Chapter

Advances in Tissue Engineering Approaches for Craniomaxillofacial Bone Reconstruction

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Abstract

Trauma, congenital abnormalities and pathologies such as cancer can cause significant defects in craniofacial bone. Regeneration of the bone in the craniofacial area presents a unique set of challenges due to its complexity and association with various other tissues. Bone grafts and bone cement are the traditional treatment options but pose their own issues with regards to integration and morbidity. This has driven the search for materials which mimic the natural bone and can act as scaffolds to guide bone growth. Novel technology and computer aided manufacturing have allowed us to control material parameters such as mechanical strength and pore geometry. In this chapter, we elaborate the current status of materials and techniques used in fabrication of scaffolds for craniomaxillofacial bone tissue engineering and discuss the future prospects for advancements.

Keywords: tissue engineering, regenerative medicine, bone regeneration, 3D printing, craniomaxillofacial, additive manufacturing, scaffold, polymer, synthetic polymer, biopolymer, bone defect

1. Introduction

The incredible capacity of human body to regenerate is governed by factors such as size of the defect, requirement of growth hormones and the type of the tissue [1]. Any injury to a tissue beyond the critical size needs external support for regeneration. A defect is considered as a critical size defect when it does not spontaneously heal on its own and requires intervention [2]. This approach of mitigating and reconstructing the damaged or injured tissue is referred to as regenerative medicine or tissue engineering [1].

Bone is considered to be the second most engineered tissue, which undergoes degeneration due to tumor surgeries, osteoporosis, trauma etc. [3]. Natural bone matrix is composed of organic (collagen) and inorganic (apatite) materials. By weight bone contains 30% collagen matrix, 60% mineral and 10% water. The unique modulus of bone, falls between conventional plastics and ceramics, and

is primarily determined by a unique interpenetrating arrangement of collagen and apatite at the nanoscale. Naturally, the process of bone healing is determined by the size of the wound. This process in turn is directed and stimulated by well-balanced biological and microenvironmental cues. In case of large bone defects the fibrous tissue regenerates faster than the bone tissue and becomes dominant at the defect site. Excess of fibrous tissue does not compensate for the loss of mechanical strength. Therefore, repair of large bone defects necessitates implantation of a replacement material to facilitate bone healing [4].

Calvarial and long bones are unique and distinct from each other in terms of development, structure and function. From a developmental point of view, intramembranous ossification is dominant in skull bone formation whereas long bone formation majorly occurs via endochondral ossification. These distinctions require customized tailoring of specific strategies for either calvaria or long bone repair. Calvarial bones are required to withstand impact forces whereas long bones must withstand the bending and twisting movements and therefore horizontal grafting and vertical bone augmentation techniques need to be developed for reconstruction purposes respectively [5, 6].

The craniofacial region includes facial skin, muscles, bone, tendons, ligaments, nerves and blood vessels. The craniomaxillofacial bones consist of cranial and facial bones. Cranial bones enclose the brain and protect it, whereas facial bones such maxillary and mandible act as load bearing bones for dental region [7]. The bone tissue thus encompasses mandible, auditory ossicles, neuro cranium (protects the brain) and splanchnocranium (supports the face) [8]. Whereas, the cartilaginous part of the craniomaxillofacial region is primarily constituted by temporomandibular joint disc, auricle and nasal regions of the craniofacial cavity [3]. Moreover, the dental tissue in the craniofacial region includes both hard structures (enamel, cementum and dentin) and soft tissue component (pulp cavity) [7], which together make up the structure of tooth. Tooth is embedded into the maxillary and mandibular bones, which together constitutes the alveolar bone. It has been found that the cortical thickness of the alveolar bone is 2.1–2.4 mm and a density is 1.64–1.75 mg/cm^3 whereas the compressive strength of cancellous bone in the mandibular region is in the range of 0.2–10.44 MPa (average 3.9 ± 2.7 MPa, depending on the bone density, age and gender) [9].

Tooth defects or tooth loss caused by endodontic diseases, periodontal disease, tumor, trauma and variety of genetic disorders require dentin and dental pulp tissue regeneration. However, the current available treatments involve replacement of lost tooth by artificial dentition or dental implants. Thus, extensive research is required to achieve reconstruction of such craniofacial and dental tissue defects [10]. The reconstruction therapy for critical bone defects should address the post-surgical side effects of slow or deficient bone recovery, graft rejection and low osseointegration [11].

Periostium is a major source for osteoprogenitor cells whereas dura mater contains multipotent mesenchymal stem cells that facilitate skull bone healing through paracrine signaling, indicating that the indigenous surrounding tissues of the craniomaxillofacial skeleton such as dura mater, periostium, suture and bone marrow themselves play an important role in healing processes [5]. As the craniomaxillofacial region is associated with a variety of vital functions such as vision, hearing, speech, mastication, breathing and normal brain function, the injuries of this region caused due to trauma, tumor surgery or genetic defects results in critical defects which are difficult to reconstruct because of complexity of anatomical structure, variety of tissue specific requirements and restoration of esthetic facial features, seeking for facial harmony and most perfect symmetry [8, 12–14]. Furthermore, maxillectomy defects are more complex when critical structures such as the orbit, globe and cranial base are involved [12]. Moreover, applications of tissue engineering procedures in this region require additional understanding of

complex developmental processes, physiology, molecular pathways and remodeling characteristics [14]. Thus, an ideal tissue engineering approach to repair craniomaxillofacial defects should result in a complete biological tissue capable of adapting to physiological cues and overcoming the limitation of prosthetics [15].

Extensive research in the field of tissue engineering over the last two decades has revolutionized our approach toward regenerative therapies. Over the years, this approach has progressed in terms of biologically relevant implant materials and technologies for fabrication of scaffolds. There have been tremendous advancements with respect to scaffold synthesis. The earlier researches had focused on replicating the 3D structure of the defect site, where as the recent researchers are capable of performing *in situ* fabrication of the implant. Moreover, further improvements in our know-how have enabled development of live grafts by utilizing stem cells for the purpose.

In the following sections the authors have attempted to summarize the need for addressing bone tissue engineering with special emphasis on craniomaxillofacial regeneration. However, the approach was not diluted and the focus was maintained on discussing the advancement in technologies over the recent years that have opened up new avenues of scaffold fabrication in the field of regenerative medicine.

Methods: After a thorough literature survey, the authors concluded to focus this chapter about the advancements in the technologies for fabrication of bone implants. The authors formulated the basic design of the chapter and targeted their search to the specific keywords, to avoid deviation from the theme. In order to provide an exhaustive but concise overview of the recent developments in methodologies for creation of craniomaxillofacial implants, the authors used various search engines including PubMed, Scopus, Google Scholar and Web of Science. The shortlisted research articles were carefully curated on the basis of their relevance. The authors selected majority of recent articles that highlight the current advances in implant fabrication techniques. To inculcate the basic concepts of materials and techniques, some of the archaic references were also incorporated in the article. The data from these reference articles was then extracted to prepare a summary of advanced techniques of scaffold fabrication, which have gained popularity due to their efficacy and cost effectiveness.

2. Craniomaxillofacial bone defects and reconstruction strategies

Earlier it was assumed that strategies developed to augment appendicular skeletal repair can be directly translated for craniofacial reconstruction. But the use of advanced techniques such as intravital imaging, fluorescence trapping and whole-body optical imaging has revealed that calvarial bone possess a larger normalized blood volume fraction and enhanced bone remodeling activity as compared to long bones [5]. However, the traditional therapeutic modalities of reconstruction such as autologous bone grafting present myriad limitations of restricted availability of donor-site, morbidity and significant complications in restoring the three-dimensional structure of craniomaxillofacial bone [14, 16].

For instance, cleft lip/palates, the most common oral and craniomaxillofacial birth defects are addressed by the standard clinical procedures of surgery involving reconstruction of the mouth roof to separate the nasal cavity from the oral cavity. Two flap palatoplasty and Furlow double-opposing Z-plasty are the two common surgical procedures that involve suturing of soft tissues to close the wound. However, complete restoration of severe cleft palate still remains a challenge due to non-availability of autologous soft tissues [17].

Furthermore, craniomaxillofacial osseous reconstructive surgeries are performed using autologous reconstruction techniques such as free flaps (fibula and

ilia crest) instead of regional flaps (pectoralis major muscle with ribs, trapezius, temporalis muscle with calvaria), because of problems associated with morbidity of regional flaps, though the regional flaps provide for the best candidate in terms of tissue matching [12]. Therefore, membranes have gained extensive importance in the field of oral and maxillofacial surgery, for their use in guided bone regeneration (GBR). These membranes function as a barrier between the fast proliferating soft tissues (fibrous connective tissue or epithelium) and slow proliferating hard tissue (bone) [18]. Membrane systems that are clinically applied do not sufficiently prevent bacterial infections. To address this problem the membranes were fabricated using film casting method, which generates a mechanical barrier to prevent bacterial transmigration through the membrane [19]. Furthermore, as these membranes are either allogenic or xenogenic, a potential risk of transmission of infection along with legal, ethical or religious limitations should be taken into consideration [18].

It has been suggested that the use of scaffold with tailored geometries and surfaces may promote bone regeneration in GBR [18]. Furthermore, finite element analysis of dental implants during mastication has revealed that the surrounding alveolar bone, that supports the dental implant, experiences a compressive stress of 62 MPa while experiencing an applied bite force of 146 N. These compressive forces may go as high as 122 MPa and therefore the bone graft is expected to fully integrate and eventually replace by the host bone tissue [9].

The last decade has seen an extensive progress in craniofacial bone tissue engineering modalities that couple biomaterials with growth factors or stem cell-based therapies [14]. Basically, the bone grafting materials can be divided into autologous, allogenic, xenogenic and alloplastic [9]. However, transplantation of autograft or allograft has limited applicability due to low availability, donor site morbidity, risk of infection, persistent pain, hemorrhage and subsequent graft failure [4, 20, 21]. Also autografts, allografts and xenografts are brittle due to the post extraction processing [9]. Additionally, the traditional procedures of implantation employed metal and metal alloys for repairing of bone defects due to their excellent mechanical properties. However, it was lately realized that the elastic modulus of these metals including stainless steel and titanium-based alloys was much higher than that of human bones, leading to stress-shielding. Moreover, corrosion and release of ionic species from these metal implants has also been found to induce inflammatory responses, cell apoptosis and foreign body reaction [22]. One of the studies demonstrated that a significant amount of time spent in contouring the titanium or absorbable scaffolds (to fit the irregularity of craniomaxillofacial bones), increases the overall risk due to extended operation time. Moreover, over-bending and lack of passive fitting of titanium eventually leads to fatigue fractures [23].

Tissue engineering has been found to address some of these limitations through development of biomimicking 3D matrices [4]. The repair of complex craniofacial bone defects is challenging and the success mainly depends on the choice of reconstructive method [24]. In order to design, develop, recreate and reconstruct a tissue defect, bioimplants (cell-based or cell-free) have emerged as a promising tool. Strategies of bioimplantations require exhaustive knowledge of diverse field such as chemistry, material science, biology, medicine, and engineering. Additionally, the actual designing requires a scaffold material, cells and cell growth factors in place. We have summarized both the knowledge based and material-based requirements in **Figure 1**.

However, placement of implants in the oral cavity encounters a major challenge of insufficient bone volume, as the dental implants cannot be placed in atrophic jaw bone. Therefore, the success of bone reconstruction/regeneration procedures extensively depends on the fact that whether the implant site can firmly support the bone graft material [25]. Moreover, the dentoalveolar defects require a rapidly resorbing matrix to avoid wound dehiscence, exposure and subsequent microbial contamination [26].

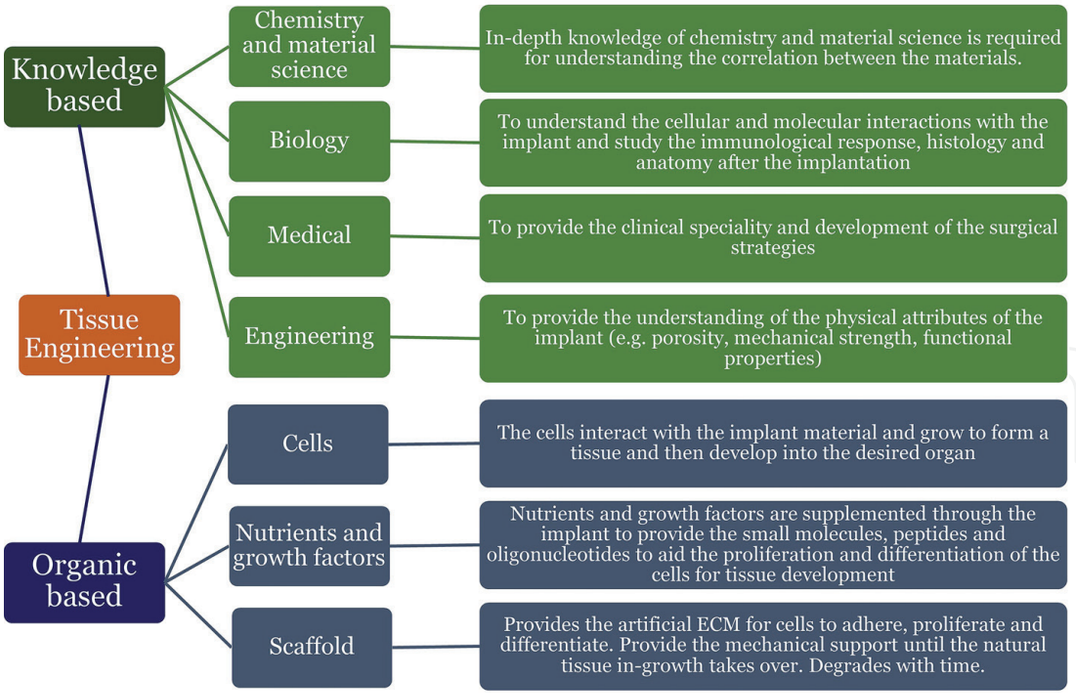

Figure 1.
Pre-requisites for tissue engineering.

Based on several such investigations and observations the specific expectations from a craniofacial scaffold can be enlisted as follows:

a. The scaffolds must have mechanical properties close to that of human bones [22].

b. The scaffold should be three-dimensional, porous with highly interconnected pore structure (to guide bone in-growth, nutrient transport, metabolic waste removal, deliver bioactive agents) to act as bioreactor for growth of cells [13, 22, 27].

c. The use of natural polymers should be preferred for the fabrication of implant to achieve better interaction with cells and eliminate the risk of immunological reaction [4].

d. The implant should appropriately fit into the complex 3D anatomical defects [13].

e. The implant should have spongy like inner part to facilitate scaffold adaptability, integration into the surrounding tissue and vascular invasion [28]. A slow or incomplete vascularization at the site of implantation could result in inadequate supply of oxygen and nutrients and problems in waste product removal, leading to hypoxia and cell death in the surrounding region [29].

f. The scaffold material should be such that it could sustain the masticatory forces of maxillofacial region and protect the implant structure from constant brain pressure until the regenerate acquires the responsibilities [13, 28].

g. The implant material should be biodegradable and bioresorbable (if the bioimplant is meant to temporarily replace an organ) [30].

h. It should be free from contamination and should be easily moldable into various shapes and sizes [30].

i. The implant should be able to develop functional groups on its surface upon appropriate treatments so that it encourages cellular attachment, differentiation and proliferation [30].

j. These implants could be an equivalent alternative to autologous bone when combined with growth factors (BMP2, TGFβ) and/or cells [4].

The choice of biomaterial and method of fabrication are the two critical factors that shape the use of scaffold. Biomaterials are the materials that interface with biological systems and can be classified on the basis of chemical and physical composition, biodegradability, type of origin and generations of modifications. Based on chemical composition biomaterials are classified into ceramics, polymers and composites [1].

i. Inorganic metal compounds and/or calcium salts are the major components of ceramics and are primarily used in orthodontal applications [1].

ii. Polymers on the other hand mimic the connective tissues and are majorly used for soft tissue engineering [1].

iii. The composites that have major applications in orthopedic and dental tissue engineering can be ceramic-based or hydrogel based; are a blend of ceramics-polymers, polymer-polymer; and can incorporate biomolecules, carbon nanotubes and metals [1, 3].

The methods of fabrication are directly dependent on the bulk and surface characteristics of the biomaterials and the projected function of the scaffold. The techniques should be capable of processing different microstructures with strict monitoring of pore size, porosity and pore interconnectivity [31]. The fabrication approach should include design techniques that can rigorously control both the exterior shape of the scaffold and interior porous architecture, to provide the right balance between load bearing strength and delivery of biomolecules [13].

3. Biomaterials for bone tissue engineering

As mentioned in the previous section the biomaterials used for bone tissue engineering are classified as ceramic, polymers and composites. According to the International Union of Pure and Applied Chemistry, the materials are further are classified into three categories: microporous (< 2 nm), mesoporous (2–50 nm) and macroporous (> 50 nm), on the basis of porosity. The porous materials suitable for fabrication of bone implants should have pore sizes ranging from micropore to mesopore scale [32]. Their porous structure provides them a higher surface area to volume ratio, thus enhancing their drug loading capacity [33].

3.1 Bioceramics

Bioceramics such as hydroxyapatite (HA), α and β tri-calcium phosphate, demineralized bone matrices, calcium carbonates, calcium sulfates and bioactive glasses have recently gained importance as novel treatment for craniomaxillofacial bone reconstruction and cleft lip/palate repair [13].

1. Hydroxyapatite (HA, $Ca_{10}(OH)_2(PO_4)_6$), due to its chemical and crystallographic similarities to inorganic components of the bone matrix is popularly used as bioactive coating on dental and orthopedic implants, where it enables adhesion and proliferation of osteoblast on the prosthetic surface and eventually results in biological fixation of implant with the bone tissues [34]. Octacalcium phosphate has also recently emerged as a biological precursor of hydroxyapatite in bone and teeth [16].

2. Calcium phosphate cement is a promising material for dental and craniofacial applications due to its injectability, moldability, bioactivity and bone replacement capability. Incorporation of RGD in calcium phosphate cement has been suggested to enhance formation of microcapillary-like structures by endothelial cells [29]. A synthetic bone substitute material fabricated by mixing alginate-fibrin microfibers and calcium phosphate cement is an aqueous phase that can be injected into osseous defects and allowed to set in-situ during dental, craniofacial and orthopedic procedures [35].

3. Tricalcium phosphate (TCP) is a commonly used bone implant material. Its low bending strength makes it suitable for engineering of maxillofacial bone instead of load-bearing bones. Particle size, depowering efficiency, binder droplet size and scaffold geometry govern the resolution, porosity and strength of 3D printed TCP scaffolds. Its partial dissolution and release of calcium and phosphate ions results in formation of biological apatite precipitate on the surface of bioceramic scaffolds [36]. One such composite scaffold of microstructure β-TCP granules embedded in glycerol matrix has been reported to induce bone formation when implanted at heterotopic sites in bilateral alveolar goat cleft model [13]. One of the major challenges while working with β-TCP is to maintain a low temperature of sintering to avoid transformation of β-TCP to chemically unstable α-TCP [26].

4. Biphasic calcium phosphate (BCP) ceramics exhibit controlled degradation rate, high porosity and low mechanical properties, thus limiting their application for non-load-bearing bones [36]. BCP implant, fabricated using custom-designed 3D microprinting, was demonstrated to induce new bone formation when implanted in critical-sized alveolar bone defects of rats [16].

5. Calcium silicate ceramics possess excellent mechanical properties and compressive strength. But their high dissolution and degradation rate results in increased pH of the surrounding environment thereby hampering cell growth and osseointegration [36]. Wollastonite ($CaSiO_3$) is an attractive bioceramic for repair of craniomaxillofacial defects because of its biological performance and improved mechanical properties by foreign ion doping [37].

6. HAB, a triphasic bioceramic developed by incorporation of hydroxyapatite, β-tricalcium phosphate, calcium silicates and traces of magnesium has been suggested to be a suitable material for craniofacial bone tissue engineering due to its osteoconductive, osteoinductive and proangiogenic properties [38].

7. Phosphate glasses (PG) contain phosphate rather than silicate and have a highly asymmetric structure. Orthophosphate (PO_4) tetrahydrone forms the basic unit of these glasses. Calcium PGs have gained importance because they can be tailored to have a composition similar to the mineral phase of the bone [39]. These bioglasses have a higher rate of dissolution in aqueous medium (due to

ease of P-O-P bond hydration), that is strongly dependent on their composition. Their dissolution rate can be tailored by adding appropriate metal oxides (TiO_2, CuO, NiO, MnO, Fe_2O_3) and these bioglasses can be utilized as controlled release vehicles [33]. Calcium PGs scaffolds have been demonstrated to regenerate bone and cementum when implanted in 1-wall intrabony alveolar defects of beagle dogs [39].

8. Mesoporous materials such as bioactive glasses (BG) are popularly used as implant materials for alveolar bone regeneration. For synthesis of mesoporous BGs, surfactant is introduced as the structure directing agent, during the sol-gel process of the glass. The surfactant is removed at the end of the process by calcination or extraction and the micelles previously occupied by the surfactant are replaced by mesopores. For the purpose of tissue engineering mesoporous bioglass can be coated on the surface of polymeric scaffold; incorporated in a polymeric matrix in the form of particles; fabricated as scaffold and coated with a polymer [33]. Mesoporous BG nanolayers (thickness 100 nm), created by spin coating on the surface of β-TCP scaffolds have been found to significantly improve osteogenesis [40].

3.2 Polymers

Polymer materials are composed of chemical compounds typically formed from monomers of carbon, hydrogen, oxygen and nitrogen. These monomer structures repeat and bind with themselves to create long molecular chains. Polymers have gained importance for fabrication of scaffolds as they are inexpensive, biocompatible, biodegradable and can be easily manipulated for their chemical, mechanical and biological properties. The commonly used polymers in craniofacial tissue engineering include natural polymers and synthetic polymers [3]. Natural polymers can be categorized into two main subgroups e.g. polysaccharides (alginate, cellulose, starch, chitosan) and polypeptides and proteins (collagen, silk fibroin, albumin) [31], whereas synthetic polymers majorly include polycaprolactone (PCL), poly lactic acid (PLA), poly(l-lactic) acid (PLLA), poly D, L-lactide-co-glycolic acid (PLGA), polyvinylidene fluoride (PVDF) and magneto-responsive polymeric systems [3, 41, 42].

Synthetic materials such as PVDF (poly (vinylidene fluoride)), P(VDF-TrFE) co-polymer of vinylidene fluoride (VDF) and trifluoroethylene (TrFE), PHBV (poly-3-hydroxybutyrate-3-hydroxy valerate), poly-l-lactic acid (PLLA) and natural polymers such as cellulose, collagen, silk and chitin, exhibit piezoelectric properties and hold a great promise in the field of bone tissue engineering [33].

3.2.1 Natural polymers

1. Collagen is extracted from animal (bovine and porcine skin or bone) and marine sources. However, animal derived collagen poses risk to public health and safety [22]. Collagen is the main organic component of dentin matrix and presents a good alternative for dental implantations [41].

Adhesion of calcium salts to a suspension of collagen and glycosaminoglycan in phosphorous acid results in precipitation of brushite form of calcium phosphate into the collagen network, which upon subsequent lyophilization form porous foam. A portion of calcium and phosphate ions from these mineralized scaffolds are released and accelerate osteogenesis. As craniofacial bones rely on intramembranous ossification, these mineralized collagen scaffolds

are shown to promote osteogenesis in rabbit calvaria and sub-critical sized porcine mandible [43].

2. Silk fibroin is a natural, low-cost, biodegradable and biocompatible polymer obtained from cocoons of *Bombyx mori*, *Antheraea pernyi* (tussah), *Antheraea mylitta* (tasar) and *Philosamia ricini* (eri). Being a natural polymer, it offers good permeation for oxygen and water but has a low compressive strength [44].

Silk can be manipulated into various forms for fabrication of scaffolds for craniomaxillofacial tissue engineering applications. Addition of methacrylate groups to amine-containing side groups of silk gives rise to silk methacrylate (SilMA) scaffolds, whereas combing methacrylate groups with amine-containing side groups of gelatin results in gelatin methacrylate (GelMA), that becomes photocross linkable in the presence of photoinitiator [9].

3. Chitosan is a deacetylated form of chitin and is the second largest natural polysaccharide after cellulose. It is extracted from crustacean shells and marine sponges. It exhibits fungicidal and anti-microbial activities [24, 41]. Its biodegradability, biocompatibility and excellent cell adhesive properties make it a popular choice for implant material [41]. But its weak mechanical strength and high rate of biodegradability requires crosslinking with other natural or synthetic polymers (e.g. blends) for orthopedic or periodontal applications [24]. For example, chitosan is crosslinked with polyethylene glycol diacrylate (PEGDA) to produce photocrosslinkable blends [9].

3.2.2 Synthetic polymers

1. PCL is a biodegradable thermoplastic (with a low melting point of 59–64°C) that is widely used for drug delivery in dental implants owing to its low cost and physico-chemical properties, enabling its application in nanometric scale processing and prototyping [41, 45]. PCL also finds its application in prevention of bacterial accumulation on dental implants [41]. Incorporation of fibroin and nano-HA in PCL have been found to enhance compressive modulus, cellular adhesion and calcium deposition after 14 weeks of implantation in large scale calvarial defect model [46].

2. PLA is a degradable polymer that can be adapted to different morphologies. However, its degradation results in production of acidic by-products and therefore requires blending with other materials such as tricalcium phosphate [41].

3. PLLA is fast degrading polymer that possesses good physical and mechanical properties and supports cell adhesion and proliferation [41].

4. PLGA is a biodegradable and biocompatible polymer that exhibit close resemblance with natural proteins and is metabolically hydrolyzed to monomers of lactic acid and glycolic acid [41]. A functionally gradient three layered PLGA construct with low macroporosity and high mechanical properties was demonstrated to successfully bring about periodontal regeneration [47].

5. PVDF is also a biocompatible, flexible material with high mechanical strength and good anti-bacterial properties [41].

6. Magneto-responsive polymeric systems are comprised of polymer networks that are physically or chemically functionalized with magnetic nanoparticles (of magnetic elements such as iron, cobalt, nickel or their oxides). When these particles are covalently immobilized or physically entrapped (by blending, in situ precipitation or dip coating), they respond to magnetic field. This property enables spatio-temporal control over the physical, structural and mechanical properties of the polymeric scaffold. Leaching out of magnetic nanoparticles (< 50 nm) from these materials and their ability to cross the biological membrane, thus inducing inflammation, generating ROS, impeding DNA function and driving cell apoptosis limits them incorporation in the polymeric networks [42].

3.3 Composites

The composites are a blend of ceramics-polymers, polymer-polymer; and can incorporate biomolecules, carbon nanotubes and metals [1, 3].

Various ceramics, polymers as well as composites are subjected to functionalization of their surfaces to improve their compatibility with the biological microenvironment. In order to modify the surface hydrophobicity of polymers, to make them more hydrophilic and biocompatible, various surface modification techniques are employed [30, 48].

Graft polymerization technique: Polymer grafting and graft polymerization are achieved by chemical, photochemical, plasma induced and enzymatic grafting methods [30].

Nanoindentation method: This method is used to increase roughness by micropatterning to promote cell adhesion, but it is difficult to implement on large scale [30].

Surface modification by self-assembled monolayer formation: Metal surface modified by ligands through metal ligand bond formation [30].

Corona discharge: Electrically induced stream of ionized air is bombarded on the polymeric surface resulting in generation of oxygen containing functional groups. As this method does not operate in vacuum, it is prone to contamination by local moisture and humidity [30].

Flame treatment: Bombardment of polymer surface with ionized air resulting in surface functionalization of top several layers of polymer with hydroxyl, aldehyde and carboxylic functional groups. Although the method increases printability, wettability and adhesiveness of the polymer surface, it reduces the optical clarity [30].

UV irradiation: UV irradiation of polymer results in generation of reactive sites and can initiate graft polymerization of bioactive molecules such as N-vinyl pyrrolidinone [30].

Wet Chemical treatment: treatment of polyethylene and polypropylene surfaces with concentrated acid such as chromic acid in presence potassium permanganate and concentrated sulfuric acid results in development of reactive oxygen of functional group. This method generates hazardous chemical waste and surface etching and is therefore difficult to scale up [30].

Plasma treatment: Plasma is a high energy state of matter in which gas is partially ionized into charged particles, electrons and neutral molecule. Such ions when bombarded on a polymer surface results in functionalization of molecules in contact [30].

Graphene coating: Graphene is a 1-atom thick film with a honeycomb structure and is composed of carbon atoms created by sp2 hybridization [10]. The materials of graphene family have been widely applied in diverse medical applications owing to their nanoscale size photoluminescence properties, large specific surface area

and anti-bacterial activity [49]. High elasticity and flexibility of graphene and its derivatives (graphene oxide (GO) and reduced graphene oxide (rGO)) presents them as a promising mechanical filler for biomaterials [34]. It is widely used as surface modification coating or dopant in scaffolds, to enhance biocompatibility and promote osteogenic differentiation of stem cells [44].

4. Fabrication techniques

Tissue engineering is a multidisciplinary field that imbibes the principles, knowledge and methods of chemistry, physics, engineering and biology. It involves three fundamental elements: cells, scaffold and cell signaling [7].

The fabrication of scaffolds requires pre-treatment of the graft material with various solvents, which facilitates the dissolution of the biomaterial. It is a general observation that processing and storage of scaffolds in the presence of solvent, hampers the control of spatial distribution of the bioactive agent in the porous structure of the scaffold. The final processing involves removal of these toxic organic solvents or any other residual porogen species by leaching or solvent extraction methods. This results in loss of the medically important bioactive agents. However, the methods employed for processing of scaffolds should also be evaluated from an environmental point of view. Therefore, E-factors (the actual amount of waste product produced in the process per gram of product) and low carbon footprint methods are the two parameters that deserve attention. Thus, processing technologies such as melt molding (compression, injection and extrusion molding); 3D printing; fused deposition modeling; sintering of solid particles (heat, compressed CO_2 and selective laser sintering); gas foaming and compressed or supercritical CO_2 foaming, operating in the absence of solvent during assembly of 3D scaffolds, present ideal strategies for development of medicated scaffolds [11].

The use of natural fiber composites over synthetic fibers also needs substantial attention from green synthesis point of view. Glass or carbon fiber-reinforced composites, belonging to the category of synthetic composites have been well researched for last 20 years. However, due to environmental and economic considerations the focus of research has diverted to natural fiber-reinforced composites. The advent of 3D and 4D printing provides huge opportunity for development of natural biocomposites, for the first time on the same time scale as their synthetic counterparts [50].

In the sections below, we will be discussing various techniques employed for fabrication of biomaterial implants for craniomaxillofacial bone reconstruction/regeneration.

4.1 Electrospinning

Electrospinning was first applied in 1934 by Anton Formhals and represents a combination of electrospray and spinning of fibers [51]. A typical electrospinning apparatus includes a capillary tube with a spinneret, a high voltage power supply and a collector (**Figure 2**). During electrospinning, polymer droplets are generated by extruding polymer solution from the electrically conductive spinneret. A high voltage is applied between the spinneret and the grounded collector. The polymer solution ejects from the spinneret when the potential between the solution breaks through the surface tension of the droplets, resulting in fibrous polymer scaffold (diameter ranging from 100 nm to several micrometers)

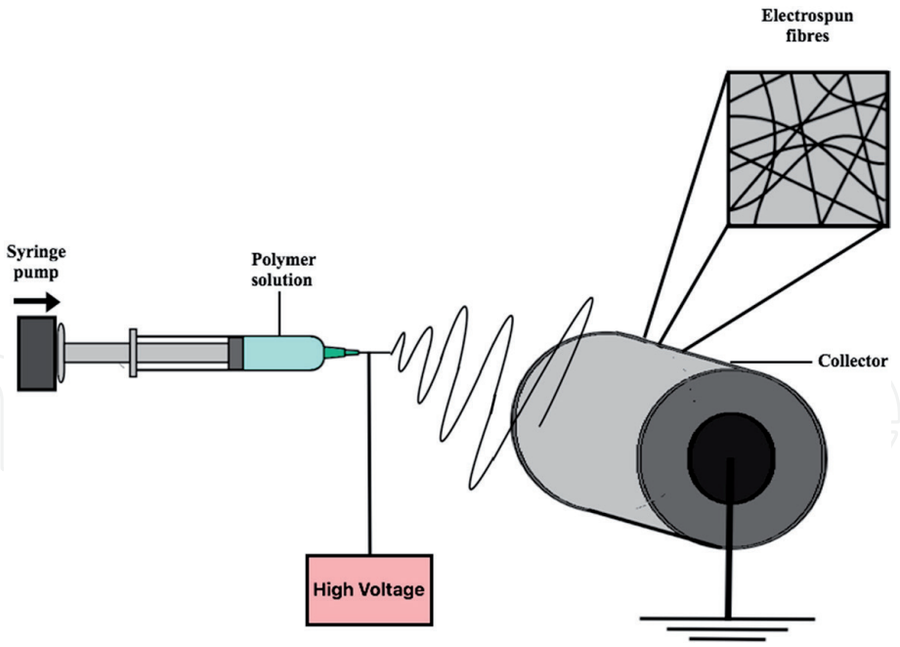

Figure 2.
A typical electrospinning apparatus for fabrication of scaffolds.

[52]. Introduction of a liquid bath collector in an electrospinning setup results in production of fluffy morphologies such as yarn or spongiform fabric. For example, the collector may contain ethanol and water-ethanol (non-solvents), for spinning of polysaccharides such as cellulose/heparin (blend) and cellulose/multiwall carbon nanotubes (core/sheath) [20]. In addition, antibiotics can also be incorporated into the electrospun scaffold to prevent bacterial colonization after implantation [53].

The electrospun scaffold is physically like a tissue paper with easy handling and therefore is well adapted for critical bone defects in craniomaxillofacial region [54]. On the basis of initial state of polymer electrospinning can be categorized as solution electrospinning, emulsion electrospinning and melt electrospinning writing [18, 55].

a. In solution electrospinning, polymer is dissolved in organic solvent (e.g. chloroform or dimethyl formamide), which evaporates when the polymer jet is ejected toward the collector [18]. A lower flow rate is preferred for proper evaporation of the solvent [51]. But the solvent residues left on the fibers limits its applications [18]. An increase in fiber diameter and pore diameter (the void portion) is observed by increasing the polymer flow rate that also alters the morphological structures [51].

b. In emulsion electrospinning core-shell nanofibers are constructed without a specific needle setup, by emulsifying the drug aqueous protein solution in the polymer solution. In this technique an emulsion is created within a single solution, where the emulsified droplets get organized in two separate phases, consequently as a result of evaporation of the solvent form the electrospun fibers [55].

c. Melt electrospinning writing utilizes melting of medical grade polymers such as PCL, thus eliminating the risk of residual toxic solvent [18]. This technique has been used for fabrication of well-defined surface layers with different

geometries, that are individualized for attachment of osteoblast on one side and keratinocytes and fibroblast on the other side [19].

Ceramic, metallic, glass-based fibers can be produced by electrospinning, by injecting the polymers to a simple syringe with metallic tip of different diameters [47]. PCL and nano-HA composite scaffolds fabricated using similar technique holds potential for repairing of critical bone in craniomaxillofacial region [54]. These scaffolds not only have superior mechanical properties but also possess ability to carry growth factors and drugs [56]. It was demonstrated that trigeminal ganglion when added to ε-PCL membranes synthesized by electrospinning and functionalized with nerve growth factor nanoreservoirs, were able to regenerate peripheral axons in the pulp cavity, two weeks after implantation [57].

Melt spinning and wet spinning: Melt spinning involves melting of the polymer followed by its extrusion through small holes resulting in formation of solidified fibers after cooling. The resulting fibers are collected by a take-up wheel to form continuous fiber strands. Wet spinning involves dissolution of polymer in appropriate solvent followed by extrusion of polymer solution through a spinneret into a coagulation bath containing a non-solvent [22].

4.2 Electron beam melting (EBM)

EBM was developed and patented by Swedish Arcam Company. The equipment is mainly composed of an electron beam gun compartment and a specimen-fabrication compartment, both kept in high vacuum. The technology employs high energy electron beam to melt the metal powder. The electron beam preheats the powder bed to reach a slight-sintering state by scanning the powder layer quickly before EBM. This step is followed by selective scanning of powder layer by electron beam based on the 3D hierarchical data, causing the preheated powder to melt and solidify together. The high beam-material coupling efficiency makes it a method of choice for processing of metals with extremely high melting points. One of the case studies demonstrated fabrication of 3D titanium scaffold with EBM for reconstruction of whole mandible defect [52].

4.3 Gas foaming

The principle of gas foaming is to generate pores in a polymeric matrix through a nucleation-growth mechanism of gas bubbles that results in formation of microporous material after venting out of the bubbles. This process is compatible with both hydrophobic and hydrophilic polymeric matrices and is usually performed under mild temperatures [11]. This solvent free technique consists of 3 steps

Step 1: dispersion of porogen (chemical blowing agent e.g. Sodium Bicarbonate and Ammonium Bicarbonate or physical blowing agent e.g. inert gas - Nitrogen, Argon or Carbon dioxide) [11].

Step 2: generation of gas bubbles due to porogen removal resulting in nucleation growth mechanism and pore formation [11].

Step 3: rapid lowering of temperature to allow vitrification of the material by freezing and avoid destabilization of resulting foam [11]. The foaming process carried out using molds can be used to cast the foam to a shape that can fit into an anatomical defect.

Supercritical CO_2 ($scCO_2$) foaming and compressed CO_2 foaming are two of the green technology processes that utilize the valorization [11] and plasticizing effect of CO_2 under super critical conditions (temperature and pressure above the critical point of CO_2, 31.1°C and 72.8 bar) for reducing the apparent glass transition (T_g) and the melting temperature of the polymers. When the pressure is reduced, CO_2 dissolved in the polymer matrix gets super saturated resulting in formation of pores from growing nucleation sites. This method employs mild conditions and avoids the use of organic solvents, thus retaining the activity of thermally sensitive compounds such as growth factors [46].

4.4 Freeze-drying technique

In this method a water-soluble polymer is frozen such that interpenetrating ice crystals are created which are later removed by sublimation, resulting in formation of porous scaffold [22].

4.5 Particle leaching and phase inversion

In this process PLGA was dissolved in DMSO, and then the PLGA solution was thoroughly mixed with CaP particles in a ratio of 1:3 (w/w). This sticky mixture of CaP/PLGA was then poured into an aluminum foil mold filled with sugar crystals at a weight of 3 times the DMSO volume. The mixture diffuses throughout the mass of porogen crystals. After 2–3 minutes the mold was transferred to a refrigerator at −18°C for 1 h to set the mixture. The PLGA was precipitated and the sugar crystals were leached out of the precipitated CaP/PLGA mixture in ddH_2O at room temperature (20°C) for 3 days, during which time the ddH_2O was changed approximately 4 times each day. Every time a scaffold block was produced [58]. Thus, PLGA and two calcium phosphate phases (first is a particulate within the structure and second is a thin ubiquitous coating) get fabricated into a composite scaffold with a pore size and interconnecting macroporosity similar to that of human trabecular bone. The osteoconductive surface of calcium phosphate abrogates the putative foreign body giant cell response to the underlying polymer, whereas the internal calcium phosphate phase provides dimensional stability. The highly interconnected microporosity and the ability to wick up blood make the scaffold a clot-retention device and an osteoconductive support for growth of host bone. Such scaffold has been implemented in human patients for maintenance of alveolar bone height following tooth extraction. These scaffolds also augment alveolar bone height through standard sinus lift approaches. It was also observed that these scaffolds regenerated sufficient bone tissue in the wound site and provided good foundation for dental implant placement [16].

4.6 Phase separation

It is a solvent based technique that employs change in temperature to induce phase separation of homogenous polymer-solvent solution through solid-liquid demixing or liquid-liquid phase separation [22, 47]. On this basis it is mainly divided into two types liquid-solid and liquid-liquid phase separation. The method is conducted by reducing the temperature of solution and extraction of solvent phase, till it reaches a porous polymer scaffold [47]. The phase separation majorly involves formation of a polymer-rich phase and polymer-poor phase upon rapid cooling of polymer-solvent solution by freeze-drying or freeze-extraction [4, 22]. As a result, the polymer-poor phase is eventually removed [22]. The solvent system utilized is usually a mixture of 1,4-dioxane and water and the temperature and

time for the process is around 60°C for two hours. It has been observed that strong polymer-solvent interaction leads to solid-liquid phase separation, whereas weak polymer-solvent interactions results in liquid-liquid phase separation. The role of non-solvent such as water is to lower the degree of polymer-solvent interaction so as to induce liquid-liquid phase separation [4].

4.7 Computer-aided techniques

The Computer-aided designing (CAD) is gaining popularity with respect to construction of model on the basis of constructive solid geometry or boundary representation principle. Models obtained by boundary representation require more storage space compared to constructive solid geometry. Therefore, as the model becomes larger or more detailed in internal structure the size of the file containing boundary-representation-derived-model drastically increases causing difficulty in operation. CAD methods are realized by utilizing various tools such as UG, CATIA and Pro/E. Some dedicated design software's such as Magics (3D printing preprocessing software developed by Belgium Materialize Company), have been recently developed in which the designer can directly instruct various integrated unit cells. MATERAILAS and computer-aided system for tissue scaffolds (CASTS) are some of the software and parametric library respectively that are used to design algorithms for minute detailing of the desired scaffold [52]. Implementation of CAD/CAM softwares along with radiology procedures for easy acquisition and transfer of DICOM3 (Digital Imaging and Communications in Medicine) data allows the surgeon to perform three-dimensional measurements and reconstruct the deformed or missing anatomy by segmentation [59].

Computer assisted textile-based technologies constitute an attractive route to strategize scaffolding (including stitching, braided, woven, non-woven and knitted) of more complex fibrous 3D scaffolds suitable for engineering of soft tissue such as ligament in the craniofacial regions [28].

With advancements in our knowledge about materials along with a boon in computer-aided technologies, several methods have evolved recently that increases the precision and accessibility of craniomaxillofacial bone reconstruction (**Figure 3**).

Recently rapid prototyping has emerged as an effective tool for 3D printing of porous scaffolds with interconnected porous network [42], complex geometries, well defined and reproducible architectures [22]. The basic concept of rapid prototyping involves presentation of cycles of cross-sectional sheets from where the data is exerted into the solid free form fabrication machine to produce the physical model. As the layers are built from bottom to top, each newly manufactured layer sticks to the previous. Several techniques originate from this principle of rapid prototyping. We have discussed some of the major types of this technique in the following sections [42].

4.7.1 3D printing

3D printing has emerged as a promising tool of additive manufacturing (AM) that enables us to optimize the processes of preoperative planning, develop intraoperative guidance tools, reduce operative time and improve bifunctional and esthetic outcome [12, 60]. Liu et.al fabricated Al_2O_3 scaffolds with a through-hole structure using 3D printing and sol-gel technology. Alumina (Al_2O_3) is a bioinert ceramic and exhibit negligible tissue reaction and therefore several researchers incorporate other components into Al_2O_3 to enhance the mechanical strength of the scaffold. Fabrication of Al_2O_3 / borosilicate glass scaffolds using urea- formaldehyde resin as in-powder adhesive by 3D printing has been demonstrated to maximize the tensile

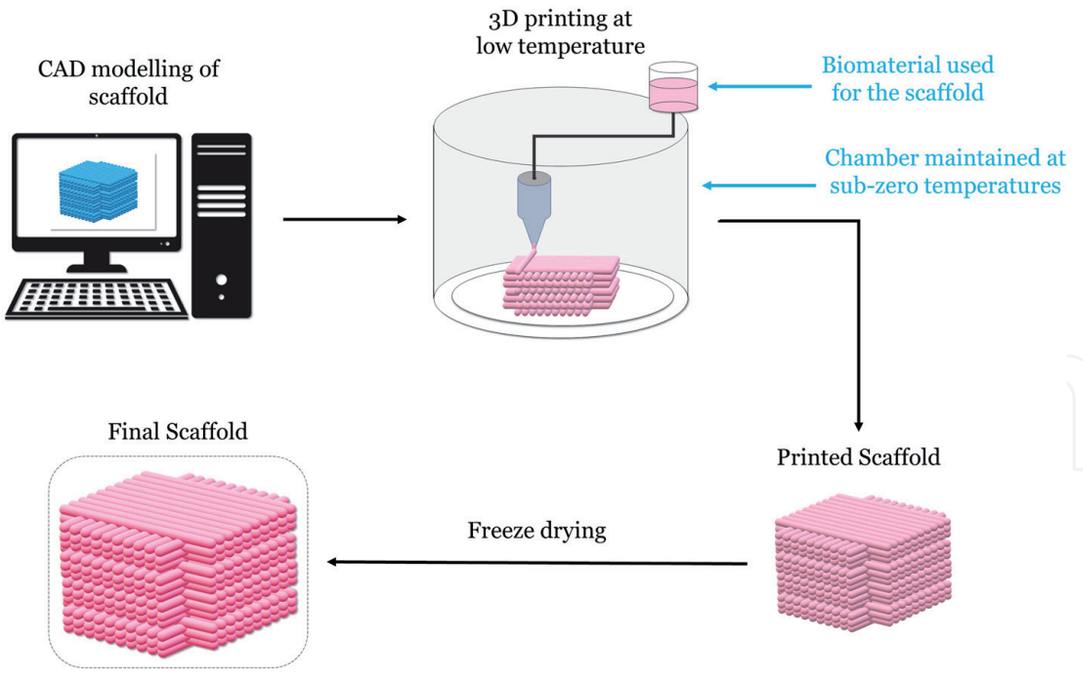

Figure 3.
Computer-assisted scaffold modeling for fabrication of implants.

strength [40]. In the last decade, investigators have reported 3D-printed prostheses of nose, ears, eyes, face, and hand [12]. With the help of direct writing technology tricalcium phosphate scaffolds have been fabricated and used to repair rabbit trephine defects [61].

Significant improvements in clinical imaging and user-friendly 3D software with progression of open source platforms, associated with recent hardware developments have enabled 3D printer to build layers as small as 16 μm thickness for stereolithography (Polyjet, Stratasys); 178 μm thickness for fused deposition modeling (Fortus 900 mc, Stratasys); 80 μm thickness for selective laser sintering (sPro 230 HS, 3D systems) and 75 μm resolution for stereolithography (3D systems) [12, 62]. These 3D printing techniques including stereolithography, multi jet modeling, selective laser sintering, binder jet technique and fused deposition modeling provide appreciation of visuospatial relationship between the anatomical structures created and craniomaxillofacial reconstructive surgery [12].

1. Inkjet printing was the first bioprinting technology of AM that was developed by Hewlett Packard company in 1970s as a 2D printing technology [42]. Later in 1992 an elevator platform that can move along Z axis was added to it to develop a 3D bioprinting system [42]. It offers the option of creating complex spatial patterns without fabricating purpose-specific lithographic masks. Structural and conformal cell printing methods have been used to create cell constructs from bottom to upward (layer-by-layer or cell-by-cell), resulting in heterogeneous cell and biomolecular 3D structure. Structural cell printing requires simultaneous or sequential printing of cells and biomolecules whereas conformal cell printing is a hybrid approach where biomolecules are printed on top of thin layers of prefabricated scaffolding. This method is beneficial for fabrication of implants that facilitates vascularization and therefore promises their role in oral and maxillofacial bone regeneration [63].

2. Piezo inkjet printing: In powder bed inkjet printing, droplets of dilute solutions or biomaterials act as binder to the bulk material positioned within the

powder bed. These droplets are dispensed and driven either by thermal or piezoelectric processes into a powder bed. The thermal inkjet printing employs temperature between 100 and 300°C to nucleate a bubble and eject the droplets, which produces shear and thermal stress on natural polymer inks, resulting in inconsistent droplet volume. Piezoelectric technology utilizes pressure or acoustic waves produced via piezoelectric actuator to generate the drops and therefore can be used with a range of polar and non-polar solvents [52].

A wide range of powder materials such as polymers, ceramics, proteins and cells can be processed using this technique. However, the ink's viscosity is limited to 5 to 20 Pa.s to avoid high ejection pressure or continuous flow of material [3].

3. Selective laser sintering (SLS) is an additive manufacturing technique which utilizes high power laser for sintering of metals or ceramic powders for scaffold synthesis [40, 42]. This technique was developed and patented by Carl Deckard and Joe Beaman in 1989 [42]. The method employs a computer-controlled CO_2 laser beam to induce a local increase of temperature (above the T_g of the selected polymer) and selectively fuse and sinter polymer composite powders in a layer-by-layer manner to build a 3D scaffold [11, 22, 40]. After the fabrication is completed excess powder is removed either by brushing or application of compressed air [42]. SLS is a single step process that offers products with high resolution due to laser precision [11]. The method is associated with certain disadvantages due to its working requirements. (a) since the scaffold is created by fusion of spherical particles, there is certain degree of roughness on its surface that requires polishing [42]; (b) use of high temperature renders it unsuitable for natural polymers [3]; (c) at industrial scale standard SLS machines require large quantities of material in adequate powder form, thus making the process very expensive [11]. This method extensively finds its application in regeneration and repair of periodontal, craniofacial bone and osteochondral defects that possess complex anatomy and can be used to work with a variety of powder materials including metals, bioceramics and synthetic polymers such as PLA, PCL, poly ethyl ether ketone and poly ether ketone ketone [3].

4. Stereolithography or vat polymerization fabricates the products through selectively curing photo reactive resin. The method involves formulation and polymerization of photopolymer liquid in a vat by ultra violet light irradiation on the surface with designed pattern. As the platform moves the parts that are built downward after each new layer are cured. The steps are repeated to form the entire object and the excessive resin is drained out. SLA utilizes two types of polymerization reaction – free radical polymerization and cationic polymerization. On the basis of irradiation type SLA is further categorized into vector scan approach (irradiation through ultra violet beam and projection on liquid surface through optics and scanning galvanometer) and mask projection approach (the radiation source creates large area pattern through digital micromirror device and harden one layer at a time) [52].

UV radiation is the most common curating agent in SLA. When a two-dimensional layer of gelatin methacrylate (GelMA) and different concentration of photoinitiator were cured with UV exposure, it was found that the concentration of photoinitiator affected the porosity of GelMA hydrogel by polymerization-induced phase separation. Similarly, fabrication of poly(propylene fumarate) (PPF) was carried out by embedding PPF/diethyl fumarate photopolymer with PLGA microspheres loaded with bone morphogenetic proteins (BMPs). PPF is known to form cross linked polymer network when combined with photoinitiator

bisacrylphosphrine oxide and exposed to UV light [9]. Cha et al. utilized nano-SLA to print micropillar and microridge patterns on the scaffold and investigated the effect of these patterns on cell adhesion, proliferation and osteogentic differentiation [36]. PolyHIPEs (poly high internal phase emulsion) are the class of material where porosity is created due to phase separation between two immiscible liquids in presence an emulsifier. 2-ethylhexyl acrylates (EHA) and isobornyl acrylate (IBOA), when mixed together and combined with the photoinitiators (diphenyl(2,4,6-trimethylbenzoyl) phosphine oxide/2-hydroxy-2-methylpropiophenone) formed a porous structure in presence of water, upon curation and photopolymerization by laser. In this method the curation is carried out by laser instead of UV radiation. Similarly methacrylated poly(D,L-lactide) (PDLLA) scaffolds were prepared using Irgacure 2959 as photoinitiator [9].

Ceramics are known to be non-photocurable and therefore, they require photocurable resin to bind the particles together [9]. Ceramic materials are primarily made up of metals with inorganic calcium or phosphate salts and are generally osteoconductive and osteoinductive [3]. The scientists investigated that bioceramic slurry of HA and TCP, mixed with photocurable FA1260T resin, cured with SLA and sintered at 1400°C (to remove the solidified photocurable resin and fuse the bioceramic particles together), resulted in fabrication of biocompatible osteoinductive scaffold. In one of the studies, researchers investigated the utility of thiol-ene reactions to produce photopolymer networks, as an alternative to the use of photoinitiators. These reactions occur between alkene and thiol-monomers to form an alkyl sulfide group that is regarded to photo-trigger the chemical reaction, thus eliminating the need of photoinitiator. A 1:1 ratio of thiol (pentaerythritol tetrakis(3-mercaptopropionate) (PETMP)) and alkene (poly (ethylene glycol)divinyl ether (PEG-DVE)), has been shown to crosslink without the presence of photoinitiator and was also biocompatible [9].

5. Laser-assisted 3D printing also known as laser-assisted bioprinting (LAB) basically has three main components (a) a pulsed laser source, (b) a transparent glass slide or ribbon, as a target, serving as a support for the printing material and (c) a receiving substrate to collect the materials. While printing, a focused laser pulse stimulates a small area of the target which is mainly made up of an energy absorbing layer on the surface and bioink solution underlay. Evaporation of a portion of the energy absorbing layer results in formation of a droplet that is collected by the receiving substrate and crosslinked [3]. Pure calcium silicate and dilute magnesium doped scaffolds of different layer thickness and macropore sizes, prepared by varying the layer deposition mode from single-layer printing to double layer printing, have been demonstrated to improve bone tissue ingrowth in craniomaxillofacial bone defect treatment [37]. Varying the layer configuration from single to double layer printed versions has been shown to significantly enhance side-wall pore size and strut thickness [37].

As, LABs are not equipped with nozzle and obviate direct contact between dispenser and bioink, they minimize the problem of material or cell clogging [3, 42].

6. Fused deposition modeling (FDM) was developed and patented by Scott Crump and is one of the most popular rapid prototyping technique [42]. It is the most widely used extrusion based additive manufacturing technique that fabricates scaffolds without the use of toxic organic solvents [22]. Extrusion based printing uses pneumatic, piston or screw driven system to create

pressure and push out the suspension, solution or emulsion [1]. FDM is a heat utilizing technique, where the thermoplastic filament is guided into a liquefier for melting through rollers and extruded from the computer-controlled nozzle in a layer-by-layer manner to create a scaffold [22, 25]. Thus, the fabrication process involves movement of computer-controlled nozzle in X-Y plane in order to create the desired pattern after which the nozzle move upward along the Z axis to a predefined distance, to print the next layer [42]. The thermoplastic polymers used for fabrication of scaffolds are called as bioinks [1]. To ensure good interlayer adhesion, the previously formed layer is kept at temperatures just below the solidification peak of thermoplastic material [34]. The process temperature depends on the melting temperature of the building material, which is generally very high for biological molecules [25].

The efficacy of FDM largely depends on the parameters such as nozzle temperature, nozzle diameter, extrusion speed, layer thickness and raster angle [22]. Its solvent free technology cost effectiveness and high speed renders some advantages to this technique [42]. Since this process is carried out at high temperature incorporation of biological molecules becomes impossible [34]. Furthermore, this technique faces a limitation in terms of availability of a medical grade biocompatible thermoplastic material with viscosity that is adequately low for extrusion but at the same time high enough for scaffolding [42]. PCL/HA bone scaffolds fabricated using CT-guided FDM have been found to exhibit cortical bone like features, displayed close mechanics to that of natural bone and integrated tightly with the surrounded tissue [52]. Scientists have developed computer-aided low-temperature deposition manufacturing system that has been successfully demonstrated to fabricate 3D scaffolds identical to the patient-specific alveolar bone defects. But as the resulting bone substitutes are in the form of blocks or granules, they face limitations in clinical applications requiring restoration of complex structure in craniomaxillofacial region [16].

7. Fused filament fabrication (FFF): Similar to other 3D printing techniques, the FFF also involves layer-by-layer deposition of thermoplastic material through a heated nozzle onto the platform or previously printed layers. FFF has certain advantages that include (a) minimizing the cost of production runs (b) reducing the production waste (c) shortening the design manufacture cycle (d) ability to build intricate geometries and (e) ability to tailor microstructure and properties in each layer. The mechanical performance of FFF is controlled by slicing and printing parameters. The slicing parameters include raster angle (in-plane angle), the inter-filament distance, layer height, filament orientation, nozzle diameter, filling pattern (e.g. honeycomb, hexagonal, triangular) and the build orientation (out-of-plane). The printing parameters include nozzle geometry, nozzle temperature, printing speed, printing trajectory, bed temperature and calibration. Though development of fiber-reinforced composites developed using FFF have enhanced mechanical properties, the major limitation of these printed composites are inherent extrusion-induced defects, such as porosity (due to poor interfacial bonding between the fibers and the polymer and between the printed beads or the printed layers) [50].

No matter which specific technique is used to produce the 3D biomodel, the following are proven advantages of using 3D printing for reconstructive surgery [12]:

a. 3D printing involves direct visualization of anatomic structures and their spatial relationships, thus improving the understanding of complex underlying

conditions which significantly enhance the quality of diagnosis and treatment planning [12].

b.3D modeling helps the plastic surgeon to provide better preoperative counseling to their patients [12].

c.3D biomodel allows the assessment of bony defects for grafting and the adaptation/prebending of reconstruction plates. This results in improvement in preoperative surgical planning by designing incisions and surgical resection margins [12].

d.3D modeling facilitates development of intraoperative guidance tools to improve communication among surgeons. This shortens the operative time; reduces time under general anesthesia; shortens the duration of wound exposure; and reduces intraoperative blood loss, errors, and risks [12].

e.3D printing helps in the production of patient-specific implants/prosthetics in everyday surgical practice such as temporomandibular joint prostheses, distraction devices, and fixation devices. This improves the esthetic outcomes as a result of individual fitting and complements individual anatomical needs. Furthermore, in contrast to the standard implants, these customized implants avoid the need for intraoperative modification and adjustments resulting in improved clinical outcomes and a decreased risk of complications, such as infections. These custom made implants yield superior functional and esthetic outcomes [12].

f. The 3D printing is more predictable and provides accurate surgical outcomes [12].

g. Typical 3D printing materials can be sterilized using chemicals, such as Food and Drug Administration–approved glutaraldehyde protocols, steam and gas for intraoperative handling [12].

h. 3D printing enables accessibility of physical models that can be realistically held and rotated and can be used as an educational tool for medical students and residents. These models can be interactively manipulated regardless of complexity and are accessible without the need for computers or advanced training [12].

i. As 3D printing allows use of a variety of materials, its utility is not only limited for bone reconstruction but can also be extended for replacing soft tissues [12].

It has been a general observation that 3D printed biocomposites have low fiber content (< 30 wf%) and a very low aspect ratio (L/d), that reduces their overall viscosity and improves printability. In addition, discontinuous or short fiber-reinforcement exhibit high porosity of biocomposite because of low pressure applied during printing. Therefore, the future trends in 3D printing are expected to deliver higher mechanical properties with improved material selection. The use of continuous natural fiber for biocomposites could bring about drastic improvements in mechanical performance due to high fiber content and better control of anisotropy by fiber orientation [50].

With advent of 3D printing technology, it is now possible to fabricate cell-based 3D scaffolds. The use of stem cells for clinical applications must fulfill Good Manufacturing Practice (GMP) requirements to ensure safety and quality of the treatment [63]. Bone marrow stromal and adipose derived stem cells find preferred applicability for orthopedic and maxillofacial tissue regeneration [61]. Hamlet and colleagues investigated a cell-based approach for alveolar bone regeneration using

hydrogel as bioink for cell delivery. Bioprinting of periodontal ligament cells has also been performed to create a 3D hydrogel microarray. The process of bioprinting the cells using a pressure-assisted valve-based bioprinting system is carried out within a sterile hood and controlled by a computer [3].

4.7.2 4D bioprinting

The director of the Self-Assembly lab at the Massachusetts Institute of Technology (MIT), Skylar Tibbits, demonstrated the concept of 4D printing for the first time in 2014 [64]. 4D printing is an invasive and robust [42] technique that involves the development of raw printing materials and design of the mechanism and multilayer architecture of printed structures that directly incorporate a pre-programmed transformation [50]. With the rapid progress of nanotechnology 4D bioprinting has been developed to incorporate time as the fourth dimension in combination to the 3D bioprinting strategies, to bring about changes in confirmation, shape and functionalities (shape, property, self-assembly or self-repair) of the printed objects [22, 50]. So, basically 4D printing can be defined as the ability of 3D printed material to actuate when an external stimulus is applied [50]. The types of stimuli can be physical (e.g. temperature, pressure, electricity, light and magnetic field), chemical (e.g. humidity and pH) [42] and biological (cell traction force-CTF). Mechanisms of CTF have been utilized for cell origami technology in which 3D constructs of cell are developed by folding two-dimensional elements into predefined shapes. Currently the stimuli-responsive biomaterials have largely replaced CTF-based and manual folding approaches for 4D printing [64].

A case study: Maxillo-orbital surgery for placement of titanium implants to anatomically reduce bone fracture presents several challenges. In many cases deep insertion of titanium mesh implant to the orbital floor may result in damage to optic nerve and vision loss. However, if the mesh is not inserted deep enough, the reconstruction of orbital floor will not deplete and the eye will lack support. Therefore, titanium mesh implants must be inserted into the orbital wall and should tightly fit the surface of orbital floor. Under certain circumstances the titanium mesh may deviate from its position, thus increasing or decreasing the volume of orbital cavity, and as a result symptom such as diplopia, exophthalmos and enophthalmos are not relieved [23]. 4D printing has revolutionized our approach to address such complicated issues by empowering the surgeons to place and modify of scaffold in real-time during the surgical procedure. This technique enables actuation of a 3D material by application of external stimulus and can be performed after the scaffold has been placed at the site of injury/defect.

A 3D printed product should exhibit smart behavior such as "Shape memory" or "Self-actuation", to be considered for 4D printing [42]. A variety of materials have been developed for this purpose such as shape memory polymer (SMP), electro-active polymer (EAP) and hygromorph composites (moisture induced morphine – that utilizes moisture induced anisotropic swelling of natural fibers to drive actuation in development of hygromorph composites) [50].

Shape memory is defined as ability of materials to "remember" and recover their original shape. This suggests that the original shape of a material can be deformed to fix into a secondary form, by application of an external physical force. The material retains this new shape until a specific stimulus (e.g. temperature, ultraviolet light, humidity, electric and magnetic field) is applied that triggers the transformation of matter and the original shape is regained. Humidity is utilized as one such stimulus for shape changing materials, and this increases the utility of hydrogels for 4D printing. Since, hydrogels have relatively low stiffness, natural fibers are preferred for fabrication of scaffolds. Hygromorphs biocomposites are the natural fiber biocomposites, making their mark as the new class of smart materials and can be used in 4D printing [50].

Currently two design strategies are employed for generating actuation with 4D printed hygromorph biocomposites: mono-material printing and multi-material printing deposition. The mono-material printing approach presumes that a given material possesses different mechanical properties and different coefficients of expansions in different directions (anisotropy), induced by the orientation of the fibers within the filament and the printing process itself [50].

Scientists have synthesized renewable bioscaffolds by utilizing PCL and cross-linkers with castor oil which displayed both shape memory and shape recovery at physiological temperatures. Additionally, scaffolds made from epoxidized acrylate material based on renewable soyabean oil, using 3D laser printing, have been shown to express temperature-responsive shape memory. The major disadvantage of solid-state SMP in terms of 4D bioprinting is that the cells can only be seeded on the surface but cannot be uniformly dispersed within it. Moreover, the incitation mechanisms utilized to trigger deformation procedures also pose substantial restrictions. For example, dramatic changes in physical and chemical parameters such as UV and pH may have possible negative effects on cell viability but variation in temperature (between 4 and 40°C) and Ca^{2+} concentration does not have any detrimental effect on living cells [64].

The main factors influencing the process of 4D printing are: (a) type of additive manufacturing process utilized; (b) the nature of the responsive material; (c) type of stimulus; (d) mechanism of interaction between the material and stimulus; and (e) mathematical modeling of the material transformation [42]. The stimuli-responsive biomaterials have made it possible to realize spatio-temporal distributions and release of bioactive cues and cells for heterogenous tissue regeneration. The Project Cyborg software designed by MIT is a platform that offers abilities to simulate self-assembly and optimization of design constructs of programmable materials [64].

4.7.3 Reverse modeling

Reverse modeling design is an image-based technique that reconstructs bone tissue microstructure based on its CT or MRI image. This technique employs binary value method to analyze slice information, where element "1" represents the solid and "0" represents the void. The 2D model thus created is transformed into STL (standard tessellation language) files and transmitted to AM equipment to construct 2D layer. Layer-by-layer method is then used to obtain the 3D structure. This method combines advanced medical imaging system, powerful image analysis software and rapid AM technique to mimic microarchitecture of bone tissue [52].

Cutting et al. in 1986 elaborated the use of 3D computed tomography (CT) images in planning virtual surgeries, and these principles have now been extrapolated to develop customized 3D scaffolds for craniofacial reconstruction [14].

4.7.4 Mathematical modeling

This method mainly utilizes shape functions to construct porous scaffold with implicit function surfaces or irregular polygonal models. Triply periodic minimal surface (TPMS) method uses trigonometric functions to derive complex porous structure with minimal surface, in which the curvature at any point is zero. It is similar to the natural surface geometries of beetle shells, butterfly wings and crustacean bones, where periodicity exists in three independent directions and no sealed cavities are present in the geometry. TPMS based method has been used for designing tissue scaffolds and a simple primitive (P-type) unit. Other types of TPMS units such as diamond (D-type) and gyroid (G-type) have also been proposed for bone scaffold designing. Capfer et al. studied two types of TPMS-based structures

including network solids and sheet solids. (a) In network solids the minimal surface makes the solid void interface, whereas in (b) sheet solids minimal surfaces to sheets with predefined thickness are inflated to construct porous solids. The latter was found to possess considerably higher mechanical stiffness and Poisson's ratio [52].

5. Conclusion

Tissue engineering is a multidisciplinary field that focuses on the development of materials and strategies for tissue reconstruction/regeneration. Recent developments in technology has improved the ways in which engineering of tissues are performed. The main requirement for tissue engineering is the selection of material and technique used for fabrication of scaffold with the given material. However, the advancements in fabrication technology have dramatically improved the amalgamation of biomaterial and cell-based scaffolds. Such drastic improvement in our understanding and implementation of material science along with cell biology has empowered surgeons to approach the challenging regions such as craniofacial sites for reconstructive surgeries.

Innovative and multidisciplinary approaches including advanced materials, nanobiotechnology, cell biology, computer assisted techniques, robotics and tools of artificial intelligence offer huge potential for augmentation of craniomaxillofacial tissue engineering [65]. Such rapid progress in technologies bestows great promise for large scale manufacturing and implementation of these scaffolds for reconstructive surgeries. This would also make the process economic and affordable for clinical applications.

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Conflict of interest

The authors declare no conflict of interest with relation to this study.

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