

A review on the challenges of 3D printing of organic powders

Daniel J. Whyte^a, Rangam Rajkhowa^a, Ben Allardyce^a, Abbas Z. Kouzani^{b,*}

^a Deakin University, Institute for Frontier Materials, Geelong, Victoria, 3216, Australia

^b Deakin University, School of Engineering, Geelong, Victoria, 3216, Australia



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ABSTRACT

3D printing technologies have enabled advances in biomedical research and development, including rapid fabrication of complex, customised constructs for personalised treatment of patients. However, there are still limitations with the 3D printing technologies in relation to the diversity of biomaterials, and the versatility of the printing methods to print such diverse materials. While many materials have been used for biomedical applications involving high load bearing forces, e.g. ceramics and metals, they struggle in areas of biodegradability, and supporting tissue adhesion. An alternative are natural polymers, which are currently being investigated in the biomedical industry and are generally biocompatible, biodegradable and bioactive. However, they lack optimal mechanical properties necessary for high load bearing applications. Natural polymers in a powdered form, also referred to as organic powders, can allow for much denser and higher integrity constructs compared to their more common bioink counterparts, whilst maintaining many of the desired biological attributes. However, many organic powders are either do not respond to light and degrade in heat, preventing their use in many light and thermal based 3D printing processes. Powder inkjet printing is a 3D printing technology that utilizes an alternative means of solidifying the powdered material. These include physical or chemical bonding, instead of photopolymerisation, melting or sintering via the exposure of heat or light. Nevertheless, the binders or solutions that are commonly used with organic powders are high in acidity which can damage the printer components, further limiting this materials printability. This paper investigates the current 3D printing technologies that can print with biomaterials. Several elements are investigated, including current biomedical applications, fusion technology, material limitations and useable biomaterials. In addition to the printing processes, a study on available biomaterials, in particular organic powders, is elaborately discussed. The origins of the biomaterials are explored, in addition to the acceptable 3D printers, current applications, and printable forms. The limitations found with the current available printing processes is also discussed, in relation to their compatibility with organic powders, and their required binder solutions.

1. Introduction

3D printing has drastically improved our ability to fabricate objects with precise dimensions, customised design and complex architecture, whilst ensuring the standardisation and reproducibility of manufacturing processes [1–3]. In the biomedical industry, patient-specific treatments are of paramount importance to optimize biological repairs, replacement and regeneration [4]. Clinical image data received from x-rays, magnetic resonance imaging (MRI) or computerised tomography (CT) scans allow 3D printers to fabricate constructs specifically tailored to each patient, with controllable micro-architecture [5–9]. For example, clinicians recently fabricated a biodegradable tracheal splint for an infant using 3D printing and CT imagery of the patient's airway [9]. Other examples

include printed artificial ears specifically designed for patients, personalised drugs, and drug delivery implants [10,11]. The use of 3D printing for prosthetic implants reduces cost and time compared to traditional fabrication methods [12].

One method of bioprinting is the printing of scaffolds which are used as a support for growing cells. This allows the construction of complex 3D living tissue, by precise allocation of biomaterials, living cells, growing factors and other biological inclusions [13]. Typical scaffold architecture designs include meshes, fibres, sponges and foams; these designs are chosen because of their high porosity, which promotes uniform cell distribution, diffusion of nutrients, and the growth of organised cell communities [14]. Several types of tissues have been generated using this technology, including; bone, cartilage, skin, vasculature, heart, and

* Corresponding author.

E-mail address: kouzani@deakin.edu.au (A.Z. Kouzani).

neuronal tissues. Bioprinting has been defined as: “the use of material transfer processes for patterning and assembling biological relevant materials, molecules, cells, tissues, and biodegradable biomaterial – with a prescribed organisation to accomplish one or more biological functions” [4,15].

Many areas of biomedical research have benefited from bioprinting technologies, however, bioprinting is still considered to be in its initial stage of development [15–18]. Currently, issues arise when developing scaffolds for high load bearing applications as the present printable materials used for these applications, such as ceramics and metals, are restricted due to lack of biodegradability and bioactivity (tissue adhesion) and some degree of inflammatory response from host tissues. Moreover, the metal and ceramics cannot be functionalised with drugs and biomolecules. A possible solution to developing scaffolds with both adequate mechanical properties, biodegradability, biocompatibility and bioactivity involves the use of organic materials and in the form of powders.

Organic powders are natural polymers in a powdered form and are only beginning to be explored for use in bioprinting. Natural polymers have many benefits as a biomaterial, such as high biocompatibility, degradability and minimal long-term inflammation, and are one of the most popular biomaterials for existing applications, excluding orthopaedics [19,20]. Most natural polymers are printed in a bioink form, a high viscosity liquid that enables extrusion printing into 3D hydrogels, however, this form generally prevents the production of scaffolds that are suitable for high load bearing applications. Whilst maintaining many of the beneficial attributes, organic powder would produce scaffolds with much denser and higher mechanical integrity compared to their bioinks counterparts, which are more suitable for high load bearing applications. Nevertheless, challenges arise when proposing the use of organic powders in printing methods. Common binders used with organic powders are acidic and would be potentially damaging for common printer components. In addition, many printing methods include the use of light or heat, which can cause problems as many organic powders don't respond to light and degrade under heat.

2. Properties of biomaterials and biomaterial scaffolds

The selection of biomaterials for fabrication of biomedical scaffolds is vital for its success. The scaffolds need to be precisely designed to complete specific tasks and must have corresponding attributes which correlate with the application at hand. These attributes are within the areas of biocompatibility, biodegradability, mechanical properties, and structure architecture.

2.1. Biocompatibility

The single most important factor that defines a biomaterial from a regular material is its ability to function within a body with a positive probability of being accepted by the living host. Biocompatibility is defined as the ability of a biomaterial to function in relation to a medical therapy, generating the most appropriate cellular and tissue response in the selected anatomical site, optimising the performance of the medical remedy, while not producing any undesired local or systemic effects to the recipient [21]. In general, biocompatibility describes a material that is harmless to the host and has the correct attributes to exist in a living body (i.e. non-toxic, non-carcinogenic), whilst retaining the properties required to perform the desired medical application (i.e. mechanical properties, degradability).

2.2. Biodegradability

Biodegradability describes the rate in which a material degrades within a living host by being broken down and replaced by regenerating natural surrounding tissue, with the degraded by-products being absorbed and released via metabolic processes of the body [22]. Biodegradable

biomaterials are slowly replaced by types of living tissue which allows for no surgical removal. The biodegradability of a biomaterial also describes the mechanical properties and structural integrity over the lifespan of the component, and therefore the type of application the biomaterial is suitable for. For example, in tissue engineering it is a critical requirement for the scaffold to have the correct rate of biodegradability in relation to the surrounding tissues regeneration rate, to allow the cells to produce their own extracellular matrix (ECM) [23].

2.3. Mechanical properties

Biomaterial scaffolds are required to have a degree of mechanical integrity in correlation to their designed application. Ideally, scaffolds should have the same consistency as the anatomical site they are placed into, while being strong enough to be handled and implanted [23]. Producing scaffolds for applications that require high mechanical properties, such as bone or cartilage, remains one of the highest challenges in the area. These applications require biomaterials that can withstand large amounts of stress/strain whilst supporting cell activity without sacrificing functionality. These scaffolds not only require the correct mechanical properties to be able to withstand high load bearing applications, they must remain functional until the completion of the healing process [23,24].

2.4. Architecture and structure

The architecture and structure of biomaterial scaffold is critically important as they have influence on the cellular adhesion, migration, proliferation, differentiation and colonization of the cells [25,26]. On a microscopic scale, a biomaterial provides a framework and capillary networks for local cells to grow. Therefore, it is important that the architecture must integrate adequate cellular penetration for nutrients supply throughout the whole structure, whilst also accounting for the removal of waste [26]. A major issue in scaffold based tissue engineering is core degradation, where there is insufficient vascularization and waste removal from the centre, affecting functionality, cell growth, mechanical properties, degradability and biocompatibility and causing toxicity [23]. To counter this, scaffolds are commonly designed with an interconnected matrix architecture with high porosity, to allow these functions to take place.

A key consideration of the scaffold architecture is the mean size of the pores, as different types of cells require specific pore sizes to allow for adequate migration, proliferation and differentiation. The mean pore size must be large enough to allow for the cells to migrate into the structure, yet small enough to establish sufficient connection pathways between the cells. In addition, the architecture must allow for sufficient structure strength to support the porosity of the scaffold whilst maintaining the required mechanical properties.

3. 3D printable biomaterials

A challenge with current scaffold research is obtaining materials with strong mechanical integrity suitable for high load bearing applications, while maintaining optimal levels of biodegradability, biocompatibility, and bioactivity. Ceramic biomaterials used in powder inkjet printers, have correct attributes to produce scaffolds for bone and cartilage applications, yet lack biodegradability, biocompatibility, bioactivity, and tend to be brittle [27,28]. Metal biomaterials, often used in Selective Laser Sintering (SLS), Selective Laser Melting (SLM) printers, are suitable for high load applications as they have high strength, elasticity and fracture toughness. However, they are only suitable for long term implants as they are non-degradable, and include no bioactivity properties [29,30].

Excluding orthopaedics applications, polymers are the most popular biomaterials, due to their mechanical versatility, biocompatibility, similarity to living tissue, biodegradability and minimal long-term

inflammation [19,20]. There has been a large amount of research into the development of polymer biomaterials which mimic tissue [31,32]. These biomaterials have been fabricated to match the biochemical properties of soft tissues, yet generally lack the mechanical properties required for high load bearing applications when compared to other biomaterials [20].

Synthetic polymers are man-made polymers which are produced in controlled environments. These materials have reproducible mechanical and physical properties, such as degradability, tensile strength and elastic modulus [20]. Unfortunately, synthetic polymers constructs have bioactivity limitations when compared to natural polymers, because of the lack of biofunctionality [33]. The most common synthetic polymers for bioprinting include Polyethylene glycol (PEG), Polylactic acid (PLA), Polyglycolic acid (PGA), Polylactide-co-glycolide (PLGA) acid and Polycaprolactone (PCL). These materials have been used in several printers for tissue engineering applications, including the growth of smooth muscle cells in vitro on PEG scaffolds, and fabricated scaffolds that contained PCL used in vivo for cartridge tissue regeneration [34,35].

Polymers with natural origin, such as from plants or animals, are one of the most attractive biomaterials, due to their similarities with the extracellular matrix found within a human body [36,37]. These polymers, also referred to as natural polymers, biopolymers or organic polymers when in powdered form, are chemically versatile, being able to be modified through chemical methods. Biomaterials made from biopolymers are often biocompatible, eliciting minimal inflammatory response and are usually biodegradable [32]. In addition, they are usually biologically active and promote excellent cell adhesion and growth [23]. Biopolymers are most commonly used in bioinks to fabricate scaffolds.

Alginate is a naturally occurring biopolymer found in seaweeds, or walls of brown algae. It is non-toxic, biodegradable, and non-immunogenic [13,38,39]. At room temperature, sodium alginate dissolved in water has a gel-like structure, which, when heated to above 90 °C, turns into a liquid. Having a liquid state at a temperature of above 90 °C makes it suitable for printing using direct write printers [13]. Many novel bioinks that contain alginate have been developed and used in direct write printers to produce scaffolds incorporating living cells [40, 41]. Investigation has been conducted to test the incorporation of alginate to CaP powder to improve the brittleness of ceramic scaffolds. Additionally, alginate has been used as a biomaterial for the construction of many types of tissue, including cartilage, bone and vascular [40–42]. Alginate has relatively low mechanical properties and is not cell adhesive, restricting its use in biomedical applications [43].

Chitosan is a biodegradable, non-toxic, non-allergenic, anti-bacterial, mucoadhesive biopolymer and has gained significant interest in biomedical applications [36,38]. Chitosan is a cationic polysaccharide, derived from material that is found in the exoskeletons of crabs and shrimps [44]. Chitosan based materials have been developed for applications including: wound healing, cartilage tissue, artificial skin, nerve tissue and bone tissue [31,38]. Research has been conducted on the use of chitosan in SLA printers to develop ear-shape scaffolds with highly defined architecture [45]. Other research includes the use of a direct write printer which was able to print a chitosan scaffold that had a flexible and organised microfiber network [46]. Like alginate, this biopolymer has low mechanical properties, and is also often used in bioinks or resins.

Collagen is the primary structural protein found in connective tissue and is the most common protein found in living animals [16]. This natural biopolymer provides strength and structural stability to tissue including, skin, blood vessels, tendon, cartilage and bone [31]. Other than hydroxyapatite, collagen is the major component of bone, and has significant potential for bone tissue engineering. Unfortunately, like the natural biopolymers previously discussed, collagen also has relatively poor mechanical properties. Even so, collagen is the most commonly and broadly used natural material in tissue engineering, and is typically printed in preformed moulds due to its limited mechanical stability [16]. Collagen scaffolds designed for soft tissue implants have been

successfully printed by a direct write printer [47]. Research has also been conducted on calcium phosphate scaffolds for bone regeneration, produced by a powder inkjet printer with a collagen infused binder [48]. Additionally, photo-crosslinked collagen based gelatine methacrylate scaffolds with complex porous architecture have been printed with a SLA printer; on which endothelial cells successfully attached and proliferated [49].

Silk fibroin has attracted attention in recent years for biomedical applications due to its advantageous mechanical properties, whilst maintaining high biocompatibility, low immunogenicity, limited bacterial adhesion and controllable biodegradability [16,50,51]. Silk fibroin is a biopolymer produced by arthropods, including silkworms, spiders, mites and select scorpions [52]. A benefit of silk fibroin is that it degrades within the body proteolytically generating non-toxic by-products, unlike synthetic polymers such as PLA, PGA, and PLGA which degrade hydrolytically producing acidic by-products [53]. Additionally, the US pharmaceutical industry has introduced silk fibroin as a non-degradable material because after 60 days of implantation it maintains approximately 50% of its structure [54]. Silk fibroin outrival other natural biopolymers in mechanical properties including; breaking strength, elongation, toughness, ductility, and Young's modulus [16,50,52]. For these reasons, silk fibroin is a better suited candidate for bone tissue engineering than other biopolymers [50].

Silk fibroin is a protein with light and heavy amino acid sequences which are connected through a disulfide bond. The heavy chain amino acid sequence consists of repetitive hydrophobic blocks of Gly-Ala-Gly-Ala-Gly-Ser and Gly-Ala/Ser/Tyr dipeptides, whilst the light chains consist of a non-repetitive hydrophilic sequence, forming 12 crystalline domains [55]. Extremely stable β -sheet nanocrystals have the ability to be tightly packed due to the high glycine content, with hydrogen bonds being the main molecular interaction. The hydrogen bonds grant silk excellent rigidity and tensile strength. Despite these properties, most constructs made from silk fibroin are brittle and weak due to the available processing methods [52]. Currently, silk fibroin is most commonly used as a bioink within direct write printers, however many of the beneficial mechanical properties are lost when used in this form [4,7,56].

Other biopolymers that are being investigated for bioprinting include: agarose, elastin, carrageenan, gelatine and fibrin [13,16,44].

4. Processes for 3D printing of biomaterials

4.1. Direct write

Direct write printers use one or more nozzles to extrude material onto a flat surface, called the building platform. The nozzle has three translation degrees of freedom along the X, Y, and Z axis (in relation to the building platform), that gives these printers the ability to deposit the material in a manner that not only allows the creation of width and depth but also height to the construct (see Fig. 1). The printer uses the freedom of the X and Y axes to deposit the material on the printer building platform with predetermined geography. After a layer is completed, the printer head or building platform travels along the Z axis, which allows the next layer to be printed on top of the previous deposition. Repeating this process allows the production of 3D constructs from the selected biomaterial.

Direct write 3D printers, e.g., bioplotters and robocasting printers, have the widest variety of biomaterials available. They can also cure materials with photopolymerisation, humidity gradients or other chemical polymerisation methods [15]. For this reason in addition to their lower cost, direct write printers are one of the most common printers used in bioprinting [15]. In addition, direct write printers have a large variety of cell friendly curing methods, and bioinks enabling their ability to print with living cells [57,58].

Direct write printing has been used to print a range of materials, from cell scaffolds, to micro stents. With the use of bioinks, various tissue types have been printed, including cartilage, bone and smooth muscle [58].

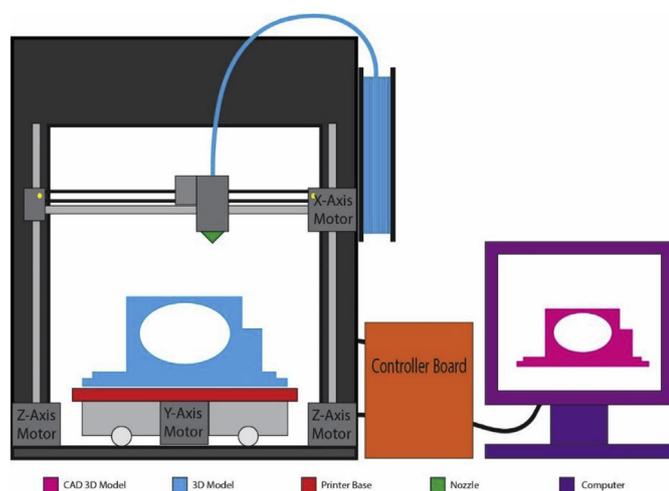


Fig. 1. General 3D Direct Write process diagram.

Though direct write has been used in a wide range of biomedical applications, constructs are limited by low mechanical properties, are often fragile, and experience damage from high shear stress during printing. To maintain shape and architecture of printed constructs, direct write printers are often required to use high viscosity inks. However, issues arise with the use of high viscosity as they are prone to clogging 3D printing nozzles and supply lines, resulting in damage to biological inclusions, such as cells.

4.2. Stereolithography (SLA)

Stereolithography (SLA) refers to a technology that uses ultraviolet light to photo-polymerise liquid resins. The material, in a photo sensitive liquid resin state, is placed within a bath and is exposed to UV light, creating a thin photopolymerised layer of solid material. Photopolymerised layers are built up consecutively, with the previous layer adhering with the next layer via chemical reactions, resulting in a 3D physical structure (see Fig. 2).

Stereolithography has been used in a number of different biomedical applications including cell scaffolds as well as implantable devices [9]. This technology is marked by having one of the highest accuracy for 3D printing, and is capable of producing constructs with an accuracy of 0.5–50 μm [33]. Even so, stereolithography is still considered to be in developmental infancy, due to the limited choice of photo-curable materials. It is also relatively expensive compared to other technologies, and

has limited ability with the inclusion of cells due to light exposure during the printing process and common post processing procedures. These issues limit its use in biomedical applications [59]. Photo-curable resins also commonly produce brittle and rigid constructs, making the method unsuitable for use in high mechanical load applications.

4.3. Selective Laser Sintering/Melting

Selective laser sintering/melting printing refers to two types of similar technologies, including selective laser sintering (SLS) and selective laser melting (SLM). These technologies are similar in operation and use a laser beam to selectively fuse powdered material within a bed, yet differ on the materials available, due to the differences in laser strength. In general, these printers have a heated bed of powder at near melting point with a laser beam moving across the X and Y axis's, selectively sintering or melting the powder particles to fuse them together, then once completed applying more powder. This process is repeated until a 3D construct is developed [29,60–62] (see Fig. 3).

SLS printers have been used to print biomaterials such as bioglass and calcium phosphate for biomedical applications that require high mechanical strength [60,62–64]. SLS is a relatively slow and costly process, and the produced constructs can have issues with non-uniform thermal field distribution, causing imperfections and cracks. Despite these issues, SLS is more commonly used than SLM [60]. SLM shares similarities with SLS, however a notable difference in processes is the melting of the powder opposed to the sintering via use of a higher-powered laser beam. This allows for a different variety of available materials and has been used to fabricate biodegradable implants using pure zinc and iron [65].

The main advantage of SLS and SLM technologies is their ability to directly print biomaterial implants with high precision (20–100 μm [66]), and the available metallic materials which can be used for high loading application implants. The implants, with customisable shape, features and size, can be tailored for specific tasks for patients to promote bone ingrowth and surrounding tissue regeneration [63]. The implants that have been printed with these technologies include: femoral head, femoral stems, knee replacements, heart valves and fracture plates [62]. The disadvantage of this technology is that it cannot be used with light or heat sensitive materials, such as natural biopolymers, living cells or biomolecules [29]. When exposed to higher radiation of light or heat, the particles in these materials don't fuse together but receive damage and degrade.

4.4. Powder inkjet

Compared to the other printers, powder inkjet printers are one of the

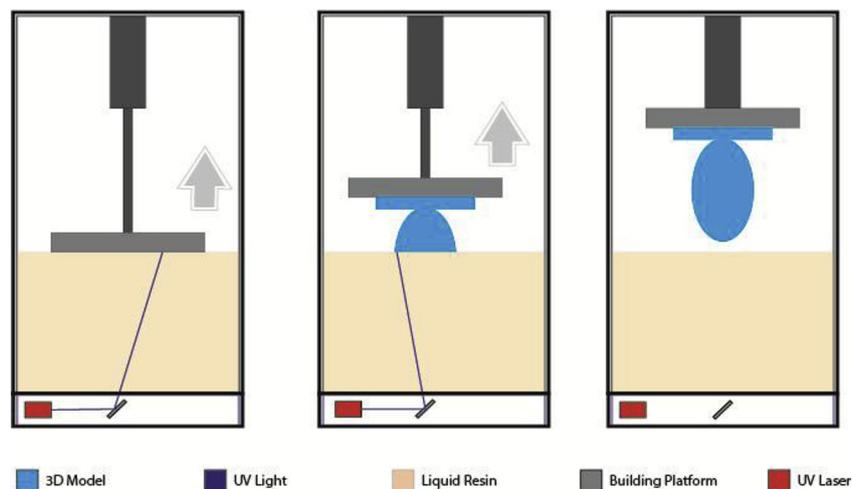


Fig. 2. Stereolithography printer process diagram.

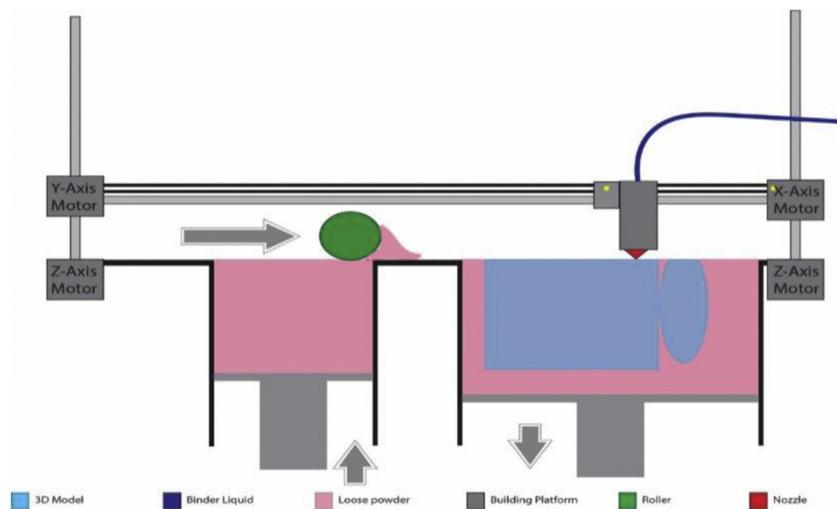


Fig. 3. Selective Laser Sintering/Melting process diagram.

least explored technologies for bioprinting [2]. The processing components of powder inkjet printers, shown in Fig. 4, are very similar to SLS and SLM printers. They both use powdered materials, a powder bed, and powder movement/management mechanisms. The technologies differ on the techniques used to fuse the powder particles, and the variety of printable materials.

Powder base inkjet printers use aqueous or low acidic solution inks to physically or chemically bind the powdered particles together [15,29]. Powder inkjet printers are most commonly used with several types of biocompatible ceramics. Thus far, scaffolds have been made of many different ceramic powders including: hydroxyapatite and tricalcium phosphate [15,29,42]. This technology shows a high potential for printing complex tissue, with its ability to integrate biological inclusion [67,68]. However, aqueous binders used in this technology produce water-soluble constructs, limiting application possibilities. When acidic base binders are used, such as phosphoric and citric acid, the scaffolds can be insoluble. Though, there are major concerns when using acid based binders as the acid can significantly damage printer components [29,69,70].

5. Biomaterial forms

Biomaterials are available in many forms, however, when these materials are used in 3D printers, they are commonly converted into bioinks or powders. There are several types of bioinks including hydrogels, cell aggregates, microcarriers and decellularized matrix components. Bioinks have been used in several biomedical applications, such as developing

contact lenses and wound dressings. They are also commonly used to print cell supporting scaffolds, or scaffold-free constructs mimicking embryonic development [57]. Though bioinks support high cell viability, scaffolds (see Fig. 5) produced from bioinks are not suitable for high load bearing applications due to their relative low consistency.

The alternative to bioinks for bioprinting, are biomaterial powders. A biomaterial in a powdered form can offer greater mechanical strengths and integrity, leading to an increase in interest of these materials in bone tissue engineering [2,69,72,73]. Other than greater mechanical properties, scaffolds produced from biomaterial powders have rougher macro surfaces which enhances cell adhesion [74,75]. Biomaterial powders have been used in a variety of high load bearing applications, ranging from orthopaedic prosthetic components to implants [72]. Currently, there are several different types of powdered biomaterials available, including ceramic powders, and organic powders.

The most widely used biomaterial powders for bioprinting are hydroxyapatite (HA) and beta tricalcium phosphate (β -TCP), which are both forms of calcium phosphate. These biomaterial powders have been used extensively in research using powder inkjet printers for bone tissue engineering applications [42,72,77–79]. They have demonstrated to support osteoblast adhesion, proliferation and possess some bioactive properties [78]. In addition to this, they also closely mimic the mineral structure and chemical composition of bone. Several binders and solvents have been used with these materials, commonly an acidic binder containing 5–30 wt% phosphoric acid [38,42,72]. When phosphoric acid is

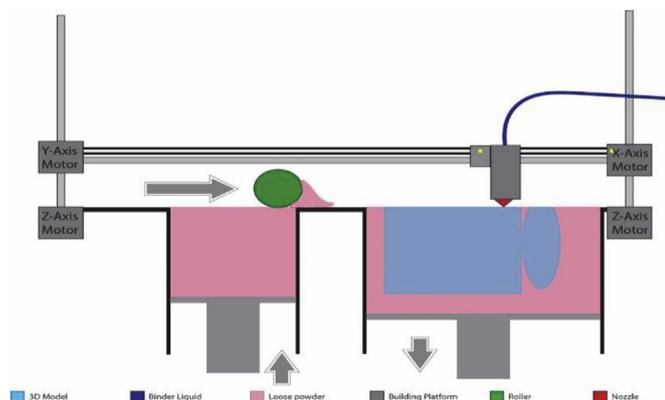


Fig. 4. Powder inkjet printer process diagram.

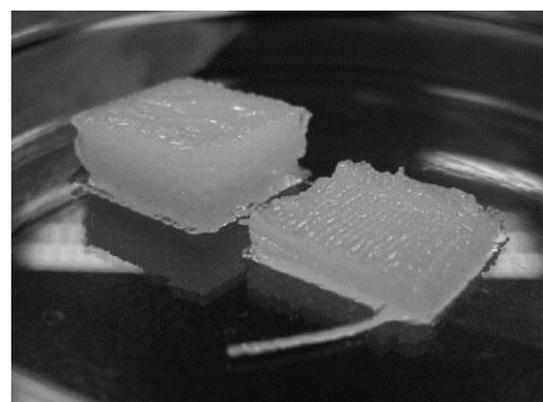
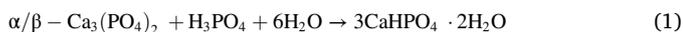


Fig. 5. Bioink scaffolds produced from chitosan composite in a direct write process (reproduced with permission from Ang, Sultana, Huttmacher, Wong, Fuh, Mo, Loh, Burdet and Teoh [71]).

applied to HA and TCP, the powder undergoes a dissolution-precipitation process via an acid base reaction between the two substances. This reaction causes nanocrystalline brushite to form, described by Equation (1) and examples seen in Figs. 6 and 7, binding the powder particles together into an insoluble construct [27,76]. This process has been used in several studies on powder inkjet printing of calcium phosphate implants for reconstruction of cranial, maxillofacial defects, and complex bone defects [72,80]. Other popular ceramic powders that have been used for bioprinting include alumina, zirconia, and bioactive glass.



Powders produced from biopolymers are often referred to as organic powders. These powders remain largely unexplored with powder inkjet printing. Most of the research conducted on 3D printing biopolymers required the polymer to be in a bioink form, chemically or physically cross-linked into hydrophilic polymeric networks having the ability to contain high amounts of fluid, known as hydrogels [57,58,71]. However, some studies have reported using biopolymers in powdered form.

Several studies reported using chitosan powder as a bioink by using acetic acid [71,82,83]. One study included the use of chitosan powder in a composite with chondroitin sulphate powder and nano-silicon dioxide (SiO_2) powder, to produce solid composite scaffold by freezing and lyophilization within a vacuum [84]. These scaffolds showed improved mechanical integrity and lower degradation rate, whilst maintaining non-toxic properties [84]. Collagen powder has shown to possess excellent cell adhesion, haemostatic properties, and adequate simulation of cell reactivity in clinical research [85,86]. Moreau et al. [87] used a collagen calcium phosphate cement composite to fabricate a self-hardening scaffold with a biomimetic, nano apatite-collagen matrix that enhanced cellular attachment and bone regeneration, while maintaining flexural strength of 8–10 MPa with high toughness properties. Another study investigated the use of collagen powder with fibril morphology and characteristic triple helix conformation to function as an easy administrable for a basic fibroblast growth factor (bFGF) fused with clostridium histolyticum collagenase. The results showed that the bFGF/collagen powder composite is a promising agent for promoting bone fracture repair [88].

Several studies have demonstrated the use of silk fibroin in a bioink form used within direct write 3D printers to construct scaffolds [4, 90–92]. When used as a bioink, many desired mechanical properties are lost due to the low density and high liquid content of the materials. Other research has been conducted on silk fibroin in alternative fabrication methods to 3D printing and have demonstrated silk fibroin potential for high load applications. A recent study investigating the performance of biodegradable screws, seen in Fig. 8, created by freeze drying silk fibroin cast within a mould found that the silk screws exhibited good shear properties, were biocompatible and could remain within rat femurs up to 8 weeks [89]. Other studies have used silk fibroin microfiber/powder as



Fig. 6. Example of tricalcium phosphate brushite samples produced by powder inkjet printing process (reproduced with permission from Gbureck, Hölzel, Klamert, Würzler, Müller and Barralet [76]).

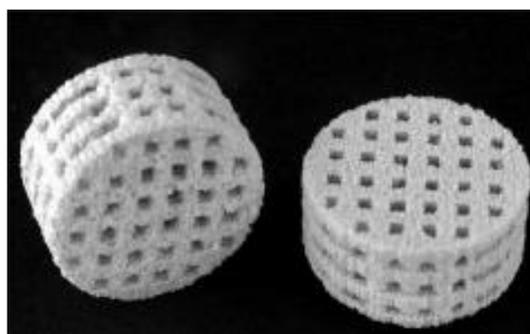


Fig. 7. Hydroxyapatite scaffolds (diameter 11.7 mm) printed using powder inkjet (reproduced with permission from Schieker, Seitz, Drosse, Seitz and Mutschler [81]).

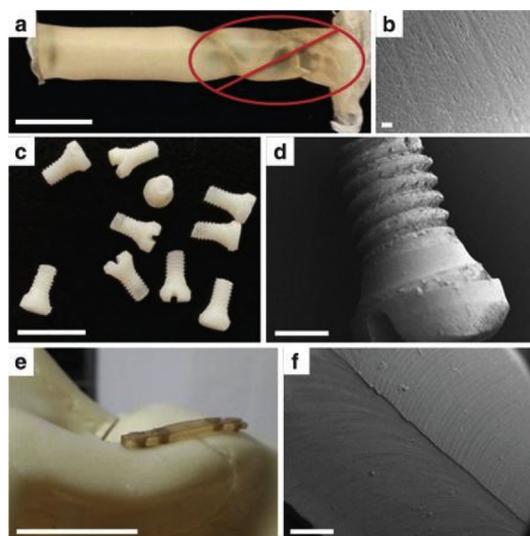


Fig. 8. Biodegradable screws that were placed within rat femurs, fabricated from silk fibroin. (a) Silk blanks prior to machining. Left portion is considered uniform; however, the right portion is considered not uniform and is discarded. (b) Scanning electron microscope (SEM) image of the surface of silk material after being machined. (c) Pure silk screws. (d) SEM image of silk screws. (e) Silk screws with metal plate on model skull. (f) SEM image of machined surface of silk plate. (reproduced with permission from Perrone, Leisk, Lo, Moreau, Haas, Papenburg, Golden, Partlow, Fox, Ibrahim, Lin and Kaplan [89]). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

a filler to enhance the mechanical properties of scaffolds [93–95]. One paper reported an increase in the scaffold's compressive modulus from 0.3 MPa to 1.9 MPa when reinforced with silk microparticles [93]. While another paper showed a 40 times increase in the compressive modulus and the yield strength of 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) based scaffolds, when reinforced with silk particles [94].

6. Challenges in 3D printing of organic powder

Many 3D printing technologies are used for biomedical purposes, though direct write printers are most commonly used as they have a large variety of cell friendly curing methods. Seen in Table 1, other technologies such as selective laser sintering, selective laser melting and stereolithography use light and heat in their solidifying process causing issues when trying to use biological inclusions, as this environment can cause damage. Even though direct write printers have this advantage over other 3D printer technologies, the technologies key disadvantage is that the producible scaffolds exhibit low mechanical properties, limiting its potential in hard tissue applications.

Table 1
Summary of material limitations and useable biomaterials of 3D printing technologies.

Printer	Fusion Technology	Material Limitations	Useable Biomaterials	Biomedical Applications	Advantages and Limitations
Direct write	-Photopolymerisation -Melting -Humidity gradients -Chemical polymerisation methods	Useable materials have low viscosity	-Bioactive glass -Alginate -Chitosan -Collagen -Silk fibroin	-Scaffolds -Medical implants -Cartilage, bone and muscle tissue engineering	+Enables bioactive components +Available commercially +Large range of biomaterials +Free form structures possible +No trapped material within constructs +Minimal material waste +Capable of multiple print heads therefore multiple materials -Scaffold produced have Low mechanical strengths +High 3D resolution +Capable of producing complex internal features -Photo induced cell damage -Limited selection of biomaterials -May require furnace postprocessing -Uses high costly and complex equipment
Stereolithography	-Photopolymerisation	Requires to be photocurable	-Bioactive glass	-Scaffolds -Medical implants	+High mechanical strength +High 3D resolution +Capable of producing complex internal features -Photo induced cell damage -Limited selection of biomaterials -May require furnace postprocessing -Uses high costly and complex equipment
Selective laser sintering (SLS) and selective laser melting (SLM)	-Particle sintering/melting via lasers	Require thermo responsive powders	-Bioglass	-Heart valves -Fracture plates -Scaffolds	+High mechanical strength +High 3D resolution compared to other powder printers -Not a large range of available Biomaterials -Not biodegradable -May require furnace postprocessing -Powder may thermally degrade during processing -Difficult to remove trapped powder
Powder bed inkjet	-Physical or chemical particle bonding via applying solutions	Require powders that can be physically or chemically bonded	-Calcium phosphate (HAP, tricalcium) -Alginate (binder) -Gelatine (binder)	-Tissue engineering -Scaffolds	+Bioactive components can be incorporated +Higher mechanical strengths +Capable of complex internal structures +Requires no supporting structures +Low cost -May require post printing treatments -Low to medium resolution -Difficult to remove trapped powder

Biomaterials in powdered form allow the material to maintain many mechanical attributes, unlike bioinks used in direct write printers as they generally have a high liquid content. Implants for hard tissue applications regularly use materials such as ceramics or metals in powdered form in selective laser sintering, selective laser melting and powder inkjet printers. However, the issue with ceramics and metals are their limited biodegradability, and bioactivity properties. Organic powders (biopolymers in powdered form) are an alternative option to metals and ceramics, though these also have several disadvantages in 3D printing.

Organic powders offer several advantages when compared to metals and ceramics. These polymer biomaterials, seen in [Table 2](#), can offer minimal inflammatory response, promote excellent cell adhesion/growth, are chemically versatile and are generally biocompatible, biodegradable and bioactive. However, biopolymers can be sensitive to heat and not respond to light, limiting the usability in several 3D printer technologies. Powder inkjet printers utilize different means of solidifying the powder, through either physical or chemical bonds which is more suitable to biopolymers. Though this could be a possible solution for

using organic powders in 3D printing, there are further concerns in the compatibility of this technology for this application.

Powder inkjet printers regularly use aqueous and low acidic solutions to bind powder particles together, such as hydroxyapatite powder and 5–30 wt% phosphoric acid. However, to fuse the particles, research indicates that organic powders will require the use of high acidic solutions that will cause damage to several powder inkjet components. For example, multiple papers have reported the use of applying 98% formic acid and 0.01% calcium chloride onto silk fibroin to form 3D nonwoven mats for biomedical research [96–98]. The high concentration of formic acid causes the hydrogen bonds in the crystalline region to partially dissolve, leading to a degree of dissolution. After a small period, the formic acid evaporates resulting in the precipitation of the silk 3D nonwoven mats. Nevertheless, one study successfully addressed this issue of using high acidic binders by integrating non-corrosive components, which include polypropylene and polytetrafluoroethylene (PTFE) fluid lines and an inert glass reservoir in a ZPrinter 450 powder inkjet printer [48].

Table 2
Polymer biomaterials.

Polymer Biomaterial	Naturally Derived/Organic	Acceptable 3D Printer	Currently Used Applications	Current printing Challenges
Alginate	Yes	Direct Write Powder Inkjet (binder infused)	Wound healing Tissue engineering	-Poor solubility -Low mechanical strength
Chitosan	Yes	Direct Write SLA	Tissue engineering Bone implants Artificial skin	-Poor solubility -Low mechanical strength
Collagen	Yes	Direct Write Powder Inkjet (binder infused) SLA	Tissue engineering Artificial skin	-Poor solubility -Low mechanical strength
Silk fibroin	Yes	Direct Write	Wound healing Tissue engineering	-Weak -Brittle -Low mechanical strength

Powder management is also a challenge, as most commercial powder inkjet printers are specifically designed for selected powder characteristics, often for powders developed by the same company. For an organic powder to be considered, investigations on the organic powder characteristics need to be conducted. Such characteristics include: flowability, stability, permeability, and wall friction properties. Establishing these characteristics is fundamental in determining the required parameters of the printer, in addition to producible resolution, accuracy, reproducibility and mechanical properties.

Fabricating scaffolds from organic powder, in particular silk fibroin, has the potential to exhibit attractive biological and mechanical attributes, such as biocompatibility, biodegradability and superb mechanical properties. Using the fabrication method of 3D printing will also allow for individual architecture and structure designs specifically tailored for the patient. Organic powders can be introduced as a replacement material for ceramics where moderate mechanical strength and higher cell proliferation is needed, such as bone repair. Additionally, organic powders can be useful in applications where biopolymers are currently being used, where an increase in mechanical properties would be beneficial. Such applications include tissue engineering and wound healing.

However, if a powder inkjet printer was to be configured for organic powder printing, a limitation of this printer, compared to the common direct write printers for biomedical purposes, is the inability to include living cells during the printing process due to the high acidic binders. A strategy to overcome this limitation is to use an existing process, and possible scaffold postprocessing, which allows the seeding of cells in vitro. The printed scaffold would be placed within a bioreactor, which is an incubator that creates the correct environment for cells to proliferate. The medium the scaffold is submerged in, would contain preselected cells and cell nourishment, encouraging the cells to lay down a matrix onto the supporting scaffold [99–101]. Bioreactors are also able to apply shear stress onto the scaffold which can further encourage cell growth. Nevertheless, depending on the scaffold architecture and structure, cells may fail dispersing throughout the scaffold, therefore affecting proliferation and networking. Printers that allow printing of cells do not have this issue.

3D printing is a known method of rapid prototyping, therefore has the potential to increase the development speed of organic powder applications. One possible advancement is research on printing organic powder composite scaffolds. As discussed earlier, silk fibroins have been implemented into scaffolds of known materials, increasing overall mechanical properties [93–95]. Furthermore, biological components such as drugs or

cell growth factors could be introduced in the 3D printing process, to increase the effectiveness of treatment. Another possible advancement is to change the chemical make-up of selected organic powders, to accept binders that allow the inclusion of cells in the printing process.

As silk, a known organic powder, is stronger than steel, in time it has the potential to replace metal materials in biomedical applications where cell growth is required. In addition, silk can be used to solve current issues in orthopaedics such as metals not being able to degrade over time repressing tissue restoration in the local area. Being able to adjust biodegradability and potentially mechanical properties of organic powders for the application at hand, will facilitate new opportunities for biomedical industry.

7. Conclusion

An issue for high mechanical load applications is finding materials that can withstand the stress/strain forces, whilst supporting high levels of biocompatibility, degradability, and bioactivity. Materials such as metals and ceramics are currently used for high load applications as they have suitable mechanical properties to withstand such forces, though have limited biological properties. Natural polymers are one of the most popular biomaterials as they are generally biocompatible, biodegradable and bioactive. However, they lack optimal mechanical properties when used in available 3D printing technologies.

Organic powders, natural polymers in powdered form, offer a unique solution to these issues. They have biocompatible properties, promote excellent cell adhesion/growth and induce minimal inflammatory responses. Silk powder in particular, offers the potential for high mechanical properties, in addition to a slow and controllable biodegrade rate, attributes desired for high load bearing applications. However, organic powders have been hardly explored in 3D printer technologies when compared to other biomaterials, limiting the producible scaffold architecture, reproducibility, and the potential of these materials. Many natural polymers are sensitive to light and heat, limiting the 3D printing processes available. Nevertheless, powder inkjet printing utilizes a non-destructive means of solidifying the powder, though the binders used for organic powders are high in acidity and can cause damage to printer components.

Further research needs to be conducted on organic powders, due to the limited data on the technology and high potential for biomedical applications. A compatible powder printer will accelerate progress in the research on organic powders by allowing custom design for specific purposes and enabling rapid prototyping.

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