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Fabrication of Tissue Engineering Scaffolds Using Rapid Prototyping Techniques

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***Abstract*—**Rapid prototyping (RP) techniques are a group of advanced manufacturing processes that can produce custom made objects directly from computer data such as Computer Aided Design (CAD), Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) data. Using RP fabrication techniques, constructs with controllable and complex internal architecture with appropriate mechanical properties can be achieved. One of the attractive and promising utilization of RP techniques is related to tissue engineering (TE) scaffold fabrication. Tissue engineering scaffold is a 3D construction that acts as a template for tissue regeneration. Although several conventional techniques such as solvent casting and gas forming are utilized in scaffold fabrication; these processes show poor interconnectivity and uncontrollable porosity of the produced scaffolds. So, RP techniques become the best alternative fabrication methods of TE scaffolds. This paper reviews the current state of the art in the area of tissue engineering scaffolds fabrication using advanced RP processes, as well as the current limitations and future trends in scaffold fabrication RP techniques.

***Keywords*—**Biomanufacturing, Rapid prototyping, Solid Free Form Fabrication, Scaffold Fabrication, Tissue Engineering

I. Introduction

TISSUE engineering (TE) or regenerative medicine is an interdisciplinary field that applies the principles of engineering and life sciences and aims at restoring or regenerating a damaged tissue by combining cells, derived from a patient biopsy, with scaffolds [1]. These Scaffolds provide a framework for cells to attach, proliferate, and form extracellular matrix. The scaffolds may also serve as carriers for cells, growth factors, and/or other bimolecular signals [2]. Successful TE scaffold should have the basic requirements shown in Fig. 1.

The ability to control scaffold architecture, material composition and porosity through design and fabrication could be a critical factor in the future clinical success of tissue engineering [3]. Because optimum scaffold doesn't obtained yet, there are many research efforts to fulfill desired scaffold requirements by enhancing scaffolds design, material and manufacturing processes. Actually, there are various conventional and manual based techniques used for scaffold fabrication such as solvent casting and gas forming. However, these methods cannot produce scaffolds with controlled internal architectural features. Additionally, the resultant scaffold architecture is highly dependent on the process [4]. As a result, RP techniques are considered the best alternatives for achieving precise control of pore size, geometry and interconnectivity.

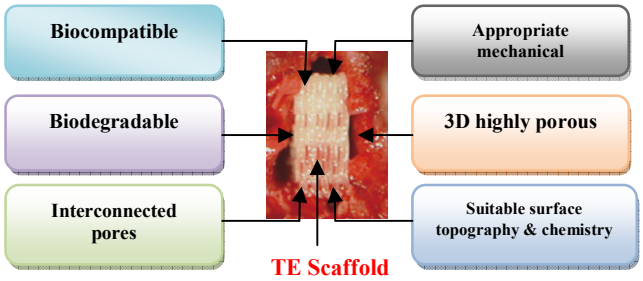

Fig. 1 Basic requirements of TE scaffold.

Rapid prototyping (RP) , generally known as solid freeform fabrication (SFF) or additive manufacturing (AM), is a group of advanced manufacturing processes in which objects can be built layer by layer in additive manner directly from computer data such as Computer Aided Design (CAD), Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) data. Scaffold fabrication is one of the earliest applications of RP and now it becomes more successful and mature area. That's because the ability of RP technologies to incorporate advanced RE and CAD techniques to produce complex models and customized parts. The main scaffold fabrication steps by RP techniques are shown in Fig. 2. Generally, the fabrication process starts with a 3D design of the scaffold, Afterwards, the design is transferred into a .STL (stereolithography) file format where it is virtually slices into thin, virtual, horizontal cross-sections and finally the file transferred to RP machine and the scaffold is directly fabricated layer by layer.

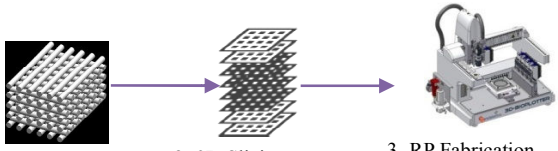

Fig. 2 Main scaffold fabrication steps using RP techniques.

The purpose of the present work is to introduce a comprehensive review of the recent developments in the area of RP technologies in direct fabrication of TE scaffolds. In the first sections we review the most successful of RP technologies in direct scaffold fabrication. Each technique will be descried and followed by recent research activities in using of this technique in scaffold fabrication. Finally, we discuss

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the existing limitation and future prospects.

II. Rapid Prototyping techniques for Tissue Engineering scaffolds fabrication

A. The extrusion-based RP techniques

The extrusion-based RP technique is also known as Fused Deposition Modelling (FDM) in which a thin thermoplastic filaments are melted by heating and guided by an extruder controlled by a computer, to form 3D objects. The material leaves the extruder in a liquid form and hardens immediately. The previously formed layer, which is the substrate for the next layer, must be maintained at a temperature just below the solidification point of the thermoplastic material to assure good interlayer adhesion [5]. The major limitations of FDM are the use of filament-based materials and the high heat effect on raw material. To overcome some of these limitations, alternative extrusion-based processes have been proposed like 3D Fiber Deposition (3DF) [6], Bioplotting [7], precision extruding deposition(PED) [8] and other techniques we will describe through the review context.

The feasibility of FDM to fabricate porous customized freeform structures of medical-grade polymethylmethacrylate (PMMA) was investigated by Espalin et al. [9]. It was found that, by enabling the use of PMMA in FDM, medical implants such as custom craniofacial implants can be directly fabricated from medical imaging data improving the current state of PMMA use in medicine. Yen et al. [10], also employed FDM in the production of poly (D,L-lactide-co-glycolide) (PLGA) scaffolds filled with type II collagen and evaluated the cellular proliferation and matrix deposition of these hybrid scaffolds. SEM of the pure and hybrid scaffolds are shown in Fig. 3.

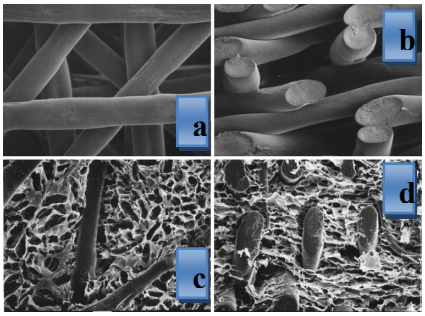

Fig. 3 SEM of pure PLGA scaffolds (a, b) and hybrid scaffolds (c, d) produced by FDM [10].

Recently, Tellis et al. [11] produced polybutylene terephthalate (PBT) scaffolds groups with various pore structures by FDM. They used compression testing and Micro CT to compare compressive stiffness, porosity, connectivity density, and trabecular separation of each scaffold to a natural bone sample. In another approach, Geffre et al. [12] compared bone ingrowths into macroscopic PBT porous scaffolds fabricated by FDM, which had either a simple pore structure or a complex pore structure mimicking the native tissue architecture.Woodfield et al. [6], on the other hand, developed the 3D Fiber Deposition (3DF) system. Which is an FDM-like technique in which a molten hydrogels, thermoplastic polymers and biomaterial pastes are extruded from a CAM controlled robotic unit on a stage in the form of a fibre. 3DF was utilized to produce poly (ethylene glycol)-terephthalate/poly (butylenes terephthalate) (PEGT/PBT) block co-polymer scaffolds with fully interconnecting pore network for engineering of articular cartilage. By varying the co-polymer composition, porosity and pore geometry, scaffolds were produced with a range of mechanical properties close to articular cartilage. The scaffolds seeded with bovine chondroccytes supported a homogeneous cell distribution and subsequent cartilage like tissue formation [6]. Li et al. [13], On the other hand, produced a porous Ti6Al4V scaffolds, (Fig. 4) with fully interconnected pore networks, highly controllable porosities, and pore sizes by 3DF. The Ti6Al4V powders (68 vol %) were mixed with an aqueous solution of methylcellulose (0.3 wt %) as binder and satiric acid (0.2 wt %) to improve the rheological properties of the slurry. They concluded that 3DF is a promising technique for the design and fabrication of custom made Ti6Al4V scaffold architectures for orthopaedic implant applications.

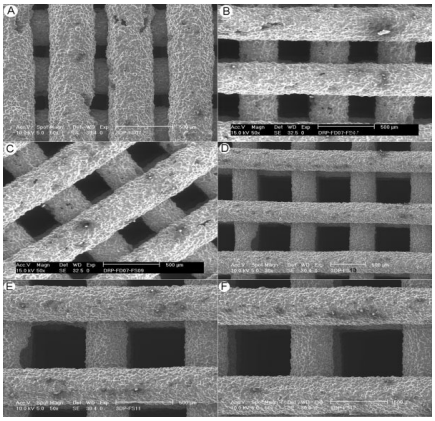

Fig. 4 Surface morphologies of 3DF Ti6Al4V scaffolds built with varying fiber spacing from 0.2 to 0.7 mm in increments of 0.1 [13].

Woodfield et al. [14] fabricated anatomical femoral and tibial cartilage constructs by 3DF. They evaluated produced scaffolds in vitro and in vivo in an autologous rabbit model and found that Porous, interconnected 3DF scaffold architectures enhanced chondrocyte attachment and re-differentiation capacity while exhibiting mechanical properties similar to native articular cartilage explants.

To overcome filament preparation problem in FDM, a variation of FDM called precision extruding deposition (PED) for fabrication of bone tissue scaffolds were developed by Wang et al. [8]. In PED , material in pellet or granule form is fed into a chamber where it is liquefied. Pressure from a rotating screw forces the material down a chamber and out through a nozzle tip. This process was used by Shor et al [15] to directly fabricate polycaprolactane (PCL) and (PCL–hydroxyapatite, HA) composite tissue scaffolds. Similar work was conducted by Yildirim et al. [16] to fabricate (PCL) scaffolds with a 0/90° strut configuration with 300 μm pore

size, 250 μm strut width and were treated with oxygen-based plasma in order to increase the cellular activity.

Low temperature deposition manufacturing (LDM) is another modified version of FDM developed by Xiong et al. [17] to overcome the heating and liquefying processing of materials. The system comprises a multi-nozzle extrusion process and a thermally induced phase separation process. LDM was recently used by Li et al. [18] to fabricate individualized tissue engineering PLGA/ tricalcium phosphate (TCP) composite scaffolds based on alveolar bone defects. Mäkitie et al. [19], also assessed the viability of (PLGA/TCP) composite scaffold generated with LDM (Fig. 5) in a 3D cell cultivation, in vitro.

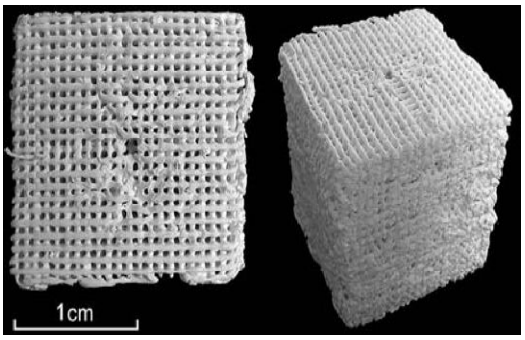

Fig. 5 PLGA–TCP scaffold manufactured by LDM [19]

Another variants of the FDM technique is Bioplotting, developed separately by Freiburg Materials Research Centre, Germany, and marketed by Envision Technologies GmBH, Germany, [20]. In bioplotter technique, a micro needle is employed as the extrusion nozzle where liquids, pastes, melts, solutions, hot melts, reactive oligomers or dispersions which are initially stored in a heated cartridge, are extruded into a temperature controlled liquid dispensing medium. The dispensing medium induces solidification of the deposited material by cooling, heating or through chemical reaction. Also, by using a dispensing solution of similar density as the building material, the buoyancy exerted by the medium on the build can prevent the collapse of complex structures thus eliminating the need for sacrificial support structures which are typical in conventional FDM systems [20]. Most recently, Various studies were carried out using bioplotting technique to control the scaffold architecture in order to obtain better results in terms of combining enhanced tissue growth with adequate mechanical properties [21]-[24]. In these studies, Scaffolds mechanical properties, cell growth, and structure morphology were evaluated and characterized. Son and co-worker [25],[26] , modified the bottom plate of a commercial bioplotter so that it was vibrated by a piezoelectric transducer (PZT). By this modification, scaffolds with rough surface strands can be obtained and as a result they fabricate 3-D polymeric scaffolds with enhanced compressive modulus, initial cell attachment and proliferation (as shown in Fig. 6) without any chemical or biological treatment. Another modification in 3D bioplotter was also introduced by Hee et al [27]. They designed an oscillating nozzle system for the 3D plotter, in order to increase the elastic modulus and yield strength of the strand in the scaffold.

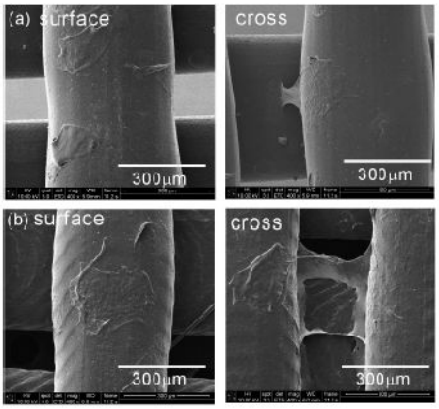

Fig. 6 Cell attachment of bioplotted PCL after 7 days (a) on the normal strand and (b) on the modified strand produced with a vibration of 30 Hz.[25], [26]

Daoud et al. [28], developed a bioplotted microfabricated PLGA Scaffolds with controlled pore structures. They optimized the structural integrity and pore size required for pancreatic islet culture and seeding. Ye et al. [29] used a Bioplotting system to fabricate nano biocomposite scaffolds of non-stoichiometric apatite (ns-AP) and poly(ε-caprolactone) (PCL) scaffolds. They reported that, scaffold with 40 wt% ns-AP contained open and well interconnected pores with a size of 400–500 μm, and exhibited a maximum porosity of 76%. Additionally, Oliveira et al. [30] Studied the nucleation and growth of biomimetic apatite layers on the surfaces of bioplotted starch/polycaprolactone SPCL scaffolds with a 0°/90°strut structure. Haberstroh et al. [31] Investigated the osteogenic effect of three different cell-seeded 3D-bioplotted scaffolds with 3 different biomaterials in an ovine calvarial critical-size defect model.

Robocasting, also is an extrusion-based RP process in which a colloidal suspension, or ink, is extruded through a micron-sized nozzle in a defined trajectory to form a three-dimensional structure [32] and is referred to in the literature as robotic deposition and direct-write assembly [33]. Recently, this technique has been used to fabricate porous β- Tricalcium phosphate (TCP) scaffolds with a controlled architecture [34]. The compressive strength of the fabricated TCP scaffolds was enhanced by polymer infiltration. The authors reported that infiltrating polymers into the porous robocasted ceramic structure was shown to considerably boost the strength and toughness of the material. The fracture modes and the strength of robocasted HA and TCP scaffolds was also identified by the same group in another related work [35].

Another novel and modified deposition techniques have been also introduced in the last 3 years. These methods were developed to increase manufacturing flexibility by enhancing deposition capability in achieving optimum scaffold requirements. The new methods include Multi-head deposition system (MHDS) [36], [37], screw extrusion system (SES)[38],[39], BioExtruder [40],Combined FDM and

electrospinning (ESP) system [41], [42], Combined plotting and (ESP) [43], [44], 3DF and (ESP) [45]. Combined rapid freezing and plotting system [46], [47], porogen-based extrusion system [48] and modified plotting system [49].

B. Three Dimensional Printing (3DP)

3D printing was the first RP technique to be proposed for biomedical and tissue engineering purposes [50]. 3D Printer uses ink-jet printing approach to accurately writes a ‘‘binder’’ solution like polymer latex or silica colloid , which moves in accordance to the CAD cross-sectional data through the inkjet print head, onto metallic, ceramics or composites powder [51]. The first step in 3D Printing is the spreading of powder onto a platform with a roller, followed by the inkjet print head printing a two-dimensional pattern, onto the powder layer. Then the next powder layer is spread and the process is repeated until the part is finished. The unused powder acts, as support for the part and is brushed or blown off afterwards. The piston chamber is lowered and refilled with another layer of powder and the process repeated. The process usually followed by a temperature treatment to burn the binder off and a final sintering step [52].

Recent researches on 3D printed scaffolds focus on evaluating Mechanical and in vivo and in vitro performance of scaffolds. Recently, Detsch et al. [53] fabricated samples from pure HA and β-TCP as well as a biphasic calcium phosphates BCP mixture with~60 wt% HA by 3DP. They studied cell development on manufactured scaffolds surfaces by analyzing cell proliferation, differentiation, and activation. Shanjani et al [54] fabricated a calcium polyphosphate structures with a 3DP system and used SolidWorks® software in the design of the porous samples. They reported that structures fabricated using the direct 3DP method may be more advantageous compared to the conventionally sintered CPP structures of equivalent percent porosity due to higher compressive strength and larger pores. Klammert et al. [55] introduced powder-printed magnesium ammonium phosphate (struvite) structures for the first time.

Ge et al. [56] investigated the mechanical properties and micro-environment of 3D printed poly-lactic-co-glycolic acid (PLGA) scaffolds and they evaluated the proliferation and differentiation of human fetal osteoblasts after 3 weeks of in vitro culture on the produced scaffolds. The results showed that the PLGA scaffolds examined had mechanical properties similar to that of trabecular bone, but was still much weaker compared to cortical bone. In addition to general porosity, the PLGA scaffolds also had micropores within macropore walls and the cultured human osteoblasts could proliferate upon seeding on the PLGA scaffolds. Warnke and co-workers [57], [58], investigated the biocompatibility of HA and TCP scaffolds produced by 3DP printing/sintering techniques and their ability to support and promote the proliferation of human osteoblasts compared with the commonly used bone replacement material, Bovine hydroxyapatite(BioOss) in vitro. They noted that both versions of 3D printed and sintered scaffolds were colonized by human osteoblasts, however more cells were seen on HA scaffolds than TCP scaffolds. Cell vitality staining and biocompatibility tests also showed superior biocompatibility of HA scaffolds to BioOss , while BioOss was more compatible than TCP. The group also evaluated the biocompatibility and osteoinductivity of individually designed HA and TCP blocks compared to BioOss for heterotopic bone induction in a rat model as shown in Fig. 7. It was found that, designed HA and TCP blocks tested as well as the BioOss blocks are suitable as matrices for endocultivation as they showed good biocompatibility in vivo.

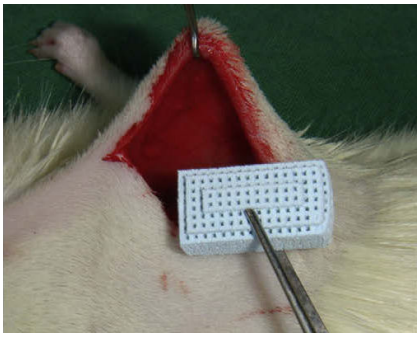

Fig. 7 Insertion of individually designed 3D-printed HA scaffold into the rat [58].

Klammert et al. [59] established a novel 3D powder printed material using calcium phosphate cement chemistry as a cell culture scaffold for osteoblastic cells. In another study, Lowmunkong et al. [60] investigated the possibility of fabricating 3D scaffolds from pure plaster of Paris (POP) powder (calcium sulfate hemihydrates) with an average particle size of 10 μm–20 μm by 3DP and to transform the fabricated object from POP to HA or other bioceramic. After HA-transformed, specimen was sintered at 1150° C for 3 h, the compressive strength increased four times when compared with HA specimen. However, its crystal structure was transformed to β-TCP due to the chemical reaction of transformed HA with remaining phosphate in the specimen.

Gbureck et al. [61] Fabricated a custom made TCP/calcium pyrophosphate bone substitutes with a well-defined architecture using 3DP. They characterized the mechanical performance and porosity contained within the fabricated samples. The feasibility of 3DP to fabricate porous pure Titanium dental scaffold was recently investigated by Wiria et al. [62]. The 3D printed Titanium dental implant prototype has been successfully fabricated and shown to have elastic modulus of 4.8–13.2 GPa. This elastic modulus is much lower than the modulus of the bulk commercially pure Titanium and is in the range of elastic modulus of natural bone.

Another processing system based on inkjet printing technology is the 3D phase change inkjet printer. This process utilises droplet deposition technique in which thermoplastic building material and a wax like support material are deposited from separate jets onto a working surface. As a result of heat conduction, the droplets induce local melting on the underlying layer and causes bonding to occur. After each layer hardens, uniform thickness is maintained by a milling head [63]. Using this printing system, Park et al. [64] designed

and fabricated composite hybrid polymeric scaffolds for targeted cell transplantation of genetically modified human cells for the formation of human tooth dentin-ligament-bone complexes in vivo.

C. Selective Laser Sintering (SLS)

In SLS, a thin layer (approximately 100 - 200μm) of powder is spread on a surface using a cylindrical roller. A laser is then scanned over the powder bed, which heats the powder locally and sinter-bonds the adjacent particles to form a single layer of the part. The non-sintered particles act as a support for any hollow section, overhangs or undercuts in the part (like in the case of 3DP). After the formation of the first layer, the next layer of powder is spread over the first layer followed by laser scanning. Upon completion of a part, it is removed from the chamber, the loose powder removed and the part is post processed, if necessary [65].

Mechanical and structural properties relationship has an important consideration in scaffold fabrication process. Sudarmadji et al. [66], [67] produced scaffolds with different structural configurations and porosity values using SLS to study the relation between scaffold porosity and compressive stiffness. Another study related to selective laser sintered scaffold mechanical and structural properties was introduced by Eshraghi and Das [68]. Additionally, Lohfeld et al. [69] examined three different SLS scan options with a view to achieve a minimum strut thickness through changing process parameters in sintered polycaprolactone PCL constructs production. They fabricated and characterised the PCL scaffolds in terms of strut morphology and mechanical properties.

In another related study by Eosoly et al. [70], the effects of SLS parameters on the dimensional accuracy and mechanical properties of HA and PCL scaffolds was investigated. It was observed that the dimensions and mechanical behavior of the fabricated parts were strongly dependent on the manufacturing direction and scan spacing. Salmoria et al. [71], also studied the influence of powder particle size and build parameters of the SLS process on the structural and mechanical properties of cellulose based scaffolds. They found that it is possible to fabricate biopolymer scaffold structures using starch–cellulose and cellulose acetate using SLS by process optimization based on the adjustment of laser power and scan speed. It was also shown that, specimens prepared with small particle size exhibit satisfactory level of porosity and mechanical properties for the design and fabrication of scaffolds with potential use in tissue engineering and drug delivery.

Nanocomposites and polymer matrix composite with nanotubes have received much attention in TE scaffold area due to their potential to achieve combination of proper mechanical properties and good biocompatibility. The concept of using selective laser sintered nanocomposites scaffolds has been recently studied by Duan and co-workers [72]-[78]. The authors have conducted extensive research on the integration of SLS and Calcium phosphate (Ca–P)/poly(hydroxybutyrate-cohydroxyvalerate)(PHBV) and carbonated hydroxyapatite (CHAp)/poly(L-lactic acid) (PLLA) nanocomposites (see Fig. 8). They reported the promising success of SLS in fabricating nanocomposite scaffolds for bone tissue engineering.

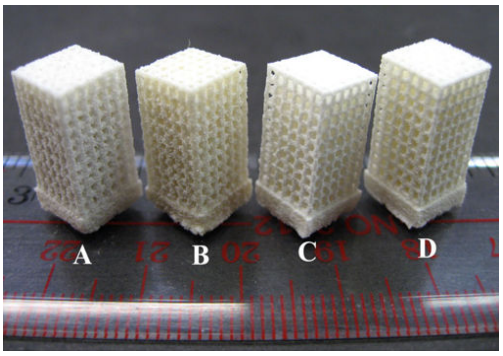

Fig. 8 scaffolds produced by SLS: (A) PHBV; (B) Ca-P/PHBV; (C) PLLA; (D) CHAp/PLLA.[76].

Zhou et al. [79] also fabricated TE scaffolds from the poly (L-lactide) (PLLA) and PLLA/ carbonated hydroxyapatite (CHAp) nanocomposite microspheres by SLS. The effects of laser power scan spacing and part bed temperature on the scaffold structure was studied. On the other hand, fabrication of β-TCP scaffolds was carried out by Li et al. [80] via SLS and mixed with Carbon nanotubes (CNTs) to enhance scaffold mechanical properties. According to their findings, the strength of scaffold mixed with 0.2% CNTs reaches 0.819 MPa which has been improved by 85.7% compared with that without CNTs and the produced scaffold has a good interconnectivity, and pore size mainly distributes in the two regions of 60–340 μm and 500–620 μm.

D. Stereolithography (SL)

SL involves selective curing of a photo-curable liquid polymer, using a laser beam directed by a computer in accordance with a CAD model. The laser scans the layers onto the surface of the resin, the first layers being attached to a platform. Successive layers are cured by lowering this platform and applying an exact thickness of liquid resin [81]. SLA Process requires support structures to be added to the model, to prevent any overhanging or unconnected features from falling to the bottom of the liquid-filled vat. After completion, the model is raised and any support structures can be removed manually.

Several research groups have utilized Stereolithography process for tissue engineering scaffolds. Recently, one research group has explored this area using a biodegradable resins comprised of poly (ethylene glycol)/poly(D,L-lactide) hydrogel, poly(D,L-lactide-co-ε-caprolactone)-based resin and poly(D,L-lactide) [82]-[85]. Their goal was to use SLA to fabricate biodegradable scaffolds with an appropriate mechanical properties and large freedom of design, Fig. 9 Shows a μCT of PDLLA scaffold with gyroid architecture showing a gradient in porosity and pore size.

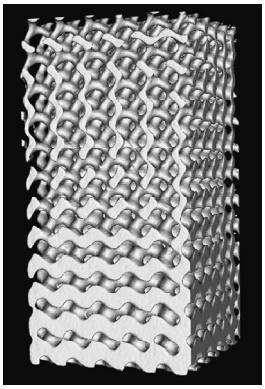

Fig. 9 PDLLA scaffold with gyroid architecture showing a gradient in porosity and pore size [83].

Another research group [86], has explored the capabilities of (SL) for fabricating multi-material spatially controlled bioactive poly (ethylene glycol) constructs through modifications in a commercial SL machine. The authors reported that Multi-material spatial control was successfully demonstrated in features down to 500 µm.

E. Microstereolithograph (µSL)

Micro-stereolithography (µSL) is a relatively new approach which shares the same principle with SL. However, to get a better resolution, the laser beam is focused more precisely in order to reduce the spot size to a few micrometers of diameter to solidify a thin layer of 1–10 µm in thickness. Lee et al.[87]-[89] evaluated the mechanical properties and cell proliferation based on internal pore size and 3D architecture of scaffolds fabricated by µSL and poly (propylene fumarate) (PPF) based materials. The authors pointed out that cell proliferation on the µSL scaffold was clearlysuperior and indicated that µSL would be a good replacement for conventional scaffolds fabrication methods. Moreover, in the authors other related work to improve the bioactivity of TE scaffolds, structures containing hydroxyapatite composites have been fabricated by µSL and resins containing dispersed hydroxyapatite particles. By mixing poly (propylene fumarate) PPF and hydroxyapatite particles in diethyl fumarate as reactive diluent, a photo-polymerisable composite resin was obtained and Scaffolds containing nano/microscale structures of PPF -HA photopolymer were successfully fabricated [90].

In the same context, to produce biodegradable and biocompatible scaffolds with controlled micro-architecture, Choi et al. [91] developed a (Digital Micro mirror Device)-based µSL system and fabricated 3D PPF based micro-scaffolds. It was reported that the developed µSL system and the use of PPF is promising in fabricating complex micro-scaffolds with prescribed micro-architectures.

F. Electron Beam Melting (EBM)

The EBM system builds parts from the bottom up by scanning the focused electron beam at ≈ 103 mm/s to selectively melt specific areas of the powder bed using a 3D-CAD system while powder is continuously added from the powder cassettes to the top of the building part in a vacuum [92].

Most of research in the area of TE scaffold fabrication using RP is mainly focused on polymer, ceramic or composite materials. However, some recent investigations have been performed in order to fabricate 3D porous metallic scaffolds by RP techniques including EBM. A titanium alloy, specifically Ti-6Al-4V, is widely used as an implant material for biomedical applications due to its relatively low modulus, good biocompatibility, and enhanced corrosion resistance [93]. The mechanical properties and characterization of EBM scaffolds were the recent mean research focus. In the studies of Li et al. [94]-[96], Ti6Al4V implants in a form of cylinders with internal honeycomb-like structure with controlled porosity as shown in Fig. 10, have been fabricated by EBM. Characterization and mechanical properties including, hardness, compression and bending tests have been investigated. The authors concluded that, the mechanical properties of the porous Ti–6Al–4V implant are compatible with those of human bone, that makes porous Ti–6Al–4V implants with a high porosity and low stiffness might be a good candidate for biomedical applications.

Fig. 10 The EBM fabricated Ti–6Al–4V implants with honeycomb structure [95].

In a similar study by Parthasarathy et al. [97], [98], an image based micro-structural analysis and the mechanical characterization of Ti6Al4V structures with porosities ranging from 49.75% to 70.32% as shown in Fig. 11, were fabricated using the EBM . Their results indicated that, the fabricated structures with porosities as high as 50%-70% satisfy the mechanical strength requirements needed for craniofacial applications.

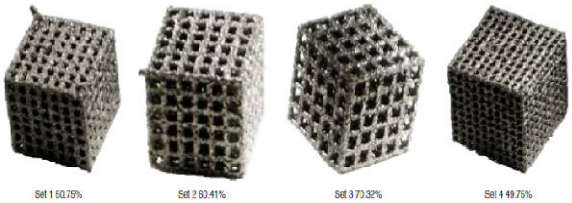

Fig. 11 Different porosities of Ti6Al4V parts fabricated with the EBM system [97].

Heinl et al. [99] also, employed EBM to fabricate Cellular Ti6Al4V structures with interconnected porosity. They have

examined the mechanical properties of these structures and performed surface modifications by a wet chemical treatment in HCl and NaOH. The authors reported that, the mechanical properties of these fabricated cellular structures were similar to those of human bone and the suggested chemical surface modification using HCl and NaOH induce in vitro apatite formation and thus is believed to provide a better fixation of the implant in the surrounding bone and improve the long-term stability of the implant.

Ti6Al4V scaffolds, on the other hand, have been a subject of in vitro and in vivo research. Haslauer et al. [100] assessed the in vitro biocompatibility of EBM Ti6Al4V structures by comparing the cellular response of solid polished, solid unpolished and porous EBM discs to the cellular response of discs made of commercially produced Ti6Al4V. Additionally, the bone regeneration as well as the ingrowths of osseous tissue into porous EBM-processed Ti-6Al-4V scaffolds in domestic pig calvaria bone were recently evaluated by Ponader et al. [101].

Other research works that have exploited the capabilities of EBM to fabricate functional custom or tailored implant components include the work of Harrysson et al. [102]. Where, a Ti6Al4V hip stems with tailored mechanical properties was designed and fabricated in addition to design, fabrication, testing and FEA evaluation of Nonstochastic mesh structures.

G.Selective Laser Melting (SLM)

SLM is similar in principle to SLS except that high power solid-state lasers are used to melt very fine metal powders in inert gas atmospheres. The full melting enables the production of solid, dense metal parts in a single process (i.e. not using binders or post-process furnace operations that have been previously used to make metal parts via SLS). A variety of metals can be used, including stainless steels, cobalt-chrome and titanium. These processes are relatively new and, whilst they are not suited to the production of models of human anatomy, their potential for producing custom fitting implants and prostheses is already evident. They also lend themselves well to the manufacture of custom surgical guides, templates and instruments [81].

Although biocompatible materials used in SLM are typically metals or metal alloys, some recent research has been conducted on the use of other compositions as SLM materials of scaffold. Lindner et al. [103] assessed the possibility of direct fabrication of β-tricalcium phosphate (β-TCP) and poly (D, L)- lactide (PDLLA) composite scaffolds using SLM. According to their findings, the SLM technique presents a high potential for manufacturing nearly any desired shape of individual scaffolds made of this biodegradable composite material. In addition, SLM process allows the integration of a defined and completely interconnected porous structure offering a regular and reproducible morphology of the pores. Fig. 12 shows a (50% β-TCP, 50% PDLLA) scaffolds produced by SLM . Another attempt for the direct production of Porous Titanium, Ti with Titanium hydride, TiH2 scaffolds utilising SLM is reported by Wang et al. [104]. The authors investigated the effects of TiH2 content and scan speed on the microstructural porosity and pores size.

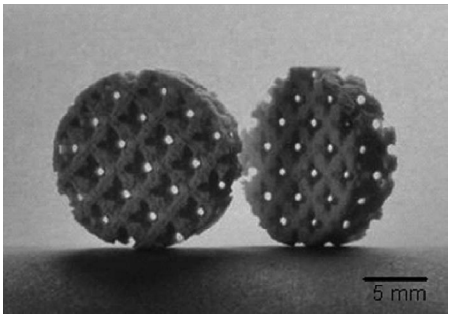

Fig. 12 scaffolds made of 50% β-TCP and 50% PDLLA by SLM [103]

III. Current Limitations

Although using RP technology in TE scaffold fabrication achieved recognised progress as noted in the previews review, RP techniques have some general limitations which can be summarized in the following points:

-The Limited clinical application due to high machine cost, design and fabrication time involved.

-The need of multidisciplinary collaboration.

-High processing temperatures in some RP techniques limits their ability to process temperature-sensitive polymers with bioactive component and affects the part mechanical strength. Further current limitations of each RP process can be found in [20], [105].

IV. Future trends

To overcome the mentioned limitations and to move the current TE scaffold fabrication by RP to the next frontier, future research should focus on three main areas.The first one is the continuous improvement of RP machines to produce mass production with cost effective precise scaffolds through enhancing machines resolution, accuracy, trapped liquid or loose powder removal techniques and developing methods for direct placements of bioactive components such as cells and proteins within the 3D structures.It is also still challenging to find a biomaterial that elicits the appropriate cell response. So, the second aspect is the development of new generation of strong and bioactive biomaterials and evaluation of the function and regenerative capability of such materials.

Finally, further improvements in scaffold's internal and external architecture in addition to incorporation of material heterogeneity within the scaffold structure are needed to obtain the optimal scaffold design. This may be achieved by creation of more advanced interaction between CAD , CAE and RP systems . For example, developing of scaffold CAD libraries, material databases and incorporating FEA tools in an integrated design environment. This can yields optimised scaffolds for clinically driven tissue engineering applications, facilitates the interaction with RP technology and eliminates the reliance on user skills and hence, the whole process can be commercialized.

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