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Tissue engineering in plastic and reconstructive surgery: fostering advances in the 21st century via an understanding of the present state of the art and future possibilities

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Tissue engineering is a subfield of regenerative medicine that has been hailed as the most cutting-edge medical and surgical achievement to date. Tissue engineering aims to restore or construct whole tissues that have been lost due to congenital disabilities, trauma, or surgery. Tissue engineering is based on the premise of obtaining mesenchymal stem cells that can be used to create an embryologically comparable organ. To regenerate an organ that resembles the intended tissue to be replaced, a complex synergistic interplay between stem cells, signaling molecules, and scaffold, is required. Tissue engineering in plastic surgery is expected to reduce surgical morbidity by integrating cell signals or bio-artificial components taken from the patient's cells, which may replace damaged bodily tissue without the need for extensive reconstructive surgery. With the advent of 3-dimensional printers for modeling scaffolds and current tissue engineering methods for the regeneration of muscle, bone, and cartilage in the laboratory, the scope of tissue engineering is no longer confined to cells and scaffolds, but also encompasses growth factors and cytokines. Although these methods seem promising, clinical success has been limited to essential tissue regeneration, with considerable difficulties remaining to overcome. This paper aims to introduce readers to tissue engineering's existing breadth, regeneration processes, limits, and prospects.

Keywords Stem cells / Regeneration / Tissue engineering / Tissue scaffolds

INTRODUCTION

The artificial regeneration of tissues, organs, or even more complex living organisms was an age-old matter of fallacy and speculation. With the concept of replacing damaged body parts with normal tissue, medical science has evolved from tissue replacement to tissue regeneration [1]. With advances in technology, it is possible now to engineer hard and soft tissue, which forms the building block of the term "tissue engineering."

The concept of tissue engineering was first introduced by W.T. Green, a pediatric surgeon, at Boston Children's Hospital in the early 1970s. Green performed a series of experiments focused on inducing cartilage from chondrocytes seeded in bony spicules. Since then, several significant experiments around the globe contributed to advances in tissue engineering. It was not until 1993 that Langer et al. [2] defined the term "tissue engineering" as "an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, improve tissue function."

The basic idea of tissue engineering is that a clinician could re-

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generate lost tissue, including bone, skin, mucosa, tendon, cartilage, or an organ as a whole. With this idea, the founding of the Tissue Engineering and Regenerative Medicine International Society in 2003 marked a significant milestone and opened up a platform for scientists across the world to share and collaborate on their research [1,3]. Tissue engineering is a combination of multiple disciplines, as it involves concepts of medicine, genetics, and engineering [4]. The key concept underlying tissue engineering is based on the patency of stem cells; in simple terms, stem cells can be defined as cells that can transform into many different types of cells in the body [5,6]. If stem cells are functionally coordinated and regulated in line with physical demands, it is possible to regrow the desired tissue in the laboratory. Keeping this in mind, plastic surgeons quickly recognized this technology's potential and have found its implications in almost all branches of plastic surgery.

This paper discusses the applications of tissue engineering in the oral and maxillofacial field, the primary mechanisms of successful regeneration, limitations, and future perspectives on tissue engineering.

PRINCIPLES OF TISSUE ENGINEERING

Tissue engineering is a highly organized process that proceeds along the three well-known steps of inflammation, proliferation, and remodeling [7]. The principles of tissue engineering are based on a complex interaction of the triad of stem cells, signaling molecules, and the scaffold or extracellular matrix. When these three components are placed *in vitro* surrounding tissue-engineered constructs, the extracellular matrix forms a natural scaffold [7,8]. This scaffold is placed in such a fashion that it can organize the cells in a three-dimensional (3D) fashion. This combination of cells, signals, and the scaffold thus forms the "classic" tissue engineering triad [7-9].

Stem cells

Stem cells are multipotent cells that have an inherent capability to differentiate into specialized tissues such as bone, cartilage, muscles, nerves, and vasculature in an appropriate environment. Research has revealed that these cells tend to have significantly more potent therapeutic effects when their linked anti-inflammatory, trophic, paracrine, and immuno-modulatory capabilities are engaged [7,9,10]. A growing amount of research indicates that mesenchymal stem cells (MSCs) have the ability to modulate the immune system and exert pro-angiogenic activity, both of which are helpful for tissue regeneration. MSCs secrete numerous immune-modulatory cytokines, which disrupt dendritic cell and T-cell activity and result in the creation of a local immunosuppressive milieu [11-13]. Stem cells acquired from culture must be coupled with the proper carriers before transplantation. They offer a 3D framework on which transplanted progenitor cells may develop and eventually form a bone or marrow organ, allowing the establishment of a vascular bed. Several appropriate materials are being developed and improved, including polyglycolic acid (PGA) and polylactic acid (PLA), as well as synthetic hydroxyapatite/tricalcium phosphates [12,14,15].

Scaffolds

Scaffolds constitute a biocompatible 3D matrix that provides stem cells with optimum conditions to migrate, proliferate and differentiate [16]. Apart from this, scaffolds also permit the transportation of vital nutrients, oxygen, and cellular metabolic residues, which are very much required for successful tissue engineering [15,17].

An ideal scaffold should have the required properties such as biocompatibility, biodegradability, good mechanical properties, and a porous structure. Biocompatibility is a critical characteristic because it averts immunological and inflammatory reactions by the host, which may hamper the biomaterial's regenerative performance [16,17]. In addition to this, a scaffold should be biodegradable, meaning that cells of the body can degrade the matrix when it is no longer needed. Moreover, a scaffold should have also optimum mechanical properties with osteoconductive and osteoinductive features to aid bone formation. The process of bone regeneration employing bone tissue engineering technology greatly benefits from the osteogenic properties of scaffold biomaterials. In particular, the scaffolds used in large bone tissue engineering can mimic the intrinsic structural properties of bone tissue. In order to support cell division and differentiation as well as tissue regeneration, the networked pore structure can carry nutrients and remove waste. However, developing materials that can fulfill all these criteria continues to be a challenge in tissue engineering. Of fundamental importance is that scaffolds must have optimum pores with adequate size and dispersion ability, since these properties aid in cell penetration, diffusion of the nutrients needed by the cells, early vascularization of the site, and the transport of residual products out of the matrix [14-17].

Several substitutes of both natural and synthetic origin are candidates for an ideal scaffold. A natural-origin matrix is composed of collagen fibrils and hyaluronic acid, while synthetic scaffolds consist of PLA, PGA and its poly-copolymer (poly(lactic-co-glycolic) acid; (PLGA) [17,18]. For bone regeneration, the most commonly used substitutes are beta-tricalcium phosphate and biphasic calcium phosphate [16,18,19].

However, when reviewing the literature, the evidence indicates that synthetic-origin matrices have shown higher efficacy than the natural types. A possible explanation for this is the freedom to control mechanical, physical, and degradation properties when fabricating a synthetic matrix.

Signaling molecules

Signaling molecules or growth factors are extracellular proteins that signal cells to coordinate cellular growth, migration, adhesion, and

differentiation. These are highly regulated molecules that bind to specific receptors in the cell membrane [20]. The rationale behind adding growth factors is that it promotes adequate vascularization, which enables the delivery of oxygen and nutrition. Various signaling molecules can be used in tissue engineering, including the following [20,21]: (1) bone morphogenetic proteins (BMPs); (2) hedgehog proteins; (3) fibroblast growth factor; (4) interleukins; or (5) tumor necrosis factor and vascular endothelial growth factor.

Among these, the BMP superfamily (including rhBMP-2 and rhBMP-7) has been extensively studied and applied for clinical use. These molecules induce ossification by activating mesenchymal cells from nearby tissues and regulating their differentiation toward bone-forming cells [17,20-22].

BONE

Bone reconstruction secondary to oncological resection, trauma, and congenital deformity in the craniofacial region is a challenging procedure in technical and functional terms [23]. Reconstruction with an autologous bone graft remains the gold-standard modality as it has withstood the test of time [23,24]. Nonetheless, donor site morbidity has always been an issue with autologous bone grafts. Tissue engineering is an alternative option that combines the benefits of the autologous graft with no requirement for secondary interventions. The most commonly applied scaffolds for bone regeneration are calcium/phosphate bioactive ceramics and polymer-based scaffolds because of their excellent osteoinductive properties and optimum pore size [24-26]. In terms of growth factors, BMP-2 has shown a high rate of clinical success both in ectopic and orthotopic bone healing, and was the first growth factor to be approved by the Food and Drug Administration (FDA) for wide applications in maxillofacial surgery [24,26,27]. Recently, isomers of BMP have been extensively investigated as a way to counteract the cost of traditional BMPs. Osteogenic cells such as pluripotent stem cells are harvested from the patient and seeded into a scaffold, which is then implanted into a patient to form a tissue engineering construct [25]. The most crucial factor determining the procedure's success is the degradation of the scaffold at the same rate of bone growth to impart adequate strength to the tissue-engineered construct [25,28]. In recent studies, bioreactors have been used to achieve additional growth in terms of the size and volume of tissue-engineered bone grafts. Clinical applications in craniofacial surgery include the reconstruction of mandibular continuity defects, maxillary sinus augmentation, and reconstruction of the maxillary alveolar cleft [1,5,11]. Early in 2004, Warnke et al. [29] were the first to reconstruct a 7-cm mandibular continuity defect in a 56-year older man using BMP-7. Evidence of bone growth was visible in subsequent radiographs, but the formed bone became infected and ultimately led to the failure of the graft. Multiple studies in the literature have pointed towards early success in terms of graft incorporation. Still, in the long run, grafts have always failed with the formation of more fibrovascular tissue and less new bone formation [2,3,30]. This has led to a more pragmatic approach. Researchers have increased the success rate by using autologous bone as a precursor and mixing it with a recombinant human BMP substrate [26,30,31]. New perspectives have been attained in cell-based tissue engineering, as confined or small maxillofacial defects have been regenerated. Maxillary sinus augmentation, alveolar ridge augmentation, and increasing the height of the posterior maxilla are a few examples of cell-based tissue engineering. Studies have shown favorable reports of clinical success in radiographic bone mineralization and superior outcomes [29,32]. Autologous bone was used symbiotically with tissue engineering, with the soaking of periosteal and osteogenic cells in polyglactin-910 fibers for use as an autologous cell polymer transplant during implant placement [30,31]. However, the clinical efficacy in terms of long-term success was not impactful enough to stand against the traditional approach of bone reconstruction, and this method warrants further short- and long-term clinical trials.

CARTILAGE

Cartilage regeneration is often challenging because growth is highly variable and cartilage may rapidly differentiate into bone [33]. In the maxillofacial region, tissue engineering could be promising for successful temporomandibular disc regeneration and repair and nasal cartilage reconstruction [33-36]. The articular disc of the temporomandibular joint is made up of fibrocartilage, which is unique relative to the hyaline cartilage of the knee and other complex joints. Two approaches have been considered to regenerate the articular disc: with and without a scaffold [34,37]. A bioreactor with dynamic loading and hydrostatic pressure can be applied to stimulate cell differentiation and growth, but the literature on the use of bioreactors remains uncertain. The available growth factors for the regeneration of the articular disc are the committed chondrocytes, dermal fibroblasts, embryonic stem cells, and MSCs; of these, MSCs are extensively used in clinical studies and have shown promising results [34,35,38]. Embryologically, the articular disc grows under optimum stress and strain, which stimulate the growth of the condyle neonatally; these properties of the cartilage pose the most significant challenge, as optimizing the size and shape of cartilage during regeneration is highly variable [36]. Internal derangement of the temporomandibular joint occurs when the articular disc loses its integrity to the articular eminence and the superolateral portion of the condylar head. The current management strategies never promise to restore the integrity of the disc to normal. Hence, however finessed surgery could be, complications will always occur in the future. Tissue engineering is a promising alternative for regenerating the articular disc [34,37]. The most common cell source for regeneration is the costal cartilage because of its relative abundance and minimal donor site morbidity. Multi-

ple animal studies have concluded that resorbable PLA infused with adipose tissue-derived MSCs has promising outcomes for disc regeneration [38]. Another *in vitro* study by Vapniarsky et al. [37] on minipigs concluded that treatment with tissue-engineered implants restored disc integrity up to 4.4-fold and induced 3.2-fold intra-laminar fusion.

Regeneration of the nasal cartilage is perhaps the second most crucial scope of tissue engineering in the craniofacial region, both in aesthetics and function. Nasal cartilage requires reconstruction secondary to craniofacial anomalies, oncological resection, and severe naso-orbital-ethmoidal trauma. Nasal cartilage, primarily hyaline cartilage, is characterized by an abundance of type II collagen content. The septal cartilage is an ideal cell growth source for engineered nasal cartilage because of its optimum mechanical and anatomical properties, with an abundance of type II collagen protein in the central zone [35,36,39]. A new paradigm has recently emerged that supersedes the traditional tissue engineering approach (i.e., cells, scaffolds, and stimuli). In the newer approach, scaffold-free engineering has been considered, wherein neocartilage is hypothesized to be regenerated by only cells from the septum cartilage and growth factors such as MSCs supplemented with insulin-like growth factor 1 and basic fibroblast growth factor, which showed histological staining of type II collagen fibers in the neocartilage [36]. Perhaps the most successful evidence came from the first human trial by Fulco et al. in 2014 [39]. The researchers recruited five patients secondary to oncological reconstruction, reconstructed nasal cartilage with engineered cartilage grafts, and concluded that the outcomes were satisfactory both in terms of aesthetics and function after a 1-year follow-up [39].

SALIVARY GLAND

Dysfunction of the salivary glands and xerostomia are common after resection and radiation therapy for head and neck cancers. These problems require maxillofacial surgeons to devise permanent solutions to the side effects of surgery. Tissue engineering could be a possible alternative, where salivary glands can be regenerated through the component-by-component strategy [40,41]. Salivary glands are highly complex and organized structures with secretory functions that make regeneration quite challenging compared to bones and cartilage [40-43]. The salivary gland primarily consists of acinar, ductal, and myoepithelial cells that mandate regeneration of every cell as a prerequisite for saliva production with the correct consistency and viscosity. Furthermore, each salivary unit must be sealed with an optimum organization to maximize its functional capacity [42]. To date, entirely regenerating the salivary gland has not been possible, but a few studies have documented the successful *in vitro* creation of a 3D-branched structure of the ducts with stem cells. Research with packed hydrogel and partially differentiated acinar cells from the submandibular gland has also been carried out [41,44]. The main aim behind this approach is to make the engineered unit less complex and organized enough to perform secretory functions optimally. The next approach involves integrating biology with engineering to reinnervate salivary glands with neural and vascular bundles [44,45]. Studies on mice have shown that to stimulate neural growth from neural crest-derived MSCs and stem cells from dental pulp that were combined in a solution of gold and iron-oxide nanoparticles, further-engineered salivary glands could be induced by beta-agonists and muscarinic agonists, which might lead to the formation of the neuro-epithelial unit [42, 43]. In terms of the vascularization of salivary glands, a layer-by-layer cell coating approach has been used to create oral mucosa models in the laboratory, in which blood vessels were engineered from human umbilical vein cells. A study by Joraku et al. [45] in a mouse model concluded that when epithelial cells from the human salivary gland are cultivated using a biodegradable polyglutamic acid polymer scaffold, they showed acinar gland-like structures with the confirmation of human amylase secretion and evidence of aquaporin 5 (a water channel protein that ensures tight junctions for the optimum flow of saliva). Further research with human subjects would be necessary to validate the performance of engineered salivary glands in the long term.

NERVE REGENERATION

Inadvertent injury to neural bundles is common during maxillofacial surgery because of the intricate anatomy of the head and neck region. The degree of nerve injuries can vary on a scale from temporary paresthesia to full-blown irreversible nerve damage. Although nerve injuries can have many possible etiologies, iatrogenic injury during the surgical procedure and high-impact trauma are more prone to cause total nerve degeneration beyond repair, which necessitates an autologous nerve graft at present. With tissue engineering, successful nerve regeneration might be possible, which would obviate the possible complications of donor site morbidity and graft rejection [46,47]. Schwann cells and the extracellular matrix have the potential to regenerate in the peripheral nervous system after trauma. Schwann cells act as a scaffold for regenerating axons that grow through empty basal lamina tubes. Although nerve regeneration from Schwann cells seems feasible, harvesting of Schwann cells itself is a challenging task because of the clinically questionable survival rate and morbidity to the harvested nerve [46-48].

On the contrary, adult stem cells, bone marrow-derived MSCs, and adipose-derived stem cells have shown promising results *in vitro* for differentiation into Schwann cells [49]. In a study on animals, the authors concluded that adipocyte stem cells could differentiate into a Schwann cell-like phenotype, expressing markers such as S-100, glial fibrillary acidic protein, and P75 neurotrophin receptor, and accelerating neurite outgrowth in an *in vitro* co-culture model [50]. Successful coaptation of a nerve gap at the distal end

hinges on the genesis of a neo-extracellular matrix scaffold, over which blood vessels, fibroblasts, and Schwann cells can migrate and progress towards the distal nerve stump for regeneration, with formation of the myelin sheath [47,51]. However, in more extended nerve defects, creating an optimum environment for the extracellular matrix is challenging, and failure occurs because the nerve ends are unable to provide a continuous layout over which regenerative elements can migrate [52]. Most studies have implied that the successful regeneration of peripheral nerve ends over a period between 4 weeks to 12 weeks, with adipocyte-derived stem cells as an ideal choice for stem cells in shorter defects [46-52].

ADIPOSE TISSUE ENGINEERING

Volumetric soft tissue loss in the craniofacial region occurs secondary to oncological resection, trauma, poorly performed facial cosmetic surgery, and congenital craniofacial syndromes. To date, an ideal filler material has not been achieved, and one of the main complications of autologous fat transfer is the volumetric resorption of the graft because fat grafts are obtained from distant sources, such as the medial and lateral thigh, abdomen, and flanks [53,54]. To circumvent the problem of resorption and donor site morbidity, engineering of adipocyte cells in culture seems feasible. Preadipocytes, which are precursors of adipocyte cells, can differentiate into a large number of cells, making it possible to obtain the tissue of interest. Preadipocytes with endothelial cells are cultured from the enzymatic degradation of fat cells during fat biopsy or liposuction [53,55]. Preadipocytes are cultured over an absorbable scaffold made of polyester-based adsorbable material, hyaluronic acid, collagen, and polyethylene glycol. Then, together with injectable microcarrier beads and growth factors, preadipocytes with the scaffold are injected into the soft tissue defect. With stimuli from growth factors, preadipocytes transform into mature adipocytes and can be identified by histological staining and fluorescence labeling [56]. One of the main problems currently facing tissue engineering of adipocytes is forming a vascular network. Growth factors do assist in neo-angiogenesis, but the duration is short, and it impedes further diffusion of nutrients across the channels [57]. Vascularization before implantation could be a possibility, but there is not enough evidence to prove the successful outcome of this technique. In a clinical study, adipose stem cells were used to reconstruct a 10-cm anterior mandibular defect. The authors concluded that adipose stem cells in combination with β-TCP granules and BMP-2 led to successful outcomes in treating significant mandibular defects [57]. A similar result was achieved by Mesimaki et al. [58] in reconstructing maxillary defects with ectopic bone formation. After 8 months of follow-up, the authors concluded that grafted flaps had developed mature bone and neo-vasculature with successful postoperative healing. The scientific community has yet to address numerous factors and challenges to develop vascularized adipocyte lineages that will maintain their volume and form over an extended time.

SOFT TISSUE ENGINEERING

In the craniofacial area, regeneration of the lips and oral mucosa is the prime focus for maxillofacial surgeons. A relative shortage of local flaps and the complex structure of lips, which are composed of mucosa, skin and muscles, makes reconstruction surgery highly challenging. Reconstruction of lips with a distant flap secondary to trauma, tumor resection, and congenital anomalies remains a mainstay mode of treatment [59,60]. Distant flaps have the disadvantages of poor color matching and texture, which compromise the aesthetic unit and functional outcomes (sucking, chewing, and creating an optimum mucocutaneous seal) [59,61]. Tissue engineering offers an alternative approach, where the *in vitro* tissue development of an *ex vivo* produced oral mucosa equivalent can be constructed; this is popularly known as an EVPOME model. The steps involved are harvesting keratinocytes from non-keratinized oral mucosa and skin and culturing them in chemicals to increase the number of cells with the desired signaling molecule (0.06 mM calcium) [62-64]. The obtained keratinocytes are seeded over full-thickness skin after decellularizing a human cadaver or autogenous donor site with a barrier to separate oral and skin keratinocytes by creating a cell-free zone. The barrier is left overnight by culturing in serum with a 0.06 mM calcium concentration, allowing the migration of cells between the skin and oral mucosa to form the basic anatomy of the lip [65]. The final step involves culturing the obtained structure in 1.12 mM calcium for at least 10 days in an air-liquid phase, then integrating it onto a donor site to allow the growth of complex units (muscle, nerve and blood vessels). The EVPOME construct has successfully cleared phase 1 clinical trials by the FDA, and more subjects are being recruited for phase 2 and phase 3 clearance. In an *in vitro* study by Izumi et al. [62,63], the authors claimed that through their experiments, one-third of the linear dimension of an upper human lip was achieved by an EVPOME construct with the scope of further refinement to engineer the entire complex unit of the upper and lower lip. Another study by Bayar et al. [60] proposed a technique where premixed keratinocytes of different ratios from oral mucosa and skin were used to create a mucocutaneous junction with a cell-free zone for a future vermillion border. Through future research incorporating advanced technology, it will be possible to create fully-functional lips and other multi-tissue units in the future.

Chronic wound healing

In the craniofacial area, the incidence of chronic wounds or wounds that fail to heal is rare because of the rich vascular supply in the head and neck region. However, wound healing can often be complicated by conditions such as hyperglycemia, long-term steroid therapy,

and old age. Tissue engineering can offer a promising scope to manage such cases where the lack of neovascularization delays wound healing [66,67]. On a microscopic level, the cell population that contributes to new blood vessels formation includes endothelial progenitor cells, a type of bone marrow-derived cells that help in blood vessel formation after cutaneous trauma [68]. Apart from this, chronic wounds lack growth factors and exhibit an unorganized extracellular matrix with a compromised blood supply. The decreased levels of keratinocytes, endothelial cells, and platelets further compromise the proliferative and remodeling stages of wound healing [66-67]. The concept of tissue engineering lies in the fact that it creates an optimum environment for wound healing by local delivery of required exogenous cells and quickly replenishes diminishing growth factors [69,70]. Clinical success has already been achieved in treating cases such as long-standing diabetic foot ulcers and reconstruction secondary to burn trauma with the decellularized dermal matrix. The complex and coordinated signaling cascades of wound healing rely heavily on endogenous stem cells [71]. The adnexal structures, notably the hair follicle bulge stem cells, constitute the most-documented epidermal stem cell population and are the most numerous skin stem cells. Most cells were found to be stem cells, comprised of bone marrow, adipose-derived stem cells, umbilical cord stem cells, and Wharton's jelly MSCs (81.9%). Fibroblasts were identified as the second most common cell type (7.1%) [66,72]. From the standpoint of wound healing, stem cells have the capacity to address biological inadequacies in chronic wounds, allowing full skin regeneration, including the restoration of skin appendages. Stem cells are administered to the wound by topical spray, direct injection, systemic distribution, and cell-seeded scaffolds after being collected [66,68,73]. Cell interactions with the natural scaffold may drive and impact cell activity within tissues. It is crucial to determine which microenvironmental components can effectively convey cells to an injury site and instruct them to help with tissue repair and regrowth [69,71].

VASCULARIZATION STRATEGIES FOR TISSUE ENGINEERING

Tissue engineering's main goal is to create tissues that can be used instead of donor tissue to replace or repair damaged tissues. A robust vascular network within the tissue construct is required for the successful engineering of each specialized tissue to enhance oxygen transfer, provide nutrients, and eliminate metabolic waste [72]. A vascular network is also necessary for promoting immune cell circulation and supplying healing and growth factors to the newly implanted transplant. In the vascular system, there are three different structures. The macro-vessels (arteries and veins) branch out into two micro-vessel types, (arterioles and venules), and then into capillaries [72-75]. Several methods for improving vascularization are presently being researched. *In vivo* prevascularization, scaffold functionalization, and *in vitro* prevascularization using microfabrication techniques to create microchannel networks that serve as templates for subsequent populations of endothelial cells result in the formation of vascular networks within engineered tissue constructs [72,75-77]. In engineered tissue, an ideal vascular network must have certain features. One of the most important functions of a vascular network is to ensure that all cells in a tissue receive enough nutrition. This indicates that all of the cells must be within 200 μm of a vessel, which is typically considered the oxygen and nutrient diffusion limit inside a tissue [78]. The engineered vascular network should be organized as a vascular tree, with larger vessels budding into smaller vessels, which sooner or later branch into capillaries that are distributed throughout the tissue volume, to achieve this fine distribution while minimizing the pressure required for blood flow [76,78]. Aside from that, the vascular network should establish a selective barrier that controls the flow of materials from the vessels to the surrounding environment.

There are four essential techniques used in tissue engineering and tissue regeneration to tackle vascularization difficulties, as discussed below. First, *in vivo* techniques include the flap technique, which involves transferring allogeneic vascularized tissue units from one donor site to another to restore damaged tissue, and the arteriovenous-loop technique, which involves incorporating a vascular conduit in a non-vascularized tissue chamber connected to an artery and vein to propagate vascularization within the tissue [77]. Second, scaffold functionalization procedures focus on improving the scaffold's architecture to increase oxygen and nutrition delivery to the cells and infusing angiogenic growth factors and/or cells into the scaffold structure [78]. Third, cell-based approaches, such as cell sheets and cell spheroids, use scaffold-free structures created by allowing cells to self-assemble via cell–cell and cell–extracellular matrix interactions for utilization as building blocks to generate larger tissues [79]. Fourth, bio-fabrication methods allow various structures to be generated ahead of time and inserted into not-yet-polymerized hydrogels utilizing sacrificial molding. Perfusable hollow structures may be created by removing the sacrificial structure from polymerized hydrogels, which can then be seeded with vascular cells. By exposing a photoresist to ultraviolet light via a photomask, photolithography allows the creation of tiny structures with very high resolution. An elastomer is put on the prepared mold, cured, and then peeled away from the stamp using soft lithography [65]. Various procedures and biocompatible materials may be utilized in 3D bioprinting to include live cells in predetermined spatial areas [64,65].

TISSUE ENGINEERING IN PLASTIC AND RECONSTRUCTIVE SURGERY

Breast reconstruction

Breast reconstruction following a mastectomy is an integral com-

ponent of treating breast cancer. The current standard of care for breast reconstruction involves either an autologous tissue flap or an implant-based (silicone or saline) treatment. However, both have the potential to cause further morbidity through flap necrosis and failure [80]. The goal of tissue engineering is to employ autologous cells to multiply and replenish the desired tissue, which could address the long-term issues related to the conventional approach [81]. Cell-assisted lipotransfer scaffolds are now either biological or synthetic. In animal studies, collagen-based scaffolds have produced substantial amounts of adipose tissue from the surrounding tissue as well as endogenous generation of growth factors necessary for adipogenesis and angiogenesis, yielding encouraging results among biological scaffolds [82]. In contrast, tissue decellularization is a solution that eliminates the cellular component, leaving only the extracellular matrix to build a scaffold. The significant limitations of biological scaffolds include rapid enzymatic and hydrolytic degradation and robust immunogenic response *in vivo* [83]. A previous study [83] used synthetic scaffolds made of hydrogel, and a poly(D, L)-lactide polymer scaffold was designed and manufactured using a 3D printer. Compared to biological scaffolds when priming with cell and tissue components, the major drawback of synthetic scaffolds is the lower cellular affinity to the synthetic material. It has been hypothesized that this disadvantage could be overcome by combining a synthetic polymer's core functionality with a biological matrix to engineer a hybrid scaffold, but this approach has met with limited success [84]. Although considerable achievements have been accomplished in refining scaffolds, stem cells, and precursors for tissue engineering, the problem lies in vascularization. There is emerging evidence for the co-development and co-regulation of adipose tissue and the blood arteries that supply it. A significant breakthrough in angiogenesis has been made in animal research. This will pave the way for future replication in human research to ensure the best possible outcomes in breast reconstruction post-mastectomy.

Burn reconstruction

Burn wounds are a catastrophic type of skin injury with dire implications for patients and the healthcare system. The most common type of induced injury is thermal burns, followed by surgical wounds and, less frequently, radiation burns. Depending on the severity of the insulting factor, a burn may involve only the epidermis or all three layers of skin (epidermis, dermis, and hypodermis) [85]. Skin grafting, skin replacements, and negative-pressure wound therapy are among the clinical procedures used to treat burn wounds. Operations for autologous skin transplantation usually follow the early removal of necrotic tissue from burn sites [86]. Unfortunately, the grafted areas have been known to leave a scar with a subpar aesthetic outcome and functional issues such as stiffness and rigidity. In the domain of wound healing, tissue-engineered skin substitutes hold enormous promise, especially in light of the limited supply of autologous skin [87]. By encouraging primary differentiation into skin tissue structures and interacting with adjacent cells to foster a more conducive environment for regeneration, stem cells can contribute significantly to tissue-engineered burn treatment [88]. Mesenchymal stromal cells are far the most broadly utilized type of stem cells for *in vitro* experiments of burn wound rehabilitation. Animal studies have indicated the ideal dose range of MSCs, and an unexpected finding was reported that excised full-thickness burn wounds could be healed with only a modest dose of 200–40,000 cells/cm^2 [89]. With its mechanical and cell-adhesive qualities, collagen is a naturally occurring protein that forms a crucial part of the skin's extracellular matrix. Collagen is frequently employed as a scaffolding material. In order to improve skin regeneration, composite scaffolds can combine the benefits of many biomaterials and make up for the drawbacks of individual materials [90]. The ability to create anatomically similar skin constructs, which can replicate the key characteristics and physiological functions of human skin, has recently been made possible by advances in fabrication technologies such as 3D bioprinting. These constructs can be used as a test model for novel skin substitutes or as a basis for developing therapeutic products [87,90]. A single intraoperative step could turn this technology, which was found to be practical and well-tolerated in experiments on mice, into a clinical treatment for people with severe burns. In preclinical models of burn wounds, it is clear that the application of stem cell-based tissue engineering techniques, complemented by biomaterials to aid skin restoration, has exhibited encouraging results, where full-thickness burns have been healed [91]. Tissue-manufactured skin has come closer to becoming a reality for the therapeutic treatment of full-thickness burns by combining cross-disciplinary breakthroughs in synthetic biology, incorporating stem cells, improved biomaterials, fabrication techniques, and nanotechnological innovations.

Face allotransplantation

The reconstruction of craniofacial deformities caused by congenital abnormalities and high-velocity trauma is complicated and frequently has poor results. In recent years, facial transplantation has been used to address the shortfall of autologous grafts; however, locating human donors and long-term immunotherapy diminish the advantages of the procedure overall.

Nonetheless, given the ability to prevent immunosuppression and circumvent the lack of human donors, the prospective benefits of transplanting face features created outside the live body are quite significant [92]. Using autologous stem cells extracted from the recipient's nasal septum, it has been possible to engineer face features *in vitro* for eventual transplantation by implanting grafts of the alar nasal lobule [92,93]. Tissue engineering will likely aid in the short-term repair of specific face abnormalities.

Recent studies have shown that extensive facial deformities, including the loss of the skeletal framework, may be successfully re-

paired using composite tissue allotransplants, which contain bone, muscle, nerve, and skin and can restore both form and function.

To create a new field of face reconstruction, the benefits of both tissue engineering and maxillofacial composite tissue allotransplantation should be merged. A flawless hard framework of tissue-engineered bone, cartilage, and teeth may ensure appropriate occlusion [94,95]. However, composite tissue allotransplantation might enable a soft tissue covering that autologous tissue cannot provide.

CURRENT CHALLENGES

There is no question that extensive tissue engineering research has been carried out over the past three decades with considerable results. Still, only a few tissue-engineered products, such as human skin, have reached the level of clinical trials or applications. There is a pressing need to build cross-disciplinary collaboration and funding possibilities [93,96]. In the future, health professionals anticipate shifting the paradigm from tissue repair to tissue regeneration for therapeutic applications. Tissue engineering is a multidisciplinary field that combines health science, engineering, and fundamental scientific fields. The scientific community has adequately addressed this subject, resulting in the broad collaboration of many research organizations throughout the world that are working together to attain their aims [89]. Another reason that makes tissue engineering difficult is the complexity and lack of scientific information regarding complex facial tissues. We still have many questions about biomimetic approaches and how these tissues are made in nature. One of the largest non-scientific hurdles in performing stem cell research is ethical issues; nevertheless, the type of ethical problems differs by geographical location. The following questions constitute just a few examples: What kind of cells should be used? What cell source should be employed, and how should researchers and collaborators handle cells? Whether embryonic stem cell research should be permitted is debatable [89,97]. Another non-scientific yet important challenge for translating technology is the cost efficiency of tissue engineering products. Once these custom-engineered items are provided to end consumers, an industrial element must be addressed as well, such as regulatory approvals and methods for preservation, storage, and distribution [89,98]. Furthermore, factors like shelf life, safety, and handling must be considered. It will be critical to pay close attention to the end user's expectations (the patient). Once these devices are in use, health professionals may get helpful input from patients about their happiness to dramatically enhance the quality and functionality [93,99]. Scaffolds are one of the most important parts of a tissue engineering strategy because they interact directly with the generated cells and tissue. Mechanical parameters (e.g., hardness and elastic modulus), physical properties (e.g., porosity and surface area), and biological properties (e.g., biocompatibility and degradation rate) must all be matched to real tissue. Another problem to address is sterilization, which is critical since no cellular tissue or biomaterial that has been infected or contaminated may be employed in the body [98]. Maintaining a sterile environment during the tissue engineering process is difficult since it might take months to create dental tissue. Sterilization procedures that can sterilize tissue modified during synthesis and shelf life must be regulated [100]. The management of patients will need significant upgrades to healthcare facilities.

FUTURE PERSPECTIVES

New methods for preparing materials or scaffolds, such as 3D printing and microfluidics, have received extensive interest as tissue engineering progresses. Cell sheets and genome editing have also been extensively investigated to create appropriate cells for tissue engineering [97,99].

Bioprinting is one of the newer techniques for creating tissue-engineered constructions. Bioprinting is a technique that involves patterning cells, biomaterials, and biomolecules to create tissue-like constructions. Lasers, droplets, and extrusion are the most common methods for the 3D bioprinting of cells, growth factors, and hydrogels. These 3D printing techniques have been used to manufacture biological substitutes with greater resolution and hierarchical structure in creative and spatial combinations of cells and biomaterials (typically in the form of gels or fibers) [100].

Another development in the field of engineering cells is in personalized tissue engineering and regenerative medicine, where cell sheet technology is a scaffold-free technique with abundant promise. Unlike standard cell treatments (cell suspension injections or cells/scaffold constructs), the cell sheet technique allows transplanted cells to remain entirely in the target areas and retain their vitality. One of the key benefits of cell sheets over other tissue engineering approaches is the lack of possible material degradation-related cytotoxicity [98,100].

Changing the genome in cells has recently become feasible because of genomic technology and genome engineering developments. Genome editing technology has advanced to the point that it can directly manipulate the genome. As a result, this approach may be used to examine the functional impact of genetic variations in a systematic manner [98]. The fundamental problem to examine is the safety of the technology. Unexpected genetic alterations, which may cause surprising physiological changes or even death in patients, are one of the safety issues facing genomic technology. Scientists are also concerned about off-target genomic and cellular processes. Furthermore, mutations in critical genes may result in cancer or organ damage. As a result, genomic technology safety evaluations must progress in lockstep with new technologies.

Various cell microencapsulation methods have been developed during the last two decades. Microfluidic devices or materials might be changed to create cells encapsulated in microbeads or microfibers. Microfluidic technology's versatility enables the creation of

multi-structured fibers, such as hollow areas, multiple layers, grooves, and so on [97,99].

It has proven difficult to bridge the gap between discoveries in 2D cell culture *in vitro* and 3D tissue culture conditions *in vivo*. The creation of *in vitro* models in tissue engineering and regenerative medicine (i.e., organs-on-a-chip) is also aided by 3D printing and microfluidic devices [99]. To better imitate the milieu of blood vessels, these 3D hydrogel-based vascular architectures might be incorporated into organ-on-chip systems. This would enable capillaries to form in an ideal setting for early angiogenesis, increasing the viability of the designed organ [100].

CONCLUSION

The area of tissue engineering has advanced rapidly in general, as seen by the massive growth in the number of research studies on issues such as material scaffolds, cells, and bone inductive cues. However, a moment of contemplation is also necessary. One finding is that only a small percentage of techniques make it to the clinical trial level. The degree to which potential scientific findings are transferred into human research is of great interest. Furthermore, increased interactions between disciplines would have benefits for promising human tissue engineering strategies, clinical efficacy, and patient safety, for example, by mobilizing clinicians in the early stages of research and development and providing measures for material scientists and bioengineers to move into the environments of biology, medicine, and healthcare. This would necessitate the involvement of healthcare systems that possess the appropriate expertise, infrastructure, and resources to critically evaluate complicated regenerative products.

NOTES

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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