3D Printing of Bioinspired Biomaterials for Tissue Regeneration

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Biological systems, which possess remarkable functions and excellent properties, are gradually becoming a source of inspiration for the fabrication of advanced tissue regeneration biomaterials due to their hierarchical structures and novel compositions. It would be meaningful to learn and transfer the characteristics of creatures to biomaterials design. However, traditional strategies cannot satisfy the design requirements of the complicated bioinspired materials for tissue regeneration. 3D printing, as a rapidly developing new technology that can accurately achieve multimaterial and multiscale fabrication, is capable of optimizing the fabrication of bioinspired materials with complex composition and structure. This review summarizes the recent developments in 3D-printed bioinspired biomaterials for multiple tissue regeneration, and especially highlights the progresses on i) traditional bioinspired designs for biomaterials fabrication, ii) biological composition inspired designs for the 3D-printed biomaterials, and iii) biological structure inspired designs for the 3D-printed biomaterials. Finally, the challenges and prospects for the development of 3D-printed bioinspired biomaterials are discussed.

1. Introduction

After millions of years of evolution, the creatures in nature have evolved almost excellent structures and functions. For example, the lotus has an unique structure with many parallel channels, which could significantly promote air exchange and nutrients transportation from external environment. The nacre and whale baleen exhibit excellent mechanical strength as well as superior toughness due to the ordered microstructures. Furthermore, human are consisted of multiple complex tissues which possess hierarchical structures from macroscale to nanoscale to maintain the operation of the body. The bone with hierarchical structure contributes the perfect combination of strength and toughness. The lamellar skin exhibits various functions for different layers. In addition, the composition is critical for biological systems to survive in the complicated environment. Creatures in nature are just like experienced materials scientists who acclimatize themselves with wonderful compositions. Nacre, bone, and whale baleen, all contain inorganic and organic components to optimize the mechanical properties for adapting the complex environment. However, due to the diversified environment, there are great differences for the proportion of the two phases. Furthermore, the excellent photothermal and tissue healing effect of oligomeric proanthocyanidins and polydopamine, which are inspired by the major constituent of grape seed and mussel, are investigated. These biological capabilities are far beyond conventional engineering systems, which inspire the scientists to design the advanced and multifunctional materials.

Biomaterials applied in tissue regeneration should possess comprehensive characteristics due to the complex human environment. For example, the repair materials for load-bearing bone and tooth should possess excellent mechanical strength. The skin repair materials should possess the functions including the tissue-regeneration bioactivity, bacteriostatic efficacy, and anti-inflammatory ability. To realize the excellent properties and multifunction, scientists have tried to design biomaterials from the perspective of bionics. For example, load-bearing bone repair materials were fabricated through imitating the lamellar structure of nacre. In addition, the enamel-inspired columnar nanocomposites were reproduced based on the structure of tooth enamel. Moreover, the artificial bioinspired heart was constructed with cells and collagen, which has similar composition to actual heart, and thereby enhanced the biocompatibility and achieved the part functions of heart. It would be useful to imitate the composition and structure of biological systems for replicating the remarkable functions in tissue regeneration field. However, the pathway to the synthesis of bioinspired materials remains a significant challenge.

At present, some strategies have been developed for biomaterials design, such as self-assembly method and bioinspired mineralization. However, these conventional technologies are
only capable of fabricating the biomaterials for the tissues that possess relatively simple structures and composition like skin and bone. However, the delicate structure and complicated composition of creatures are far beyond the capability of fabrication technologies, which restricts the biomimetic research of tissue regeneration.\[1,19\] 3D printing has shown great promise in designing biomaterials with multiscale, multitematerial, and multifunctional architecture. It could easily realize the architecture design from macroscale to microscale and control the complicated composition of biomimetic objects. In decades, plenty of 3D printing technologies have been successfully developed such as direct ink writing (DIW),\[20\] fused deposition modeling (FDM),\[21\] selected laser sintering (SLS),\[22\] and stereolithography (SLA).\[23\] However, the challenging issue is how 3D printing technology can be applied in the design of advanced biomaterials for multiple tissue regeneration based on biomimetic strategy.

In this review, we will present an overview of recent advance of 3D-printed biomaterials for multiple tissue regeneration from the perspective of bioinspiration (Figure 1). We briefly summarize the traditional bioinspired designs in tissue regeneration. More importantly, recent achievements and progresses on 3D-printed bioinspired biomaterials will be highlighted, including 1) biological composition inspired designs of the 3D-printed biomaterials. Inspired by the composition of fancy creatures and repair tissues, the bioinspired biomaterials with multifunctionality are fabricated through 3D printing technology; 2) biological structure inspired designs of the 3D-printed biomaterials. The microstructure accounts for the excellent mechanical properties of the biomimetic objects like nacre, tooth, and lobster claw. Furthermore, some other interesting structures, such as the parallel channels of lotus, the sausage embedded in bread structure of hot dog have been replicated into the biomaterials design for tissue regeneration. Finally, current challenges and future developments on 3D printing of bioinspired biomaterials are discussed.

2. Traditional Designs of Bioinspired Materials

Constructing the extracellular matrix (ECM) which entirely mimics the composition and structure of tissues would be one of the best biomimetic strategies. However, due to the complicated tissue environment, it is a great challenge to replicate natural ECM into biomaterials.\[24\] Thus, various biomimetic methods are explored in the design of biomaterials for tissue regeneration, such as mimicking tissue composition and bioinspired mineralization (Figure 2).\[25–27\]

2.1. Mimicking Tissue Composition

So far, autologous tissue grafting is an optimal choice for repairing tissue defects, like bone,\[28\] skin,\[29,30\] nerve,\[31,32\] and so on.\[33\] However, autologous tissue grafting will cause secondary injury to the patients, making it not suitable for the large/refractory tissue repair. Hence, the scientists have paid more attentions to biomaterials design by imitating the tissue composition to replace autologous tissue grafting.\[34–36\]

2.1.1. Mimicking Bone Composition for Defect Regeneration

Repairing the massive bone defect remains challenging in clinic. In recent years, various types of biomaterials have been fabricated for improving bone replacement/regeneration, such as titanium alloy, bioactive polymers, and bioceramics.\[19\] The ideal

Biomaterials for bone replacement/regeneration are designed to mimic the structure and biological performances of the native bone tissue in terms of both biological composition and hierarchical architecture. Considering biological composition, bioceramic scaffolds have attracted increasing attention for application in bone regeneration due to their biocompatibility, bioactivity, osteoconductivity, osteoinductivity, and mechanical load-bearing capability as well. It is discovered that the bone with hierarchical structures is composed of 35% collagen and 65% hydroxyapatite (HA), which contributes the brilliant mechanical and biological properties. To date, various biomaterials containing collagen have been fabricated such as collagen hydrogels, collagen hierarchical micro/nanofibrous, and nanosilica-collagen scaffolds to repair the bone defects. For example, the nanosilica-collagen scaffolds were composed of collagen matrix and nanosilica coating, which could effectively induce in situ bone regeneration, indicating a great potential for clinical translations. The collagen grafts played significant roles in bone regeneration due to their ability to mimic the biochemical composition and mechanical properties of native bone ECM. The nanosilica on the scaffold surface could further trigger osteogenic differentiation of MSCs. In addition, the HA-based composites have been widely studied for bone regeneration due to their excellent biocompatibility and mechanical strength. Recently, Zhu et al. had prepared micro/nanostructured calcium phosphate bioceramics which could induce mesenchymal stem cell immunoregulation and accelerate the regeneration of segmental bone defects. By mimicking the bone components...
of collagen and HA, plenty of biomimetic materials have been fabricated for bone defect repair. Such biomimetic composition strategy is of great potential in biomaterials fabrication for hard tissue regeneration.

2.1.2. Mimicking Tissue Composition for Skin and Neural Tissues Repair

The healing of soft tissues requires the synergistic functions of cells and ECM. Conventional regeneration biomaterials like hydrogels and electrospinning scaffolds (mostly collagen, gelatin, chitosan) have been widely attracted due to their biocompatibility and degradability.[48–51] For better regeneration and imitating the composition of tissues, skin-related cells were introduced into the biocompatible materials.[53–56] As main cells in the skin, human fibroblasts (hFBs) and keratinocytes (hKCs) were loaded in the fibrin matrix to prepare the skin regeneration materials. In the inducement of biomaterials, fully differentiated human skin came into being in vivo, indicating that such biomaterials were promising in clinic for large skin regeneration.[57]

The spontaneous regenerative ability of neural tissue is inadequate after injury.[57,58] Unfortunately, there are no satisfied biomaterials to enhance endogenous regeneration for neural tissue repair.[59] Nerves are composed of hierarchal collagen bundles, which contain kinds of neural cells.[60] Lai et al. had constructed neural stem cell (NSC)-loaded collagen sponge scaffolds to reconstruct the neural defects in a canine model. After 72 weeks of repair, the canines regained the abilities of weight-bearing locomotion and coordinated stepping, revealing that the biomaterials mimicking the composition of nerves had excellent regeneration ability for neural tissues. In addition, the studies demonstrated that the collagen in biomaterials would significantly stimulate the adhesion and proliferation of neural stem cells.[61–63] Imitating the composition of neural tissue to construct the biomaterials containing the pluripotent stem cell-derived cells and collagen may be a future direction to regenerate neural tissue.

2.2. Bioinspired Mineralization

Bioinspired mineralization is translating the self-assembly characters which found in natural living systems to a general method for modifying bioactive molecules within protective covering.[64,65] Such bioinspired mineralization strategy has been widely exploited in the synthesis of photonics materials, biomedical implantation, industrial catalysis, and biopharmaceutical delivery.[64] The applications of bioinspired mineralization in tissue regeneration are mostly focusing on improving the bioactivity and mechanical strength for bioactive molecules.[15,134]

2.2.1. Improving the Bioactivity of Materials

The inorganic phase plays a key role in the bioactivity improvement of biomaterial matrices.[66,67] Recently bioinspired mineralization has been applied in multiple biomaterials to enhance the bioactivity of materials system.[68–71] For example, graphene, as a 2D material has been considered as a potential biomaterial in tissue regeneration due to its large surface area, brilliant mechanical properties, and potential to modulate the biological performance.[70,72] For further improving the bioactivity of graphene, we proposed to introduce the inorganic calcium silicate into the multilayered graphene oxide/chitosan/calcium silicate biomaterials to repair the bone defect through vacuum filtration self-assembly method (Figure 3a,b). Using this bioinspired mineralization strategy, the calcium silicate was embedded in the lamellar layers of graphene and further improved the bioactivity of the system. In Figure 3c, the cross-section morphology of the material exhibited the distribution of inorganic phase on the surface of the layered graphene oxide homogeneously. The in vitro and in vivo results indicated that inorganic calcium silicate enhanced the bioactivity of the composite and further promoted the cell adhesion, proliferation, the expression of osteogenic genes, and the formation of new bone (Figure 3d,e).[16] In addition, we have confirmed that such composite with porous microstructure was in favor of skin regeneration. Therefore, it could be concluded that bioinspired mineralization strategy is of great significance for the fabrication of tissue regeneration biomaterials.[15]

2.2.2. Nacre-Mimicking Design for Mechanical Enhancement

Another significant purpose for bioinspired mineralization focuses on the mechanical reinforcement of the organic matrix. Nacre possesses excellent mechanical performance, which is composed of 95 wt% plate-like CaCO₃ and 5 wt% organic polymers after the bioinsORIZATION for long time.[73] Brilliant mechanical performances are required for the regeneration of tissues like load-bearing bone, tendon, tooth, and skin.[15] Therefore, plenty of biomaterials were fabricated by
imitating the “brick-and-mortar” structure. Bai et al. developed the nacre-mimetic HA-based composites by a bidirectional freezing method, which exhibited similar mechanical performances to cortical bone, indicating a great potential for bone regeneration.\cite{74} The bidirectional freezing method has been applied for the dental materials design, and the nacre-like zirconia-based composites displayed exceptional damage tolerance, unprecedented mechanical properties, and excellent machinability. Such materials have been demonstrated as a potential candidate for new-generation tooth replacements.\cite{75} Moreover, the nacre-mimic composites with enhanced mechanical strength were designed to detect the environment, translate the solar energy, which opened up a new approach for the next wearable and flexible electronic skin.\cite{76,77} Nacre-mimicking design is an effective strategy to enhance the mechanical properties of biomaterials. However, such designs are limited to the shape and size accuracy, and some additional process should be operated to match the requirements before the application.

3. 3D-Printed Biomaterials with Biological Composition Inspiration

A current challenge in conventional designs of bioinspired materials is to focus on the preparation of single heterogeneous architectures. The complexities of tissue composition and structure are exceeding the capacity of traditional design strategies. 3D printing, as a rapidly developing new technology that could accurately achieve multimaterial and multiscale fabrication, has been applied in the fabrication of bioinspired biomaterials with complex composition and architectures. Furthermore, 3D printing would be more potential for application in tissue regeneration, because it could fabricate the biomaterials with the matched shape of defects. Moreover, the biomaterials with different components would be easily prepared, especially for cell-containing biomaterials. Mimicking the environment of tissue defects as much as possible would be an effective strategy for different tissue or organ regeneration. Hence, in this section, 3D bioprinting which transfers the various cells to the tissue defects will be mainly introduced for tissue regeneration. The 3D-printed biomaterials inspired from specific biological composition are further discussed.

3.1. 3D-Bioprinted Biomaterials for Mimicking Composition of Multiple Tissues

The seed cells, scaffolds, and growth factors are three most key components in tissue engineering.\cite{78} The application of seed
cells which are related to the injured tissue is a promising biofabrication strategy to accelerate the regeneration. However, it remains a challenge for traditional strategies to fabricate different artificial tissues because the seed cells are difficult to achieve a homogeneous distribution in biomaterials. 3D bioprinting opens new avenues for constructing the desired 3D functional tissues with single or multiple cells distributed in biocompatible materials. To achieve the complex artificial tissue fabrication, several bioprinting technologies are developed, such as inkjet-based bioprinting, stereolithography bioprinting, and magnetic bioprinting. Using the advanced bioprinting technologies, various complex artificial tissues like bone, cartilage, skin, heart, and lung have been successfully fabricated.

3.1. Bone and Cartilage

Due to the limited self-repairing capability of bone and cartilage, plenty of regeneration biomaterials were applied to accelerate the repair process of bone defects, such as bioactive glasses, bioceramics, and bioactive polymers. The tissue cells play critical parts in the defect regeneration. Therefore, combining native tissue cells and bioactive materials would distinctly promote the regeneration of hardly self-healing tissue like bone and cartilage. Replicating the different cell composition of native articular zones, Idaszek et al. fabricated the 3D-printed hydrogel scaffolds which contain human articular chondrocytes and human mesenchymal stem cells (MSC) by emulating the heterogeneous layer structures of native articular tissue to achieve the integrated regeneration of bone and cartilage defects. The 3D-printed scaffolds exhibited remarkable capabilities in vitro and in vivo, but it still remained a challenge to regenerate functional articular cartilage capable of sustaining high load-bearing environments. Rathan et al. fabricated a special high-strength scaffold, which was composed of 3D-printed PCL framework and bioinks with MSC, growth factors, and hydrogel. They firstly printed the PCL framework and then filled the empty pores with the bioinks in sterile conditions. Compared with the elastic modulus of hydrogel scaffold of 0.01 MPa, such scaffold was successfully reinforced with an elastic modulus of 0.33 MPa, similar to that of native cartilage. The scaffold possessed brilliant compressive mechanical property and bioactivity, indicating a great potential for cartilage regeneration in the complex load-bearing microenvironments. For mitigating the infection incidence after craniotomy, macrophages and antibiotics were integrated into the 3D-printed scaffolds to establish a persistent anti-infection biomaterial. The macrophages presumably promoted biofilm dispersal and effectively transformed biofilm-associated bacteria into metabolically active cells which were sensitive to antibiotic action.

3.1.2. Skin

As the largest organ of human body, skin plays an essential role in sensation, moisture retention, and defending the pathogen as the first defense barrier. However, skin could be damaged easily by various factors such as scratches, burns, and dermatoenecrosis, which requires numerous skin substitutes for regeneration. Skin healing demands the synergistic function of various cells and ECM. Hence, numerous bioprinted biomimetic biomaterials (mostly collagen, gelatin, and fibrin) have been constructed for skin replacement/regeneration. However, most of single-component bioinks have to overcome the limited mechanical strength and shrinkage issues through introducing additional polymers like alginate in bioink formulations, which requires the multicomponent bioinks with increased complexity, and consequently reduced control over cellular responses. Recently, Pereira et al. developed a single-component pectin-based bioink with controlled mechanical and rheological properties, which could be biofunctionalized with relevant peptide ligands for bioprinting of complex 3D skin constructs without the need of additional polymers. The biofunctionalized pectin-based bioinks as vehicles for cell entrapment supported the adhesion and differentiation of human neonatal dermal fibroblasts, indicating a great potential in skin regeneration. Contrary to the extrusion bioprinting, stereolithography (SLA) bioprinting has the advantages of high resolution (around 25–100 µm) and high speed. Furthermore, the SLA bioprinting could ensure high cell viability, because no external forces are applied to the cells during the printing process. However, most of the SLA bioprinting systems rely on UV light that might damage the DNA of cells. Fortunately, Wang et al. have developed a biocompatible eosin Y (EY)-based photoinitiation with a wavelength between 500 and 600 nm to print a fibroblast-laden EY-gelatin methacryloyl hydrogels. It was found that this bioprinting system could fabricate the cell-laden hydrogel with complex architecture easily, and the cells inside the hydrogels could achieve numerous proliferations to form 3D intercellular networks. It demonstrated that the visible-light-based bioprinting system was suitable for long-duration bioprinting and potential for skin regeneration.

3.1.3. Heart

Congenital heart defects are presented in 35 000 newborns annually. Unfortunately, most of the patients would develop symptoms of right-ventricular (RV) dysfunction. It was reported that cardiac progenitor cells (CPCs) played important roles in improving the failing RV of juvenile rats. Hence, Bejleri et al. developed a bioprinted cardiac patch that constituted of CPCs, cardiac extracellular matrix hydrogel (cECM), and gelatin methacrylate (GelMA). The CPCs exhibited high proliferation activity in the cardiac patch up to 7 d, and improved the differentiation and angiogenic abilities of GelMA patches, showing their excellent reparative functionality. To some extent, decellularized tissue-based inks from different sources could meet the heart repair requirements. However, to ensure minimal immune response, completely autologous biomaterials are preferred. Noor et al. extracted an omentum tissue from the patient and separated the cells from the tissue. Decellularized tissue was prepared into a thermoresponsive hydrogel. The tissue cells were reprogrammed to develop into pluripotent and then differentiated to become cardiomyocytes and endothelial cells, followed by encapsulated in the thermoresponsive hydrogel to generate the bioinks for bioprinting the engineer vascularized cardiac patches and artificial heart.

Transplanting the autologous engineered tissue
into the patient would be an effective strategy to avoid the risk of immune rejection during repair or replacement of injured heart. However, it was a great challenge to print the whole organ with multicomposition and multifunction.\textsuperscript{[103]} A key problem for bioprinting artificial organs is to achieve the 3D structure and function replication through the soft and dynamic biomaterials.\textsuperscript{[104]} Lee et al. developed a kind of freeform reversible embedding of suspended hydrogels (FRESH) method to fabricate an artificial human heart. Although the functions of artificial heart were not fully evaluated, some models like collagen vessels and microporous collagen scaffolds composed of collagen and cells have been printed to verify the biocompatibility of the artificial heart. In addition, tri-leaflet heart valve with cyclical opening and closing property has also been printed, which indicated the brilliant mechanical performance of biomaterials.\textsuperscript{[105]} The 3D bioprinting technology opens a new direction for the artificial tissue construction by recapitulating the structure, mechanical, and biological performances.

### 3.1.4. Lung

Another great challenge for 3D bioprinting is to achieve the fabrication of artificial lung, due to the fact that the lung is composed of complex macro/microscale architectures with different cell types.\textsuperscript{[106]} Many patients suffered from serious lung diseases that urgently needed the artificial lung transplantation due to donor scarcity for lung transplantation.\textsuperscript{[106]} Stereolithography could achieve the fabrication of complicated structure with high resolution, making it potential for the fabrication of artificial lung. However, most of photosensitive additives which play significant roles in the polymerization are of toxicity, making them not suitable for biological application.\textsuperscript{[107–109]} Grigoryan et al. reported that tartrazine was a biocompatible photoabsorber with low toxicity and broad utility in the food industry, which made it possible for the stereolithographic production of hydrogels. Using the stereolithography, the vascularized alveolar model was printed, which possessed similar functions to human lung, that is, mixing the oxygen with red blood cells when the air sac was ventilated with $O_2$.\textsuperscript{[110]} Although there are some limitations for the artificial lung such as the size and composition change; the strategy plays a very important role in the further development of artificial lung and regenerative tissues.

### 3.2. 3D Printing of Drug-Inspired Functional Biomaterials

There are lots of drugs which come from the inspiration of creatures and play important roles in the disease therapy.\textsuperscript{[111,112]} Nowadays, the effects of the bioinspired drugs in tissue regeneration have attracted more and more attentions.\textsuperscript{[112,113]} Icariin is the main ingredient of a plant—Epimedium; Lai et al. prepared a PLGA/TCP/icariin scaffold using 3D printing technology for bone regeneration. The scaffold could achieve a sustained release of icariin over two weeks and exhibited excellent biocompatibility in vitro. After two months of scaffolds implantation into bone tunnels of steroid-associated osteonecrosis rabbits, it was found that the scaffold could guide osteogenesis in the implanted region, which was promising for massive bone defect regeneration. Furthermore, the PLGA/TCP/icariin scaffolds exhibited better bone regeneration ability than the PLGA/TCP scaffold, suggesting the important role of bioinspired icariin in improving the bone bioactivity.\textsuperscript{[114]}

Some bioinspired components extracted from plants could not only promote tissue regeneration, but also possess many other functions to achieve the multimode treatment. Inspired from the grape seed extracts, our group applied oligomeric proanthocyanidins (OPC)-containing hydrogels for the treatment of melanoma and wound healing (Figure 4a).\textsuperscript{[111]} The OPC possessed photothermal effect to remove the tumor cells and simultaneously facilitated the crosslinking of hydrogels (Figure 4d–f). The 3D-printed OPC-containing hydrogel scaffolds could effectively support the proliferation and migration of dermal fibroblasts and umbilical vein endothelial cells, as well as accelerated angiogenesis and skin regeneration in chronic wounds (Figure 4g,h). As an active component, the OPC played multiple roles in the hydrogel scaffolds, such as a photothermal agent for tumor therapy, a crosslinking agent for hydrogels, and a carrier of bioactive ions for skin regeneration.

Inspired from the specific composition of creatures, more tissue regeneration biomaterials could be further developed. 3D printing, as an advanced technology would also play significant role in the composition design for the regeneration materials, which endows them with more interesting functions.

### 4. 3D-Printed Biomaterials with Biological Structure Inspiration

The structures have significant effects on biomaterials such as tissue induction activity\textsuperscript{[83,115]} and mechanical properties.\textsuperscript{[116]} The biological system inspires the scientists to design the advanced structured biomaterials for tissue regeneration. In recent years, several bioinspired structures have been replicated in the fabrication of both high-bioactivity and high-strength biomaterials. However, traditional methods to fabricate bioinspired structure are limited because of the inaccuracy of shaping and inadequate ability of mass production. It is well known that 3D printing could achieve the structure control of multiple materials easily from macroscale to microscale.\textsuperscript{[117,118]}

Therefore, 3D printing would be highly effective for preparing bioinspired materials which are applied for tissue regeneration. 3D-printed porous structures are firstly introduced due to the significant effects of structures on the formation of new bone and blood vessel in the defects. Furthermore, the complex tissues at the interfaces are hard to regenerate due to the structure and composition complexities. It would be efficient to produce biphasic structure layers by 3D printing for repairing the tissue interface. Moreover, 3D printing could provide a wonderful strategy to fabricate the delicate structures with excellent mechanical properties for load-bearing tissue regeneration.

#### 4.1. Porous Structures for Tissue Regeneration

Porous structures are very important in the creatures. For example, the skin surface is full of micropores for the heat
exchange and metabolism.\cite{lotus_channels}

lotus is filled with connected channels for the nutrients transportation and air exchange. Some studies demonstrated that the tissue repair efficacy was dependent on the pore structures significantly.\cite{pore_dependence,lotus_pores}

Therefore, various pore structures inspired from the creatures have been fabricated to explore the relationship between the structures and regeneration effects.

Traditional 3D-printed scaffolds are stacked by the solid struts without channel architecture which limits the transportation of oxygen and nutrients and further the rudimentary vasculature and new bone formation in the center of struts.\cite{traditional_scaffolds,hotdog_scaffolds}

Due to the advantages of hollow-channel materials to vascularization and inspired from the lotus root with plenty of parallel channels for promoting air and moisture exchange to the environment, lotus root-like scaffolds were proposed to fabricate for repairing critical-sized bone defects, which possessed the struts with parallel multichannels via a modified 3D printing strategy (Figure 5a,b).\cite{hotdog_scaffolds}

The physicochemical properties of the 3D-printed biomimetic scaffolds could be effectively regulated and the biomimetic structure could facilitate to support cell delivery (Figure 5c). The in vivo results showed that the lotus root-like scaffolds could significantly induce formation of the blood vessels and new bone in the lotus root-like structure, promoting the osteogenesis and angiogenesis (Figure 5d). In addition, the results also indicated that the lotus root-like biomimetic scaffolds have more potential for regeneration of massive bone defects.

To promote the bone-forming ability inside the struts of the 3D-printed scaffolds, the design strategy for hierarchical structure was introduced into the scaffolds. As a popular food, hot dog is composed of bread and sausage which provides the energy and nutrients, respectively. Inspired by the structure and function of hot dog, we fabricated the hot dog-like scaffolds with hierarchical structure by combining 3D printing and bidirectional freezing method (Figure 6a).\cite{hotdog_scaffolds}

The scaffolds were consisted of hollow tube embedded by bioceramic rods with ordered lamellar microstructures (Figure 6b). The specific surface area of scaffolds was significantly enhanced with the hot dog-like structure, leading to the enhanced cells adhesion inside the scaffolds (Figure 6e). Moreover, the hierarchical rods in the scaffolds exhibited high loading capacity and long sustained release period for osteogenic drugs, which further facilitated the cell differentiation and new bone formation (Figure 6c,d). More interestingly, the new bone could be simultaneously induced to grow in the macro-pores of tubes and the interior of lamellar structure of hot dog rods, and thereby extremely promoted new bone formation in the scaffolds (Figure 6f,g).

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Figure 4. 3D-printed grape seed-inspired scaffolds. a) Schematic illustration of the functions of the smart scaffolds. b) Photograph of the smart scaffold. c) The compressive strength of the smart scaffold after the laser irradiation, suggesting that the crosslinking performances of the OPC-containing scaffolds respond to irradiation time. d) Photothermal ability of the scaffold. e) Treatment of the tumor. f) Tumor volume during 14 d. g) Skin wound of blank, CS+SA, and CS+SA+4%OPC during 16 d. h) Skin wound area statistics of different samples. The results suggested that the smart hydrogel scaffolds provided a possibility for tumor therapy and skin regeneration. Reproduced with permission.\cite{smart_scaffolds} Copyright 2019, American Chemical Society.

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Figure 5. a) Schematic of the physicochemical properties of the 3D-printed biomimetic scaffolds. b) Therapeutic effects of the scaffold. c) In vivo results showed that the lotus root-like scaffolds could significantly induce formation of the blood vessels and new bone. d) The results also indicated that the lotus root-like biomimetic scaffolds have more potential for regeneration of massive bone defects.

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Hydrogel, as a biocompatible material, is usually used for constructing the 3D cell microenvironment by 3D printing. However, due to the limited mechanical property, it is still a challenge to fabricate 3D hydrogel scaffolds with controlled microstructures and open channel structures under mild conditions. Inspired from the vascular structures, Luo et al. developed a vascular-like 3D hydrogel scaffold by 3D printing with the help of a special core–shell nozzle. The produced vascular-like 3D hydrogel scaffolds exhibited mechanical performances comparable to that of normal hydrogel scaffolds, while the microstructure of the hydrogel scaffolds played significant roles in the promotion of the attachment and spreading of hBMSC. Gao et al. proposed a specific method to create hollow calcium alginate filaments by using a coaxial nozzle-assisted printing system. The microchannels of the scaffolds can be formed by controlling the crosslinking time to control the structures, which show great potential in nutrients delivery. The viability of fibroblasts in the hollow channels was much higher than that in alginate structures without hollow microchannels. Compared with the hydrogel scaffolds without hollow microchannels, 3D-printed hydrogel scaffolds with hierarchical pores exhibited distinct capabilities for tissue regeneration. Wei et al. reported a facile strategy to fabricate 3D-printed hydrogel scaffolds with micropores in the scaffold struts by using sacrificial recrystallized sodium chloride. Such micropores of the hydrogel scaffold could significantly facilitate the drug/protein delivery. Hence, this strategy might be efficient for 3D-printed artificial tissue constructs mimicking the properties of different tissues using hydrogel bioinks.

4.2. Complex Tissue Regeneration

Due to the complex environment at the interface for different tissues, it remains a challenge to regenerate the defects which contain various tissues. Osteochondral defect is considered a thorny problem in clinic. The cartilages lack blood vessels for the transport of nutrients, leading to the limitation of the proliferation and differentiation of chondrocytes. The cartilages are integrated with the subchondral bones which could help to promote cartilage regeneration. However, for treating osteochondral defects, the simultaneous regeneration of both articular cartilage and subchondral bone is of great difficulty due to their different structures and functions.
Figure 6. 3D printing of hot dog-like scaffold. a) The schemata of preparation of hot dog-like scaffold (HD-AKT). b) Morphology of the HD-AKT. The struts of the scaffolds were composed of hollow tube and hierarchical rod with lamellar structures. c) The osteogenic drug loading and release properties of the scaffolds. HD-AKT could achieve efficiently drug loading and sustain release beyond 90 d, indicating the important roles of the hierarchical rods in the drug delivery. d) SEM images of the hierarchical rods after the drug release for three months. Lots of nanostructures appeared in the surface, which accounted for the high loading efficient and long release time of HD-AKT. e) The cell adhesion situations of four kinds of scaffolds. f) Bone-forming bioactivity of scaffolds in vivo. HD-AKT could significantly induce the new bone forming in the struts of scaffold, as well as the hierarchical structure of the rods (blue circle). g) Magnification of the blue circle. Hierarchical structures of the scaffold efficiently promoted the new bone forming, indicating great potential on bone regeneration. Reproduced with permission. [123] Copyright 2019, Wiley-VCH.

By imitating the bilayer structure of osteochondral defects, lots of scaffolds have been designed to achieve the simultaneous regeneration of bone and cartilage.[128,130,131] Gao et al. designed the gradient hydrogel scaffolds to repair the osteochondral defects.[129] The bottom layer of the gradient scaffold contained bioceramic nanoparticles to enhance the bioactive bonding with the host bone tissue. Meanwhile, growth factors were loaded in the upper layers to promote the cartilage regeneration. The in vivo results further demonstrated that the bilayer structures could simultaneously accelerate the regeneration of cartilage and subchondral bone in the defects, respectively. Some previous studies revealed that the micro/nanostructured surface of biomaterials could facilitate the reconstruction of bone and cartilage tissue.[132,133] We constructed the 3D-printed bioceramic scaffolds with different micro/nanostructured surfaces, including nanograin, nanolamella, and microrod (Figure 7a). The micro/nanostructure of surface not only enhanced the mechanical strength of scaffolds by healing the microcracks (Figure 7b–e), but also accelerated the differentiation of chondrocytes and rBMSVs in vitro (Figure 7f,g). It could be explained that the micro/nanostructured surface stimulated the differentiation of chondrocytes by activating integrins αvβ1 and α5β1, and further promoted osteogenic differentiation of bone marrow stem cells owning to the synergistic effects of integrin RhoA and α5β1. The micro/nanostructure could efficiently enhance the regeneration of both cartilage and subchondral bone in vivo (Figure 7h). The results suggested that 3D-printed scaffolds with biphasic layer could be a smart biomaterial for the regeneration of osteochondral defects. It is a great challenge to repair the complex interface tissue,
Figure 7. 3D printing of biphasic scaffold for osteochondral defect. a) The photographs and SEM images of the different scaffolds suggested that various nanostructures grew on the surfaces of samples nanograin, nanolamella, and microrod. b–d) The samples for mechanical tests, compressive strength, and the curves of strength with the deformation of scaffold, respectively. e) BRT scaffolds were hydrothermally treated for different time. Microcracks were healed attributing to hydrothermal treatment, which accounted for the improvement of compressive strength significantly. f) The adhesion of rBMSC and chondrocyte on the scaffolds. g) Adhesion rates of chondrocytes, rBMSCs, and adsorption amounts of BSA protein and FN protein. h) In vivo bioactivity of scaffold for cartilage and subchondral bone regeneration. The scaffolds with biphasic structures had induced more cartilage and new bone formation than the CTR and BRT which was without the nanostructures. Reproduced with permission. [128] Copyright 2018, John Wiley and Sons.

while the strategy of mimicking the structure of injured tissue through 3D printing would provide new insight for multitissue regeneration.

4.3. High Strength of Bioinspired Structure

Load-bearing biomaterials are urgently pursued in multiple tissue repair like bone,[134,135] tooth,[17,136] tendon,[137,138] and muscle[139,140] to match the complex mechanical environment. The fabrication of biomaterials, which simultaneously possess great mechanical performances and distinct bioactivity, remains a challenge because these properties are usually mutually exclusive. After millions of years of evolution, biological systems like nacre and bamboo, have achieved the perfect combination of subtle structures and superior mechanical performances to adapt to the environment. Therefore, it would play a significant role for the repair of load-bearing tissues by translating the high-strength structures of biological systems to the biomaterials design.

4.3.1. Nacre

Nacre possesses brilliant strength and toughness due to the delicate architecture between inorganic phase and organic phase.[141] To date, plenty of technologies have been developed to fabricate nacre-like materials, such as freeze casting,[74] self-assembling,[142] and mineralization.[143] However, conventional methods are difficult to satisfy the microstructure control and brilliant mechanical performances simultaneously. As a new fabrication technology, 3D printing is a useful tool to fabricate complicated architecture and high-performance biomaterials, which could be an effective strategy to address the challenge for the regeneration of load-bearing tissue.

Yang et al. constructed nacre-like hierarchical composites with aligned graphene nanoplatelets using an electrically assisted 3D printing technology (Figure 8a). In the guidance by an electric field, the graphene nanoplatelets were assembled in the resin slurry to form a layered structure.[144] The 3D-printed hierarchical structures with aligned graphene nanoplatelets exhibited better mechanical performances compared to those with random graphene nanoplatelets. Importantly, the artificial nacre with aligned graphene nanoplatelets showed similar specific toughness and bending strength to the natural nacre, indicating a great potential in the biomedical application (Figure 8b).

4.3.2. Bouligand Structure

Bouligand structure is composed of superimposed layers of parallel aligned fibers with special angle deviation, which widely exists in the "weapon" or "armor" of animals such as the claws of crabs,[145,146] fish scales,[147,148] beetle wings,[149,150] and Mantis shrimp.[151] Crack propagation paths of Bouligand structure would be longer than “brick-and-mortar” structure due to the superimposed layers of fibers with special angle deviation. The 3D printing technology can be an effective strategy to fabricate Bouligand structures with controllable fiber angles. However, the fabrication of Bouligand structure using conventional methods, such as compression molding, is impossible.
Figure 8. 3D printing of high-strength bioinspired materials. a) 3D-printed high-strength nacre with aligned graphite. b) Mechanical testing of the sample rGN and sGN suggested that the aligned lamellar structures could efficiently increase the load resistance of materials. Reproduced with permission.\textsuperscript{[144]} Copyright 2019, AAAS. c) 3D-printed biomimetic Bouligand architectures. Reproduced with permission.\textsuperscript{[153]} Copyright 2017, John Wiley and Sons. d) Bowhead whale and whale baleen. e) The structures of whale baleen were distinguished into solid shell and tubular layers, leading to the excellent mechanical performances. f) Model of the tubular layer. g) 3D printing of whale baleen-like structure. h) Compressive strength of the samples indicated that the whale baleen-like structure possessed best mechanical property. Reproduced with permission.\textsuperscript{[3]} Copyright 2018, John Wiley and Sons.

to the larger interfacial area per unit crack length, leading to more energy dissipation and a high damage tolerance. Recently, Chen et al. prepared twisted plywood structure with hydroxyapatite and sodium alginate through a brushing-induced assembly method.\textsuperscript{[152]} The bulk composites exhibited comprehensive imitation of both the structures and mechanical performances of natural creatures. It is indicated that artificial materials with twisted plywood structure were able to enhance the toughness, and have potentials in repairing the load-bearing bone defects. However, the assembly process to achieve Bouligand structure is complicated and time-consuming. Therefore, Yang et al. fabricated aligned multiwalled carbon nanotubes (MWCNT) composites with Bouligand structure by using electrically assisted 3D printing technology.\textsuperscript{[153]} The aligning direction of MWCNT in resin matrix could be accurately controlled in an electrical filed. Through adjusting rotation angles of the tank which contains polymer/MWCNT composite resin at different layers, Bouligand-type anisotropy could be introduced in the final composites (Figure 8c). The twisted plywood structure exhibited extremely reinforced effects on the composites. When the fracture has taken place, the composites possessed a greater energy dissipation and impact resistance. The work will improve the structure design of biomimetic biomaterials. Besides, the study provides a feasible strategy for fabricating artificial meniscus with excellent mechanical properties. The elastic modulus of artificial meniscus with Bouligand structure (0.79 MPa) is matched with the human meniscus (0.69 MPa). This result shows that the printed meniscus can play a role as a shock absorber and improve the tear resistance to enhance the lifetime of usage. The enhanced modulus and improved fracture energy demonstrate that the 3D-printed meniscus is promising as a replacement for injured or diseased meniscus. The researches about the Bouligand structure give promising insights into how to enhance the mechanical properties of engineered structural materials from the perspective of biomimetic, which would further promote the development of load-bearing biomaterials.

4.3.3. Other Interesting Structure

The tooth of whale is replaced by baleen after long time evolution. Whale baleen plays key role in separating prey from water. Therefore, it has to withstand the strong forces from water flow and prey. Some studies demonstrated that the whale baleen possessed novel sandwich-tubular structure with a solid...
shell enclosing a tubular layer, which contributed the excellent mechanical properties (Figure 8d–f). Inspired by the whale baleen structure, Wang et al. replicated three principal structures of the baleen plate including hollow medulla, mineralized tubules, and sandwich-tubular structure into composite design by 3D printing (Figure 8g). Several structural models (Model I, II, III, and IV) as shown in Figure 8h were fabricated to elucidate the roles of structures under the compressive forces. The results demonstrated that Model IV with the incorporation of stiff “mineral” component provided the improved mechanical performance compared to Model I with solid shell, Model II with tubules, and Model III with hollow tubules. By comparing the compressive behavior of each groups, it further demonstrated the role of the structure of hollow tubules composed of filament-matrix lamellae and mineralization, and a solid shell played a role in determining its mechanical performances (Figure 8h).[31] The study provides new insights on how biomimetic structure promotes the mechanical behavior. Therefore, the structure inspired from the whale baleen is of great interest for designing and creating load-bearing biomaterials.

Balsa wood is the best engineering material with excellent specific bending stiffness and specific bending strength.[135] By the guidance of wood structure with hierarchical aligned channels,[136] scientists have fabricated epoxy-based cellular composites with multiple controlled alignments and oriented fiber reinforcement by 3D printing.[137] Several architectures with varying cell geometry were created to demonstrate 3D printing of cellular composites. In addition, the cellular composites with fibers exhibited higher specific strength compared to pure epoxy samples. 3D printing could offer unparalleled flexibility on achieving controlled geometric shape, composition, function, and complexity over conventional manufacturing technologies. Therefore, creating structures mimicking balsa wood by 3D printing is ideal for fabricating lightweight structural materials with controlled mechanical properties. Furthermore, it could provide insight into potential application in biomedical field. The biological structures which possess novel functions have inspired scientists to fabricate advanced structural biomaterials. However, there are still lots of interesting structures to explore for the progress of materials design.

5. Conclusions and Outlook

In conclusion, an overview of the 3D-printed bioinspired biomaterials for tissue regeneration is summarized. The conventional bioinspired designs are mainly limited for biomaterials in tissue regeneration because of the inaccuracy of shaping and inadequate ability of mass production. Nowadays, 3D printing is introduced for bioinspired materials design to overcome the limitations due to its ability for precise multimaterial and multiscale fabrication.[137,138] The bioinspiration of 3D-printed materials design in tissue regeneration mainly includes the biological inspiration of both composition and structure. For biological composition inspiration, it is significant to mimic the cell compositions and ECM of the tissues, and 3D bioprinting could meet the requirements to fabricate biomaterials for tissue repair. In addition, some drugs extracted from the creatures would also play important roles in tissue regeneration. As we know, traditional Chinese medicine contributes a great deal to the development of medicine.[139] The combination of traditional Chinese medicine and multimaterials fabrication of 3D printing is promising in tissue regeneration, and would be a new driving force for the developments of Chinese medicine and tissue regeneration. For biological structure inspiration, 3D printing could achieve the precise mimicking structures from macroscale to microscale, which offers great possibility to design and fabricate biomimetic structures. The pore structures are very important in creatures for multiple functions. For imitating the pore structures and functions in tissue regeneration, a variety of studies have demonstrated that 3D printing technology was a powerful method to achieve the matched biomaterials for defect repair. Besides, biological structure inspiration also provides new insight for complicated tissue regeneration. 3D printing technology can replicate the functional structures to stimulate complex tissue regeneration. Moreover, high strength, one of the basic properties for clinical biomaterials, was discussed from biomimetic perspective, and some interesting bioinspired structures, such as “brick-and-motor” structure and Bouligand structure, are highlighted in tissue regeneration.

Although the 3D-printed bioinspired biomaterials have been successfully developed in tissue regeneration, there are still many issues that need to be further addressed (Figure 9). 1) It is convenient to fabricate the bioinspired structure from macroscale to microscale by 3D printing technology. However, some delicate structures at the nanoscale are hard to be replicated. Hence, it is urgent to enhance the resolution of 3D printing technology to satisfy the biomimetic structures from macroscale and microscale to nanoscale for better functions replication of creatures. 2) Most of the 3D-printed tissues and organs only contain insufficient cell types. They are small in scale and composed of relatively simple architectures, and exhibit limited functionality. In addition, the nutrient supply of the bioprinted tissues usually depends on physical diffusion. Hence, 3D bioprinting of more complex tissues with robust mechanical performances and biocompatible materials needs to be explored for the derivation and expansion of various types of functional and supporting cells, as well as new strategies for constructing vascular networks for nutrient supply. 3) The amazing nature endows us with plenty of inspirations to facilitate the biomaterials with excellent characteristics. However, more biomimetic objects with novel compositions, structures, and interesting characters are still not explored. It would be meaningful to find the original structures, and thereby further accelerate the progress of engineering biomaterials. 4) Complex tissue regeneration and treatment would be an important research direction in biomedical engineering. However, it remains a challenge to design multifunctional biomaterials to imitate the structure, composition and functions through 3D printing technology. Much effort is dedicated to exploring fabrication techniques in combination of 3D printing, which aims to solve the complex tissue regeneration and treatment under ambient conditions. 5) The materials used in bioinspired 3D printing for tissue regeneration are usually limited to polymers and certain bioceramics. These materials have limitations, such as unsatisfactory tensile strength and low temperature tolerance, compared to the advanced composites. The research to explore new biomaterials that can be applied in 3D printing as well as to develop advanced composites is significant in the future. Meantime, it remains a great...
challenge for the current 3D-printing technology to process materials in different categories due to the incompatible forming conditions. The strategy combining the traditional 3D printing with other strategies like self-assembly to achieve better performance of composite materials might be efficient to broaden the materials categories. Bioinspiration strategy has provided great potential for the development of multissue regenation. Meanwhile, 3D printing technology as a useful tool could support bioinspiration strategy to come true. Therefore, 3D printing of bioinspired biomaterials would provide a new insight for the development of biomedical engineering.

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Conflict of Interest

The authors declare no conflict of interest.

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