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AN APPLICATION OF RAPID PROTOTYPING IN SCAFFOLD BASED TISSUE ENGINEERING

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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. The most interesting and challenging applications of Rapid Prototyping technologies are in the field of medicine. RP medical models have found application for planning treatment for complex surgical procedures, training, surgical simulation, diagnosis, design, and manufacturing of implants as well as medical tools. One such area, where the application of Rapid Prototyping has been rapidly developing in the field of Tissue Engineering (TE) and guided tissue repair. Tissue engineering scaffold is a 3D construction that acts as a template for tissue regeneration. Although several conventional techniques are utilized in scaffold fabrication but these processes are shown 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— Rapid prototyping, 3D Printing, Tissue engineering, Scaffold, Medical application

I.Introduction

Scaffold based Tissue Engineering (TE), is an interdisciplinary technology which emerged thirty years ago, has progressively drawn significant attention from numerous scientists, engineers, technologists, and physicians around the world due to its ability to construct biological substitutes to repair and replace diseased and damaged tissues. An important branch of tissue engineering has been the combination of new techniques such as micro fabrication and additive manufacturing that will enable future innovations.

The aim of using tissue substitutes is to replace the diseased tissue. There are two modern treatments, which are organ transplantation and use of medical devices [1]. The enormous success of organ transplantation has increased demand for transplantable solid organs. Although the fact that the numbers of organ donors and transplantations have doubled during the last two decades, the demand still far outstrips the supply, leading to a worldwide organ shortage[2]. In general, medical implants are referred to tissues or devices that are purposefully placed inside or on the surface of the body on purpose, typically for replacing damaged or missing parts of the body, e.g., knee implants in prosthetics. However, certain surgical risks should be addressed. There could be unexpected risks during implant placement and removal. Also, implant failure and induced infection to the body can be very harmful to the recipient. Other potential difficulty includes bruising, swelling, redness and even infection due to skin contamination during surgery. Extra care and medication will be required when complications occur, which, in the worst case scenario, would require the removal of the implant. This leads to find an alternative approach like scaffold based tissue engineering [1].

II.Scaffold Based Tissue Engineering

"Tissue Engineering is the implementation of the principles and methods of engineering and life sciences toward the fundamental understanding of structure-function relationships in normal and pathologic mammalian tissue and the development of biological substitutes to restore, maintain, or improve function" [3].

When tissues or organs have been so severely diseased or lost by cancer, congenital anomaly, or trauma that conventional pharmaceutical treatments are no more applicable, artificial organs (including tissues) or organ transplantation is the first choice to reconstruct the destroyed tissues or organs. However, these surgical treatments have been facing some challenges at the moment. Artificial organs have been improved by remarkable advances in the biomedical engineering in the past decades, but still, need better biocompatibility and bio functionality. Problems in current organ transplantation include a shortage of donated organs and immune rejection, although immunosuppressive therapy has recently much advanced. Approximately three decades ago a new paradigm emerged as an alternative approach to tissue and organ reconstruction that is tissue engineering. A unique feature of tissue engineering is to regenerate patient's tissues and organs that are entirely free of poor biocompatibility and small bio functionality as well as

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severe immune rejection. Due to the outstanding advantages, tissue engineering is considered as an ultimate ideal medical treatment. To regenerate new tissues, this biomedical engineering utilizes three essential tools; cell, scaffold and growth factor. These three are not always simultaneously used.

A scaffold is referred to a highly porous three-dimensional substrate. Cells that are donated or taken from the patient himself/herself are significantly expanded in culture and then transferred to the scaffold, which provides a surface where cells adhere, proliferate and generate essential elements that make up living tissue. The behaviour and health of the cells seeded inside the scaffold are controlled by not only the scaffold material but also the internal architecture such as dimensions of the pores, walls, struts, and channels.

Some techniques for scaffold fabrication have been developed over the past 40 years [5, 6].

The top priority consideration for all TE applications is always the patient's safety. It is also crucial that the selected processing method does not have negative impacts on the scaffold regarding biocompatibility and biodegradability. The scaffold should perform two primary functions, which are (1) enabling and directing cell growth within it before implantation; and (2) guiding cell migration into the defect. The scaffold should also contain surface chemistry since this may encourage cell attachment and proliferation of cells. Porous structures are essential for cell adhesion, transport of nutrients and metabolic wastes, confluent tissue formation and sufficient vascularisation of new tissue [7].

In respect of the mechanical properties of a scaffold, they are determined by scaffold geometry, inherent properties of the material and the fabrication technique. For instance, polymers with higher crystallinity regularly show higher tensile strength. If the crystallinity of polymer chains is reduced due to the processing method used, the resulting scaffold strength is diminished and the scaffold's lifetime is also reduced [5].

In the selection of scaffold fabrication techniques, material properties (such as bulk and surface properties), as well as the function that the scaffold is expected to perform, should be considered. Also, the time and cost for fabricating scaffolds relating to the viability of the treatment for the particular patient should also be taken into account. Most fabrication techniques involve applying pressure and heat to the material or organically dissolving it followed by a molding process to obtain the desired final scaffold geometry. However, harsh conditions are sometimes induced during certain fabrication processes, which are detrimental for cells and bioactive molecules. Therefore, these harsh conditions should be reduced. In fact, each manufacturing technique has both distinct benefits and drawbacks, and hence, the selection of an appropriate method should be based on the requirements of the exact type of tissue. There are some requirements and considerations for the engineeried scaffold listed below;

Should possess interconnecting pores of appropriate scale to favour tissue integration and vascularisation.

Should be made from materials with controlled biodegradability or bio-resorbability.

Have appropriate surface chemistry to favor cellular attachment, differentiation, and proliferation.

Possess adequate mechanical properties to match the intended site of implantation and handling.

Be easily fabricated into a variety of shapes and sizes.

Not induce any adverse response.

III. Scaffold Fabrication

There are many techniques used in making scaffolds that are divided into two categories, non-designed manufacturing techniques, and designed manufacturing techniques. Non-designed manufacturing techniques include conventional methods. Designed manufacturing technique involves rapid prototyping of solid free-form technologies [8].

Even though many conventional methods have successfully produced viable scaffolds, clinical disadvantages exist in that most of these procedures require long processing time to remove the organic solvents used. These processes are also limited to producing scaffolds with simple geometry which may not satisfy the geometric requirements of the defect [9]. This has prompted researchers to turn to RP techniques [3,6,10].

IV. Rapid Prototyping Techniques For Fabrication Of Scaffold

Rapid prototyping of solid free-form is a method designed manufacturing process that fabricates three-dimensional scaffolds with the fully interconnected porous network. These techniques require a computer model of the desired scaffold architecture from Computer-Assisted Design (CAD) or Computed Tomography (CT) (Figure 2). The advantages of these methods are their capability to fabricate scaffolds with complex architectures in micron scale μm [3].

According to the working principle, the direct AM technologies can be classified into three types; Laser-based, Nozzle-based, Hybrid.

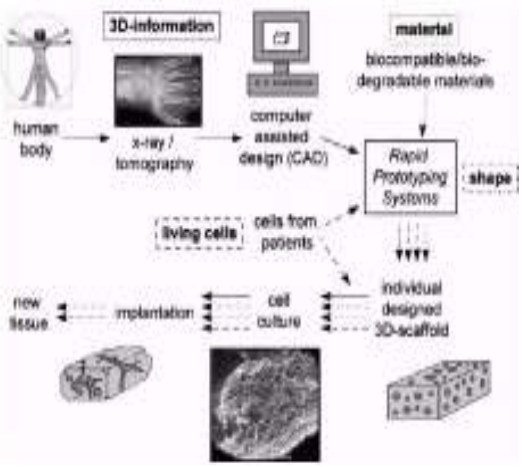

Fig. 1: Scaffold based tissue enginneirng [8]

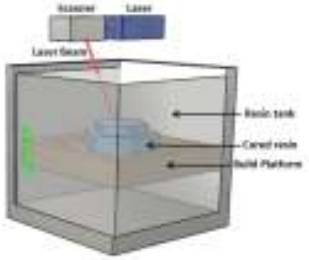

Fig. 2: Stereolithography apparatus (SLA) [1]

A. Laser-based technology

At present, laser-based AM techniques are used in TE include Stereolithography (SLA), Selective Laser Sintering (SLS) and Selective Laser Melting (SLM) [11].

Stereolithography

SLA was introduced by 3D Systems in 1986 and is the first commercially available AM technique (Figure 3). Based on the spatially controlled solidification of a liquid resin by photopolymerization, 3D objects are manufactured by SLA. Using a computer-controlled laser beam or a digital light projector with a computer-driven building stage in SLA, a pattern is illuminated on the surface of the resin. After photopolymerization of the first layer, the platform is moved away from the surface, and the built layer is recoated with liquid resin. A pattern is then cured in this second layer. As the depth of curing is slightly larger than the platform step height, good adherence to the first layer is ensured [11].

Regarding accuracy and resolution, stereolithography is greater to all other SFF techniques.

Chua et al., [1] used this technique to manufacture HA-based porous implants. The crucial step was the casting of HA-acrylate suspension into a mold, by which the scaffold with interconnected channels of 366 μm in resolution was generated. Bian and Li et al., [13] presented a strategy to design and fabricate biphasic biomimetic osteochondral scaffolds that overcome the traditional difficulty for osteochondral TE, delamination of cartilage scaffold and bone scaffold. The engineered scaffolds were made from beta-tricalcium phosphate (β-TCP) through SLA and gel casting. Lee et al., [3] made-up scaffolds having nano/micro scale structures using micro-SLA technology and a Poly (propylene fumarate) (PPF)/diethyl fumarate (DEF) - hydroxyapatite (HA) photopolymer.

Selective laser sintering

In SLS process, a laser beam is used to selectively scan the surface of the powder according to precise cross-sectional profiles. The powder is heated to its glass transition temperature, leading to material deformation and fusion. SLS is appropriate for fabrication of porous ceramic matrices for bone implantation (Figure 4) [15].

Lohfeld et al., [15] studied the use of SLS for the generation of bone TE scaffolds from PCL and PCL/TCP. Sharma, [16] illustrates an application of SLS; a 3D-printed robotic suit has given a woman who was paralyzed from the waist down after a ski accident the ability to walk again. Salmoria et al., [17]

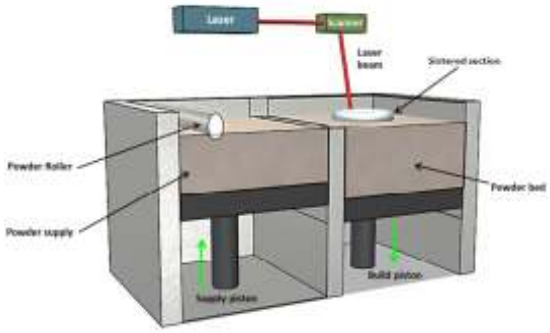

Fig. 3: Selective laser sintering technique [1]

presented a study on the effect of HA on the microstructure and mechanical properties of HDPE/ HA composites and the fabrication of functionally graded scaffold of HDPE/HA by SLS. Recent advances of SLS have been the ability to produce lower stiffness scaffolds and higher resolution features.

B.Nozzle-based technology

The class of nozzle-based systems is characterized as describes as follows;

Fused deposition modeling

Fused deposition modeling (FDM) is the deposition of molten thermoplastic materials through two heated extrusion heads with a small orifice in a particular lay down pattern. One nozzle deposits the thermoplastic material and the second deposits temporary material to support cantilevers. In FDM, one of the traditional

methods melts thermoplastic polymer into a semi-liquid state, and the head extrudes the material onto the build platform. The part is formed in a layer-by-layer fashion where the layers are fused together (Figure 5) [3].

Some biodegradable materials have been used in FDM, including PCL, polycaprolactone (PCL)/ bioactive glass (BAG), polylactide acid (PLA), poly (L-lactide -co-e-caprolactone) (PLC) [19]. Park et al., [20] fabricated scaffolds for bone TE with poly (e-caprolactone) (PCL) and poly (D, L-lactic-glycolic acid) (PLGA). However, certain limitations should be addressed. FDM requires input material to be of a particular size. Moreover, any changes to material properties require recalibration of the roller feeding parameters. The resolution of the FDM process is 250 µm, which is lower than other AM methods. Available materials for FDM are quite limited. Natural polymers cannot be used since materials have to be in the filament form and melted during extrusion [1]. To avoid the above limitations improvements are continuously made, leading to the emergence of variants of the FDM technique.

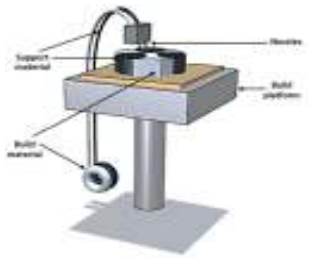

Fig. 4: Fused deposition modeling [1]

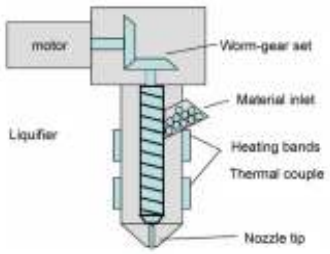

Fig. 5: Precision Extrusion Deposition system [21].

Lower operating temperatures can be applied, and precursor filaments are not necessarily required. These options include precision [1].

To avoid the problems of time-consuming precursor step of filament fabrication and brittle material's thick filament buckling failures, PED is proposed, which consists of a mini-extruder mounted on a high-precision positioning system (Figure 6). PED can be used with bulk material in granulated form, which avoids most of the material preparation steps in a filament-based system. This configuration opens up the opportunity for the utilization of a wider range of materials, making the PED a viable alternative manufacturing process for composite scaffold materials [5].

Shor et al., [21] fabricated PCL and PCL/HA (PCL–HA) composite scaffolds, having 25 per cent HA by weight using PED system. Hamid et al., [22] expands PED's library of biopolymers by introducing an assisting cooling device which allowed the PED to utilize a wider range of biopolymers with higher melting points, increased the working extrusion temperature from 120 to 250°C.

Due to the elevated temperatures involved in the melting process, scaffold bioactivity may be damaged. New systems have been developed to replace the melting process with material dissolution.

Xiong et al., [23] proposed to fabricate PLLA/TCP composite scaffolds for bone TE with Low-temperature deposition manufacturing. This happens in low-temperature (Figure 7) environments under 0°C. The LDM process can better preserve bioactivities of scaffold materials because of its non-heating liquefying processing of materials. Sun et al., [24] use LDM to fabricate scaffolds with three different materials, which are poly(L-lactic-co-glycolide)/TCP (PLGA/TCP), poly(L-lactide-co-D, L-lactide)/TCP PDLLA/ TCP) scaffold, poly(L-lactic acid)/TCP (PLLA/TCP). The scaffolds were used to repair the 15 mm segment defect of rabbit radius.

To reduce precursor time of changing filament Yan et al., [25] designed a four-nozzle LDM system, which is referred to as Multi-Nozzle (Low-Temperature) Deposition Modeling (MDM, M-LDM) (Figure 7). Different liquid materials will be fed into different material supplies. It is an enhanced version of LDM. Each material supply is a vessel that at its bottom has a soft pipe connected to a nozzle. Because different materials have varied hydrodynamic properties, nozzles have various designs. Four nozzles at most can work orderly in the same system controlled by a computer. The four-nozzle MDM system can also perform single-nozzle the deposition process, a bi-nozzle deposition process, and tri-nozzle deposition process by leaving the spare nozzle(s) unused according to the

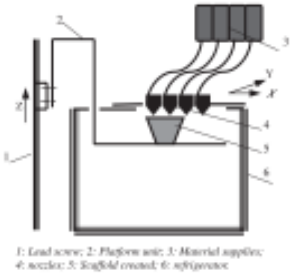

Fig. 6: M-LDM [25]

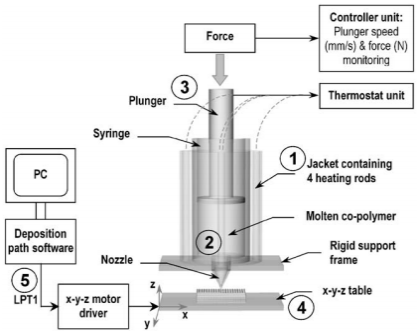

Fig. 7: 3D fiber-deposition device [26]

requirements of manufacturing different scaffolds.

3D fiber-deposition technique

Particularly, titanium and its alloys have been widely used in orthopedic and dental devices because of their superior mechanical properties and biocompatibility. 3DF is a technique that allows the development of metallic scaffolds with accurately controlled pore size, porosity, and interconnecting pore size. 3DF deposition, being a layer-by-layer (Figure 8) manufacturing technology, can be used to manufacture prototypes in which each layer may have a different fiber diameter, thickness, and fiber space and fiber orientation [3].

Li et al., [26] successfully fabricated 3D porous Ti6Al4V scaffolds by 3DF technology

Pressure-Assisted Microsyringes (PAM)

Giovanni Vozzi, [27] developed PAM. The system, illustrated in Figure 9, consists of a stainless-steel syringe with a 10-20 mm glass capillary needle. A solution of the polymer in a volatile solvent is placed inside the syringe and expelled from the tip by the application of filtered compressed air (Figure 9). The needle is mounted on the z-axis of a three-axis micro positioning system which was designed and built at the University of Pisa and has a resolution of 0.1 mm. A supporting substrate, usually glass, is placed on the two horizontal motors and is moved relative to the syringe.

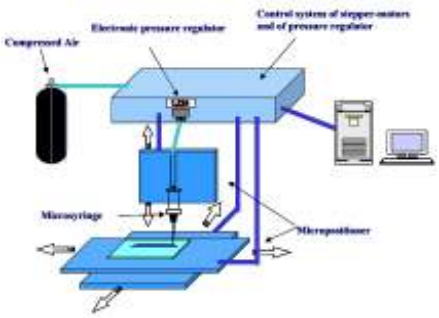

Fig. 8: Schematic of microsyringe method [27]

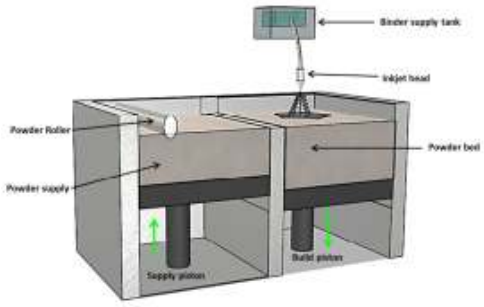

Fig. 9: 3D printing process [1]

Vozzi et al., [27] use PLGA to fabricate TE scaffolds. Tirella et al., [28] proposed a piston-assisted microsyringe (PAM2) system, which was designed to fabricate well-defined 3D scaffolds using materials with high viscosity, typically hydrogels, and can be considered as an evolution of the pressure-activated microsyringe (PAM).

3D printing

3D Printing was developed at the Massachusetts Institute of Technology. In the 3D printing process, powder particles are bonded together with a binder or solvent for the powder, which is delivered via an inkjet print head (Figure 10) [29].

Seitz et al., [18] used a modified HA powder for the fabrication of 3D printed scaffolds because of the safety of HA as biocompatible material and efficacy for bone regeneration.

3D bioplotting

The processing conditions of laser based processes prevent the usage of hydrogels. Furthermore, hydrogel scaffolds have not been fabricated via 3D printing or fused deposition modeling. These standard rapid prototyping techniques do not meet the requirements of soft tissue scaffolds, so the appearance of the new 3D dispensing method was long awaited. The key feature of this 3D Bioplotter (Figure 11) developed at the Freiburg Materials Research Center, is the dispensing of a viscous plotting material into a liquid medium with a matching density. As a result of the gravity force compensation, complex architectures can be fabricated without any temporary support structures. Developed specifically for the biofunctional processing 3D Bioplotter makes it possible for the first time to integrate aqueous Biosystems, e.g., living cells, into scaffold fabrication process. Most industrial rapid prototyping machines fail to prepare biological, temperature-sensitive materials. The office format of the 3D Bioplotter device enables easy production of scaffolds in a sterile laminar flow hood [3].

Fig. 10: 3D Bioplotting process [29]

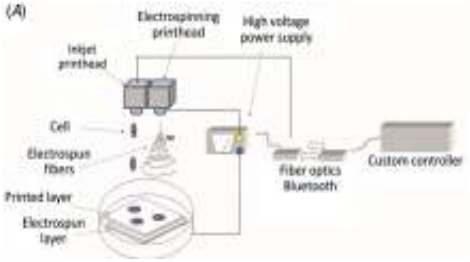

Fig. 11: Hybrid printing system (inkjet print head and the electro spinning) [30]

Hybrid AM technology

Hybrid printing, which combines multiple methods for scaffold fabrication, was proposed by Xu et al., [30]. To achieve simultaneous high-throughput cell patterning and controlled scaffold production, thus overcoming one of the major limitations of inkjet printing alone, a novel hybrid printing system by incorporating an electro spinning apparatus into an inkjet printing platform was developed. Figure 12 shows the schematic drawing of this hybrid printing system.

V. Conclusion

Any AM technique is material dependent, and there is no general AM process. Materials that are AM-able for TE applications are limited. A typical AM process starts with a CAD model. Replicating this reality in the CAD model is challenging. An ideal scaffold should be an equivalent of an ECM. So it requires a fabrication resolution at nano scale (<100 nm). No AM can fabricate an ECM yet.For both SLS and 3DP; there is a challenge with creating stronger structures without increasing dimensions. SLA can reach extremely high resolutions; there are a limited number of biodegradable, biocompatible resins. Advances have been made to synthesize new macromers with biodegradable moieties, however, these materials have not been FDA approved. Although macro and micro architecture has made great strides in the past five years, additional work should focus on the Nano architecture. Due to harsh processing conditions of SFF methods (e.g., Heat, organic solvent), biochemical molecules are not incorporated directly into the scaffold. In a nutshell, there is still a lot of work to be done before AM technologies are used in every hospital and to fabricate new tissue or organs on demand. Several issues need to be addressed before clinical application, including mechanical reliability of scaffolds, induction of vascularization and tailored degradability.

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