Three-Dimensional (3D) printing and bioprinting for orthopaedic biomaterials – A short review

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Abstract. Tissue engineering is a non-conventional approach in creating artificial organs and tissues to heal from bone fracture or organs malfunction by regenerating the damaged tissues. This review paper focuses on bone scaffold fabrication methods using three-dimensional (3D) printing and bioprinting for tissue engineering applications. Various types of biomaterials and fabrication techniques of bone scaffolds aimed for tissue engineering were thoroughly discussed. Suitability of biomaterial and polymer for bone scaffolds is deliberated as part of the review paper. Several biomaterials, mechanical properties and porosity of scaffolds were reviewed to give an in depth understanding on 3D printing and tissue engineering field.

Keywords: Bone Scaffold; Tissue Engineering; Three-Dimensional (3D) Printing; Bioprinting

1. Introduction
Bone tissue generally comprises of 65-70% inorganic crystals, with hydroxyapatite (HA) as the main constituent. On other hand, due to unique hierarchical structural organization with multi-scales, bone is considered as a natural solid biocomposite which contributes to fracture, toughness, and high strength. Bone injuries and defects are among the significant clinical problems faced by the patients that needs to be treated before it could deteriorate one’s health. These defects can be treated by various methods including autografts, allografts and alloplastic materials. Additional surgical site is needed for autografts which also carries the risks of postoperative infections or donor-site illness. Meanwhile, allografts normally exhibit the potential risk of disease transmission and immunologic rejection upon its treatment [1,2]. However, the advancement in bone tissue engineering could provide an alternative approach to repair the bone defects and expedite the healing process. Bone tissue engineering conceivably gives elective opportunities to bone substitutes rather than allograft. This is on the grounds that the artificial bones would give a structure for the cells to attach, differentiate, proliferate, and form new bone tissues. Moreover, these artificial bones would go about as cell transporter, factors of development, or other signals of biomolecular and most importantly, an ideal material is crucial to
be chosen for the tissue engineering in order to get a notable healing process. Table 1 summarizes the composition and mechanical properties of cortical and cancellous bones.

Table 1. Bone composition and mechanical properties [4].

| Bone Type | Composition (wt %) | Mechanical Properties |
|-----------|--------------------|-----------------------|
|           | Protein | Calcium Phosphate (CaP) | Water | Strength (MPa) | Elasticity (GPa) |
| Cortical  | 28      | 60                    | 12     | 70-200         | 3-30             |
| Cancellous| 26      | 54                    | 20     | 0.1-30         | 0.02-0.5         |

Bones generally possess compressive strength which is high ~170 MPa, tensile strength which is low ~104-121 MPa and shear strength which is very low ~51.6MPa [16]. High compressive strength is well known to be an essential aspect in order to withstand any loads/stresses subjected to the bones. Bone repair can be executed in three potential methodologies, (1) substitute of cells that supply a necessary capacity; (2) conveying the substances that induce tissue, for example, development factors to be focused on the spot; and (3) a three-dimensional (3D) scaffold that develop cells. Nonetheless, the initial two methodologies are more suitable for modest and all around contained damages, while the 3D scaffold is appropriate for creating a bigger tissue blocks with predesigned shapes, customized to the patient’s type of injury [3,4]. Therefore, this paper discusses the basic concepts of tissue engineering, the suitable biomaterials and method of printing for fabrication of bone scaffolds.

2. Tissue Engineering

The term “Tissue Engineering” was introduced by the National Science Foundation (NSF) in the year 1988 (USA) as “the application of principles and methods of engineering and life sciences toward fundamental understanding of structure-function relationships in normal and mammalian tissues and the development of biological substitutes to restore, maintain or improve tissue function”. The three major interactive elements fundamental for tissue engineering to succeed are cells, signaling factors, and scaffolds. Tissue engineering is a field of multidisciplinary whereby it facilitates the advancements in chemistry, physics, clinical sciences and life, and engineer in solving the basic therapeutic issues problem, for example loss of organ failure or tissue. The broad areas of tissue engineering applications are orthopaedics, skin development, cartilage regeneration and reconstruction of neurons and organs [5, 6].

Optogenetic is an emergent technology among the most recent research fields, such as three-dimensional (3-D) bioprinting, nanotechnology, and stem cells. For the TE systems to be fruitful, a mixture of scaffolds, growth factors, and cells should be the character of the utilized material. In order to ensure the success of the method, the scaffolds average pore size should be 300-400 μm range in order to allow osteons to grow into the scaffold. Fig.1 shows the chronological development of the tissue engineering scaffolds [7].

Tissue engineering and regenerative medication (TERM) has an extraordinary part as it gives a significant idea in planning more effective unions with the point of upgrading osteoconduction, osteoinduction, osteogenesis and osteointegration in bone deformities [8].

Figure 1. Scaffolds in Tissue Engineering.
The investigation of novel ideas in designing bone scaffolds and other complex biomedical models, for example, prostheses and implants can be cultivated by the use of computational modelling. This model will utilize patient-specific anatomical information and can be emerged by Additive Manufacturing (AM) procedures to ease and expedite the treatment process [9].

3. Biomaterials

Biomaterials from a health care perspective can be defined as “materials that possess some novel properties that makes them appropriate to come into immediate contact with the living tissue without eliciting an adverse immune rejection reaction.” [10]. Biomaterials can give the necessary mechanical and chemical prompts, just as other regulatory flagging significant for cell self-restoration or obligation to a separation heredity [11]. For a better biocompatibility properties, biomaterials ought to be non-antigenetic, non-cytotoxic, high cell suitability properties that can induce cell movement and proliferation, just like the requirements for an extracellular matrix needed for tissue growth. Depending on the tissue to regenerate, there are now many options for biomaterials in orthopaedic [12].

Osteogenic biomaterials are an option in contrast to conventional bone repair strategies, prompting quicker healing of the elaborate joint. Biomaterials utilized for artificial bones are partitioned into two kinds; natural materials and synthetic, for example, PLA, polycaprolactone (PCL), collagen (COL) and gelatin (GEL) [13]. Calcium orthophosphates, a sort of inorganic biomaterials is right now standing out enough to be noticed for its expected clinical applications in numerous territories such as in bone substitutions and other interior obsessions. A past work revealed that HA shows the ideal properties such as nontoxic, biocompatible, bioactive, non-immunogenic, non-inflammatory, osteoconductive and possesses good osteointegration properties [14].

In applications of orthopaedic, the usage of biomaterial is usually intended to replace the injured bone or reestablish the structural integrity. Fig.2 shows some of the important factors to be considered in designing orthopaedic materials. The classical biomaterials can be additionally arranged into: (i) metals and alloys (ii) non-metals such as polymers, ceramics, porcelain, glass, and composites [15]. In a modern context of orthopaedic biomaterials are sub-categorized into two significant classifications namely, nanophase biomaterials and classical biomaterials as shown in Fig.3.

![Figure 2. Important factors for orthopaedic biomaterial design.](image-url)
4. Three-Dimensional (3D) Printing

The 3D printing is an emerging technology that can be used to construct complex structures inexpensively for modelling, prototyping, or production through deposition or solidification. This technology was developed by the Massachusetts Institute of Technology (MIT) during 1990s’ and it was utilized for prototype fabrication and design validation purpose. The concept of 3D printing is the deposition or addition of materials in molten form by one layer over the other in 2-dimensional area in succession through an automated layer-by-layer process to construct a 3D structure [16, 17]. The 3D printed object structure is customized by a computer-aided design (CAD) model which is connected to a 3D printer. The CAD models designate 3D objects in a series of cross-sectional layers, and permits 3D printers to reproduce models through an additive process. By converting computed tomography (CT) or magnetic resonance imaging (MRI) of clinical defects into a CAD software, the specific CAD models of the patients can be developed. A slicing software is then used to section CAD models into layer by layer information using G-code, which encodes the 3D CAD models in a 3D printer controllable format. The G-code will then modify and optimize the required parameters such as print head speed, nozzle temperature, layer height and pressure [19, 20]. The schematic diagram of a 3D printer is illustrated in Fig.4.

Figure 3. Categories of orthopaedic biomaterials: classical and nanophase biomaterials [15].
In 3D printing, the architectures of scaffolds at every angle including its porosity, pore shape and interconnectivity can be discretely controlled to achieve patient-specific demands besides having high structural complexity and flexibility. Once incorporated in the patient’s body, the 3D printed scaffolds would provide a growth-directing structure that facilitates cell migration and proliferation to regenerate the bone tissue. In addition, the fabrication of scaffolds using 3D printing is a quick process and could decrease experimental errors [21]. A previous study by Fahimipour et al. (2017) constructed avascular endothelial growth factor (VEGF)-loaded with gelatine/alginate/ Beta-TCP composite scaffold by method of 3D printing with usage of a computer-assisted design (CAD) model. The scaffolds compressive modulus, 98 ± 11 MPa, was seen to be in the cancellous bone range, with suggestions of their potential applications for craniofacial tissue engineering [22].

Hassanajili et al. (2019) applied the 3D printing approach to develop bone scaffolds using polylactic acid, polycaprolactone and hydroxyapatite (PLA/PCL/HA) composites. A dissolvable 3D printed negative mold was used to caste the composite suspensions to achieve simultaneous macro- and micro-porous composites using freeze drying/particle leaching method. They found that the scaffold with PLA/PCL 70/30 w/w and 35 wt% of HA composition showed better properties in terms of its mechanical properties, microstructure, pore size and porosity [23]. With the existence of various 3D printing processes that are currently evolving, designers need to fully understand the merits and demerits of these concepts and associated materials in order to make a better decision and choice in selecting the 3D printing process to optimize the bone scaffolds for tissue engineering purposes [24].

5. Bioprinting

Bioprinting is one of the progressing and inventive innovation of this century that has got developing intrigue around the world such as in reformed drug industry, tissue engineering, and regenerative prescriptions areas, and so on. It suggests the usage technology of three-dimensional (3D) printing to print distinctive biomaterials with joined suitable cells. The triumph of bioprinting technology depends upon the profitability of 3D bioprinters and availability of the bioink (involved polymer arrangement and feasible cells) and its primary, physio-chemical, mechanical, thermal, and biological features [25]. The contrast between “3D printing” and “3D Bioprinting” must be differentiated clearly as both of these terms are invariably used by the scientific community. Both of these processes construct a 3D structure layer-by-layer from a 3D CAD model. In any case, 3D Bioprinting includes the utilization of
cell-laden bioinks and other living cells to develop a structural tissue model whereas 3D printing technologies utilizes only materials in molten form without any biologics [26, 27]. The technology of bioprinting itself has been the focal point of research for almost 15 years and will keep on growing to cater the tissue engineering needs for quite a long time to come [28]. The overall process of bioprinting is summarized in Fig.5 while Fig.6 shows the three different techniques of bioprinting.

![Figure 5](image1)  
Figure 5. The overview of bioprinting [27].

The three major modalities of bioprinting technologies are categorised into extrusion-based bioprinting (EBB), droplet-based bioprinting (DBB) and laser-based bioprinting (LBB), which have been utilized for vascular or vascularized tissue fabrication [29].

![Figure 6](image2)  
Figure 6. Mechanisms of bioprinting techniques: A) extrusion-based bioprinting, B) droplet-based bioprinting, C) laser-based bioprinting [30].

Demirtas et al. (2017) studied the effect of blending of cells of chitosan solution and it’s composite with nanostructured bone-like hydroxyapatite (HA) using 3D bioprinter. MC3T3-E1 pre-osteoblast
cell laden chitosan and chitosan-HA hydrogels, utilize extruder-based bioprinter and were characterized by comparing these hydrogels to alginate and alginate-HA hydrogels. In addition, the cell viability, proliferation and osteogenic differentiation had improved in the presence of bone-like nanostructured HA in alginate and chitosan hydrogels and it was also been proved by the authors [31].

At first, exosomes were considered as futile cellular metabolic wastes, but then it had been recognized that they have numerous significant cellular capacities. One strand of the exosomal miRNAs contains miRNA-induced silencing complex (miRISC) which are normally grouped into Argonaute protein structure and interatomic fusion with target mRNA transcripts which leads to the restraint of comparing gene expression. Some biomolecules found in exosomes are usually treated as biomarkers for diagnosis of any disease, prognosis, and damage conditions, since their levels or substance might change its conditions in response to few illnesses or wounds [32, 33]. Wei et al. (2019) investigated BMP2/macrophage-derived exosomes to improve the bio-functionality for titanium implants and to modify titanium nanotube implants to favour osteogenesis. As an emerging functional material for bone tissue engineering, the exosome-integrated titanium nanotube may promote and serve the tissue regeneration [34].

6. Summary and future perspective

As it has been discussed in this review, orthopaedics is one of the areas in the tissue engineering field. There are different strategies/methods and wide selection of materials that can be utilized in fabricating scaffold. Essentially, scaffolds are utilized as a site in connecting the cells, proliferating, separating and migrating by considering the protein synthesis and developing factors. Scaffold plays an important part in giving mechanical support and conveying cells or particles that are inductive to the influenced site. In addition, scaffolds offer assistance in controlling the tissues that are recently shaped in their organizing and functioning. Each strategies or procedures have their parameters that ought to be considered such as the materials utilized, temperature, pressure applied and time retained that will result in a prodigious scaffold. On the off chance that there's any changes in those process parameters, it may result in a scaffold with some microstructural defects. Other than that, the chemical composition for scaffold fabrication, surface roughness, material-cell interaction and degradation play an important part in the characteristics of the scaffold surface. To overcome this, a vast knowledge and reliable data from the distinctive field is required such as biomedical designing and chemical engineering field. This could be taken into thought in the future for the improvement of the tissue engineering field and its applications.

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Author’s contribution

S.A.P. Sughanthy1 – first draft of the manuscript, data collection, analyses and review of the data, M.N.M. Ansari2,* - Idea & concept generation, suggestion and advice on the review manuscript, supervision of the project and manuscript editing, Noor Afeefah Nordin2 – project management and co-supervision, suggestion and advice on the analyses, manuscript editing and revision, Ng Min Hwei3 – suggestion and review manuscript editing, co-supervision of the project.

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