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Review on Engineering of Bone Scaffolds Using Conventional and Additive Manufacturing Technologies" for 3D Printing and Additive Manufacturing

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3D Printing and
Additive Manufacturing
3P D+

Review on Engineering of Bone Scaffolds Using Conventional and Additive Manufacturing Technologies

Abstract:	Bone is a complex connective tissue that serves as mechanical and structural support for the human body. Bones' fractures are common, and the healing process is physiologically complex and involves both mechanical and biological aspects. Tissue engineering of bone scaffolds holds great promise for the future treatment of bone injuries. However, conventional technologies to prepare bone scaffolds can not provide the required properties of human bones. Over the past decade, three-dimensional printing or additive manufacturing technologies have enabled the control over the creation of bone scaffolds with personalized geometries, appropriate materials and tailored pores. This paper aims to review the recent advances in the fabrication of bone scaffolds for bone repair and regeneration. A detailed review of bone fracture repair and an in-depth discussion on conventional manufacturing and three-dimensional printing techniques are introduced with an emphasis on novel studies concepts, potentials and limitations.

1 **Review on Engineering of Bone Scaffolds Using Conventional and Additive**

2 **Manufacturing Technologies**

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26 **Abstract**

27 Bone is a complex connective tissue that serves as mechanical and structural support for the

28 human body. Bones' fractures are common, and the healing process is physiologically

29 complex and involves both mechanical and biological aspects. Tissue engineering of bone

30 scaffolds holds great promise for the future treatment of bone injuries. However, conventional

31 technologies to prepare bone scaffolds can not provide the required properties of human

32 bones. Over the past decade, three-dimensional printing or additive manufacturing

33 technologies have enabled the control over the creation of bone scaffolds with personalized

34 geometries, appropriate materials and tailored pores. This paper aims to review the recent

35 advances in the fabrication of bone scaffolds for bone repair and regeneration. A detailed

36 review of bone fracture repair and an in-depth discussion on conventional manufacturing and

1 three-dimensional printing techniques are introduced with an emphasis on novel studies

2 concepts, potentials and limitations.

3 **1. Introduction**

4

5 Bone injuries have recently increased due to ageing, traumatic injuries, and congenital diseases,

6 making them a global health issue. It's estimated that the number of people aged more than 65

7 years will increase from 323 million to 1.55 billion by 2050 worldwide. Age intensifies the risk

8 of osteoporosis and consequently has dangerous effects on people's healthy life, disability,

9 countries' healthcare systems, and loss of productivity . Globally, over 200 million people have

10 osteoporosis, with an increased number of patients receiving hospital treatment every year due

11 to fragility fractures and bone loss, accelerating the demand for bone tissue surgeries. Efficient

12 and cost-effective strategies to treat bone injuries will help to improve people's quality of life

13 and relief the economic burden on governments (1, 2).

14 A bone defect is generally defined as the lack of bone tissue in an area of the body, which results

15 in pseudarthrosis. Usually, the human body is capable of self-repair, yet when a segmental bone

16 fracture exceeds a size of 10 mm, the body fails to self-repair (3). Therefore, external

17 interventions are essential to assist in the self-repairing process by creating bone scaffolds.

18 These scaffolds act as bridges over bone defect sites and facilitate repair (4). The design of the

19 bone substitutes must be controlled to avoid excessive bone tissue removal at defect sites and

20 to allow cell activity and proliferation (5, 6). The latter is facilitated by designing a scaffold

21 with a porous and linked pore structure. Thus, manufactured bone scaffolds are a promising

22 solution for treating bone fractures, but this comes with some challenges.

23 Regarding bone scaffolds manufacturing techniques, several methods have been investigated

24 to create porous scaffolds for bone repair, such as salt leaching, gas foaming, self-assembly,

25 phase separation, electrospinning, and freezing drying methods. Although these approaches are

26 capable of fabricating porous structures, they have certain drawbacks, such as restricted pore

27 structure control and a limited ability to customise for particular defect sites (7). Additionally,

28 many of these techniques leave behind organic residues of the pore-forming agent, impairing

29 the scaffolds' biological characteristics and lowering the quality of bone healing. Thus,

30 developing fabrications technique for scaffolds that are not restricted to obtaining the desired

31 external shape but also precisely control the pore structure is critical for their future orthopaedic

32 application.

33 Given this context, additive manufacturing (AM) technologies are becoming a good alternative

34 for manufacturing scaffolds as they can create porous scaffolds with customised external design

1 and a porous inner structure (8). The use of 3D printing technology for the generation of bone

2 scaffolds has been gaining more attention from researchers and the biomedical industry in

3 recent years. The near future of bone regeneration and healing is closely linked to developments

4 in tissue engineering. Polytherapy, which combines scaffolds, stem cells, and healing promoters

5 with new advances in tissue-engineered constructs in three-dimensional printing, may be

6 capable of overcoming current challenges in treating bone injuries. In this review paper, we will

7 focus on scaffolds as an established treatment for bone fracture using 3D printing technologies

8 and compare them with conventional manufacturing techniques (Figure 1).

9

2. Bone Fracture Repair

11

12 Bone tissue can undergo biological remodelling as a function of a dynamic process that involves

13 osteoclasts absorbing mature bone tissue and osteoblasts forming new bone tissue (9, 10). Bone

14 is a complex connective tissue made up of osteoblasts, osteocytes, bone lining cells, and

15 osteoclasts. The outer layers of bones are mineralised, giving them significant strength and

16 rigidity to support the body structure and allow skeletal movement. Bones composition includes

17 the inorganic phase (60% - 70% of the tissue), (22% - 35%) organic matrix and liquid (5% –

18 8%), where collagen represents the majority of the organic matrix and only 10% non-

19 collagenous proteins (11). Bones strength and stiffness are mainly provided by hydroxyapatite

20 crystal $(Ca_{10}(PO_4)_6(OH)_2)$ with carbonate ions (12) which are found within and between

21 collagen fibres in the form of needles, plates and rods with an average diameter of 20–80 nm

22 and 2–5 nm in thickness [2]. Bone can also modify its structure according to body requirements,

23 such as in repair, modelling, remodelling, and growth (13, 14).

24 Bone tissues can be classified into cortical and trabecular (Figure 2). Both have the same matrix

25 composition; however, they vary in structure and function as well as in relative distribution

26 between bones. Cortical bone (dense or compact) is composed of layers surrounded by lamellar

27 bone and vascular channels. This arrangement is known as the Haversian or osteon (15). An

28 osteon's central channel contains cells, vessels, nerves, and osteon-connecting Volkmann's

29 channels (15). On the other hand, trabecular bone (spongy or cancellous) is located in the

30 epiphysis and metaphysis of long bones and inside small or flat bones. Trabecular bone has a

31 wide network of individual trabeculae, small and interconnected plates and rods guided by

32 external loading (15). Typically, cortical and trabecular bones have Young's moduli of

33 approximately 17 and 1 GPa, respectively (15). Cortical bone is a dense structure representing

34 about 80% of the total skeletal tissue. Yet, cortical bones have some microscopic pores (about

1   10% of the total cortical bone volume) to allow vascular and neural supply and enable the

2   delivery of nutrients (16). The porosity of cortical bone is critical as an increase in intracortical

3   porosity can reduce the bone strength and consequently increase the chances of fracture (17). It

4   is evident that cortical bone becomes more fragile at very high strain levels reflecting high-

5   impact trauma (Figure 2). As the strain rate increases, cortical bone shows a ductile to brittle

6   transition (18), and like any material, the cortical bone could be prone to fatigue failure.

7   On the other hand, trabecular bone has a lower mass and high porosity when compared to

8   cortical bone. Pores represent 50%-90% of total trabecular bone volume (19). This makes it

9   considered an open cell porous foam with a reduced compressive strength to about one-tenth

10   of the cortical bone (20). It does, however, provide a large surface area that is necessary for red

11   bone marrow, blood vessels and bone-connected tissues and facilitates hematopoiesis and

12   homeostasis of minerals. The trabecular bone's physical and mechanical properties vary widely

13   depending largely on the anatomical location, age and orientation of the cell structures (21, 22).

14   Depending on the type and orientation of these basic cell structures, the mechanical properties

15   can differ by a factor of 10. A comparison between the compressive properties of trabecular

16   and cortical bones is shown in (Figure 3) (21). As shown, the cortical bone acts as a typical

17   brittle material at which the stress steeply increases at a low strain in the elastic region, and the

18   fracture occurs without a noticeable change in the strain.

19   On the other hand, trabecular undergoes a ductile behaviour under compression loading with a

20   substantial increase in the plastic deformation before fracture. Individual trabeculae bone

21   damage and repair is a physiological process that occurs throughout life and increases with age

22   (23).

23   Bone fracture healing process can be enhanced using several techniques, such as grafts, which

24   replace defected bone with another bone from the patient's own body (autografts) or from a

25   donor, or by using healing growth materials in fabricating bone implants or scaffolds (24).

26   Autografts are currently the bone regeneration golden standard (24). However, Autografts

27   techniques have several disadvantages, such as surgical complications and the limited supply

28   of natural tissue. Tissue-engineered bone scaffolds are a suitable alternative to autografts as

29   they improve fracture healing and enhance the incorporation of grafts (25, 26). Requirments of

30   tissue-engineered scaffolds are to have properties close to those of autografts irrespective of

31   their limitations (27).

1 Bone scaffolds need to have high porosity with sufficient sizes of pores across all sites of the

2 scaffold in order to create an ideal environment for the formation of new tissue matrix and bone.

3 Moreover, growth factors like the basic growth factor of fibroblasts (28, 29) can influence cell

4 functions, proliferation, or differentiation; Promotive healing agents, for example, Human

5 platelet-rich plasma (hPRP) (30-32); and also Tarantula cubensis extract (33) could be

6 incorporated into the scaffolds to improve the damaged connective tissue's ability to repair.

7 The scaffold's vascularity is important as if not present, ischemia will occur in the scaffold, and

8 hence the cells would die. Therefore, it would be useful to incorporate growth factors such as

9 FGF, PDGF, and VEGF to promote angiogenesis in scaffolds and grafts (34, 35). A

10 combination of stem cells and scaffolds with growth factors can be one possible approach

11 providing all the required characteristics to enhance bone repair and regeneration. Currently,

12 none of the grafts provided all the desirable requirements such as biocompatibility, size

13 limitation, cost, osteogenic, osteoconductive, osteoinductive, and angiogenic properties (Table

14 1). Tissue engineering seeks to provide all or most of these characteristics (36, 37). Also, tissue

15 engineering can cause bone defects to be repaired and reconstructed (27). Incorporating the

16 basics of orthopaedic surgery with knowledge from various sciences such as biology,

17 engineering, chemistry, materials science and physics could overcome current treatments'

18 shortcomings (25). Advances in biomaterials and tissue engineering can provide more

19 appropriate tools to support the differentiation and proliferation of bone cells and improve bone

20 fracture healing. Although there are a large number of studies in the literature on the effects of

21 different agents on bone healing, it is certain to investigate the best manufacturing techniques

22 for fabricating the desired scaffolds (38-40).

23

3. Conventional manufacturing techniques

26 Conventional techniques used to prepare bone scaffolds are based on subtractive procedures to

27 get the desired shape by removing sections of the material from an original block. The inability

28 to manage complex shapes and geometries, as well as to incorporate interior architecture,

29 cavities or curved channels, is a major disadvantage of these techniques (41). This is of special

30 importance in the biomedical industry, where complex and organic shapes are usually needed

31 for the implants to fit well. Additionally, cell viability and function can be affected by the use

32 of organic solvents, even if only residues remain (42). In order to obtain those geometries, until

33 now, several conventional manufacturing methods, such as salt leaching, gas formation, phase

34 separation, freeze-drying, electrospinning, and self-assembly, have been employed in the

5

1 fabrication of porous bone scaffolds despite their limitations (Figure 4). The principles of each

2 procedure are covered in the following sections. The prevalence of research on each technique

3 is summarised in (Figure 5). It is evident that more phase separation technique has attracted the

4 attention of researchers over the last 10 years.

5

3.1. Salt leaching

8 This process was widely used in the manufacture of tissue-based scaffolds. In this technique,

9 salt crystals or porogen (e.g. sodium chloride) are put in a mould, and the remaining gaps are

10 filled with a polymer. The polymer is then solidified, and salt crystals are dissolved in a suitable

11 solvent like alcohol or water by dissolution (43-45). After all the salt leaches out, a solidified

12 polymer with porosity is created, as illustrated in (Figure 4a) (46).

13 β-chitin and collagen have been successfully used to prepare using salt leaching technique. The

14 prepared membranes achieved a porosity of 77.81% and an average pore size of 260-330 μm.

15 β-chitin membranes were prepared with NaCl salt-leaching, and then collagen solution crossed

16 membranes by lyophilisation at −75 ° C (Figure 6a-b) shows the scaffold's cross-sectional and

17 surface morphologies. In vitro cell culture demonstrated that the human fibroblasts attached to

18 the collagen sites after 3 days, and proliferation took place within 14 days of cultivation (47).

19 The salt leaching technique enables the customisation of the pore size by adjusting the porogen

20 size employed. It is also possible to control the porosity and pore size of the scaffold by

21 manipulating the volume and size of the salt particles used, respectively (43, 48). Despite the

22 mentioned benefits of salt leaching for scaffold fabrication, this process has some limitations.

23 For instance, it is not possible to control the pore distribution or the shape of scaffolds created

24 (43). Additionally, this technique requires scaffolding to be manufactured only in the form of

25 tubes and flat sheets, which means that it is ideal for the manufacture of membrane scaffolds.

26 Besides, the use of organic solvents can negatively impact the viability of cells and their

27 biological functions (49). Although the residues of any cytotoxic solvents could be detected

28 (50), they pose limitations for general applications of salt leaching scaffolds.

29

3.2. Gas foaming

32 Gas foaming is a manufacturing approach in which a polymeric material is filled with a foaming

33 agent such as carbon dioxide, water or nitrogen at high pressure (51-53). Solid polymer disks

1   like polyglycolide (PGA) and poly-L-lactide (PLA) are created at high temperatures before

2   spreading high-pressure carbon dioxide gas through the disks for a few days before decreasing

3   the pressure down to the ambient level (Figure 4b) (54).

4   Gas foaming was used by Kim et al. (55) to fabricate porous biphasic calcium phosphate (BCP)

5   scaffold by using gas-foamed polyurethane as a model achieving a porosity of 75% to 85% and

6   pore size around 300-800 μm. The BCP scaffold was biocompatible and successfully

7   differentiated and regenerated bone according to both the in vivo and in vitro experiments

8   conducted in this study (55). In another study, a biodegradable poly (L-lactic acid) (PLLA)

9   scaffold with high open porosity was fabricated using a gas-foaming technique along with salt

10   leaching (56). The scaffold had a porosity of around 90% with pore sizes around 300-400 μm,

11   which is ideal for high-density seeding of cells (Figure 7). Upon seeding rat hepatocytes into

12   the scaffold, 40% viability and around 95% seeding efficiency were achieved within 24 hours

13   (56).

14

15   The key advantage of using the gas foaming technique is that it does not require the use of

16   chemical solvents, thus reducing the overall manufacturing time. Nonetheless, it is challenging

17   to control the internal structure of the scaffolds in terms of pore size and high connectivity using

18   this technique (57, 58). In addition, high temperatures during the creation of disks often prevent

19   the use of bioactive molecules in scaffolds (59). Although this technique has the ability to

20   fabricate scaffolds with 93 % porosity and pore sizes up to 100 μm (59), it has been noted that

21   the scaffold interconnects only 10–30 % of the pores which may limit the proliferation of

22   encapsulated scaffold cells (54).

23

24   **3.3. Phase separation**

25

26   In phase separation, a polymer is generally dissolved in an appropriate solution and then

27   deposited in a mould that is gradually cooled till the solution freezes. The solvent is then

28   removed by freezing, leaving behind a porous matrix, as illustrated in (Figure 4c). Various types

29   of phase separation methods are available, including thermal-induced, solid-liquid and liquid-

30   liquid phase separation (60, 61).

31   The study by Kim et al. employed thermally induced phase separation (TIPS) for the

32   manufacture of polyethene-glycol (PEG) poly(l-lactic) acid (PLLA) scaffold to support

33   MC3T3-E1 osteoblast-like cells (62). It was shown in this study that ageing times and

34   temperature have a significant effect on the pores morphology of the fabricated scaffold,

1   quenching temperatures of 25°C, 30°C, and 35°C (<mark>Figure 8a-c</mark>). The TIPS technique allowed a

2   simple control of the scaffold pore size between 100 – 300 μm. Authors noted that MC3T3-E1

3   cells could proliferate successfully within 4 weeks after being seeded on the microporous

4   scaffold of PEG-PLLA (62).

5

6

7   The main merit of using the phase separation technique is that it does not require extra leaching.

8   However, the use of organic solutions like ethanol or methanol during the scaffold

9   manufacturing process can prevent the integration of bioactive compounds or cells.

10   Furthermore, the small pore diameters generated are another constraint for phase-separation

11   scaffolds. (43, 59).

12

3.4. Freeze-drying

14

15   Freeze-drying technique is based on a frozen liquid that sublime directly into the gas phase

16   leaving behind a porous hydrogel (<mark>Figure 4d</mark>) (63). The manufacturing approach was first

17   explored by Whang et al. to produce PLGA scaffolds (64). The literature demonstrates that

18   fabricated scaffolds' porosity and pore diameter are highly influenced by variables like the

19   water-to-polymer mixture ratio and the viscosity of the emulsion (59). Also, altering the cooling

20   temperature can control the scaffold's internal pore structure (65).

21   In a study conducted by Park et al. (66), freeze-drying was used to fabricate collagen and

22   hyaluronic acid (HA) membranes and then crosslinked using 1-ethyl-3-(3-

23   dimethylaminopropyl) carbodiimide (EDC). Porosity and pores' size were measured to assess

24   the effect of freezing temperature and crosslinking on the internal structure of the scaffolds,

25   freezes dried temperature used were at –20 ° C, −70 ° C, and −196 ° C (<mark>Figure 9a-c</mark>), (Table 2).

26   The higher the freezing temperatures, the larger the pore size and the porosity percentage. Also,

27   the use of EDC has significantly increased both the porosity and pore size. The prepared

28   membranes were safe and did not exhibit significant toxicity to L929 fibroblast cells upon

29   testing (66).

30

31   The main benefit of the freeze-drying technique is that it eliminates many rinsing processes by

32   immediately removing scattered water and polymer solutions (64). In addition, polymer liquids

33   can be utilised directly instead of any monomer crosslinking. Nevertheless, in order to increase

1 scaffold homogeneity, the freeze-drying method must be managed to minimise heterogeneous

2 freezing (65). Moreover, this approach is associated with high energy consumption, long

3 timescales, small irregular pores, and cytotoxic solvents (67, 68).

4 **3.5. Electrospinning**

5

6 Electrospinning is an innovative electrochemical technology that utilises an electrical charge to

7 create solid, nano-sized fibres from a liquid solution (69). As illustrated in (Figure 4e), the

8 electrospinning process begins with a syringe filled with a solution containing a precursor for

9 the nanofiber material and a connecting polymer being loaded onto a regulated syringe pump.

10 A metallic needle is attached to the syringe and is connected to a high-voltage power source

11 (70). As the solution flows through the metallic tip, it becomes electrified, generating a

12 deformed conical shape known as a Taylor cone. The Taylor cone's tip releases an electrified

13 fibre jet. As the solution travels to a grounded collector, the solvent evaporates, and the fibres

14 harden (70).

15 Wutticharoenmongkol et al. used electrospinning to create a 12% w/v PCL fibrous scaffold

16 with HA nanoparticle concentrations ranging between 0.5 and 1.0%. The porosity of the

17 constructed fibre scaffold increased by 82 and 90 %, and had pore sizes ranging from 4.3 to 5.6

18 μm. The prepared fibrous scaffolds had a tensile strength between 3.6 and 3.8 MPa (71).

19 Another study by He et al. reported the fabrication of a PCL/HA scaffold with different ratios

20 of PCL/0.3 HA, PCL/0.4 HA, and PCL/0.5 HA (Figure 10a-c) and an average pore size of 167

21 μm which is suitable for osteoblasts, by stacking meshes using near-field electrospinning (72).

22

23 Electrospinning has the advantage of being able to manipulate both the mechanical properties

24 and the porous structure of the fibre by regulating the voltages and distance between the syringe

25 and the collectors (73, 74). Pores generated by this approach, however, are often fewer than a

26 few tens of micrometres in size, making them unsuitable for tissue growth and cell culturing

27 (72). Also, fabricating complex geometry can be challenging using this technique.

28 **3.6. Self-assembly**

29

30 Self-assembly is the process by which the components of a system, whether molecules,

31 polymers, colloids, or macroscopic particles, arrange into ordered and/or functional structures

32 or patterns without external direction as a result of specific, local interactions among the

33 components (75). Collagen should be examined to better understand the origins and

9

significance of these structural features, as it is one of the most common proteins in the human body. Collagen is formed within the cell as a triple helix structure by the assembly of three distinct alpha strands (procollagen) (Figure 4f) (76). Procollagen is enzymatically broken to generate tropocollagen, which combines and crosslinks with other tropocollagen molecules to create the characteristic 67 nm banded fibrils after vesicle transit to the exterior of the cell (76). This fibrillar structure is retained in collagen types I (skin, tendon, and bone), II (cartilage), and III (skin, muscle) (76).

A recent study conducted by [88] demonstrated the self-assembly of a 3D porous Reduced graphene oxide (RGO) composite scaffold that is composed of graphene oxide (GO) and nano-hydroxyapatite (nHA) with pore sizes ranging from 20–100 μm (Figure 11) (77). The scaffold significantly improved the proliferation, alkaline phosphatase activity (ALP), and osteogenic gene expression of rat bone mesenchymal stem cells (rBMSCs) (77). Another study used a combination of self-assembly and electrospinning techniques to create a hybrid scaffold with a honeycomb using Polyhydroxybutyrate/poly(-caprolactone)/58S sol-gel bioactive glass (PHB/PCL/58S) (78). The scaffold was created by changing the solution composition and concentration during a single electrospinning process (78). The nanofiber contained pores as small as a few micrometres in diameter, while the structure had pores ranging from 200 μm to 1000 μm. This facilitated the cell ingrowth and infiltration of MG-63 osteoblast-like cells into the honeycomb-like scaffold (78).

The self-assembly mechanisms are frequently triggered by the mixing of two elements or by an external stimulus (pH, ionic strength, or temperature), allowing these materials to be injected or even used directly to encapsulate cells, compared to the complex processing needed for other conventional manufacturing methods to fabricate a scaffold (76). In comparison to other manufacturing processes, the mechanisms governing the development of self-assembled nanofibers are generally more complex, requiring more careful molecular design and more intricate synthesis. (Table 3) summarises the main applications, advantages and drawbacks of the manufacturing techniques presented in this section.

From the literature review presented above, it can be concluded that conventional manufacturing methods such as gas formation, salt leaching, freeze-drying, and phase separation do not allow for accurate regulation of the internal scaffolding design or the manufacture of complicated structures, which can be accomplished through AM modelled with

computer-aided design (CAD) (79). Besides, these conventional methods require good manufacturing skills to maintain a consistent architecture of scaffolds. In addition, special care must be taken to use toxic solvents that can lead to the death of cells if they are not removed completely (80). Another limitation is that scaffolds manufactured in accordance with these conventional processing methods have poor mechanical properties (81). Therefore, alternative techniques such as 3D printing offer a good opportunity to avoid these issues.

4. Three Dimensional Printers

Industry 4.0, commonly known as digital technology, is revolutionising industries by making factories smarter and assisting manufacturers in increasing quality, productivity, and profitability. 3D printing is a manufacturing tool that has advanced over the last three decades and is an essential component of digital technology. Charles Hull invented the technology in 1986, employing UV-sensitive polymers and ultraviolet light (UV) to generate three-dimensional structures (82). Stereolithography Apparatus (SLA) was the name given to the technology later on. Scientists and engineers have since developed a variety of unique 3D printing techniques. The main advantage of these new technologies is that they enable the fabrication of complex organic shapes and internal features and cavities in components that were difficult or even impossible to fabricate with conventional techniques (83). Additional benefits of 3D printing can include; reduced lead time, elimination of extra processing required for mass customisation, develop supply chain expertise, printing systems and assemblies, fabricating complicated designs in functional components, lightweight production of cellular structures, material recycling and environmentally friendly production, scalable workflow, on-demand production and enhanced service quality (83, 84). (Figure 12) summarises the most employed 3D printing techniques. Binder Jetting, Fused Deposition Modelling, Selective Laser Sintering and Stereolithography are the most employed for the manufacturing of scaffolds and, therefore, will be discussed in detail in the following sections.

In regards to the bio-medical applications, the most significant benefit of 3D printing technologies is allowing the fabrication of completely customised components. In 3D printing, different materials such as polymers, metals, or ceramics can be created layer by layer to produce the desired shape according to a computerised model, in contrast to typical manufacturing or foaming procedures that demand removing and/or adding, such as cutting, bending, and drilling (85). 3D printing technologies have been used in many industries, such as biomedical, automotive, aerospace, defence, and many others. This is due to the capacity of AM technologies to rapidly build complicated structures with precision and accuracy, as well

as the ability to recycle materials. Numerous researchers and industrial organisations have focused their efforts on enabling the widespread implementation of 3D printing and investigating its potential and limitations. (86-89). Therefore, 3D printing can play a major role in the future of tissue engineering in general and bone scaffolds in particular. This is evident as the number of studies employing 3D printing technology for bone scaffolds has increased over the last decade (Figure 13).

4.1.   Binder jetting

Binder jetting starts with a powder bed, the composition of which varies according to the materials employed, which is dispersed over the building platform and flattened with the aid of a roller system (90). Following that, the printer nozzle spreads binder solution in the powdery regions indicated by the CAD. The excess powder is extracted (blown off) after the binder solvent and powder are mixed. The building platform is then lowered, allowing for the deposit and levelling of a new powder sheet (91). Following that, the process will be repeated till the required design is fully fabricated (Figure 12a). After the layer deposition, the part generated that is known as the green part usually has high porosity. In order to reduce the number of pores and to improve its integrity, the component is subjected to cleaning and post-processing operations: depowdering, debinding and finally, a sintering process in a furnace with densifying and strengthening purposes.

In a study conducted by Zeltinger et al., chitosan and hydroxyapatite biocomposite scaffolds were printed using a Z-Corp, Z-510 3D printer to create dense (solid, nonporous, 37.1% porosity) and cylindrical scaffolds (92). These scaffolds were fabricated by applying a 40 wt% lactic acid binder solution to various chitosan/hydroxyapatite composites (20 wt%, 25 wt%, and 30 wt% chitosan) followed by a post-hardening process. The authors observed that the scaffolds printed with 25% chitosan had good mechanical properties, as evidenced by their compression strength of 16.32 MPa and 4.4 GPa Young's modulus (92). Nevertheless, only the fabrication of nonporous scaffolds has achieved the desired mechanical strength. In another study, CALPHAD (Ca) and biodegradable Fe-Mn alloy were used to achieve higher decomposition rates (Figure 14a-b) (93). The achieved ultimate tensile strength was 228.1 MPa for the Fe-Mn and 296.6 MPa for the Fe-Mn-1Ca (93). During tensile testing, a brittle fracture occurred in a porous Fe-Mn-1Ca scaffold with 52.9 % open porosity. Fe-Mn scaffolds with an open porosity of 39.3 % had higher ductility than Fe-Mn-1Ca, demonstrating that scaffold Fe-based alloys with less porosity have higher ductility (Figure 14c-d) (93). This is a concern since porosity is a crucial feature as it promotes the diffusion of oxygen, nutrients, and cellular waste. The

availability and diversity of the powder-binder solutions make the binder jetting attractive for manufacturing bone scaffold (90). On the other hand, a drawback of this technique is that it needs post-processing, which may include heat treatment to assure durability (94).

4.2. Materials jetting

Materials jetting Printing (Bioplotter) is one of the most used 3D printing technologies for cellular research due to the low temperature it requires and the low volume it uses (between 3 and 5 mL) (95). This technology is designed for high-precision printing of small objects using small nozzles with a minimum diameter of 250 μm and low volume (96). The process starts by loading the printing material in a semi-liquid or liquid form into the syringe. Then, pneumatic pressure is applied to extrude the material through the printing nozzle (Figure 12b). The materials are deposited in a layer-by-layer manner, and the process enables the combination of different materials in each layer.

In the study by Poldervaart et al. VEGF was incorporated into a 3D printed matrigel-alginate scaffold to promote vascularisation using BioScaffolder pneumatic dispensing system (97). The incorporation of gelatin microparticles (GMPs) to sustainably regulate the release of VEGF led to higher vascularisation compared to scaffolds with no growth factors and rapidly released VEGF scaffolds when implemented in murine models (Figure 15). In another recent study, a biphasic scaffold model was fabricated with the BioScaffolder by combining the unmodified calcium phosphate cement (CPC) paste with a highly concentrated alginate-based hydrogel paste that was embedded with VEGF by two-channel plotting within a single scaffold (98). The scaffold was designed and manufactured to be used for evaluating a femur defect of size in the range of 200 μm with a macro porosity of 57%. The scaffolds' size and high porosity made them suitable for enhancing bone regeneration (98).

A unique feature of materials jetting processes is that it allows the printing of cell-laden gels to deliver viable and usable scaffolds, often including other polymeric materials like PCL (99, 100). Another advantage of materials jetting is that it enables the growth factors such as platelet-derived growth factor (PDGF) or vascular endothelial growth factor (VEGF) to be added to the bio-ink to improve cell proliferation and differentiation which promotes angiogenesis (101). Adding these growth factors will increase the tissue formation rate in scaffolds and generate robust tissue as a result of increased differentiation. On the other hand, the shear stress from the nozzles of various sizes can negatively impact cell viability (102).

4.3.   Materials extrusion

In materials extrusion, thermoresponsive polymers are heated above their glass transition temperature and then placed on a solid surface. It uses a winding thermoplastic polymer filament that is unwound and extruded through a heated nozzle on a fabrication platform. The polymer solidifies and sets after contact with the platform (103). Upon depositing a layer, the process is repeated in a layer-by-layer process until the part is fully fabricated see (Figure 12c) (104).

Hong et al. employed a multi-head deposition method to combine PCL and PLGA to fabricate a multi-material scaffold with high compressive strength of 3.2 MPa and pore size of around 300 μm with 66.7% porosity (Figure 16). In combination with mussel adhesive proteins as a functional material, the fabricated scaffolds facilitated high cell attachment and proliferation of stem cells derived from human adipose tissue (105). It also yielded positive outcomes in vivo tests, where increased bone regeneration was observed in a calvarial defect of a rat model (105). Overall, FDM was mainly used in combination with other techniques or in indirect 3D printing for tissue engineering purposes.

The key disadvantages of FDM are that it enables multi-material and multi-colour fabrication processes within one component, and the accuracy can go down to ±0.5 mm (106). However, it prevents any possible toxicity caused mainly by organic solvents that are required for the solubilisation of certain polymers, like dichloromethane, used to solubilise PLGA. The thermoplastic criterion for this technique restricts its application and adaptability in the production of scaffolds, as acrylonitrile butadiene styrene (ABS) is the most often utilised material. Other polymers have been used in FDM, like polycarbonate (PC), polyphenylsulfone (PPSF), and polyetherimide (PEI). However, these materials are not mainly used in tissue engineering applications. (107). Further investigation is needed to determine whether alternative thermoplastics, like polyesters, are suitable scaffolding materials for tissue engineering. Despite this drawback, FDM has been demonstrated to be a viable approach for manufacturing scaffolds for tissue engineering. Polyester, PLA, PCL, as well as PCL and PLA composites like PCL-TCP, HA - PCL and HA - PLA are the main option for FDM printed scaffolds (108-111).

4.4. Powder bed fusion

The laser powder bed fusion (L-PBF) technology begins with the powder layer being smeared on the surface of the base plate, followed by melting powdered particles together using a laser beam (normally a CO_2 laser) in the desired pattern (112). The process is repeated after the first layer is deposited, and then another layer is added on top of the pre-existing one (Figure 12d) (113, 114). The L-PBF technique was used to make scaffolds from biocompatible and biodegradable polymers like poly(lactic acid), polyvinyl alcohol, polycaprolactone, and polyetheretherketone (115). With the development of metal 3D printing, this technology is also employed for the fabrication of metallic scaffolds that can be created out of biocompatible metal alloys such as Ti6Al4V for the fabrication of implants (116).

The use of L-PBF for the manufacturing of scaffolds has been studied by many researchers in the literature. Liu et al. utilised hydroxyapatite (HA), sodium tripolyphosphate and silica sol biocomposite slurry to manufacture scaffolds using L-PBF with different heat treatment temperatures at ambient temperature, 1200 °C, 1300 °C, and 1400 °C (Figure 17a-d). These scaffolds showed significant mechanical strength (up to 43.26 MPa) but had low porosity with a pores size of 5-25 μm. The in vitro research, however, suggested the possibility of using these scaffolds for osteoblast growth, such as cells (117). In another study by I. Gibson, the authors optimised the laser beam power, scan spacing and laser thickness to fabricate a nanocomposite scaffold made of poly(hydroxybutyrate-co-hydroxyvalerate) and calcium phosphate (118). The analysed parameters were found to have a substantial effect on the mechanical properties of the scaffold; compressive properties, precision, and durability (119). Nevertheless, the scaffold's efficiency and utility must be evaluated in vitro and in vivo. Other scholars have fabricated a scaffold using bioresorbable polycaprolactone (PCL) at high precision and high compression moduli ranging from 52-67 MPa. The scaffold was loaded with bone morphogenetic protein-7 (BMP7), and has demonstrated bone generation in vivo (120).

When low porosity and high mechanical strength are needed, the use of L-PBF processes can be beneficial; nonetheless, the need for powdered material to be able to withstand laser heat and resistant shrinking throughout the melting process are some limitations of this technique. Another drawback of L-PBF is the pre-heating and post-heating treatments of the powdered material among the crystallisation glass transition or melting temperatures to lower the shrinking of the scaffolds induced by the laser (117, 121).

4.5. Vat photopolymerization

Vat photopolymerisation or stereolithography (SLA) technique is based on the fabrication of components from a liquid polymer via a chemical reaction mediated by light. A photocurable polymer is placed on a surface medium and then subjected to UV radiation in the 300–400 nm wavelength range, forming the first layer (Figure 12e) (122). After the initial layer has been hardened, the process is repeated, overlaying the preceding layer until the part is completely fabricated (123, 124). SLA biomaterials include polypropylene fumarate (PPF) with photocross linkers and polyethylene glycol acrylate.

A study conducted by Cooke et al. used the (SLA 250/40) stereolithography for Printing (PPF) scaffolds together with Irgacure 819 photoinitiator. The manufactured scaffolds had a porosity of 90% and a pore size range of 150–800 μm (125, 126). Their study demonstrated the possibility of fabricating scaffolds using (PPF) material (Figure 18). However, in vitro and vivo studies must be carried out to determine the scaffold cytotoxicity and biocompatibility. Despite the magnificent results that SLA can achieve in terms of complex geometries fabrication, Various novel biodegradable and biocompatible photocurable polymers must be developed. In addition, designing and improving visible light-based STA systems is important in order to have a list of polymeric materials (125).

The advantage of the SLA technology is that it allows for precise control and fabrication of high-resolution detailed scaffold geometries that almost perfectly mimic the CAD model. Nonetheless, due to the use of an extra curing phase to enhance the properties of the prototype, the final resolution is affected by the shrinkage usually occurring in the post-processing phase (127, 128). However, the drawback of SLA techniques is that it uses only photopolymers that use photoinitiators (129). In addition, the majority of photoinitiators include radical photopolymerisation by photocleavage, hydrogen extraction, and cationic photopolymerisation, with cationic photoinitiators being incompatible with biomedical applications because of the formation of toxic byproducts. Also, the widely used ultraviolet light source for the polymerisation process poses another risk as reports indicate that this light source is harmful to our DNA cells and might be a potential cause of skin cancer (130, 131). (Table 4) summarises the various 3D printing techniques used in preparing bone scaffolds.

4.6 Directed energy deposition and Sheet lamination

The directed energy deposition (DED) technology fabricates the required object by melting materials using a laser beam while using a nozzle to deposit the material in specific locations, as demonstrated in (Figure 12f) (132). It mostly uses metal types of materials, such as stainless steel, aluminum, or copper, in the form of powder or wire (133). This technique usually require post-processing due to distortions in the fabricated part (134). Due to the limited types of materials that can be processed and the poor quality of the fabrication, this technique has not been utilized much in biomedical applications

In sheet lamination (SL), a sheet material is laminated in a layer-by-layer manner and cut using a laser beam to fabricate the required object as demonstrated in (Figure 12g). It uses different types of materials such as paper, metal, and plastic (135). Similarly to directed energy deposition, sheet lamination has not been widely used in biomedical applications due to its poor fabrication quality, the need for post-processing, and the difficulty in fabricating complex shapes using this technique (136).

In general, both manufacturing technologies have different advantages and disadvantages, primarily depending on the application of the fabricated object. For example, when complex geometries and designs are required, additive manufacturing technology has proven to be a better option than conventional manufacturing as it allows for the creation of internal structures (137). In contrast, conventional manufacturing is more precise, can handle a wider variety of materials, and is better suited for large-scale production than additive manufacturing (138). Additive manufacturing is a relatively new technology that is still being developed and has limitations regarding the types of materials it can process and the size of the components it can produce (138). In fact, researchers have combined conventional and additive manufacturing technologies into a single machine for bone scaffold fabrication, effectively combining their respective advantages.

The research conducted by Jiankang He and his team, a new printing method was developed that combines FDM and electrospinning technologies to create 3D tissue-engineered scaffolds with intricate curved shapes and microscale fibrous structures (Figure 19a). The melting temperature was optimized to print PCL filaments of around 10 μm, which were stacked to create 3D walls with smooth surface (139). By adjusting the stage movement speed and direction, they were able to print PCL scaffolds with curved outlines, predefined fiber spacing, and orientations at 90° and 45° (Figure 19b-g). Biological experiments demonstrated that the printed microscale scaffolds were biocompatible and promoted in vitro cellular proliferation and alignment (139). In another research conducted by H. Hassanin et al., they successfully produced micro implantable components with the highest density and best surface quality possible by utilizing a hybrid microfabrication technology that incorporates the design flexibility of SLM and the exceptional surface quality of μ-EDM (140). Another group of researchers has developed a new approach to create three-dimensional graphene (3DG) composites scaffold by combining selective laser melting (SLM) and chemical vapor deposition (CVD) techniques (141). They fabricated a 3D porous copper template using SLM and grew graphene in-situ via CVD on the template. This technique allowed for accurate control of the design and regulation of 3DG, resulting in enhanced electromagnetic interference (EMI) shielding and improved thermal diffusion (141).

5. Conclusions

This paper presents a literature review of the most relevant works and recent advances concerning manufacturing bone scaffolds. Conventional manufacturing techniques have been

1   reviewed, and their main benefits and shortcomings have been addressed. Additionally, 3D
2   printing technologies that have emerged in the last years have proved to be a feasible alternative.
3   In this context, the review demonstrated that 3D printing technologies enable the customisation
4   of bone scaffolds to meet individual patients' unique needs and health situations. Progress in
5   this area is facilitated by advancements in computer-aided design (CAD) and computer-aided
6   manufacturing (CAM), which enable rapid and precise organ scanning and design. The
7   scaffold's structural properties, such as pore size and porosity, have a direct influence on their
8   functionality in both vitro and vivo. In general, interconnected porous scaffolding networks that
9   allow nutrient transport and waste disposal and promote cell migration and proliferation are
10   significant. Pore size and porosity affect the behaviour of the cells and determine the overall
11   mechanical properties of the scaffold. Presently, the concept of fabricating scaffolds is
12   concentrated on generating materials with suitable pore size, structure, and porosity for specific
13   uses. Typically, scaffolds are 3D printed, and cells are grown in/on these scaffolds. One of the
14   challenges of 3D printing is using non-biocompatible materials in several 3D printing
15   techniques, such as binders or photoinhibitors, even after the high-temperature debinding or
16   sintering process. These components cannot remove entirely after heating or sintering processes
17   and may compromise the biocompatibility of the constructs. Also, applying the temperature in
18   some of the technologies restricts the applicability of materials. Incompatibility of the cellular
19   application with the scaffold would gradually cause the entire scaffolding system to fail.

20   In addition, 3D printing technology has altered the way bone fractures and has enabled the
21   utilisation of drug-loaded implants and/or scaffolds of complicated geometries and high
22   resolution to accelerate the healing procedure and recover bone structure and toughness. Bone
23   scaffolds have been extensively manufactured using techniques like FDM and binder jetting.
24   FDM has been demonstrated to be capable of processing a wide variety of scaffolds with
25   complicated structures and a variety of polymeric materials. On the other hand, techniques like
26   directed energy deposition and sheet lamination (Figure 12f-g) were not explored in this field
27   due to their processing characteristics or the quality of their products or materials. Clinical trials
28   conducted by academia or commerce on the developed systems demonstrate significant
29   potential. However, challenges such as materials recycling, quality control, and the effect of
30   inherited issues of 3D printing such as surface roughness, internal defects, and post-processing
31   are still lacking.

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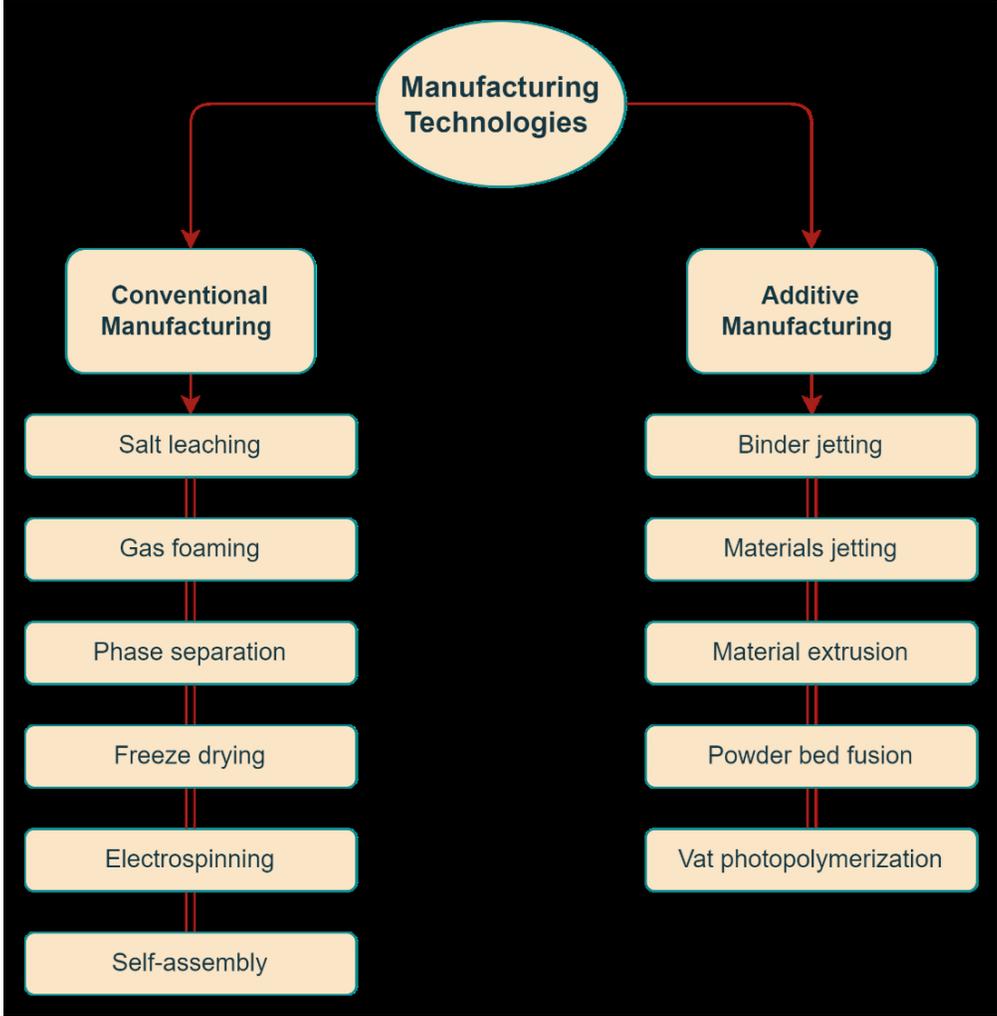
Figure 1. Flowchart of manufacturing technologies.

127x129mm (220 x 220 DPI)
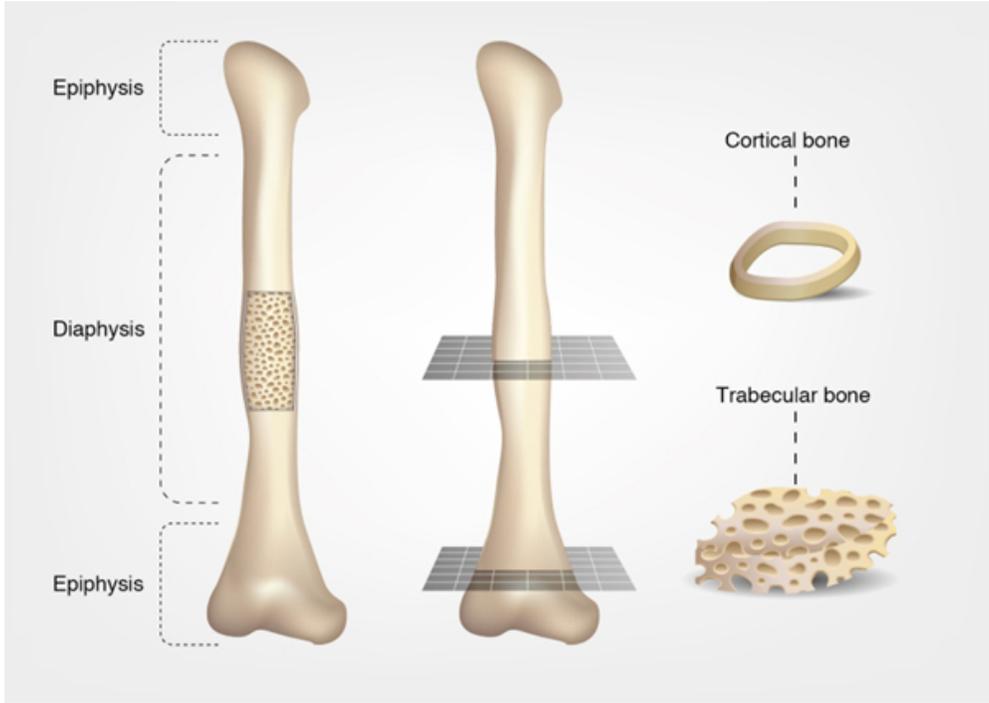
Figure 2. A long bone's macroscopic structure.

160x113mm (96 x 96 DPI)

Figure 3. A comparison between the stress-strain properties of trabecular and cortical bones. Adapted from Damien Lacroix (21).

140x92mm (113 x 113 DPI)
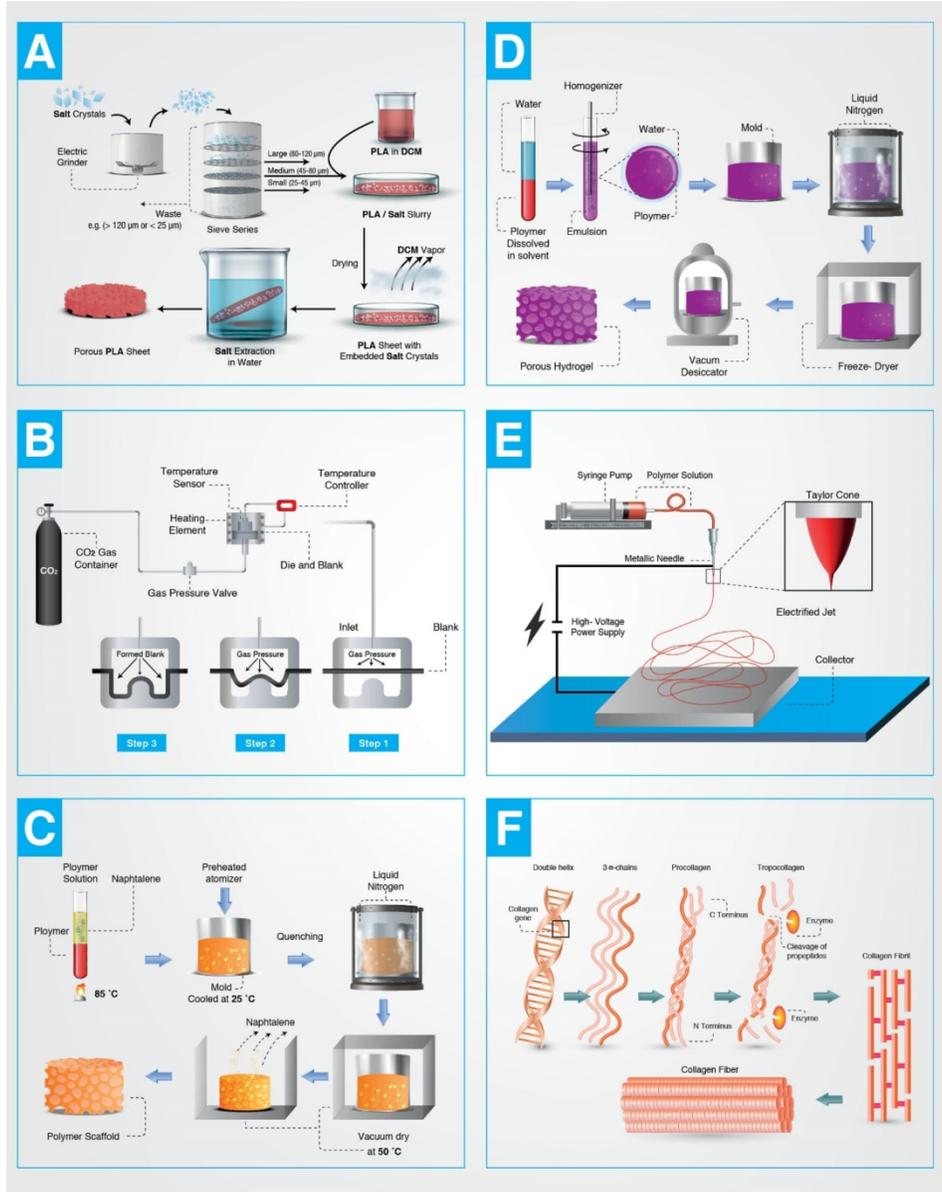
Figure 4. Conventional manufacturing techniques of bone scaffolds, (a) Salt leaching, (b) Gas forming, (c) Phase separation, (d) Freeze-drying, (e) Electrospinning and (f) Self-assembly.

169x213mm (220 x 220 DPI)

Figure 5. Number of papers published on bone scaffold fabricated by each conventional manufacturing technique over the last 10 years.

150x97mm (330 x 330 DPI)
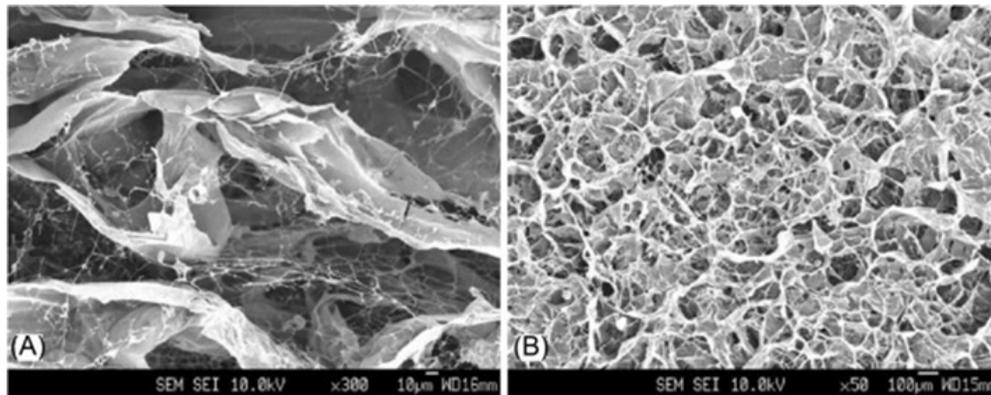
Figure 6. Collagen-coated chitin scaffold morphologies: (a) cross-section and (b) surface. Adopted from Sang Bong Lee (47).

160x63mm (113 x 113 DPI)

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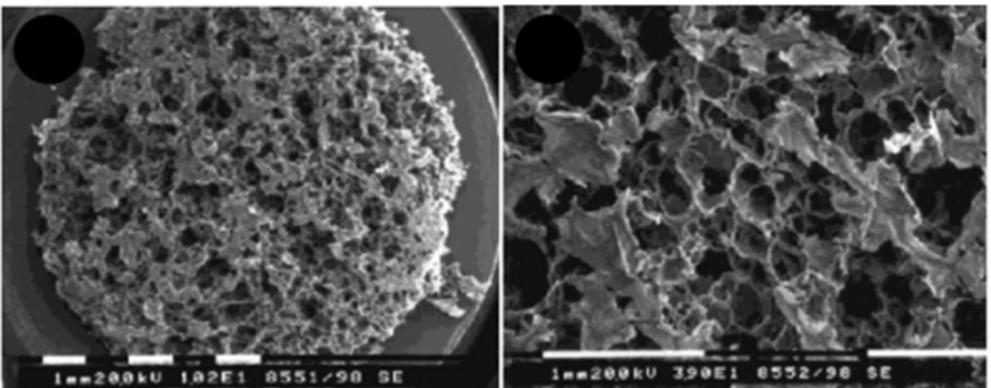
Figure 7. SEM images of surface morphology of PLLA scaffolds. Adopted from Nam (56).

159x64mm (96 x 96 DPI)
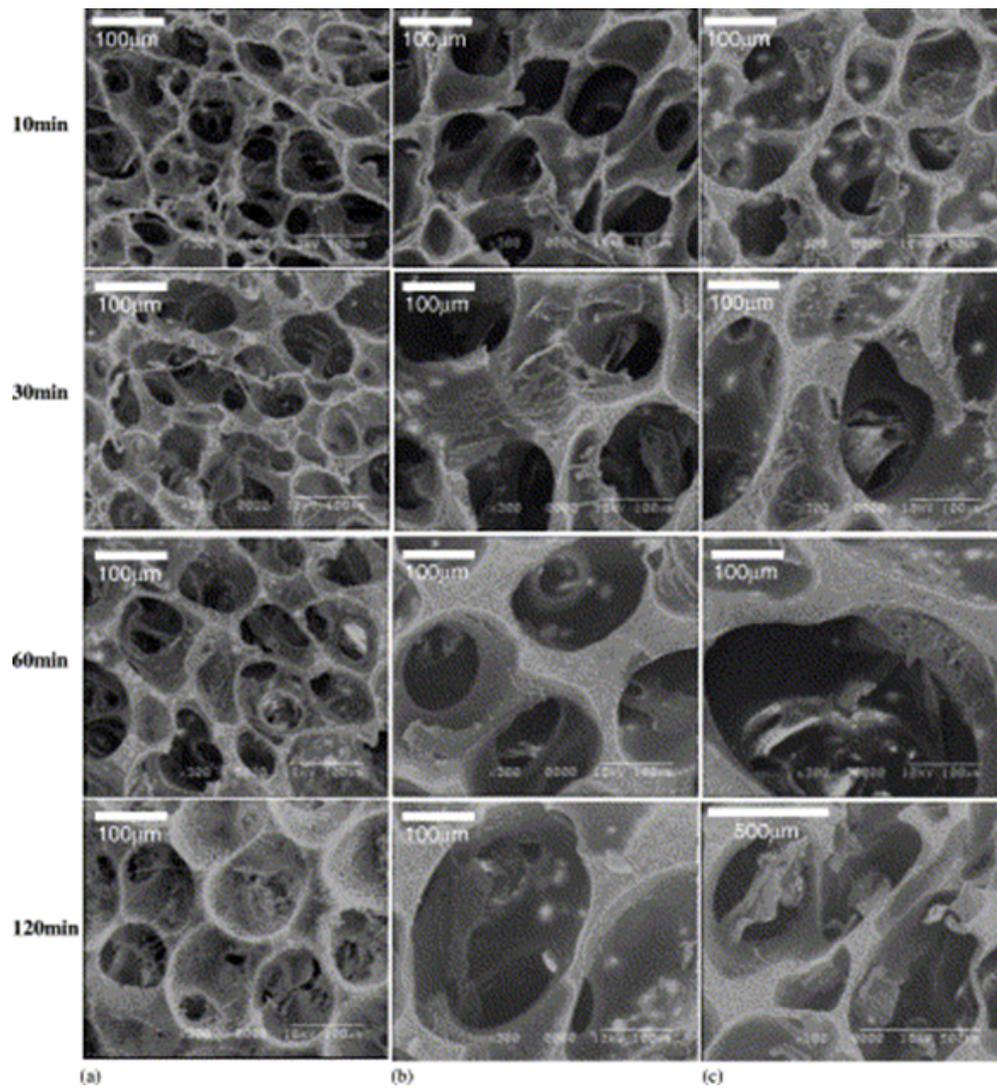
Figure 8. scanning electron micrograph of PLLA membranes as a function of aging time at quenching temperatures of 25°C (A), 30°C (B), and 35°C (C). Adopted from H Do Kim (62).

155x167mm (96 x 96 DPI)

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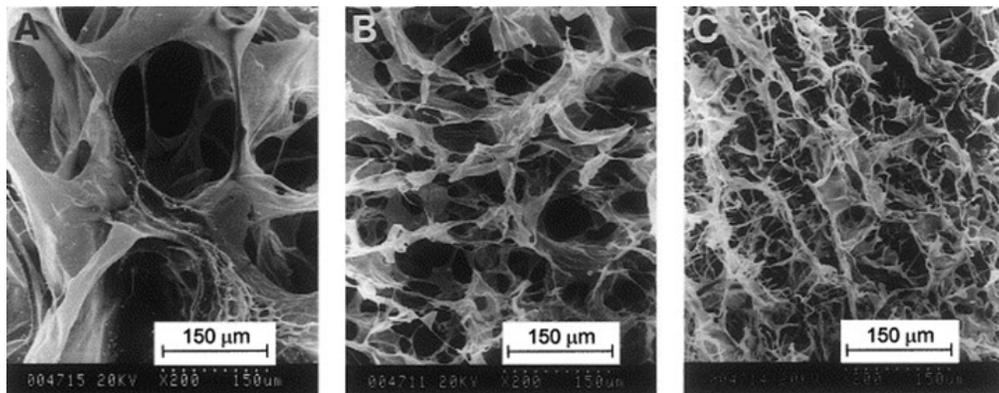
Figure 9. Effect of freezing temperature on morphology of the matrix. Collagen-hyaluronic acid scanning electron micrograph freezes dried at −20 ° C (A), −70 ° C (B) and −196 ° C (C) (magnification ×200). Adopted from SN Park (66).

161x62mm (113 x 113 DPI)
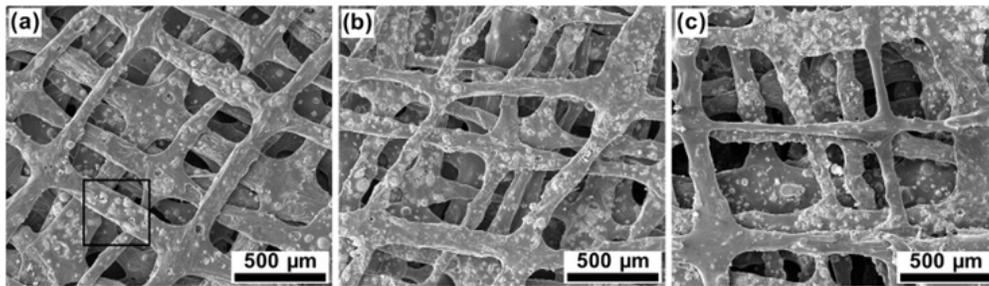
Figure 10. Morphological characterisation of the PCL/HA composite scaffolds. SEM image of (a) PCL/0.3 HA scaffold, (b) PCL/0.4 HA scaffold and (c) PCL/0.5 HA scaffold. Adopted from Feng-LiHe (72).

166x48mm (113 x 113 DPI)
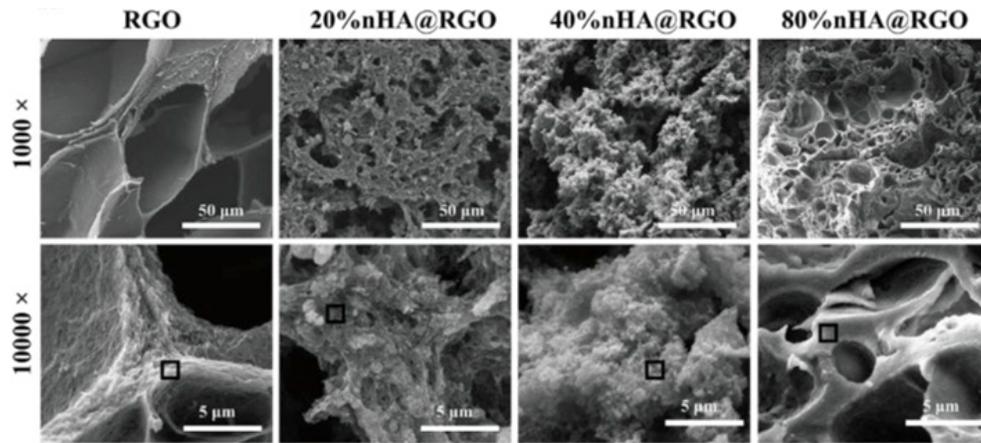
Figure 11. SEM of the nHA@RGO scaffold with the different nHA loading ratios. Reduced graphene oxide (RGO) and nano-hydroxyapatite (nHA). Adopted from WeiNie (77).

162x71mm (113 x 113 DPI)
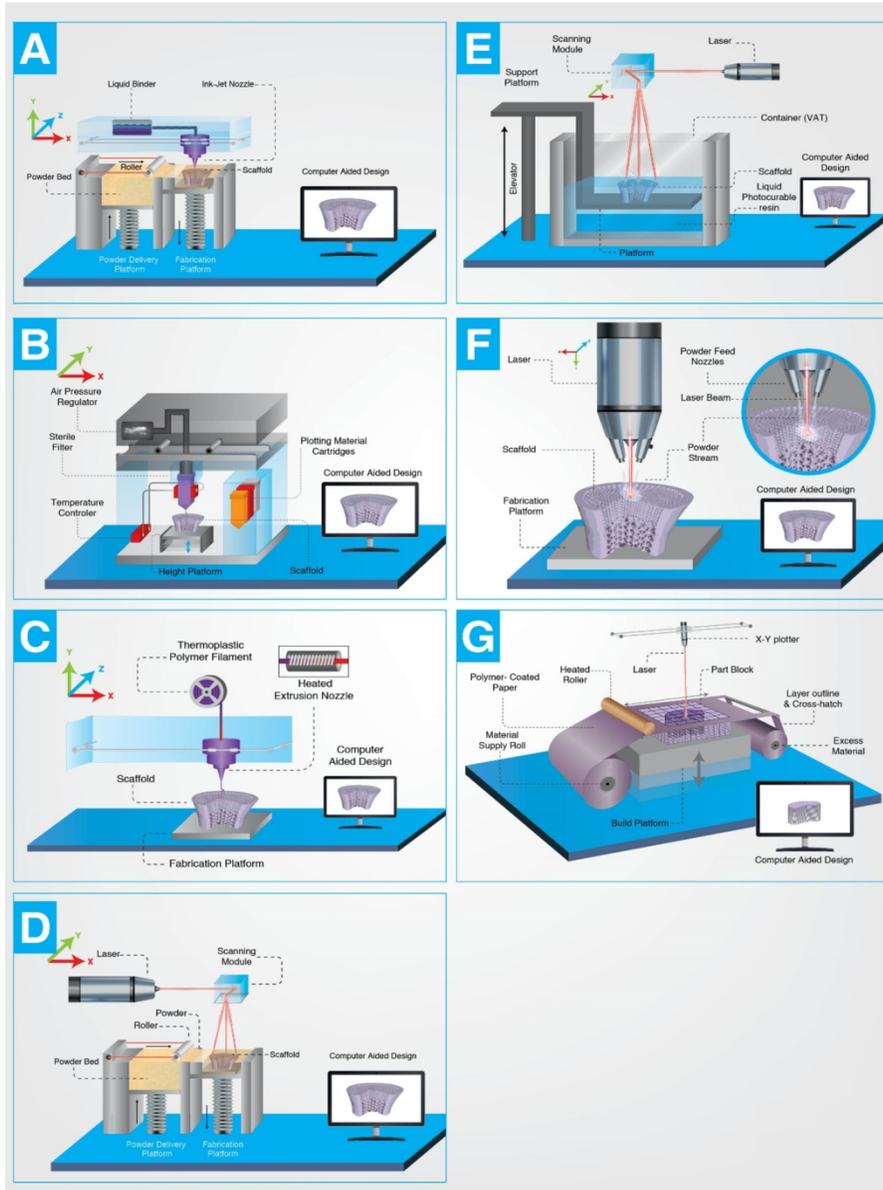
Figure 12. 3D printing techniques, (a) Binder jetting, (b) Materials jetting, (c) Materials extrusion, (d) Powder bed fusion, (e) Vat photopolymerisation, (f) Directed energy deposition, (g) Sheet lamination.

164x219mm (220 x 220 DPI)

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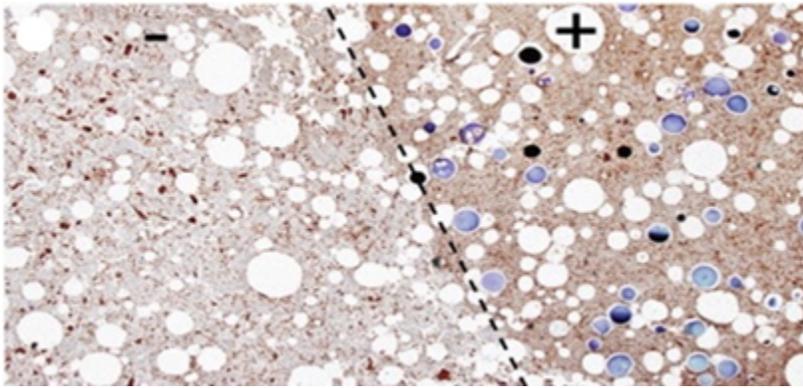41
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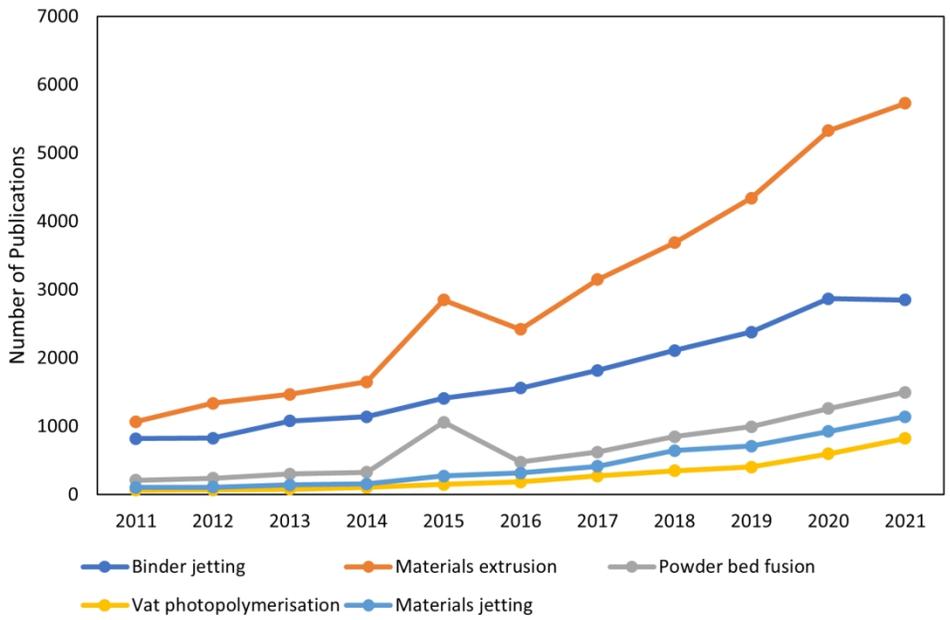
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153x99mm (330 x 330 DPI)
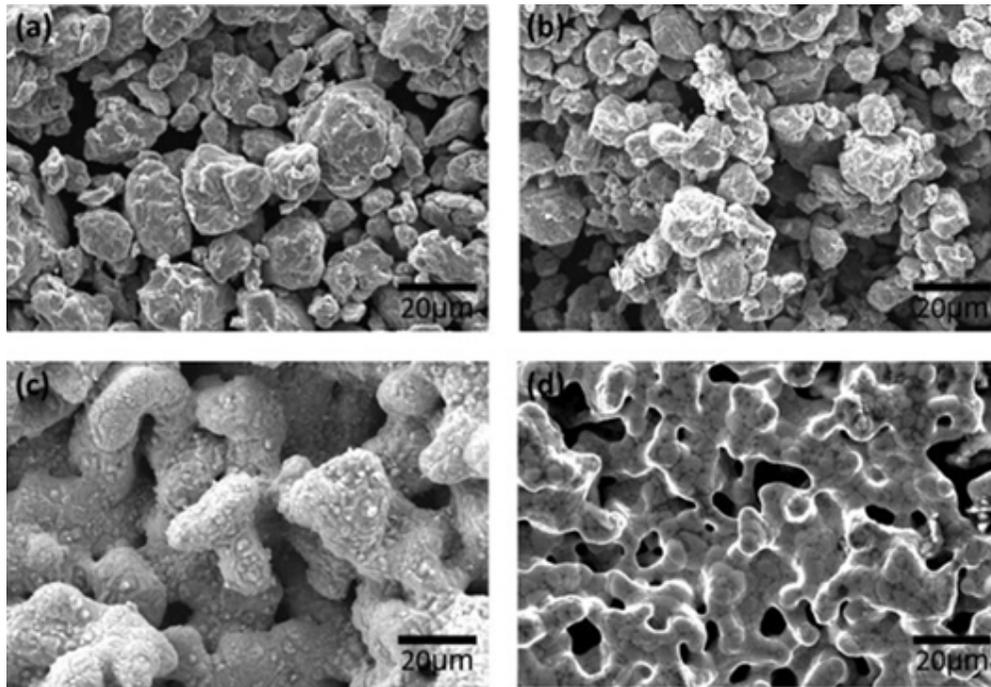
Figure 14. Morphology of (a) Fe-Mn powders (b) Fe-Mn-1Ca powders, (c) 3D printed Fe-Mn sample, and (d) 3D printed Fe-Mn-1Ca sample. Adopted from Hong D (93).

119x82mm (113 x 113 DPI)

Figure 15. 3D printed matrigel-alginate scaffold the two regions (− without VEGF, + VEGF-laden GMPs). Adopted from M. T. Poldervaart (97).
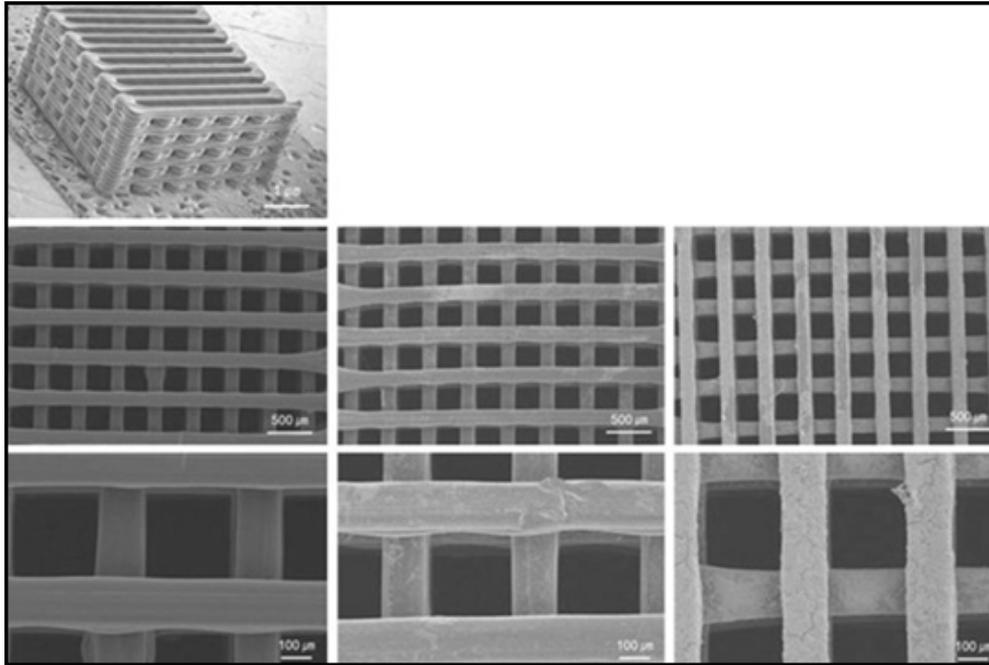
107x51mm (96 x 96 DPI)

Figure 16. SFF-based 3-D PCL/PLGA scaffold. Adopted from J. M. Hong (105).
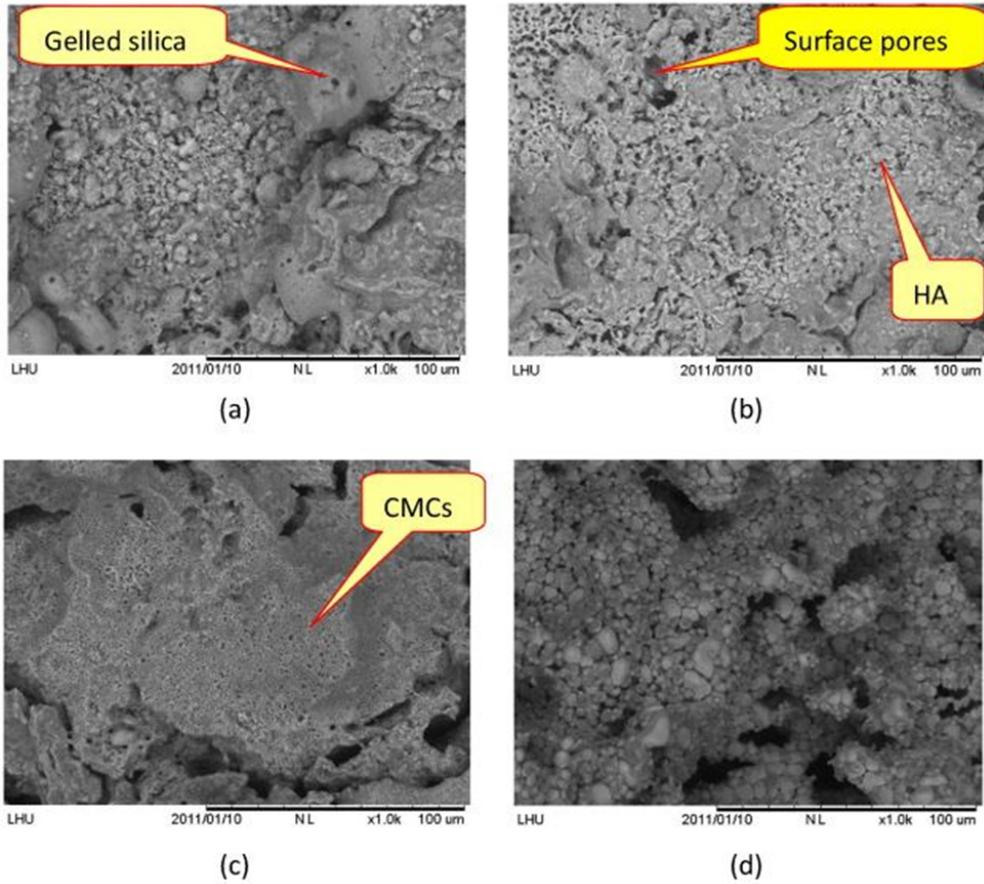
122x81mm (113 x 113 DPI)

Figure 17. SEM images of the scaffold at (a) ambient temperature, (b) 1,200 °C, (c) 1,300 °C, and (d) 1,400 °C. hydroxyapatite (HA) and Ceramic-matrix composites (CMCs). Adopted from F.-H. Liu (117).

114x104mm (216 x 216 DPI)

Figure 18. fabricated scaffold using SLA. Adopted from M. N. Cooke (124).
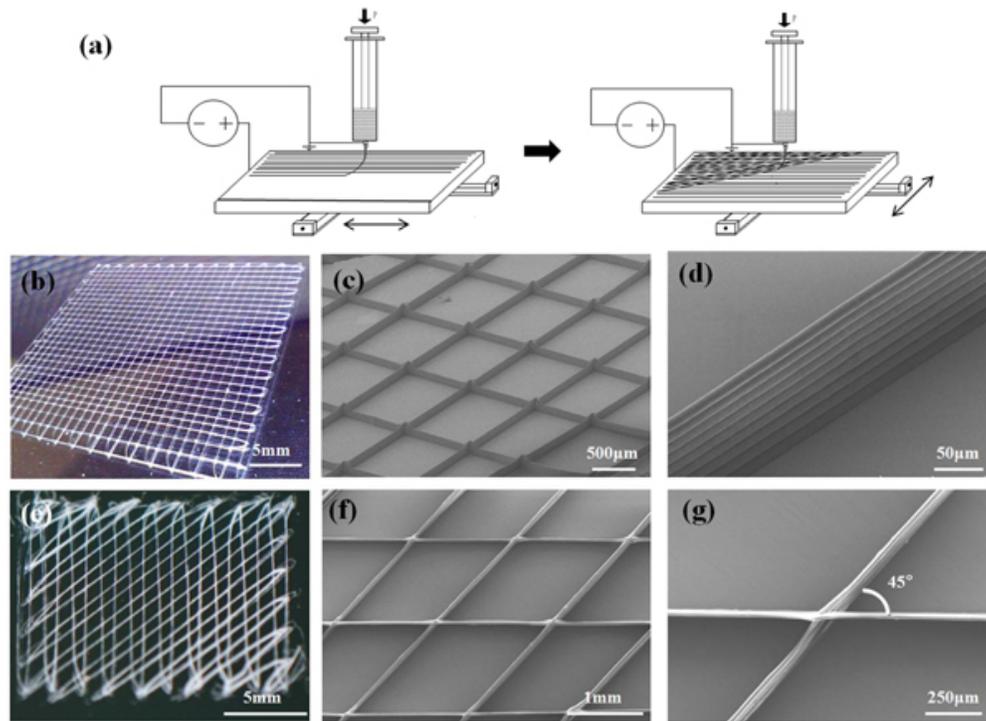
113x84mm (100 x 100 DPI)

Figure 19. Fiber orientation manipulation during the melt electrohydrodynamic printing process. (a) Schematic illustration of manipulating fiber orientation through directing stage movement, (b-d) microscopic images of the printed scaffold, which have fibers spaced at 1 mm intervals and oriented at 90° and (e-g) oriented at 45°. Adopted from Jiankang He (139).

159x115mm (96 x 96 DPI)

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Table 1. Terms, definitions and examples of bone repair [40].

Term	Definition	Example
Osteogenesis	The process by which new bone is synthesised using donor cells taken either from the host or the graft donor.	Stem cell, autografts transplants
Osteoconduction	The passive ingrowth of host vasculature, tissue, and cells into an implanted scaffold.	Phosphate cements or calcium sulphate resorption
Osteoinduction	Exogenous growth factors enable host mesenchymal stem cells (MSCs) to differentiate into osteoblasts and chondroblasts capable of producing new bone.	Proteins involved in bone morphogenesis

Table 2. Morphological properties of collagen-HA membranes. Adopted from SN Park [66].

Freezing temperature	Porosity (%)		Pore size (µm)	
	Before crosslinking	After crosslinking	Before crosslinking	After crosslinking
−196°C	58.1±3.4	61.95±3.8	40±7	84±20
−70°C	59.28±4.9	62.3±4.8	90±16	186±29
−20°C	66.46±2.6	64.93±2.3	230±52	190±42

Table 3. summary of conventional manufacturing.

Manufacturing technique	Main applications	Advantages	Disadvantages	Ref.
Salt leaching	Forming porosity in part by employing salt particles	• Controlled pore size • Suitable for manufacturing of membranes	• Lack of pores distribution control • Lack of scaffold shape control	[43, 48] [50]

Gas foaming	Forming porosity in part by applying high pressure	• It does not use chemical solvents • It is capable of fabricating parts with very high porosity	• Pore sizes are difficult to control • It employs a high temperature • Lack of scaffold shape control	[57, 58] [59]
Phase separation	Forming porosity in part by employing chemical solvents	• It does not require post-processing	• Pore sizes are very small • Lack of scaffold shape control	[43, 59]
Freeze-drying	Forming porosity in part by freezing the liquid mixture	• Pore sizes can be adjusted by controlling the temperatures	• Lack of scaffold shape control • high energy consumption • uses cytotoxic solvents	[67, 68] [66]
Electrospinning	Forming solid nano size fibres	• Mechanical and porosity properties of the fibre can be controlled by regulating the voltage and distance	• Lack of scaffold shape control	[73, 74] [72]
Self-assembly	Forming a pattern by the interactions of two components without external direction	• It does not require the use of cytotoxic solvents	• Pore sizes are very small • It necessitates greater attention to molecular design and intricate synthesis	[76] [77]

Table 4. summary of the 3D printing techniques.

AM technology	Resolution (µm)	Material	Strength	Weakness	Refs
Binder jetting	200-300	PLGA, PLLA, PEEK-HA, PCL, starch-based polymer	• No support structure is required • Fast processing • Uses a variety of materials	• Can require post-processing • Powdery surface finish • Trapped powder	[142, 143]
Materials jetting	10-1000	PCL, PLLA, TCP, Hydrogel, Organic ink	• Uses an enhanced range of materials • Can incorporate biomolecule	• Low mechanical strength • Smooth surface • Low accuracy • Slow processing • Complex design requires support structure	[144-149]
Materials extrusion	250	PCL, PP-TCP, PCL-HA, PCL-TCP, PETG-PBT, PLLA-TCP, PLA	• Good mechanical strength • Preparation time is reduced	• High temperature • Need to produce filament material • Narrow processing window • Complex design can require support structure	[108, 150-152]
Powder bed fusion	500	PEEK-HA, PCL, titanium, Stainless steel, cobalt-chromium alloys	• Microporosity induced in the scaffold • Uses an enhanced range of materials • No support structure needed • Fast processing	• Material must be in powder form • High temperature • Powdery surface finish • Trapped powder • Thermal damage can occur during processing	[153-155]
Vat photopolymerisation	366	Resin, PPF, polyethylene glycol acrylate, HA	• Control of both external and internal morphology • Uses an enhanced range of materials • High accuracy • Fast processing	• Multistep involved • Poor mechanical strength • Damages cell during photo curing • UV blue light can be toxic to cells	[156, 157]

Poly(lactic-co-glycolic acid) (PLGA), Poly(L-lactide) (PLLA), (polyetheretherketone) (PEEK), Hydroxyapatite (HA), Polycaprolactone (PCL), Tricalcium phosphate (TCP), Polypropylene (PP), Polyethylene terephthalate glycol (PETG), Polybutylene terephthalate (PBT) and Paint protection film (PPF).