Cell–scaffold interactions in tissue engineering for oral and craniofacial reconstruction

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A R T I C L E   I N F O

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A B S T R A C T

Tissue engineering (TE) is critical in oral and craniofacial reconstruction. One of the most popular topics on the biomaterial-based tissue regeneration process may be the interaction between cells and scaffolds. An increasing number of studies have identified the variables affecting cell–scaffold interaction. The creation and investigation of new scaffolds for TE and regenerative medicine based on specific interactions have become possible owing to these findings. This review discusses the effects of various types of scaffold materials on cells in TE. Because the intrinsic properties of scaffolds are essential, the influence of the physical, chemical, mechanical, and biological characteristics of scaffold materials on cell–scaffold interaction that has been discovered in recent research is elaborated in this review. The components carried by scaffolds, the degradation process, and the role of degraded products in cell–scaffold interactions are examined. Further, the roles of cells, including cell types, functions, and adhesion mechanisms, and extracellular matrix are discussed. Finally, the latest research progress on cell–scaffold interactions among various engineered tissues or organs in the oral and craniofacial region is summarized. A deeper understanding of cell–scaffold interactions is anticipated to benefit the development of TE and regenerative medicine.

1. Introduction

The oral and craniofacial region, an integral part of the human body, performs functions, such as mastication and aesthetics. Numerous materials (including bioinert or bioactive materials) have been used to restore missing oral and craniofacial tissues. For example, recent archaeological findings indicated that dental implants were found among Mayans in the 2nd century AD [1]. From the perspective of regenerative medicine, the substitutes provided by these conventional techniques do not regenerate tissues capable of fully performing their original functions. In recent decades, tissue engineering (TE) has been introduced and widely investigated as an innovative alternative to conventional biomaterials for reconstructing the tissues and organs in the oral and craniofacial region, as shown in Fig. 1 [2]. It is a multidisciplinary science that applies biological and engineering principles to investigate and develop biological substitutes for restoring, maintaining, improving, and replacing tissues [3]. It may represent a future prototype of biomedicine.

Scaffolds, as an indispensable part of TE, are versatile in promoting cell–scaffold interactions, cell adhesion, and extracellular matrix (ECM) deposition. They also enable the transport of nutrients, wastes, and regulatory signals (RSs) to permit cell proliferation and differentiation. They are supposed to trigger minimal inflammation or cell toxicity [4]. Under certain culture conditions, scaffolds can be degraded at a controllable rate. Degraded molecules or ions that interact with cells are worth investigating. In addition, scaffolds are continuously undergoing development; they are mainly combined with growth factors (GFs) and stem cells (SCs) [5]. Notably, the application of bioactive and
biodegradable inorganic or polymer scaffolds with SCs to regenerate injured tissues can provide an opportunity to promote the repair process. SCs are essential in TE because they are capable of proliferating under appropriate conditions. A complex environment where cells thrive in vivo consists of numerous biological components and physico-chemical features that can provide a wide range of possibilities for intervention [6]. In particular, the state of the cells is crucial for imposing interactions on the scaffold material. Cells proliferate, secrete various cytokines and ECMs, and finally form corresponding tissues or organs to repair wounds and rebuild functions.

Therefore, this review surveys and summarizes the recent developments in the research on the interaction between cells and scaffolds, emphasizing scaffolds’ physical, chemical, mechanical, and biological characteristics in regulating cell behavior. The factors influencing cell interaction and the latest research progress on the oral and craniofacial region are also discussed.

2. Roles of scaffolds in interactions with cells

2.1. Types of scaffold materials

According to the source of scaffold materials, they can be classified as natural polymers, natural inorganics, synthetic polymers, and synthetic inorganics, which are shown in Table 1.

2.1.1. Natural polymers and inorganics

Natural polymers involved in TE can be mainly summarized as proteins and polysaccharides. They present low cell toxicity and relatively low inflammatory response because they were extracted from organisms. However, the complex structure of natural macromolecules issues low mechanical strength [7].

Chitosan (CS) is a typical linear polysaccharide used in TE. A recently published paper reported that CS scaffolds were fabricated and characterized for dental root TE [8]. Results revealed that the.

CS source and crosslinking agent (genipin) significantly affected the cell behavior of human dental pulp SCs (hDPSCs) and scaffold properties. However, the osteoblast (OB) compatibility of CS was unsatisfactory because its cell affinity was not as excellent as expected [9]. In this regard, gelatin (GEL) is a biodegradable protein with excellent biocompatibility, plasticity, and non-antigenicity [10]. Hence, the existence of GEL in the CS scaffolds was favorable [11]. For instance, bioglass (BG) ceramics combined with CS–GEL membranes prepared by lyophilization and containing ethanol possess the prerequisites for jawbone TE [12]. As a modified derivative of GEL, photo-cross-linkable GEL methacryloyl (GELMA) has been widely used because of its excellent biocompatibility, biodegradability, and moldability [13]. Light-curing GELMA hydrogels are adequate for improving the viability of odontoblast-like cells, representing an essential step toward regenerative dentistry [14].

Silk fibroin (SF) scaffolds could enhance the osteogenesis of mesenchymal SCs (MSCs) or heal bone defects in rat models [15]. Besides, Hong et al. fabricated chondrocyte-loaded SF hydrogels with glycidyl methacrylate (GMA) for three-dimensional (3D) printing and investigating chondrogenic differentiation in vitro and in vivo [16]. In vitro cultivation for four weeks ensures the proliferation, viability, and chondrogenic differentiation of NIH/3T3 mouse fibroblasts. Moreover, a new cartilage-like tissue surrounding SF–GMA hydrogels have been found in the partially defected rabbit cartilaginous model. Hyaluronic acid (HRA), the most common glycosaminoglycan (GAG) with the highest molecular weight, plays a vital role in the ECM, regulating receptor-driven detachment and migration [17]. Because HRA could rapidly dissolve and degrade in vivo, it has to be modified, crosslinked, or combined with another material to form stable structures supporting favorable cell adhesion and proliferation [18]. In addition, cellulose and its derivatives have also been extensively used for delivering GFs and other antibiotic agents directly to the site of the impaired bone tissue to promote tissue repair [19].

The scaffolds made of natural inorganics possess circumstantial biological or physiological functions. Hydroxyapatite (HA) is the main component of bone and teeth. However, the sintering temperature required for scaffolds is increased to effectuate the desired mechanical strength, rendering HA extremely brittle during low-temperature processing [20]. Except for inadequate mechanical strength, the low fracture toughness and slow degradation rate of HA also retain bone formation and possibly increase the risk of unexpected results, such as infection [21]. Therefore, HA is invariably combined with other biocompatible inorganics, such as ZrO₂ and Al₂O₃, to improve mechanical properties and promote bone tissue regeneration. Nevertheless, these bioinert materials may decrease the bioactivity of HA [22].
Table 1
Common scaffold materials in TE of oral and craniofacial reconstruction and their main degradation products.

| Classification                  | Abbreviation/chemical formula | Degradation products | References |
|---------------------------------|------------------------------|----------------------|------------|
| Natural polymer materials       |                              |                      |            |
| Gelatin                         | GEL                         | Polypeptide          | [10]       |
| Gelatin-methacryloyl            | GELMA                       |                      | [13]       |
| Silk fibroin                    | SF                          |                      | [16]       |
| Collagen                        | COL                         |                      | [50]       |
| Fibrinogen                      |                             |                      | [47]       |
| Elastin                         |                             |                      | [51]       |
| Keratin                         |                             |                      | [52]       |
| Actin                           |                             |                      | [53]       |
| Alginate                        | ALG                         |                      | [54]       |
| Myosin                          |                             |                      | [55]       |
| Chitosan                        | CS                          | Oligosaccharide      | [10]       |
| Cellulose                       |                             |                      | [56]       |
| Agarose                         | AGA                         |                      | [57]       |
| Amylose                         |                             |                      | [58]       |
| Dextran                         |                             |                      | [59]       |
| Fucoidan                        |                             |                      | [60]       |
| Glycosaminoglycans              | GAGs                        |                      | [61]       |
| Hyaluronic acid                 | HRA                         |                      | [62]       |
| Starch                          |                             |                      | [63]       |
| Pectin                          |                             |                      | [64]       |
| Carrageenan                     | CRG                         |                      | [65]       |
| Natural inorganic materials     |                              |                      |            |
| Hydroxyapatite                  | HA/Ca₁₀(PO₄)₆(OH)₂           | Ca²⁺, PO₄³⁻          | [21]       |
| Zirconia                        | ZrO₂                        | Zr⁴⁺                 | [22]       |
| Alumina                         | Al₂O₃                       | Al³⁺                 | [22]       |
| Mg based metals                 |                             | Mg²⁺                 | [66]       |
| Fe based metals                 |                             | Fe³⁺                 | [67]       |
| Ti based metals                 |                             | Ti⁴⁺                 | [9]        |
| Ti based metals                 |                             |                      |            |
| Synthetic polymer materials     |                              |                      |            |
| Poly (caprolactone)             | PCL                         | Caprolactone         | [68]       |
| Poly (α-hydroxy acid)           | α-PHA                       | α-hydroxy acid       | [23]       |
| Poly (lactic acid)              | PLA                         | Lactic acid          | [24]       |
| Poly (glycolic acid)            | PGA                         | Glycolic acid        | [25]       |
| Poly (γ-glutamic acid)          | γ-PGA                       | γ-glutamic acid      | [25]       |
| Poly (l-lactide)                | PLL                         | l-lactide            | [69]       |
| Poly (l-lactide-co-ε-caprolactone) | PLCL                      | l-lactide-co-ε-caprolactone | [26] |
| Poly (methyl methacrylate)      | PMMA                        | Methyl methacrylate  | [27]       |
| Polyethylene glycol             | PEG                         | Ethylene glycol      | [30]       |
| Poly (3-hydroxybutyrate)        | PHH                         | 3-hydroxybutyrate    | [70]       |
| Polyhydroxyalkanoate            | PHA                         | Hydroxyalkanoate     | [71]       |
| Poly (ether-ester urethane)     | PEEU                        | Ether-ester urethane | [72]       |
| Poly (propylene fumarate)       | PPF                         | Propylene fumarate   | [73]       |
| Poly (ester urethane urea)      | PEUU                        | Ester urethane urea  | [72]       |
| Poly (vinyl alcohol)            | PVA                         | Vinyl alcohol        | [74]       |
| Poly (vinylidene fluoride)      | PVF                         | Vinylidene fluoride  | [75]       |
| Poly (3, 4-ethylenedioxythiophene) | PEDOT                    | 3, 4-ethylenedioxythiophene | [74] |
| Poly (styrene sulfonate)        | PS                           | Styrenesulfonate     | [76]       |
| Polyurethane                    | PU                          | Urethane             | [77]       |
| Poly (D, l-lactide-glycolide)   |                             | D₁, l-lactide-glycolide | [78]   |
| Poly(anhydrides)                |                             | Anhydrides           | [29]       |
| Poly (phosphazene)              |                             | Phosphazene          | [79]       |
| Poly (vinyl alcohol)            |                             | Vinyl alcohol        | [80]       |
| Poly (N-isopropylacrylamide)    | PNIPAAm                     | N-isopropylacrylamide | [81] |
| Polypyrrole                     |                             | Pyrrole              | [82]       |
| Polyaniline                     |                             | Aniline              | [82]       |
| Synthetic inorganic materials   |                              |                      |            |
| Bioglass                        | BG                          | SiO₂, Ca³⁺, Na⁺, PO₄³⁻ | [12] |
| Dicalcium/tricalcium phosphate  | DCP/TCP                     | Ca³⁺, PO₄³⁻          | [83]       |
| Calcium silicate                |                             | Ca³⁺, SiO₄²⁻         | [84]       |
| Calcium aluminate               |                             | Ca³⁺, Al³⁺           | [84]       |
| Carbon nanotubes                | CNTs                        | CO₂                  | [45]       |
| MXenes                          | Ti₃C₂Tₓ/NbₓCTₓ             | Ti⁴⁺/Nb⁺             | [49,49] |
| Graphene                        | GP                          | CO₂                  | [46]       |
| Graphene oxides                 | GPO                         | CO₂                  | [85]       |
| Reduced graphene oxides         | rGPO                        | CO₂                  | [86]       |
| Nanofillers                     |                              |                      |            |
| nano-hydroxyapatite             | nHA                        | Ca³⁺, PO₄³⁻          | [87]       |
| nano-fluorohydroxyapatite       | nFHA                       | F⁻, Ca³⁺, PO₄³⁻       | [88]       |
| nano-zirconia                   | nZr                        | Zr⁴⁺                 | [87]       |
| nano-calcium zirconate          | nCZ                        | Ca³⁺, Zr⁴⁺           | [87]       |

(continued on next page)
2.1.2. Synthetic polymers and inorganics

Table 1 (continued)

| Classification          | Abbreviation/chemical formula | Degradation products | References |
|-------------------------|-------------------------------|----------------------|------------|
| Nano-silica             | nSi                           | SiO$_2^+$             | [80]       |
| Silver nanoparticles    | AgNPs                         | Ag                   | [90]       |
| Nano-titanium dioxide   | nTiO$_2$                      | Ti$^{4+}$             | [91]       |
| Silicon carbide         | SiC                           | SiC                  | [92]       |
| Gold nanoparticles      | GNPs                          | Au                   | [93]       |

Poly (α-hydroxy acid) (α-PHA) families are widely used synthetic polymers (SPs) [23]. Grémare et al. fabricated polyactic acid (PLA) scaffolds with varying pore sizes via fused deposition modeling [24]. They found that this process could decrease the biodegradation temperatures of the scaffolds. After several days of cell culture, human bone marrow MSCs (hBMSCs) presented increased viability and homogenous porous distribution regardless of the pore size. Poly (γ-glutamic acid) and glycerol composite hydrogel could present superior proliferation and higher adhesion of L929 fibroblast cells than gellan gum [25]. Poly (l-lactide-co-ε-caprolactone) and polyglycolic acid have also been applied as vascular conduits [26].

In addition to α-PHA, poly (methyl methacrylate) (PMMA) is commonly utilized in prosthodontic restorations and bone TE because of its incomparable properties, such as low density, aesthetics, inexpensiveness, and easy manipulation [27]. Atila et al. optimized hydrophobic and brittle PMMAs combined with hydrophilic and flexible SF fibers via electrospinning to provide an ECM-like microenvironment for new bone tissue formation, resulting in excellent mechanical characteristics and wettability under dry or wet conditions [28]. Additionally, hDPSCs were able to attach to and proliferate in the composite scaffolds.

Despite their advantages, hydrophobic polymers can be detrimental in TE because of their limited wettability, resulting in inadequate cellular attachment and subsequent interactions [29]. To overcome the limitations of α-PHA and PMMA, degradable polymers with various desirable features are used. Polyethylene glycol (PEG) is typically used as hydrogel because of its excellent water solubility [30]. For instance, GEL/PEG/poly (D, ε-caprolactone)/TGF-β1 hydrogels supported the cell proliferation of hDPSCs in vitro [31]. Cell-encapsulating nano-composite hydrogels also promoted the adhesion, viability, and chondrogenesis of hDPSCs.

In contrast, synthetic inorganics exhibit outstanding biological and osteoinduction properties because they can induce the propagation, adhesion, differentiation, and tissue regeneration of bones and cartilages [32]. When implanted into a bone defect, BG could interact with the bone tissues [33]. After doping Nb$_2$O$_5$, the composite scaffolds could approximately regenerate a critical-size calvarial defect superior to pure BG.

Calcium silicate-based ceramics are the most frequently used synthetic inorganics in clinics. Mineral trioxide aggregate (MTA), which is a bioactive composite inorganic, is used as an endodontic material with excellent biological and physical properties [34]. It could induce the mineralization of exposed pulps, seal the root end, and maintain pulp vitality. Accordingly, in most cases, MTA has become a substitute for Ca (OH)$_2$ in endodontics [35]. Moreover, MTA can enhance the osteogenic–odontogenic potential of adipose-derived SCs (ADSCs). Thus, it is considered a favorable source for regenerating the dentin-pulp complex [36]. A recent study found that nano-MTA induced favorable tissue response and osteogenic differentiation in a rat bony defect [37]. In addition, MTA can upregulate the expression of dentin matrix acidic phosphoprotein 1 in pulp-capped rat molars [38].

However, there are some disadvantages of MTA which limit the clinical applications, such as long setting time and discoloration. In addition, after the exposed pulp is capped with MTA, the calcified tissue is not primarily the product of odontoblast differentiation, which suggested that the newly formed mineralized hard tissues after pulp capping with MTA may not be a regeneration response [39]. Therefore, new generations of MTA-based cement, such as commercialized iRoot BP Plus and Biodentine™, were introduced. In pulp capping procedures, these materials could modulate the secretion of factors, such as TGF-β1, thus improving the regeneration potential of the pulp [40]. Biodentine™ could induce reparative dentine formation, as indicated by the formation of dentin bridge formation and layers of well-distributed odontoblasts after six weeks [41]. Investigations showed that iRoot BP Plus was more effective than MTA in preserving pulp vitality as well as proliferating and mineralizing hDPSCs [42]. Moreover, iRoot BP Plus probably promotes the osteogenic and odontogenic differentiation of hDPSCs by activating the MAPK and NF-κB signaling pathways. It can also upregulate the expression of osteogenic markers, including collagen-I (COL-I), bone sialoprotein (BSP), and osteocalcin (OCN) [43].

In addition to bioceramics, environmentally friendly and renewable carbon-based nanomaterials were introduced with an important role [44]. Carbon nanotube (CNT) is an excellent bone substitute because it possesses excellent mechanical properties, increases surface roughness, and mimics the structure of a human trabecular bone. Hence, it complements HA and improves the cell adhesion to the implant’s surface [45]. Furthermore, the growth and differentiation of bone marrow stem cells (BMSCs) have been studied using graphene (GP)-based scaffolds [46]. After seeding on GP scaffolds, BMSCs strongly promote osteogenic differentiation by expressing osteogenic markers, such as osteopontin (OPN), OCN, and Runx2. The foregoing results suggest the ability of BMSCs in inducing osteogenic differentiation rather than odontogenic differentiation because of the high Young’s modulus of GP, while the soft substrates are indispensable for odontogenic differentiation [47].

MXenes are a family of two-dimensional inorganic composites. They mainly contain transition metal nitrides, carbides, or carbonitrides with a combined hydrophilic nature [48]. Zero-dimensional Ti$_3$C$_2$ MXene-based quantum dots have been reported to improve the repair of injured tissue by reducing the CD4+$^+$ T cell activation and spontaneously promoting the expression of immunosuppressive CD4+$^+$ CD25+ FoxP3$^+$ regulatory T lymphocytes [49]. These MXene scaffolds, which are compatible with BMSCs and fibroblasts, can also enhance the physicochemical properties for SC delivery and tissue repair.

2.2. Components carried by scaffolds

Owing to the 3D structure with numerous pores, the scaffolds are always carried with other components. They could be filled with nanofillers (NFs) to improve the physical properties and enhance mechanical characteristics. Moreover, it could load RRs to induce and regulate tissue formation.

2.2.1. NFs

Different from scaffolds, the use of nanofillers (NFs) induces wider surface areas, higher mechanical strength, and preferred stability; it can also improve cell adhesion, proliferation, and differentiation [50]. Due to their uniform distribution, NFs probably enhance the storage modulus. However, the addition of NFs also hinders the degradation rate of scaffolds because of enhanced intermolecular interactions, which cover up or block the lysozyme cutting sites [56].

In bone TE, nHA has been commonly used as NF in CS-based composite scaffolds [87]. Cell proliferation studies reveal that the low response rate from OBs has been resolved, and 3D bioactive CS/fucoidan
scaffolds filled with nHA via freeze drying contribute to high porosity (90%). They could facilitate the vascularization, cell proliferation, biocompatibility, and biomineralization of periosteum-derived MSCs [60].

Adding silver nanoparticles (AgNPs) can also enhance the mechanical properties and biomineralization as well as provide an antibacterial microenvironment [95]. Saleh et al. found that AgNPs could improve the microstructure and hydrophilicity as well as enhance the resistance against degradation [90]. Moreover, AgNPs can trigger a mild inflammatory response after implantation and be used as dressing dressing [96]. In addition to AgNPs, gold nanoparticles (GNPs) possess several advantages, including easy preparation, easy control of particle size, high surface area, and biocompatibility [93]. These nanoparticles (NPs) could interact with proteins in the cytoplasm and ECM through mechanical stress and activate the p38/MAPK signaling pathway, which could upregulate osteogenic genes and downregulate adipogenic genes [97]. The capability of GNPs with calcium phosphate cement (CPC) for the osteogenic induction of hDPSCs has been investigated [98]. The composite scaffolds improved cell adhesion and function. Furthermore, the GNPs enhanced the protein adsorption of CPC, indicating that CPC-GNP scaffolds significantly improved the osteogenesis of hDPSCs. In addition to inorganic NFs, nanocellulose and CS NPs can also be applied as NFs [99].

2.2.2. RSs

As key components of achieving new tissues, the addition of single or multiple RSs has direct or indirect effects on tissue development. Besides the morphogenesis of tissues, RSs, including biological, biochemical, and biophysical stimuli, also play a crucial role in maintaining their structure and function both in vivo and in vitro [100]. Appropriate control of these signals topically may lead to control over a specific treatment or regenerative process.

2.2.2.1. GFs. Naturally occurring GFs can stimulate cell proliferation and differentiation [101]. They are mainly proteins or steroid hormones that gradually lose bioactivity due to environmental changes, such as damage in the bioactive functional groups and the active sites or enzymolysis [102]. Scaffold materials, such as hydrogels and hydrophobic polymers, can preserve their native properties to promote cell activity. Currently, most GFs, which are critical in regulating various cellular activities, can be delivered by scaffolds. They generally enhance cell growth and differentiation, which vary among their types [103]. They are also crucial to cell microenvironment interactions. Generally, cell fate is influenced by the chemical identity, concentration, and duration of GFs. The main GFs in the TE of the oral and craniofacial region as well as their primary functions and involved signaling pathways are summarized in Table 2.

Individual GF proteins emerge as members of a large family of structurally and evolutionarily related proteins. Because the effectiveness of delivery mechanisms and other pharmacokinetic parameters considerably impacts the uses and efficacy of GFs [104], designing dedicated delivery systems of optimal activity and stability is essential. It is also noteworthy to design the scaffolds to mimic the 3D structure of ECM and deliver GFs that favorably affect and control cell response [105,106]. Azzizian et al. fabricated a nanocomposite scaffold based on CS and GEL incorporated with CS NPs loaded with basic fibroblast growth factors (FGFs) and bovine serum albumin [107]. Results revealed that CS NPs could affect the physical properties of the scaffolds and enable the sustained release of GFs to enhance the proliferation of fibroblast cells significantly.

2.2.2.2. Drugs. There are other functional agents which are not proteins or steroid hormones secreted by mammalian cells. These non-GFs are collectively classified as drugs in this review. To prevent the release of drugs to non-target locations and promote tissue regeneration, which typically requires a considerable amount of time, the topical and sustained administration of bioactive molecules from scaffolds during TE is necessary. The development of a drug carrier that can maintain sustained release, control the associated drug’s bioavailability, improve target cell specificity, and effectively deliver macromolecules to target cells is among the key components of TE drug delivery [108]. Theoretically, porous scaffolds are beneficial for drug loading [109]. The characteristics of degradation are also conducive to the release of drugs. In general, most locally released drugs are aimed at promoting osteogenesis, angiogenesis, anti-bacteria, or other purposes. For example, Yao et al. incorporated 3D fibrous GEL scaffolds with mesoporous silicate NPs, bone morphogenetic protein 2 (BMP-2), and deferoxamine (DFO) into the biomimetic osteogenic microenvironment [110]. Their study results indicated that DFO could be released for 10 d and maintain angiogenesis and osteogenesis. Additionally, both mouse C2C12 and human MSCs were found to be affected by the dose/duration of the released DFO, which significantly increased the BMP-2-induced osteogenic differentiation.

Antibacterial property is also a primary function required for TE to achieve better cell–scaffold interactions. It can be easily achieved by loading drugs, such as antibiotics, antibacterial peptide motifs, or anti-bacterial NPs. Layer-by-layer (LBL) PLA nanocoatings loaded with vancomycin exhibited considerable antibacterial activity and had no cytotoxic effects on mouse L929 fibroblasts [111]. Edhilarasu et al. found that combining aloe vera and tetracycline hydrochloride with polycaprolactone (PCL) hybrid nanofibrous mats is safe for human fibroblasts and may even exhibit noteworthy qualities, including anti-inflammatory, antioxidative, and anti-bacterial actions, to support skin TE [112]. Wang et al. formulated thermo-reversible polyisocyanopeptide hydrogel by incorporating doxycycline and lipoxin A₄ for antimicrobial and anti-inflammatory treatment [113]. The antibiotic mechanisms of NPs and metal ions released from scaffolds are illustrated in Fig. 2.

2.2.2.3. Physical stimuli. Except for regular molecular delivery, varied physical stimuli, such as pH [114], temperature [115], light [116], and magnetic fields [117], may be necessary RSs to alter the characteristics, interactions, structures, and dimensions of scaffolds and subsequently affect the interactions between cells and scaffolds [118]. To achieve the objective, the stimuli may either be accumulated at the site of action or administered externally. Additionally, polymers can be further tailored to react to diverse stimuli because they can be combined to produce the appropriate responsive building blocks and numerous functional groups to activate specific features [119]. Consequently, the concept of four-dimensional bioprinting is proposed by simulating a dynamic environment that responds to stimuli and corresponds to physiological activity [68].

As mentioned in Section 2.1.1., GMA is a common photo-crosslinker that can respond to light as a stimulus. Poly (N-isopropyl acrylamide) (PNIPAAm) is the most commonly studied synthetic thermo-sensitive polymer for biomedical applications [120]. It is typically utilized due to its aequous solubility and physiologically relevant low critical solution temperature (32 °C). Ahmad et al. fabricated PNIPAAm–spiropyran thermo-responsive ECM-mimicked coatings with surface-initiated atom transfer radical polymerization [121]. They found that the grafted layers were non-toxic with temperature changes. The cell adhesion, growth, and proliferation of mouse L929 fibroblasts do not significantly differ from those of the control group. Regarding the magnetic stimuli, the formation of a composite hydrogel consisting of Fe₃O₄ NPs filled with nHA (m-nHA) and polyvinyl alcohol (PVA) solutions was reported [80]. In addition to biocompatibility, strong mechanical capabilities, and slow degradation rate, the concentration of m-nHA significantly improved the adhesion and proliferation of OBs.
RSs in TE regarding the oral and craniofacial region.

| Category | Abbreviation/Aliases | Function with cells | Main signaling pathways | References |
|----------|----------------------|---------------------|-------------------------|------------|
| Adrenomedullin | AM | Angiogenesis | PI3K/akt | [134] |
| Angiopoietin |  | Angiogenesis and improves repair of bone defects | Autophagy | [135] |
| Artenin | ARTN | Survival neurons | AKT/β-catenin | [136] |
| Autocrine motility factor protein families | | | | |
| Bone morphogenetic protein-2 | BMP-2 | Cartilage and bone morphogenesis | JNK, Smad2/3, YAP/TAZ | [139] |
| Bone morphogenetic protein-4 | BMP-4 | Cartilage and bone morphogenesis | G-CSF, TSP-1, PI3K/PDK-1/Akt, | [140] |
| Bone morphogenetic protein-6 | BMP-6 | Bone morphogenesis | p21/CIP, p18, p19, JNK | [141] |
| Bone morphogenetic protein-7 | BMP-7/OP-1 | Cartilage and bone morphogenesis | PI3K/PDK-1/PKC | [142] |
| Bone morphogenetic protein-9 | BMP-9 | Bone morphogenesis, development of cholinergic | Notch, retinoid, IGF | [143] |
| Ciliary neurotrophic factor | CNTFs | Neurotransmitter synthesis and neurite outgrowth | ERK1/2/MAPK, JAK, STAT, p91 | [144] |
| Colony-stimulating factor | CSFs | Proliferation and differentiation into blood cells | JAK/STAT3/5, p21/MAPK, PI3K | [145] |
| Epidermal growth factor | EGFs | Stimulation in the growth of epithelial cells | MEK/MAPK | [146] |
| Ephrins | EP | Axon guidance, angiogenesis, and SCs differentiation | SRC-2, FAK, PI3K, MAPK, Small GTPases | [147] |
| Erythropoietin | EPO | Stimulation in developing erythropoietic cells | JAK2/STAT5, RAS/MAPK, PI3K | [148] |
| Fibroblast growth factors | FGFs | Angiogenesis, keratinization and proliferation, angiogenesis | Wnt, RAS/MAPK | [149] |
| Fibroblast growth factors 1 | FGF1/aFGF | Endothelial cell migration and proliferation, angiogenesis | MAPK/ERK | [150] |
| Fibroblast growth factors 2 | FGF2/βFGF | Proliferation of fibroblasts and initiation of angiogenesis | p42/p44 MAPK, MEK/ERK, PI3K | [151] |
| Fibroblast growth factors 4 | FGF4 | Proliferation of limb mesenchyma | Shp2/SFK/RAS/ERK | [152] |
| Fibroblast growth factors 23 | FGF23 | Regulation of phosphate concentration by osteocytes | ERK1/2/S6K, AKT, p42/p44 MAPK | [153] |
| Glial cell line-derived neurotrophic factor | GDNFs | Survival of neurons | ERK1/2/MAPK, PI3K/akt | [154] |
| Growth different factors | GDFs | Stimulation in the growth of epithelial cells | MEK/MAPK | [155] |
| Growth differentiation factors 5 | GDF5 | Osteogenic and chondrogenic differentiation, cellular condensation, survival of neurons | BMPR-1A/ALK-3, | [156] |
| Growth differentiation factor 10 | GDF10/BMP-3b | Axonal sprouting | PI3K, SMAD1/2/3/5/8, NF-κB | [156] |
| Hepatocyte growth factor | HGF | Myogenesis and wound healing | MAPK, PI3K | [157] |
| Hepatoma-derived growth factor | HDGF/HHGF-1L2 | Pro-angiogenesis | MAPK, PI3K | [158] |
| Insulin | | Vascularization, increase of the growth rate and the GAG fraction of chondrocytes, provision of nutrition for bone formation | Insulin-IR, Insulin/IGF1R PI3K, PIP2/3, GSK, PKB | [159] |
| Insulin-like growth factor 1 | IGF-1/I | Stimulation in incorporation of sulfates into cartilage, excretion of insulin-like action on cells, wound healing | IGF-IR, AKT/PI3K, JNK, ERK1/2/MAPK, IGF-1 | [160] |
| Interleukins | ILs | Development and differentiation of T and B lymphocytes, and hematopoietic cells | IGF1R, IGF-IGFBPs, PI3K/akt/mTOR | [161] |
| Interleukin 1α | IL-1α | Proliferation of lymphocytes fibroblast, SMCs, and keratinocytes | VEGF/VEGFR-2, NF-κB, JNK, p38/MAPK | [161] |
| Interleukin 1β | IL-1β | Attenuation of bone formation and reduction of osteostogenesis | NF-κB, AKT, ERK, p38/MAPK | [161] |
| Interleukin 2 | IL-2 | Proliferation of responsive T cells | JAK3/STAT5, MAPK, PI3K | [162] |
| Interleukin 4 | IL-4 | Proliferation, induction of T cells to differentiate into helper T2 cells | JAK2/STAT6, KARAP/DAP12 | [163] |
| Interleukin 6 | IL-6/SF2-2/FN-β-2 | Plasmatocyte growth, nerve cell differentiation | JAK1/STAT3, RAL, ELK1 | [164] |
| Interleukin 8 | IL-8/CXCL8 | Induction of chemotaxis on neutrophil granulocytes, endothelial cells, etc., angiogenesis | CCR1/2, MAPK, PKC, LIMK2, GNG2, PIK3CB | [165] |
| Interleukin 10 | IL-10/CSIF | Activation of B cells, promote macrophages and inhibit Th1 cells production of cytokines | JAK1/TK2/STAT3 | [166] |
| Interleukin 13 | IL-13 | B cell formation and differentiation, inhibit Th1-cells and macrophage inflammatory factor production | STAT6, Src/PI3K/Akt/mTOR | [166] |
| Interleukin 17 | IL-17 | Osteoclast formation, angiogenesis, proliferation of keratinocytes and proinflammatory cytokines | TRAF2/ERK5, NF-κB, MAPK, ILK | [168] |
| Interleukin 18 | IL-18/IGF | Upregulation of IFN-γ and NK cell activity | NF-κB, MAPKs, PI3K/AKT/GATA4 | [168] |
| Interleukin 21 | IL-21 | Generation and activation of CD8+ T cells and increase the cytotoxicity of NK cells | JAK/STAT, MAPK, PI3K | [169] |
| Interleukin 33 | IL-33 | Induce helper T cells to produce type II cytokine | MyD88/IRAK/TRA6, NF-κB, ERK1/2, JNK, p38, STAT2L, PI3K/ACT | [170] |

(continued on next page)
2.3. Role of physical properties of the scaffolds interacted with cells

Scaffolds with heterogeneous pores possess complex 3D structures and mass transportation characteristics. Therefore, simplifying the structural complexity and function to render cells suitable for tissue reconstruction is important. Hierarchical pores (10–1000 \( \mu \)m in diameter) at different physical scales must be designed [122].

2.3.1. Porosity

The term porosity involves pore size, distribution, and tortuosity. Porosity is mainly governed by the solid-phase volume, the volume of immobile pores, and the volume of water-containing mobile pores that can move freely in a saturated system [123]. Ma et al. prepared a PEG terephthalate fibrous matrix via thermal compression to modify the pore size and porosity [124]. They found that the initial cell proliferation rate of trophoblast cells in the low-porosity matrix is higher than that in the matrix with high porosity. Additionally, the cells cultured in low-porosity matrices can spread more efficiently in adjacent fibers, resulting in increased cell proliferation [125]. Studies found that OBs were more prevalent in small pores (40 \( \mu \)m). However, large pores (100 \( \mu \)m) favored cell migration [78]. Porosity also affects angiogenesis and the mechanical properties of scaffolds. The minimum porosity required for angiogenesis is approximately 30–40 \( \mu \)m, enabling the exchange of metabolic productions and facilitating the entry of endothelial cells [126]. High porosity possibly favors nutrient and oxygen transport and promotes cell growth. It can also influence mechanical properties because of the considerable void volume [127].

2.3.2. Surface roughness

Surface roughness regulates the biological response of cells by directly influencing cellular adhesion, proliferation, and phenotype expression [128]. Any surface roughness modification can change the surface topography and the structure’s local and integral mechanical properties. Studies indicate that the cells grown on rough surfaces are stimulated toward differentiation based on the results of gene expression [129]. Chen et al. investigated the effect of the surface roughness of electrospun scaffolds on the osteogenic differentiation of hMSCs [130]. The results showed that high surface roughness increased the induction rate of OPN, BMP-2, and Runx2. Conversely, low surface roughness indicated the high expressions of osteogenic and cartilage genes in hMSCs.

2.3.3. Tortuosity and permeability

Pore structures typically consist of irregularly shaped voids and interconnected channels because of the integration of adjacent voids, resulting in fenestrations in void walls [131]. Pore tortuosity is also critical in optimizing and designing TE scaffolds because it affects molecular diffusion and cell migration beyond the fundamental requirements for sufficient pore size and interconnectivity.

However, the term permeability instead of tortuosity is preferred to describe the ability of cell penetration in scaffolds [125]. Permeability can be used to estimate the scaffold mass transport and characterize its topology, enabling an appropriate assessment of the overall performance of scaffolds [81].

Generally, permeability is mainly related to the tortuosity of scaffold materials [132]. Miki et al. reported the invention of an efficient customized spherical indentation-based testing approach to evaluate the hydraulic permeability of GELMA hydrogels methodically. This technique may improve the prediction of the permeability behaviors of various additional hydrogel types [133].

2.4. Role of mechanical properties of the scaffolds interacted with cells

2.4.1. Surface stiffness

Cell attachment, proliferation, and differentiation also depend on substrate stiffness to a certain extent [183]. Furthermore, cells can modulate the implant surface at the interface between the tissue and implant, altering the stiffness of their own or other cellular microenvironments [184]. Some studies suggest that an initial soft substrate promotes axonal elongation. The subsequent hardening of the matrix can promote the growth of more primary dendrites of neurons, thereby enhancing synaptogenesis [185]. Soft substrates can modify cell morphology and inhibit the proliferation of dental pulp SCs (DPSCs) [186]. The expression of markers related to osteogenic/odontogenic differentiation, including BMP-2, OPN, ALP, OCN, and DMP-1, significantly increased with the substrate stiffness; this was related to the Wnt signaling pathway.
2.4.2. Mechano-transduction

Until the tissue is repaired or regenerated and normal mechanical function is restored, maintaining the mechanical integrity of the defects is crucial. Additionally, the adherence to cell mechano-transduction, which is crucial to regeneration processes, is impacted by the mechanical strength of the scaffolds. For instance, the mechano-transduction in bone TE has been suggested associated with prospective osteoinductive characteristics, and structural mechanics is related to osteoconductive properties [187]. Osteoinduction is considerably influenced by the mechanical stimulation of cells resulting from scaffold deformation.

The mechanical properties of scaffolds mainly originate from their structure and composition. Mechano-transduction occurs when cells transduce or convert the biochemical signals induced by physical forces that combine with cell responses [188]. Innumerable molecules and cellular and extracellular components are involved in this transduction. In general, integrins could mechanically link the ECM and actomyosin cytoskeleton, then trigger signaling cascades and induce localized adhesion formation. The application of force to bind integrins promotes actin filament polymerization, local adhesive assembly, and cytoskeletal contraction [53]. The force is transmitted to the substrate and enables cells to sense the surroundings, distinguishing minor alterations in matrix elasticity [189]. Concurrently, individual mechanosensitive molecules enable cells to detect stress and react. Thus, the mechanical force produced by the cell ECM or intercellular adhesion could affect tissue formation [190]. Cambria et al. explored strategies for improving agarose-GEL hydrogel’s biological characteristics [191]. The composite hydrogels exhibit approximately five-fold higher pFAK/β-tubulin when not compressed, and increased pFAK/FAK values after compression.

2.5. Role of chemical properties of the scaffolds interacted with cells

2.5.1. Surface charges

Surface charges can affect cell behavior; as the charge density increases, further cell adhesion biocompatibility, affinity, differentiation, and proliferation are observed [192]. For example, negatively charged PEG fumarate hydrogels increase the degree of chondrocyte differentiation (e.g., GAG and collagen (COL) expressions) [193]. The surface charge can regulate protein adsorption and directly affect integrin binding, thus modulating cell adhesion. Tang et al. reported that adding negative charges might favor protein adsorption, which could enhance cell adhesion and other responses [194]. Particle size, pH, and charge stoichiometry are all affected by charge imbalance, porosity, and pore

Fig. 2. Antibacterial mechanisms of AgNPs or other metal ions: (1) released metal ions, (2, 3) interaction between metal ions or nanoparticles with the cell membrane by electrostatic interactions; (4) formation of intra- or extracellular reactive oxygen species (ROS), and damage of lipids, DNA, and proteins through oxidative stress; (5) high-levels of metal-ions or ROS lead to damages to the plasma membrane and the leakage of the cell content; (6, 7) once absorbed, AgNPs or other metal ions can directly interfere with proteins and DNA, disturbing their function and cellular metabolism because of altered ROS production [66].
2.5.2. Surface hydrophobicity

Surface hydrophobicity can be evaluated by measuring the water contact angle. The degree of hydrophilicity is directly proportional to cell adhesion [196]. For example, when the surface contact angle is increased from 0° to 106°, the adhesion of OBs decreases [197]. Furthermore, reports showed that the most excellent fibroblast adhesion occurred at contact angles between 50° and 60° [198]. However, the hydrophobic nature of synthetic scaffold materials limits their use in TE because it is disadvantageous to cell adhesion, proliferation, and migration [199]. In contrast, cell signaling molecules are present in natural scaffold materials, attracting cells to their hydrophilic surfaces for biological functions.

2.5.3. Protein adsorption

When biomaterials interact with the environment, the surfaces are initially reached by water molecules and inorganic salt ions, followed by protein molecules in body fluids, blood, or culture medium; finally, cells reach the surface of the material. Therefore, typically, an adsorbed protein layer is present between the surfaces of the material and cells. The cells adhere and then spread to the surface of the material through the mediation of the protein layer [200]. Protein interaction forces are classified into four types: ionic or electrostatic interactions, hydrophobic interactions, hydrogen bonding forces, and charge transfer or particle donor–acceptor interactions. The first two forces are more common. Adsorbed protein molecules must come into contact with a surface through diffusion, thermal convection, bulk flow, etc. [201]. Moreover, the protein’s access to solid surfaces is influenced by protein transport, protein size, temperature, concentration gradients, and flow rate [202].

When the scaffolds are implanted in vivo, the host ECM and plasma proteins bind to the substrate to regulate, neutralize, or block the harmful agent; this is the beginning of an immunity response [203]. In this process, protein adsorption is essential to describe the aggregation molecules on the substrate surface [204]. Numerous binding sites exist between the surface and amino acids, facilitating the adsorption and adherence of large proteins to the surfaces. In general, GEL surface modification, such as PEG-based coating [205] or polymer brushing [206], has been utilized to reduce protein adsorption.

When the energy released exceeds the obtained energy, protein adsorption occurs according to Gibbs free energy formula:

$$G = H - ST < 0,$$

where $G$, $H$, $S$, and $T$ denote the Gibbs free energy, enthalpy, entropy, and temperature, respectively. When considering protein transport, the protein access to the substrate is influenced by the concentration gradient, temperature, protein size, and flow velocity [207]. The adsorption rate can be estimated based on protein concentration, time, and diffusion coefficient at low flow rates with a slight temperature difference.

The primary driving forces behind protein adsorption in addition to surface energy include hydrophobicity, intermolecular forces, and electrostatic interactions [208]. The interactions between charges and transfers are critical in surface interactions and protein stabilization. Generally, the donor–acceptor process can be attributed to electrophilic species, which are mainly caused by orbital electron effects in aqueous media. Other factors, such as temperature, ionic strength, surface roughness, and structure, can also affect protein adsorption, thus influencing cell–scaffold interactions [209,210].

2.6. Role of biological properties of the scaffolds interacted with cells

2.6.1. Biocompatibility

The capacity of a material to react to the host in a certain situation is known as biocompatibility [211]. Appropriate materials for preparing scaffolds are limited by biocompatibility because they must fit into the host and encapsulated cells [212]. Scaffolds with inadequate biocompatibility produce invasive foreign body responses (FBRs) in vivo. Accordingly, immunomodulatory biomaterials must be developed to ensure the host tolerance to foreign body scaffolds or modulate the immune environment for cell survival [213].

When scaffolds are placed in vivo, several reactions, such as extensive cytotoxic effects, fibrosis, microvascular changes, activation of the coagulation cascade, and platelet response, may occur [211]. They may constitute fundamental determinants of the host response and involve the interaction between proteins and other molecules as well as scaffold surfaces, resulting in immune responses, followed by regeneration processes [214]. Naahidi et al. reviewed the biocompatibility of hydrogel-based scaffolds used in TE applications. They found that predicting the toxicological reactions of materials and characterizing the structural and chemical properties of the scaffolds to reduce the immune response in vivo were possible [215].

2.6.2. Immune response

The immune response is typically stimulated by the transplantation of cells, implantation of scaffolds, or delivery of inducing factors. As a necessary process, this response can remove the cellular debris from an injury and limit bacterial infection [216]. Nevertheless, the initial immune response to injuries may result in further tissue damage, thus inhibiting tissue regeneration [217]. Blocking the infiltration of macrophages can contribute to extensive damage and decreased regenerative capacity. To promote regeneration, exploiting the beneficial aspects of immune response and limiting potentially harmful factors may be necessary.

Scaffolds are designed to create a local microenvironment that can enhance tissue growth. However, tissue damage during implantation and the inflammatory response of the host and the implanted material can hinder the creation of such a microenvironment [218]. In addition to repairing damage, local progenitor regeneration can produce functionally intact tissues concurrently. Immediately after tissue damage, the immune response can severely affect wound repair or regeneration. Primary injury activates complement proteins through classical or alternative mechanisms and triggers cellular pattern recognition receptors in the presence of pathogens or cellular injury [219]. Consequently, inflammatory cytokines are produced at the injury site by polymorphic nuclear neutrophils (PMNs), fibroblasts, and monocytes. After penetrating the wound’s surface, PMNs phagocytose the pathogens and damaged cells, generating reactive oxygen species (ROS) and pro-inflammatory cytokines, such as interferon (IFN), tumor necrosis factor, and interleukin-1 [220]. Furthermore, PMNs could eradicate pathogens by phagocytosis and release ROS and pro-inflammatory factors, causing secondary damage to the surroundings. Monocytes can remain at the damage site for months before differentiating into macrophages. Although macrophages secrete GFs and phagocytic cell debris, they also create cytokines, ROS, and other substances that are necessary for regeneration.

The existence of scaffolds and other foreign substances, such as exogenous cells, can enhance inflammatory responses by triggering FBR and then leading antigens to the injury sites. Furthermore, the interaction between the blood and scaffold results in the absorption of protein through the scaffold surfaces, affecting subsequent cell adhesion [221]. Therefore, the physicochemical properties of scaffold surfaces are primarily causative for FBR [222]. Phagocytes are attracted to the implant by the chemokines (CKs) released by the matrix and surrounding cells and then adhere to the scaffold surfaces, leading to the increased secretion of pro-inflammatory factors.

When PMNs and some macrophages depart from the injury site, the inflammation progresses and becomes chronic [216]. Fibroblasts also proliferate and remodel the local ECM to repair the wound. Concurrently, the remaining macrophages continue to produce CKs, which...
dependent on the immunophenotype [223]. The changes in the helper T cells of CD4⁺ are related to the transition from a pro-inflammatory (M1) to an anti-inflammatory (M2) phenotype, thus promoting the resolution of inflammation. Macrophages have also been shown to develop the M2 phenotype after the phagocytosis of debris. Therefore, reducing the immunogenicity of scaffold materials is essential. For example, as a common scaffold material, COL has been previously considered an inert protein without immunogenicity. Although it can be deemed as a weak antigen, recent investigations have revealed that it can interact with antibodies [224]. Atelocollagen was developed by Jeevithan et al. to reduce immunogenicity by removing terminal telopeptides using proteolytic enzymes, such as pepsin [50]. This modified material is promising for more profound TE applications.

2.7. Role of bioactive molecules and ions degraded from scaffolds

2.7.1. The biodegradation of scaffolds

Biodegradation refers to the breakdown of substances caused by cells or microorganisms; it can be divided into deterioration, fragmentation, and assimilation [225]. The deterioration process starts from the surface by changing the physicochemical and mechanical properties of a material. This occurs when the scaffold material is exposed to the external environment and further deteriorates, causing the structure to degrade over time [226]. Deterioration can occur concurrently with fragmentation and may occur first in the degradation process [227]. Fragmentation is a lytic process that breaks down scaffolds and generates oligomers or monomers in situ [228]. At this stage, water may penetrate the scaffold’s internal structure, swell the scaffolds, and finally activate the breakdown of product [229]. Enzymes are the catalysts of water-induced hydrolysis [230]. In addition to hydrolysis, enzymes perform an essential function in the breakdown of scaffolds known as enzymolysis.

Finally, the products resulting from the fragmentation are integrated into the cell during the assimilation stage. Some fragmented products are easily intracellularly transported via membrane carriers [231]. However, some cells further require biotransformation reactions to produce products that can be transported into the cell. Inside the cell, the product enters the catabolic pathway, resulting in the creation of adenosine triphosphate.

Most scaffold materials that can be degraded rely on passively controlled degradation mechanisms [232]. Hence, hindering the degradation process of a scaffold after its implantation in vivo is necessary. Additionally, the process of tissue regeneration supported by scaffolds is unpredictable because it depends on the interactions between cells and scaffolds. Therefore, the development of technology for specific degradation rate control is anticipated [232]. According to reports, the addition of glutathione, an antioxidant, can trigger and accelerate the breakdown of reduction-sensitive biodegradable elastomeric polyurethanes, which include disulfide linkages. The degradation of scaffolds containing many disulfide bonds is rapid in vivo subcutaneous implantation scenarios [77].

2.7.2. Effect of biodegradation products on cells

In general, inorganic scaffolds biodegrade into positively charged metal ions or negatively charged acid radicals. In contrast, polymers mainly degrade into monomers. These active groups have analogous internal structure, swell the scaffolds, and finally activate the breakdown of products [229]. Some fragmented products are easily intracellularly transported via membrane carriers [231]. However, some cells further require biotransformation reactions to produce products that can be transported into the cell. Inside the cell, the product enters the catabolic pathway, resulting in the creation of adenosine triphosphate. Most scaffold materials that can be degraded rely on passively controlled degradation mechanisms [232]. Hence, hindering the degradation process of a scaffold after its implantation in vivo is necessary. Additionally, the process of tissue regeneration supported by scaffolds is unpredictable because it depends on the interactions between cells and scaffolds. Therefore, the development of technology for specific degradation rate control is anticipated [232]. According to reports, the addition of glutathione, an antioxidant, can trigger and accelerate the breakdown of reduction-sensitive biodegradable elastomeric polyurethanes, which include disulfide linkages. The degradation of scaffolds containing many disulfide bonds is rapid in vivo subcutaneous implantation scenarios [77].

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Degraded bioactive ions perform unique functions in tissues. For example, Ca, Zn, Mg, and Sr ions can modulate cellular functions in bone formation, whereas Ca, Ag, and Zn ions have been reported to promote angiogenesis [66,67]. Some inorganic ions degraded from dissolved minerals have been found to impact the phenotype of SCs [235]. Liu et al. reviewed and concluded that these ions from inorganic NPs could also control redox balance besides regulating cellular behaviors and specific RSs [236]. Consequently, external inorganic ions can directly or indirectly affect SCs by regulating their states through selective ion release properties.

The enhancement of the proliferation of hMSCs by Ca²⁺ has been reported, whereas both Ca²⁺ and PO₄³⁻ can positively regulate the osteogenic differentiation of hMSCs [237]. Another study has revealed that local PO₄³⁻ can initiate osteoinductive responses through COL mineralization. Moreover, sustanably releasing Ca²⁺ can promote angiogenesis in vivo and in vitro [238]. Silicate bioceramic ion extract has been shown to activate osteogenic marker genes, such as Runx2, Col-I, BSP, and other bone markers promoting the osteogenic differentiation of hMSCs [229]. However, determining the effect of each type of ion on scaffolds can be complicated because controlling the ratios and concentrations of various ions under sophisticated cell culture conditions is difficult. Numerous studies have revealed that SiO₂⁻ can promote the capacity of MSCs for differentiation and tissue regeneration [239].

In the microenvironment of SCs, GFs and CKs, which are typically incorporated into scaffolds, are imperative interventions for release. Studies have revealed that silicate bioceramic ionic extracts can stimulate the expression of GFs without osteogenic mediators. For instance, akermanite (Ca₂MgSi₂O₅) extract, including bioactive ions, such as Ca²⁺, Mg²⁺, and SiO₂⁻, can increase the expression of alkaline phosphatase (ALP) and OCN as well as stimulate BMP-2 in ovariectomized rat BMSCs [240]. In addition, ionic extracts of inorganic scaffolds stimulate angiogenesis by affecting the vascular endothelial GFs (VEGFs) and epidermal GFs [241]. Moreover, ECM is considered a potential regulator of the cellular environment to modulate SC behaviors [242]. The mechanical and thermal properties of polyacrylic acid (PLA) as a biodegradable polymer are suitable. When applied in vivo, PLA is directly hydrolyzed without the aid of any catalysts or enzymes [243]. Poly (lactide-co-glycolide) (PLGA) also degrades gradually, but pure PLGA has low osteoconductivity despite its considerable biocompatibility, thus limiting its applicability to bone regeneration [244]. After the seeding of tamarind seed polysaccharides nHA–CS nanocomposites exhibit prominent biodegradation and enhanced biomineralization [245].

2.7.3. Synergetic effects of scaffolds and biodegradation products on intercellular interactions

During native tissue regeneration, cells typically encounter the structural and chemical features of scaffolds [246], which may synergistically affect cells. Because simultaneously controlling the surface structure and chemical properties of scaffolds are challenging, the studies examining the effects of the chemical composition and surface topography of scaffolds on cells are few. The nanotopography of scaffold surface has been reported to enhance the attachment and proliferation of rat BMSCs as well as stimulate the expression of osteogenesis-related genes. Active SiO₂⁻ can further enhance cell proliferation and osteogenic differentiation, indicating that the combined interactions of scaffolds have additive effects on the stimulation of SC behavior [233]. Kim et al. showed that 3D scaffolds containing BGs and PLA nanofibers could synergistically enhance the osteogenesis and angiogenesis of rat MSCs to promote bone formation [247]. Additionally, a recent study showed that the intercellular interactions between epithelial cells (ECs) and fibroblasts are influenced by the chemical characteristics of BG ion products and the structures of electrospun nanofibers [248]. Another study found that the activation of paracrine effects and gap junction communication by electrospun poly...
(D, L-lactide)/PCL nanofibrous structures and bioactive ions from BG promoted intercellular interactions between human umbilical vein endothelial cells and human dermal fibroblasts [249]. By combining the trapping effect of graphene oxide (GO) nanosheets and the pernicious effect of Ag⁺, Shuai et al. prepared a co-dispersed GO–AgNPs system exhibiting synergistic antibacterial effects (more than 95%) [85].

In addition, electrically stimulated PVA conductive scaffolds mixed with poly (3, 4-ethylene dioxythiophene) can improve the cell response of MSCs [74]. Therefore, the combination of surface structure and bioactive molecules or ions probably affects cells synergistically. A recent study also described the bone repair effect of electroactive composite PLGA/HA/PLA scaffolds with a local osteoinductive factor expression under electrical stimulation [250]. The study results reveal that the composite scaffolds and BMP-4 carrier complex can effectively improve the in vivo bone repair of the rabbit radial bone defect model and synergistically promote the proliferation and differentiation of rabbit radial bone defect model cells in vitro.

3. Roles of cells in interaction with scaffolds

3.1. Cells in the oral and craniofacial TE

Generally, traditional TE utilizes cells for replacing new tissues, as shown in Fig. 3. Theoretically, cells can be aided by matrices or applied without support. An appropriate microenvironment for promoting cell adhesion, proliferation, differentiation, and integration with the surrounding tissue is indispensable. The cells used in TE can be classified as autologous, allogenic, xenogenic, syngeneic, and isogenic depending on their source [251].

Although somatic cells provide an ex vivo model of cell behavior, they are frequently terminally differentiated, implying that they are difficult or even impossible to proliferate or differentiate. In contrast, based on their differentiation potential, undifferentiated SCs can also be classified as multipotent, pluripotent, and totipotent SCs [252]. In general, SCs in dental and craniofacial reconstruction are mainly applied to odontogenesis, osteogenesis, neurogenesis, and angiogenesis.

The SCs involved in TE are grouped considering their location in the oral and maxillofacial region, as summarized in Table 3.

3.1.1. MSCs

As pluripotent stem cells have all the common characteristics of stem cells, namely self-renewal and multidirectional differentiation ability, MSCs can be readily isolated from adult tissues utilizing minimally invasive approaches. As the multipotent adult SCs in multiple tissues, MSCs are capable of self-replication to several passages and can self-renew and differentiate into somatic cell lineages, such as osteoclasts (OCs), OBs, adipocytes, chondrocytes, fibroblasts, myocytes, and neuroblasts.

In TE, secretomes of MSCs can induce tissue regeneration. Exosomes are specific extracellular vesicles secreted by MSCs and other cells that transport information from MSCs to target cells, affecting cellular proliferation, migration, differentiation, and apoptosis [253]. They function in paracrine signaling and could horizontally transfer proteins and RNAs. As intercellular communicators, exosomes are a promising candidate in bone TE. They may be isolated from osteoblastic cells, dendritic cells, or monocytes and used for craniofacial repair and regeneration [254]. For example, the exosomes derived from hADSCs with PLGA scaffolds could accelerate the healing of mouse calvarial defects with a critical size [255]. These types of exosomes are capable of osteogenesis, proliferation, and migration in vitro and could optimize osteoinduction. In addition to modulating the osteogenic differentiation process of target cells, exosomes and corresponding angiogenesis-promoting factors could create an appropriate niche for bone regeneration [256].

3.1.1.1. BMSCs and ADSCs. Although both BMSCs and ADSCs exhibit morphologies resembling fibroblasts and express several comparable markers, they are generally collected from different tissues. Human
BMSCs could express more markers, such as CD50, CD56, CD120a, and CD124 [257]. In TE, BMSCs are the frequently studied MSCs. However, the osteogenic differentiation potential and proliferation rate of BMSCs determined from different tissues were found to differ. Mandibular-derived MSCs showed significantly higher mineralization and osteogenic differentiation potential than femur-derived or ilium-derived MSCs; the foregoing might be significant for bone/dental TE [258,259]. However, the low extraction yield and number of BMSCs as well as the loss of proliferative and differentiation capabilities during cell expansion distinguish their therapeutic applications [260].

Adipose tissue, an easily accessible and abundant source of TE, contains a large population of multipotent progenitor cells called ADSCs, which are reported to be more available than BMSCs in multipotent differentiation abilities [261]. However, Li et al. reviewed and compared the differentiation potential of BMSCs and ADSCs in cartilage TE [257]. They found that as most studies verified, the chondrogenic potential of BMSCs was higher than that of ADSCs. The research on the therapeutic use of ADSCs is expected to continue expanding given that ADSCs are relatively abundant and easy to culture. Moreover, their production is higher compared with BMSCs [262]. The development of extensive and efficient differentiation procedures for various cell types, optimization of in vivo transport strategies, and immune response reduction in allogeneic transplantations are some of the challenges that must be overcome for ADSCs.

### 3.1.2. Somatic cells

In the process of tissue regeneration, SCs may come from outside the defect with or without scaffold material or from the periphery of the defect. In contrast, somatic cells are mainly differentiated from SCs or may also be sourced from the periphery of the defect. Although SCs perform a significant function in the tissue regeneration process, most regenerative tissues consist of somatic cells. Further, immune cells are involved in immune control.

#### 3.1.2.1. Osteoblasts and osteoclasts

The specialized terminally differentiated products of MSCs include OBs. They produce specific proteins, such as OCN and OPN, which comprise the organic matrix of bones [269]. Bioceramics have been found to promote osteoinduction, osteo-conduction, and osteointegration. According to recent research, BG can increase the expression levels of cyclin C and E, which induce OBs to enhance the expression of microRNA-30c, BG could promote OB differentiation, thereby enhancing Runx2 expression [271].

| Applications | Stem cells | Abbreviation | References |
|--------------|------------|--------------|------------|
| Bone         | Bone marrow-derived MSCs | BMSCs | [259] |
|              | Adipose-derived SCs | ADSCs | [255] |
|              | Dental pulp SCs | DPSCs | [266] |
|              | Buccal fat pad MSCs | BFPP MSCs | [273] |
|              | Periodontal ligament SCs | PDLSCs | [274] |
|              | Gingiva-derived MSCs | GMSCs | [275] |
|              | Dental follicle SCs | DFSG | [276] |
|              | Tooth germ SCs | TGSG | [275] |
| Cartilage    | Bone marrow-derived MSCs | BMSCs | [277] |
|              | Adipose-derived SCs | ADSCs | [278] |
|              | Synovium-derived SCs | SSCs | [279] |
|              | Periosteum-derived SCs | PSG | [280] |
|              | Dental pulp SCs | DPSCs | [31] |
|              | Tooth germ SCs | TGSCs | [281] |
|              | Dental follicle SCs | DFSG | [276] |
|              | SCs from human exfoliated deciduous teeth | SHED | [282] |
|              | Periodontal ligament SCs | PDLSCs | [283] |
|              | Gingiva-derived MSCs | GMSCs | [284] |
|              | Dental pulp SCs | DPSCs | [284] |
|              | SCs from human exfoliated deciduous teeth | SHED | [263] |
|              | SCs from apical papilla | SCAP | [263] |
|              | Periosteum-derived SCs | PSG | [286] |
| Mucosa       | Dental follicle SCs | DFSG | [263] |
|              | Tooth germ SCs | TGSG | [263] |
|              | Gingiva-derived MSCs | GMSCs | [263] |
|              | Oral epithelial SCs | OECS | [285] |
|              | Gingiva-derived MSCs | GMSCs | [283] |
|              | Periosteum-derived SCs | PS | [286] |
| Skin         | Adipose-derived SCs | ADSCs | [262] |
|              | Bone marrow-derived MSCs | BMSCs | [277] |
|              | Epidermal SCs | EG | [287] |
| Salivary gland | Salivary gland-derived SCs | SGSCs | [288] |
|              | Adipose-derived SCs | ADSCs | [278] |
|              | Bone marrow-derived MSCs | BMSCs | [277] |
|              | Dental pulp SCs | DPSCs | [264] |
|              | SCs from human exfoliated deciduous teeth | SHED | [281] |
| Nerve        | Ductal progenitor cells | DPCs | [288] |
|              | Adipose-derived SCs | ADSCs | [278] |
|              | Bone marrow-derived MSCs | BMSCs | [277] |
|              | Dental pulp SCs | DPSCs | [289] |
|              | Neural SCs | NE | [290] |
|              | Bone marrow-derived MSCs | BMSCs | [277] |
|              | Dental follicle SCs | DFSG | [276] |
|              | SCs from apical papilla | SCAP | [281] |
|              | SCs from human exfoliated deciduous teeth | SHED | [283] |
| Muscle       | SCs from apical papilla | SCAP | [280] |
|              | Muscle-derived SCs | ADSCs | [57] |
|              | Dental pulp SCs | DPSCs | [264] |
|              | Adipose-derived SCs | ADSCs | [278] |
|              | Dental follicle SCs | DFSG | [276] |
| Tendon       | Tendon-derived SCs | TDSG | [291] |
|              | Bone marrow-derived MSCs | BMSCs | [277] |
|              | Adipose-derived SCs | ADSCs | [278] |
| Vessel        | Endothelial progenitor cells | EPCs | [292] |
|              | Bone marrow-derived MSCs | BMSCs | [277] |
|              | Dental pulp SCs | DPSCs | [268] |
|              | SCs from apical papilla | SCAP | [281] |
|              | Periodontal ligament SCs | PDLSCs | [285] |
A specific type of bone cell, OC, which can destroy bone tissues, is crucial for maintaining, repairing, and remodeling bone tissues. To ensure the dynamics of bone remodeling, typically, bone replacement materials must be capable of supporting both OB and OC functions. Osteoclastogenesis is influenced by the extent of tissue calcification, and the composition, roughness, crystallinity grain size, and dissolution rate of scaffold materials [272].

Through the RANK–RANKL–OPG signaling pathway, OBs and OCs interact in a complicated manner [293]. The coupling relationship between osteoclasts and OBs is implemented not only by CXCL12/CXCR4 but also by direct communication via ephrinB2/4. Moreover, Wnt signaling can activate osteoprotegerin expression, further inhibiting osteoclast differentiation and promoting osteogenesis. Notch signaling can also inhibit osteoclasts [272].

3.1.2.2. Immune cells. The scaffolds are perceived as foreign and may cause FBRs, which are characterized by fibrosis and chronic inflammation. Phagocytes are led to the implantation site as part of the initial acute inflammatory response, with neutrophils arriving first, followed by long-lived macrophages. When attempts to phagocytose the foreign material fail, chronic inflammation develops. This occurs because phagocytes erroneously consider the scaffolds as foreign due to surface-adsorbed proteins; subsequently, they release pro-inflammatory cytokines [167]. Then, because the macrophages are prevented from progressing, they combine to form foreign body giant cells (FBGCs). Macrophages and FBGCs persist at the defects covered by the fibrous capsule for the duration of the scaffolds, sustaining low-grade chronic inflammation. After the scaffolds deteriorate, the fibrous capsule gradually degrades [294].

3.2. Cell adhesion on scaffolds

Ideally, scaffolds are resorbed by cells and replaced by the ECM and cells [295]. Hence, for cells to function in scaffolds, cell adhesion is essential. Non-receptor-mediated cell adhesion (NRMCA) is the term used to describe the non-specific interactions between cells and materials. These interactions are caused by weak chemical bonds, such as electrostatic and hydrogen bonds, or ionic interactions between the functional chemical groups of polymers and molecules in the cell membrane [296]. The survival of anchorage-dependent cells and the sufficiency of signal transmission from the extracellular environment are not guaranteed by NRMCA.

Molecules regulate receptor-mediated cell adhesion on scaffolds in the ECM, including integrin, fibronectin, vitronectin, or laminin [297]. These RSs can naturally adsorb onto the scaffold surfaces from the surroundings. Furthermore, typical anchorage-dependent cells can bind the specific amino acid sequences of these compounds. One of the adhesive ECM molecules involving at least three amino acids is Arg-Gly-Asp (RGD) [298]. In the new generation of more sophisticated biomaterials, receptor-binding ligand-cooperative oligopeptides can be directly attached to scaffolds. They are ideally situated to mediate mechanotransduction and conduct mechanical stress. Research indicates that the adhesion mediated by integrins and cadherins is fundamentally mechanosensitive [299]. The main mechanism of mechanotransduction may be the strengthening of adhesion in response to mechanical stimuli [300].

3.2.1. MSCs adhesion

The studies on the cell adhesion of MSCs typically focus on the function of fibronectin compared with other ECM proteins. Fibronectin can bind to 20 different integrins; this explains the increase in the ratio of cells that adhere to it [301]. Surfaces that are ECM-modified have implications beyond enhanced cell adherence [302]. In this regard, MSCs have been shown to respond to COL-I and vitronectin in a dose-dependent manner, with fibronectin eliciting the strongest response. According to reports, cytoskeletal stress, which is important in determining whether MSC lineages commit to adipogenesis or osteogenesis, may be reflected by cell adhesion and subsequently spread. Osteogenesis is supported by spreading, whereas adipogenesis is aided by unspreading [303]. The matrix is deformed as a result of MSC contact with elastic ECM, which also promotes adipogenesis. The stiffer matrix efficiently resists the stresses produced by the cells and enhances cell spreading, which assists in the differentiation of MSCs into the osteogenic lineage [304]. However, the stiff matrix’s geometrical restrictions may potentially prevent osteogenesis and promote the development of adipocytes [305].

3.2.2. OBs and OCs adhesion

Nanostructured scaffolds have been reported to enhance the adhesion of OBs. However, they deteriorate the adhesion of fibroblasts and ECs [306]. For example, nHA fibrous scaffolds improve OB adhesion, proliferation, and differentiation as well as induce mineralization for bone formation [307]. Furthermore, OPN may be a factor in cell adhesion that initiates the crystallization of HA. However, OPN with thrombospondin binds to the integrins in osteoclasts, mediating their adherence to bone surfaces before performing resorptive activity [308]. In contrast, the adhesion of OCs is mainly mediated by F-actin, talin, and...
vinculin [309]. Most of the biomaterials applied to bone TE support the adhesion of OCs [272].

3.2.3. Immune cells adhesion

The adhesion of immune cells, particularly macrophages, is also affected by the topography and surface chemistry of the scaffolds. For example, compared with the nano-architecture, the micro-architecture is more capable of limiting cell fusion in flat control surfaces and developing a macrophage phenotype from M1 to M2 [216]. After grafting microparticle hydrogels onto polymeric materials, protein adsorption and monocyte adherence decreased; the levels of inflammatory cytokines after implantation also decreased [310].

3.3. Effects of ECM

As a heterogeneously connected network of fibrous GAGs that coordinate in vivo, the ECM provides physical scaffolding, mechanical stability, and biochemical cues for tissue morphogenesis and homeostasis. It interacts with cytoskeletal networks with cells and is crucial to tissue maintenance and regeneration. Accordingly, a ubiquitously distributed ECM plays a critical role in TE [311]. Investigations show that ECM proteins are critical to SC niches [312]. Because anatomical structures consist of cellular and acellular components, SC niches can regulate cell adhesion, proliferation, and differentiation. These niches are essential in promoting cell self-renewal and replenishing the body with differentiated cells.

Hence, the ECM can be viewed as a native scaffold secreted by cells. The purpose of designing scaffolds in TE is to mimic the ECM of the target tissue structurally and functionally to the extent possible [313]. Although considerable research has been devoted to exploiting mechanical or biochemical cues to fabricate fibers and fabrics composed of fibrous ECM proteins, the effect of the local distribution of proteoglycans and protein cooperation on fiber formation remains unclear [314]. The primary scaffolding options for the tissue-specific consideration of TE typically include cell-seeded pre-fabricated porous scaffolds, cell sheets with secreted ECM, decellularized ECM (dECM), and cells encased in self-assembled hydrogels. With native 3D architectures and diverse bioactive components, dECM scaffolds have been explored to imitate a non-immune environment [315]. Different decellularization procedures, including physical, chemical, enzymatic, and combined treatments, have been established by researchers [316]. In the procedures, dECM is invariably ground into particles containing ECM components that are inherent to the tissue type and provide binding sites for cells [316]. These particles can be combined with biologically inactive synthetic or biological scaffolds to form composite scaffolds with tissue-specific biological activity. Moreover, decellularization may enhance cellular material clearance while minimizing harm to the ECM [317].

High mechanical strength, reasonable degradation rate, and satisfactory pore structure can be achieved in the fabrication of 3D bio-printed SF–dECM bioinks [318]. Their constructs appropriately support BMSC proliferation and promote chondrogenesis, especially when loaded with TGF-β. Nokhbatolfoghahaei et al. compared four decellularization protocols: trypsin, Triton X-100, sodium dodecyl sulfate, and a combination for detecting remnant cells and COL [83]. They found that the Triton X-100 group exhibited a higher amount of residual ECM. Combination or biological scaffolds to form composite scaffolds with tissue-specific biological activity. Moreover, decellularization may enhance cellular material clearance while minimizing harm to the ECM [317].

Various methods have been applied to remove the cellular components of the dECM and retain their natural native components to the extent possible. However, exogenous SCs are necessary for the dECM to function in traditional TE. Therefore, authentic cell-free approaches for TE have been introduced [320]. Notably, cell-free or scaffold-free TE simply means that no exogenous scaffolds or cells are involved. In fact, during tissue regeneration, cell-free TE extracts SCs around the defect, whereas SCs secrete ECM to function as scaffolds in scaffold-free TE. These processes are illustrated in Fig. 3 (B and C). To achieve the purpose of cell-free TE, the following must be available: (1) scaffolds, (2) RSs, and (3) SCs recruited from resident populations within the peripheral region. Through SC homing factors or chemotaxatrans, circulating SCs or exogenously administered SCs can locate and enter an environmental niche [321]. For improving cell homing to defects, numerous techniques have been investigated [322–324]. These methods either use scaffold-based or cell-based techniques (such as employing modified SCs to improve signal reception). According to a study, the ability of cell-free PU/SDF-1 scaffolds to stimulate cartilage regeneration was confirmed when these scaffolds were implanted in rabbits with articular cartilage defects [325].

3.3.1. MSCs interacted with ECMs

Matrix elasticity, which influences cell–ECM contact, can regulate how MSCs differentiate into distinct lineages by regulating MSC destiny and direct gene expression [326]. For instance, soft matrices promote neurogenesis, stiffer matrices enhance myogenesis, and rigid matrices stimulate osteogenesis [327]. In contrast to standard culture, the BMSCs grown on the dECM derived from a bone marrow exhibit increased proliferation. Moreover, the MSCs cultured with the ECM displayed enhanced bone formation in vivo [328]. Another study reported coating Ti with ECM before implementing MSC seeding. After the confluent MSC layer had secreted the ECM and was decellularized, they were seeded on Ti meshes and then reseeded with MSCs. The scaffolds functionalized with the ECM exhibited increased calcium deposition compared with the uncoated Ti [329].

3.3.2. OBs and OCs interacted with ECMs

Numerous proteins secreted by OBs or OCs, including COL, OPN, OCN, and other glycoproteins, comprise a major portion of the ECM in bone tissues [330]. Dong et al. reported that bioactive proteins from the ECM of OCs (mainly CXCL12 and IGFBP5) can promote the migration, adhesion, and osteogenic differentiation of MSCs in vitro [331]. Additionally, the OBs cultured on the COL-I matrix, which is an important part of the ECM, exhibit a higher level of osteoblastic phenotype than the cells grown on plastic surfaces.

3.3.3. Immune cells interacted with ECM

Immune cells can be regulated by ECM proteins. Under disease conditions, these cells receive physical and physiological signals from the alterations in the ECM’s composition and structure, consequently affecting how they are activated. Immune cell activation and chemotaxis are affected by both enzymatically degraded ECM components and intact ECM proteins [332]. Modo et al. tagged peripheral immune cells using perfluorocarbon nanomethylcations by 19F magnetic resonance imaging (332). After implanting the ECM hydrogel for 24 h, 35% of the immune cells within the peri-infarct area were macrophages, and 11% were neutrophils. In contrast, in the ECM hydrogel, 66% of the immune cells were neutrophils.

3.4. Secreted GFs

When cells adhere to the scaffolds, they start to secrete GFs to regulate cellular processes. In diverse environments, cells secrete various GFs through the regulation of different signaling pathways. For example, FGFs, a family of cell signaling proteins secreted by macrophages, are essential for development in TE and involved in a wide range
of other activities [61]. The hepatocyte GFs produced by MSCs have protective effects against lipopolysaccharide-induced vascularization, which is mediated by the mTOR/STAT-3 pathway [334].

4. Latest studies of cell–scaffold interactions for TE in the oral and craniofacial region

The oral and craniofacial region is complex and consists of different tissues or organs, such as teeth, bones, cartilages, skins, mucosae, muscles, and vessels. The specific signaling pathways involved in TE at these sites are shown in Fig. 5. In-depth studies on TE as a promising technique in regenerative medicine have recently been conducted.

3.5. Dental pulp and dentin TE

As a complex composite, tooth healthcare has been one of the most noticeable concerns worldwide [335]. Dentin and dental pulp regeneration are now viable, and alternative therapies for endodontics to restore damaged teeth by applying the advancements in TE are available. Regarding the feasible techniques of dental pulp and dentin regeneration, BMPs are important wherever in vivo or ex vivo [336]. Chakka et al. revealed that transfection of DPSCs with BMP-2–polyethyleneimine complexes lead to increased expression of BMP-2, promoted proliferation, and enhanced mineralization compared to MTA [337]. Cordeiro et al. firstly demonstrated transplanting poly-L-lactide scaffolds seeded with SHED from immunodeficient mice into human tooth slices. The produced bioengineered tissues showed cellular architecture which resembled physiologic dental pulps [69]. The result includes the transformation of SHED into cells similar to odontoblasts and ECs. When administered with VEGF, SHED generated CD31 and vascular endothelial cadherin; SHED was also structured into sprouts resembling capillaries.

Numerous studies on pulp and dentine TE using scaffolds and SCs have been conducted [338]. For instance, the in vivo root maturation of non-vital young permanent teeth with apical periodontitis can be improved by the incorporation of DPSCs and GFs into CS hydrogel, which can produce dentine-pulp-like tissue [339]. Komichi et al. found that the S100-A7 protein released from dentin by MMP-20 was critical in dentin-pulp regeneration, which could be potentially achieved by the scaffolds [340]. Furthermore, Zhang et al. found that with the induction of dentin matrix extract, human umbilical cord blood MSCs (hUMSCs) displayed certain odontoblast markers (DSP, DMP-1, and DSPP) [341]. In contrast, the co-cultured hUMSCs and VEGF enclosed in 3D injectable COL scaffolds produced dental pulp-like regenerated tissue and vessel-like structures. A recent study investigated the functions of amphiregulin (ARG). It revealed that amphiregulin promoted odontoblastic differentiation and facilitated the regeneration and mineralization processes in hDPSCs via the MAPK, JNK, ERK, and PI3K/AKT signaling pathways [342].

The original theory for COL intrafibrillar mineralization in bone and dentin was ion-mediated crystal nucleation. Recent research on the mechanism of particle-mediated crystallization indicates that non-collagenous proteins or their analogs sequester calcium and phosphate ions to create prenucleation clusters [343]. Aggregated clusters penetrate COL fibrils, self-assemble, and undergo crystallographic alignment within the gap zone of the generated COL molecules. The incorporation
of calcium hydroxide could also enhance the formation of a mineralized dentin matrix of hDPSCs, irrespective of the presence of fibronectin [344].

The regeneration of dental pulp and dentin and restoration of tooth vitality may be possible with the use of scaffolds and SCs, which can be both safe and effective [266]. Numerous techniques have highlighted the variety of TE applications in endodontics. Despite these treatment modalities, traditional pulp tissue regeneration over the entire root canal was generally limited. Additionally, the regenerated tissues were repaired by the synthesis of fibrous tissue, cement, or bone, instead of retaining their original pulpal architecture and function [338]. Because the tissue is coated with dentin and receives low blood flow outside the root apical end, pulp regeneration is also restricted. Xuan et al. have recently implanted ex vivo autologous tooth SCs from deciduous teeth in animal models and patients [345]. The results revealed the regeneration of functional dental pulp containing odontoblasts, vasculature, and nerves in the teeth and the recovery of sensitivity to stimuli like temperature. According to their research, the implantation of tooth SCs can aid in the partial regeneration of traumatized teeth.

3.6. Enamel TE

Due to caries, trauma, or other diseases, the enamel is easily defeeted. This must be restored using artificial materials, such as resin, to resemble its mechanical properties. Notwithstanding the necessity for enamel regeneration, the main obstacles to enamel TE are the intricate protein modifications for crystal growth and the precise movements of ameloblasts during the organization of HA crystals into enamel rods [346,347]. Cells originating from the non-dental epithelium, such as gingival ECs and human keratinocyte SCs, have been regulated to differentiate into ameloblasts, which are gradually lost after tooth development.

To culture a patterned enamel correctly, 3D COL sponges with NIH 3T3 mouse fibroblasts have been found to support and compensate for the interactions between ECs and MSCs that occur during early tooth development [348]. Enamel proteins, such as ameloblastin, amelogenin, KLK-4, and MMP-20, were produced by primary enamel organ cells that are co-cultured with NIH 3T3 cells [347]. Tall columnar ECs, which are present on enamel and dentin surfaces, expressed amelogenin when enamel organ ECs and hDPSCs are combined in the scaffolds to form enamel. To mimic epithelial–mesenchymal interactions, hDPSCs and HAT-7 dental ECs were seeded into a 3D multilayered macroscale biomimetic co-culture system utilizing CS and COL [349].

The research related to tooth enamel regeneration mainly focuses on biomimeralization. Five techniques for enamel TE may be implemented: 1) enamel synthesis via physicochemical methods; 2) cell-based enamel engineering; 3) enamel surface remineralization; 4) enamel crystal formation guided by matrix; 5) enamel regeneration resulting from tooth morphogenesis induction de novo [350]. Nevertheless, the available reports regarding specific scaffolds and their impact on possible SC-mediated enamel regenerative outcomes are limited [351]. Enamel cannot renew on its own; thus, engineering methods must be applied. For example, researchers developed a calcium phosphate ion cluster-based material that may be utilized to create a precursor layer that will cause enamel apatite to grow epitaxially, which simulates the crystalline-amorphous biomimeralization of hard tissues in vitro [352]. Despite precisely duplicating the hierarchical and complex structure of enamel, the enamel-identical repair layer was approximately 2.0–2.8 μm for 48 h, much less than the average thickness of tooth enamel (2.0–2.5 mm). Enamel regeneration has been proven possible using recombinant enamel proteins, such as amelogenin, surfactants, or calcium phosphate-based materials [353]. However, the TE procedures appear to be challenging to apply clinically or they only produce a small amount of regenerated enamel on existing natural enamel. Therefore, the use of artificial materials is probably necessary for the final step of tooth restoration even after pulp and dentin regeneration.

3.7. Periodontal ligament TE

The periodontium comprises various specialized tissues that surround the tooth root as well as support and attach the tooth to the jaw. The periodontal ligament (PDL) anchors the mineralized cementum-lined root surface to the alveolar bone, and the gingiva covers the entire root surface. The prevalence of periodontal pockets among the elderly was the highest compared with those among adults and adolescents [354]. Moreover, periodontal inflammation will eventually result in tooth loss. Therefore, devising a reliable and effective therapy to repair PDL is crucial.

The potential for TE-based therapies for disturbed periodontium was highlighted by the discovery of PDLSCs. For instance, Basu et al. cultivated PDLSCs as a cell sheet that showed two structurally different tissues [355]. Similar to the cementum, the central tissue was mineralized; moreover, it expressed ALP and BSP. In contrast, the uncalcified peripheral tissue expressed peristin and PDL-associated protein-1, which are features of the PDL. With the support of the cell sheet, PDLSCs can self-assemble into a structured cementum PDL-like complex. Scaffolds made of poly (3-hydroxybutyrate) and CNTs may also resemble the PDL [70]. In the composite scaffolds, CNTs enhanced the mechanical characteristics equivalent to those of the PDL obtained from a 23-year-old human. An investigation of a rat model in vivo revealed that the CNTs in the scaffolds had acceptable biocompatibility; they also induced mild inflammation and moderate vascularization.

Although PDLSCs are auspicious in TE, their rarity restricts their use in PDL regeneration. Moreover, due to the severity and prevalence of periodontitis, more older people require PDL regeneration than adults or teenagers. However, as donors age, the multipotent and self-renewal abilities of PDLSCs decline. To devise a PDL regeneration therapy, the collection of PDLs from elderly patients must be simplified [356]. Other less differentiated MSCs, including induced.

Stapar et al. reported that the presence of fiber-guiding architecture appeared to promote the level of PDL maturation with highly ordered ECM and high peristin expression; however, cementogenesis is insufficient [357]. Farag et al. reported that decellularized PCL scaffolds were capable of inducing cell differentiation in vitro and potentially facilitating PDL regeneration in vivo [358]. This verifies that decellularized PDL scaffolds have the regeneration potential for PDL TE [359].

3.8. Whole tooth TE

The successful bioengineering of an entire tooth requires appropriate cell sources and scaffolds as well as the induction of the cascade expression of particular genes involved in tooth development [360]. Traditional SC-based, biomaterial-based, and bioengineered tooth germ-based strategies as well as other novel approaches (such as cell sheet technology, biofunctionalization, and GF delivery) have also been investigated [361].

For an in-depth understanding of whole tooth TE, signaling pathways and in vitro culture conditions were thoroughly investigated to study the development of an embryonic mouse tooth bud [362]. However, embryonic tooth germ cells are not the foremost for therapeutically applicable tooth replacement therapy because of their inaccessibility as well as the drawbacks identified above. The ability of adult tooth cells to regenerate for producing bioengineered teeth has thus been the subject of extensive research. Adult dental SCs from deciduous or adult exfoliated teeth are appropriate for tooth regeneration applications because they are readily available and can be collected and stored for subsequent use. There have been some challenges in whole tooth TE: creating the functionality of teeth with a predetermined size and shape, producing full-size bioengineered teeth, controlling infection, maintaining oral health, and achieving complex tooth tissue regeneration [363]. Therefore, certain regenerative dentistry approaches, such as iPSCs, may have potential applications. To study whole tooth regeneration and development, some studies have developed mouse models to investigate the
mesenchymal–epithelial interactions in whole tooth regeneration. Wu et al. characterized the odontogenesis of cells from early-stage tooth germs of miniature pigs with COL/GEL drop in vitro [364]. The explants in the pig jawbones survived and grew, according to radiographic evidence. It was also revealed that the explants developed entire tooth structures and had dental components that were properly ordered, including organized dentin, cementum, PDL, dental pulp, vascular, and nerves that closely resembled those of actual teeth, according to histological analysis.

3.9. Bone TE

Bone TE is possibly the most popular field of research on the oral and craniofacial regions. A critical-sized defect may result from systemic or local causes. In this situation, a scaffold with MSCs or osteoprogenitor cells can be implanted into the bone defect site for bone reconstruction. For bone TE, bioceramics are renowned for their biocompatibility, biodegradability, osteoconductivity, strong mechanical strength, and stiffness. Their biocompatibility and similarities with the ECM can be further improved by the addition of natural polymers [365]. Additionally, a crucial stage in the construction process is incorporating cells onto scaffolds, whether through 3D bioprinting or directly seeding cells onto prefabricated scaffolds. Fig. 6 shows important fabrication methods for bone TE, including 3D bioprinting.

Tissue thickness restricts nutrition and oxygen diffusion, which are necessary to enable osseointegration and osteogenesis during bone healing and regeneration. Hence, vascularization is the most critical problem in bone TE [366]. Some studies have investigated the appropriate vascular network in engineered scaffolds, including using more biocompatible materials with nano-structure or micro-structure, improving the morphology and porosity of scaffolds, adding angiogenic GFs (such as FGFs and VEGF), and utilizing co-culture cell systems under dynamic or static conditions [367,368].

Furthermore, SCs have not only the capacity to regenerate or differentiate in cases of chronic tissue defects but also the ability to regulate the immune microenvironment [369]. Bone tissue regeneration begins with acute inflammation and then shifts to a regenerative or degenerative phase primarily because of immune cells and their interaction with the other cells involved in bone regeneration [370]. Wang et al. incorporated SrFe$_{12}$O$_{19}$ NPs into HA/CS scaffolds doped with magnetic lanthanum to derive SCs and facilitate endogenous bone TE [371]. By stimulating macrophage polarization into the M2 phenotype in vitro, the scaffolds successfully obtained rat BMSCs and altered host immune responses. By increasing the phosphorylation of the SMAD 1/5/9 pathway, the scaffolds may also promote the differentiation of rat BMSCs toward OBs.

Inadequate volumes or dimensions of bone or gingiva are insufficient to achieve proper function. Accordingly, esthetic or prosthetic restoration, guided bone regeneration, and guided tissue regeneration are introduced as common dental surgical procedures to direct the formation of bone or gingival tissue in situ with barrier membranes (such as Bio-gide®) [373]. The delivery of sufficient blood and undifferentiated MSCs for angiogenesis is crucial during the early phases of regeneration [374]. In the case of extensive bone defects, although COL-resorbable barrier membranes are widespread, regenerative, osteoinductive, and osteoconductive bone substitutes are indispensable [375]. Bone substitutes (e.g., Bio-Oss or InterOss®) are mainly inorganics rich in Ca$_{2+}$ and PO$_{4}^{3-}$; the specific mechanism of cell inorganics is discussed above. In the clinic, bone substitutes are typically mixed with blood, platelet-rich plasma, fibrin, or concentrated GFs to improve treatment [376]. These additives contain high amounts of GFs, which can accelerate the healing of soft and hard tissues [377]. With the assistance of bone substitute scaffolds and RSs, enhancing the localized ingrowth of MSCs from the surrounding tissue in the bone defect is possible.

Fig. 6. Fabrication techniques for bone TE materials [372].
Although bone TE has been widely used in implant and orthopedic surgery, the majority of the literature focused on the nature of materials and the fabrication technology for potential applications in bone TE, such as short-term aspects of bone formation, including cellular attachment, proliferation, differentiation, and early mineralization [378]. There is insufficient coverage of large-scale repair situations with recovery and biodegradation profiles. Moreover, simple, secure, and effective techniques for SC isolation and expansion (including an improved understanding of MSC phenotype bioinformatics) are required. Additionally, the structure and mechanical properties of bone tissue are impacted by various loading situations and in vivo implantation sites. One of the greatest challenges in bone TE is probably developing mechanically strong porous scaffolds that preserve ideal vascularization and host integration properties.

3.10. Cartilage TE

Through intramembranous ossification, the craniofacial bones are mainly generated from the cranial neural crest or paraxial mesoderm. The most studied cartilaginous structure in the craniofacial region is the temporomandibular joint disc [292]. Therefore, the restoration of cartilage abnormalities or deformities is challenging due to cartilage’s avascularity and low regeneration ability.

Injectable hydrogels administered into multiple scaffolds have received increasing attention because of their physical properties similar to those of a native ECM, tolerance to mechanical stresses (especially in load-bearing joints), and minimally invasive injection procedures. They can also be tuned to change their properties according to the requisites of the application [379]. The hydrogel’s composition, level of cross-linking, crosslinking techniques used to create the structure rigid, and cell density are some of the variables that can be modified. thermo-sensitive hydrogels have been popular in cartilage TE because they are facile to embed into cell ECMs, suitable for irregular cartilage defects, and easily triggered under mild physiological conditions [380]. The addition of articular cartilage ECM components, such as HRA [62], chondroitin sulfate [381], and COL [54], is an attractive substitute. Ngadimin et al. reviewed papers on GAG-based hydrogels and concluded that they are the most viable compared with other hydrogel types. Moreover, PEG-based hydrogels have the highest Young’s modulus. However, their modulus remains insufficient compared with the compression modulus of the articular cartilage in a natural state [382]. Changes in material stiffness and composition have an impact on the ability of chondrocytes to fabricate the matrix.

In vivo and in vitro research has found that hydrogels can offer a favorable environment for the development of chondrocytes and MSCs. Some challenges limit the large-scale application of hydrogels, such as inevitable degradation, lack of affinity between scaffolds and cells, absence of a well-designed structure, and lack of clinical application standards. Additionally, more studies on the effects of hydrogel chemistry and matrix stiffness imposed on cells must be implemented.

3.11. Mucosa and skin TE

The oral mucosa is the most abundant tissue lining the oral cavity. However, it can be injured or destroyed due to various disorders, including gingival recessions, vestibuloplasties, cleft palate, traumas, and tumor excisions, which all result in the abnormalities of the buccal mucosa [383]. Healing these soft tissue defects in the oral cavity that result in the abnormalities of the epithelium [385]. There is insufficient coverage of large-scale repair situations with recovery and biodegradation profiles. Moreover, simple, secure, and effective techniques for SC isolation and expansion (including an improved understanding of MSC phenotype bioinformatics) are required. Additionally, the structure and mechanical properties of bone tissue are impacted by various loading situations and in vivo implantation sites. One of the greatest challenges in bone TE is probably developing mechanically strong porous scaffolds that preserve ideal vascularization and host integration properties.

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displayed a maximum loading and final stress comparable to those of natural tendons. The composite SF scaffolds may also increase the expressions of COL1, SCX, and TNMD. Wang et al. constructed a biomimetic parallel-aligned COL scaffold combined with tendon SCs and periostin [399]. Through the regulation of Sox2 and Oct4 by periostin, the scaffolds can activate the stemness and tendon differentiation ability of tendon SCs and achieve the satisfactory repair of tendon defects in terms of structure and function.

Thus far, in the community involved in tendon TE, the precise functional role of material cues in directing the tenogenic differentiation of SCs is not well-established. It is difficult to properly adapt these material cues and incorporate them into a scaffold for creating sophisticated tendon grafts. Furthermore, correctly targeting the appropriate immune cell populations at the appropriate point in time is difficult. This is because of the incomplete understanding of the immunological processes governing the tendon healing process, particularly the influence of functional and phenotypic alterations on immune cells [291]. As the complexity of cell types and lineages included in muscle and tendon TE constructs develops, improved concentration on the biomaterial characteristics, both pre- and post-printing, needs to be taken into consideration.

### 3.13. Nerve TE

In the craniofacial region, peripheral nerve injury (PNI), including numbness or lack of feeling in the tongue, gingiva, cheeks, jaw, or face, is a prevalent clinical disorder that frequently results in functional loss and aberrant patient appearance. The engineering of PNI with the advantages of DPSCs produced from the neural crest, including abundant supply, easy extraction, moderate immunogenicity, and high rate of in vitro proliferation, has been reported [400]. This is shown in the hematoxylin and eosin staining of the histological section illustrated in Fig. 7. Schwann cells can develop and release important neurotrophic factors that support the repair of damaged nerves.

Although synthetic materials are increasingly becoming popular due to their mechanical strength and versatility in manufacturing, synthetic polymers combined with natural polymers have been found to function extremely well as conduits and grafts [401]. Neurite development and extension have significantly progressed because of conductive polymers [402]. Nanofibers have been proven more successful than microfibers by significantly boosting cell adhesion, growth, and proliferation. Cells, such as Schwann cells and different SCs are chosen to increase the biological activity of scaffolds. In addition, to enhance the sticky properties of scaffolds (which promote cell growth and differentiation), cell adhesion molecules (e.g., integrins or cadherins) have been added. Growth factors (such as nerve GFs or glial cell line-derived neurotrophic...

![Fig. 7. The HE images of cross-sections and longitudinal sections of the regenerative nerve fibers after 3-month surgery. (A) Cross-sectioned nerve conduit; (B) Longitudinally split nerve conduit. CSM: Cellulose/soy protein isolate composite membrane; G: GelMA hydrogel; GFD: GelMA hydrogel combined with recombinant human bFGF and DPSCs; NA: nerve autograft. Blue arrow: CSM conduit; Yellow arrow: newly regenerated blood vessels [289].](image-url)
3.14. Vascular TE

Early attempts to create replacement blood vessels were centered on using bypass grafts made of MPs, such as polytetrafluoroethylene or Dacron® [405]. However, these grafts barely satisfy the biological challenges that occur at the blood–material interface where thrombotic events may rapidly occur. In addition, the blood vessels in the oral and maxillofacial areas are mostly small [406]. Therefore, the use of vascular TE is more promising. It can also ensure the survival and regeneration of the grafts. Sasaki et al. revealed the generation of functional blood vessels of DPSCs for vasculogenetic differentiation that was seeded in tooth slices/scaffolds (mainly HA). Results revealed that DPSCs could be regulated by the ERG transcriptional activity and MEK1/ERK signaling pathways [407]. CD301b+ macrophages could also regulate the angiogenesis of bioconstructs via the axis of CaN/-NFATc1/VEGF [408].

Vascularization is also crucial in managing PNIs, particularly in preventing central necrosis in nerve grafts for sizable and protracted nerve defects. For instance, Li et al. fabricated an HRA-based hydrogel modified with fibronectin motifs that enhanced the ECs to bind to integrins and improved the vascularization in a mouse PNI model [409].

The challenges in developing the optimum design of vascular substitute are considerable. Moreover, multifunctional materials with optimized bioactive molecule release as well as presentation for in situ vascular regeneration remain considerably insufficient. A deeper comprehension of the biology of vascular progenitor cells is necessary to fully utilize their potential in the endothelialization of artificial grafts [410].

3.15. Salivary gland TE

Xerostomia is frequently identified in patients with head and neck cancer based on salivary gland (SG) epithelial damage. The regeneration of the SG epithelium or even repairing its secretory function has become possible through regenerative medicine. Except for the cell modalities of the spheroid, organoid, 3D microfluidic cell culture system, and dECM scaffolds, the bioprinting technique has been a candidate for SG regen[411]. In addition, polymers (such as PLA, PGA, PEG, poly(lactic-co-glycolic acid) hydrogels, and HRA-catechol conjugates) have been used as scaffolds for TE in the field of SG [412]. However, ADSCs are the only engineered cells that have reached clinical trials to enlarge the SG epithelium and improve salivary flow [288]. Furthermore, the identification of RSs, cells, and matrices may improve the maintenance of the SG secretory function in vitro [413].

Despite the promising polarization provided by matrix mimetics, improving the secretory function remains limited. This difficulty highlights the necessity for combinatory methods that optimize the matrix with other paradigms. The impact of matrix and media conditions on the acinar and duct must also be investigated. Moreover, methods based on the secreteme and 3D organotypic cells may be adequate to regenerate the SG and replace traditional cell transplantation techniques.

4. Conclusion and perspectives

Currently, xenogeneic, allogeneic, and autologous cells are primarily used in TE research. The use of xenogeneic and allogeneic cells is typically confronted with several drawbacks, such as immune response or ethical issues. In contrast, MSCs and iPSCs derived from somatic cells are easy to obtain and only cause slight damage to the donor. Because these cells have a strong differentiation ability, researchers and clinicians have gradually favored their use.

As previously discussed, all single scaffold materials have certain restrictions. Although the majority of natural polymers and inorganics exhibit excellent biocompatibility in vitro and in vivo, the immune system continues to consider them as non-autologous foreign bodies. As immune reactions play an important role in cell–scaffold interactions, the number of studies investigating scaffold materials with immune regulation or delivery of growth factors/agents with immune regulation functions has gradually increased. Moreover, considering the instability of biomaterials and variation in molecular structures among batches is important. In contrast, previous investigations have shown that synthetic materials possess weak cell affinity. To enable future practical applications, more trustworthy data from animal studies are required because of the intricacy of structures and the absence of specific control.

Furthermore, developing multilayer scaffolds that may result in coordinated tissue regeneration and regulation of the degradation rate of the scaffolds to match the rate of defect healing may be important for future improvement. New technologies, such as rapid prototyping (RP), are introduced to overcome the obstacles in accurately preparing the scaffold structure that is encountered in traditional technologies [414]. The construction process is typically implemented via 3D printing or additive manufacturing (AM) techniques, which gradually accumulate materials to create solid assemblies. The continuous development of manufacturing technologies enables the printing of biofunctional scaffolds similar to the ECM (such as 3D-printed microgel [415]), acting as a microenvironment for cell adhesion, proliferation, and differentiation. The RP techniques for medical and TE purposes can be applied to non-cellular scaffold fabrication [416]. For clinical applications, AM scaffolds have considerable potential [2]. In contrast, 3D bioprinting enables the harnessing of cells, tissues, and organs (which are considerably in demand for regeneration) based on the precise localization of biological components and cells by the LBL technique. Through different bioprinting techniques, desired AM structures and properties for advanced tissue regeneration can be achieved.

Bioink utilizes 3D printing techniques to fabricate artificial living tissues. It consists of cells and other carrier materials around the cells. Such carrier materials are typically polymer hydrogels that serve as scaffolds. Cells can grow, proliferate, and differentiate after attaching to the hydrogels [417]. The printability of bioink is crucial in hydrogel fabrication. In addition to biocompatibility and biodegradability, bioink must also exhibit deformability and flowability. It must be stable after printing to preserve the structure and shape of a model. Because the components of hydrogel bioink (such as GEL and HRA) are more advantageous than inorganic ceramics, they have received considerable attention.

Organoids are characterized as 3D-structured in vitro biological complexes that contain single or multiple cell types and partially reproduce the shape and functions of their in vivo counterparts [418].
Even if their maturity and complexity vary, it has been demonstrated that practically all organs are generated from the three germ layers, including the definitive endoderm, mesoderm, and ectoderm. In vitro and in vivo simulations are used to create organoids with intricate biological functions. Recently, bone and dental organoids have demonstrated a wide range of potential applications in the research of drug screening, organ development, and mechanism study [419,420].

Traditional scaffold materials, such as inorganics or polymers, are all insulting. However, the demand for conductive scaffold materials has also started to increase, especially in skin, nerve, and muscle TE. Moreover, the successful use of engineered constructs is currently restricted to tissues requiring less or no vascularization, such as skin or cartilage, for which the post-implantation vascularization from the host is sufficient to provide oxygen and nutrients. Therefore, facilitating vascularization after implantation is necessary to apply TE successfully to large tissues, such as bones and muscles.

Cells, scaffolds, and RSs form a triad in traditional TE. The number of studies investigating the addition of only two components (i.e., cells and scaffolds) continues to increase. In addition, the conduct of cell-free or scaffold-free research is gradually increasing. Hence, the use of fewer components with advanced regeneration effects has become an important direction of TE research, providing a new opportunity to understand the interaction between cells and scaffolds. Although more new technologies and biocompatible materials are expected to be used to achieve TE and tissue regeneration in the future, the current regeneration technique is time-consuming. Researchers must explore methods for applying GFs and other materials to accelerate the process and the specific molecular mechanisms involved. Single-cell genomic techniques could also be applied to engineer cells to generate atlases of cell diversity [421]. Studies related to this technique concentrate on characterizing engineered cells at the level of chromatin organization and epigenetic markers in addition to transcriptome [422]. The understanding of intricate multicellular biological systems has substantially increased as a result of recent developments in spatial transcriptomics, which tries to integrate gene expression data spatially. Although the technique is regarded as a promising tool to reveal the cell–scaffold interactions in the oral and craniofacial region, the methodologies demonstrate method-specific drawbacks, including sensitivity, tissue-type dependence, labor extensiveness, tissue-type dependence, and a limited ability to obtain particular single-cell information [423].

Cell–scaffold interaction is indispensable in TE and scaffold design. Numerous studies have reported on the interaction between cells and scaffolds. By combining inorganic and polymeric composite scaffolds, satisfactory biocompatibility can be achieved, and the immune response can be minimized. In addition, most current studies focus on the interaction between scaffolds and seeded SCs. In contrast, only a few investigations on the interaction between scaffolds and recruited somatic cells around defects have been conducted. Furthermore, research on the synergetic effect of scaffold structures and degraded bioactive molecules or ions on cells has not been conducted. With the use of new technologies, the study of cell–scaffold interactions can be improved. Cell adhesion is a complex process. With the synthesis of physical, chemical, and mechanical features. In TE, cell differentiation and other responses are controlled by scaffold properties, enabling superior regeneration. Novel devices can detect and screen diseased cells as well as analyze the interaction processes. The clinical application of TE in the oral and craniofacial region is crucial because the ultimate goal of TE technology is the development of regenerative medicine. Therefore, more well-designed clinical trials need to be carried out to 1) further confirmation of in vitro and in vivo studies; 2) determination of the clinical relevance; 3) comprehensively compare with existing techniques; 4) standardization of the trial. Additionally, prolonged follow-up periods should be taken into account to confirm the therapeutic effect.
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