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Advances in growth factor-containing 3D printed scaffolds in orthopedics

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Abstract

Currently, bone tissue engineering is a research hotspot in the treatment of orthopedic diseases, and many problems in orthopedics can be solved through bone tissue engineering, which can be used to treat fractures, bone defects, arthritis, etc. More importantly, it can provide an alternative to traditional bone grafting and solve the problems of insufficient autologous bone grafting, poor histocompatibility of grafts, and insufficient induced bone regeneration. Growth factors are key factors in bone tissue engineering by promoting osteoblast proliferation and differentiation, which in turn increases the efficiency of osteogenesis and bone regeneration. 3D printing technology can provide carriers with better pore structure for growth factors to improve the stability of growth factors and precisely control their release. Studies have shown that 3D-printed scaffolds containing growth factors provide a better choice for personalized treatment, bone defect repair, and bone regeneration in orthopedics, which are important for the treatment of orthopedic diseases and have potential research value in orthopedic applications. This paper aims to summarize the research progress of 3D printed scaffolds containing growth factors in orthopedics in recent years and summarize the use of different growth factors in 3D scaffolds, including bone morphogenetic proteins, platelet-derived growth factors, transforming growth factors, vascular endothelial growth factors, etc. Optimization of material selection and the way of combining growth factors with scaffolds are also discussed.

Keywords: 3D printing, Growth factors, Scaffolds, Bone regeneration

Introduction

Growth factors are water-soluble polypeptides, has a positive effect on the proliferation and differentiation of many cells [1]. For bone tissue, the proliferation and differentiation of osteoblasts also require stimulation of growth factors [2]. The instability of growth factors and the challenges of their effective delivery have limited the application of growth factors in orthopedic clinics [3]. However, Liu et al. [4] found that the loading of growth factors with microspheres not only ensured the stability of growth factors but also allowed for their slow release to specific sites. However, growth factor-containing microspheres can only promote bone regeneration but not early bone repair, so Liu et al. [5] loaded growth factor-containing microspheres into 3D-printed scaffolds to personalize scaffolds that promote bone repair and bone regeneration. 3D printing technology is

to upload data to computer software, and then the computer controls the 3D printer to superimpose the material layer by layer, and finally make a specific form of three-dimensional products [6]. 3D printing can precisely incorporate growth factors into individually customizable scaffolding structures and achieve controlled release. This method not only improves the stability of growth factors and avoids their explosive release, but also promotes the regeneration of bone tissue at the site of bone defects or fractures [5, 7]. Studies have shown that 3D printing is used in surgery, and orthopedic applications account for 45.18% of the total. 3D printing technology has a wide range of development prospects in orthopedics [8].

The treatment of bone tissue defects and fractures has always been one of the important problems in orthopedics [9]. Traditional treatments have some limitations and problems (The side effects of autologous bone grafting are: increased trauma to the patient, which may lead to infection and bleeding in the donor area. The side effects of allogeneic bone grafting are: immune rejection, spreading of disease, and poor bone healing.), autologous and allogeneic bone grafts often suffer from a lack of donors, while personalized 3D printing can provide more suitable and sufficient stents according to the patient's defective site [10, 11]. By continuously improving the 3D printing process and selecting more ergonomic and biocompatible materials, 3D printed scaffolds containing growth factors will make a great difference in orthopedics.

3D printed scaffolds containing growth factors

3D printing technology

3D printing technology was first reported in the late 1970s [12]. So far, 3D printing technology can be divided into non-biological 3D printing technology and biological 3D printing technology [13]. Biological 3D printing technologies mainly include inkjet printing, laser-assisted printing and extrusion printing, while non-biological 3D printing technologies mainly include stereolithography (SLA), fused deposition modeling (FDM), selective laser sintering (SLS), selective laser or electron beam melting (SLM or EBM), laminated object manufacturing (LOM), etc. [13–15].

Non-biological 3D printing technology

Stereolithography (SLA) is the use of a computer for data input and instruction output, through a computer-controlled laser beam or digital light projector in a liquid resin operating box for curing printing [16]. By irradiating the pattern in the liquid resin with a laser, a curing resin of the corresponding pattern is formed. The first layer is printed by laser, then the platform is lowered, and then the resin is coated on the first layer to continue laser irradiation curing, and the 3D shape is solidified from bottom to top [16]. SLA has high printing accuracy and can print more complex shapes. However, SLA limits its development in the biological field due to the lack of biocompatible resin materials, high cost, and slow speed [13, 16].

The most common type of 3D printing technology is fused deposition modeling (FDM), which mainly melts thermoplastic materials into semi-fluids, then prints them into a layer of 2D form through nozzles, and finally superimposes them layer by layer to form a 3D structure [17, 18]. The semi-fluid material solidifies naturally at room temperature. FDM has low cost, simple operation, fast printing speed, etc., and can also be

mixed printing by dual nozzles, and the porosity can be adjusted by printing speed, wire walking sequence, etc. [13, 19]. However, FDM requires a high temperature and is not suitable for printing materials with biological activities such as cells and growth factors [13]. Therefore, FDM bioprinting is still a big challenge.

Selective laser sintering (SLS) is the process of sintering adjacent powder particles together by irradiating a specific pattern on a powdered material with a laser beam, and then superimposing them layer by layer to form a 3D form [20]. Selective Laser Melting (SLM) operates in the same way as SLS, except that SLS allows adjacent particles to reach glass transition temperature and sinter them together, while SLM melts and fuses the particles together [21]. Electron beam melting (EBM) is very similar to SLM, with the only difference being that SLM uses a laser while EBM uses an electron beam [21]. They all have the advantage of high mechanical strength, but the complex dispersion of molecules limits the choice of materials, and the high temperature is not suitable for biologically active substances [13, 20, 21].

Laminated object manufacturing (LOM) is less commonly reported in the literature, in which materials are pasted together layer by layer and then cut out by laser to create the corresponding form [22]. The main materials are paper, wood boards, etc. It is easy to print and inexpensive, but the material limitations make it difficult to use in biomedicine [23].

Biological 3D printing technology

Inkjet printing is the first 3D printing technology to be used for bioprinting [24]. Inkjet printing was widely used in 2D printing before entering the era of 3D printing, and it is commonly used for font printing on paper [13]. There are two forms of inkjet printing, as shown in Fig. 1a and b. Inkjet printing is a non-contact jet of digital information delivered by a computer, forming a specific pattern on the base plate, and then layering it layer by layer from bottom to top until the desired object is printed. The nozzle for inkjet bioprinting can be as small as 50 μm, so single-cell printing can be achieved [25]. Materials used for inkjet printing are hydrogels (alginate, polyethylene glycol, chitosan, collagen, silk), powders (tricalcium phosphate, polyvinyl silicate, polylactic acid, peptides), polymers, small molecule substances (cells, growth factors) [25, 26]. Inkjet printing is suitable for printing biologically active scaffolds, but it is also a concern that some cells die due to extrusion during printing [26].

Laser-assisted bioprinting (LAB) consists of a pulsed laser, a substrate on which the material is placed, and a substrate that collects the printed material [27]. The laser acts on the substrate where the material is placed, creating a high-pressure bubble that pushes the material onto the receiving substrate to form a three-dimensional structure [13], as shown in Fig. 1c. LAB printing does not require a printhead, so the pressure on the cells is reduced by mechanical stress. Laser-assisted printing has high resolution and high cell deposition density, but it has high requirements for cross-linking and high price, and the effect of laser on cells is still unclear [13, 28].

Extrusion printing is done through pneumatic or mechanical extrusion, so that the printed material is extruded through the nozzle, and the three-dimensional structure is printed layer by layer. There are three types of extrusion prints, as shown in Fig. 1d, c, and f. Extrusion printing is similar to inkjet printing, where inkjet printing

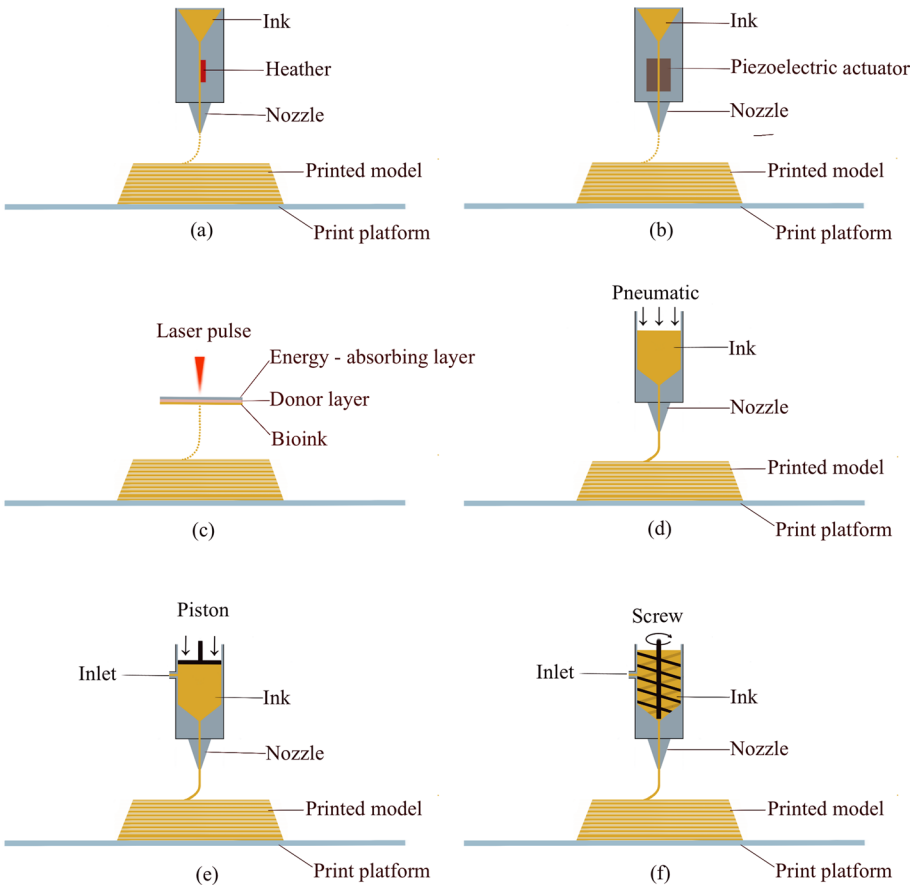

Fig. 1 Schematic diagram of three bio-3D printing technologies: **a** heated inkjet printing, **b** piezoelectric inkjet printing, **c** LAB, **d** pneumatic extrusion printing, **e** piston extrusion printing, **f** Spiral extrusion printing

produces discontinuous ink droplets, while extrusion printing produces continuous materials [13]. Although the accuracy of extrusion printing is not as high as inkjet printing, it can print a wider range of materials, and it can print a wider range of material viscosity, while inkjet printing cannot print materials with high viscosity [28]. Murphy et al. [29] have shown that the mechanical stress of extrusion printing can affect cells. However, Koons et al. [30] showed that embedding growth factors in polymer microparticles protects growth factors.

Growth factors

In recent years, there has been increasing interest in loading growth factors and cells in scaffolds to improve the regeneration and repair of bone defects [31]. At present, the growth factors used for scaffolds mainly include bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor-β (TGF-β). In addition, there are insulin-like growth factor (IGF), stromal cell-derived factor (SDF-1), etc.

BMPs

The BMPs family of proteins was first discovered in 1965 by Dr. Marshall Urist et al. and demonstrated to have the effect of inducing bone growth [32]. BMPs are a member of the TGF-β family, and the subtypes used for bone regeneration are mainly BMP-2, -4, -6, -7 and -9 [33]. These growth factors are analyzed below.

BMP-2 is the most widely studied bone morphogenetic protein family and plays an important role in osteogenesis, osteoinduction, and bone repair. BMP-2 plays a role mainly through the Smad1/5/8 signaling pathway, enhancing the expression of alkaline phosphatase and osteocalcin, thereby promoting the proliferation and differentiation of bone marrow mesenchymal stem cells (MSCs) and osteoblasts, and achieving osteogenesis [34]. Draenert et al. [35] investigated the release and osteogenesis of BMP-2 by physical transient adsorption of BMP-2. However, this method cannot avoid the influence of materials and environment on the survival rate of growth factors, nor can it control the release rate well, and there may be the possibility of explosive release. Studies have shown that too little BMP-2 can induce bone regeneration, and too much can lead to a variety of side effects, such as ectopic osteogenesis, inappropriate inflammation, and cancer induction [31]. Kim et al. [36] further confirmed the osteogenic effect of BMP-2 by loading BMP-2 with composite hydrogel compared with the control group, and at the same time achieved sustained release and reduced environmental effects on growth factor activity. However, the mechanical properties of hydrogels are not ideal and are often not suitable for the treatment of weight-bearing bones. Seok et al. [37] encapsulated BMP-2 in polymer particles, which were then combined with alginate to form a bioink, which was printed by 3D extrusion to form a bone scaffold. Growth factor survival, sustained release, and mechanical properties of the stent are addressed, and personalized treatment can be provided. Wei et al. [38] loaded two growth factors in the scaffold and found that SDF-1 had a synergistic effect on BMP-2. At the same time, studies have shown that PDGF, VEGF, etc. also have a synergistic effect on BMP-2 [39]. According to the latest research, Song et al. [40] have developed a multifunctional microsphere system that can respond to ultrasound and the environment, so as to release and reverse the microenvironment of bone damage on demand, which provides a new idea for the slow release of growth factors and bone regeneration and repair.

BMP-4 has a strong correlation with cartilage growth and development and can be used to treat cartilage defects [41]. BMP-4 stimulates the production of cartilage matrix components, including type II collagen and aggrecans, and promotes the differentiation of bone marrow mesenchymal stem cells (MSCs) into chondrocytes [41, 42]. Similar to the BMP-2, the BMP-4 has a short half-life and is susceptible to environmental influences. Sarsenova et al. [42] treated rabbit cartilage defects by hydrogel-loading with BMP-4 and TGF-β, confirming that BMP-4 can indeed enhance cartilage regeneration and repair. Sun et al. [43] fabricated 3D bio-printed scaffolds containing MSCs, macrophages, and BMP-4-loaded mesoporous silica nanoparticles (MSNs). The treatment of bone defects in rabbits with diabetes has confirmed that it is an effective treatment to improve diabetic bone damage.

BMP-6 also can promote osteogenesis, and studies suggest that BMP-6 may promote osteogenesis by enhancing IGF-1 and epidermal growth factor (EGF) expression [44]. The combination of IGF-1 and BMP-6 has a greater ability to regenerate bone

than BMP-6 alone. Toprak et al. [45] developed an electrospun scaffold embedded with BMP-6 embedded in a metal–organic framework, which has a better protective effect on growth factors and is also applicable to various growth factors, and confirmed the osteogenic role of BMP-6 and its potential for bone tissue engineering.

BMP-7 has been widely demonstrated to have osteogenic induction capabilities. However, Tsuji et al. [46] found that the lack of BMP-7 growth factor did not affect the normal growth and development of bone by knocking out the BMP-7 gene in mice. This shows that BMP-7 is not required for normal bone growth and development, but it also can promote bone growth. BMP-7 is necessary for the growth and development of soft tissues such as kidneys and skin [47]. The healing status of goat dental implants by Hunziker et al. [48] with different concentrations of BMP-7 showed that the slow release of BMP-7 at physiological doses can cope with severe bone deficiency and promote new bone formation. BMP-7 is the second BMP family member to be approved by the U.S. FDA for clinical bone induction therapy after BMP-2. BMP-7 is the second BMP family member to be approved by the U.S. FDA for clinical bone induction therapy after BMP-2 [49].

BMP-9 has strong osteoinduction capabilities both in vitro and in vivo [50]. BMP-9 has only emerged in recent years, so its specific role in the skeletal system is unclear, but it has been found to differ from other known BMP family members in the mechanism by which bone is induced [50]. Park et al. [51] studied BMP-9 by inhibiting the p53 osteohomeostatic pathway, PI3K/Akt/MDM2 pathway, and increasing p53 activity, thereby promoting osteoblast fraction. BMP-9 is an effective inducer in the adjuvant treatment of bone defects, according to the latest studies show that it has the strongest osteogenic effect in the BMP family, but its actual efficacy and safety need to be further studied, and with the continuous deepening of research, it may play an important role in bone tissue engineering in the future.

FGFs

FGFs are peptides that are involved in a variety of reactions. Studies have shown that FGF-2, FGF-8, FGF-9, and FGF-18 have osteogenic induction effects [52, 53]. Stamnitz et al. loaded FGF-2 and BMP-2 into a hydroxyapatite hybrid scaffold and found that FGF-2 could enhance the proliferation and differentiation of bone marrow mesenchymal stem cells, and the combination of FGF-2 and BMP-2 would enhance this effect [54]. The osteogenic effects of these other growth factors have also been demonstrated, but there are few studies on their loading onto scaffolds for the treatment of bone injury. In recent years, there have been many studies on FGF-21 and FGF-23, but their mechanisms need to be further clarified.

PDGF

PDGF is also one of the growth factors approved by the US FDA for clinical use. It is composed of homodimers (AA, AB, BB, CC, and DD), and PDGF-BB is the most active in bone and is key to promoting osteogenesis, which not only promotes angiogenesis but also aids in osteogenesis [55, 56]. The formation of blood vessels is also essential in the process of bone regeneration, so PDGF has a dual role as an adjuvant therapy. PDGF-BB can stimulate the release of VEGF, thereby promoting neovascularization,

transforming pericytes into MSCs, promoting the proliferation and differentiation of pericytes and free MSCs, and regulating MSCs, VEGF, pericytes, and endothelial cells [57, 58]. Lee et al. [59] established a dual growth factor sustained-release system by loading PDGF and BMP-2 with microspheres, and the results showed that bone regeneration was promoted. Novak et al. [60] showed that PDGF-BB inhibited BMP-2 osteogenesis by inhibiting the Smad pathway. Therefore, when choosing a growth factor for conjugation, attention should be paid to the special relationship between the two. Mohan et al. [61] loaded PDGF-BB into polylactic acid-glycolic acid copolymer microspheres for 3D scaffold printing, thereby achieving sustained release of PDGF-BB and inducing bone regeneration. Daniels et al. [62] compared the efficacy of PDGF with autografts through prospective randomized controlled studies and confirmed that PDGF is superior to autografts in ankle fusion and can be replaced. However, the need to select the appropriate base material when loading PDGF also determines the effect of treatment.

VEGF

Bone and blood vessels are inseparable, and in bone formation, blood vessels develop before bone, and only when blood vessels can provide enough nutrients for bone can bone be successfully formed [63]. VEGF plays an important role in the development of blood vessels, as well as in the formation of bone. VEGF is composed of homodimers (VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor) [63]. Fitzpatrick et al. [64] fabricated a sustained-release scaffold containing VEGF and a variety of growth factors through 3D printing, confirming the osteogenic effect of VEGF and its synergistic effect with other growth factors. However, VEGF has been shown to be associated with tumor angiogenesis due to its excellent pro-angiogenic ability, and too high a VEGF amount is at risk of promoting cancer formation [65]. Although VEGF has a unique effect on osteogenesis, it cannot be the mainstream of osteogenesis induction due to the limitation of its use concentration, and further research is still needed to make reasonable use of its properties.

Other growth factors

In addition to the above factors, there are many factors that also have their own role in bone regeneration, but the use and research in 3D printed bone scaffolds are not clear, so they are only briefly explained. IFactor (P-15) is a factor approved by the US FDA for clinical use. Hasan et al. [66] used it for post-spine treatment and found that bone healing was significantly accelerated. Since iFactor has recently been marketed, its research has mostly been used to treat radiculopathy, and it has been confirmed that it has the same effect as autologous bone grafting [67]. IGF-1 has the effect of promoting cell proliferation, inhibiting apoptosis, and promoting musculoskeletal proliferation, and studies have shown that it also has a repair effect on cartilage. Wei et al. [68] used 3D printed scaffolds to load IGF-1 for sustained-release treatment of cartilage, proving that IGF-1 has a good ability to form cartilage. SDF-1, also known as C-X-C chemokine ligand12, has been shown to mobilize and recruit bone marrow mesenchymal stem cells by activating the SDF-1/CXCR4 signaling pathway, leading to osteogenesis at the site of injury [69]. The growth factors and their mechanisms that have been extensively studied in 3D printed scaffolds are shown in Table 1.

Table 1 Growth factors associated with bone regeneration

Growth factors	Subtype	Function
BMP	BMP-2 BMP-4 BMP-6 BMP-7 BMP-9	Enhances the expression of alkaline phosphatase and osteocalcin, thereby promoting osteogenesis By promoting chondrocyte differentiation, it promotes cartilage growth and development Promotes osteogenesis by enhancing IGF-1 and EGF expression Induce intrachondral ossification to promote osteogenesis and enhance the expression of related proteins By inhibiting P53 activity, it promotes osteogenesis
FGFs		Induces intrachondral ossification to promote osteogenesis
PDGF	PDGF-BB	Promote osteoblast proliferation and differentiation Promotes angiogenesis Suppression BMP-2 adult bone
VEGF		Promote osteoblast proliferation and differentiation, and promote neovascularization
IGF-1		Promote cell proliferation, inhibit apoptosis, and promote musculoskeletal proliferation
SDF-1		Bone marrow mesenchymal stem cells are mobilized and recruited by activating the SDF-1/CXCR4 signaling pathway to promote osteogenesis

Table 2 Common 3D printing materials and their advantages and disadvantages

Category	Name	Advantages	Disadvantages
Metals	Ti Mg	Good mechanical properties, corrosion resistance, good biocompatibility Degradable, magnesium ions promote osteogenesis	Non-degradable, poor elasticity Poor mechanical properties and fast degradation rate
Polymers	Gelatin PLA	Good biocompatibility and good degradability It has good plasticity and can be degraded	The mechanical properties are very poor The degradation products are harmful and have poor biocompatibility
Bioactive ceramics	Bioactive glass HA	It has good biological activity and histocompatibility and can promote bone regeneration It has good biocompatibility and has a certain ability to promote bone regeneration	The mechanical properties are insufficient, the brittleness is large, and the plasticity is not high Pure HA has poor degradability and is not suitable as a single printing material
Composite materials		The structure is closer to bone tissue and can be adjusted as needed	The proportions of each component are difficult to determine

Material properties

As one of the indispensable components of bone tissue engineering, bone scaffold needs to have good biocompatibility, strong mechanical properties, good biological activity, and degradability. Different materials have their own different properties (Table 2), the following is an explanation of the characteristics and selection of materials.

Metals

Non-biodegradable metals

So far, the non-biodegradable metals used for 3D printing scaffolds mainly include titanium alloys, tantalum stents, stainless steel, and tungsten alloys. They all have

good histocompatibility and mechanical properties and are often used for joint replacement and fracture internal fixation implantation [70]. Zhao et al. [71] adjusted the porosity of the titanium scaffold to load two types of cells by 3D printing for angiogenesis and osteogenesis.

However, non-biodegradable metals exist as foreign substances in the body and need to be removed by secondary surgery, which not only wastes medical resources but also makes patients suffer from secondary surgery [72].

Biodegradable metals

At present, the degradable metals used in bone scaffolds are mainly magnesium, iron, and zinc. With the development of bone tissue engineering, the study of degradable metals has become more extensive. Biodegradable metals can not only play a supporting role in the healing process of bone defects, but also gradually disappear with the growth of bone, reduce the retention of foreign bodies in the body, and gradually release bioactive substances to promote bone regeneration [73].

Magnesium is essential in the human body, magnesium and its alloys have a density and elastic modulus similar to that of human bone, and more importantly, the magnesium ions produced by degradation can promote neovascularization and osteogenesis [74, 75].

According to numerous studies, pure magnesium has poor mechanical properties and a fast degradation rate, and the load of bioactive substances can easily lead to explosive release. However, Dong et al. [76] confirmed that the coating can reduce the degradation rate and improve biocompatibility by adding magnesium fluoride and calcium phosphate coating to the surface of the 3D extruded printed pure magnesium scaffold and comparing it with the pure magnesium stent. The research on magnesium alloy is still ongoing, some scholars have made magnesium–calcium alloy into absorbable screws, and some researchers have made magnesium–calcium–zinc alloy with better degradability [77]. Magnesium is flammable and oxidized, so it needs to pay special attention when choosing the printing method [78].

Iron is abundant in nature, and it is involved in the reaction of hemoglobin in the body to carry oxygen, oxidize the respiratory chain, and promote enzymes. The degradation rate of iron is slow, but the degradation rate can be adjusted by ferroalloys, surface modifications, compounding with polymers, etc. Putra et al. [79] fabricated a porous iron scaffold through 3D extrusion printing, confirming that it can be made into a personalized scaffold with similar mechanical properties to bone.

Zinc is important for reproductive function and is a component of enzymes. Zinc is degraded faster than iron and slower than magnesium, which is closer to bone regeneration [80]. So far, there are few studies on the 3D printing of pure zinc bone scaffolds, and the research on zinc is mostly compounded with other metals, polymers, bioceramics, etc. Cockerill et al. [81] fabricated a porous zinc scaffold by fused deposition method, which regulates the degradation rate through porosity, while having similar stiffness and strength to bone, with a cell loading survival rate of > 75%, in addition to strong antimicrobial effects.

These metals are mechanically stronger than polymers and are more malleable than bioceramics. Of the above three biodegradable metals, iron has higher mechanical

properties. Magnesium, iron, and zinc are prone to oxidation and have a low ignition point, so SLM is not recommended when choosing a printing method, as it will lead to high material loss due to material evaporation [81]. According to several studies, the use of fused deposits is more recommended.

Polymers

Natural polymers

The natural polymers used in biomaterials mainly include collagen, gelatin, hyaluronic acid, chitosan, sodium alginate, etc. Collagen is the most abundant protein in mammals, and it is used in various industries, including food, pharmaceutical, cosmetic, and biomedical [82, 83]. It has become the biomedical material of choice due to its biocompatibility, low antigenicity, and good degradability [82]. Collagen is also a component of bone, and gelatin is a special form of collagen, so they both have the same effect, but the cost of collagen is higher than that of gelatin [83]. Gelatin is a water-soluble molecule, which can be divided into acid and alkali benign type A and B gelatin due to different preparation methods. Gelatin comes from a wide range of sources, but it is susceptible to degradation and has insufficient mechanical properties. Huang et al. [84] mixed gelatin and hydroxyapatite and made a scaffold with loaded cells for the treatment of articular cartilage injury by 3D extrusion printing, confirming its feasibility. Diba et al. [85] made gelatin into raspberry-like gelatin microspheres loaded with vancomycin, showing a good sustained-release effect. Chitosan is a natural polysaccharide that is a cationic polysaccharide produced by the deacetylation of chitin. Chitosan is a natural polysaccharide that is a cationic polysaccharide produced by the deacetylation of chitin [86]. Chitosan hydrogels can be used for a variety of purposes through 3D printing, such as drug delivery by injection, scaffolding, etc. [87]. Sodium alginate is also a cationic polysaccharide, which can be a component of scaffolds or as a hydrogel, it also has good biocompatibility and degradability, but it also lacks mechanical properties and can be improved by compounding with other materials. The disadvantage of natural polymers is that it is difficult to change or adjust their degradation rate, and at the same time, their mechanical properties cannot be modified by chemically modification, because chemically modification will destroy the integrity of its polymer chain and thus destroy its biological activity.

Synthetic polymers

Synthetic polymers, also known as artificial polymers, are a type of polymer that is chemically synthesized by man. The most commonly used synthetic polymers are polylactic acid (PLA), polycaprolactone (PCL), polylactic acid-glycolic acid (PLGA), polyglycolic acid (PGA), and polyethylene glycol (PEG). Among them, PLGA, PLA, PGA, and PCL have been approved by the FDA [88], and these are widely studied and used in bone tissue engineering. PLA has three isomers, and being biocompatible can improve its performance in various ways, but degradation products are not good for tissue. Donate et al. [89] coated the 3D-printed PLA scaffold with calcium carbonate to make the surface of the scaffold rough, so that the non-hydrophilic PLA scaffold has hydrophilicity, and calcium carbonate can neutralize the acidic substances produced by PLA degradation to improve the damage microenvironment, and the degradation rate can be adjusted. PCL

is also biocompatible and better degradable. Wei et al. [68] 3D-printed PCL scaffold was loaded with IGF-1 by PLGA particles, which achieved the slow release of growth factors, and the released substances were non-toxic, which played a good role in promoting the growth of cartilage. PLGA not only has the advantages of good biocompatibility, but it can also adjust the degradation rate by adjusting the ratio of polylactic acid and glycolic acid. PLGA can be used not only as a particle for loading growth factors, as in Wei et al. [68], but also as a scaffold 3D printed like Mironov et al. [90]. It was shown that PLGA had no cytotoxicity and showed good adhesion. Compared to natural polymers, the degradation rate and mechanical properties of synthetic polymers can be artificially controlled. Synthetic polymers are less bioactive than natural polymers, and degradation such as polylactic acid creates an acidic environment that is not conducive to cell survival. Synthetic polymers also cause more inflammatory and immune responses than natural polymers. The challenge of synthetic polymers is to create scaffolds with similar mechanical properties to bone without causing inflammation.

Polymers are often printed by extrusion because they do not affect their physical or chemical properties due to heat sensitivity, photosensitivity, etc. [10]. Polymers can be used alone as materials for growth factor-loaded microspheres, hydrogels, substrate materials for scaffolds, etc., or can be mixed with other materials to make hybrid scaffolds.

Bioactive ceramics

Bioactive glass

Bioactive glass is a synthetic multifunctional inorganic material, the main components of which are SiO2, CaO, and P2O5. Bioactive glass can promote bone regeneration, mainly through a series of chemical reactions in the body through bioactive glass to form carbonated hydroxyapatite on the surface, thereby promoting bone regeneration. Daskalakis et al. [91] made bioactive glass particles incorporated into polycaprolactone 3D printed scaffolds, indicating that the addition of bioactive glass particles improved the mechanical properties of scaffolds and played a good role in cell dispersion. Fazeli et al. [92] compared the 3D-printed PCL scaffolds with hydroxyapatite (HA)/bioactive glass surface modifications, and found that HA and bioactive glass modified increased the surface roughness of the scaffold, increased hydrophilicity, and increased cell adhesion. The surface modification had a greater number of osteoblasts than the control group and showed more durable performance. Bioactive glass has good biological activity and histocompatibility, and the performance can be adjusted by adjusting the proportion of each component, but the overall mechanical properties cannot reach the degree of bone, and the brittleness is large. These are all things that need to be further studied and improved in the future.

Hydroxyapatite

Hydroxyapatite (HA) is a calcium-based inorganic substance and is the most abundant inorganic component in bone tissue. HA has good biocompatibility, bioactivity, and the properties of promoting bone regeneration. Pure hydroxyapatite is less degradable in tissues but can be mixed with other materials (including gelatin, polymers, and biodegradable metals) and can change the rate of degradation by adjusting the ratio of the

mixture. HA is now widely used in bone defect repair, dental fillings, and 3D stent printing, among others. Wei et al. [93] made hydroxyapatite into microspheres and added them to the PLGA scaffold, and the results showed that when the hydroxyapatite microspheres were added to 45%, the mechanical strength of the scaffold reached a maximum of 40Mpa, and the addition of HA microspheres made the adhesion and proliferation of bone marrow mesenchymal stem cells stronger. Fitzpatrick et al. [64] mixed hydroxyapatite with silk to make bone cement, and then printed hydroxyapatite scaffolds loaded with three growth factors through 3D extrusion printing, which showed good biocompatibility, mechanical properties, and degradation properties, and the growth factors could also be sustained, which further confirmed the feasibility of hydroxyapatite for 3D printing bone scaffolds.

Tricalcium phosphate

Tricalcium phosphate (TCP) is similar to HA in that it is also an inorganic substance, mainly calcium and phosphorus compounds. TCP has good histocompatibility and biodegradability, and is one of the most widely studied inorganics. TCP has two different structures, including β-TCP and α-TCP. β-TCP is a hexagonal crystal structure, while α-TCP is a triangular crystal structure. α-TCP is more susceptible to degradation, while β-TCP is relatively stable [94]. β-TCP will be converted into α-TCP at more than 1125 °C [95], α-TCP is often used in 3D printed scaffolds due to its rapid degradation and instability, and is often used in composites with other materials, while β-TCP is more used in bone tissue engineering. Shu et al. [96] made a β-TCP scaffold by 3D printing, which showed the feasibility of β-TCP scaffold, and its degradation products are phosphate and calcium ions, which can participate in bone formation. At the same time, in order to use zinc and cobalt metal for the treatment of osteoarthritis, the researchers functionalized the β-TCP scaffold and successfully made it have the function of reactive oxygen species (ROS) clearance. Zinc-cobalt functionalized β-TCP scaffold can promote bone growth while scavenging ROS and anti-inflammatory, providing a new protocol for the treatment of osteoinflammatory diseases.

Composite materials

The above simple materials are important, but they all have their own shortcomings, and if you want to find a scaffold material that is closer to the structure of bone tissue, it must be a composite. Under the different limitations of various materials, the composite can form a more suitable 3D printing bone scaffold material through the combination and ratio of different components. Since the bone itself is complex, the composite material may be closer. With the extensive research of 3D printing and growth factor loading scaffolds in recent years, composite materials have gradually become the focus of research.

The purpose of metal composites is to solve the problem of excessive degradation rates and insufficient mechanical properties such as zinc. The magnesium-titanium composite materials made by Yang et al. [97] showed good mechanical properties and osteoinductiveness by infiltrating the magnesium melt into the titanium scaffold without pressure after 3D printing the pure titanium stent, but the degradation rate of magnesium was faster than that of the pure magnesium stent, which the researchers said may be related

to galvanic corrosion. Ali et al. [98] composited PLA and magnesium and then 3D printed a porous scaffold and showed that a good honeycomb structure was produced at a concentration of 5% Mg, and a porous structure at 10%. The composite scaffold has good cell adhesion and osteoinduction, but the addition of Mg will make the degradation rate faster, which is still a problem.

The polymer complex is mainly designed to address the acidic microenvironment and degradation rate generated by the decomposition of polymers. Yazdimamaghani et al. [99] modified the surface of the magnesium scaffold with a mixture of PCL and bioactive glass, which not only slowed down the degradation rate of the magnesium scaffold but also formed a layer of hydroxyapatite on the surface to promote osteogenesis. At the same time, it also improves the overall mechanical properties and biological activity of the stent. The complexes of polymers and bioactive ceramics are now widely studied, including gelatin microspheres and hydroxyapatite composite scaffolds, as well as polymers and bioactive ceramic particles, and the plasticity of polymers plus the biocompatibility and mechanical properties of bioactive ceramics are a kind of complexes worth studying.

The purpose of bioactive ceramic composites is to alter the degradability of bioactive ceramics. The research on nano-hydroxyapatite in bioactive ceramics continues to deepen, Xu et al. [100] printed a porous composite scaffold based on nano-hydroxyapatite, polylactic acid, and nano-magnesium oxide, which had good mechanical properties, biocompatibility, and the ability to stimulate bone regeneration. Magnesium oxide also adjusts PH during degradation, improving the microenvironment. It has shown great potential in the treatment of bone defects.

A complex may be made up of two different substances in the same class, or it may be composed of two or more different substances. The components complement each other to compensate for the shortcomings between the components so that a composite material can be compounded closer to the bone tissue. Composites are widely studied in bone tissue engineering today and are expected to be suitable bone graft materials in the future.

Mechanism and control of growth factor release

Growth factors are essentially peptides or proteins. The factors that affect its stability mainly include physical factors, chemical factors, and biological factors [101]. Physical factors are mainly temperature and light. High temperatures tend to cause denaturation and inactivation of growth factors, while low temperatures inhibit the activity of growth factors but do not cause loss of activity [30]. Light, mainly UV light, causes growth factor chemical bonds to break thus affecting growth factor stability. Chemical factors are mainly PH and oxidants, etc. Growth factors are usually only stable within a specific PH range, when the PH is too high or too low it will cause the growth factor charge to change and thus destabilise [102]. Oxidants can oxidise certain groups of growth factors, therefore affecting their stability. Biological factors are mainly proteolytic hydrolysis, where specific proteases hydrolyse the corresponding growth factors and inactivate them outright [103]. Therefore ensuring the stability of growth factors is also a key issue. At the same time, the main role of growth factors in bone scaffolds is to stimulate the proliferation and differentiation of bone marrow mesenchymal stem cells

and osteoblasts to promote osteogenesis, so it is also crucial to maintain an appropriate concentration of growth factors. The release of growth factors depends mainly on how they are bound to the scaffold. The main methods of bonding are physical adsorption or embedding, chemical bonding, microsphere encapsulation, etc. (Table 3).

Physical binding is the easiest way to load growth factors, but simple physical adsorption may result in explosive releases that can have side effects. Kanematsu et al. [104] loaded basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), PDGF, and VEGF into the collagen matrix by direct physical adsorption, indicating that these growth factors exhibited different release curves. This may be related to the interaction of growth factors with the collagen matrix. Although different growth factors have different release profiles, they all have an initial burst of release. This further suggests that burst releases are susceptible to occur both by simple physical adsorption on the surface of the scaffold, for example, and by various means of binding to scaffolds that have a rapid rate of degradation. It can be controlled by adjusting the physical structure of the scaffold (porosity, cross-linking), or by modifying the surface of the scaffold with a fast degradation rate, and at the same time, the growth factor can be encapsulated by microspheres and then loaded on the scaffold to achieve delayed release [105]. Or some of the more widely studied non-covalent bonding methods, including electrospinning, hydrogel bonding, and polyelectrolyte multilayer film coating. At present, there are also many studies on hydrogel binding, because hydrogels can be used as both a carrier for growth factor binding and one of the substrate materials for composite scaffolds. Lv

Table 3 Key findings and key insights from different methods of loading growth factors with different materials

Combination method	materials	Key findings	Critical insights
Physical adsorption	Scaffold Hydrogel	Simple, fast, with a noticeable burst of release Simple, fast, with sudden release	Growth factors are exposed and their activity is susceptible to environmental influences and uncontrolled release rates Growth factors are not directly exposed, they are more active, and the sustained-release rate is poorly controlled
Chemical bonding	PCL PVA-Tyr	Poly(oligo (ethylene glycol) methacrylate) (POEGMA) modified PCL was covalently bound (amide bonded) to growth factors Extensive covalent binding (bisphenol bonding) to growth factors	Chemical modification is required to provide the active moiety first, and the effect on growth factor activity is unknown Multiple growth factors can be achieved by combining multiple growth factors to achieve multiple growth factors together for slow-release therapy
Microsphere encapsulation	Gelatin PLGA Chitosan	Biocompatible and can be loaded with a wider range of growth factors depending on the charge carried by the A and B types Long sustained release time. Degradation produces acidic substances that produce undesirable effects It can be made into nanoscale microspheres, but 80% of it is released abruptly in the first 4 h	The combination of types A and B allows for the co-retardation of multiple growth factors Neutralisation of acids in combination with alkaline materials, e.g. simultaneous use with MnO2 microspheres It can be combined with PLGA, etc. to prepare novel composite microspheres

et al. [106] then achieved rapid release of PDGF-BB and slow release of BMP-2 through a temperature-controlled hydrogel, which provides new ideas for the treatment of bone defects using multiple growth factors at different release concentrations. In short, physical bonding, though simple, has lost its research fervor.

Compared with direct physical binding, chemical binding can effectively reduce explosive release. Covalent binding, as the name suggests, is a chemical reaction between two chemical groups to bind together, and the binding of growth factors and scaffolds requires modification of the growth factors and scaffolds so that they can be covalently bound with active functional groups. This method can be released slowly, mainly due to hydrolysis and reduction reactions, or catalyzed reactions by enzymes [107]. Covalent binding to scaffolds or chemical application on the scaffold surface can affect the active site of the protein and may affect the biological activity of the growth factor. However, Di Luca et al. [108] covalently combined BMP-2 and TGF-β with polycaprolactone to 3D printed scaffolds, which showed that the growth factor activity was not affected and could be released gradually, effectively promoting bone regeneration. Atienza-Roca et al. [109] used tyraminated poly-vinyl-alcohol (PVA-Tyr) to form bi-phenol bonds with growth factors, which were then prepared into hydrogels to achieve long-term sustained release. In conclusion, although covalent binding contributes to slow release, the drawbacks are obvious; covalent modification is difficult, the preparation process is time-consuming and labor-intensive, and most importantly, the effect on growth factor activity is still unclear and no good improvement methods have been reported. This is a difficulty and a priority that needs to be addressed in future research.

Microsphere encapsulation is currently the most widely studied approach. The materials prepared by microspheres include gelatin, hyaluronic acid, chitosan, artificial polymer materials (polylactic acid, polylactic acid-glycolic acid copolymer), hydroxyapatite, etc. The preparation methods of microspheres include the emulsification method, secondary coagulation method, solvent volatilization method, electrospray method, etc. [110]. The small size and large surface area of the microspheres are conducive to the loading of growth factors, the microspheres can protect the growth factors from reaching the site of action, the microspheres can prolong the release of growth factors and release them slowly with bone growth, and the microspheres can also be used as the constituent materials of the scaffold and mix with other substances to make the scaffold. Azizian et al. [111] prepared chitosan nanospheres loaded with basic fibroblast growth factor and bovine serum albumin by ionic gel method, and then fabricated porous chitosan gelatin scaffolds. The results showed that the addition of chitosan nanoparticles slowed down the degradation rate of chitosan gelatin scaffolds, and the growth factor release of chitosan particles alone reached 80% within 4 days and reached 80% after incorporation into the scaffold, and the release of growth factors was significantly slowed down. Although Azizian et al. achieved slow release by microsphere loading of growth factors, the time of slow release was still unsatisfactory, whereas Scheiner et al. [112] loaded both VEGF, FGF, and IGF via negatively charged PLGA microspheres, and IGF exhibited rapid release, whereas VEGF and FGF sustained release for 4 weeks. While they improved the slow release of growth factors, they could not selectively control the sequential release of growth factors when loading multiple growth factors. This problem was solved by Liu et al. [113] by creating a shell-and-core microsphere that achieves

control of the order of release of the two growth factors by loading different growth factors into the outer shell and the inner core. However, the release rate of growth factors loaded into the shell in the shell-core microspheres they prepared was still fast, and could perhaps be improved by adjusting the materials used to prepare the microspheres or by preparing a microsphere with a three-layer structure.

With the deepening of research on bone tissue engineering, some studies have shown that the degradation rate can be changed through external interference to regulate the release of growth factors. These external factors may include temperature, ultrasound, infrared light, and mechanical stress. Badeau et al. [114] fabricated hydrogels that respond to multiple factors, which can be delivered in multiple cells in a continuous space–time manner under the stimulation of enzymes, reduction, and light, and expressed potential for the treatment of highly restrictive diseases. Song et al. [40] developed a multifunctional polylactic acid-glycolic acid (PLGA) microsphere, loaded with BMP-2, which was released on demand in response to the damage microenvironment and external ultrasound, which provided a new idea for controlling the release of growth factors. 3D printing technology can more precisely control the distribution of growth factors in the scaffold, so that the distribution of growth factors is more uniform, and then continue to release growth factors with the gradual degradation of the scaffold, compared with traditional technology, the release rate of growth factors is more stable [5]. Combining this intelligent control method with the personalized treatment of 3D-printed stents will solve many difficult problems. However, the release of multiple substances at the same time and the selection of response materials are issues that need to be further studied and improved.

Clinical application

Bones have a high regenerative potential and support the organism of living organisms. Bone defects can deprive a person of the most basic support, which can lead to a range of problems. The treatment of bone defects remains a clinical challenge, and although autologous transplantation is an optimal treatment, it is often not possible to meet the demand due to the limited number of autologous bone grafts. With the continuous research of bone tissue engineering, 3D printed scaffolds have emerged, which solves the shortage of graft materials and the need for personalized customization, but its osteogenic effect still cannot meet the needs of clinical treatment. Therefore, 3D-printed scaffolds loaded with growth factors have been born.

Before the advent of 3D-printed bone grafts, bone grafts had a long history, dating back to 2300 BC. Archaeological finds in Peru of Inca skulls, covered with gold or silver plates, confirm that early transplantation of the skulls of wounded soldiers was carried out at that time, according to relevant literature, as well as coca, gourd, coconut, but all failed [115]. In the first bone graft in 1668, the Dutch physician Jon van Meekeren used a canine skull to repair a soldier's skull injury, but it was removed due to opposition from the Christian church [116]. In 1881, Macewen performed the first allogeneic bone graft in Scotland [117]. In 1885, autologous bone grafting was popularized, in 1892, the synthetic material calcium sulfate gypsum, and in 1965, titanium was used [118]. Bone tissue engineering was proposed in the 90 s of the twentieth century and began extensive and in-depth research, which is the basis of bone tissue engineering now, and 3D

printing began to be used by researchers to print bone scaffolds during the same period [119, 120]. Since then, the connection between 3D printing and bone tissue engineering has been deepened, and 3D-printed biological scaffolds have ushered in a research boom.

Kim et al. [121] fabricated a temperature-sensitive 3D printed scaffold containing growth factors, which can be used to adjust the mechanical properties of the scaffold by temperature during printing, and showed good performance in animal experiments. These intelligent and controllable stents are still in the experimental stage, and no relevant treatments have been reported in clinical trials. 3D-printed titanium stents have long been clinically reported for clinical use in distal tibia defects and foot surgery, and have also been approved by the FDA [122]. 3D printing in orthopedic clinical practice from model printing to help doctors intuitively understand the bone structure and make surgical planning, customized prostheses, accurate printing of personalized prostheses to meet the individual needs of patients, surgical assistance and navigation, printing special auxiliary tools and models to make surgery safer, bone tissue engineering 3D printing, bringing new hope for bone regeneration and bone repair. 3D printing technology has been widely used in clinical practice, but 3D printed scaffolds loaded with growth factors are still in the experimental stage as shown in Fig. 2, and are rarely reported in clinical literature, and are promising to be applied to clinical applications in the future.

Challenges and future directions

Bone tissue engineering is an effective treatment for bone defects and other diseases, and scaffolds containing growth factors are the most widely studied. Although the research on 3D printed scaffolds loaded with growth factors is becoming more and more mature, there are still many problems that need to be solved.

Bone tissue engineering is a multidisciplinary project, so it is difficult and necessary to ensure the accuracy of printing. The pore structure and complex personalized geometry of the scaffold are required, but printing trabecular structures that resemble bone tissue remains a challenge. At the same time, the selection and concentration control of growth factors is also a challenge. The growth factor is a protein that promotes tissue growth and differentiation, and it is easy to lose its activity in vitro, so it is also important to

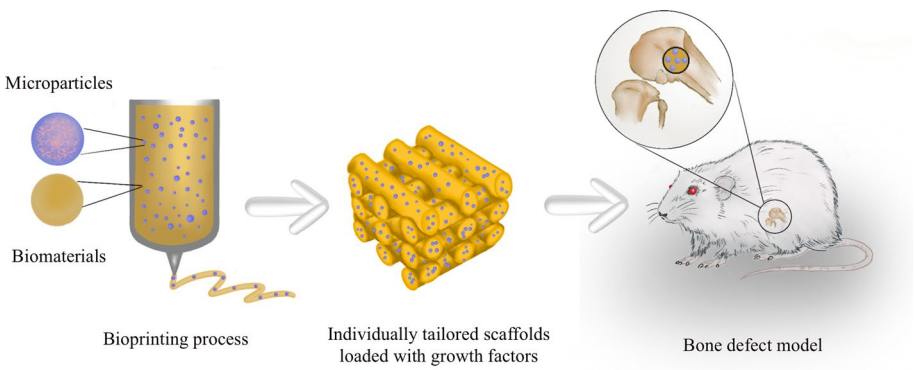

Fig. 2 Schematic diagram of 3D printing of growth factors and biomaterials for preparation of scaffolds and their application

choose the right carrier, although various hydrogels, polymer particles, etc. are currently used, the guaranteed activity is still limited. At present, the degradation rate of many scaffolds can be controlled by various methods such as material ratio, but the degradation rate of scaffolds close to the bone growth rate still needs to be studied. 3D printed composite materials still need to find a more suitable composite composition and the ratio of each component. It is necessary to have a high degree of plasticity, and according to the mechanical strength of the bone in different parts, print scaffolds are similar to their mechanical properties. The degradation rate needs to be highly consistent with the regeneration rate of bone, and it needs to be continuously degraded with the growth of bone, and the degradation products have no adverse effect on tissue. 3D printing materials have their limitations, so it still takes effort to choose the right printing method and printing material to print a bracket with a high degree of biomimicry. The controllable release of multiple growth factors from the same scaffold is a promising way to treat bone defects in the future, which is 4D printing. 4D printing adds a temporal dimension to the 3D basis, making a scaffold by responding to some stimuli with materials, and then controlling some of the properties of the scaffold through specific stimuli. Stimuli are physical (temperature, light, magnetic, electric), chemical (PH), and biological (enzyme) [123]. 4D printing can achieve human intervention and control so that the stent is dynamic. However, due to the limited material response to external stimuli and the possible similarity of molecular weights and isoelectric points of multiple growth factors, 4D printing with on-demand gradual release still needs further research.

Conclusion

Bone tissue engineering is an important engineering project to solve bone grafting, and 3D-printed bone scaffolds are a product of a new era of bone tissue engineering. At the same time, it can also replace normal bone tissue to support the organism. The most important thing is that the loaded growth factors can activate and promote the regeneration of bone tissue, and reduce the occurrence of poor bone healing and nonunion.

The first step in 3D printing bone scaffold is to choose the right printing material, and the bone graft material has gradually changed from non-degradable to biodegradable, from a single metal to polymer, bioceramic, and then to a composite. The material is gradually approaching the functional properties of normal bone tissue, not only having the mechanical properties, biocompatibility, and biological activity of normal bone but also now the research can achieve in vitro intelligent response and release bioactive substances on demand. The development of 3D printing technology has also brought more choices for personalized scaffolds, and different materials are suitable for different printing methods, such as metal multi-purpose fused deposition method, polymer multi-purpose extrusion printing, and bioceramic commonly used SLM. 3D printing technology can control the porosity during printing to adjust the performance of the stent. Growth factors for bone tissue engineering are also being clarified, and the most common and effective ones are the BMP family.

Through 3D printing, the scaffold is accurately manufactured to fit the bone and provides the necessary mechanical support, and the growth factor promotes bone growth, and with the growth of bone, the scaffold is constantly degraded and disappears. Although many studies are still in the experimental stage, their potential is obvious

to all, and 3D-printed scaffolds containing growth factors are expected to become an important tool in the treatment of orthopedic diseases soon.

Limitations

Although there are many studies suggesting that 3D-printed scaffolds containing growth factors are an effective solution to difficult problems such as bone defects, there are still some problems and limitations in the current research. First, there are limited biomaterials that can be used for 3D printing with good mechanical properties and degradation, and current materials still do not fully satisfy clinical applications and long-term needs. Second, the rate and duration of growth factor release is currently difficult to precisely control, and realizing the on-demand release of multiple growth factors is even more difficult. Finally, studies have been conducted in animal models or in vitro, and there is still a lack of research and data to support the safety and efficacy of growth factor-containing 3D-printed scaffolds in humans. Coupled with the high cost of 3D printing and biomaterials, clinical translation remains problematic. In conclusion, although growth factor-containing 3D-printed scaffolds have great potential in the treatment of orthopedic diseases, further research is needed to address these issues and limitations.

Author contribution

Longwen Zhan: write articles, ideate, and graph. Yigui Zhou: article revision, polished. Ruitang Liu: article revision, polished. Ruilong Sun: provide ideas, polished. Yunfei Li: provide ideas, polished. Yongzheng Tian: provide ideas, polished. Bo Fan: article revision, polishing, review.

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Competing interests

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