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Techniques in scaffold fabrication process for tissue engineering applications: A review

Abinash Kumar, Anu Jacob*
Department of Biotechnology, Karunya Institute of Technology and Sciences, Coimbatore, India.

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ABSTRACT

Tissue engineering is a highly complex process with goals to replace, restore, and regenerate tissues. Tissue engineering combines multidisciplinary fields such as biochemistry, clinical medicine, biological science, and materials science. It has application in personalized drugs, organ transplantation, and as a drug transporter. The scaffold fabrication process for tissue engineering depends on numerous factors such as biodegradability, mechanical possessions, scaffold architecture, and manufacturing process. The scaffold properties based on its biological aspects, structural requirements, material composition, conventional and advanced fabrication technologies, and extrusion-based scaffold fabrication techniques are analyzed and discussed in the current review. Further studies for the development of bio-scaffolds will provide a broader roadway into a new dimension of various tissue engineering techniques and provide greater advancement in medical and clinical research.

1. INTRODUCTION

Tissue engineering is an interdisciplinary field that connects biomedical engineering, mechanical engineering, clinical medicine, genetic engineering, and biotechnology. It is a complex process as tissue regeneration depends upon various factors such as maintenance of cell-cell interactions, surface properties, porosity, mechanical stability, solubility, and degradability of biomaterials used. Accidents and conditions, such as osteomyelitis, arthritis, anaemia, cancers, hereditary multiple exostoses, and hereditary bone marrow failure syndromes, cause severe damage to the bones cartilage and tissues[1–5]. With the increase in demand for bone grafts in organ transplantation and substitute surgery, the market value is expected to be 11.5 billion US dollars by the end of 2025 [6]. Tissue engineering is the development of porous scaffolds to provide a favourable environment for the regeneration, growth of tissues, and complex organs. Scaffolds are three-dimensional structures that are porous, fibrous and permeable biomaterials. It helps in the transport of body fluids, promotes cell-cell interaction, deposition of extracellular matrix, viability against various pathogens with minimum inflammation, and toxicity rate. In the 3D scaffolds fabrication technique, the materials are generally classified into chemical factors such as synthetic and natural polymers, hydrogels, metals and non-metals, composites, ceramics, and non-ceramics [7]. The development of biologically synthesized scaffolds depends on various factors such as pore sizes, interconnectivity between the pores, biodegradability, ability for cells to produce their extracellular matrix, machine limitation, biocompatibility, clinical status, instrumentation choice, good manufacture practices, and mechanical properties. In addition, the modifications considered during fabrication of bio-scaffolds are bio-mimicking with various cellular components, delivering of different bioactive molecules and ameliorative agents like antibiotics, cytokines, drugs, inhibitor, growth factors, proteins, which provide an anchorage for great importance in stacking to the scaffolds [8,9]. With the advancement in technology and various architectural demands, various approaches have been developed for the fabrication of scaffold materials.

Although the conventional methods are widely used and are evolving with decades, advanced prototyping techniques are also being adopted. The advanced techniques use high- end computer-aided designing software for the bio fabrication process with micro, macro, and nanoscale architecture [7,8]. With the recent development of biologically active agents, natural biopolymers

*Corresponding Author
Anu Jacob, Department of Biotechnology, Karunya Institute of Technology and Sciences, Coimbatore, India. E-mail: anujacob @ karunya.edu

Table 1: Type of materials used in scaffolds fabrication.

Types of bio-materials	Examples	Advantages	Disadvantages	Application	Fabrication technique
Ceramics	Hydroxyapatite, β-TCP, α-TCP, calcium silicate, calcium sulphate, TCP, bioactive glasses.	Non-toxic, biocompatible, anti-inflammatory, osteo-conductive.	Slow degradation rate, low mechanical strength, compact in nature, brittle.	Bone and dental tissues applications	Inkjet printing, gel casting binder, salt leaching, SLS, stereolithography, extrusion type, fused deposition modelling.
Metals and alloys	Tantalum, Co–Cr, Iron magnesium alloys, Mg-RE Mg-Ca, Fe-Mn alloys, stainless steel, Fe foam, Titanium, Ti, Ti–6Al–4V.	Non-toxic, corrosion resistance, light-weight, biocompatible, osteo-conductive	Poor osteo-integration with the nearby bone. non-biodegradable, release toxic ions which causes inflammatory responses	Bone, and dental application, knee replacement surgeries and artificial hearing applications	SLS, stereo lithography, vacuum foaming, electron beam melting, selective laser melting.
Natural polymers	CS, alginate, dextran, collagen, gelatine, glycosaminoglycan, agarose fibrinogen, actin, keratin, cellulose, hyaluronic acid.	Biodegradable, non-toxic, biocompatible, support cell-cell attachment and differentiation, anti-allergenic, osteo-conductive, viscoelastic.	Rapid degradation, complex structure, less mechanical strength, water-soluble, less cell attachment	Drug delivery, bone and tissue applications, gene therapy	Electro-spinning, inkjet printing, solvent casting, emulsification, photolithography.
Synthetic polymers	Polyesters, polycarbonates polyether, polyhydroxy acids, polysiloxanes, polylactones, PCL, polyurethanes, polyorthoesters polyanhydride.	Biodegradable, good mechanical strength, biocompatibile, non-toxic, low melting point, easy to manufacture and design, the unwanted product can be degraded and can be removed	Toxic, hydrophobicity, high production cost, slow degradation, antigenic, less cell-cell interaction.	Bone, tissues, cartilage and dental application, drug delivery	Freeze drying, electro spinning, gas foaming, electron beam melting, selective laser melting phase separation, fused deposition modelling, SLS, stereo lithography.
Composites	Different polymers, ceramics and metals are blended. Calcium silicate, calcium sulphate, tricalcium, iron magnesium alloys, CS, alginate, dextran, collagen, gelatine, polysiloxanes, polylactones.	Good mechanical strength, biocompatibile, non-toxic, osteo- conductive, corrosion resistant, lightweight	Slow degradation, less cell-cell interaction, compact in nature.	Bone, tissues, cartilage and dental application	Freeze-drying method, fused deposition modelling, stereo lithography.

are replacing synthetic polymers. The natural polymers are either plant-derived or animal-derived and are biodegradable, non-toxic, and provide customized pore sizes and renewability [10]. Moreover, the various types of natural bio-polymers play an important role in biocompatibility, influence cell behaviour, possess high surface area, with pre-existing vascular networks, porosity, and rapid biodegrading properties [11].

The biomaterials and fabrication methods are selected for processes depending upon the analysis and complexity of targeted tissues. Different type of materials used in scaffolds fabrication for various tissue engineering applications is compiled in the Table 1.

Recently, various studies reported on advancement in biomaterials fabrication technique and development of scaffolds from biomaterials which enhanced cell proliferation, cell viability, and printability without any stress [7,12–15]. Plant-based biomaterial such as plant proteins, lignin, polysaccharides, and plant extracts have various bioactive products that are useful in various restoration, regeneration and improvement of scaffolds fabrication. However, the synthetic and animal-derived biomaterials used in regeneration and restoration applications, have some disadvantages such as scarcity, expensiveness, high cell deaths, and poor biocompatibility [11].

This review describes different techniques of advanced rapid prototyping (RP) and conventional 3D fabrication for bio-scaffolds preparation. These techniques can create porous 3D structures with controlled mechanical possession, pore size, interconnected pores, and porosity. The paper summarizes research status of each of the methods and the opportunities and challenges are analyzed.

2. FABRICATION OF SCAFFOLDS BASED ON THEIR REQUIREMENTS

The scaffold fabrications and designing consider various properties, such as mechanical, biological, and physicochemical based on its feasibility and requirements. Additionally, the interconnectivity within the pores, shape, pore size, porosity, strength, and degradation rate are the important factors based on which the scaffolds fabrications depend. The 2D scaffolds technique possesses several advantages by providing higher resolution and

accuracies with control over physical and chemical properties. The imaging and characterization are easier with automated lab facilities and high processing screening methodologies [16,17]. The 3D bio fabrication techniques for scaffolds design uses advanced bio-printing and bio-assembly methodology that include cells for the fabrication process in an automated manner [18]. It uses various types of computer-assisted designing software packages to create virtual cross-sections of cell-loaded matrices by consecutive layers formation with a computational fabrication process. The 3D scaffold designs are a form of sponges, foams, and meshes and can resist the external pressure caused by various factors such as different tissue interaction with the extracellular matrix, mechanical stiffness, rapid degradation, cell deaths, toxicity, and biocompatibility. Nevertheless, the designing of 3D scaffolds develops proper homogeneous mixture, cell-to-cell contacts, cell proliferation and cell attachment. The emerging 3D technology has revolutionized into 4D printing depending on the type of biomaterials and environmental factors [19]. However, the 4D printing technique is quite expensive as compared to other 3D printing technologies [20]. The scaffolds prints are prepared with advanced processing through multiscale finite element analysis and computational neuromusculoskeletal evaluation to obtain load-holding capacity, *in vivo* cyclic stress and biocompatibility [21].

Different types of scaffolds and fabrication techniques are used depending upon their biomaterials' composition (Table 2). For example, in bone tissue engineering, nanofibrous scaffolds are used that mimics extracellular matrices and collagen fibres [32–34]. Gelatin and Fibrin are natural biopolymers that have been widely used in scaffold fabrication as it has high biodegradability and biocompatibility [35,36]. Alginate has been widely used for bone and cartilage tissue engineering and capable of scaffold-reinforcement and non-immunogenic property [37].

The design of scaffold for tissue engineering involves high interconnectivity within the nano-vascular networks of extracellular matrix, transport of oxygen, nutrient, and various soluble factors that are responsible for removing metabolic wastes [7,11]. Based on its complexity, construct materials source, geometrical distribution of structure and the process of fabrication technique, the 3D scaffolds are available in various forms Table 3.

3. DIFFERENT TYPES OD SCAFFOLD FABRICATION TECHNIQUES

The conventional tissue engineering scaffold production techniques include thermally induced phase separation (TIPS), fiber bonding, electrospinning, solvent casting and particulate leaching, membrane lamination, freeze-drying, and gas foaming [49]. The recent development in scaffold fabrication mainly comprises integration with computer-aided design (CAD) software through RP technology such as stereolithography, bio-plotting, solvent-based free forming, combination modelling technique, fused deposition modelling, 3D printing, and selective laser sintering (SLS) [8,9]. The techniques retain the ability to maintain pore structures, cell–cell interaction, reduction in mechanical instability, and control over mitigation of the cellular matrix. Advantages and disadvantages of different type scaffolds fabrication technique for tissue engineering applications are compiled in Table 4.

4. CONVENTIONAL METHODS

There are various conventional methods of scaffold fabrication (Fig. 1) and have been developed for drug delivery, 3D cell culture and tissue engineering. However, the conventional scaffold fabrication techniques sometimes remain incompatible as they deviate from the optimal environment for cell attachment, multiplication, and

Table 2: Different scaffolds fabrication for various tissue engineering applications.

Biomaterial composition	Technique	Cells type used	Result	References
Poly (ethylene glycol) dimethacrylate hydrogels	Inkjet printing	Articular chondrocytes	Cartilage tissue formation	[22]
PCL/gelatin scaffold	Electrospinning	L929 mouse fibroblast cells	Tissue engineering applications	[23]
Hydrogel-filled polylactide porous scaffold	Thermally-induced phase separation	Chondrocytes	Bone and cartilage tissue engineering	[24]
3D bioprinting nanocellulose and alginate scaffolds	Extrusion	Chondrocytes	Cartilage tissue engineering	[25]
Poly (ethylene glycol) dimethacrylate hydrogels	Stereo-lithography	NIH-3T3	Vascular endothelial growth factor secretion	[26]
Alginate-PVA-hydroxyapatite hydrogel	Bioprinting	MC3T3	Best for osteo-conductivity and tissue engineering	[27]
Laminated hydroxyapatite nanoparticle layer on polyhydroxybutyrae fibrous scaffold	Electrospinning	MSCs	Cellular regeneration and attachment, osteogenic phenotypic generation	[28]
CS polyelectrolyte complex porous scaffolds	Freeze drying	feline fibroblast cells	Good cytocompatibility and Cartilage tissue engineering	[29]
Hydrothermal cross-linked CS porous scaffolds	Freeze drying	L929 mouse fibroblast cell	Cartilage tissue engineering	[30]
Electrospun nanofibrous polyurethane/poly(glycerol sebacate) scaffolds	Electrospinning	Swiss mouse NIH 3T3 fibroblasts	Vocal fold tissue engineering	[31]

Table 3: Various forms of 3D scaffolds.

Types of scaffolds	Materials	Advantages	Disadvantages	References
Porous scaffolds	Poly(lactic-co-glycolic acid), poly-L-lactic acid, PCL, polybutylene terephthalate, polyglycolic acid	High porosities and homogeneous interconnected pore structure, nutrient and gas transportation through channel networks, yield space for the extracellular matrix with the cells	Pore size dependant	[38,7]
Fibrous scaffolds	Poly(lactic) acid, Poly-caprolactone, cellulose, silk fibroin, gelatine, collagen	Great potential for neurite growth by depending on cell separation, mimic the extracellular matrix depending on surface volume ratio, porosity and cell infiltration, induces greater cellular attachment as compare to microfibers	Limited material selection, inadequate resolution	[39,40]
Solid free-form scaffolds	Synthetic and natural biopolymers	Have controlled architecture and reproducible properties, high precision geometrically complex scaffolds with controlled pore size forming high interconnectivity within the pores	Limited to photoresist, residual toxic moieties, post-processing challenges	[7,41]
Natural-biopolymer scaffolds	Soya protein, camelina protein, gluten, zein cellulose, pectin, starch, lignin, plant extracts	Inexpensiveness, high surface areas, chemical signalling, biocompatibility, porosity, degradable, vascular networks, liable mechanical characteristics, high polarity and due to hydrophilic potentialities, greater cell attachment effects, greater mimics capacity with the extracellular matrixes.	Low immunogenicity potentials, low molecular weight, time-consuming. less dense, chemical complexity	[7,11]
Alloplastic-synthetic scaffolds	Synthetic polymers, glass, ceramics, metals	Has great control over the architecture of the construct and various mechanical properties	Limits to form a proper extracellular matrix, degradation of scaffolds, toxicity, poor biocompatibility and expensive in natures	[7,42]
Hydrogel scaffolds	Gelatine, fibrin, agarose, alginate, synthetic polymers, natural polymers, Fibrinogen, collagen, CS, hyaluronic acid	Semi-crystalline, amorphous, high flexibility, biocompatibility, hydrophilicity, degradability, abled to survive in harsh environment	Have limited mechanical properties, small pore size	[7,43]
Micro-sphere scaffolds	Collagen, CS, gelatine, poly-lactic acid-glycolic acid	Widely used for gene therapy, tissue engineering, site-specific drug delivery, growth factors	Cells tendency depends upon the type of materials and methods incorporated, costly	[44,45]
Bio-ceramics scaffold	α-TCP, β-TCP, bioactive glass	Good compression and corrosion resistance, bio-resorbability and good biocompatibility	Reliability, slow degradation rate, poor fidelity	[46,47]
Polymer-ceramic composite scaffold	PLA, polylactic co-glycolic acid, Polyglycolic acid	Great biocompatibility and biodegradation, absorbability, high toughness, low price, workability	Poor cell affinity and cell-matrix interaction, acidic degradation	[46,48]

reproduction. In the case of skin tissue engineering, it forms a non-homogeneous structure and is limited to the only internal design of the scaffolds. Additionally, the technique is limited to manual intervention as it involves a time-consuming, multistage process that is labor intensive and requires high skill and experience. Conventional methods do not result in the regularity of pore shape, reproducibility and sufficient pore interconnectivity [9,49].

4.1. Freeze-Drying

Freeze-drying, also known as lyophilization, is a widely utilized fabrication technique. In this technique, it freezes a synthetic polymer dissolved in selective solvent at a temperature between −20°C and −80°C, resulting in the solid solvent. The frozen sample then evaporated by sublimation with help of a lyophilizer to form a solid scaffold with various interconnected pores [68]. Soumya *et al.* [69] have successfully developed an osteoinductive herbal scaffold by freeze-drying. They blended medicinal plant extracts with natural biopolymers [O-carboxymethyl chitosan (CS) and alginate] by lyophilization process. Later, fabricated scaffolds with desirable properties for tissue engineering applications. The cytocompatibility studies on the developed composite scaffolds with human mesenchymal stem cells (MSCs) proved its biocompatible nature. The scaffolds fabricated with plant extract showed a remarkable difference in cell attachment and cell reproduction as compared to scaffolds fabricated without extract. The developed scaffold showed promising porosity and water absorption properties. The freeze-drying technique is mostly favorable for biomedical application as water solvents are used

Table 4: Advantages and Disadvantages of different types of scaffolds fabrication techniques for tissue engineering application.

Technique	Advantages	Disadvantages	References
Freeze-drying	1. Possess higher porosities 2. Adjustable scaffolds structure and pore size 3. Greater interconnectivity of the porous structure with the extracellular matrix.	1. Process is high energy and time consuming 2. Use of cytotoxic solvents 3. Leads to shrinkage of the tissue	[50]
Solvent casting and particle leaching	1. Ease in production 2. Adjustable porosity of the scaffolds and the pore size. 3. High porosity, develops a 3D cell structure	1. Use of cytotoxic solvent and leads to the denaturation of molecules and cells 2. Bio-inductive property of the molecules decreases. 3. Requires a longer time to degrade and a time-consuming process due to the use of thin membranes	[51–53]
Gas foaming	1. Due to the use of organic solvents, they are non-flammable 2. Processing cost is cheap 3. Carbon dioxide is used as porogen gas	1. Process cannot be used as a hydrophilic material 2. Carbon dioxide used is of low solubility	[51]
Thermal-induced phase separation	1. Porous polymer membrane of anisotropic and tubular 3D scaffolds 2. A low probability of defects 3. Low temperature is used for bioactive molecules integration	1. Process is not much suitable for seeding of osteoblast cell and maintaining the pore size of the bone tissue growth. 2. Use of organic solvents 3. Used for thermoplastic utilization.	[51]
Electrospinning	1. Can be used in large scale productions 2. Has control over the diameters of the micro and nanoscale thin fibres 3. Abilities to generate homogeneous mixtures with nanoscale fibres 4. Develop polymers of high tensile strengths.	1. Process is limited in producing 3D scaffolds due to poor control over pore structural size and shape 2. Due to the use of the wide range of biomaterials, the solvents used sometimes might be cytotoxic	[8,15,54]
SLS	1. The operating cost of this technique is low. 2. Process provides high precision and control over the microstructure of the complex scaffold structure and high porosity 3. Has great mechanical strength. 4. Post-processing is not required	1. Requires high operating temperatures where thermally stable materials can be used and are limited to small pore size 2. Removal of excess trapped material is complex as it remains in powdered form. 3. Post-sinter stage required in this process 4. Poor control over surface topography.	[12,55,56]
Stereolithography	1. Provides high accuracy and high resolution. 2. Forms a complex 3D structure where interconnectivity and pores structure maintains uniformity. 3. Excess liquid and photopolymer are removed by heating 4. The cell patterning and growth factors are maintained in this process.	1. Requires the post-polymerization stage to maintain the strength of the scaffolds 2. Requires photo-polymerization materials of the low range 3. Requires structure support and the abundant amount of monomers 4. Uses quite expensive materials.	[12,57]
Fused deposition modelling	1. Cost-effective process that has high mechanical strength and production rate 2. Simple process that uses multiple nozzles and allows moderate temperature deposition of scaffolds 3. Solvent-free process. 4. High porosity where the structure and size of the pores can be adjusted	1. Requires a high operating temperature 2. Limited variety of materials range and size of pore structures 3. Low utility with non-thermoplastic polymers due to thermal degradation of the polymers 4. During the process, pre-formed consistent-sized fibres are needed to feed through rollers and nozzle and are limited for the application in biodegradable polymers [58].	[12,58,59,60,61]
Multiphase jet solidification	1. Performs contiguous high resolution	1. Material is quite expensive 2. A good rheology control	[62,63]
Precise extrusion deposition	1. Used for the fabrication of high scale precision micro and complex scaffold 2. Materials used are in the form of a pellet	1. Requires high temperature	[62]
3D Bioplotting	1. The pore size of the scaffold are well connected 2. Use of abundant amount of biomaterials and biomolecules	1. The scaffold developed have low mechanical strength 2. Difficult in operating due to slow speed	[51,62]
Inkjet printing	1. A high-speed tractable o droplet size precision process 2. Vast amount of biomaterial are available 3. A low-cost process with high resolution, high-throughput capability, reproducibility, and easy to use	1. Lacks in the precision of droplet deployment and adequate size 2. It requires low viscosity for the bio-ink.	[14,64,65]

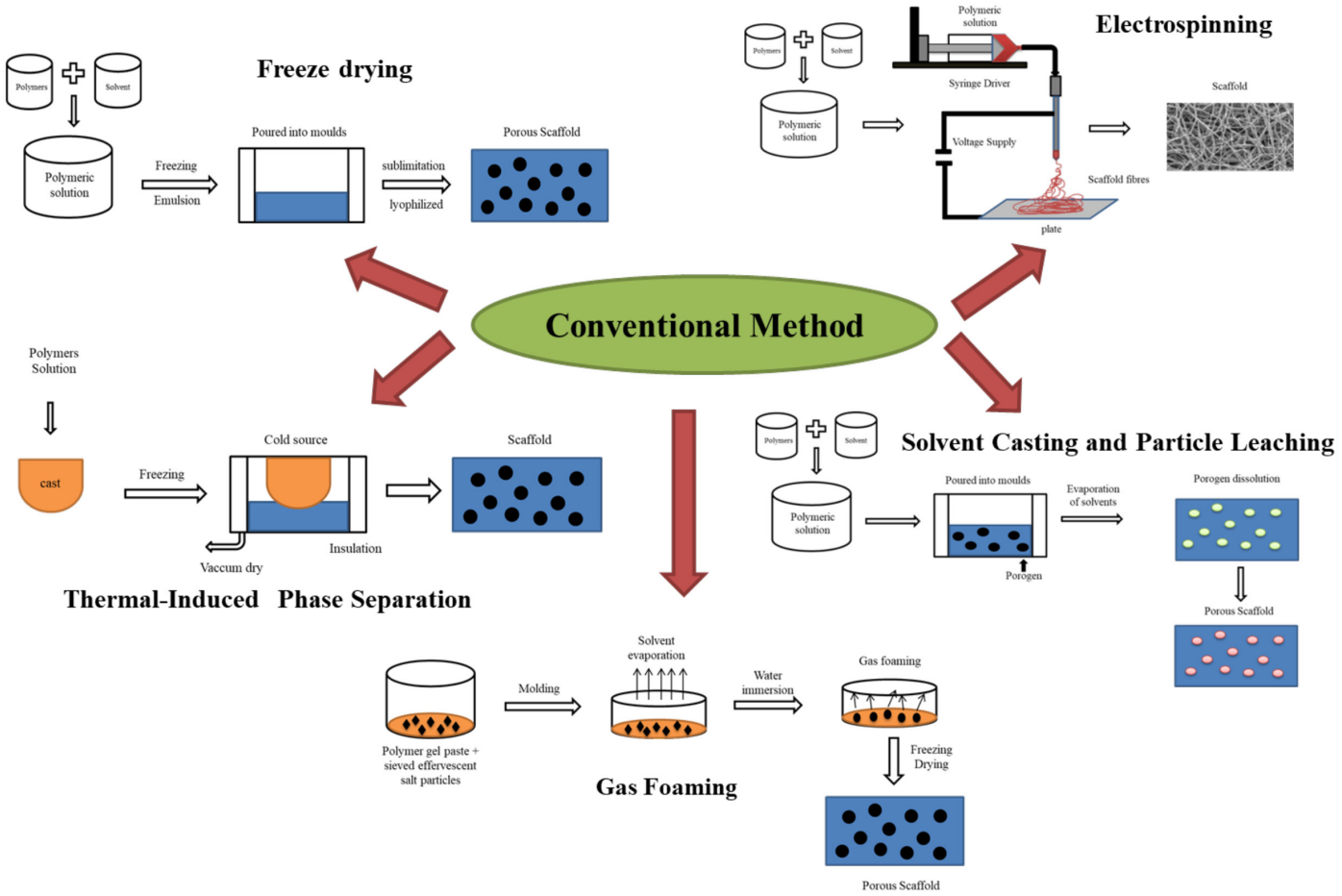

Figure 1: Conventional methods of scaffold fabrication [66,67].

rather than other organic solvents for the fabrication of scaffolds but remain challenging for the fabrication of classified structured scaffold-like vascular systems used in biomedicine applications [70]. The cytotoxic solvents used for the mixing of the polymers consume high energy. The developed scaffold requires washing several times to remove the toxic solvents and hence declines cell death as well as triggers the irregularities in pore sizes for a longer time- span [71,72].

4.2. Solvent Casting and Particle Leaching

This method is generally used for Bone and cartilage tissue engineering applications [73]. In this technique, a highly volatile solvent solvates the polymer and cast in molds along with porogen. The solvents evaporate with a matrix consisting of salt particles and the formed composite matrix contains porogen and polymers. The matrix submerged in water allows salt leaching to develop scaffolds structure with high porosity. The solvent combined with uniformly distributed organic amalgams like glucose, gelatine microsphere and otherwise soluble inorganic salts like potassium chloride is selectively leached to get a certain pore size which is used as porogen to dissolve in the polymer solution [74]. The scaffolds prepared through this technique have a porosity of 50% to 90% and are of a low-cost process [75]. Zeng *et al.* [66] successfully fabricated and developed 3D porous polylactic acid (PLA) scaffold with high porosity using methods like phase separation, particle leaching techniques, vacuum-assisted solvent casting, and solvent extraction. In this process, the surface is modified with the help of a CS/osteogenic growth peptide (OGP) coating layer that can be potentially applied in bone, cartilage regeneration and tissue engineering purposes. The experiment proved that the higher the porosity of the PLA scaffolds developed, the lower the PLA solution concentration and the temperature. The hydrophilicity and mechanical properties of CS/OGP/PLA developed scaffold were higher as compared to the uncoated PLA scaffold.

4.3. Gas Foaming

Gas foaming utilizes natural cytostatic solvents and high temperature. In this method, the fabrication model uses inert gas foaming agents like methane, carbon dioxide, hydrogen, nitrogen to pressurize with a biodegradable polymer like fluoroform or water solvents until it reaches saturated conditions to form gas bubbles [76]. The gas foaming techniques and fused deposition methods are combined where, the porous PLA scaffolds dominate 1 to 10 μm micro pores sizes structures. Gas foaming process generates micropores (<10 μm) while in conventional 3D process, it is barely developed. The fused deposition method incorporates macro porosity through attached channels that have developed the

saturation capabilities by reducing the time consumptions in the process. This technique achieves generally 85% porosity and 30 to 700 μm pore size for the developed structure which is sponge in nature [58]. Song *et al.* [67] developed tailored macro/micro-porosity architectures scaffolds by combined technology of gas foaming and fused deposition modelling process for applications in tissue engineering technology.

The PLA is blended with poly(vinyl alcohol) (PVA) to fabricate composite filaments by fused deposition modelling. After the fabrication process, the developed scaffolds were dominated by the gas foaming process to create micropores of size less than 10 μm. The outcomes of this process revealed that without further dense skin layers, interconnected pores attained micropores size of 2 to 10 μm. Hence the scaffolds developed have great potentialities for cartilage and bone tissue regeneration. Manavitehrani *et al.* [77] have studied polyester-based poly propylene carbonate (PPC)-starch bioscaffolds in tissue engineering technologies. In this process, to develop a porous scaffold, the bi-products are degraded and fabricated by gas-foaming technique with PPC blended with starch and bioglass particles. The pore sizes developed were ranged from 100 to 500 μm, with high pore interconnectivity.

4.4. Thermal-Induced Phase Separation (TIPS)

In the TIPS process, various polymeric solutions of solvent and non-solvent are demixed, quenched and consist of different polymeric phases. This demixing process takes place either by evaporation or extraction process, solvent is removed resulting in pores formation [74]. In this technique, the mixing of various types of selective solvent, additive blenders, and multicomponent polymer are used at low temperature for developing force separation process, where the homogeneous polymer solution at high-temperature environment settled down to induce phase separation by achieving variant polymeric phases [75,58]. The scaffold structures are attained, as the solvent gets eliminated by the freeze-drying process with relatively porous and nano-scale fibrous meshes. The thermoplastic crystalline polymer scaffolds are generally used for construction and low temperature is mostly used for blending bioactive materials with the fibrous scaffold [9]. In this method, the porosity of the fibres is achieved 98% higher than the surface to volume ratio of the scaffold constructed. Biswas *et al.* [78] have developed porous CS scaffold by using combined technology with mechanical foaming and thermal-induced phase separation technique and obtained 80% porosity, 2.6 to 25 kPa adjustable compacting parameters with 120 mm pore size. The scaffold showed great potential for tissue engineering and the foaming process incorporated by air bubbles, functioned as a mould for the macro-porous construct of the developed scaffold. In this technique, materials are limited to the fabrication process, inadequate resolution and very selective minimal materials are used in the phase separation process with uniform porous structures [70].

4.5. Electrospinning

Electrospinning is extensively used in nanofibers polymers and for scaffolds fabrication process from the selective solution by using electric current [79]. In this technique, the nanofiber scaffolds exhibit great porosity, high surface area, and biomimetic like natural extracellular matrix. During this process, the polymeric droplet is executed by the stress at the needle tip by using an electrically charged jet. Then at high voltage, the charging solvent gets dominated by an interaction between electrostatic repulsion and surface tension, where the spinneret droplets erupt and gets stretched by passing through grounded collector from the spinneret tip. Finally, the jet solidified into nanofibers as the solvent starts evaporating [80]. For the production of nanofibers, various parameters are followed such as surface tension, conductivity, flight distance, type and concentration of polymeric solvents, viscosity, spinneret diameter, interactions between the molecules, the electric current supplied, rate of flow, types of collector [81]. Yuan *et al.* [82] has successfully developed nanofibrous scaffolds with polyethylene oxide (PEO) and CS using the electrospinning fabrication technique. In this study, with the reduction of mass of CS and PEO from the scaffolds, degradation is exhibited. The bactericidal study shows stronger growth inhibition and cytotoxic effects. This fabrication technique is categorized into three depending on the types of manufacturing methods consisting of solution materials by changing electrospinning materials, setting up of collector by using liquid-assisted collectors with perfunctory setup, post-processing operation after electrospinning [8]. Hejazi and Mirzadeh [83] have developed 3D scaffold with electrospraying and electrospinning combined technique by using polycaprolactone (PCL). The 3D scaffold was fabricated using macro and nanofibres particle with optimized pore size, porosity, interconnectivity within the pores and biomimetic the extracellular matrix structure. Although electrospinning is widely used, there is various limitation such as distribution of sufficient homogeneous pores sizes, limited to some applications in biomedicine [70], and the solvent used is toxic and depended on various variables.

5. RAPID PROTOTYPING (RP)

Due to various limitations in the conventional methods, the use of RP technique was introduced to develop 3D porous scaffolds with great architecture advantages, higher porosity and interconnectivity within the pores. This process, also known solid free-form fabrication technique, is a strong fabricating tool and immensely used for the preparation of scaffolds in tissue engineering. The scaffolds are developed by using computer-aided designing tools to provide a perfect fit architectural structure with physio-chemical properties. In the RP process, closely attached materials are combined with powder, sheet or liquid and the fabrication process is employed by the addition of consecutive layers to produce 3D scaffolds utilizing the computer-generated models [84]. Initially, development takes place for a 3D volumetric computer model which is derived from yield information produced by surface digitizers or by clinical imaging frameworks. Later, the digital model extracted is stacked and fused on top of each other to make user-defined structures that statistically cut into layers with a consistent thickness [49]. The main advancement of these fabrication techniques is that they can maintain the porosity, pores shape and size of the scaffolds and have highly interconnected pore structure. It additionally empowers for development of patient-specific customizable scaffolds that are appropriate for tissues and organs designing technology [58]. There are various

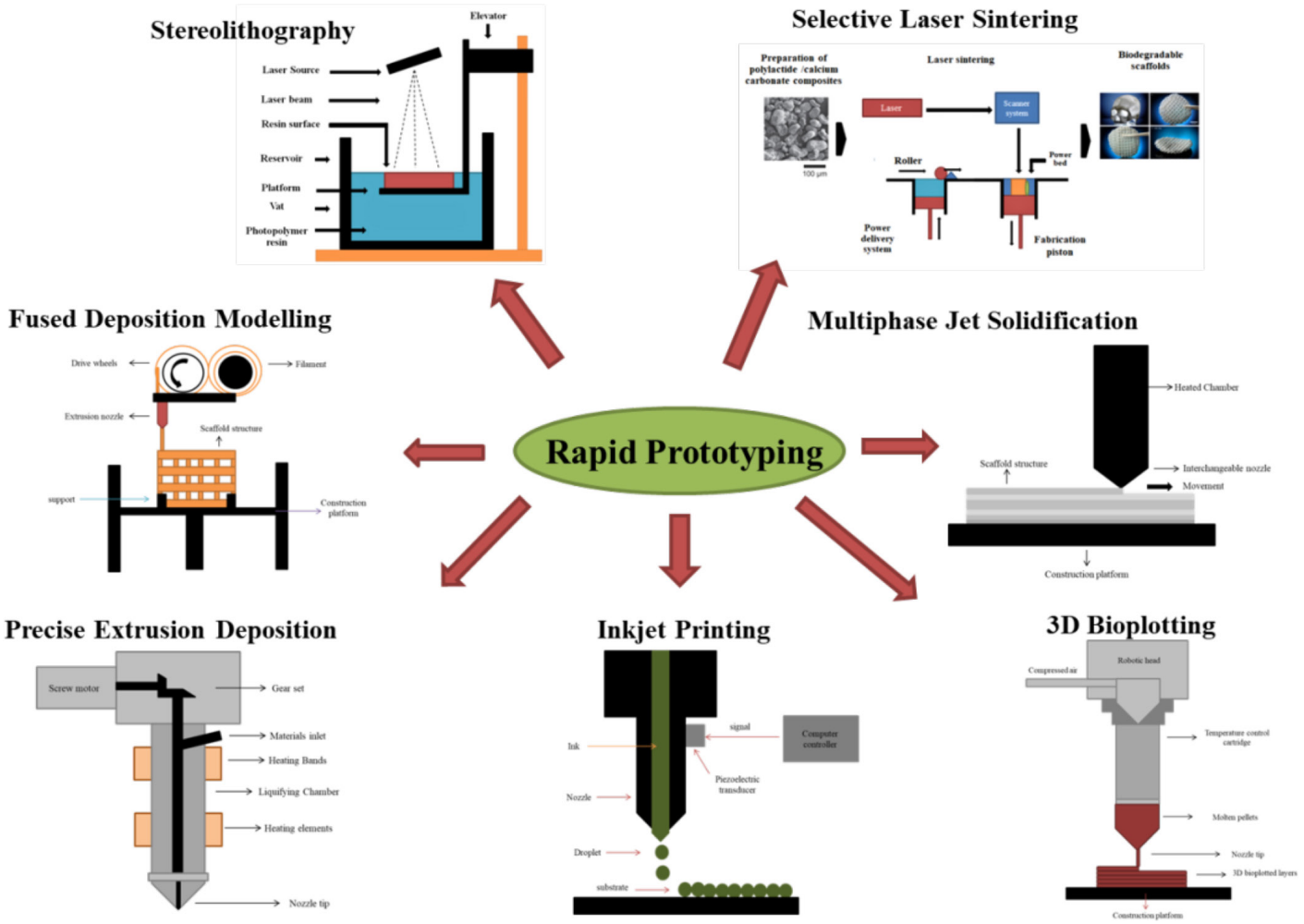

Figure 2: Types of RP fabrication techniques for tissue engineering [85].

types of RP fabrication techniques for tissue engineering purposes (Fig. 2) such as fused deposition modelling, stereolithography, electron beam melting, 3D printing and SLS [9].

5.1. Selective Laser Sintering (SLS)

SLS is a powder-based fabrication technique that uses laser technology to sinter powdered material such as polymers, ceramics, and metals. It is utilized for manufacturing and developing the scaffolds for fabrication in tissue engineering [9]. Patient-specific implants are developed with composite interconnectivity pore structures to advance bone ingrowth [86]. Although there are various process for the treatment and developing solutions to treat bone imperfections such as with titanium and polyetherketone ketone inserts as they are non-degradable but have the chance of getting several complications in future [85]. The productivity of this method is that it provides outstanding dominance over the microstructures of the developed scaffolds under various parameters like compositions of percentage by physically mixed polymers or by composite powder mixes to get the properties of the favoured scaffold [87]. Gayer *et al.* [85] have successfully developed and fabricated solvent-free polylactide-calcium carbonate composite scaffolds by using the SLS technique. In the cycle, four distinctive composite powders with about 75% (wt%) polylactide (PLLA and poly(D,L-lactic acid) and 25% (wt%) calcium carbonate (calcite) composite were set up by milling process dependent on Good Manufacturing Practice principles, where the four different grades of polylactide were selected for shelling the broad inherent viscosity range of 1.0–3.6 dl/g. The composite material with the most minimal inherent viscosity at (1.0 dl/g) showed the best processability by SLS process where the biaxial twisting quality of up to 75 MPa was accomplished. While the cell culture measures showed great feasibility of MG-63 osteoblast-like cells on the SLS exhibits. At last, the 3D scaffolds with great pore structure and interconnectivity with the pores were developed [87].

5.2. Stereolithography

Stereolithography technique is the process of developing solid 3D scaffolds based on structurally regulated solidification of the fluid resin by photo-polymerization measure. In this process, the printing of thin layers of ultraviolet curable material is done layer by layer using photolithography pattern [88]. The four main components in this process are UV laser for radiating resin, photosensitive liquid

resin, transferable built platform, and dynamic mirror system [9]. The process starts by depositing the surface layer of photosensitive liquid resin with a UV laser and solidified to a characterized depth.

The initial layer is solidified and the platform is lowered down vertically layer by layer through photo-polymerization methods. The assisted platform is moved from the surface and the assembled layer is recoated with fluid resin. At that point, the subsequent layer is put over the principal layer where the process persistent until the 3D scaffold is created. Lastly, the uncured sap wiped out and the scaffolds are post cured under UV light with controlled exposure time, light intensity [9]. Farzan *et al.* [89] developed an elastic 3D-printed scaffold by stereolithography and solvent-free methods followed for the fabrication process using PCL and polyethylene glycol (PEG). The outcomes demonstrated that PEG-containing PUs had higher degradation rates, and the elasticity of PU/PCL/PEG was 1.4 and twice higher than that of PU/PEG and PU/PCL, separately. The 3D printed PU/PCL/PEG demonstrated high appropriateness in delicate tissue engineering during the scaffold development process. Elomaa *et al.* [90] developed photo-cross linkable PCL based resin utilizing solvent-free stereolithography methods. In this process, the photo-cross linkable macromers was set up by methacrylating three-outfitted oligomers alongside methacrylic anhydride where the porous scaffolds were developed by stereolithography technique using PCL resin. The ideal resin viscosity was formed during the curing process by heating the resin. Due to absence of solvent, the scaffolds developed provide the best fits with CADs as the material shrinkage do not take place. The interconnectivity of the pores was high in the photo-cross linkable biodegradable PCL resin and has a great potential for tissue engineering scaffolds [91].

6. EXTRUSION-BASED SYSTEM

Extrusion based techniques are the advanced fabrication technique because of various advantages like high resolution, drug-loading, cell-friendly environments, high physical properties, feasibility controlled over drop-on-demand high precision deposition [92]. This technique is mechanically accessible and are less expensive with correlation to other solid freeform manufacturing process. The extrusion-based system depends on material melting where computer-aided designing software is used and scaffold is formed layer after layer. This technique is categorized based on material melting methods and type, however, the heat method is commonly used, some of the other methods like premixed pastes and inks methods are also used [93]. The various process of the extrusion-based system includes fused deposition modelling, multiphase jet solidification, precise extrusion manufacturing, and 3D plotting.

6.1. Fused Deposition Modelling

It is a hot melting extrusion process wherein, the casting of the solid polymer takes place through a nozzle which is ejected and melted on the surface of 3D patterns by utilizing a controlled computer-aided tool for extrusion and deposition process [9]. In this process, the 3D scaffold is made up of layer-by-layer method, where multiple layers of adjacent microfilaments take places. In this technique, the tailoring of bone scaffolds is done with controlled cross-hatch microarchitecture depending on various printing parameters like printing temperature, infill angle, and layer thickness. It is then adjusted directly along with the use of readily available filaments and can be used in developing pharmacological products of good auxiliary properties with the least post-processing requirements. Kalita *et al.* [94] developed and fabricated 3D particulate-reinforced polymer-ceramic composites of polypropylene (PP) polymer and tricalcium phosphate (TCP) ceramic by high shear blending process with adjusted porosity. The PP-TCP composite fibers are prepared to utilize a solitary screw extruder followed by fused deposition to manufacture permeable structures. In this process, the strength of the composite was decreased as pore volume increased to identify through compression testing. The porous scaffolds developed were later characterized to use as bone grafts. Chen *et al.* [95] manufactured PVA/β-TCP composite scaffolds with a combination of solid-state shear milling and fused deposition methods. For the development of bioactivity and osteo-conductive properties, β-TCP bioceramic material is used as the fortifying filler while PVA as the polymer grid. The composite scaffolds formed demonstrated that β-TCP particles homogeneously scattered into the PVA grid with the help of solid-state shear processing. The outcomes firmly demonstrate the capability of the fabricated scaffolds in tissue engineering applications. The deposition takes place at low temperature though encapsulation of cells during the fabrication process was not allowed [59].

6.2. Multiphase Jet Solidification

In this technique, high thickness metallic and ceramics parts are created by utilizing low melting point compounds or powdered blender by compressing out through computational controlled spout to fabricate the part layer-by-layer [49]. The very important parts in this process are controlled with computational positioning arrangements and a warmed chamber with a stream and a pulling framework. The materials in this technique are stacked in the structure of material-binder mixture, powder, bar, and pellets which are heated in a reservoir system or by the processed chambers exceeding the melting point of the binder. Gradually throughout the process, the binder gets liquefied where the heated paste is propelled out through a heated jet spout and settled onto a controlled computational system [59]. Xiong *et al.* [96] developed and fabricated porous PLLA/TCP scaffolds through a computer-aided low-temperature deposition manufacturing process for bone tissue engineering applications. The developed scaffolds showed great porosity of 89.6% besides that scaffolds has controlled interconnectivity pores, great biocompatibility, great bone conductivity, and suitable *in vivo* biodegradable property which make them suitable for tissue engineering.

6.3. Precise Extrusion Deposition

The Precise Extrusion Deposition technique developed scaffolds with controlled structural adaptations and pore sizes that can provide strength, structural integrity, and microenvironment for tissue regeneration and tissue engineering [97]. This method is more applicable in the pharmaceutical field. PCL, one of the most widely used biodegradable polyester has a low melting point and are approved by the Food and Drug Administration with peculiar

applications which are particularly utilized for longer run embeds and controlled drug delivery applications [97]. The precision extruding deposition process is the variations of fused deposition modelling technique, where without filament preparation the scaffolding material are directly deposited [98,99]. Shor *et al.* [100] has developed scaffolds using a precision extrusion deposition process by fabricating PCL-hydroxyapatite composite through computer-aided tissue engineering technique. In this study, the capability of the precision extrusion deposition fabrication process of the scaffolds with structural integrity, controlled microstructure, pore interconnectivity pore size, mechanical property is required for cartilage and bone tissue engineering have been characterized. The developed scaffolds formed 60%–70% porous and with 100% interconnectivity. This technique presented great advantages for high-precision on the micro-scale for complex scaffold fabrication as compared to the conventional fabrication of scaffold methods.

6.4. 3D Bioplotting

3D Bioplotting is one of the versatile and widely used techniques. It involves the mapping of a 3D moving head extruder which is controlled by the use of packed air to compel out fluid or glue-like plotting medium to produce 3D solid scaffolds. Then the characterization is done based on different compositions of material such as tailor-made internal structures, pore interconnectivity, and complex shapes. The scaffolds developed in this technique are fabricated layer-by-layer, where the plotting materials are stored in the form of a movable dispenser. Then with the help of a heating jacket and with nozzle, the gaseous tension is controlled into fluid plotting medium are plotted and maintained [59]. In this process, the polarity and density of the fluid medium are in a limited manner along with plotting material for prevention of gravity-induced structural collapse and to get temporary support structures [101]. Naghieh *et al.* [102] have developed alginate scaffolds by an indirect-bioprinting process that is categorized for nerve tissue engineering applications. These low-concentration alginate scaffolds are developed through the indirect-bioprinting process. It involves various methods that include printing a conciliatory system from gelatin, impregnating the structure with low focused alginate, and eliminating the gelatin system by incubation process, and shaping low-fixation alginate scaffolds. The mechanical and biological properties of the developed scaffolds are influenced by the accumulation of alginate and the disinfection method used which give a viable method of adjusting scaffold properties during the backhanded bioprinting measures. Gómez-Lizárraga *et al.* [103] have developed a 3D bioplotting process for scaffolds fabrication which is made up of a composite of PCL and ceramic micro-powder. The scaffold was manufactured in a cell grid structure. The scaffold developed had a porosity of 32% and a pore size of 323 μm and that indicated a potential use in tissue regeneration and tissue engineering applications.

6.5. Inkjet Printing

This technique is also known as drop-on-demand or electro-dynamic inkjet printing. It is a non-contact technique that is controlled by the physical properties depending on the jet dispensing process where ink droplets or liquid material are discharge into the substrate with predetermined patterns [88,97]. The liquid material is forced through the nozzle by pressurizing the stream of droplets to form the bio-ink solution which is then made up for cell culture medium or the hydrogel [104,64]. In this technique, print heads are provided by the printer that is connected to nozzles through ink chambers. The droplets stipulated are signaled in the form of the pulse from the electrostatic, thermal, and piezoelectric actuators which produce pressure in the liquid materials [104]. While the surface tension of liquid material gets surpass by the pressure formed in the nozzle orifice and finally ink droplet gets emitted out through the nozzle tip. Later through the nozzle, high voltage is applied to the generated substrate. The liquid material gets encircled by the meniscus which prises the ink with the substrate where the electrical power is constrained by surface pressure, that deposit on the substrate [97]. Generally, there are two different methods for this type of technique i.e. piezoelectric-actuated ink jet printing and thermal inkjet printing. Zamanifard *et al.* [105] have developed and fabricated natural polyhydroxybutyrate biocompatible polymer scaffolds by using electro-spun technology and computer-aided software. The modelling of scaffolds was based on data received from the software with response to the exterior and artificial neural matrix strategies. The various data generated are compared with experimental results. In this inkjet process, the natural polymer polyhydroxybutyrate was electrospun and bioscaffolds with high biocompatibility were developed of 224 to 360 nm breadth range. However, the final results confirmed that the scaffold developed by printing polyaniline nanoparticles and oxygen plasma methods with defined designs has a great effect on cell attachment and cell development. In addition, was non-harmful for human fibroblasts and is reasonable for considering the impact of electrical incitement on human fibroblasts. The developed scaffold exhibits various uses in nerve and tissue engineering technologies established by the degradation studies.

6.6. CAD Technology

The CAD technology makes scaffold fabrication a cheap, safe and time convenient by replacing conventional drawing board methods. The CAD process uses multidimensional coordinating system where three-dimensional data are generated digitally with the uses of noncontact 3D laser scanner and rapid-measuring technology. It is then viewed in broad array of perspectives and results are procured with the uses of RP process. The CAD based scaffolds (Table 5) are the advanced integrated fabrication techniques. The computer-aided designing software include AutoCAD [116], FreeForm Plus software [117], MathMod, Meshmixer, Netfabb, and Cura [107] and used libraries such as Visualization Toolkit (VTK), numpy and wxPython libraries that provide complexity geometric primitives [118]. AutoCAD (Autodesk) is a specialized CAD application to develop 2D and 3D model precisely with respect to its dimensions from micro to macro structures and arrangements with the pores sizes. Similarly poncad [119], meccad [120], solidwork [121] and blockscad [122] are associated with designing of scaffolds. Ansys Fluent [123] is to visualize the scaffold with respect to fluidic properties

Table 5: List of Software used in Scaffold design and fabrication.

Software used and purpose	Method of fabrication	Material	Application	Reference
Abaqus assembly Module: geometrical files in stereolithography format were exported MathMod: to visualize and animate parametric surfaces.	Fused filament fabrication	PCL	Soft biological tissue scaffolds	[106]
Mesh- mixer: a3D design software to change porosity of structure. Netfabb: to measure porosity Cura: to change the dimensions of the whole structure.	Extrusion-based 3D printing,	PLA,	Bone tissue engineering	[107]
SOLIDWORKS 17.0: for initiation of geometry was initiated. finite element ANSYS 15.0 software programs for modeling and analysis.	Powder metallurgy process	Titanium dioxide (TiO_2) and Alumina (Al_2O_3) nanoceramic particles	Femur bone	[108]
CAD to obtain Geometry suitable for fabrication	Direct ink writing	Ceramic hydroxyapatite	Ceramic bone scaffolds	[109]
Mathmod (V3.1) software: to generate files to describe the surface of Gyroid. Rhinoceros, netfabb software: for scaling to create various unit cell sizes and final model preparation.	Electron beam melting (EBM)	Ti-6Al-4V gyroid scaffolds	Bone implant applications	[110]
CAD and finite element analysis for designing. COMSOL Multiphysics software: to validate CAD scaffold design.	3D bioplotting.	Of poly(L-lactic-co-glycolic acid), type I collagen, and nano-hydroxyapatite	Bone tissue engineering	[111]
CAD system 3D design software SolidWorks®2012 and exported as an STL file	Powder-based three-dimensional printing stere-olithography	A high-performance composite material (Zp150)	Bone tissue engineering	[112]
MakerBot Replicator 2 FDM modeler for RP	Fused deposition modeling	Polymer ABS material poly(lactic-co-glycolic acid)	Bone tissue engineering	[113]
SolidWorks software to create scaffold patterns. ABAQUS/CAE software: for FE simulations	Robocating or direct ink writing,	Hydroxyapatite (HA)	HA bone scaffolds	[114]
RPTools software for RP	SLS	PCL 4% HA	Bone tissue engineering	[115]

and Abaqus [124] applied in modelling and finite element analysis. VTK [125], is an open-source, freely available software system for visualization, processing of the image and developing three dimensional computer graphics. wxWidgets/ wxPython [126] is a freely available program that allows to develop highly graphical user interfaces. NumPy [127] is specialized program in data analysis and numerical calculation for large dimensions of data using array processing Python package.

7. CONCLUSION

Tissue engineering is a vast multidiscipline area with a wide range of applications. The fabrication of scaffolds is a very complex, dedicated, and sensitive process due to its various factors and parameters. Based on literature surveys, it can conclude that the RP techniques as an advanced fabrication technique that uses computer aided software and tools for scaffold development in tissue engineering applications. The 3D scaffold should able to provide an innate cellular microenvironment and able to create great cellular interaction which plays a vital role in tissue engineering. Although, with recent progress in this research, many challenges have been overcome such as porosity, pore size, expensiveness, mechanical properties, suitable biomaterial selection, inter-connectivity within the pores. Resolving these kinds of problems during the fabrication of 3D scaffold can lead to improved tissue regeneration, gene therapy, drug delivery, bone repairing, tissue-related various engineering processes.

8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

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