well as patient-dependent factors influencing the healing process.

Keywords Bone fracture healing · Non-union · Compromised healing · Autologous bone graft · Cell therapy · Immune therapy

Introduction

The repair process after bone injury requires the participation of several cell and tissue types to achieve a successful healing outcome. Bony tissue represents an impressive biomaterial due to its ability to completely regenerate under normal healing conditions. Despite this efficacy, still up to 10–15% of the fracture patients show an impaired healing process, leading to a delayed healing outcome or even to a non-union [1]. This is not only a burden for the patient's life due to additional surgical treatment and hospitalization time but also for the socio-economic and health care systems [2]. Hak and colleagues reported in 2014 that a treatment of an established non-union of long bones costs over $10,000 (average costs: Canada $11,800, USA $11,333, and UK £29,204) [3].

The process of bone fracture healing can be divided into five distinct but overlapping phases: hematoma formation with an accompanying inflammation (which is separated into a pro-inflammation [1] and anti-inflammation [2]), soft callus formation [3], hard callus formation [4], and remodeling [5]. A hematoma is formed in the fracture area due to the blood influx after vessel disruption [4, 5]. Cells of the innate immune system are one of the first cells infiltrating the fracture area [6–8]. Based on their secretion profile, they create a pro-inflammatory state, which induces the recruitment of cells of the adaptive immunity and mesenchymal stromal cells (MSCs). For the progression of the repair process, the pro-inflammation has to switch to an anti-inflammation. The

No benefit of any kind has been or will be received either directly or indirectly by the authors

This article is part of the Topical Collection on *Orthopedic Management of Fractures*

✉ Georg N. Duda
georg.duda@charite.de

[1] Julius Wolff Institut and Center for Musculoskeletal Surgery, Charité-Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany

[2] Berlin-Brandenburg Center for Regenerative Therapies, Charité-Universitätsmedizin Berlin, Berlin, Germany

switch to the anti-inflammatory state initiates the revascularization of the fracture area, which is another prerequisite for a successful healing outcome [9••]. In the next step, fibrocartilage tissue refills the fracture area and a soft callus is formed leading to a first stabilization of the injury site. The cartilaginous tissue matures, becomes hypertrophic, and starts to mineralize. An external hard callus is build composed of newly formed woven bone, which replaces the hypertrophic chondrocytes. The last step is the remodeling of the fracture area [10]. In humans, this remodeling process can last up to several years, depending on the general condition of the patient and on the fracture type/location.

The question is: When is an impaired healing fracture called a non-union? There is still no standard definition how to define in general a non-union. Among others, it depends on the site of injury. Based on the definition of the US Food and Drug Administration (FDA) for long bones like the femur, a non-union is present when the fracture is not healed within the first 9 months after injury and showed no signs of healing progression for at least 3 months [11].

The definition of non-union is not simple or satisfying implying that also the diagnosis of a non-union is difficult. This is due to the fact that not two non-union cases are alike as causes are multi-factorial. The affected bone has to be considered, the type of injury, the bone quality, the soft tissue cover, the patient with its habits and comorbidities, and the environmental factors. This not only hinders clear treatment guidelines but also research into non-union to gain a better understanding. To overcome these difficulties, a non-union scoring system (NUSS) has evolved similar to the Injury Severity Score or the scoring system to grade joint disease. Early scoring systems relied solely on radiographic assessments; however, this was proven to be insufficiently reliable—being compared with tossing a coin [12]. The NUSS exceeds the radiographic evaluation to also consider the bone quality, the history of the fracture as being open or closed, the clinical interventions up to the current point, the soft tissue state, and uses the American Society of Anesthesiologists (ASA)-grade system for patients to include the individual patient constitution. The score for the individual patient situation would provide the surgeon with a treatment guideline. Low scores would receive a standard treatment of refreshment and fixation revision, medium low scores would require a more specialized treatment including a supplementation with osteoinductive factors (autologous spongiosa or growth factors) while fixation is revised. The higher score would indicate that a specialized treatment in form of a viable bone graft or segment transport is necessary in addition to applying osteoinductive factors (autologous spongiosa or growth factors) with a specialized fixation. The highest score would indicate that an amputation has to be considered a final solution. The NUSS (Table 1) has been proposed in 2008 [13], giving a guideline for the classification of non-union fractures and indicators for the clinical treatment but lacking in validation at that time. Bastenberg et al. recently evaluated the scoring system, confirming that the NUSS score led to a high agreement in classifying non-union fractures between observers [14•]. Earlier performed evaluation studies in 2011 [15] and 2014 [16] also support the validity of the NUSS system to evaluate non-union fractures.

Reasons for Non-union

The underlying causes for the occurrence of fracture non-unions are various and depend among others on the mechanics (e.g., site of injury), associated concomitants (e.g., infection), patient-dependent factors (e.g., age, lifestyle, chronic diseases), and the type and severity of the fracture itself.

Treatment in cases of a non-union often includes an optimization of the fracture stabilization (Fig. 1). Movement of the bone fragments exceeding a certain window will lead to an arrest of the healing (Fig. 2). However, a too rigid fixation will also affect the healing negatively. The mechanical stimulus to form bone and to repair a fracture would be missing and the bone-forming process would cease. This phenomenon led to an intensive study of the optimal fixation stiffness and technique to treat broken bones [17–25]. In addition, the concept of using a mobilization of the fractured bone during the healing cascade has been extensively investigated [26–29]. So far, no defined mobilization treatment strategy has reached the clinic, however, and early weight bearing is favored more and more in lieu of prolonged bed rest and immobilization of the affected limb.

Non-unions, arrests in the fracture repair process, can be classified as:

- Septic non-unions (bacterial infection impeding the healing)
- Pseudoarthrosis (atrophic, non-viable bone ends forming an artificial joint capsule)
- Hypertrophic non-union (viable bone ends, indicating problems with the fixation rather than the biology)
- Atrophic non-union (dysvascular bone ends, indicating problems with the biology)
- Oligotrophic non-union (an intermediate of the above)

While in most of these cases the first step is an optimization of the fixation, the most critical distinction is the viability of the bony ends of the fracture. If the bone ends are still viable then the biology to heal the fracture is still available to aid the healing process and once stabilization is regained, healing will proceed. However, if the bone ends are no longer viable, changing fixation will not gain a progression in healing—in these cases, the bone ends have to be removed. The best prognosis can be given for those cases, where the bone has closed the bone marrow

Table 1 Non-union scoring system (NUSS) is first proposed by Calori et al. in 2008

NUSS adapted from Calori et al. [13]			
The bone			
Quality of bone	Good	0	
	Moderate	1	For example, mild osteoporosis
	Poor	2	For example, severe osteoporosis or bone loss
	Very poor	3	Necrotic, avascular, septic
Primary injury	Closed	0	
	Open 1° grade	1	
	Open 2–3° A grade	3	
	Open 3° B–C grade	5	
Number of previous interventions	None	1	
	< 2	2	
	< 4	3	
	> 4	4	
Invasiveness of previous interventions	Minimally invasive	0	Osteosynthesis which includes bone grafting
	Internal intramedullary	1	Nailing
	Internal extramedullary	2	Plate
	Osteosynthesis which includes bone grafting	3	Ilizarov
Adequacy of previous intervention	Inadequate stability	0	
	Adequate stability	1	
Weber & Cech group	Hypertrophic	1	
	Oligothrophic	3	
	Atrophic	5	
Bone alignment	Non-anatomic alignment	0	
	Anatomic alignment	1	
Bone defect	0.5–1 cm	2	
	1–3 cm	3	
	> 3 cm	5	
Soft tissue			
Status	Intact	0	
	Minor scarring	2	Uneventful surgery
	Soft tissue defect—previous treatment	3	Skin loss, local flap cover, compartment syndrome
	Soft tissue defect—complex previous treatment	3	Free flap
	Poor vascularity	5	No distal pulses, poor capillary refill, venous insufficiency
	Presence of actual skin lesion	6	Ulcer, sinus, exposed bone
The patient			
ASA grade	1 or 2	0	
	3 or 4	1	
Diabetes	No	0	
	Yes—well controlled	1	HbA1c < 10
	Yes—poorly controlled	2	HbA1c > 10
Blood tests	FBC: WCC > 12	1	
	ESR > 20	1	
	CRP > 20	1	
Infection status	Clean	0	
	Previously infected or suspicion of infection	1	
	Septic	4	
Drugs	Steroids	1	
	NSAIDs	1	
Smoking	No	0	
	Yes	5	

This scoring system includes the Weber-Cech classification, but besides the X-ray, evaluation takes also into account several risk and patient-related factors. The scores are added up, multiplied by two, thus resulting in a score between 0 and 100. Scores of 0–25 will receive standard treatments, 26–50 require specialized care, 51–75 need specialized care and specialized treatment, and scores above 75 require the consideration of an amputation as a treatment option. Factors are weighed according to their importance for the classification

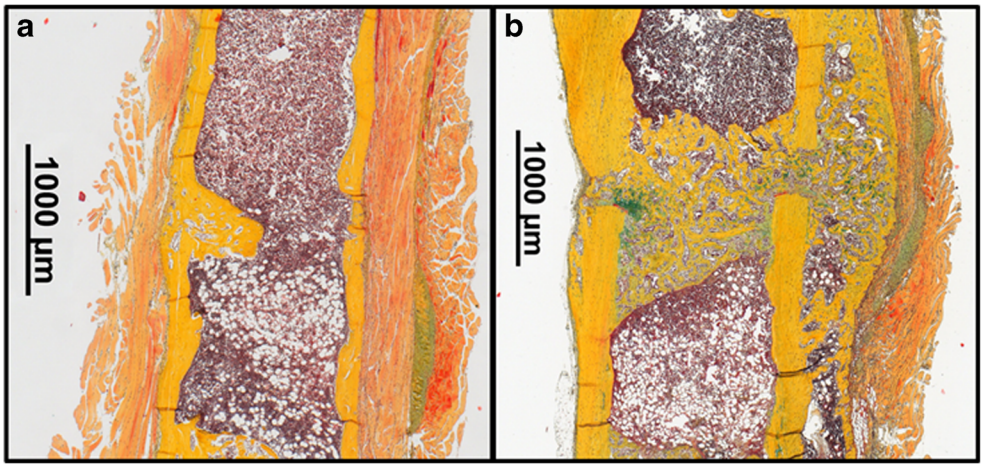

Fig. 1 Non-critical osteotomy gap in the femur of BL6 female 12-week-old mice were fixated with an external fixation (RISystem) with a rigid (**a**) and a semi-rigid (**b**) stabilities. Twenty-one days after osteotomy, the healing outcome was measured histologically (Movat Pentachrome staining: bone-yellow, cartilage-green, bone marrow-dark red, muscle-orange). The optimal fixation stability (**a**) allowed complete bridging and progression of remodeling while the unstable fixation (**b**) led to a larger callus formation to compensate for the missing stability thus leading to a delayed healing were at the 21-day time point bridging has occurred; however, the bone marrow cavity is still closed and remodeling has not yet succeeded. While the lack of stability in this case leads to a delayed healing, the bone has been able to overcome the lack by forming a larger callus and compensate for the non-optimized stabilization

cavity in an attempt to "heal" the bone (Fig. 3). The bone has an active healing capacity but has not been able to overcome the gap between the bone fragments—narrowing the gap between the bone ends after reopening the bone marrow cavity will enable a bridging.

The most common reason for an insufficient biology in case of occurring non-union in bone is the lack in the revascularization and thus of angiogenesis in the fracture area. During the fracture healing cascade, this process occurs twice: first at the very beginning of the healing, after the injury

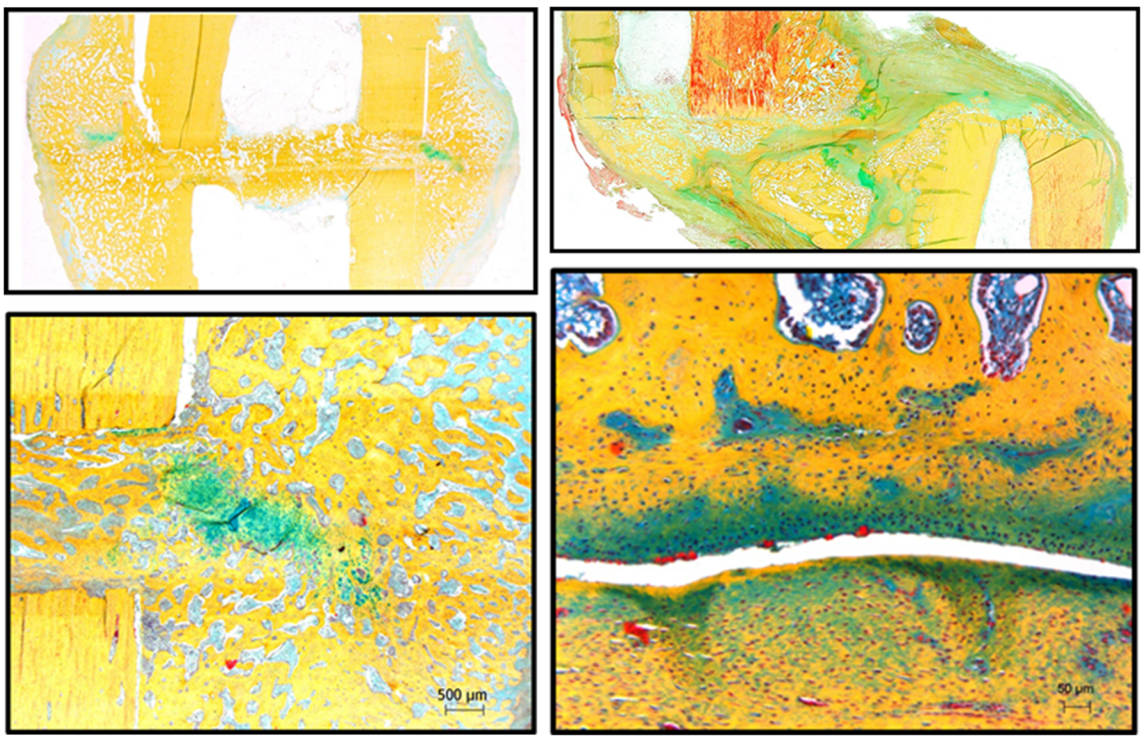

Fig. 2 In a sheep tibia osteotomy model, a 3-mm gap was stabilized with a rigid external fixator (left) or with a rotationally instable fixator (right) (Movat Pentachrome histology). In this case, the mechanical instability was so high that healing was not possible (visible in the displaced bone ends on the right upper image). While under stable fixation at day 42 post-surgery, a woven bone callus bridged the gap (left lower panel), rotational instability caused the formation of a pseudo joint (right lower panel)—thus a non-union ensued

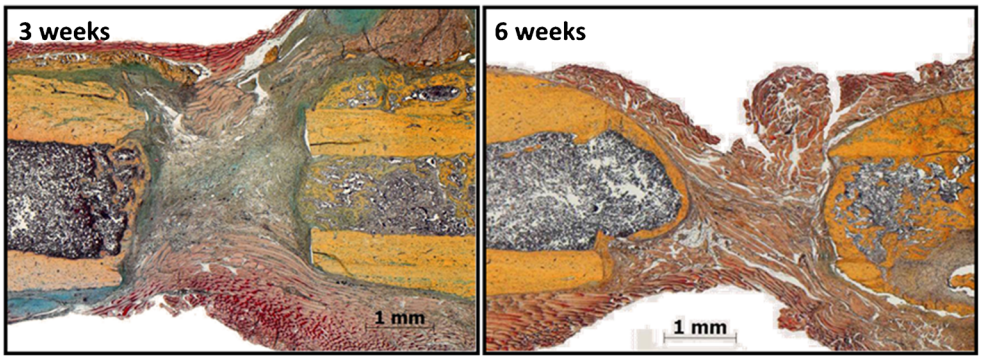

Fig. 3 Healing in a critical-sized defect (5 mm) in a rat femur osteotomy model stabilized with an external fixation (custom made) has been analyzed histologically (Movat Pentachrome staining) after 3 and 6 weeks, respectively. Already at the 3-week time point, the closing of the bone marrow cavity at the bone ends is detectable. While the attempt to close the gap is visible in the cone formation at the left bone end in the 6-week sample, healing clearly has stopped and a non-union has formed. To enable healing, the bone cavities would have to be reopened and the bone fragments moved closer together to allow contact

disrupts the blood vessels and a hematoma is formed, the hematoma matures to an organized granulation tissue were the newly formed blood vessels reestablish the adequate supply of the fracture area. Angiogenic signaling during this phase is closely coupled with the inflammatory reaction, and both processes are interlinked [30]. An upregulation of the angiogenic signaling cascade occurs during the undisturbed healing when the first initial pro-inflammatory reaction abates. Therefore, the timely termination of the pro-inflammatory cascade is a key element of successful bone healing [9••, 31]. A second revascularization step is needed upon the transformation of the cartilage-dominated avascular callus towards the mineralized woven bone callus [32, 33] (Fig. 4). Through these newly formed vessels, cells important for the remodeling of the callus infiltrate.

Non-unions with a lack in the biology, showing a disturbed revascularization require additional treatment to enhance the biological healing capacity of the bone to achieve a bony bridging and thus a functional bone structure. To date, several treatment options are available.

Treatment Strategies

A bone injury prone for non-union problems is the fracture of the lower extremity. For Germany, over 220,000 cases were reported for the year 2015 (Statistisches Bundesamt 19 Nov 2017) with an about equal distribution of male and female patients. About 90,000 patients were released from the clinic within 3 days. The German statistic Department listed between 13,400 and 14,800 cases of non-unions of fracture ends per year between 2010 and 2015 (Statistisches Bundesamt 19 Nov 2017) (Fig. 5).

Current clinical treatment options for a non-union that affects the lower extremity are reported here with an example of a patient who suffered from a III° open fracture of the lower leg with a resulting bone defect (Fig. 6a). The bone was

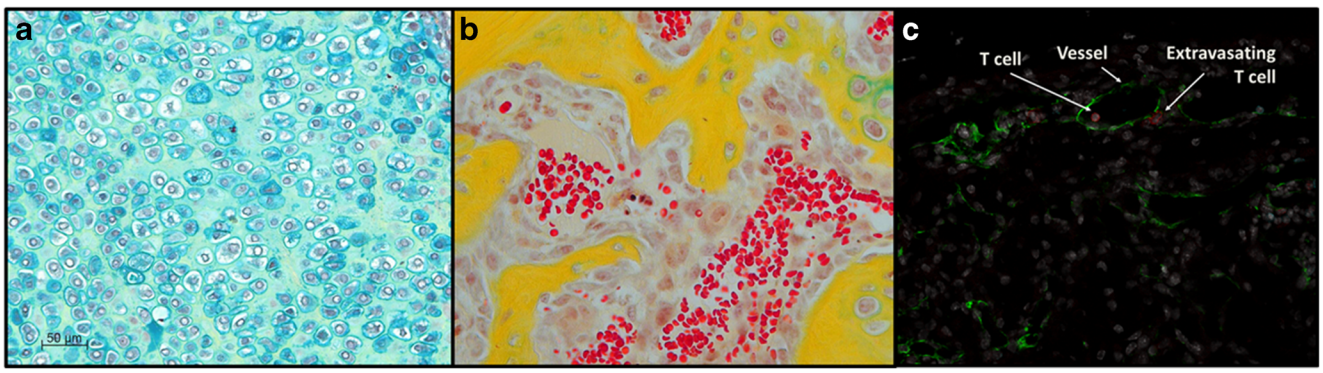

Fig. 4 Endochondral ossification in a mouse fracture model was evaluated histologically 14 days after three point bending fracture and internal fixation with an intramedullary nail. Hyaline cartilage transformed to hypertrophic cartilage (**a**) occurring blue green in the Movats Pentachrome staining. Cartilage consist of one cell type only, chondrocytes, and there are no blood vessels apparent. Consecutive formation towards woven bone proceeds with a revascularization step. Woven bone is highly vascularized (**b**). While bone appears yellow, bright red erythrocytes filling the newly formed vessel structure are clearly visible within the bone marrow interspersing the bony columns. Immunohistological staining of the vessels (**c**) (laminin staining, green) and T cells (CD3, red) shows that immune cells infiltrate the callus via the newly formed vessels at the border of cartilage and woven bone (cell nuclei, white)

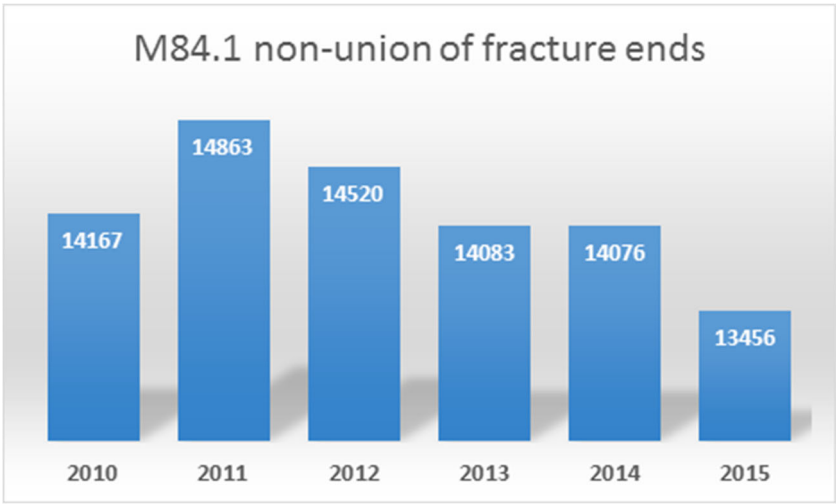

Fig. 5 ICA classification M84.1 lists cases of non-union of fracture ends; numbers refer to patients treated in German hospitals from 2010 to 2015. These numbers were released by the Statistisches Bundesamt, 19 Nov 2017

stabilized with an external fixator executing the Ilizarov principle that allows to transport a bone segment (Fig. 6b). To bridge the critical bone defect at the distal end of the tibia shaft, an osteotomy was performed mid-tibial. The bone fragment thus created was then translocated distally. To enhance the healing, the ensuing gap was filled with autologous bone graft supplemented with bone morphogenetic protein 7. To account for the missing soft tissue coverage of the fractured bone, a muscle flap was performed. The gap constructed by the segment transport mineralized successfully; however, the docking site developed an infection and in consequence a non-union (Fig. 6c). During surgery, the infected bone was

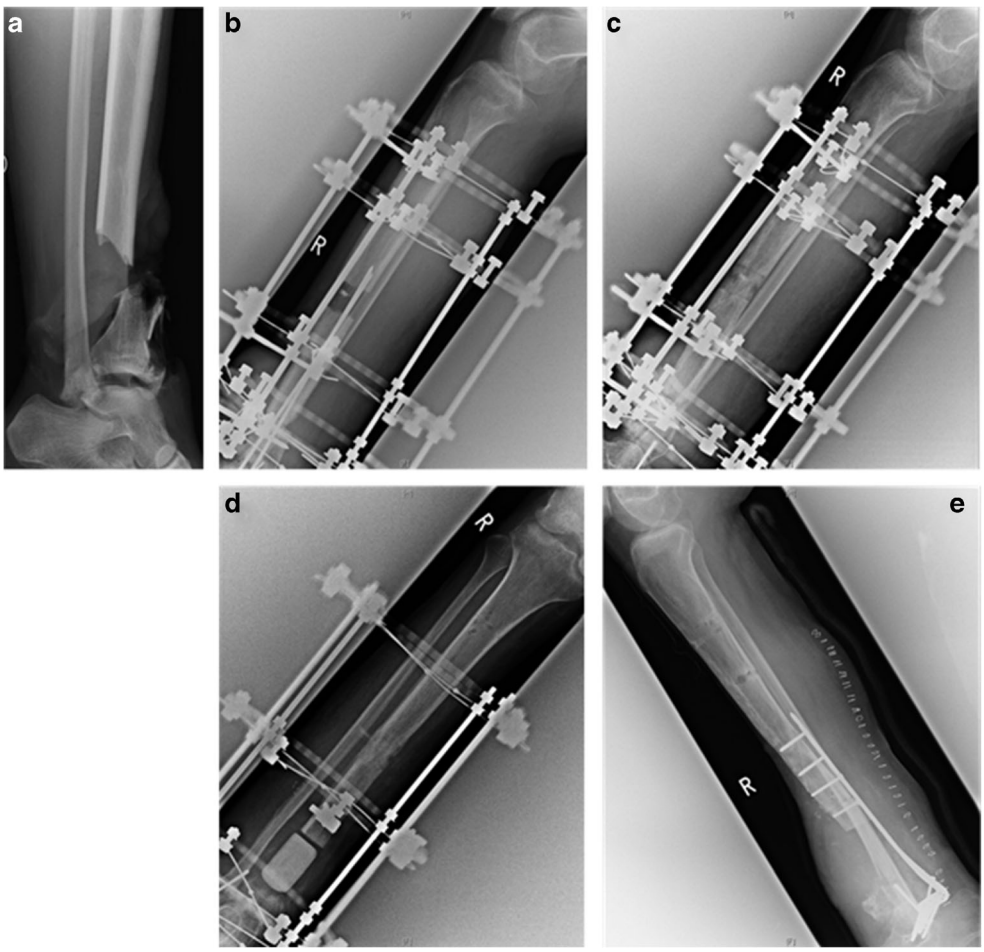

Fig. 6 A critical-sized bone defect in the lower leg ensued in a non-union. Upon treatment of the defect, **a** several techniques were used to reach a satisfactory healing outcome. This included a segmental transport (**b**), augmentation with autologous spongiosa and growth factor bone morphogenetic protein 7 (**c**), debridement after infection and a cement spacer application (**d**), and the transplantation of the vascularized fibular segment (**e**)

Table 2 Summary of the diagnostic, classification, risk factors, clinical treatment options, and future innovative treatment approaches of impaired or even non-healing fractures

Non-union fracture	A fracture that has no potential to heal without further intervention
Diagnostic	9-month elapsed time without healing progress for 3-month radiologically assessed
	Non-union scoring system after Calori et al.
	Persistent pain, swelling, and lack function
	Computer tomography in case of comminuted fractures with multiple bone fragments
	Magnet resonance imaging to determine soft tissue interference in the bone gap
	Stress radiography to gain a status on the fixation stability
Classification	Septic non-union
	Hypertrophic non-union (callus forms, "elephant's foot" = abundant callus or "horse's foot" = less abundant callus, vascularized, lack in fixation stability)
	Oligotrophic non-union (minimal callus = not completely void of biologic activity)
	Atrophic non-union (no callus formation—impaired vascularization/metabolic causes)
	Pseudoarthrosis (adequate vascularity, excessive motion/instability, formation of a false joint)
Risk factors	Instability (lack in fracture fixation)
	Infection (open fracture, osteomyelitis)
	Poor vascularity (multiple causes)
	Comorbidities (age, hyperparathyroidism, diabetes, neurofibromatosis 1, osteoporosis, paraplegia, etc.)
	Fractures with low soft tissue coverage, high-energy fractures, extensive soft tissue damage, open fractures, considerable bone loss, comminuted fractures
	Habitual risk factors (smoking, chronic alcoholism, obesity, etc.)
	Drugs (NSAIDs, steroids, chemotherapy, bisphosphonates)
	Malnutrition (vitamin D insufficiency, inadequate protein and energy supply)
Clinical treatment options	Principle: cure infection, debridement if necessary, correct deformity, provide stability, add biological stimulus
	Biology − good + stability − lacking: provide stability, correct deformity, no bone graft required
	Biology − poor + stability − lacking: provide stability, bone graft
	Biology − lacking + stability − lacking: provide stability, bone graft, other reconstruction
	Biological stimuli: autologous spongiosa, autologous bone graft, bone marrow aspirate, allograft bone, demineralized bone matrix, vital bone graft, growth factors (platelet derived, recombinant BMPs)
	Significant bone loss: segment transport
	Non-surgical treatments: electromagnetic stimulus, ultrasound, extracorporal shock wave therapy
	Soft tissue damage: muscle flap
Future innovative treatment approaches	Immunomodulation
	Potential prognostic biomarker to identify patients with impaired fracture healing: level of circulating CD8+ TEMRA cells
	- Ongoing studies:
	1. Multicenter trial to evaluate CD8+ TEMRA cells as a biomarker of impaired fracture healing
	2. Pharmocological blocking of CD8+ TEMRA cells to improve bone regeneration
	Stimulation of potential favorable immune cells in bone repair (i.e., CD4+ Treg)
	Mechanotherapy
	Loading vs. unloading of the fractured limb → appropriate loading to foster bone regeneration
	Appropriate choice of the right material for implantation to stabilize the fractured bone → optimal support by the implant of the load onto the fractured bone to improve the regeneration process

removed and a new gap was thus created. This gap was filled with a cement spacer loaded with antibiotics to eradicate the infection (Fig. 6d). While treating the infection, this method also created a fibrous sleeve resembling an artificial periosteum spanning the gap section. The Masquelet technique was therefore applied when the cement spacer was removed during the next surgical procedure. Due to the size of the defect, a vascularized fibular bone segment was implanted within the bone defect and the defect was again treated with autologous spongiosa that was inserted into the Masquelet membrane (Fig. 6e). At this stage, the Ilizarov fixator was removed and exchanged against a plate osteosynthesis.

This case combines a multitude of available treatment options due to the complication that occurred over the healing period. The critical-sized bone defect offered additional problems that allow the presentation of the available treatment strategies within one case report. The critical-sized defect however occurs during non-union treatment due to the

debridement necessary in the case of avital or infected bone ends and therefore should be considered within this context.

This case shows the necessity of a change in the fixation of a non-union patient, albeit at a late time point. The external fixation system is exchanged for a plate osteosynthesis method. Changing the fixation is often necessary if difficulties in the bone healing process occur and is often the first step to counter healing difficulties.

The application of osteoinductive autologous spongiosa is still considered the gold standard to treat non-unions, gaining the best results. In this case the spongiosa was additionally enhanced by adding a growth factor, bone morphogentic protein 7 (BMP7). BMP2 and BMP7 are the only growth factors that gained approval for very specific non-union treatments. As they proved very successful in enhancing the bone formation, off-label use often occurs within the clinic in problematic bone healing cases [34, 35].

Tibial fractures often result in a lack of soft tissue coverage of the fracture area. In cases of a missing soft tissue coverage healing is delayed, mostly because of an impaired revascularization step [36–38]. Even today, the optimal time point for the muscle flap application in open fractures is controversial [39] mostly because of the high probability of infection due to the open-fracture scenario.

In the here-presented case, the non-union occurred due to an infection at the docking side after the successful bone segment transfer. In case of an infection, a thorough debridement is a necessity [40, 41] together with a thorough antibiotic treatment. Such an antibiotic treatment is often applied with a cement spacer in the orthopedic setting [42]. Ceramic biocomposites might offer an alternative that is biodegradable. Newly formed bone following the degradation after eradication of the infection could obliterate the second surgical intervention needed to remove the cement spacer [43].

In this case, however, the cement spacer was used to produce an artificial periosteum as described for the Masquelet technique [44]. The Masquelet technique represents a two-stage surgical treatment often used to treat patients with a severe open fracture [45]. It is independent of the size of the bone defect which is filled with an antibiotic impregnated cement spacer to induce a so-called periosteal membrane. Even though this membrane is equivalent to a fibrous capsule, the positive healing results support the reference to a periosteal membrane. During the second surgical intervention, a longitudinal incision is made through the periosteal membrane and the spacer is removed. After freshening the bone ends, the hollowed periosteal cavity is filled with autologous bone graft.

In the case of the here-presented patient, the filling of the periosteal cavity was further enhanced by transplanting a vascularized fibular bone segment into the gap [46]. A technique first reported in 1975, reported to have a 95% success rate, and used to enhance bone healing in bones from head to toe [47].

This clinical case demonstrated the multitude of treatment options currently available to treat non-unions; however, they all are time intensive and require a high compliance from the patient and a surgical specialist. Therefore, further improvement of the non-union treatment is desirable and several research approached are being investigated to date Table 2.

Treatment Strategies: Research Approaches

A multitude of studies was already conducted in order to elucidate a biological biomarker that determines a non-healing fracture when the injured patient is coming into the clinic. The finding of such a suitable biomarker would enable the development of appropriate treatment strategies for non-healing conditions at the time of the initial fracture stabilization procedure. Due to the complexity of the patient-related and non-related risk factors for a non-union, several research studies using biological approaches have been initiated and evaluated for their potential to improve bone healing. These will be considered in the following paragraphs.

Mesenchymal Stromal Cells

The best approach would be the use of the patient's own biological material to foster bone regeneration. MSCs are the precursors of bone-forming osteoblasts and thereby represent a potential cell population to improve bone regeneration under compromised healing conditions. Results obtained from several animal studies already confirmed the potential use of MSCs to enhance bone formation in general [48–52]. Intravenously injected MSCs were able to reach the site of injury, already implicating an attraction of bone-forming precursor cells to the fracture gap. Dreger et al. evaluated the competence of CD127-MSCs in a murine unilateral closed femur fracture model [49]. They showed that the time point of MSC application is critical for the success of the treatment outcome. One study analyzed the impact of MSCs in a non-union mouse model [50]. Expanded murine MSCs were used and an intravenous injection 24 h post-fracture led to improved bone formation thus restoring the impaired healing characteristics. In the human situation, the question arises where to get the MSCs from (bone marrow vs. adipose tissue derived MSCs) and how many MSCs would be needed to overcome impaired healing. Hernigou and colleagues analyzed the needed number of progenitor cells in human autologous bone marrow grafts for successful treatment of non-unions [53]. Bone marrow aspirates with > 1500 progenitors/cm^3, corresponding to a total average number of 60,000 progenitor cells, were needed in order to overcome non-unions. However, in this study, not only osteoblast progenitor cells were present in the aspirates but also other, mononuclear cells, further supporting bone regeneration.

Bone Morphogenetic Protein

Bone morphogenetic proteins (BMPs) are a group of growth factors belonging to the TGF-β superfamily. BMPs are characterized by the stimulation of bone and cartilage tissue formation [54, 55]. Thus, BMPs are potent agents to foster bone regeneration under compromised conditions. In 2001, the FDA approved BMP7 for the treatment of long bone non-unions. One year later, in 2002, the FDA approved BMP2 for the use in tibia shaft fractures. Until today, there is no approval of BMP2 to treat non-unions. Both, BMP2 and BMP7, are the most reported BMP members for the treatment of bone injuries.

Govender et al. reported the positive impact of BMP2 in a prospective study with 420 patients having an open tibial fracture [56]. The patient group receiving the higher BMP2 dosage (total dosage of 12 mg) at the time point of trauma management showed significantly more healed fractures 12 months post-operative as well as less infection and hardware failure with regard to the control group. In contrast, Aro et al. published a higher infection rate in BMP2-treated patients with an open tibial fracture in comparison with the control group [57]. In addition, the authors did not find a significantly increased healing rate with BMP2. In the context of non-unions, Friedlaender et al. compared the outcome of BMP7 usage in a collagen type I carrier to the gold standard usage of autologous bone graft [58]. Both treatment strategies showed comparable healing rates (81% (BMP7) vs. 85% (bone graft)). This is in accordance with a report published by Desmyter and colleagues in 2008, reporting the healing rate of non-unions in Belgium after the use of BMP7 [59]. Giannoudis reported in 2009 a 100% healing rate of 45 patients with aseptic atrophic non-unions after the treatment of BMP7 together with bone autograft [60]. A case study including 175 patients compared the usage of BMP2 vs. BMP7 in 214 limb segment non-unions [61]. At an overall result, the BMP2-treated group displayed a better healing outcome (higher rate of radiographic healing and faster weight bearing) with regard to the BMP7-treated group. The BMP2 group showed a lower complication rate, although this was not statistically significant. Although a multitude of studies showed a positive impact of BMP2 and BMP7 in treating bone fractures and non-unions, also several unwanted side effects are reported for BMP2 in non-spinal bone applications [62]. This included local infections, wound complications, and heterotopic bone formation. However, the authors also stated that the reported observed side effects of BMP2 usage vary depending on the type and location of the fracture and the performed surgical procedure. The nature of fracture non-unions is very diverse and complex. The function and success after BMP treatment in bone healing is still under debate due to the consistently seen and reported site effects. Thus, using BMP for the treatment of bony non-unions is a risky option and can lead to more unwanted complications in the fracture treatment and thus to even higher costs and more pain and hospitalization time for the patient. In addition, BMP7 is no longer available for a clinical application has it has been withdrawn from the market [63].

Vascular Endothelial Growth Factor

Another possibility to foster bone regeneration is to stimulate angiogenesis, thus the rebuilding of the vessel network in the fracture area needed, i.e., sufficient nutrition supply [64]. The vascular endothelial growth factor (VEGF) is one of the main proteins stimulating vessel formation.

Animal studies already reported a positive effect of VEGF application in the treatment of bony non-unions [65, 66]. Although the experimental setup was different (VEGF application directly during setting the fracture [65] vs. in an already established non-union [66]), both studies showed improved healing outcome in comparison with the non-treated animal groups. Garcia and colleagues analyzed vascularization pattern in a non-union rat model and observed an even higher revascularization in the fracture zone of non-union animals in comparison with the normal healing group [67]. However, they found a decreased expression of pro-osteogenetic factors BMP2 and BMP4. Thus, the authors concluded that the ratio of expressed pro-angiogenetic and pro-osteogenetic factors could determin non-union formation. One study compared the application of BMP2, VEGF, and platelet-derived growth factor (PDGF) in an atrophic non-union model in rats [68]. Kaipel et al. used a silicon spacer for 4 weeks in order to impair the revascularization of the fracture area. After removing the spacer, growth factors were applied and included into a fibrin clot. The control group only received a fibrin clot. The PDGF and VEGF groups failed to increase bone formation over the observation duration. Whereas, the BMP group displayed increased bone regeneration. The time point of application could explain the non-functional treatment of the pro-angiogenetic factors. Both, PDGF and VEGF, mainly interact in the acute early fracture healing phase by promoting angiogenesis which is a pre-requisite for bone formation. However, Kaipel and colleagues applied these factors 4 weeks after the setting of the fracture and further impaired the healing cascade due to the silicon spacer. Therefore, the administration of PDGF and VEGF could have at least partial anti-osteogenetic effects when used in the later healing phases. This result is even more important for a potential clinical application.

Platelet-Rich Plasma

Another biological material stimulating angiogenesis is platelet-rich plasma. Activated platelets are known for the secretion of growth factors stimulating bone regeneration

[69]. One of these growth factors is PDGF. For PDGF, it was already shown that it acts on human osteoblasts and stimulates their proliferation [70]. In the context of non-unions, Labibzadeh et al. and Centeno et al., respectively, reported two small case studies with human fracture patients with long bone non-unions [71]. In these studies, the combination of platelet lysate and autologous MSC application for non-union treatment was analyzed. At least for the study reported by Labibzadeh, all analyzed patients tolerated the treatment approach of the cell-lysate complex well. In four out of seven (Labibzadeh) and four out of six (Centeno), respectively, it even led to a bridging of the non-union gap after 12/6+ months (Labibzadeh/Centeno). For both studies, one explanation for the non-responder could be the long duration of the non-union from 16 months to several years. Although both studies used different platelet lysates (autologous (Labibzadeh) vs. allogenic (Centeno)) and different expansion time and implanted passage of cultured MSCs, both studies demonstrated the potential and feasibility of using both, platelet lysate and autologous, culture expanded MSCs, in combination to treat non-unions. However, the used MSCs were isolated out of bone marrow aspirate obtained from the iliac crest. This means a second surgical intervention with a potential risk of an infection and further pain for the patient. In 2016, a one-patient study reported the sole use of autologous platelet lysate to treat a non-healing tibia and fibula fracture that failed the initial stabilization by an internal metal plate [72]. Injection of autologous platelet lysate led to bony union after 8 months post-injection without any signs of discomfort for the patient or infections or other complications. Based on the reported findings in the literature, autologous platelet lysate represents a potential option treating bone defects without any reported negative side effects so far.

Low-Intensity Pulsed Ultrasound

Low-intensity pulsed ultrasound (LIPUS) is reported as a possible non-invasive technique to stimulate bone formation (company site: http://www.exogen.com/). This method is based on the emission of ultrasound waves through the skin to the fracture site. These waves will then stimulate the activity of cells and thus foster bone regeneration. In vitro analysis already showed that the application of low-intensity ultrasound stimulates periosteal cells as well as MSCs to proliferate and to differentiate into the osteogenetic lineage [73, 74]. Furthermore, it was already shown that LIPUS enhances the migration of MSCs to the fracture site. However, this was not significant in comparison with the control group. Zura et al. reported a case study including 767 patients with established non-unions from more than 1 to 10 years. LIPUS-treated patients showed a healing rate of 86.2%. This is in accordance with published findings from other studies, stating an increased healing rate of delayed healing or non-union fractures [75–78]. Although there exist a multitude of reported cases showing the positive outcome after LIPUS treatment, one important disadvantage becomes obvious after having a closer look into the patient selection of the studies. Often, complicated delayed or non-healing cases were not included into the study design, thus falsifying the real existing patient cohort suffering from impaired bone healing. Furthermore, a control group receiving standard surgical procedures does not accompany the follow-up of LIPUS-treated patients. Thus, the outcome-healing rate after LIPUS application cannot be compared relative to standard treatment approaches for non-unions. However, the National Institute for Health and Care Excellence (NICE) in the UK published a cost saving of around $1726 per patient when using LIPUS instead of surgical intervention to treat non-union [79]. LIPUS seems to be a promising and potential alternative to treat non-invasively impaired healing fractures. However, clinical trials including a broader patient cohort and well-defined control groups are still missing and have to be initiated and done in order to correctly judge and interpret the observed putative beneficial results after LIPUS treatment.

Future Innovative Treatment Approaches Conducted in our Institute

Immunotherapy

The immune system plays a key role in bone regeneration [33]. The interdisciplinary research field “osteoimmunology” combines both research areas, the bone and the immune system [80]. The bone and the immune system share a multitude of factors and molecules and thus are interdependent on each other, meaning, intervention in the one system will also influence the other one. Therefore, immune therapy represents another promising research approach to overcome impaired healing. Terminally differentiated CD8+ effector memory T cells (T_{EMRA}), a subpopulation of the T cell compartment of the adaptive immunity, could be a promising biomarker determining impaired healing fractures. We already showed a positive correlation between a higher amount of pro-inflammatory CD8+ T_{EMRA} with a poorer healing outcome after closed tibia head fracture [81••]. This has been accounted to a prolonged pro-inflammatory state in the fracture area due to the higher CD8+ T_{EMRA} population. CD4+ regulatory T cells (Treg), another subset of the adaptive immunity, could be one possible counterpart to CD8+ T_{EMRA} cells. An in vivo study in a murine caldaria defect model demonstrated the pro-osteogenetic effect of Treg application together with bone marrow MSCs. We also analyzed the effect of Treg in the treatment of a fracture in long bones. Our results support the positive modulating impact of Treg in bone regeneration, whereas this was dependent on the status of the adaptive immune system of the recipient (manuscript in preparation). Our

observation further highlights the sensitive and complex interplay between the bone and the immune system in the context of immune therapy. Besides the adaptive immunity, also macrophages as part of the innate immunity play a crucial role in bone regeneration. Stimulating macrophages in the "alternative" M2 lineage at the time point of fracture by the administration of IL-4 and IL-13 led to improved healing outcome with regard to the control group in a murine osteotomy model [82].

Immune therapy in bone-related diseases comes more and more into the clinical focus. However, we are still at the beginning to understand the mechanism how immune cells interact in the bone systems and vice versa, patient-based immunotherapeutical approaches are a very promising treatment approach to determine and overcome impaired healing outcome.

Mechanotherapy

Another direction seeing reasonable considerations are approaches using mechanobiological cues to induce regeneration and considered to enable "mechanotherapy" in musculoskeletal healing [83]. With the help of patient data, pre-clinical animal models and computational models, patient-specific mechanical constrains can be modulated at bone defects, fracture zones, or joints [84]. Such mechanobiologically optimized conditions or the active mechanical stimulation allow to enable endogenous regenerative cascades to overcome impaired bone regeneration.

Conclusion

Looking for actually registered clinical trials corresponding to the keyword "non-union" in the US and EU clinical trial registers (www.clinicaltrials.gov and www.clinicaltrialsregister.eu) revealed 124 (US) and 8 (EU) hits, respectively. Among the listed clinical trials, 15 (US) and 4 (EU), respectively, evaluate the impact of autologous stem cells, mostly MSCs, in bony non-unions. A few studies apply for low-intensity ultrasound or electromagnetic field stimulation to foster bone healing (7/124). Only six trials use BMP2 (2/124), BMP7 (1/124; 1/8), or platelet lysates (2/124). The other listed clinical trials deal with (autologous) bone grafts, different fixation, and screw types, the effect of vitamin supply and fluid lavage or reaming-irrigator aspirates (RIA). The wide and diverse range of the at the-moment registered clinical trials concerning non-unions highlights the complexity of the nature of a non-union and therefore the multitude of treatment options. It further shows even more the importance of ongoing research in order to (1) understand the underlying pathomechanisms leading to the formation of a non-union and to (2) develop appropriate treatment strategies to improve the patient's life and simultaneously to decrease the burden for the socio-economic system.

Funding Information Funding was provided by the DFG FOR2165 and the Berlin-Brandenburg School for Regenerative Therapies.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
• of importance
•• of major importance

1. Haas NP. [Callus modulation—fiction or reality?]. Der Chirurg. Zeitschrift fur alle Gebiete der operativen Medizen. 2000;71(9):987–8.
2. Zeckey C, Mommsen P, Andruszkow H, Macke C, Frink M, Stubig T, et al. The aseptic femoral and tibial shaft non-union in healthy patients—an analysis of the health-related quality of life and the socioeconomic outcome. Open Orthop J. 2011;5:193–7.
3. Hak DJ, Fitzpatrick D, Bishop JA, Marsh JL, Tilp S, Schnettler R, et al. Delayed union and nonunions: epidemiology, clinical issues, and financial aspects. Injury. 2014;45(Suppl 2):S3–7.
4. Schell H, Duda GN, Peters A, Tsitsilonis S, Johnson KA, Schmidt-Bleek K. The haematoma and its role in bone healing. J Exp Orthop. 2017;4(1):5.
5. Kolar P, Schmidt-Bleek K, Schell H, Gaber T, Toben D, Schmidmaier G, et al. The early fracture hematoma and its potential role in fracture healing. Tissue Eng Part B Rev. 2010;16(4):427–34.
6. Opal SM. Phylogenetic and functional relationships between coagulation and the innate immune response. Crit Care Med. 2000;28(9 Suppl):S77–80.
7. Hoff P, Maschmeyer P, Gaber T, Schutze T, Raue T, Schmidt-Bleek K, et al. Human immune cells' behavior and survival under bioenergetically restricted conditions in an in vitro fracture hematoma model. Cell Mol Immunol. 2013;10(2):151–8.
8. Gaber T, Haupl T, Sandig G, Tykwinska K, Fangradt M, Tschirschmann M, et al. Adaptation of human CD4+ T cells to pathophysiological hypoxia: a transcriptome analysis. J Rheumatol. 2009;36(12):2655–69.
9.•• Schmidt-Bleek K, Schell H, Lienau J, Schulz N, Hoff P, Pfaff M, et al. Initial immune reaction and angiogenesis in bone healing. J Tissue Eng Regen Med. 2014;8(2):120–30. **This study provided the proof of the strong interdependency of the switch to the anti-inflammatory phase and the starting revascularization, which is a prerequisite for a successful healing outcome, in the early fracture healing phase.**
10. Schmidt-Bleek K, Petersen A, Dienelt A, Schwarz C, Duda GN. Initiation and early control of tissue regeneration—bone healing as a model system for tissue regeneration. Expert Opin Biol Ther. 2014;14(2):247–59.

11. Bishop JA, Palanca AA, Bellino MJ, Lowenberg DW. Assessment of compromised fracture healing. J Am Acad Orthop Surg. 2012;20(5):273–82.
12. Bhattacharyya T, Bouchard KA, Phadke A, Meigs JB, Kassarjian A, Salamipour H. The accuracy of computed tomography for the diagnosis of tibial nonunion. J Bone Joint Surg Am. 2006;88(4):692–7.
13. Calori GM, Phillips M, Jeetle S, Tagliabue L, Giannoudis PV. Classification of non-union: need for a new scoring system? Injury. 2008;39(Suppl 2):S59–63.
14.• van Basten BM, Houben IB, Blokhuis TJ. The non-union scoring system: an interobserver reliability study. Eur J Trauma Emerg Surg. 2017; **This study evaluated and confirmed the reliability of the NUSS score (introduced by Calori and colleagues) to determine the severity category of a fracture and the definitive treatment**
15. Abumunaser LA, Al-Sayyad MJ. Evaluation of the Calori et al nonunion scoring system in a retrospective case series. Orthopedics. 2011;34(5):359.
16. Calori GM, Colombo M, Mazza EL, Mazzola S, Malagoli E, Marelli N, et al. Validation of the non-union scoring system in 300 long bone non-unions. Injury. 2014;45(Suppl 6):S93–7.
17. Huber E, Pobloth AM, Bormann N, Kolarczik N, Schmidt-Bleek K, Schell H, et al. DBM as a carrier for BMP-2: burst release combined with long term binding and osteoinductive activity evaluated in vitro and in vivo. Tissue Eng Part A. 2017;23:1321–30.
18. Manjubala I, Liu Y, Epari DR, Roschger P, Schell H, Fratzl P, et al. Spatial and temporal variations of mechanical properties and mineral content of the external callus during bone healing. Bone. 2009;45(2):185–92.
19. Schell H, Thompson MS, Bail HJ, Hoffmann JE, Schill A, Duda GN, et al. Mechanical induction of critically delayed bone healing in sheep: radiological and biomechanical results. J Biomech. 2008;41(14):3066–72.
20. Epari DR, Kassi JP, Schell H, Duda GN. Timely fracture-healing requires optimization of axial fixation stability. J Bone Joint Surg Am. 2007;89(7):1575–85.
21. Trepczik B, Lienau J, Schell H, Epari DR, Thompson MS, Hoffmann JE, et al. Endochondral ossification in vitro is influenced by mechanical bending. Bone. 2007;40(3):597–603.
22. Epari DR, Schell H, Bail HJ, Duda GN. Instability prolongs the chondral phase during bone healing in sheep. Bone. 2006;38(6):864–70.
23. Lienau J, Schell H, Duda GN, Seebeck P, Muchow S, Bail HJ. Initial vascularization and tissue differentiation are influenced by fixation stability. J Orthop Res. 2005;23(3):639–45.
24. Schell H, Epari DR, Kassi JP, Bragulla H, Bail HJ, Duda GN. The course of bone healing is influenced by the initial shear fixation stability. J Orthop Res. 2005;23(5):1022–8.
25. Kaspar K, Schell H, Seebeck P, Thompson MS, Schutz M, Haas NP, et al. Angle stable locking reduces interfragmentary movements and promotes healing after unreamed nailing. Study of a displaced osteotomy model in sheep tibiae. J Bone Joint Surg Am. 2005;87(9):2028–37.
26. Willie BM, Blakytny R, Glockelmann M, Ignatius A, Claes L. Temporal variation in fixation stiffness affects healing by differential cartilage formation in a rat osteotomy model. Clin Orthop Relat Res. 2011;469(11):3094–101.
27. Claes L, Blakytny R, Besse J, Bausewein C, Ignatius A, Willie B. Late dynamization by reduced fixation stiffness enhances fracture healing in a rat femoral osteotomy model. J Orthop Trauma. 2011;25(3):169–74.
28. Claes L, Blakytny R, Gockelmann M, Schoen M, Ignatius A, Willie B. Early dynamization by reduced fixation stiffness does not improve fracture healing in a rat femoral osteotomy model. J Orthop Res. 2009;27(1):22–7.
29. Bartnikowski N, Claes LE, Koval L, Glatt V, Bindl R, Steck R, et al. Modulation of fixation stiffness from flexible to stiff in a rat model of bone healing. Acta Orthop. 2017;88(2):217–22.
30. Schmidt-Bleek K, Kwee BJ, Mooney DJ, Duda GN. Boon and bane of inflammation in bone tissue regeneration and its link with angiogenesis. Tissue Eng Part B Rev. 2015;
31. Lienau J, Schmidt-Bleek K, Peters A, Haschke F, Duda GN, Perka C, et al. Differential regulation of blood vessel formation between standard and delayed bone healing. J Orthop Res. 2009;27(9):1133–40.
32. Konnecke I, Serra A, El Khassawna T, Schlundt C, Schell H, Hauser A, et al. T and B cells participate in bone repair by infiltrating the fracture callus in a two-wave fashion. Bone. 2014;64:155–65.
33. Schlundt C, Schell H, Goodman SB, Vunjak-Novakovic G, Duda GN, Schmidt-Bleek K. Immune modulation as therapeutic strategy in bone regeneration. J Exp Orthop. 2015;2:1–10.
34. Poon B, Kha T, Tran S, Dass CR. Bone morphogenetic protein-2 and bone therapy: successes and pitfalls. J Pharm Pharmacol. 2016;68(2):139–47.
35. Ratko TA, Belinson SE, Samson DJ, Bonnell C, Ziegler KM, Aronson N. Bone morphogenetic protein: the state of the evidence of on-label and off-label use. Rockville (MD): AHRQ Technology Assessments; 2010.
36. Gopal S, Giannoudis PV, Murray A, Matthews SJ, Smith RM. The functional outcome of severe, open tibial fractures managed with early fixation and flap coverage. J Bone Joint Surg Br. 2004;86(6):861–7.
37. Francel TJ, Vander Kolk CA, Hoopes JE, Manson PN, Yaremchuk MJ. Microvascular soft-tissue transplantation for reconstruction of acute open tibial fractures: timing of coverage and long-term functional results. Plast Reconstr Surg. 1992;89(3):478–87. **discussion 88-9**
38. Cierny G 3rd, Byrd HS, Jones RE. Primary versus delayed soft tissue coverage for severe open tibial fractures. A comparison of results. Clin Orthop Relat Res. 1983;178:54–63.
39. Wood T, Sameem M, Avram R, Bhandari M, Petrisor B. A systematic review of early versus delayed wound closure in patients with open fractures requiring flap coverage. J Trauma Acute Care Surg. 2012;72(4):1078–85.
40. Dym H, Zeidan J. Microbiology of acute and chronic osteomyelitis and antibiotic treatment. Dent Clin N Am. 2017;61(2):271–82.
41. Taj-Aldeen SJ, Gamaletsou MN, Rammaert B, Sipsas NV, Zeller V, Roilides E, et al. Bone and joint infections caused by mucormycetes: a challenging osteoarticular mycosis of the twenty-first century. Med Mycol. 2017;55(7):691–704.
42. Haddad S, Corona PS, Reverte MM, Amat C, Flores X. Antibiotic-impregnated cement spacer as a definitive treatment for post-arthroscopy shoulder destructive osteomyelitis: case report and review of literature. Strat Trauma Limb Reconstr. 2013;8(3):199–205.
43. Ferguson J, Diefenbeck M, McNally M. Ceramic biocomposites as biodegradable antibiotic carriers in the treatment of bone infections. J Bone Jt Infect. 2017;2(1):38–51.
44. Saxer F, Eckardt H. Reconstruction of osseous defects using the Masquelet technique. Der Orthopade. 2017;46(8):665–72.
45. Chadayammuri V, Hake M, Mauffrey C. Innovative strategies for the management of long bone infection: a review of the Masquelet technique. Patient Saf Surg. 2015;9:32.
46. Qi Y, Sun HT, Fan YG, Li FM, Lin ZS. Do stress fractures induce hypertrophy of the grafted fibula? A report of three cases received free vascularized fibular graft treatment for tibial defects. Chin J Traumatol. 2016;19(3):179–81.
47. Taylor GI, Corlett RJ, Ashton MW. The evolution of free vascularized bone transfer: a 40-year experience. Plast Reconstr Surg. 2016;137(4):1292–305.
48. Cheung WH, Chin WC, Wei FY, Li G, Leung KS. Applications of exogenous mesenchymal stem cells and low intensity pulsed

ultrasound enhance fracture healing in rat model. Ultrasound Med Biol. 2013;39(1):117–25.
49. Dreger T, Watson JT, Akers W, Molligan J, Achilefu S, Schon LC, et al. Intravenous application of CD271-selected mesenchymal stem cells during fracture healing. J Orthop Trauma. 2014;28(Suppl 1): S15–9.
50. Obermeyer TS, Yonick D, Lauing K, Stock SR, Nauer R, Strotman P, et al. Mesenchymal stem cells facilitate fracture repair in an alcohol-induced impaired healing model. J Orthop Trauma. 2012;26(12):712–8.
51. Qi Y, Zhao T, Yan W, Xu K, Shi Z, Wang J. Mesenchymal stem cell sheet transplantation combined with locally released simvastatin enhances bone formation in a rat tibia osteotomy model. Cytotherapy. 2013;15(1):44–56.
52. Xue G, He M, Zhao J, Chen Y, Tian Y, Zhao B, et al. Intravenous umbilical cord mesenchymal stem cell infusion for the treatment of combined malnutrition nonunion of the humerus and radial nerve injury. Regen Med. 2011;6(6):733–41.
53. Hernigou P, Poignard A, Beaujean F, Rouard H. Percutaneous autologous bone-marrow grafting for nonunions. Influence of the number and concentration of progenitor cells. J Bone Joint Surg Am Vol. 2005;87(7):1430–7.
54. Chen D, Zhao M, Mundy GR. Bone morphogenetic proteins. Growth Factors. 2004;22(4):233–41.
55. Urist MR. Bone: formation by autoinduction. Science. 1965;150(3698):893–9.
56. Govender S, Csimma C, Genant HK, Valentin-Opran A, Amit Y, Arbel R, et al. Recombinant human bone morphogenetic protein-2 for treatment of open tibial fractures: a prospective, controlled, randomized study of four hundred and fifty patients. J Bone Joint Surg Am Vol. 2002;84-A(12):2123–34.
57. Aro HT, Govender S, Patel AD, Hernigou P, Perera de Gregorio A, Popescu GI, et al. Recombinant human bone morphogenetic protein-2: a randomized trial in open tibial fractures treated with reamed nail fixation. J Bone Joint Surg Am Vol. 2011;93(9):801–8.
58. Friedlaender GE, Perry CR, Cole JD, Cook SD, Cierny G, Muschler GF, et al. Osteogenic protein-1 (bone morphogenetic protein-7) in the treatment of tibial nonunions. J Bone Joint Surg Am Vol. 2001;83-A(Suppl 1(Pt 2)):S151–8.
59. Desmyter S, Goubau Y, Benahmed N, de Wever A, Verdonk R. The role of bone morphogenetic protein-7 (osteogenic protein-1) in the treatment of tibial fracture non-unions. An overview of the use in Belgium. Acta Orthop Belg. 2008;74(4):534–7.
60. Giannoudis PV, Kanakaris NK, Dimitriou R, Gill I, Kolimarala V, Montgomery RJ. The synergistic effect of autograft and BMP-7 in the treatment of atrophic nonunions. Clin Orthop Relat Res. 2009;467(12):3239–48.
61. Conway JD, Shabtai L, Bauernschub A, Specht SC. BMP-7 versus BMP-2 for the treatment of long bone nonunion. Orthopedics. 2014;37(12):e1049–57.
62. Woo EJ. Adverse events after recombinant human BMP2 in nonspinal orthopaedic procedures. Clin Orthop Relat Res. 2013;471(5):1707–11.
63. Sreekumar V, Aspera-Werz RH, Tendulkar G, Reumann MK, Freude T, Breitkopf-Heinlein K, et al. BMP9 a possible alternative drug for the recently withdrawn BMP7? New perspectives for (re-)implementation by personalized medicine. Arch Toxicol. 2017;91(3):1353–66.
64. Hankenson KD, Dishowitz M, Gray C, Schenker M. Angiogenesis in bone regeneration. Injury. 2011;42(6):556–61.
65. Eckardt H, Ding M, Lind M, Hansen ES, Christensen KS, Hvid I. Recombinant human vascular endothelial growth factor enhances bone healing in an experimental nonunion model. J Bone Joint Surg Br Vol. 2005;87(10):1434–8.
66. Ogilvie CM, Lu C, Marcucio R, Lee M, Thompson Z, Hu D, et al. Vascular endothelial growth factor improves bone repair in a murine nonunion model. Iowa Orthop J. 2012;32:90–4.
67. Garcia P, Pieruschka A, Klein M, Tami A, Histing T, Holstein JH, et al. Temporal and spatial vascularization patterns of unions and nonunions: role of vascular endothelial growth factor and bone morphogenetic proteins. J Bone Joint Surg Am Vol. 2012;94(1): 49–58.
68. Kaipel M, Schutzenberger S, Schultz A, Ferguson J, Slezak P, Morton TJ, et al. BMP-2 but not VEGF or PDGF in fibrin matrix supports bone healing in a delayed-union rat model. J Orthop Res. 2012;30(10):1563–9.
69. Oprea WE, Karp JM, Hosseini MM, Davies JE. Effect of platelet releasate on bone cell migration and recruitment in vitro. J Craniofacial Surg. 2003;14(3):292–300.
70. Graves DT, Valentin-Opran A, Delgado R, Valente AJ, Mundy G, Piche J. The potential role of platelet-derived growth factor as an autocrine or paracrine factor for human bone cells. Connect Tissue Res. 1989;23(2–3):209–18.
71. Labibzadeh N, Emadedin M, Fazeli R, Mohseni F, Hosseini SE, Moghadasali R, et al. Mesenchymal stromal cells implantation in combination with platelet lysate product is safe for reconstruction of human long bone nonunion. Cell J. 2016;18(3):302–9.
72. Jiang HJ, Tan XX, Ju HY, Su JP, Yan W, Song XG, et al. Autologous platelet lysates local injections for treatment of tibia non-union with breakage of the nickelclad: a case report. SpringerPlus. 2016;5(1):2013.
73. Kusuyama J, Bandow K, Shamoto M, Kakimoto K, Ohnishi T, Matsuguchi T. Low intensity pulsed ultrasound (LIPUS) influences the multilineage differentiation of mesenchymal stem and progenitor cell lines through ROCK-cot/Tpl2-MEK-ERK signaling pathway. J Biol Chem. 2014;289(15):10330–44.
74. Leung KS, Cheung WH, Zhang C, Lee KM, Lo HK. Low intensity pulsed ultrasound stimulates osteogenic activity of human periosteal cells. Clin Orthop Relat Res. 2004;418:253–9.
75. Gebauer D, Mayr E, Orthner E, Ryaby JP. Low-intensity pulsed ultrasound: effects on nonunions. Ultrasound Med Biol. 2005;31(10):1391–402.
76. Jingushi S, Mizuno K, Matsushita T, Itoman M. Low-intensity pulsed ultrasound treatment for postoperative delayed union or nonunion of long bone fractures. J Orthop Sci. 2007;12(1):35–41.
77. Mayr E, Frankel V, Ruter A. Ultrasound–an alternative healing method for nonunions? Arch Orthop Trauma Surg. 2000;120(1–2):1–8.
78. Schofer MD, Block JE, Aigner J, Schmelz A. Improved healing response in delayed unions of the tibia with low-intensity pulsed ultrasound: results of a randomized sham-controlled trial. BMC Musculoskelet Dis. 2010;11:229.
79. Higgins A, Glover M, Yang Y, Bayliss S, Meads C, Lord J. EXOGEN ultrasound bone healing system for long bone fractures with non-union or delayed healing: a NICE medical technology guidance. Appl Health Econ Health Pol. 2014;12(5):477–84.
80. Arron JR, Choi Y. Bone versus immune system. Nature. 2000;408(6812):535–6.
81.•• Reinke S, Geissler S, Taylor WR, Schmidt-Bleek K, Juelke K, Schwachmeyer V, et al. Terminally differentiated CD8(+) T cells negatively affect bone regeneration in humans. Sci Transl Med. 2013;5(177):177ra36. **This study was the first to clearly show the high interconnectivity of the amount of potential unfavorable CD8+ terminally differentiated effector memory T cells (TEMRA), and thus of the adaptive immunity, and of impaired bone regeneration in fracture patients.**

82. Schlundt C, El Khassawna T, Serra A, Dienelt A, Wendler S, Schell H, et al. Macrophages in bone fracture healing: Their essential role in endochondral ossification. Bone. 2015.
83. Cezar CA, Roche ET, Vandenburgh HH, Duda GN, Walsh CJ, Mooney DJ. Biologic-free mechanically induced muscle regeneration. Proc Natl Acad Sci. 2016;113(6):1534–9.
84. Pobloth, A., Checa, S., Razi, H., Petersen, A., Weaver, J. C., Schmidt-Bleek, K., Windolf, M., Tatai, A. Á., Roth, C. P., Schaser, K. D., Duda, G. N., Schwabe, P. Mechano-biologically optimized 3D titanium-mesh scaffolds enhance regeneration in large segmental bone defects. Cience Transl Med, 2018, in press.