Tissue Engineering for Facial Reconstruction

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Contents

Abstract Craniofacial structures are essential for many physiological functions, including vision, olfaction, hearing, and food intake. In addition, facial features are critical for the development of personal identity, communication, and social interaction. Thus, damage to the face resulting from traumatic injury or disease can be particularly devastating to a patient's quality of life, and the development of methods to restore normal craniofacial structures is essential. In recent years, facial transplantation using microsurgical techniques has become a reality, but this technique is limited by a shortage of donor tissue and the need for chronic administration of immunosuppressive drugs to prevent graft rejection. Recent advances in tissue engineering and regenerative medicine provide opportunities to create biological substitutes that can be used in reconstructive surgery. This field applies the principles of cell transplantation, material science, and bioengineering to develop tissues and organs in the laboratory that can then be implanted into a patient to replace damaged or missing structures. In this chapter, we will discuss these techniques in detail, and we will illustrate how they can be used to revolutionize the concepts of facial reconstruction.

Abbreviations

AFPS	Amniotic-fluid and placental-derived stem
BSM	Bladder submucosa
EC	Endothelial cells
ECM	Extracellular matrix
ES	Embryonic stem
FDA	Food and Drug Administration
iPS	induced Pluripotent State
MEFs	Mouse embryonic fibroblasts
PGA	Polyglycolic acid

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PLA Polylactic acid
PLGA Poly(lactic-co-glycolic acid)
SIS Small-intestinal submucosa
VEGF Vascular endothelial growth factor

43.1 Introduction

The face is the most prominent part of the body, and it plays many important roles. Physiologically, it is the entry point of both the respiratory and digestive systems, and contains structures that are essential in respiration and food intake. In addition, the sensory organs for vision, olfaction, hearing, and taste are housed in the craniofacial area. Psychologically, the face is essential for development and maintenance of personal identity, which is critical for social communication, expression of emotion, and mutual interaction. Therefore, when the facial area is damaged or disfigured due to disease processes or injury, a patient's quality of life is severely decreased and the ego is frequently disturbed.

Conditions involving birth defects, injuries, diseases, or certain therapeutic modalities such as surgery or radiation therapy often cause facial disfigurement, which result in severe physiological and psychological trauma.[1,2] Facial reconstructive surgery aims to restore the function and esthetics of each subunit of the face using tissue substitutes. As such, the reconstruction is usually customized for each individual patient. Various surgical methods have been used in facial reconstruction, including grafts, vascularized tissue flaps, microvascular free flaps, or combinations of these.[3,4] When larger facial defects are encountered, extensive reconstruction might be the treatment of choice, and this often requires multiple surgeries and different reconstructive approaches. Despite the recent progress in reconstructive surgery, the results of large-scale facial reconstruction remain unsatisfactory.[5] The application of free tissue transfers, expanders, and tissue prefabrication allows for facial defect coverage; however, functional deficits are not restored to the normal state. Moreover, regardless of the methods used, the structure and composition of the tissues that make up the face are specific, and it is not easy to transfer tissues from other parts of the body. In addition, tissues in the facial area are composite structures consisting of multiple tissue types that require coordinated function.

In order to achieve replacement of the tissues, according to Gille's rule of replacing "like tissues with alike," transplantation of the face from human donors is currently a novel approach for reconstruction of severe defects in patients after trauma, burn injuries, cancer, or congenital malformations. Since the first human face transplant was performed, several additional cases have been reported worldwide.[6,7] Despite the current success in face transplantation, several challenges must be addressed before the technique can be applied in mainstream clinical medicine. These include the availability of a suitable donor, complex surgical techniques, use of immunosuppressive medication, and ethical and psychosocial issues.

In recent years, advances in tissue engineering and regenerative medicine have provided various opportunities in medicine. The science of tissue engineering aims to generate tissues that would replace the structure and function of failing organs.[8-10] Using techniques from cell biology, material science, and transplantation, investigators have been able to construct tissue substitutes in the laboratory that can restore the normal structure and function of missing or diseased organs. This has been accomplished by combining cells, biomaterial "scaffolds" on which the cells can attach and grow, and appropriate signaling molecules in bioreactors. Although this field is still in the early stages of development, some successful approaches have already been applied to the human body, which suggests that this type of treatment is promising and may be used in the future.[11-13]

The use of tissue engineering techniques for the craniofacial area has several advantages. First, replacement tissues could be custom-designed for different individuals depending on their needs. For example, the use of an appropriate scaffold to guide tissue growth might simplify the reconstruction of a variety of facial contours and shapes, and this could lead to reconstructed tissues that are more similar to a patient's natural facial structure. New interactive biomaterial scaffolds are now being investigated in order to accomplish this goal.[14] In addition, if autologous cells were used to engineer replacement facial tissues, the use of immunosuppressant medications after tissue transplantation could be reduced or eliminated.[15] However, the generation of complex tissues such as those present in the craniofacial area remains a challenge. Normal tissue is made up of numerous cell types that are arranged in a specific and well-organized manner, and this structural complexity is often difficult to replicate in the laboratory. Within the

living body, cell–cell and tissue–tissue interactions are dynamic and topographically oriented, and thus in order to engineer a functional tissue, it is necessary to establish an environment that is capable of providing temporal and spatial cues appropriate for tissue formation. In addition, in order for a tissue to be functional, it must be integrated to the vascular and nervous systems within the body. Tissue engineering provides an encouraging and exciting basis toward reconstructive options in facial reconstruction. This chapter will introduce the basic principles of tissue engineering and outline the current advances in this field. In addition, evolving methods for using tissue engineering technologies to reconstruct craniofacial tissues and organs will be discussed.

43.2 The Basics of Tissue Engineering

Tissue engineering employs aspects of cell biology and transplantation, materials science, and engineering to develop biological substitutes that can restore and maintain the normal function of damaged tissues and organs. It includes techniques such as the injection of functional cells into a nonfunctional body site to stimulate regeneration and the use of biocompatible materials to create new tissues and organs. These biomaterials can be natural or synthetic matrices, often termed scaffolds, which encourage the body's natural ability to repair itself and assist in determination of the orientation and direction of new tissue growth. Often, tissue engineering uses a combination of both of these techniques. For example, biomaterial matrices seeded with cells can be implanted into the body to encourage the growth or regeneration of functional tissue.

43.2.1 Biomaterials Used in Tissue Engineering

The design and selection of a biomaterial for use in regenerative medicine is critical for the proper development of engineered tissues. The selected biomaterial must be capable of controlling the structure and function of the engineered tissue in a predesigned manner by interacting with transplanted cells and/or host cells. In addition, it should be biocompatible, able to promote cellular interaction and tissue development, and it should possess the proper mechanical and physical properties required for tissue support and function in the body site of interest.

Appropriate biomaterials should be biodegradable and bioresorbable to support the reconstruction of a completely normal tissue without inflammation. Thus, the degradation rate and the concentration of degradation products in the tissues surrounding the implant must be maintained at a tolerable level.[16] Such behavior avoids the risk of inflammatory or foreign-body responses that are often associated with the permanent presence of a foreign material in the body. In addition, the biomaterial should provide appropriate regulation of cell behavior (e.g., adhesion, proliferation, migration, differentiation) in order to promote the development of functional new tissue. Cell behavior in engineered tissues is regulated by multiple interactions with the microenvironment, including interactions with cell-adhesion ligands[17] and with soluble growth factors.[18] Cell-adhesion promoting factors (e.g., Arg-Gly-Asp [RGD]) can be presented by the biomaterial itself or incorporated into the biomaterial in order to control cell behavior through ligand-induced cell receptor signaling processes.[19,20] In vivo, the biomaterials must provide temporary mechanical support sufficient to withstand forces exerted by the surrounding tissue and maintain a potential space for tissue development. The mechanical support of the biomaterials should be maintained until the engineered tissue has formed sufficient structural integrity to support itself.[21] This can be achieved by an appropriate choice of mechanical and degradation properties of the biomaterials.[22]

Finally, the chosen biomaterial must have properties that allow it to be processed into specific configurations. A large ratio of surface area to volume is often desirable to allow the delivery of a high density of cells. A high porosity, interconnected pore structure with specific pore sizes promotes tissue ingrowth from the surrounding host tissue. Several techniques, such as electrospinning, have been developed, and they allow precise control of porosity, pore size, and pore structure.[23-28]

Various biomaterials have been used in tissue engineering and regenerative medicine. These include naturally derived materials, such as collagen and alginate; acellular tissue matrices, such as bladder submucosa (BSM) and small-intestinal submucosa (SIS); and synthetic polymers, such as polyglycolic acid (PGA), polylactic acid (PLA), and poly(lactic-*co*-glycolic

acid) (PLGA). Naturally derived materials and acellular tissue matrices have the potential advantage of biologic recognition. However, synthetic polymers can be produced quickly and reproducibly on a large scale with controlled properties of strength, degradation rate, and microstructure.

Collagen is the most abundant and ubiquitous structural protein in the body, and it may be readily purified from both animal and human tissues with an enzyme treatment and salt/acid extraction.[29] Collagen has long been known to exhibit minimal inflammatory and antigenic responses,[30] and it has been approved by the US Food and Drug Administration (FDA) for many types of medical applications, including wound dressings and artificial skin.[31] Collagen contains cell-adhesion domain sequences (e.g., RGD) that exhibit specific cellular interactions. This may help to retain the phenotype and activity of many types of cells, including fibroblasts[32] and chondrocytes.[33]

Alginate, a polysaccharide isolated from seaweed, has been used as an injectable cell delivery vehicle[34] and a cell immobilization matrix[35] owing to its gentle gelling properties in the presence of divalent ions such as calcium. Alginate is a family of copolymers of D-mannuronate and L-guluronate. The physical and mechanical properties of alginate gel are strongly correlated with the proportion and length of the polyguluronate block in the alginate chains.[34] Efforts have been made to synthesize biodegradable alginate hydrogels with mechanical properties that are controllable in a wide range by intermolecular covalent cross-linking and with cell-adhesion peptides coupled to their backbones.[36]

Acellular tissue matrices are collagen-rich matrices prepared by removing cellular components from tissues. The most common tissue that has been used for this purpose has been bladder tissue. The matrices are prepared by removing the cellular material from a segment of bladder tissue using mechanical and chemical processes.[37-40] The resulting matrix can be used alone or seeded with cells. The matrices slowly degrade after implantation and are replaced and remodeled by extracellular matrix (ECM) proteins synthesized and secreted by transplanted or ingrowing cells. Acellular tissue matrices support cell ingrowth and regeneration of many tissue types with no evidence of immunogenic rejection.[40,41] Because the structures of the proteins (e.g., collagen, elastin) in acellular matrices are well conserved and normally arranged, the mechanical properties of the acellular matrices are not significantly different from those of native bladder submucosa.[37]

Polyesters of naturally occurring α-hydroxy acids, including PGA, PLA, and PLGA, are widely used in regenerative medicine. These polymers have gained FDA approval for human use in a variety of applications, including sutures.[42] The degradation products of PGA, PLA, and PLGA are nontoxic, natural metabolites that are eventually eliminated from the body in the form of carbon dioxide and water.[42] Because these polymers are thermoplastics, they can easily be formed into a three-dimensional scaffold with a desired microstructure, gross shape, and dimension by various techniques, including molding, extrusion,[43] solvent casting,[44] phase-separation techniques, and gas-foaming techniques.[45] More recently, techniques such as electrospinning have been used to quickly create highly porous scaffolds in various conformations.[25-27,46]

Many applications require a scaffold with high porosity and a high ratio of surface area to volume. This need has been addressed by processing biomaterials into configurations of fiber meshes and porous sponges using the techniques described previously. A drawback of the synthetic polymers is lack of biologic recognition. As an approach toward incorporating cell recognition domains into these materials, copolymers with amino acids have been synthesized.[19,20,47] Other biodegradable synthetic polymers, including poly(anhydrides) and poly(ortho esters), can also be used to fabricate scaffolds with controlled properties.[48]

43.2.2 Cells Used in Tissue Engineering Applications

When cells are used for tissue engineering, donor tissue is removed and dissociated into individual cells, which are then implanted directly into the host or expanded in culture, attached to a support matrix, and then implanted as a cell-scaffold construct. The donor tissue can be heterologous, allogeneic, or autologous.

Autologous cells are the ideal choice, as their use circumvents many of the inflammatory and rejection issues associated with a nonself donor. In the past, one of the limitations of applying cell-based regenerative medicine techniques to organ replacement was the inherent difficulty of growing certain human cell types in large quantities. However, the discovery of native

targeted progenitor cells in virtually every organ of the body has led to improved culture techniques that have overcome this problem for a number of cell types. Native targeted progenitor cells are tissue-specific unipotent cells derived from most organs. By noting the location of the progenitor cells, as well as by exploring the conditions that promote differentiation and/or self-renewal, it has been possible to overcome some of the obstacles that limit cell expansion in vitro. For example, urothelial cell culture has been improved in this way. Urothelial cells could be grown in the laboratory setting in the past, but only with limited success. It was believed that urothelial cells had a natural senescence that was hard to overcome. Several protocols have been developed over the last 2 decades that have improved urothelial growth and expansion.[49-52] Using these methods of cell culture, it is possible to expand a urothelial strain from a single specimen that initially covers a surface area of 1 cm^2 to one covering a surface area of 4,202 m^2 (the equivalent area of one football field) within 8 weeks.[49]

An advantage of native targeted progenitor cells is that they are already programmed to become the cell type needed, and no in vitro differentiation steps are necessary for their use in the organ of origin. An additional advantage in using native cells is that they can be obtained from the specific organ to be regenerated, expanded, and used in the same patient without rejection, in an autologous manner.[39,49,53-68] However, a major concern has been that, in cases where cells must be expanded from a diseased organ, there may no longer be enough normal cells present in that organ to begin the expansion process. Recent research suggests that this may not be the case, however. For example, one study has shown that cultured neuropathic bladder smooth muscle cells possess and maintain different characteristics than normal smooth muscle cells in vitro, as demonstrated by growth assays, contractility, and adherence tests in vitro.[69] Despite these differences, when neuropathic smooth muscle cells were cultured in vitro, and then seeded onto matrices and implanted in vivo, the tissue-engineered constructs showed the same properties as the constructs engineered with normal cells.[70] It is now known that genetically normal progenitor cells, which are the reservoirs for new cell formation, are present even in diseased tissue. These normal progenitors are programmed to give rise to normal tissue, regardless of whether they reside in a normal or diseased environment. Therefore, the stem cell niche and its role in normal tissue regeneration remains a fertile area of ongoing investigation.

Most current strategies for tissue engineering depend upon a sample of autologous cells from the diseased organ of the host. In some instances, primary autologous human cells cannot be expanded from a particular organ, such as the pancreas, or there is not enough normal tissue remaining in the diseased organ to use for the procedures described above. In addition, the use of autologous cells from tissues containing malignancies is not recommended, as abnormal cells could be harvested and would grow within the newly generated organ as well. In these situations, pluripotent human stem cells are envisioned to be an ideal source of cells, as they can differentiate into nearly any replacement tissue in the body.

Embryonic stem (ES) cells exhibit two remarkable properties: the ability to proliferate in an undifferentiated, but still pluripotent state (self-renewal), and the ability to differentiate into a large number of specialized cell types.[71] They can be isolated from the inner cell mass of the embryo during the blastocyst stage, which occurs 5 days postfertilization. Many protocols for differentiation of ES cells into specific cell types in culture have been published. However, many uses of these cells are currently banned in a number of countries due to the ethical dilemmas that are associated with the manipulation of embryos in culture.

Adult stem cells, especially hematopoietic stem cells, are the best understood cell type in stem cell biology.[72] Despite this, adult stem cell research remains an area of intense study, as their potential for therapy may be applicable to a myriad of degenerative disorders. Within the past decade, adult stem cell populations have been found in many adult tissues other than the bone marrow and the gastrointestinal tract, including the brain,[73,74] skin,[75] and muscle.[76] Many other types of adult stem cells have been identified in organs all over the body and are thought to serve as the primary repair entities for their corresponding organs.[77] The discovery of such tissue-specific progenitors has opened up new avenues for research.

A notable exception to the tissue-specificity of adult stem cells is the mesenchymal stem cell, also known as the multipotent adult progenitor cell. This cell type is derived from bone marrow stroma.[78,79] Such cells can differentiate in vitro into numerous tissue types[80,81] and can also differentiate developmentally if injected into a blastocyst. Multipotent adult progenitor cells can

develop into a variety of tissues including neuronal,[82] adipose,[76] muscle,[76,83] liver,[84,85] lungs,[86] spleen,[87] and gut tissue,[79] but notably not bone marrow or gonads.

Research into adult stem cells has, however, progressed slowly, mainly because investigators have had great difficulty in maintaining adult non-mesenchymal stem cells in culture. Some cells, such as those of the liver, pancreas, and nerve, have very low proliferative capacity in vitro, and the functionality of some cell types is reduced after the cells are cultivated. Isolation of cells has also been problematic, because stem cells are present in extremely low numbers in adult tissue.[84,88] While the clinical utility of adult stem cells is currently limited, great potential exists for future use of such cells in tissue-specific regenerative therapies. The advantage of adult stem cells is that they can be used in autologous therapies, thus avoiding any complications associated with immune rejection.

The isolation of multipotent human and mouse amniotic-fluid and placental-derived stem (AFPS) cells that are capable of extensive self-renewal and give rise to cells from all three germ layers was reported in 2007.[89] Undifferentiated AFPS cells expand extensively without a feeder cell layer and double every 36 h. Unlike human embryonic stem cells, AFPS cells do not form tumors in vivo. Lines maintained for over 250 population doublings retained long telomeres and a normal complement of chromosomes. AFPS cell lines can be induced to differentiate into cells representing each embryonic germ layer, including cells of the adipogenic, osteogenic, myogenic, endothelial, neural-like, and hepatic lineages. Since the discovery of the AFPS cells, other groups have published on the potential of the cells to differentiate to other lineages, such as cartilage,[90] kidney,[91] and lung.[92] Muscle differentiated AFPS cells were also noted to prevent compensatory bladder hypertrophy in a cryo-injured rodent bladder model.[93]

Recently, exciting reports of the successful transformation of adult cells into pluripotent stem cells through a type of genetic "reprogramming" have been published. Reprogramming is a technique that involves dedifferentiation of adult somatic cells to produce patient-specific pluripotent stem cells, without the use of embryos. Cells generated by reprogramming would be genetically identical to the somatic cells (and thus, the patient who donated these cells) and would not be rejected. Yamanaka was the first to discover that mouse embryonic fibroblasts (MEFs) and adult mouse fibroblasts could be reprogrammed into an "induced pluripotent state (iPS)."[94] iPS cells in this study possessed the immortal growth characteristics of self-renewing ES cells, expressed genes specific for ES cells, and generated embryoid bodies in vitro and teratomas in vivo. Another study shows that teratomas induced by these cells contained differentiated cell types representing all three embryonic germ layers. More importantly, the reprogrammed cells from this experiment were able to form viable chimeras and contribute to the germ line like ES cells, suggesting that these iPS cells were completely reprogrammed.[95] It has recently been shown that reprogramming of human cells is possible.[96,97] However, despite these advances, a number of questions must be answered before iPS cells can be used in human therapies. One concern is that these cells contain three to six retroviral integrations, which may increase the risk of eventual tumorigenesis. Although this is an exciting phenomenon, our understanding of the mechanisms involved in reprogramming is still limited.

43.2.3 Generation of Tissue-Engineered Constructs

The basic strategy for engineering a tissue or organ involves seeding a biomaterial scaffold with appropriate cell types. This construct is then incubated for a period of time to allow the cells to attach to the scaffold and begin to grow. However, simply placing the cells onto a scaffold in a culture dish may not be the most efficient or effective method for growing tissue in vitro. For example, it has been shown that a number of cell types, such as muscle, may require exposure to mechanical forces in order to mature and develop the proper cellular orientation required for a functional tissue. In addition, oxygen and nutrient exchange is limited in a culture dish, and this may hamper the development of normal tissue. Finally, in order to engineer more complex tissues and organs that are made up of a number of different cell types, it is necessary to place each cell type in a very specific spatial orientation within a construct, and the simple culture dish method of in vitro tissue culture does not provide this capability. Therefore, over the last few years, a number of new technologies for creating a tissue-engineered construct have been developed.

Bioreactors for tissue engineering applications are designed to provide mechanical stimulation and mimic physiological conditions in vitro, as exposure to stimuli such as pulsatile flow and pressure changes has been shown to enhance tissue formation, organization, and function.[98-100] These components work in concert to provide an environment that allows preconditioning of cells on scaffolds in vitro and promotes the enhancement of cell–matrix interaction, cellular proliferation, and organization. Various tissues have been grown using bioreactors. For example, Lee and colleagues have shown that engineered heart valves become more completely endothelialized when they are preconditioned in a bioreactor system that mimics physiological blood flow,[101] and similarly, several studies have shown that a more complete endothelial layer forms in engineered blood vessels when they are preconditioned in a bioreactor.[26,102] In addition, bioreactors have been shown to improve the function of engineered muscle tissue, including bladder muscle.[103-105] Ladd and colleagues have shown that human skin can be expanded through a gentle stretching process in a bioreactor system to generate significantly larger pieces of skin for grafting.[9] Further development of bioreactors for both skin and muscle is particularly important in facial reconstruction using tissue engineering, as these are integral components of most craniofacial structures.

Another concern in tissue engineering is appropriate nutrient and gas exchange for the growing tissue, both during culture and immediately after implantation but before the new tissue becomes fully vascularized. While bioreactors can address this concern in vitro, it has been difficult to resolve this issue in vivo, and the size of most tissue constructs has been limited by the diffusion distance of oxygen within them. While the biological approach using vascular endothelial growth factor (VEGF) and endothelial cells is able to stimulate and promote neovascularization, it is unable to provide vascular supply to a large tissue mass within a short period of time. Recently, Oh et al. have developed a novel scaffold material that generates a sustained release of oxygen over a period of time, and their research indicates that use of this material may allow for prolonged cell survival and growth in the period after implantation but before adequate vasculature has developed (Fig. 43.1).[106]

It is known that the cells within a tissue have a very specific spatial organization, and this organization is required for appropriate tissue function. Recently, a bioprinting technique was developed to deliver cells and biomaterials to target locations to achieve spatial orientation of tissue constructs (Fig. 43.2). Natural materials such as alginate and collagen have been used as "bioinks" in this technique, which is based on inkjet technology.[107,108] Using this technology, these scaffold materials can be "printed" into a desired scaffold shape using a modified inkjet printer. In addition, several groups have shown that living cells can also be printed using this technology.[109-111] This exciting technique can be modified so that a three-dimensional construct containing a

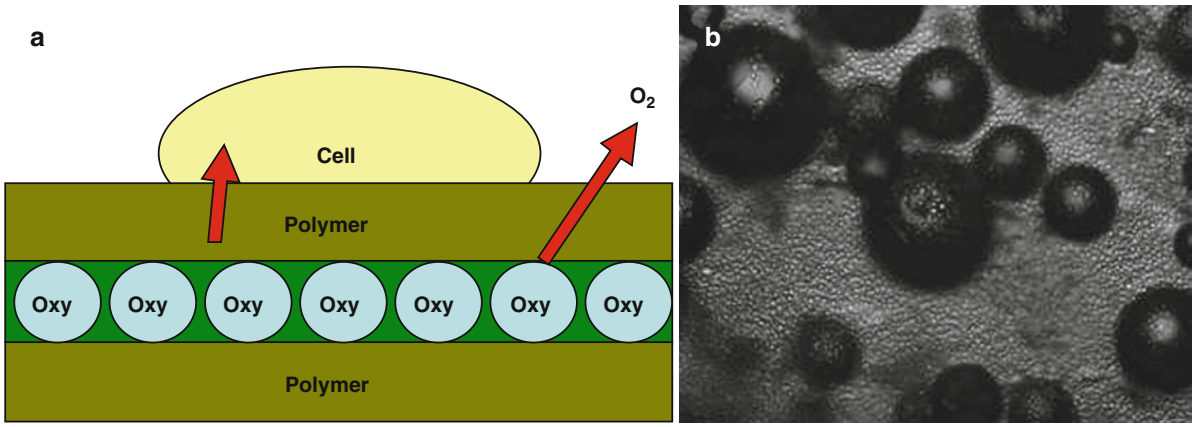

Fig. 43.1 (**a**) Oxygen-generating particles are incorporated into a polymeric biomaterial. This material is designed to generate a sustained release of oxygen over time. (**b**) Oxygen bubbles are released from a polymeric material containing oxygen-generating particles

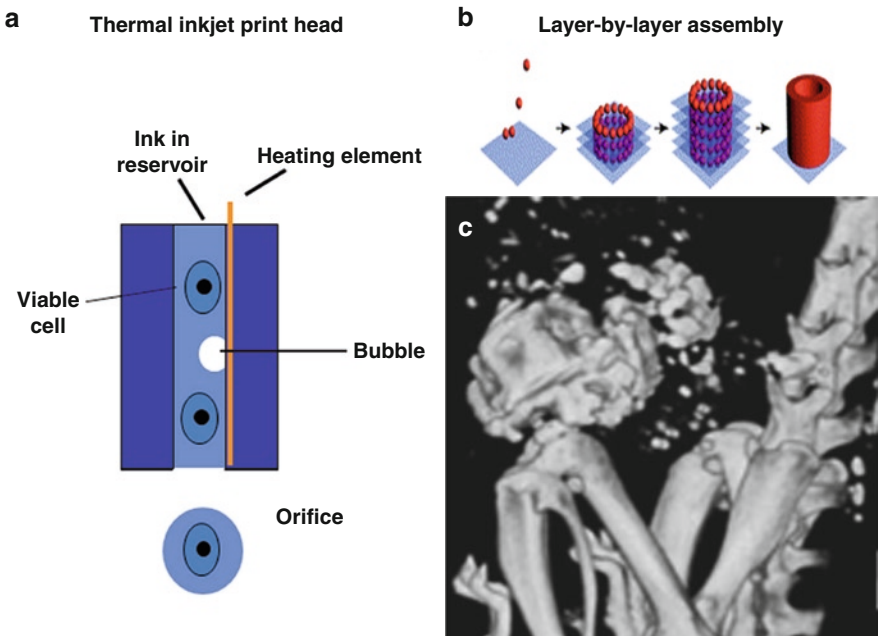

Fig. 43.2 (**a**) A schematic drawing of cell delivery system. Individual cells are pushed through the nozzle (orifice) by the bubbles generated by the heating element of the thermal inkjet print head. (**b**) Single cells are delivered to target locations layer by layer to form a three-dimensional structure. (**c**) Micro CT scan of a mouse 18 weeks after implantation of printed constructs consisting of amniotic-fluid stem cell-derived bone cells

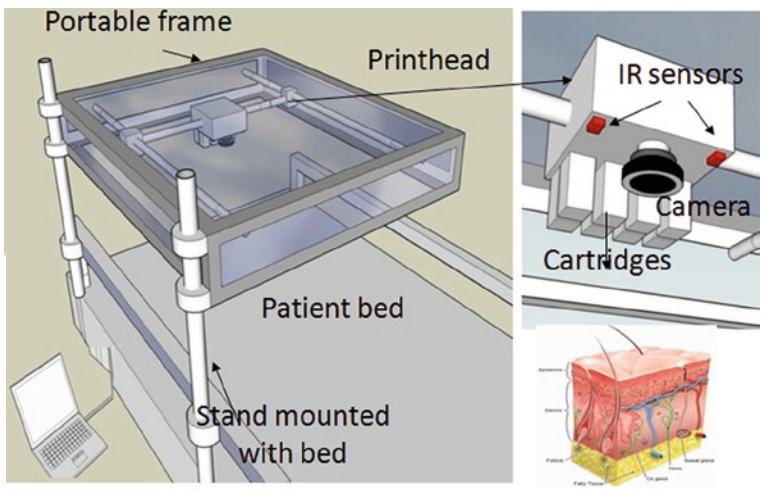

Fig. 43.3 A schematic drawing of a portable skin bioprinter. A multi-nozzle printhead, a digital camera, and infrared sensors are integrated into a portable printing operation frame. The printhead consists of multiple cartridges loaded with various skin printing materials. The portable operation frame has a computer-driven moveable module

precise arrangement of cells, growth factors, and extracellular matrix material can be printed.[111-113] Such constructs may eventually be implanted into a host to serve as the backbone for a new tissue or organ. Our group is currently developing a bioprinting system that will allow for on-site, in situ repair of burn injuries using tissue-engineered skin grafts produced with a portable skin printing system (Fig. 43.3). This printing system will deliver several dermal cell types and matrices simultaneously onto the injured skin to generate anatomically and functionally adequate dermal tissues. The amount and ratio of cells and matrices, as well as the thickness of the skin layers to be printed, can be precisely controlled using the inkjet bioprinter. The delivery of major skin tissue elements onto the injured site will allow for a rapid restoration of the skin and may minimize scarring

and enhance cosmetic recovery. Such a system could eventually be modified to print skin grafts for other applications, including restoration of skin during facial reconstruction.

43.3 Reconstruction of Specific Craniofacial Structures Using Tissue Engineering

43.3.1 Skin

The skin, along with the mucosa, is the outermost layer of tissue in the facial area. The skin and the mucosa serve as barriers against exogenous pathogens and irritants, and they prevent the loss of body fluid and other components. In clinical situations, the loss of this covering layer, as in burn wounds, can cause severe metabolic disturbances that lead to increased morbidity and mortality. These issues are encountered in facial transplantation as well. If allogeneic skin grafts are used, the risk of immunologic rejection and pathogen transmission are always present. In addition, the shortage of donor skin and mucosa limits the clinical application of this technique. Tissue engineering techniques could potentially resolve some of these issues. Currently, many research groups are attempting to construct composite skin equivalents.[114-117] By culturing epithelial cells and dermal tissues, it is possible to generate a functional skin or mucosa equivalent that could be used clinically.[118,119] Skin equivalents produced by these approaches seem to have intact dermal–epithelial junctions, and have been studied both in experimental and clinical settings. The epithelium of these skin equivalents usually contains several stratified cell layers resembling normal skin.[120] Moreover, skin equivalents that contain both epidermis and dermis could be grafted using a simple procedure. Similar progress has been reported in the development of mucosal equivalents as well.[121-123] Engineered-mucosal equivalents used for intraoral reconstructions are able to promote vascular network formation. Histologically, the differentiation pattern of the epithelium mimics that of the original tissue.[124] However, the current engineered-mucosal tissue seems to be thinner than normal tissue and lacks prominent epithelial ridges.[125] Methods for improving microstructure formation within the tissue equivalent are under investigation.

43.3.2 Soft Tissue and Bone

In addition to the skin coverage, underlying soft tissue and bone play important roles in maintaining the facial contours and function. Application of tissue engineering methods in regenerating musculoskeletal tissue has demonstrated that both bone and cartilage can be generated both in vitro and in vivo.[13,126,127] In addition, in order to engineer tissue with appropriate physiological and mechanical properties for use in the facial area, a significant effort must be made to integrate different tissues into the reconstructive process. The esthetics of the face and the ability to express emotion are mainly governed by the underlying musculoskeletal function. To ensure that muscle and skeletal tissue work coordinately, soft tissue, cartilage, and bone must be integrated properly so that they will function together. In order to achieve this using regenerative medicine and tissue engineering, numerous distinct cell types must be combined with scaffolds composed of different materials and structural designs so that tissue heterogeneity that exists in native organs can be developed in a controlled manner. In such cases, tissue interface engineering techniques could be applied.[128,129] Using these techniques, scaffolds are created to minimize stress on the implant by effectively balancing the weight and strength loads between various tissues.

It is well known that the wound healing process in the body employs different strategies than the regenerative process. In wound healing, a fibrovascular tissue layer, or scar tissue, is generated in the defective region rather than normal tissue. The formation of scar tissue results in inadequate fixation at the junction between soft tissues and bone, which leads to restricted movement. Similarly, in engineered tissue, to provide a layer where the muscle and tendon can directly connect with cartilage and bone could promote tissue integration. In addition, the approach might be ideal for cell–cell interactions that mediate interface regeneration.[130,131] Moreover, the interaction between osteoblasts and fibroblasts is associated with recruitment and differentiation of progenitor cells into fibrochondrocytes, which could facilitate the formation of tissue structure.

On the other hand, in order to successfully engineer an integrated tissue composed of both soft tissue and bone, the structural features and material

characteristics of the implanted scaffold must be well identified. The development of a biomimetic scaffold serves as a critical benchmark for the outcome of engineered tissue.[132-134] An optimally designed supporting scaffold should be able to provide structural and mechanical support as well as an appropriate environment for facilitating cell growth and differentiation.[135,136] Triphasic scaffolds composed of three different regions designed for soft tissue, fibrocartilage, and bone, respectively, might be a way to accomplish this.[137] The feasibility of the triphasic scaffold has been demonstrated both in vitro and in vivo. It is suggested that the application of integrative scaffolds might play a decisive role in functional tissue engineering.[138,139] Finally, in order for engineered tissue to be used in clinic, the issue of scale-up challenges must be addressed. Tissue engineering approaches used to repair small defects may not necessarily be ideal for use in repairing larger defects, which are frequently encountered in the clinic. Larger defects require tissue grafts with greater dimensions, but the nutrient diffusion through the immature vascular system in these large grafts might be limited. The ability to ensure that the engineered tissue is supplied with sufficient metabolic exchange is the first step to animate the transplanted tissue graft. In addition, the differences between various animals and humans must be considered since most successful tissue engineering approaches are first demonstrated in animal models. Moreover, for the purpose of facial reconstruction, the assembly of different tissue-engineered products designed for specific parts of the face might be another challenge. Although some attempts have been made toward this direction, few successful reports have been noted. The ability to regenerate a complex tissue on a large scale is an extraordinary achievement, and will revolutionize the next generation of facial reconstruction.

43.3.3 Vascularization

Most structures in the face are highly vascularized. In particular, the craniofacial area contains predominantly skeletal muscle, which is vascularized by numerous branching vascular networks nearby. To maintain the function of the tissues around the craniofacial area, adequate blood supply is required to meet the metabolic demands of the structures. Thus, the creation of highly vascularized tissue is required to allow engineered grafts to remain viable in vivo.[140] Currently, it has been shown that smaller engineered tissues are able to recruit vascular support from the host to maintain their physiological demands.[11,141,142] However, for larger implants, vascular structures derived from host cells cannot develop quickly enough to support the entire implant. The development of a new approach to the vascularization of tissue-engineered implants is required. However, the growth of a new microvascular system has been one of the major limitations to the successful introduction of tissue engineering products to clinical practice.

Numerous efforts have been made to overcome this limitation and attempts to enhance angiogenesis within the host tissue have been pursued using several approaches. These include the delivery of growth factors and cytokines that play central regulatory roles in the process of angiogenesis, which is thought to induce ingrowth of capillaries and blood vessels into an engineered implant, thus diminishing hypoxia-related cell damage. The delivery of such angiogenic factors has been achieved either by incorporating the desired factors into the scaffold material to be used or by genetic modification of the cells to be used in the engineering process, which forces the cells to express factors such as vascular endothelial growth factor (VEGF). VEGF is one of the most potent angiogenic factors.[140] A recent study by Mooney's group evaluated controlled release of VEGF by incorporating VEGF directly into PLGA scaffolds or by incorporating VEGF encapsulated in PLGA microspheres into scaffolds.[140] VEGF incorporated into scaffolds resulted in rapid release of the cytokine, whereas the pre-encapsulated group showed a delayed release. These studies demonstrated the delivery of VEGF in a controlled and localized fashion in vivo. This angiogenic factor delivery system was applied to bone regeneration and its potent ability to enhance angiogenesis within implanted scaffolds was followed by enhanced bone regeneration. This outlines a novel approach for engineering tissues in hypovascular environments.[140] In another study, VEGF delivery was tested in a study in which human vascular endothelial cells (EC) and skeletal myoblasts transfected with adenovirus encoding the gene for VEGF were injected

subcutaneously in athymic mice.[140] The transfected cells formed a vascularized muscle tissue mass, while the non-transfected cells resulted in less angiogenesis and led to the growth of a significantly smaller tissue mass. This study demonstrates that the use of cells producing biological factors can be another powerful tool in tissue engineering. Another approach involved the development of a method to form and stabilize endothelial vessel networks in vitro in engineered skeletal muscle tissue.[143,144] This study used a 3D multiculture system consisting of myoblasts, embryonic fibroblasts, and EC co-seeded on highly porous biodegradable polymer scaffolds. These results showed that prevascularization of the implants improved angiogenesis and cell survival within the scaffolds. Moreover, they emphasize that cocultures with EC and muscle cells may also be important for inducing differentiation of engineered tissues. A breakthrough in engineering vasculature could provide a solution to regenerate bulky skeletal muscle, which increases the potential for clinical application to facial reconstruction.

43.3.4 Innervation

In the craniofacial area, proper innervation of muscle is critical. For example, the ability to form facial expressions, as well as many physiological functions such as mastication, requires functional coordination between many different muscles. Under normal circumstances, most of the muscles in the face are innervated by the facial nerve. If the facial nerve is damaged, these muscles would atrophy and lose the capacity for conscious movement. Therefore, when engineering components for facial reconstruction, the establishment of proper nerve connections between the muscular tissues of the host and the implanted tissues is required.

In some cases, the peripheral nervous system has been shown to regenerate and achieve functional recovery when injury occurs. Nonetheless, nerve recovery often takes a long time and functional recovery might be incomplete. Using tissue engineering, it may be possible to restore proper innervation of the muscle. However, there are several important issues to be addressed. The first issue is the regeneration and elongation of the motor axon, and the second is the regeneration of the neuromuscular junction. For successful regeneration, the neuron itself must survive and be able to restart the axonal growth process after injury. If the axon fails to regrow, then the connection between the central nervous system and the musculature will not be reestablished, and control over muscular function will not be restored. For this to happen, the growing axon must receive adequate nutritional and trophic support from the distal nerve stump. Next, the regenerated axon must be able to reinnervate the target muscle by forming a neuromuscular junction. Once this occurs and signaling is restored, the muscle must regenerate from atrophy caused by denervation.[145] The process is complicated by the fact that even if the axon is successfully regenerated, misdirected axonal guidance might cause a muscle to become reinnervated by an inappropriate axon.[146] This usually results if an axon is misrouted along the improper fascicle or if a muscle is simultaneously reinnervated by several motor neurons.[147] Thus, a number of situations might lead to dysfunctional innervations of muscle and make complete recovery impossible.

Currently, microsurgical treatment with nerve grafts is sometimes effective in repairing nerve damage.[148] Autologous nerve grafts are regarded as the treatment of choice in grafting procedures. Nonetheless, even with this treatment, residual disability is often encountered. Furthermore, there are several problems with this approach, including functional deficit and functional impairment at the donor site created by the graft harvest, and the frequent shortage of suitable graft nerve tissue.

In light of these disadvantages, the development of a tissue-engineered nerve conduit that serves as an alternative to the autologous nerve graft is the subject of intense interest. In order to prepare an artificial nerve guide that is suitable for nerve regeneration, several concepts should be considered. For example, the nerve guidance conduit must provide an appropriate scaffold for axon regeneration. Based on numerous clinical experiences, it is well known that physical support is vital for axon regeneration. In addition, because trophic support from the distal stump is important, the engineered nerve conduit must be permeable to these critical factors. Researchers are currently studying methods of controlling the interaction between the surrounding environment and the growing axons in the conduit. It has been shown that the application of permeable scaffolds for engineering nerve conduits might facilitate nerve regeneration. In this

design, metabolic exchange and diffusion of growth and trophic factors could be achieved. Moreover, after a functional axon has regenerated, the scaffold must degrade in a controlled manner. The scaffold should provide a stable conduit for support and directional guidance during axon regeneration, so that the nerve is able to grow and reorganize its connections. If the degradation rate of this scaffold is too fast, the regenerated nerve might undergo biological, mechanical, or chemical damage. Therefore, the appropriate material for a nerve conduit must fit the above criteria to be suitable for clinical application.

Numerous studies have investigated the application of synthetic materials in nerve grafting. Scaffolds made of silicone were the first synthetic material employed in this manner. The silicone scaffold was developed for nerve reconstruction 2 decades ago[149] and it was shown to be useful in several studies. However, silicone is not a biodegradable material, and a foreign-body response to it may occur after implantation. Nerve grafts made of biodegradable materials are a more promising alternative for this reason. A variety of biodegradable materials have been tested in nerve regeneration. For example, conduits made of collagen, PGA, PLLA, and PLCL have been examined.[150] In addition, acellular matrices obtained from the decellularization of donor nerve tissue have also been successfully applied in nerve regeneration.[151] Successful results using these conduits have been reported clinically.[152] These results suggest that biodegradable conduits are a promising treatment for promoting the repair of motor nerve defects. More importantly, the results demonstrated that the axon regeneration assisted by biodegradable materials is similar to that achieved by autologous grafts. Many of these materials are now approved by the US Food and Drug Administration for human application, and they are frequently used in other clinical treatments.

Another key to reestablishing the innervation of a muscle is the formation of a neuromuscular junction between the motor neuron and the target muscle fiber. Experimentally, it has been shown that in coculture of myotubes and neural cells, neuromuscular-like junctions could be generated.[153] This indicated that the regeneration of neuromuscular junctions might be possible. Nonetheless, until now, the development of neruomuscluar junctions using tissue engineering strategies is still under investigation. It is well known that the formation of the neuromuscular junction is affected by numerous factors, such as chemotropic and electrical stimulation,[154] but it is not yet known how these stimulatory factors should be delivered. It is critical that these factors are delivered in a proper temporal and spatial manner for the successful establishment of a functional neuromuscular junction. Elucidation of this process is the next step in investigating the factors involved in complete regeneration of muscle innervation. It is likely that this process will require application of appropriate developmental and trophic factors, as well as the application of electrical, chemotactic, and mechanical stimulation.

43.4 Conclusions

Tissue engineering techniques have the potential to revolutionize reconstructive surgery, including facial reconstruction. The ability to generate new tissue structures that are genetically matched to each individual patient would render the current concerns in organ transplantation, such as donor shortages and the need for immunosuppressive therapy, obsolete. Some engineered tissues and organs, such as skin substitutes and urinary bladders, have already been introduced to the clinic, and the design of new tissue engineering approaches that one day may restore the original architecture and function of other, more complex tissues is still underway. However, although many advances have been made in the field to date, there are still numerous challenges that need to be addressed before the use of engineered tissue can be made a reality in facial reconstruction. In order to engineer fully functional facial structures, further research into the fundamental mechanisms of cellular interaction within facial tissues, including skin, muscle, cartilage, and bone, must be performed. In addition, the developmental biology and the intricate interactions between cells, scaffolds, and growth factors must be defined in order to generate the complex composite tissue structures required for facial reconstruction. In addition, adequate oxygen and nutrients to a newly implanted engineered tissue construct is critical, and this might be accomplished either by designing novel oxygen-generating biomaterials or by accelerating angiogenesis through the use of angiogenic growth factors and cytokines. Importantly, the mechanisms governing the establishment of new nerve signaling pathways between the host tissues and the implant

must be studied in more detail, as proper innervations are required for the facial structures to work together to provide natural movement and facial expression.

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