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Applications of 3d printing in promoting bone tissue regeneration

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Abstract. 3d printing has been a recent research hotspot because of the performance of printing accurately, easy to operate and can complete the more complex objects, there are many advantages missing in subtractive manufacturing, and at the same time match the requirements of orthopaedic implant scaffolds, orthopaedic implant scaffolds as one of the main applications of bone tissue regeneration (BTR) has also been commonly studied, the current literature is mostly in its raw materials to do research. This paper introduces the three main materials and gives some examples to illustrate the role of scaffolds or implants obtained after they have been 3d printed in promoting BTR, emphasizing the potential appeal of 3d printing (3DP) in BTR.

Keywords: 3d printing, Bone regeneration, Metals, Ceramics, Organic polymers.

1. Introduction

Bone tissue is widely known to contain a rich vascular network plays an essential character in project for instance, nutrient transport, metabolism, and maintenance of bone homeostasis. For effective functional bone regeneration, it is essential to simultaneously restore bone tissue and restore blood vessels. 3d printing scaffolds have the potential to improve vascularized bone regeneration by facilitating associated cell activity or improving cell distribution capacity by adjusting composition or structure[1].

In the early 1990s, the Cambridge Institute of Technology (Cambridge, Massachusetts) developed 3DP technology for the manufacture of 3D printed products [2].

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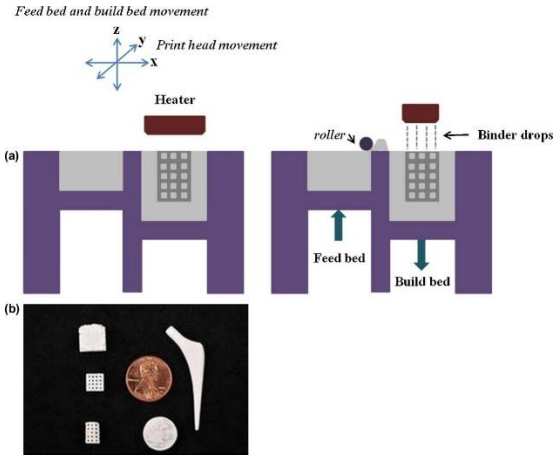

Figure 1. Schematic of the 3DP process: (a) Schematic of 3DP using an inkjet printing system. (b) 3D printed CaP sintered structure fabricated at WSU[3].

3d printing technology (applicable scenarios and pros and cons)

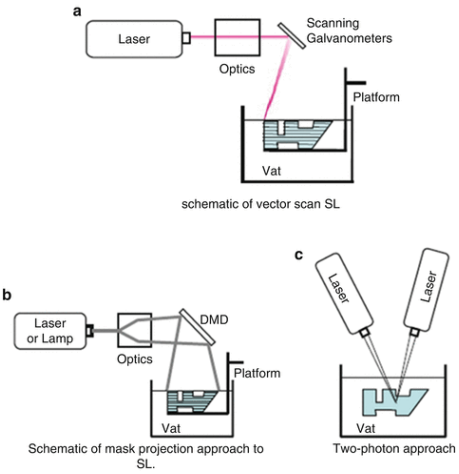

Figure 2. Schematic diagrams of three approaches to photopolymerization processes[4].

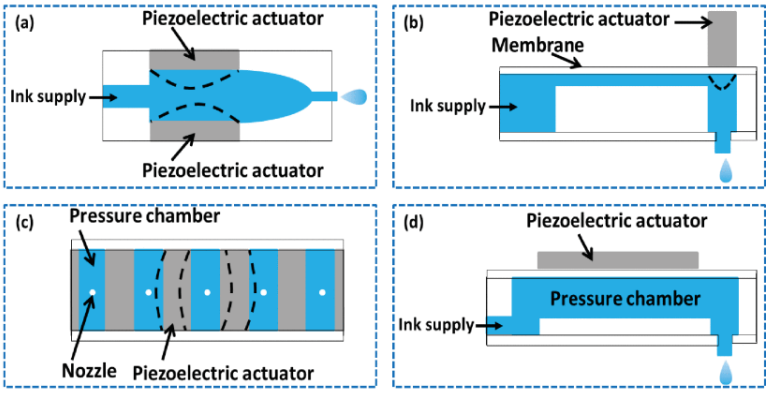

Figure 3. Piezoelectric-driven inkjet 3DP: (a) squeezing, (b) pushing, (c) shearing, (d) bending [5].

In the biomedical field more commonly used 3DP technology there are four, respectively: vat photopolymerization (VP), fused deposition modeling (FDM), inkjet printing (IP) and selective laser

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sintering (SLS), their applications have their own advantages and disadvantages. sintering), their applications have advantages and disadvantages, the accuracy of the VP is higher, you can print a more complex structure, but it can only be used in photosensitive polymers and printing speed is slow[6]; FDM operation is convenient molding speed, but must be in the ink in the molten state can be printed[7]; IP is simple and low-cost operation, but the printing accuracy is poor, the mechanical strength of the printed object is lower[8]; SLS molding efficient and material utilization high, print out the mechanical properties of the model and good strength, but its cost is also higher[9]. These four printing techniques can be ceramics and organic polymers as raw materials, while metals can only use FDM and SLS two printing methods.

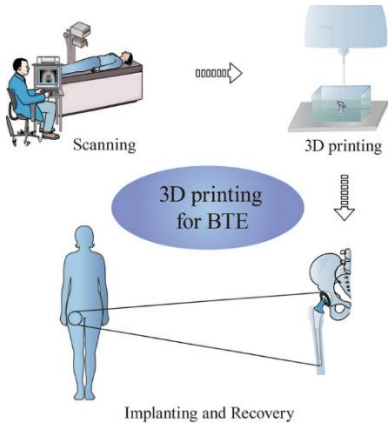

Figure 4. A brief flowchart of 3DP applied to BTE [10].

3DP and BTR as the current research hotspot, there are many scholars in the relevant aspects of research and write a review. Their purpose is mainly focused on a particular material, the use of a particular 3DP method for BTR to bring positive results. The content of this paper in addition to a brief introduction to the 3DP, will focus on the clinical and scientific research in the more commonly used three kinds of materials as well as the use of them as raw materials for 3DP scaffolds and other effects on BTR. This review combines and expounds 3DP and BTR in multiple directions and aspects, which provides a channel for practitioners to understand the current scientific research status in the industry, and also provides a broader reference vision for scholars who will be engaged in research in this field.

2. Materials

Ceramics, metals and organic polymers each show unique advantages in 3D printed BTR: ceramic materials are biocompatible and chemically stable. They mimic the inorganic components of bone, such as hydroxyapatite (HA) and bioboron glass (BBG), which have excellent osteoconductivity and can effectively help regenerate new bone tissue [11]. Excellent mechanical qualities and corrosion resistance are found in metallic materials. They are generally used to repair highly loaded bones and can be customized with different alloys to optimize and improve their biocompatibility[12] [13]. Materials made of organic polymers, often divided into synthetic and natural polymers. Natural polymers are like hyaluronic acid collagen combined with sulfates, they have good bioactivity but they're much more difficult to manufacture because of their reproducibility and controllability whereas synthetic polymers like polycaprolactones you can control their molecular weights but they don't have as good bioactivity[14].

3. Metals

Metallic materials usually have good mechanical properties and mechanical properties that make them useful in support or replacement therapy of bone tissue, while certain metal ions also play a role in various processes of BTE, so metals are commonly used in BTE, orthopedic implants, dental materials, wound healing and scaffolds[15]. However, since their stability and biocompatibility can be improved,

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recent research has focused on enhancing material properties through surface modification, alloying, and various other techniques [16]. At the same time, metals are very malleable and combinable, so they can be combined or alloyed as raw materials for 3DP.

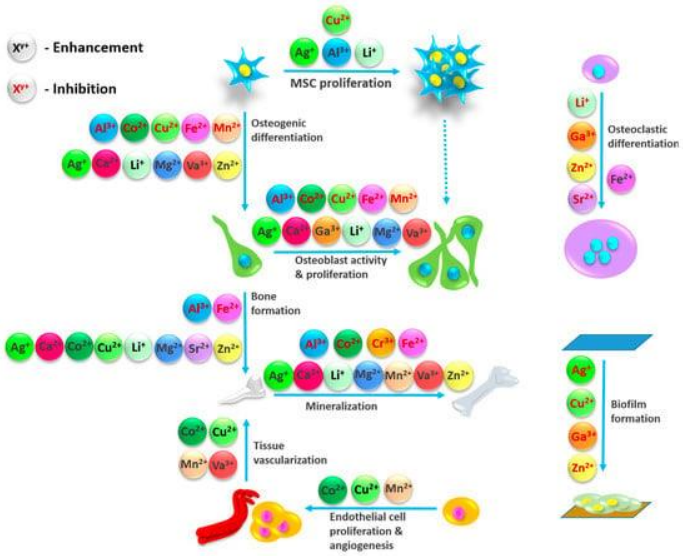

Figure 5. Influence of metal ions on different processes involved in BTR [17].

3.1. Alloys/metal compounds used as feedstock for stents

The aim of using scaffolds in BTR is to restore damaged bone tissue to its original state by employing bionic scaffolds, which serve as a platform to promote cell adhesion and spreading. Bionic bone scaffolds must be porous., featuring interconnected penetrating structures and pores of different sizes in order to sustain osteoblasts growth and provide sites for blood vessel formation[18, 19] .

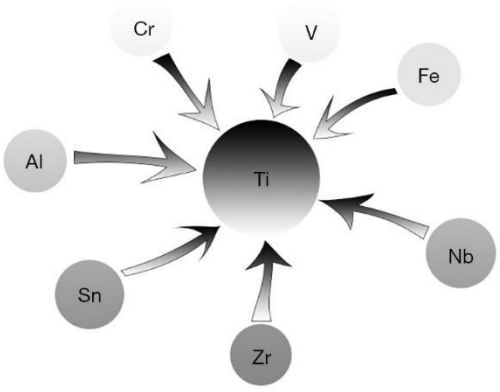

Figure 6. Compositional variations in titanium alloys. Materials science researchers work on adding different proportions of elements to titanium, which can build different alloy phases and obtain different physical or biological activities[20].

Titanium is among the most commonly used metals in medical practice, and arpart from the oral cavity titanium is also widely used in BTR. Perforated scaffolds fabricated using commercially pure titanium powder through SLS can exhibit different retention of live cells at different pore sizes, leading to increased cell retention within small pore scaffolds, potentially influencing their growth. This indicates that in vitro cell performance can be partially regulated by adjusting the pore size., with 200 μm pore scaffolds exhibiting the greastest cell retention rates[21] The greatest cell retention rates were

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observed with 200 μm pore sizes. Titanium can also combine with other metals to improve properties. In addition to titanium, cobalt-chromium alloys have become a hot topic of research due to their properties and are also used as a substitute for Ni-Cr alloys. When Ni-Cr is used as a dental alloy, cytotoxicity has been reported (16).[22] Cobalt-chromium (Co-Cr) has been used as a replacement for gold alloys in cermet crown frameworks; Co-Cr is also generally used in orthopedic implants [23] Chromium provides excellent corrosion resistance in printed materials[24]. while the graphene coating on Co-Cr alloys enhances their bioactivity[25] . The And the scaffolds made by slm showed higher cell viability (vs. Co-Cr scaffolds made by milling)[26] . The Co-Cr bioscaffolds, also interact with osteogenic precursor cells in an efficient manner, especially in case of endocrine or metabolic condition disorders[27]. Besides the metals mentioned above, there are a variety of metals that have good properties to act as 3d printed bone scaffolds, tantalum Ta, which has superior osteogenic bioactivity, as well as elevated density and elastic modulus[28] ; magnesium Mg, a biodegradable material, possesses an elastic modulus comparable to that of natural cortical bone[29] etc.

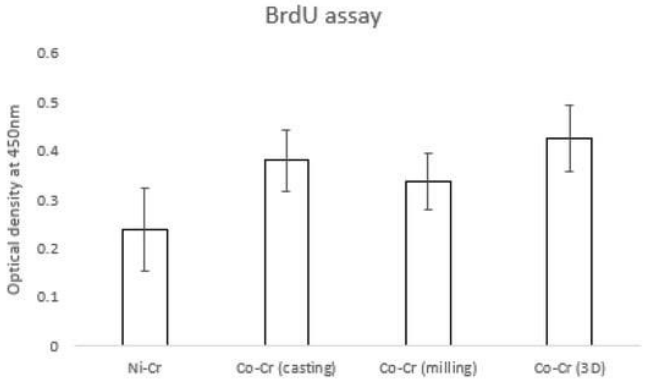

Figure 7. Proliferation of hADSC in intervertebral discs (3D printing significantly higher than other groups)[26].

3.2. Coated metals

In addition to being used as raw materials for 3d printing, metals can also be used as coatings attached to the surface of 3d printing materials, of which gallium is a widely used coating. Gallium (Ga) is a biomultifunctional element with immunosuppressive, tumor imaging, anticancer, antibacterial, anti-inflammatory, and osteoclastogenesis inhibiting properties[30]. Gallium Nitrate. Low doses of gallium nitrate not only decrease bone resorption but also enhance bone mineralization and can even eliminate bacteria by disrupting their iron metabolism. [30-32]. Gallium-doped biomaterials can enable in situ sustained release of gallium ions, thereby partially mitigating the side effects associated with the systemic administration of the drug. [33, 34]. Metallophenolic networks (MPNs) have then been constructed as a simple, fast and low-cost coating technology[35, 36] Gallium (III)-phenolic networks have been developed as a multifunctional coating. It can be prepared by submerging the implant in a mixed solution of tannic acid (TA) and gallium ions. The coating enhances the ability of the implant to osseointegrate by promoting bone and inhibiting osteoclastogenesis[37]. The coating enhances implant osseointegration by promoting bone and inhibiting osteoclastogenesis . In addition to gallium, vanadium is more commonly used as a coating, and vanadium-containing organometallic compounds have greater implements, such as longer half-lives, higher adsorption capability, and higher steadiness[38] Vanadium ions have antimicrobial properties that release the growth of various pathogens and reduce the risk of infection associated with implanted biomaterials. Furthermore, vanadium has demonstrated a significant capacity to enhance bone formation. Vanadium ions have been found to stimulate osteoblast activity and facilitate proliferation, differentiation, and mineralization, leading to accelerated bone formation and the development of a robust bone matrix[39] . In addition to the two metals previously discussed, strontium ions are crucial for promoting osteoblast proliferation, which increases the quantity of bone

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cells. Additionally, strontium ions stimulate mineralization and enhance the deposition of calcium and phosphate, both of which are essential components of the bone matrix [40] .

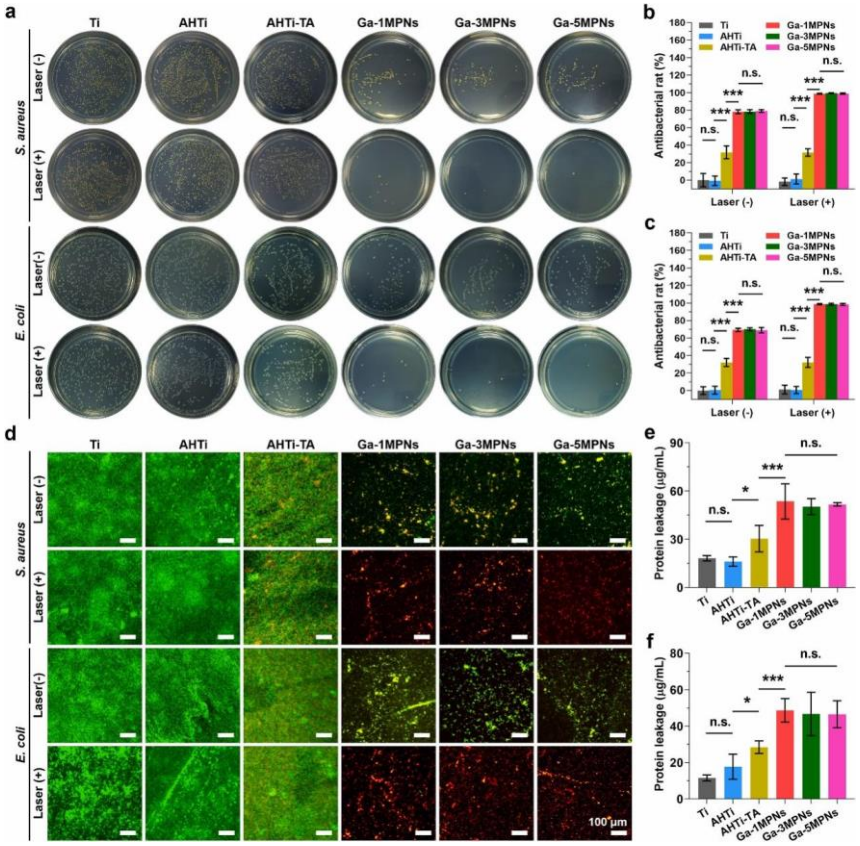

Figure 8. Antibacterial properties of Ti, AHTi, AHTI-TA, Ga-1MPNs, Ga-3MPNs, Ga-5MPNs substrates in vitro. a-f was the result of colony culture, Ga-3MPNs and Ga-5MPNs were significantly better than other groups.

4. Ceramics

4.1. Bioinert ceramics

Figure 9 (a) shows various technologies to produce additives (am) for bioinert ceramic stents, such as PBF-LB, compression of materials, targeted energy deposit, glue, addition of valuable photopolymers, lining, materials manufacturing. But the most frequently method for creating biological inertia of a Spicewood is PBF-LB, compression of materials, glue, addition of valuable photopolymer (see figure 11). In this technology, in the production of building scaffolding PBF-LB has a significant advantage, so skeletal building; the record on (b) figure 2, it is in 100 v, the largest steepen ball is wide pore size 1000 MKM. This staircase causes internal pores that do not dissolve and is known for its ability to absorb and maintain water from 20 to 100% of total body weight. Second, the drug administration system (DDS), which grafts on tablets for personal medicine, is a widely used approach to enhancing the function of support. As specified in section 4, DDS, including cells will be drugs, active compounds and embedded in generated through PBF-LB shelf. Another reason for writing this comment is that in the context of the PBF-LB is the most suitable technology for the manufacture of additives for biologically inert ceramics.

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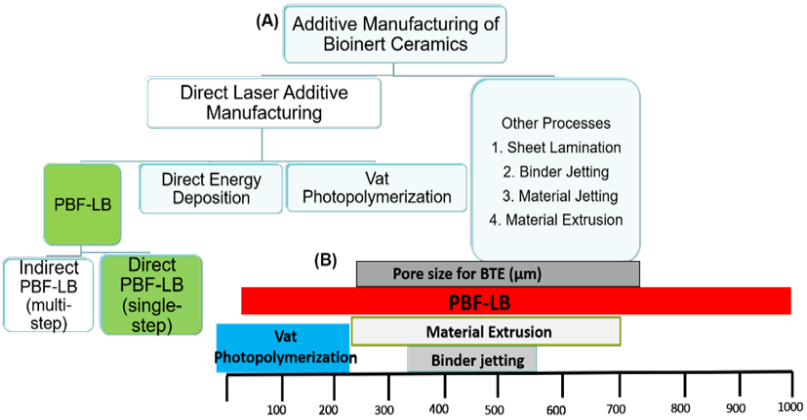

Figure 9. (A)Method of the production methods of biological cerotics additives and green leading-edge techniques, (B) Benefits of PBF-LB over other am methods for the BTE.

Figure 10 Uses two different keywords. There is also no review in Scotland on the keywords "biologically inert ceramic scaffold" and "fusion of laser dust bed". This is the main driving force that drives our review [41]. This study is to investigate lasers for hardening ceramic materials with biological nuts. These materials are mainly used to make tofu on thigh bones, as well as to treat serious plastic surgery applications such as spinal and bone defects. They exist as oxides, such as al2o3 or YSZ, or as non-oxides, such as si3n4, produced directly from PBF-LB. Ultimately, the study showed that PBF-LB, an important bone disease from 1 cm to 4.5 cm, was able to manufacture ceramic pedals with DDS of biological nuts. Figure 12 shows the number of articles on bio-nut ceramic pedal materials over the past 13 years.

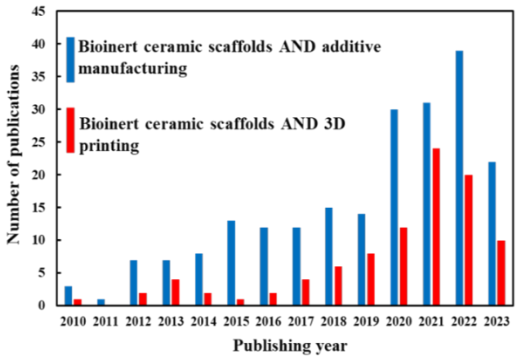

Figure 10. Number of publications announced in the last 13 articles using different keywords.

4.2. Bioactive ceramics

It is important to change the printed and desirable properties of biological materials for samples with a high degree of complete mechanical safety. Water gel also does not protect against mechanical cell damage. To improve the resolution, mechanical mass and viability of cells, it is necessary to change the pressure, temperature, viscosity and diameter of the nozzle. Figure 13 shows the number of articles in 3d printing.The choice of information in different areas, a summary of policies to support 3d printing[42].

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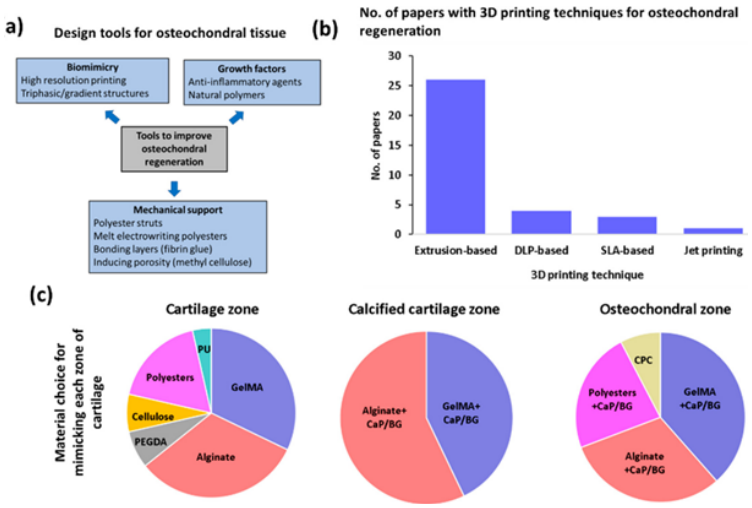

Figure 11. 3DP technology applied to ceramics.

5. Organic polymers

5.1. natural polymers

Natural polymers have several benefits in BTR, chief among them being their superior biocompatibility and biodegradability.They can mimic the structure and function of the body's extracellular matrix to promote cell regeneration and differentiation, while providing a suitable growth environment for these cells.

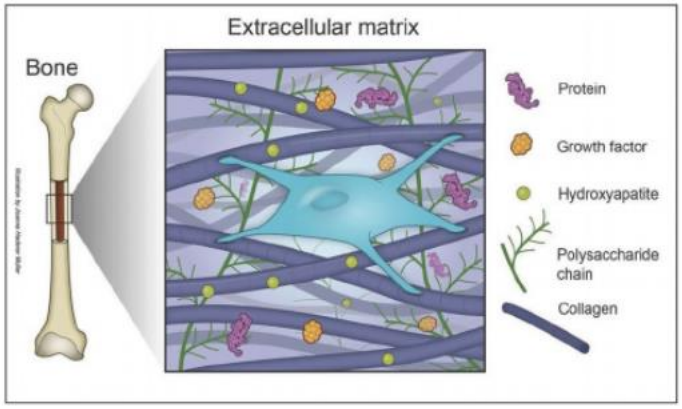

Figure 12. Extracellular matrix (ECM) of human bone[43].

For example, collagen, chitosan, gelatin, silk protein, alginate, etc., which have a high degree of similarity to human tissues, can provide scaffolds that are closer to the natural physiological environment to promote the adhesion of human cells, proliferation, and the formation of new bone tissue

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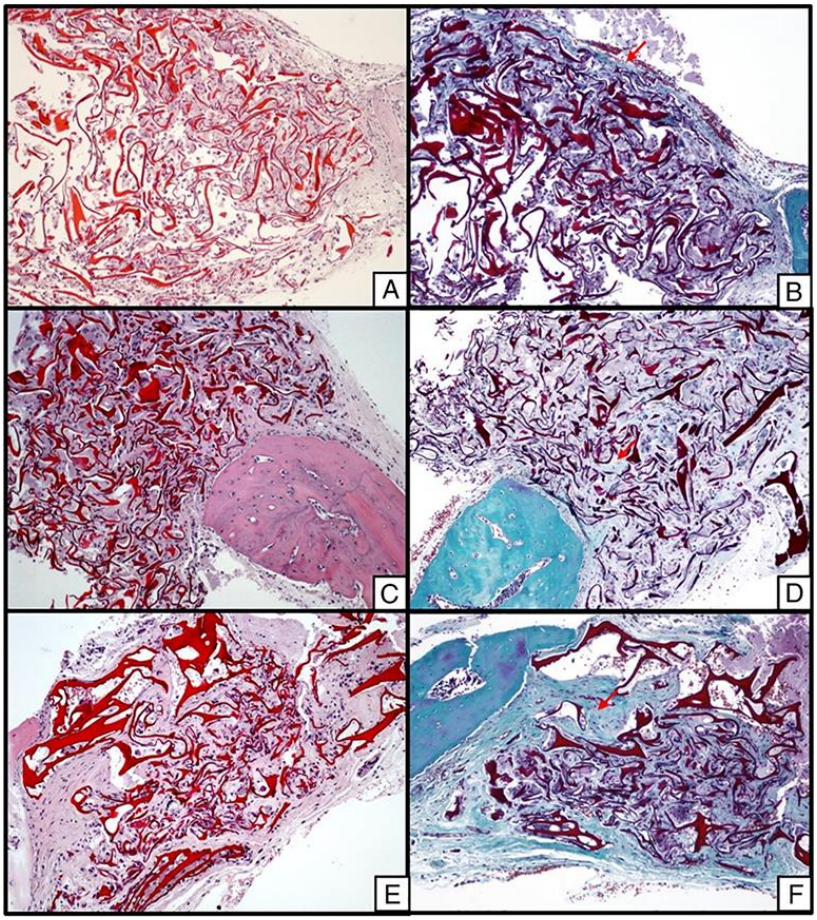

Figure 13. Histological photos of composite scaffolds made of nSI and CS. In A (two weeks), C (four weeks), and E (eight weeks), H&E staining was done. Three groups underwent Mallory trichrome staining: B (two weeks), D (four weeks), and F (eight weeks)[44, 45].

Chitosan, for example, is a natural polysaccharide that is widely available and sustainable in nature.

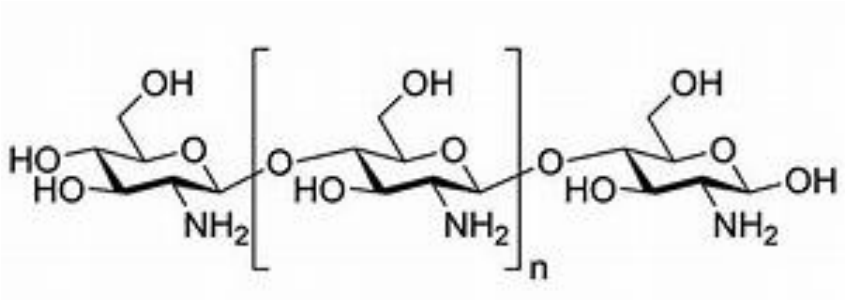

Figure 14. The structure of chitosan.

Numerous amino groups found in chitosan's chemical structure can help human cells adhere to one another and proliferate in an environment that is conducive to the differentiation of bone cells. Furthermore, the antibacterial qualities of chitosan can aid in preventing infection following scaffold implantation.

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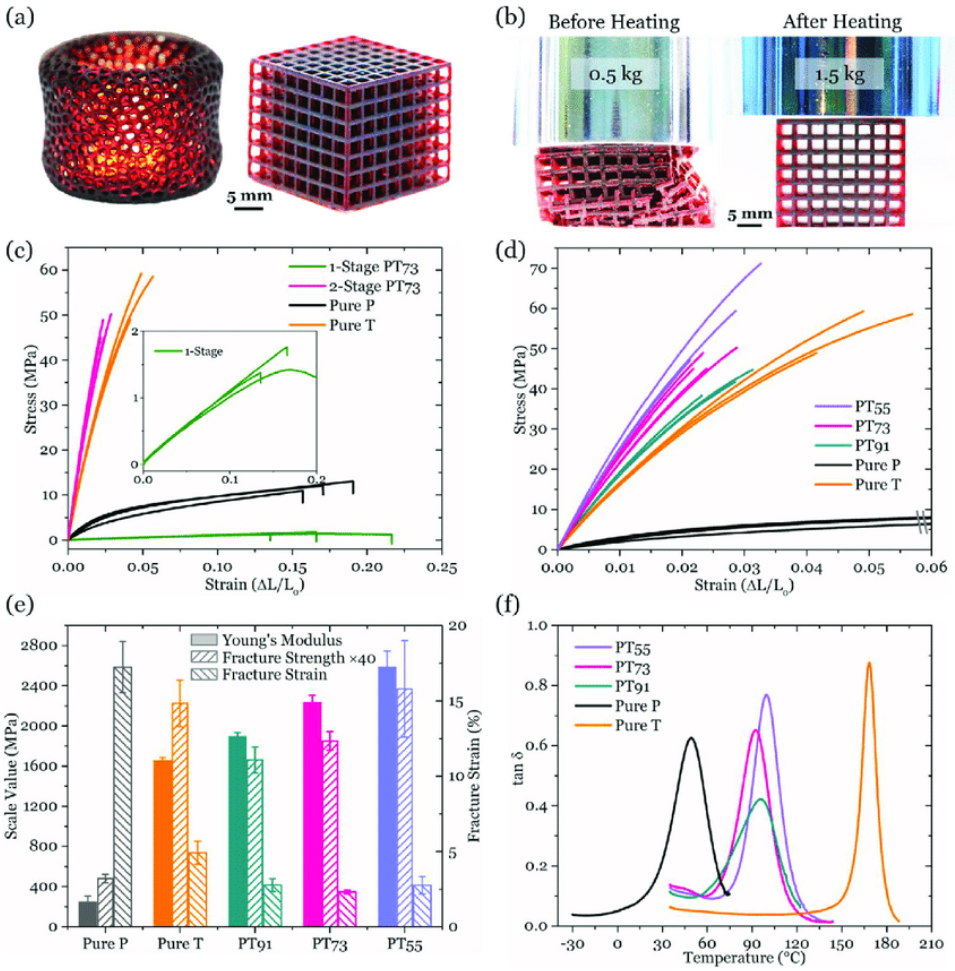

Figure 15. Mechanical characteristics of resins with pure components and polymers manufactured in 3D. (A) Lattice and hollow 3D printed structures. (b) Variations in stiffness prior to and following heat treatment. c) How heat treatment affects the tensile characteristics. (D-F) Polymer tensile and DMA results. Take note that the left axis is used to display the numbers for "fracture strength × 40[46].

Furthermore, natural polymers possess customizable physical characteristics. Their components can be modified or processed to adjust mechanical attributes such as elastic modulus and durability, allowing them to be tailored for various applications and requirements.

Some disadvantages of natural polymers are their low mechanical qualities and significant potential for contamination. It is also difficult to synthesize natural polymers on a large scale and to change their properties.

5.2. Synthetic polymers

The advantages of synthetic polymers in 3d printed BTR are their customizability, plasticity and controllability of mechanical properties. This allows them to be customized to specific scaffolds or attachments according to clinical needs. In the case of polylactic acid-hydroxyacetic acid copolymers (PLGA), for example, the advantage of this polymer is that it is chemically tunable, allowing the body to adapt to the framework of bone tissue repair by altering the ratio of monomers and precisely controlling the rate of degradation. At the same time, the biocompatibility of PLGA ensures its safety in the human body, as its degradation products can be directly metabolized by the body naturally. Moreover, the mechanical properties of PLGA can be optimized by processing techniques and used to match the strength and toughness of bone tissues, and the porous structural design of PLGA further

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enhances its potential for application in bone tissue engineering as the pores not only provide space for cell growth, but also promote cell migration and angiogenesis, which are essential for BTR and repair.

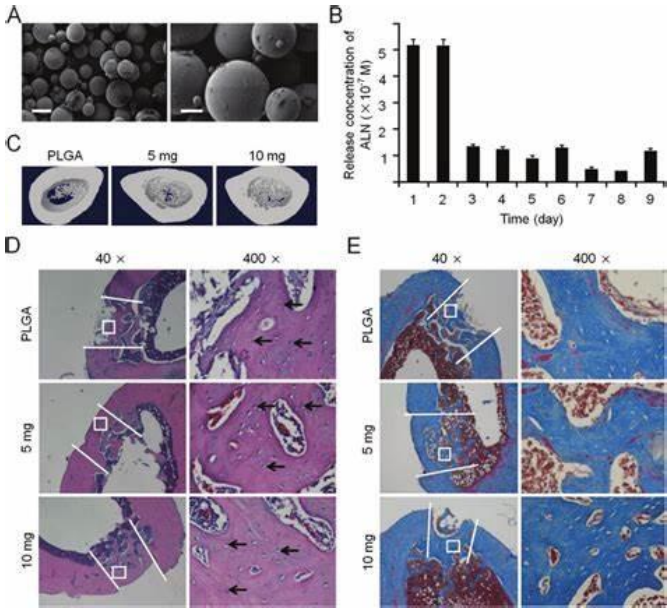

Figure 16. A) Scanning electron microscopy (SEM) images of PLGA bonded to ALN (left image scale bar: 100 μm; right image scale bar: 50 μm). B) In vitro ALN discharge from PLGA-ALN microspheres. C) Three-dimensional micro-CT scans were captured and examined six weeks after implanting 10 mg of PLGA, 5 mg of ALN-conjugated PLGA mixture, and 10 mg of ALN-conjugated PLGA mixture into rat femoral defects. D,E) Histological analysis using H&E and Masson's trichrome staining for each group, six weeks post-implantation in femoral defects. White lines demarcate the bone defect areas, with 400x magnified views shown in white frames.The osteoblasts found in the regenerated bone's lacunae are shown by black arrows. Reproduced with permission[47, 48].

In addition, synthetic polymers in 3d printing can be used to precisely print the shape and internal structure of scaffolds or attachments to meet the needs of personalized implants that can be adapted and matched to a patient's specific anatomy and therapeutic application, which can improve the probability of a successful surgery and the patient's recovery.

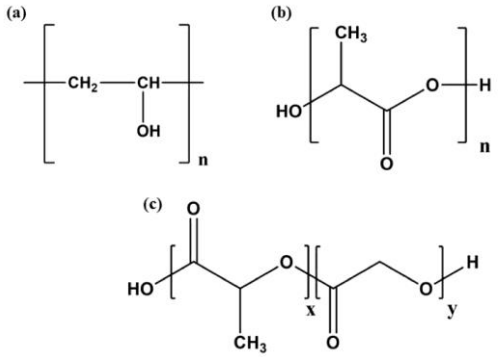

Figure 17. Synthetic polymer molecular structures: (a) PVA (polyvinyl alcohol), (b) PLA (polylactic acid) and (c) PLGA (polylactic acid-hydroxyacetic acid copolymer)[49].

To further improve the mechanical strength and bioactivity of these synthetic polymer scaffolds, nanoscale materials including hydroxyapatite (HA), bioactive glass (BG), and carbon nanotubes (CNTs) can be included.

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6. Applications

Internal fixation is crucial for fractures in weight-bearing bone regions. Current fixation methods mainly involve metallic and biodegradable materials. The stiffness of metals like stainless steel , titanium (Ti), and their metal compounds significantly differs from human bone tissue. A higher elastic modulus may not align with bone biomechanics, hindering callus formation and fracture healing, potentially requiring additional surgery. The use of 3D printing to create porous, tantalum (Ta)-coated bone plates for permanent implantation presents a novel approach to fracture healing.

The process begins by using Ti6Al4V ELI spherical powder[50] to fabricate a porous titanium bone plate in an argon atmosphere. A Ta layer was applied to the surface of the pTi bone plate using the chemical vapor deposition (CVD) method.[50].

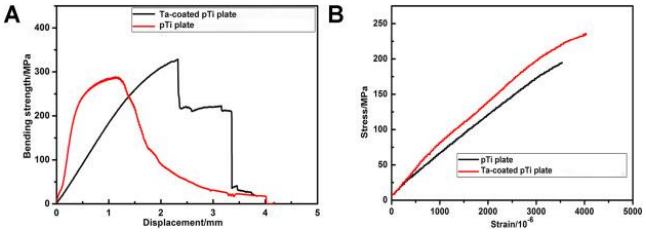

Figure 18. Testing of mechanical properties of bone plates: (A) Bending strength of pTi plate and Ta-coated pTi plate. (B) Tensile strength of pTi plate and Ta-coated pTi plate.

Radiography revealed that all three groups demonstrated good fracture stabilization four weeks after surgery, with no displacement and visible fracture lines. New bone formation was observed around the fractures in the pTi and Ta-coated groups, while the Ti group showed limited or no development near the bone plate (Fig. 19 down the A1 group). By eight weeks, all groups exhibited excellent fracture healing, with no displacement or visible fracture lines, and significant callus formation and osseous growth near the bone plates (Fig. 19 down the A2 group). After 12 weeks, fractures in the Ta-coated group recovered, the fracture line disappeared, bone density increased, callus formation around the bone plate and contralateral side reduced, and the pulp cavity remained largely unobstructed (C3 in Fig. 19). However, despite ossification in the titanium and porous titanium metal groups, fracture lines were still visible in Fig. 19 A3 and Fig. 19 B3. At 16 weeks, fractures in the Ta-coated group fully healed, showing optimal bone plasticity and complete retubation of the pulp cavity (Fig. 19 C4). In the Ti and pTi groups, fractures healed, but dense bone encircled the fractures, and full plasticity was not achieved in Fig. 19 A4 and Fig. 19 B4. At four, eight, twelve, and sixteen weeks post-surgery, Lane-Sandhu scores for the porous Ta-coated group exceeded those of the Ti and pTi groups.

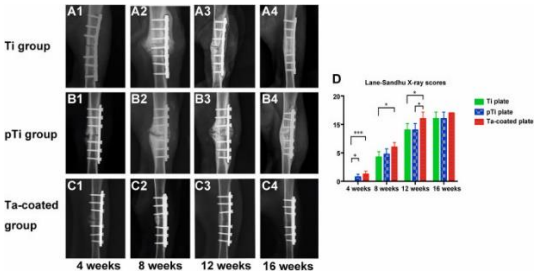

Figure 19. X-ray assessment of Bone regeneration status: (A1 to A4) The recuperation process of fractures in the titanium plate cohort. Twelve weeks post-surgery, the fracture in the normal titanium plate group has healed, although the pulp cavity remains unhealed. Strains B1 to B4 generated a significant quantity of Cali following the procedure on pTi plates. A quick regenration process of the fracture was observed in the group with Ti plates coated with porous Ta, with fracture repair completed 12 weeks post-surgery and an unobstructed pulp cavity remaining.The Lane-Sandhu radiographic scores demonstrated enhanced bone healing between 4 and 12 weeks post-surgery in the porous titanium plate group relative to the other two groups ($P < 0.05$).

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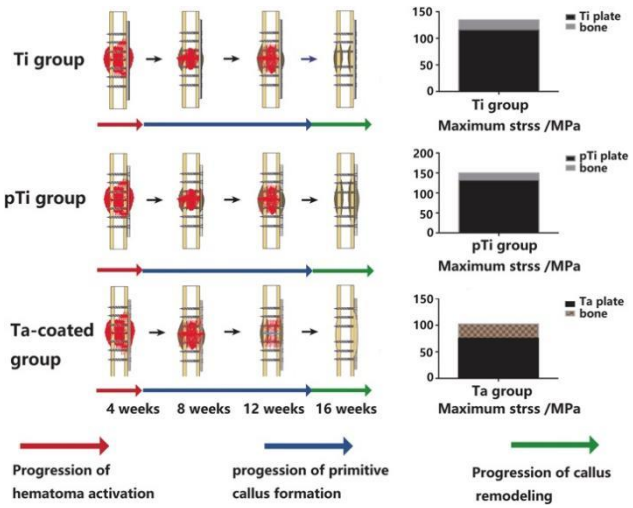

Figure 20. The process of bone healing and the study of mechanical conduction after fracture filling. In this diagram, the red arrow represents the development of the haematoma, the blue arrow the formation of the initial callus, and the green arrow the progress of the callus remodelling. Both the initial callus formation and the fracture healing process were accelerated in the porous Ta-coated plate group compared to the others. After a fracture has healed, the force can be distributed uniformly across the bone and porous Ta coating to prevent stress concentration and offer ongoing support.

Bone plates coated with porous tantalum have several uses and advantages, such as a low elastic strength and the capacity to lessen stress shielding while the body heals. In addition to being highly biocompatible, bone plates coated with porous tantalum can be permanently implanted into the body. Bone plates coated with porous tantalum have better osseointegration capabilities and speed up the healing process after a fracture.

The purpose of this study was to develop a custom-made bone plate that shows beneficial biological activity, speeds up the healing process of fractures, and can be kept in vivo for a long time. The plate should have low elastic modulus and high mechanical strength, so it can effectively stabilize fractures without causing stress shielding.There is no need for bone-inducing biomolecules when using biomaterials with appropriate porosity architectures to encourage new bone formation in non-osseous locations, according to previous research. When cells undergo osteogenic differentiation, a crucial biological feature in the production, secretion, mineralization, and maturation of bone matrix is the differentiation of osteoblasts, which is necessary for the formation of predominant osteoblasts. Research in vitro shows that cells adhere and multiply more quickly on tantalum coated surfaces due to their coarse shape. This finding is in line with previous studies.

7. Promoting BTR

7.1. Nano hydrogel

Hydrogels can be categorized into natural and synthetic types. Natural hydrogels originate from biological sources and has the benefit of intrinsic biological activity. Generally, researchers prefer natural hydrogels for bone and cartilage tissue engineering due to their exceptional biocompatibility and the biodegradability of hydrogels, which is comparable to that of natural bone or cartilage.

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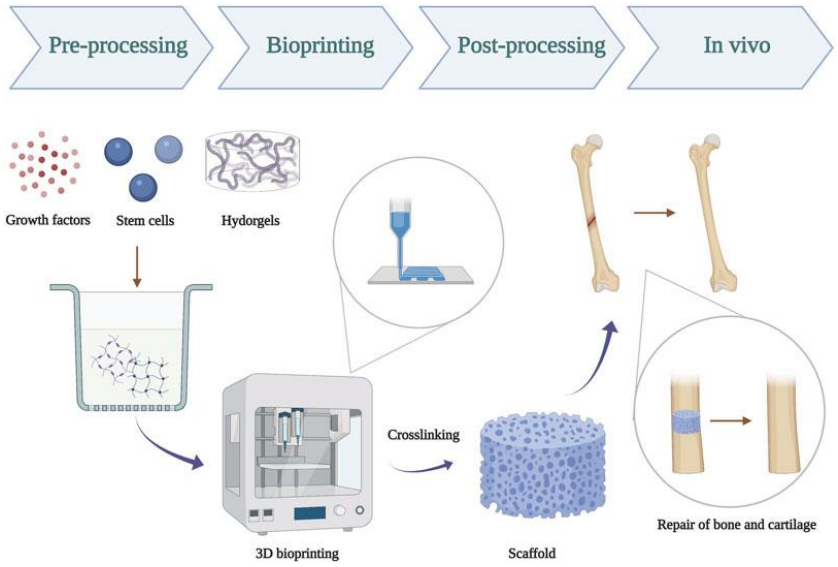

Figure 21. Application flow of hydrogel in BTE.

Bioinks, a printable variant of biomaterials, serve as the foundational substance utilised in bioprinting. Hydrogels are currently the predominant biomaterial employed in 3D bioprinting, exhibiting considerable advantages in tissue engineering. In 1954, Wichterle and Lim synthesised the inaugural hydrogel. Seven The prevalent definition of a hydrogel is "a polymer network that swells and crosslinks upon exposure to water, resulting from a straightforward reaction involving one or more monomer, polymer, or crosslinker units." Temperature, light, pH, ions, and other physiological signals are only a few examples of the many physical and biological stimuli that can easily alter their highly hydrated polymer structure.

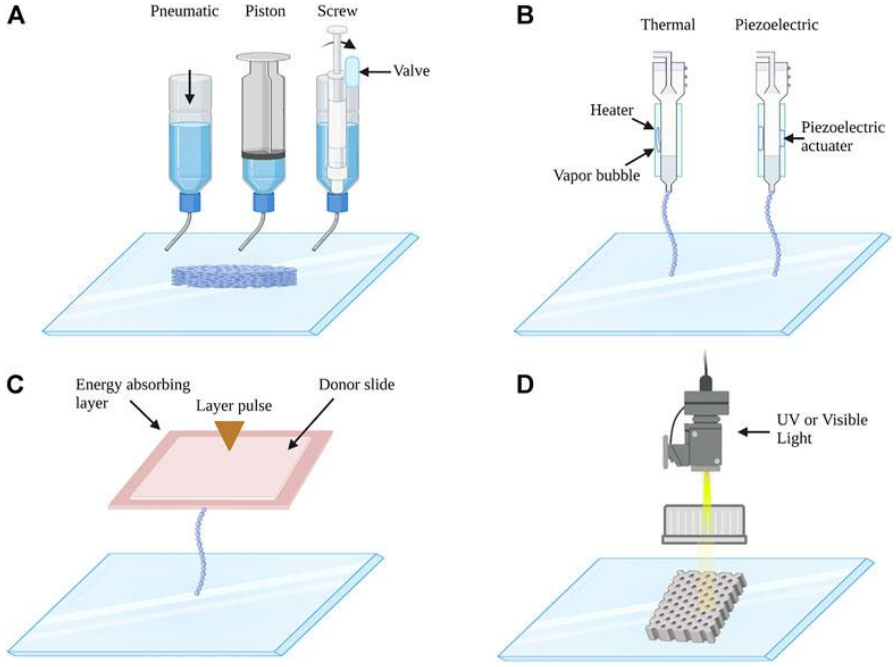

Figure 22. Schematic illustrations of (A) extrusion-based, (B) inkjet-based, (C) laser-assisted, and (D) stereolithographic 3D bioprinting systems.

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Alginate, a polymer derived from brown algae and microorganisms, contains two distinct glyuronic acids found in algae cell walls and within *Azotobacter* and *Pseudomonas* [8, 9]. Due to its favorable physiological properties—such as biocompatibility, biodegradability, and gelation—alginate is widely used in biomedical fields like wound dressings, drug delivery, and tissue engineering. Adding type I collagen (COL I) and agarose to alginate enhances mechanical strength compared to pure alginate gels, with COL I also supporting cell adhesion, proliferation, and the expression of cartilage-specific genes. Incorporating graphene oxide into alginate further improves printability and structural integrity in composite bio-inks. Studies show that graphene oxide enhances printing quality, structural properties, mesenchymal stem cell (MSC) functions, proliferation, and osteogenic differentiation.

Natural and synthetic hydrogels differ in their origin. Natural hydrogels, derived from biological sources, possess inherent biological activity. These hydrogels are preferred for bone and cartilage tissue engineering due to their excellent biocompatibility and biodegradability, resembling natural bone or cartilage.

Hyaluronic acid (HA), a major component of cartilage, is a hydrophilic macromolecule and glycosaminoglycan found in the extracellular matrix (ECM) of various tissues. HA exerts its biological effects through antioxidant activity, biocompatibility, and interactions with cell receptors. Additionally, HA regulates MSC growth, migration, and adhesion, making HA-based hydrogels suitable carriers for bone tissue engineering (BTE) and cartilage tissue engineering (CTE).

7.2. Pearl powder

Bone abnormalities resulting from trauma, infection, malignancies, and osteomyelitis are prevalent orthopedic conditions. Specifically, substantial bone lesions exceeding the crucial threshold for autologous repair cannot undergo spontaneous healing through organic stimulation. Pearl powder products are generally composed similarly to natural mineralized substances found in bone tissue. This study demonstrates a bioactive pearl powder (PP) scaffold for bone regeneration utilizing microfluidic-assisted 3D printing, as illustrated in the figure. PP is a renowned herbal remedy extracted from pearls. Owing to its exceptional biocompatibility, antioxidant properties, and osteogenic capabilities, it has been utilized in medical aesthetics, food additives, and particularly in bone tissue creation.

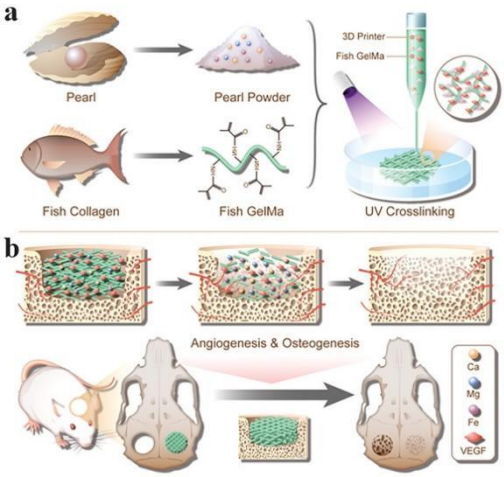

Figure 23. Schematic diagram of microfluidic 3D printed PP hybrid bioactive scaffolds for bone regeneration. a) Composition of PP hybrid bioactive scaffolds and microfluidic 3D printing. b) PP hybrid bioactive scaffolds for bone regeneration applications[51].

They attempted to 3D bioprint scaffolds using pearl powder mixed with bioactive materials, inspired by the architectural use of printing materials into shapes such as Figure 24 thereby stacking them layer by layer on top of the bone-deficient level and promoting bone regeneration (Figure 23b). The bioink is a mixture of 25% GelMa, 2% PP and 0.5% LAP. The bioink is initially extruded from a capillary nozzle

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to create geometric shapes such as squares, triangles, and circles, which are subsequently solidified using UV light (Figure 23a).

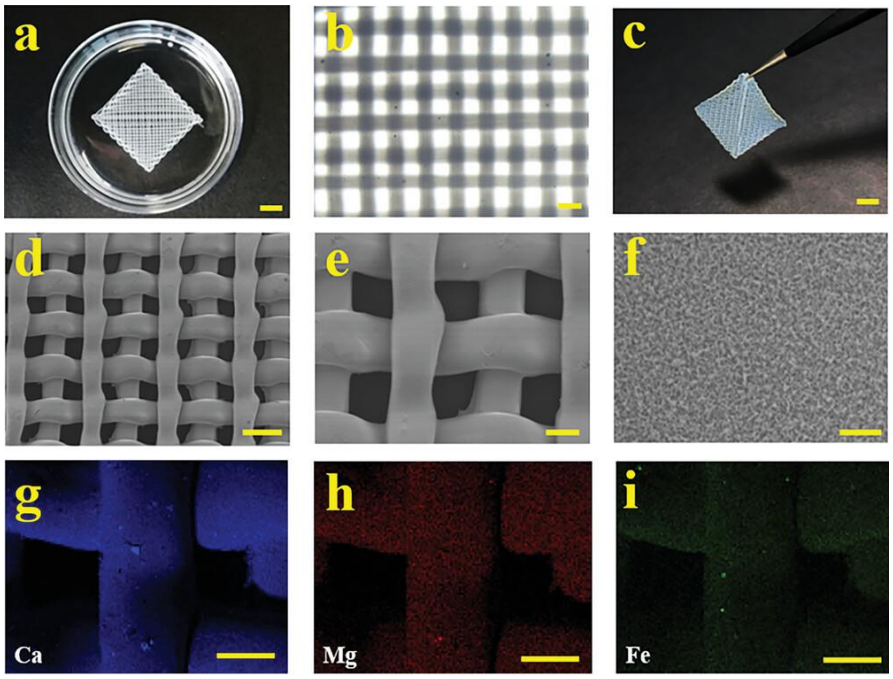

Figure 24. Characterization of PP scaffolds:

a) Digital images showing PP hybrid scaffolds.
b) Microscopic images showing their stacked structure.
c) Digital images of dehydrated PP hybrid scaffolds.
d-f) SEM images of (d) PP scaffolds, (e) fiber stacks, and (f) PP scaffold surfaces.
g-i) Energy dispersive spectroscopy (EDS) analysis showing the elements of (g) Ca, (h) Mg, and (i) Fe in the PP scaffolds. (Scale bars: (a-c) are 5 mm, 1 mm, and 5 mm, respectively; (d-e) are 500 μm, 200 μm, and 10 μm; (g-i) are 200 μm).

To validate in vivo bone regeneration potential, experiments examined the application of scaffolds in a rat cranial bone defect model to assess osteogenesis. Post-surgery, rats were assigned to GelMa, GelMa-PP, GelMa-PP&VEGF scaffold groups, and a PBS control group. After eight weeks, cranial bones were harvested for micro-CT and histological analysis. As seen in Fig. 5a, bone tissue regeneration (BTR) occurred in the GelMa, GelMa-PP, and GelMa-PP&VEGF groups compared to the control. The GelMa-PP&VEGF group showed the most pronounced bone regeneration. Hematoxylin-eosin (H&E) and Masson's trichrome staining confirmed new bone formation in scaffold-treated groups (Fig. 24b; Fig. S9, Supporting Information). Micro-CT results highlighted the GelMa-PP&VEGF group as the most effective in bone repair, with the highest bone volume to tissue volume (BV/TV) ratio and bone mineral density (BMD), accelerating bone regeneration relative to other groups.

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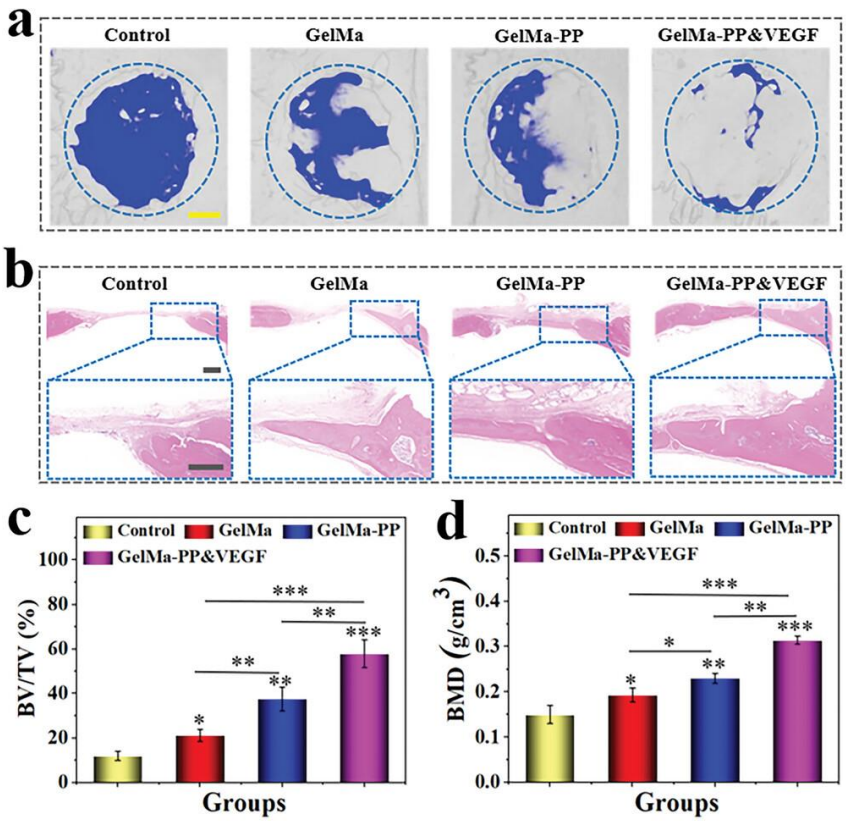

Figure 25. [51] Promoting effect of pearl powder on bone regeneration.

8. Bone implant materials

Magnetic implants exhibit angiogenic, osteoconductive and osteoinductive properties when coupled to biomaterials. Moreover, these scaffolds can be coupled with magnetic fields to enhance their regenerative potential. And magnetic nanoparticles do not have any toxic effect on bone cells. There have been studies combining magnetic nanoparticles (MNPs) with calcium silicate (CS) and using 3d printing to fabricate BTR implants. Additive manufacturing offers the capability to produce porous scaffolds with intricate geometries and flexibility, surpassing the limitations of traditional manufacturing methods[52] . Unification of mnps into CS scaffolds profoundly increased cell viability and proliferation. cell attachment and proliferation of Fe2.5 and Fe5 were remarkably better than Fe0, with Fe5 in turn being better than Fe2.5. also FeCS promotes up-regulation of the activity of ALP, which plays an important role in osteogenesis.

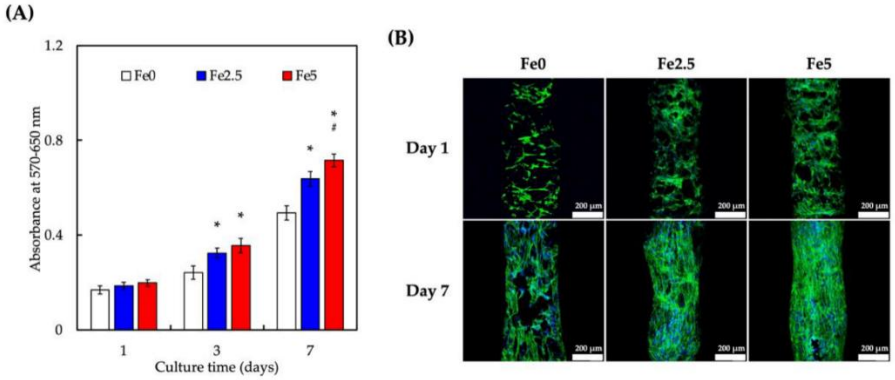

Figure 26. (A-B) Fe2.5 and e5 are superior to Fe0 in promoting WJMSCs' propagation.

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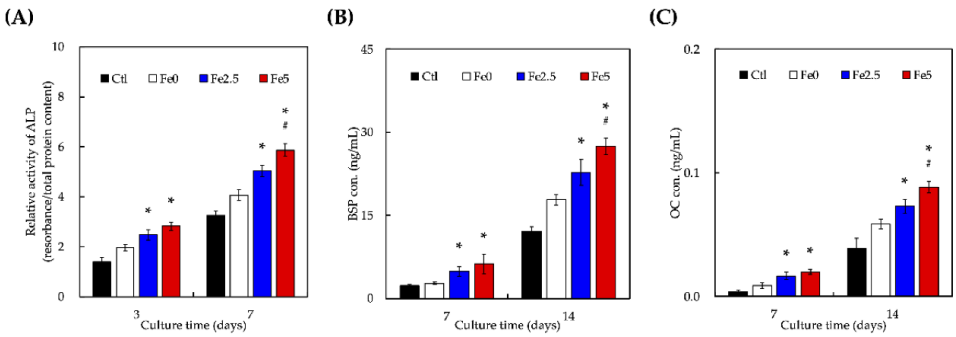

Figure 27. The expression levels of osteogenic differentiation markers (ALP, BSP, OC) in WJMSCs cultured in Fe2.5 and Fe5 at different time points were greater than those in control group and Fe0 group.

Biphasic calcium phosphate (BCP) is considered to be an important candidate for the manufacture of bone graft substitutes. Different properties can be obtained by doping different metals into it. Currently there are studies on adding strontium and magnesium to biphasic calcium phosphate to go as raw material for 3d printed bone implants.Mg doping can stimulate the growth of bone-like apatite in simulated humoral medium, preserve or improve cell adhesion and proliferation, and can also have antibacterial effects against a variety of bacteria. Scaffolds made using 3d printing have good porosity, with microporosity of 5% and 15% yielding excellent cell proliferation rates[53] . Low cytotoxicity and good cell proliferation and growth were detected by cell culture experiments.

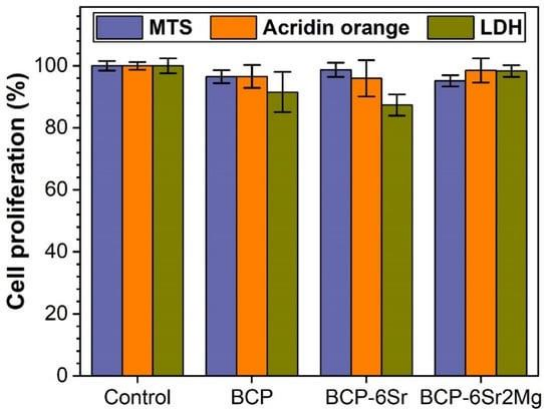

Figure 28. After 14 days of culture, cell proliferation of hFOB 1.19 grown on BCP-based scaffolds was assessed by MTS, LDH, and acridine orange assays [54].

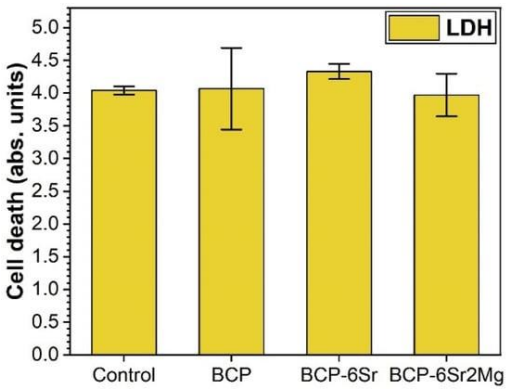

Figure 29. hFOB 1. 19 The cytotoxicity of BCP-based scaffolds was assessed by LDH testing after 14 days of cell culture[54].

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Organic polymers are also one of the more commonly applied raw materials for 3DP of BTR, with the existing study of Ilia Averianov et al. on a novel 3D printed BTR scaffold based on polyglutamic acid-modified nanocrystalline cellulose in poly (epsiloncaprolactone) (PGlu-NCC)[55] . In mechanical property tests, the material containing 5 wt% PGlu-NCC showed the optimal mechanical properties, especially in promoting BTR compared to pure PCL scaffolds, with a larger volume of mineralized bone tissue noted in the PCL/PGlu-NCC + rMSCs group. In the case of PCL/PGlu-NCC scaffolds with adherent rMSCs, the growth of new bone trabeculae in the scaffolds, the connection of defective areas, and the formation of bone healing tissues confirmed their biocompatibility and osteogenic properties.

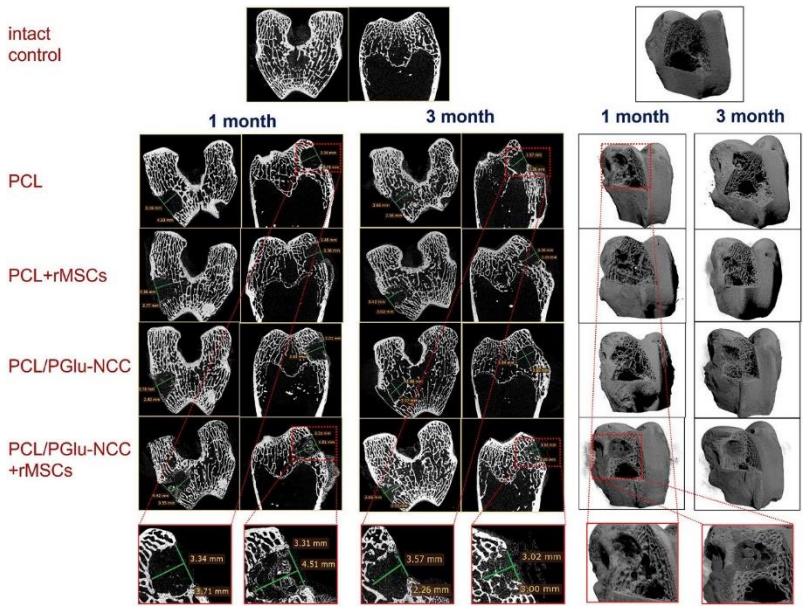

Figure 30. Images of the different groups at 1 and 3 months postoperatively were obtained by micro-CT analysis.

9. Conclusions

This paper briefly introduces BTR mechanisms and several commonly used 3DP techniques, detailing the different properties of the three most commonly used materials in medical 3DP, including mechanical and biological properties. In the application process, they are usually not independent, but are combined and weighted according to the desired properties as well as their respective properties.

In addition, we have selected the latest research results to illustrate the different effects of different materials combined with scaffolds made by 3DP in BTR. The pore size of the scaffold implant is very important, which is related to the ability of bone cells to attach and proliferate, which needs to be constantly adjusted and tried to find the most suitable pore size through 3DP. In addition, different coatings can also inject different vitality into the stent implant, in the selection of coatings should not only refer to the properties that need to be met, but also consider whether the material used with the stent implant is compatible, therefore, this field is still to be continued in-depth exploration will help these 3d printed stent implant really applied to the clinical benefit of patients.

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