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Review Article

Recent advances in a three-dimensional fabrication of hydroxyapatite-based biomaterials for craniofacial bone regeneration

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Abstract

Surgical treatment of craniofacial bone abnormalities, caused by head and neck cancers, bone fractures, congenital deformities and periodontal diseases, involves the reconstruction using bone graft implantation. Due to limitations and complications from the use of autogenic and allogenic bone grafts, synthetic bonelike ceramics have therefore become widely used, particularly for hydroxyapatite (HA), which has been used for a multitude of medical applications. Because of an advanced technology in the field of stem cell research and tissue engineering, highly sophisticated and functionalized tissues can be constructed using 3-dimensional (3D) fabricating technology in order to replace the use of human structures or organs. Combination of 3D fabricating and tissue engineering offers a state- of-the-art technology that revolutionizes the current treatment of craniofacial bone defects. It is known that HA is one of the most promising biomaterials used to fabricate osteogenic 3D scaffolds for bone tissue engineering, including the reconstruction of craniofacial bone defects. This review aims to provide a succinct review of the most commonly available 3D fabricating methods for fabrication of HA-based 3D constructs used in craniofacial bone regeneration. Advantages and limitations on each of the 3D fabricating methods are also summarized.

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Introduction

Surgical treatment of craniofacial bone abnormalities, caused by head and neck cancers, bone fractures, congenital deformities and periodontal diseases, involves the reconstruction using bone graft implantation. Several methods of bone reconstruction are available. The gold standard of bone reconstruction is the use of the autogenous bone graft (Rogers, Greene, 2012). However, autogenous bone graft harvesting is known to cause a considerable risk of several complications, including limited donor bone supply, donor site pain, hematoma formation, infection and nerve injury and also it is a time consuming procedure (Finkemeier, 2002; Laurencin, et al., 2006). Due to these complications, the allogenic bone substitute can be used as an alternative treatment. In general, the disadvantages of allografts are poor mechanical properties, compromised osteogenic potency, increased risk of disease transmission and sometime increase the cost of treatment (Vaccaro, 2002; Mankin, et al., 2005; Hou, et al., 2005).

In biomedical applications, natural biomaterials are generally used as bone grafting because they are highly biocompatible. Certain natural biomaterials have structures mimicking bone microenvironments, have mechanical properties similar to natural bone and are biodegradable. However, natural biomaterials including allografts and xenografts have not been widely used possibly due to their serious drawbacks, such as the risk of viral infection, antigenicity, uneven supply of materials and batch-to-batch property variation (Ige, et al., 2012).

Synthetic bone graft biomaterials, made from metal, polymers, ceramics including calcium phosphate and their combinations, have thus been introduced to use for bone reconstruction (Finkemeier, 2002). They have been shown to possess considerably improved biocompatibility. Among these, ceramic has been used widespread for the fabrication for bone regeneration applications such as hydroxyapatite (HA) and tricalcium phosphate (TCP). Although it is difficult to control the degradation rate of HA compared to that of TCP (Shimazaki, Mooney, 1985), this type of ceramic is a maincomponent of bone and may be ideal as a bone graft substitute. New bone formed in a porous HA network has been reported (Shimazaki, Mooney, 1985). Implanted composites of porous HA and cultured osteoblastic cells have osteogenic ability *in* vivo. (Dong, et al., 2001). HA possesses a wide range of properties including high stiffness, low elasticity and hard-brittle surface. The chemical structure of HA is similar to the mineral phase of native bone and exhibits good biocompatibility, thus being a good candidate forbone graft materials. Certain ceramics have been shown to increase osteoblast differentiation and proliferation (Hutmacher, 2000; Wang, 2003). HA has also been used to obtain an enhanced bone-to-implant material anchorage (Carotenuto, et al., 1999; Cochran, 1999; Lind, et al., 1999; Kilpadi, et al., 2001). In addition, a range of newly developed materials, such as modified phosphate-based glasses and glass-reinforced HA have recently been proposed as effective repair and regenerative materials (Lopes, et al., 1998; Salih, et al., 2001). Although improved bone integration is observed when HA-coated and HA-uncoated biomaterials are both implanted in vivo (Tisdel, et al., 1994; Pazzaglia, et al., 1998), clinical trials suggest the benefits of HA coating, such as improved implant survival and radiographic stability, minimized postoperative pain and reduced implant movement (Capello, et al., 1997; Nelissen, et al., 1998). HA has also been shown to accelerate osteoblast differentiation of cells of osteogenic lineage (Ozawa, Kasugai, 1996; Shu, et al., 2003; Knabe, et al., 2004).However, load-bearing limitation of HA, due to its relatively poor mechanical properties, restricts its use. In order to increase the mechanical properties of HA, various modifications have been introduced, for example, glass-reinforced HA composites. These composites enhance two key bone matrix proteins, bone sialoprotein and osteonectin, compared with pure HA (Salih, 2001).

Many studies have aimed to combine HA with natural polymers to produce composite materials with better mechanical strength, elasticity and toughness. (Adnan, et al., 2017). Such data strongly suggest that HA is one of the most promising biomaterials used to fabricate osteogenic 3-dimensional (3D) scaffolds for bone tissue engineering, including the reconstruction craniofacial bone defects.

Among several advanced technologies in scaffold construction for tissue engineering, 3D manufacturing technology is emerging as a powerful tool for tissue engineering, including bone tissue engineering for bone regeneration following the surgical treatment of large bone defects. This technology truly has a potential to change medicine and healthcare worldwide to the next level. The general benefits of 3D fabricating application include its ability to produce rapid and individualized material to use for bone reconstruction. Customization allows for a precise and reproducible reconstruction of patient-specific anatomy, either for surgical planning or informative purposes. These 3D-manufactured samples can be used and served as a scaffold or an internal splint fixation. Bioprosthetics are unique among 3D constructs in that biological materials eventually integrate or degrade within human body. Because of an advanced technology in the field of stem cell research and tissue engineering, highly sophisticated and functionalized tissues can be constructed using 3D fabricating technology in order to replace the use of human structures or organs. Combination of 3D fabricating and tissue engineering offers a state-of-the-art technology that decreases donor site morbidity-a serious complication following the gold standard treatment of craniofacial bone defects. HA is one of the most employed biomaterials for bone reconstruction. HA scaffolds can be additive manufactured using a 3D-fabricating system in combination with suitable binder solution when is being grinded to a powder format. 3D-fabricating is a technology where a 3D component is created with the layers of a binding material. Compared with traditional ceramic processing methods, a powder-based 3D-manufacturing allows the production of custom-made models with high biomimetic properties. The mechanical property improvement of 3D-fabricated HA constructscan be achieved by adding an elastomeric component to the brittle ceramic structure. Those hybrid structures can be obtained by infiltration of the 3D-printed and sintered HA scaffolds with biodegradable polymers, such as, gelatin, polyvinyl alcohol (PVA) or polycaprolactone (PCL) (Stevanovic, 2013). This review aims to provide a succinct review of the most commonly available 3D fabricating methods for fabrication of HA-based 3D constructs used in craniofacial bone regeneration. Advantages and limitations oneach of the 3D fabricating methods are also summarized.

Possible 3D fabricating technologies in 3D HA scaffold construction

There are different technologies that may be used to generate 3D HA constructs, i.e., 1) conventional and 2) additive layer fabricating (ALM) techniques. The conventional techniques include solvent casting, freeze casting, sol-gel technique, foam reticulation, gel casting. ALM, a solid freeform fabrication (SFF), technology is a group of technologies that generates physical structures from two-dimensional (2D) computerized information (Colasante, 2016). This system is superior to the conventional technique in many ways, such as an issue of pore interconnectivity. The ALM process begins with a creation of a 3D computer model which can be obtained by medical imaging data (such as computed tomography or magnetic resonance imaging) of patients; then the 3D computer model will be sliced by a computer software into 2D images. Subsequently, a 3D construct is fabricated by a computer-controlled layer-by-layer process, and finishing with an appropriate post-

processing (Chia, Wu, 2015). The ALM system can produce 3D scaffolds with superior mechanical properties compared with those manufactured by conventional techniques due to the higher level of the structural control. Moreover, the ALM technologies can create aseptic fabricating of tissue engineering 3D grafts that perfectly match with the patients by using patient-specific imaging information. This system is able to produce 3D scaffolds with a rough surface via 3D fabricating that may improve osteointegration.

The limitations of ALM systems include the resolution is restricted by machine specific parameters, loose powder or liquid that can be trapped in internal holes, the sterility of commercial ALM systems and also a restricted range of materials that can be used for individual techniques because it requires a specific input form of materials (powder, filament, solution) (Savalani, Harris, 2006).

The ALM systems can be classified into 3 main groups: (1) laser-based systems (such as stereolithography and selective laser sintering), (2) printer-based systems (such as 3D printing), and (3) nozzle-based systems (such as fused deposition modeling and 3D plotting). The key factors to be considered when selecting the most cost-effective technology system for the required application are benefits and disadvantages/limitations. A summary of ALM/SFF technologies applied for bone tissue engineering is shown in Table 1. A sample of HA manufactured by a 3D printing system in our collaborative center is illustrated in Figure 1. A. Comprehensive information regarding detailed processing and structural features of 3D constructs of each techniquehas already been reviewed elsewhere (Chia, Wu, 2015; Chae, et al., 2015) and is therefore not included in the present review.

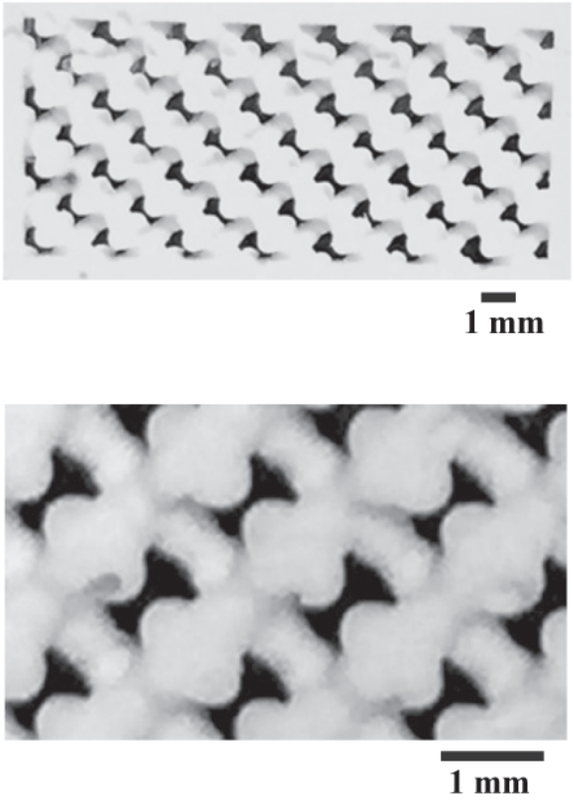

Figure 1: A sample of HA manufactured by a 3D printing system.

Table 1: A summary of common ALM/SFF technologies applied for 3D construct fabrication in bone tissue engineering

	Stereolithography	Selective Laser Sintering	3D Printing	Fused Deposition Modeling	3D Plotting
Principle	Photo-polymerization	Powder sintering	Deposition of powder and binder	Liquid extrusion	Liquid extrusion
Types of Biomaterials	Polymers Hydrogels Ceramics Composites	Polymers Ceramics Composites	Polymers Hydrogels Ceramics Composites	Polymers Hydrogels Ceramics Composites	Polymers Hydrogels Ceramics Composites
Live cell encapsulation	Applicable	Not Applicable	Applicable	Applicable	Applicable

Stereolithography (SLA)

SLA, which is a laser-based ALM system, is the first rapid prototyping process and was first developed in the late 1980s (Dowler, 1989). The SLA process involves the deposition of a photopolymer or epoxy resin and polymerization of a photocurable resin by a bottom-up system with scanning laser or a top-down setup with digital light projection (Chae, et al., 2015). The advantages of SLA are the ability to produce complex shapes with internal architecture, extremely high product resolution up to approximately 1.2 um and non-polymerized resins can be removed easily (Dowler, 1989; Zhang, et al., 1999; Savalani, Harris, 2006; Chae, et al., 2015; Chia, Wu, 2015; Colasante, et al., 2016). However, the disadvantages of SLA that potentially limit the use of SLA is that only a few biocompatible resins with proper SLA processing properties are available, with those available having poor mechanical properties, the non-polymerized resin must be removed manually and this system requires the use of photo initiators and radicals which may be cytotoxic (with long processing time) (Chia, Wu, 2015; Colasante, et al., 2016). It is noteworthy that currently, a wider range of resins compatible to SLA has been successfully employed with improved biodegradable moieties and the benefit of cell encapsulation during the processing. The novel macromers have recently been synthesized include segments of PCL or poly (d, l-lactide) (Chia, Wu, 2015). SLA is the gold standard in 3D fabricating requiring the resolutions of up to 0.025 mm, high reliability in producing complex shapes with internal architecture and the fabrication of larger objects (Ono, et al., 1994). The applications of SLA in the biomedical aspect include the creation of 3D models for surgical planning or informative purposes (Park, et al., 2014) as well as the fabrication of synthetic medical devices and titanium dental implant components. The use of SLA in tissue engineering has shown favorable outcome for stimulation of bone/cartilage regeneration in rat cranial defects and in conjunction with bovine chondrocytes for cartilage tissue engineering (Chia, Wu, 2015).It has been reported that the 3D HA bioimplant fabricated by SLA technique is successfully used as a craniofacial prosthesis (Levy, et al., 1997). Nevertheless, as previously stated, the clinical applications of SLA are markedly

influenced by the cytotoxicity of some photoinitiators and the difficulty to remove supporting structures that needs to incorporate into the computer model to assist the fabricating process (Chia, Wu, 2015).

Selective laser sintering (SLS)

SLS was first developed by the University of Texas in 1989. It is similar to 3D printing in binding together powder particles in thin layers except that a CO_2 laser beam is used (Pattanayak, et al., 2011). The SLS process utilizes a sequential layering of a laser-sintered reusable powder with thermoplastic, metal, glass, or ceramic materials to create a 3D scaffold construct (Chae, et al., 2015). SLS has many advantages include the fabrication of metallic endosseous implants that promote osseointegration and bone regeneration with good mechanical properties, such as high strength and fracture toughness, of the metal are still maintained. SLS technique can create anatomy specific structures from medical data such as a mandibular condyle scaffold (Williams, et al., 2005). This advantage is considered a key strength of SLS technique (Liu, et al., 2013). Recently, US food and drug administration (FDA) has approved the use of SLS to process a medical grade polyether ether ketone (PEEK) to generate custom craniofacial implants (Nickels, 2012). However, the disadvantages of this technique are only a limited number of materials can be used, i.e., certain materials that are not decomposed under high temperatures and the removal of remaining entrapped powder is required (Chia, Wu, 2015). Common materials used in SLS 3D manufacturing to fabricate scaffolds for bone tissue engineering include HA, polyether ketone, PEEKTCP, PVA, PCL, poly (L-lactic acid) (PLLA), and collagen-coated β-TCP (Ono, et al., 1994; Levy, et al., 1997; Tan, et al., 2005; Williams, et al., 2005; Savalani, Harris, 2006; Pattanayak, et al., 2011; Nickels, 2012; Liu, et al., 2013; Park, et al., 2014; Chia, Wu, 2015).

3D printing

The 3D printing technique was first invented and introduced by Charles Hull in 1986, and it was initially used in the industry for fabricating polyurethane frameworks to produce models and instruments (Cunningham, et al., 2005). It generates 3D structures by inkjet printing liquid binder solution into a powder bed (Cima, et al., 1994). The process of 3D printing requires an input of 3D digital information obtained from acquired imaging or a Computer Aided Design (CAD) program. The 3D dataset is converted into 2D data since the 3D printing fabricates physical models layer by layer. The processing technique begins with a deposition of a defined HA granulated layer on a building platform. Binder is then ejected by a microdispensing valve onto the powder surface and bonds the granules in the selected regions. Following each printed layer, the platform level is lowered proportional to the layer thickness and a new ceramic powder layer is further deposited on the former layer. After the whole process is completed, airflow is employed to eliminate unbound powder remained in the internal structure of the part(Leukers, et al., 2005).

Nowadays, HA scaffolds manufactured by 3D printing technology are suitable for bone reconstruction because the cells can migrate in between the HA granules (Leukers, et al., 2005). 3D printing can be used in tissue engineers to create porous ceramic 3D scaffolds with high interconnection directly from HA powder for bone reconstruction (Seitz, et al., 2005). HA has now become a candidate material because it has apatite structure that is capable of incorporating ionic substitutions which play an important role in the response of bone cells. The advances in tissue engineering has enabled the use of 3D printing technology for bioprinting, creation of hybrid structures consisting of organic tissue blended with synthetic materials to produce subtotal and total organs specifically tailored to the needs of target recipients

(Ventola, 2014). Other applications of 3D printing in craniofacial reconstruction also include, but not limit to, the 3D surgical models used as templates to harvest bone grafts, surgical guiding in dental implant, reconstruction plate bending, cutting guides for surgical osteotomies, and surgical splints. The narrow ranges of printable materials limit the application of implantable biomedical devices produced by a 3D printing technology. A range of materials compatible with 3D printing technology is currently available. These include PVA, PLGA, calcium polyphosphate, HA, TCP, TCP doped with SrO and MgO, apatite-wollastonite glass ceramic, calcium phosphate/collagen composite, and Farringtonite powder ($Mg_3(PO_4)_2$) (Chia, Wu, 2015). The resolution of 3D printer is dependent on the powder particle size and the adhesive. The material characteristics, *e.g.* morphology, particle size distribution, bulk and tapped density, and specific surface area, also affect the flowability and wettability of precursor powders (Butscher, et al., 2012).

Fused deposition modeling (FDM)

FDM possesses the same principle to SLA by building models on a layer-by-layer basis. FDM deposits thermoplastic materials of low melting temperature along a two-dimensional X-Y plane with biocompatible polymers. Subsequently, the thermoplastic material is heated into a liquid and extruded through a nozzle in a specific lay-down pattern to generate the scaffolds (Crump, 1992).

Heat transfer characteristics, melting temperature, rheology (behavior of liquid flow) and biocompatibility are the key criteria for FDM material selection. The materials that can use this technology to create 3D constructed models include poly(ethylene glycol) terephthalate/poly(butylene terephthalate) or polypropylene/TCP. In biomedical applications, other biocomposite materials such as PCL/TCP or PCL/HA are also able to be used with FDM due to their favorable biochemical and mechanical properties for bone regeneration (Rai, et al., 2004). The main biocompatible polymers used to fabricate scaffolds are poly(L-lactide/e-caprolactone) (PLC) and poly (e-caprolactone)/bioactive glass (PCL/BAG) which is highly biocompatible (Korpela, et al., 2013). The commonly used biomaterial for biomedical-related applications is PCL because of its low melting temperature, low glass transition temperature and high thermal stability (Chia, Wu, 2015).

The advantages of FDM are high porosity, good mechanical strength and better cost-effectiveness compared with the other fabricating methods (Sinn, et al., 2006). When compare FDM to SLA, FDM is faster, more accurate, and less expensive. FDM can produce surgical planning models to guide contouring of a mandibular reconstruction, decrease operating time, less blood loss, and less exposure time to anesthesia (Cohen, et al., 2009). The disadvantage for FDM is that it is yet unable to efficiently combine living cells or temperature sensitive biological agents due to the high processing temperature (Chia, Wu, 2015). A number of in vivo animal studies and clinical cases in human of the use of FDM have been reported. These include wound healing in animal models, treatment of human osseous craniofacial defects and rabbit bone defects, bone and cartilage tissue engineering and antibiotic delivery system (Chia, Wu, 2015).

3D Plotting

In 2000, 3D plotting, also known as direct-write bioprinting, was developed to create soft tissue scaffolds (Landers, M?lhaupt, 2000). The principle of 3D plotting involves the use of computer-guided design and processing combined with computer-assisted dispensing of polymers together with reactive oligomers and monomers to fabricate 3D complex-shaped constructs with custom-made internal structures. 3D plotting works by initiating the extrusion of a viscous

liquid material (generally a solution, paste, or dispersion), such as polymers, from a pressurized syringe into a matched-density liquid medium. For bioprinting, small balls of bio-ink consisting of viable cells and hydrogel materials, such as decellularized matrix and alginate, are capable of being printed in a wide range of desired shapes. The compatible biomaterials for this technique include PLGA, TCP, chitosan, collagen/chitosan composite, HA-coated collagen-alginate-silica composites, soy protein and agarose/gelatin gel (Chia, Wu, 2015).

The advantages of 3D plotting are material flexibility, practical temperature processing, and direct incorporation of cells and homogenous distribution of the encapsulated cells. The disadvantage of 3D plotting is, nevertheless, associated with its difficulty in fabricating complex shapes since a temporary, sacrificial material is needed and the hydrogels created by this method have limited mechanical stiffness which may result in a collapse of structures or limitations on the complexity of shapes (Chia, Wu, 2015).

A summary of advantages and disadvantages of different technologies used to generate 3D constructsis shown in Table 2.

Table 2: A summary of advantages and disadvantages of different technologies used to generate 3D constructs.

	Advantages	Disadvantages
Stereolithography	Ability to produce complex shapes with internal architecture	Only a few biocompatible resins with proper SLA processing properties are available
	Extremely high product resolution	Requires the use of photo initiators and radicals which may be cytotoxic
	Ease of removal of unpolymerized resins	
Selective Laser Sintering	Direct fabrication of metallic endosseous implants that promote osseointegration and bone regeneration with good mechanical properties	Only a limited number of materials can be used
		Removal of remaining entrapped powder is required.
	Create anatomy specific structures from medical data	
3D Printing	Use as bioprinting to produce subtotal and total organs specifically tailored to the needs of target recipients.	Limited by a narrow range of printable materials available.
Fused Deposition Modeling	High porosity	Unable to efficiently combine living cells or temperature sensitive biological agents due to the high processing temperature
	Good mechanical strength	
	Better cost-effectiveness	
3D Plotting	Material flexibility and practical temperature processing	Hydrogels created by this method have limited mechanical stiffness which may result in collapse of structures or limitations on complexity of shapes.
	Direct incorporation of cells and homogenous distribution of the encapsulated cells	

Conclusion

In biomedical applications, 3D manufacturing technology is a fairly new, rapidly growing fabrication technique that has been initiated numerous new applications in healthcare, including those used in craniofacial bone regeneration. Although vast improvement in 3D fabricating methods hasbeen made thus far, additional progress in 3D manufacturing technologies, especially for HA-based 3D constructs, is undoubtedly needed for improving resolution without compensating the favorable shape, strength, biological properties and hand ability of scaffolds. Moreover, enhanced osteogenic efficacy and supplementary antimicrobial property of HA-based 3D fabricating scaffolds may be new challenges in this field.

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