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3D printing of biomaterials: a review

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ABSTRACT

Fabrication of complex biological devices is one of the promising applications of 3D printing. 3D printing technology was employed initially to produce visualization models aiding in surgical process and also for tooling molds. Since then the 3D printing technology has assumed a wider role creating implants, diagnostic platforms, drug delivery systems and scaffolds for tissue engineering. The recent developments have aroused interest amongst the scientific community to combine stem cells with the customized 3D scaffolds. However, there are several technological limitations and restrictions that must be addressed with due concerns before using 3D printing technology for complex organs and regeneration of complex tissues. The present work reviews the major technological advances taken place with the common 3D printing technologies: Fused Deposition Modeling, Stereo lithography, Selective Laser Sintering, Three Dimensional Printing and 3D Plotting/Direct-write/Bioprinting. The review highlights the advances in the field of tissue engineering of each of the aforementioned technology through examples and also highlights the key limitations. The authors hope that the review motivates the future research and therefore the scientific community advances the engrossing field for application in the field of biomaterials.

Keywords: *Tissue engineering, Biomaterials, Bioprinting, 3D Printing, Stereolithography, Selective laser sintering, 3D plotting, Fused Deposition Modeling, 3D Plotting.*

1. INTRODUCTION

One of the critical aspects in tissue engineering is the design and fabrication of complex biomedical devices. Biomedical devices has a wide range of applications such as construction of scaffolds for differentiation of stem cell, reconstruction of complex organs such as liver, lymphoid etc. and restoration of anatomical defects such as craniomaxillofacial complex that is caused by congenital defects, trauma and cancer. The defect requires functional nerves, bone, cartilage, glands, lymph nodes and muscles for its proper restoration.

Restoration of other functional tissues for regeneration of tissues of maxillofacial complex has been in recently explored via investigation of various approaches on tissue engineering. Scaffolds are the critical elements in tissue engineering providing space for extracellular matrix generation and remodeling, for infiltration and proliferation of cells, physical connections for injured tissue and to direct the cell behavior using biochemical cues. The design of scaffolds at the macro, micro and nano levels is also critical to ensure proper transport of nutrients and suitable conditions for interaction between cell and matrix [1-3]. The overall shape of the device i.e., anatomical features and organs constitutes the macro-architecture, the micro-architecture is reflected upon by the tissue architecture such as porosity of the structure, spatial distribution of the powdered material, shape and interconnection between the created pores and the nano-architecture is reflected in the surface modifications such as proliferation and interaction phenomenon.

There are number of challenges relating to the scaffolds such as suitable slection of the biomaterial to be used with a particular printing technology and the desired 3D shape. The commonly used biomaterials are metals, ceramics and natural and synthetic polymers with each biomaterial being unique in material, mechanical and chemical properties, method of processing, FDA approval and cell material interaction. Some of the common methods of producing wide range of pore sizes and hence porosity are electrospinning, gas forming, freeze drying and solvent casting with particle leaching. Although with the aforementioned methods, the micro-architecture is well understood and therefore is well controlled, the macro-architecture is confined to only 3D shapes and geometries determined by manual processing and molds. Another limitation is the ability to incorporate internal architecture such as curved channels.

The fabrication of patient specific 3D complex structures has been made possible using the solid free form fabrication technology (SFF). The fabrication of objects with control at macro and micro levels has been made possible using the integration of computer aided design, techniques for imaging such as computer tomography, magnetic resonance imaging etc., and rapid prototyping. The builds for individuals can be customized with the use of patient specific imaging [4, 5]. Surface modifications are taken care off in the post processing stage. Although there are certain conventional techniques of material techniques for effective scaffold engineering, the tissue engineering of complex maxillofacial tissues is highly effective using the SFF technology. However with certain advantages there are also limitations with the fabrication technologies the selection of which depends on

materials of interest, requirements of scaffold and limitations of the machine used.

Before proceeding further, the authors of the manuscript would like to clarify the usage of the terminology "3D printing" for the rest of the article. In the present work, 3D printing reflects upon all the SFF technologies as well as liquid-binder based inkjet technology.

The potential of 3D printing technology for the fabrication of biomedical implants and other features for tissue engineering is limited by the availability of the printable materials that are biodegradable and biocompatible. Therefore, alternative techniques for material processing are required to work with materials that are hard to be printable with the existing 3D printing technologies. However, for printable materials the 3D printing technology is advantageous in fabrication of customized patient specific devices with complex geometries the production of which is costly with the conventional methods of manufacturing such as that of injection molding.

Although, there have been tremendous advancements in the capability and resolution of the industrial 3D printers, the capability of the machines for biomaterials hasn't transformed to that extent. The 3D printers are now capable to build layers with thickness such as 16 μm with SLA and as high as 178 μm with FDM. However, these systems have still not been optimized for the biomaterial applications particularly for in vitro and in vivo interests and therefore advancements are still under progress to improve the SFF technologies for the biomaterial applications.

Since most of the advances are either home-made setups or the differnt available commercial machines modified by engineers of the modern world, it becomes difficult to compare the cost of each of these technologies. The comparison for the cost becomes easier when the available materials are adapted on a large scale by 3D printers. With the large scale adaption it is also easier to compare the ease of use for printing as well as post-processing. Most of the available printers today require sacrificial support materials with the available modeling materials requiring careful removal.

There has been an explosion in the popularity of FDM which is one of the SFF methods. The technology finds a wider scope of applications for home, small businesses and schools, producing parts with lower costs and lesser manufacturing complexity. The fabrication at home using the SFF technology has also become possible with the advent of low-cost scanners and the free CAD software. Since the inception of these technologies, the use has now expanded to mainstream use from the earlier industrial and academic use.

The present work focuses on the advanced 3D printing technologies used for the fabrication of tissue engineering scaffolds. Many of these technologies are in use for the fabrication of models. Important advances made in this field in the past few years have been highlighted in the presnt review work.

2. TISSUE ENGINEERING SCAFFOLDS AND 3D PRINTING

Layer-by-layer process is the key building process adopted for the fabrication of 3D biomedical devices by most of the SFF technology. The procedural steps involved in the fabrication include: (1) 3D model generation using CAD software that can be generated using CT scans or X-rays, (2) using a software to slice the 3D CAD model into build file of 2D images, (3) fabrication of the part by layer-by-process controlled by a computer, (4) surface modifications using any post finishing operations. Some of the complex 3D structures such as voids, undercuts, cantilevers etc. are reduced to piles of simple two dimensional structures such as circle, points and lines. The additive technologies are free from the tool path restrictions and therefore a higher level of shape complexity can be dealt with. The flexibility of the SFF technologies to produce complicated structures has led to its scope expanding from primary industrial applications to that for the biomedical engineering. A number of SFF techniques have been developed that have a very precise level of control for fabrication of macro-architectures as well as microstructures for applications in tissue and biomedical engineering. The different SFF technologies have the freedom in form in combination with the appropriateness to deposit material. This flexibility results in a better direction for the spatial distribution of the cells, scaffolding substrates and signals during the fabrication and thereby leading to a better control over triads in tissue engineering. The technologies also allow for better integration of the medical imaging data with the computer aided design models [5, 6]. The integration allows for the fabrication of the patient specific tissue engineering grafts that are an accurate match with that of the patients contour. Further, the advantages also allows for the fabrication of scaffolds meeting the structural, mechanical and nutritional requirements [7].

The present work reviews the five most popular SFF technologies with their applications for tissue engineering. The recent advances made in machine capability as well as printable biomaterials for each of the technologies are also presented in the review work.

2.1. Three Dimensional Printing

2.1.1. Technology and applications. Three dimensional printing (3DP) was invented at Massachusetts Institute of Technology. The 3DP technology uses inkjet to fabricate three dimensional structures by printing the solution of liquid binder on to the powder bed [8-10]. Since most of the biomaterials exist in liquid or solid state, a wide range of materials are available for use with the 3DP technology. The 3DP technology incepts with spreading of fine powder material across the piston evenly. The desired 2D pattern is produced with the synchronization of printhead with the X-Y positioning system depositing selectively the liquid droplets of the binder onto the powdered layer [11]. The next layer of powder is spread by lowering the piston, powder bed and the finished layer. The process is repeated until the completion of the entire part. The unbound powder removal reveals the fabricated structure. Manipulation of the local composition within the under process structure can be accomplished by controlling the printhead to deposit the required quantity of the binder material. The

alteration of printing parameters during the part fabrication can aid in controlling the local microstructures [12]. The additional seeding surfaces can be effectively distributed throughout the devices' interior by incorporation of micro-channels. The incorporation of the micro-channels increases the effective seeding uniformity and the density. The spatial control of the cell distribution is offered by patterned surface chemistry. The technology is however limited by the conflicting nature of the reliability of the printhead and resolution of the feature. This is because the small nozzles are capable to produce finer features but at the same time are more prone to clogging. The resolution for one-dimensional feature is limited to 100 μm and 290 μm for three dimensional features.

The 3DP technology can fabricate complex scaffolds such as hanging features or internal channels. This is because unbound powders are there to support such features. A highly porous scaffold was produced by Kim et al. [13] by 3DP technique and through this part they demonstrated the phenomenon of cell ingrowths into the scaffolds. The temperature sensitive materials for instance the different biological and pharmaceutical agents can also be added into the scaffolds because of the room temperature processing conditions [10]. Starch based scaffolds were printed using distilled water by Lam et al. [14] demonstrating the use of living cells and biological agents during fabrication. The multi-color printing is another major capability of the 3DP technology for tissue engineering applications where each color ink can be precisely positioned as desired. This feature of the 3DP technology provides it capability to deposit extra multiple cellular matrix materials, simultaneous arrangement of multiple cell types and providing for point-to-point control over the bioactive agents. Thus 3DP technology is relatively more flexible then the other SFF technologies. 3DP technology has printed a wide range of biological agents such as proteins, peptides, DNA plasmids, polysaccharides and even living cells. However, machine modifications are required for using these biological agents for printing. The cells are required to be kept in suitable environment that has favorable temperature, oxygen and supply of nutrient.

Some of the other prominent materials that have been employed in direct 3DP technology consists of powder that are composed of synthetic polymers such as poly (ε-caprolactone) or poly(L-lactic acid) or polylactide-coglycolide with binder material as organic solvents [10,13,15]. The natural polymeric powders such as dextran, gelatin and starch with water as binder have also been in use for 3DP technology [14, 16]. In the case of indirect 3DP technology the mold is printed and is then casted with polymeric and prominent porogen materials. The available plaster powder in the market such as calcium sulfate hemihydrates powders with water as binding agent is used for printing the mold. The printed mold is then cast using slurry made up of biodegradable polymer dissolved in porogen material such as polylactide-coglycolide in chloroform in NaCl solution [17, 18]. Hydroxyapatite (HA) powders have been used for the fabrication of ceramic scaffolds with interconnected channels that have been used for bone replacement [16]. The 3DP technology allows for the fabrication of customized HA structures on the basis of patient specific medical information. Temperomandibular joints (TMJ) an example of hybrid tissue systems can be regenerated with the fabrication of biphasic scaffolds. Osteochondral composite structure has been fabricated by Sherwood et al. [19]. The lower region of the structure is composed of LPLGA/TCP composite whereas the upper region from D,L-PLGA/L-PLA. Thus the upper region functions for regeneration of cartilage and the composites' lower region promotes bone ingrowth. The potentiality of indirect 3DP technology to fabricate zygoma scaffold using the data obtained from CT scans was demonstrated by Lee et al [17]. Therefore the patients with zygomatic bone fractures can be treated using the 3DP technology.

The indirect 3DP technology allows for casting of different materials under the similar printing process parameters while in the case of direct 3DP technology, the optimization of individual process parameters is required to maximize the build resolution. One of the major advantages of the conventional or direct 3DP technology is the direct control over macroarchitecture and the microarchitecture. A higher interconnectivity amongst the pores, defined pore size and uniformity in porosity are achievable for prints using porogen as the powder. The scaffolds supporting hepatocyte ingrowth can be fabricated using this method [13]. The direct 3DP technology doesn't require any demolding which is the case with indirect 3DP. However, the tendency of the organic solvents to dissolve printhead is one of the major limitations for direct 3DP technology. Therefore to mitigate the aforementioned limitation, stencils are used to pattern the polymeric solutions onto the porogen particles for the fabrication of scaffolds [13]. The use of stencils however results in restriction to the fabrication of small and complex shaped features. The printheads compatible with the organic solvents and of high precision are now available but they are modified for a limited range of polymeric solutions. To maintain the strength of the printed part, the thickness of the layer must be more than the porogen particle size but must be smaller than the maximum threshold of 150 μm [12]. Therefore larger pore size must be printed that can overcome the porogen particle size limit. The limited availability of pore size is another limitation of 3DP technology which occurs while incorporating porogens into powders prior to the construction [15]. Further, while using degradable polymers as powdered material, the shape complexity of the scaffolds also gets limited. Therefore the 3DP technology requires the use of organic solvents as binders for the powder in the form of degradable polymer.

Many of the limitations suffered by direct 3DP technology are overcome by the indirect 3DP process. The indirect 3DP eliminates the need of stencils as the indirect 3DP technology makes use of aqueous binder and therefore the inkjet printers commonly used can be conveniently employed [17]. The interconnectivity between the layers and the resolution of the final part is not affected by the porogen size. The indirect 3DP technology can be used for the production of small features with higher aspect ratio as for instance small intestine villi and also for the fabrication of highly porous scaffolds on a bulk scale as for instance anatomically shaped zygoma scaffolds [18].

As a whole, the main advantages of the 3DP technology are the availability of the materials to be printable at room temperature conditions, the capability to print internal features and overhanging constructs and the process has a control over the microstructure. The main disadvantage associated with the 3DP technology is the removal of unbound powders from small or curved channels.

2.1.2. Recent advances in material and technology. The prominent materials available for the use with 3DP technology includes HA and TCP [21-25], calcium polyphosphate and PVA [20], TCP with doping of SrO and MgO [30, 31], TCP [26-29], calcium phosphate with collagen incorporated in binder [33], HA and apatitite-wollastonite glass ceramic with binder as water or water-based [32], Ferringtonite powder [35] and PLGA [34]. In vitro studies have been conducted with bovine chondrocytes [20], monocytic cells [22], bone tissue engineering [21, 22, 25-26, 37], human osteoblasts [23, 29, 32, 34], bone marrow stromal cells [36] and C2C12 pre-myoblastic cell line [24]. The in vivo studies on the other hand include rabbit calvarial bone [26], rat femoral defects [28, 30], rabbit tibia bone [24], rabbit femoral bone [31] and mouse femoral defects [33].

2.2. Fused Deposition Modeling

2.2.1. Technology and applications. The Fused Deposition Modeling (FDM) technology uses extrusion heads for the deposition of molten thermoplastic materials. The extrusion heads consists of a small orifice for laying down the specific pattern [38]. Out of the two extrusion heads, one is used for the deposition of thermoplastic material whereas the other is used for deposition of temporary material. The temporary material provides support to the cantilevers. In one of the conventional approaches used by FDM technology, the thermoplastic material is melted to semi-liquid state and then deposited by the extrusion head on the build platform [39]. The part is created adopting the layer-by-layer process. The layers are fused together to produce the final product. The FDM technology offers the usage of multiple extrusion heads and therefore there is no restriction for the compositional gradients in all three dimensions. The use of multiple extrusion heads is however still a restriction for the practical applications. The choice of suitable material for use in FDM depends on heat transfer characteristics and behavior of fluid flow. The most commonly used material is thermoplastics that have low melting temperatures. The other materials that have been used successfully include nylon, PVC, wax and ABS. PLC has low melting temperature of approximately 60℃, possesses the similar glass transition temperature and also has high thermal stability [38, 40]. These characteristics make PLC one of the suitable materials for biomedical applications. Another material that has been in use for the fabrication of scaffolds is the PLGA. However, PLGA has higher glass transition temperature which demands for the higher extrusion temperature for its processing [41, 42]. For creation of better flow characteristics for material from the extrusion nozzle and also for better fusion, material is required to be heated ranging 110-140℃. Rheological modifiers if used must be biocompatible. The input process variables that are controllable are raster gap width, raster thickness, layer thickness and raster angle. The inputs controlled will aid in yielding scaffolds with the required pore size, interconnectivity and morphology. In order to ensure a proper fusion of the new extruded material with the previously deposited material, the extruded molten material must have the required level of hotness. Further, the viscosity of the material is also another critical parameter for ensuring that the extrusion process takes place effectively. The flexibility in controlling the movements along the x-y directions of the extrusion head has lead to the fabrication of scaffolds using the biocompatible materials with the desired pore sizes and morphology [38]. Combination of materials such as poly(ethylene glycol) terephthalate/poly(butylene terephthalate) or polypropylene/TCP can also be used for the fabrication [43, 44]. Combination such as PCL/TCP or PCL/HA can be used that results in the mechanical and biochemical properties favorable for the regeneration of the bone [45]. The FDM technology produces components with high porosity and good mechanical strength. The major limitation of the FDM technology is to the use of only thermoplastic materials that possesses relatively good viscosity. The good viscosity results in regular biological scaffolds [40]. The FDM technology is also incapable to include during extrusion the living cells or certain biological agents that are temperature sensitive. This is because of higher processing temperature.

2.2.2. Recent advances in material and technology. The commonly used materials by the FDM technology are the biocompatible polymers that possess low melting temperatures. Some of the biocompatible polymers used for the fabrication of scaffolds include: TCP-PCL with gentamicin [48], PLGA with collagen infiltration [47], PCL and bioactive glass composites [46], PCL-TCP [49], L-lactide/e-caprolactone [46], PCL-PLGA-TCP [50], PLGA-TCP and coated with HA [42], PLGA-PCL [51], PMMA [55], PCL [53, 54], PCL coated with gelatin [52], PLA [46]. The in vitro cases have been conducted for the following: porcine chondrocytes [47], mesenchymal stem cells derived from bone marrow [53] and mouse pre-osteoblasts [52]. On the other hand the in vivo studies were done for craniofacial defect [49], murine animal models for healing of wounds [48] and rabbit bone defect [42, 50]. Some of the typical applications include: antibiotic delivery system [48], cartilage tissue engineering [48], bone tissue engineering [13] and osseous craniofacial defects [49, 55]. The filaments for FDM technology are gradually increasing in number but the material choices for the biomedical application and compatible with FDM are inferior in comparison to that for the conventional injection molding. One of the feasible options and future research scope to increase the number of material alternatives is the integration of the precision injection molding with the 3D printing technology. The integration has the potential to process most of the thermoplastic materials existing as injection molding pellets without undergoing the pre-processing phase of conversion to fine powders or traditional FDM filaments. The integration is basically a mold free injection molding producing patient specific and customized medical devices [52].

2.3. STEREOLITHOGRAPHY (SLA)

2.3.1. Technology and applications. Stereolithography (SLA) is one of the oldest rapid prototyping processes which were

developed in the late 190s [57]. The spatial control of the polymerization of the photocurable resins is accomplished using HeCd-laser beam [58]. The uncured layer then deposits next on the cured layer. The patterning then starts from the top layer. In case of top-down approach [59] a transparent plate is positioned below the vessel holding the resin. Light is projected onto to the plate that is transparent in nature. The layered pattern is formed onto the transparent plate. The cured layered structure is raises from the transparent plate and the new uncured resin fills the space between the cured layer and the plate. A masked lamp technique was then employed to cure the entire layer of the material at a time. This technique is applied in replacement to the conventional method of rastering laser beam and is used for the fabrication of large parts. The unpolymerized resin is removed by draining on completion of the entire structure. The structural part is further strengthened by post-curing in an oven that converts any unreacted groups [60].

One of the critical aspects is the kinetics of the curing reactions during the polymerization process. The thickness of the polymerized layer and the time taken to cure it depends on the kinetics of the curing reactions. The kinetics can be controlled by the scanning speed, power of the light source and amount and chemistry of the monomer and photointiators. Further, the depth of polymerization can be controlled by addition of UV absorbers to the resin [61].

The materials used with SLA for the structural fabrication must possess photocurable moieties for photo-crosslinking. Acrylics and epoxies are examples of materials used in SLA. However, only scant materials are available for tissue engineering applications that are biodegradable, biocompatible and are show dimensional stability towards photo polymerization. Poly(propylene fumarate) (PPF) is one of the materials used with SLA for the fabrication of scaffolds. The use of PPF produces scaffolds with controlled microstructure and used for the reconstruction of rabbit cranial defects [62]. To reduce the viscosity of resin, reactive diluents in the form of N-vinyl-2-pyrrolidone or diethyl fumarate is to be added with PPF. The low viscous resin ensures proper conditions of processing [63]. However, a significant amount of non-biodegradable component is introduced with the diluents. SLA has also processed resins with and without bio-ceramic dispersions.

Fabrication of models for better planning of surgery is another medical application of SLA process. SLA patterns for molds are also in use for indirect fabrication of medical devices [64, 65]. Electrical discharge machining of ingots from titanium has led to the fabrication of titanium dental implants, the machining process being carried on the basis of SLA model. SLA has numerous advantages such as the ability to fabricate complex shapes featuring internal architecture, high resolution for features and easy removal of unpolymerized resin [66]. The availability of biocompatible resins with the required properties suiting SLA process is one of the main limitations for the SLA process. The other disadvantages are the use of cytotoxic radicals and photointiator and inability to form compositional gradients along horizontal planes. Further, the residual photointiator and the unreacted monomers may get entrapped in the final structure. The photopolymers used for the tissue engineering applications possesses poor mechanical properties which also add to the disadvantage for SLA process. The SLA process requires the temporary support structures for the fabrication of unsupported features and the removal of these structures is a difficult task.

2.3.2. Recent advances in material and technology. The photocrosslinkable polymers are growing in numbers with the advances in the field of SLA technology. The recent improvements has allowed for the use of multiple types of resins for one build. Polymers with aliphatic polyesters have been synthesized and therefore adding to the list of biodegradable polymers. The patterning of PEG-DA and PEG-DMA with fluorescently labeled bioactive PEG or dextran for the fabrication of scaffolds shows the capability of SLA to use multiple types of resins for one build [67]. For the use of more than one resin material, the scaffold is removed from the container with resin and is rinsed with distilled water while a new resin material replaces the old resin material in the container. A lateral resolution of approximately 2 μm and vertical resolution of approximately 1 μm is now achievable for PPF resin using dynamic mask projection SLA [68]. Detailed microstructures are producible using the technology the challenges for the creation of horizontal channels and prevention of shrinkage are still major hurdles.

The resins with biodegradable moieties have also added to the library of available materials to be processed with SLA. Examples of novel macromers that have been synthesized are PCL [69] or poly(D,L-lactide) [63, 70-71]. The PLLA (Photo-curable poly(D,L-lactide)) resins have been developed and used by the SLA technology [70]. The photo-crosslinking capability is being possible by modification of end groups to acrylate or methacrylate. PPF-DEF [74] and PPF-DEF with BMP-2 loaded PLGA microspheres are another resins that have been used for the fabrication of scaffolds using SLA technology [72, 73]. For μSL i.e., for resolution less than 5 μm PPF-DEF or PPF-DEF with HA has been used [68, 75]. For Cartilage tissue engineering applications [77] that require materials that are elastic and flexible with stiffness ranging 22-156 kPa, macromers such as poly(trimethylene carbonate) are used [76]. Application of more stiffer structures include human umbilical vein endothelial cells [78], MC3T3-E1 pre-osteoblasts [63, 72, 74], rat bone marrow cells [73], human mesenchymal stem cells [71] and in vitro with mouse pre-osteoblasts [70]. Bone tissue engineering [79] is one of the major applications of SLA and the studies for in vivo cases have shown an accelerated regeneration of bone for cranial defects in rats [74]. The in vitro studies have been conducted with bovine chondrocytes that are part of softer and flexible tissue engineering applications [77]. The SLA-controlled architecture for pore network produced scaffolds have improved cell seeding and culturing in comparison to the scaffolds made from salt leaching with PEF-DEF and poly(D,L-lactide) [73, 80]. PEG-DA with NIH/3T3 cells [81] has shown cell encapsulation during SLA.

2.4. Selective Laser Sintering

2.4.1. Technology and applications. Texas University is credited with the development of the selective laser sintering (SLS)

technology. The SLS process is similar to the 3DP technology using powder particles to form a layer. The use of CO2 laser beam by SLS process is the only distinguishing factor that distinguishes the SLS process from the 3DP process [83]. The powdered polymeric particles are scanned by the laser beam in a specific 2D pattern which is then sintered above the glass transition temperature. Neck formation occurs between the neighboring particles as a result of molecular diffusion that takes place along the outermost surface of the polymeric particles. The piston containing the structure is lowered on completion of one layer and a fresh layer of powdered material is then spread over the completed layer. The new layer is then bounded with the previously formed layer. Once the entire part is completed the remaining unbound powder is removed and the finished part is heated so that the finished part acquires the desired density. The unbound solid particles are capable to support any cantilever structure and therefore the SLS process doesn't require any temporary support structures. The porosity between the original particles is preserved since the sintering process doesn't completely melt the powdered particles completely.

For most of the materials, the solid state sintering can be achieved for temperature ranging (0.5-1) $T_{melting}$. The electron beam melting (EBM) and selective laser melting (SLM) melts the powdered particles above $T_{melting}$ by using energy intensive beam. As a result of melting above $T_{melting}$ the particles completely fuse to form a fully-dense structure. The melting is easily accomplished if all the powdered particles have the similar melting temperature. Therefore the usage of pure metallic powders leads to easy melting of the powdered metallic powder in comparison to the alloy powdered particles.

The factors determining the resolution of the final feature produced are the size of the powdered particles, heat transfer in the powder bed and the diameter of the laser beam. The particle size is limited to 10 μm because of the poor spreading of the particles and too quick sintering leading to the edge inaccuracies. The most commonly used materials are combination of Hydroxyapatite and polyether ether ketone and PLC [84-87]. Thin solid disks are commonly obtainable using biomaterials.

Thermoplastic and previously coated ceramic powders are used with the SLS process. The extremely high melting point and glass transition temperature of ceramics demands the addition of intermediate binding materials. The melting of the binding material takes place before the ceramic powdered particles and therefore the binding particles fuses with the ceramic particles. Calcium phosphate bone implants were fabricated by Tan et al. [88] by sintering of calcium phosphate powders with polymeric material. The post processing operations such as extra sintering in an oven will increase the strength of the finished part but at the same time shrinkage of the parts takes place. Hydroxyapatite with polyvinyl alcohol (PVA) forming a biocomposite blend was also used in the SLS process [89]. Spray drying or physical blending was adopted to coat HA particles using water soluble PVA. The biocomposite was then used for the applications involving joint and craniofacial defects. PCL scaffolds with porous architecture were fabricated by William et al. [90]. The fabricated scaffolds had excellent mechanical properties and were used for bone tissue engineering applications.

The SLS technology is also used to create patient specific structures using the available medical data. The CT scan data from the pig condyle [90] was used for the fabrication of mandibular condyle scaffolds. The scaffolds with porous interior structures and anatomically shaped external architectures are now easier to fabricate with the integration of SLS techniques and computational design. A titanium mandible was created using the SLM process which was patient specific and is used for supporting the mandibular denture [91].

The ability to make metallic implants directly is one of the key advantages of SLS/EBM/SLM process. The implants produced have sufficient mechanical strength and fracture toughness. These metallic implants are used for promoting bone regeneration and ingrowth for the different load bearing applications. The compositional gradients across the vertical planes are easily achievable in SLS and SLA processes. However, the gradients of the compositional structure in the horizontal plane are limited. Availability of the materials that can fuse together and doesn't decompose under the influence of laser beam is one of the key disadvantages of the SLS technology. Further, the resolution achievable in the final product is restricted because the diffusion of the latent heat results in unwanted fusion of neighboring powdered particles. Also the smallest pore size achievable is limited because the created pores are dependent on the particle size of the powder used. Usage of too small powder size leads to poor spreading of the powder because of the particle clumping.

2.4.2. Recent material and technology advances. The major advancement in the field of SLS technology is the ability to fabricate features with higher resolution and scaffolds with lower stiffness. The PLC scaffolds are now producible with stiffness ranging 300-450 kPa [87] which was earlier used to be in the range 14.9-113 MPa [85, 86, 90, 92]. The lower stiffness makes these scaffolds suitable for the applications of soft tissue engineering such as cardiac tissue.

Fabrication of functionally graded scaffolds with the controlled porosity is another advancement which has been achieved using polyhedrals. The processed porosity is then demonstrated with PCL in SLS [93]. There has been a thorough review on design and development of the microarchitecture [94]. The design and prediction of the properties for the fabricated microarchitecture has been possible with the integration of FEA with SLS technology [92, 95].

The commonly used materials for SLS technology are PCL and HA [92, 96-97], CHAp/PLLA and Ca-P/PHBV [99, 100], β-TCP and PLC with collagen coating [98] and PVA [101]. The encapsulation of biomolecules was demonstrated by processing of BSA encapsulated in Ca-P/PHBV microparticles [102]. The in vitro studies have been performed on human bone marrow stromal cells [103], C2C12 myoblast cells for the application of cardiac tissue engineering [87], human osteoprogenitor cells [52], SaOS-2 cells [99], MG-63 [101] AND porcine adipose derived stem cells [98, 104]. The in vivo studies conducted on mice have shown better vascular and woven bone formation [98]. The applications

include interbody cages for spinal fusions and bone tissue engineering [97].

2.5. 3D Plotting/Direct-Write Bioprinting

2.5.1. Technology and applications. Freiburg Materials Research Center has been credited with the development of 3D plotting in the year 2000. This was used for the fabrication of soft tissue scaffolds. Viscous liquid material such as paste, solution or dispersion is extruded from a pressurized syringe into a liquid medium. The desired three dimensional shape of polymers, hydrogels or ceramics are obtained by deposition of the material from the syringe nozzle either in the long continuous strand form or in the form of individual dots [105]. The 3D plotting process can be carried either at room temperatures or at elevated temperatures and doesn't involve any thermoplastic as in FDM.

The 3D plotting process is mainly used for the creation of hydrogels from natural biomaterials. Thermoreversible natural polymers were employed by Landers et al. [106, 107]. In this case, the solution consisting of agar and gelatin is heated and extruded at a temperature of approximately 80°C into a cooler liquid medium, approximately at a temperature of 20°C. The cooler liquid medium of gelatin or silicone oil quickly solidifies the extruded material. Another approach is the extrusion of polymers into liquid medium consisting of reactants promoting crosslinking as for instance the extrusion of gelatin into a reservoir of Ca2+ ions [108]. For materials such as TCP, the solution is made with water and is then extruded from the syringe nozzle. The liquid is then removed following lyophilization process to produce a strut with diameter of approximately 400 μm [109].

The main advantage of the 3D plotting technology is the availability of wide range of materials and the processing conditions. The limitation is to produce complex shapes with overhang features is one of the key disadvantages of the technology. Further, the low stiffness of the created hydrogels may result in collapse of the structures.

Bioprinting is another SFF technology that fabricates hydrogels with direct incorporation of the cells. The different cell printing strategies incorporates the cells directly into the printing process. The prominent examples include alginate cell solution extruded from syringe [110], printing of bovine vascular endothelial cells using electrostatically driven ink jet printing in culture medium [111], printing of embryonic chick spinal cord cells using laser writing technique [112] and forward transfer of cells suspended in alginate solution using laser-induced printing strategy [113]. The bioprinting technology offers for the controlled spatial distribution of the cells. The fabrication technology is only limited to the use of hydrogels such as fibrin and alginate which are not suited to implantations in biological environment that demands for stronger mechanical properties. Further, the technology suits the materials with low viscosity. The collapse of the structures is prevented as a result of buoyancy created due the density match between the extruded material and the cooler liquid medium. The thickness of the strand can be controlled by controlling the viscosity of the material, speed of deposition, diameter of extrusion tip and pressure applied.

The key advantages of the bioprinting process involve the room conditions for processing, homogeneous spatial distribution of cells and direct incorporation of cells. Some of the major disadvantages of the bioprinting technology is the low resolution of the finished parts, limited mechanical stiffness, specific matching of density of the extruded material and the liquid medium and controlling of the critical timing for gelatin time.

2.5.2. Recent advances in material and technology. Some of the commonly used materials for bioplotting are PLGA, collagen and chitosan [109], TCP, chitosan [115], soy protein [117, 118], agarose with gelatin [117] and Collagen-alginate-silica composites coated with HA [116]. Some of the in vitro studies conducted include that on human mesenchymal stem cells [117] and mouse pre-osteoblasts [116]. Ovine cavalarial defects are one of the examples for in vivo studies [109]. Tissue regeneration [118] and bone tissue engineering [109, 116] are the typical applications.

The commonly used bioprinting materials are human skin fibroblasts and human umbilical vein smooth muscle cells (HUVSMCs) [119], rat primary bladder smooth muscle cells in droplets of collagen [114], gelatin-HA-tetraPEG-DA with NIH 3T3s [120], alginate droplets [108] and human micro-vascular endothelial cells in fibrin [121]. Vascular tissue engineering is the typical application of bioprinting [108, 119-121].

The ability to print a single cell and cell-laden scaffolds from hydrogels are the recent advancements. Block-Cell-Printing demonstrates the high throughput printing of single cell arrays [122]. The trapping of single cells was accomplished using microfluidic arrays of hook-shaped traps. The cell communication was then studied by separating the trapped cells by 5 μm or by pairing the trapped cells. Culturing of the trapped primary rat cortical neurons was studied. High density cell-laden hydrogels were bioprinted for creating a structure by Ahn et al. [123] by extruding the cell-alginate solution at 4°C onto a -10°C stage. The strength of the structure was enhanced by crosslinking of alginate and incubating the structure in a CaCl2 solution. A 3D structure was formed by layer-by-layer deposition of chondrocytes laden hydrogels droplets and PCL alternatively [127].

Simultaneous printing of the different pharmaceutical and biological agents is one of the recent advancements in the bioprinting technology.

3. FUTURE SCOPE

The advances are still required in the field of 3D printing technologies for producing higher resolution structures that have sufficient strength, shape and handability. A resolution limit of 200 μm in scaffolds for transporting oxygen to cells has been observed in case of diffusion consumption modeling. This has resulted into maximum of 395 μm diameter features for survival of cellular features [128]. The challenges for creation of stronger structures without increasing the dimensions still exist for the 3DP and SLS technology. The powdered particles must be properly bounded together for the small features to survive the fabrication process. With the increasing laser power or the quantity of binder material, the dimensions of the part will grow undesirable as more

powdered binder particles will bind with each other. Some additional efforts are required to enhance the capability of the SLS and 3DP processes to fabricate the features with resolution below 400 μm. Further, the size of the powdered binder particle should also be investigated so that no trapping takes place in small channels. One of the approaches may be to create powdered particles in spherical shape which will facilitate in their removal from light spaces.

The number of materials with biodegradable and biocompatible characteristics is limited in numbers. Advances have led to the development of macromers with biodegradable moieties but still clearances from various authorizing institutions and organizations remains a challenge.

The different SFF technologies highlighted in the present work have the potentiality to fabricate macroarchitecture, but the future work is required to extend their capability to nanoarchitecture such as fabrication of biochemical molecules. The future demands to develop strategies for the incorporation of the biochemical molecules directly into the tissue engineering scaffolds.

4. CONCLUSIONS

The present work although reviews the different SFF techniques and the biomaterials used for fabrication of different biological structures, work on byproducts of the material and degradation kinetics cannot be ignored. However, the authors of the work are of the opinion that the present review will provide an exposure of the fabrication techniques used for the biomaterials in fabrication of features of biological importance. With the advancements the focus has now shifted towards development of inks for bioprinting of bioengineering structures and several studies are now being conducted in this regard [129-131]. These have led to the creation of ultra low resolution features for bioengineering applications.

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