3D hydroxyapatite scaffold for bone regeneration and local drug delivery applications

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ABSTRACT

Bone tissue engineering is the technology of healing bone defects in critical clinical conditions using functional tissue-engineering substitutes. Hydroxyapatite (HAp), as a biomaterial, received extensive attention for biomedical applications in the last 15 years. HAp has been utilized systematically as a filling material for bone defects, artificial bone grafting, and as a scaffold material in prosthesis revision surgery. In this brief review, we discuss on the fundamental aspects of porous HAp scaffolds, which define their utility in bone-tissue engineering and orthopedic drug delivery applications. The review contains six sections. Section 1 provides a brief introduction on tissue engineering, history of using bio-ceramics in tissue engineering, and the present state-of-the-art scenario of tissue engineering. In section 2, we provide a brief survey of biomaterials of different kinds utilized for tissue engineering. Section 3 provides a brief review on conventional scaffold fabrication techniques and their advantages and disadvantages. In section 4, the essential physio-chemical and biological cues to the development of HAp scaffolds and their compatibility with the surrounding cells and tissues, along with their application potentials for drug loading and site-specific drug releasing are discussed. Sections 5 & 6 provide the prospects of HAp scaffolds in biomedical applications, and conclusions, respectively.

1. Introduction

Tissue engineering is an interdisciplinary field, which applies the principles of science and engineering for the development of biological substitutes that restore, maintain, or improve tissue functions. Tissue engineering integrates cell biology, medical science, materials science, and biological engineering. As has been stipulated by Hench et al. [1], biomaterial research desires to focus on rejuvenation of tissues instead of replacement. In this context, Kokubo et al. [2] investigated several novel bioactive materials of different mechanical properties. The broad areas of tissue engineering application are orthopedics, skin development, cartilage regeneration and reconstruction of neurons and organs. The evidence of pre-historic practice of tissue engineering is evidenced in ancient manuscripts, paintings, and body part remainings such as skeleton, mummy, etc. The famous painting "Healing of Justinian" (278 AD) depicting the transplantation of a homograft limb onto an injured soldier, is an early instance of the vision of regenerative medicine. The history and development of biomaterials since ancient civilizations dated beyond the past 4000 years has been described nicely by Dorozhkin [3]. At the beginning of the modern era (twentieth century), Plaster of Paris was the most popular bio-ceramic. The knowledge of toxicity and invention of aseptically surgical techniques boosted the practice of artificial prosthetic implantation. Body systems are made of organs and tissues. Cells are the building blocks of tissues. Tissue represents the cellular organizational level intermediate between cells and a complete organ. Different types of tissues such as epithelial, connective, nervous, muscle tissues, etc. exist in the animal system. When it comes to the repairment of damaged tissues, cell growth is often uncontrolled, hindering the healing process. One of the most convenient approaches adapted for controlled tissue engineering is the use of structural support to facilitate and guide the healing and growth of damaged body parts. In fact, biological cells can be implanted or 'seeded' into an artificial structure, known as "scaffold", capable of supporting three-dimensional tissue formation. The scaffold is a porous structure, adequate for cell colonization, and formation of new tissues through the reproduction of specific cells. Until 2008, an estimated 800,000 hip and knee arthroplasties have been used annually in the United States and Europe annually. In the literature, the properties and

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function of biomaterials have been discussed frequently in the context of hip endo-prosthetic implants made of popular bio-ceramic materials such as alumina, yttria stabilized zirconia (YSZ) and calcium phosphates (e.g. hydroxyapatite) [4]. Bioactive composite materials have also been tested and experimented rigorously for tissue replacement purpose during the past few decades, with special interest on some of them [5,6]. To fabricate scaffolds for tissue engineering, a comprehensive study is needed around the ideal material with necessary characteristics. An ideal scaffold material should have following properties for utilization in tissue engineering: (i) **Biocompatibility** is an important characteristic of scaffold materials which deals with inflammatory response or toxicity in the patient. The scaffold materials must bear nontoxic and non-inflammatory characteristics. The scaffolds made of such materials must sustain cell adhesion and proliferation. (ii) **Interconnected porous morphology** is an essential parameter for the design of porous scaffolds, which are responsible for the nutrient and essential body fluid supply to the transplanted and regenerated cells. Scaffolds with interconnected pore structures enhance nutrient and fluid diffusion rates and allow a better vascularization. (iii) **Adequate mechanical properties** with enough mechanical strength and stiffness to support the tissue under growth, until the newly grown tissue acquires strength to support itself. (iv) **Biodegradability** is an optional property for different types of scaffold. Most of the scaffold materials manifest degradation properties, leaving the space for new cell growth. Scaffold materials should have the capacity of breaking and dispersing in the biological fluid, even though there is no proof of elimination from the body due to macromolecular degradation. (v) **Appropriate surface chemistry for cell attachment, proliferation, and differentiation** makes a scaffold to be a successful prosthetic device for biomedical application. For example, some cells–osteocytes are exclusively located on the surface of the bone matrix. Cell adhesion in this case is essential, as it will allow further cellular functions such as spreading, proliferation, migration, and biosynthetic activities. Moreover, scaffold materials should allow the cells to differentiate blood vessels from other tissues at their surfaces to heal the traumatized organs. Biomaterials utilized for scaffold fabrication can be principally categorized in four major groups, such as: (1) ceramics, (2) natural or synthetic polymers, (3) metals, and (4) composites of these three or either two of them (Table 1).

Till date, different types of materials have been used to treat diseased, damaged or traumatized bone tissues. These materials include polymers (natural and synthetic), metals, ceramics and their combinations. Bio-ceramics are highly stable materials with superior bioactivity, which make them attractive for tissue engineering applications. On the other hand, biopolymers can be natural or naturally derived (e.g. collagen, chitosan fibrin etc.), and synthetic (e.g. poly (glycolic acid) (PGA), poly (lactic acid) (PLA), and their copolymers PLGA] [8]. Biomaterials synthesized or processed from natural origins have potential advantages due to their good biocompatibility, along with cell adhesion and function supports [16,18,19]. However, sometimes a direct use of naturally derived biomaterials is not possible due to immunogenicity or pathogenic microbe contamination, which requires further post-processing [20–22]. In this respect, synthetic materials have advantages due to their reproducible and tunable properties without microbial contamination. These are the reasons for the wider usage of synthetic materials in biomedical and tissue engineering applications in comparison to their natural counterparts [23,24]. Collagen is a natural fibrous protein, which is the main component of the extracellular matrix of mammalian tissues such as skin, bone, cartilage, tendon, ligament, etc. As has been demonstrated by Mizuno et al. [25], bone marrow stromal cells can differentiate osteoblasts in type I collagen matrix under *in vivo* conditions. On the other hand, use of hyaluronic acid-based polymers as cell carriers for tissue-engineered repair of bone and cartilage has been presented by Solchaga et al. [9]. Use of bio-degradable synthetic polymers based scaffolds such as PEG-based hydrogel scaffolds have been reported by several research groups [26] for

Table 1
Four major types of scaffold materials with advantages and disadvantages.

Materials	Example	Advantages	Disadvantages	References
POLYMERS	Nylon, Silicone rubber, Polyurethanes, Chitosan, Collagen, etc.	Bending ability with complex shapes, biocompatible	Not strong enough, deform with time, may degrade	[7–9]
METALS	Titanium, Stainless steels, Cobalt-chromium alloys, Gold etc.	Strong, tough, ductile	Not corrosion resistant, high density, poor bioactivity, costly	[10–14]
CERAMICS	Aluminium oxide, Titanium dioxide, Zirconia, Bioglass, Hydroxyapatite, Calcium phosphate, etc.	Highly biocompatible, chemically inert, high modulus and compressive strengths, excellent bioactive properties	Brittle, difficult to make their scaffolds	[4,15,16]
COMPOSITES	Combinations different materials, e.g. Ceramic-ceramic, ceramic-polymer, Metal-ceramic, etc.	Strong, tailor-made	Difficult to fabricate their scaffolds	[5,6,17]

bone regeneration. In fact, development of novel biodegradable polymeric biomaterials for tissue engineering applications is a great challenge in current biomedical research. Development of polymer based scaffolds with controlled architecture for cellular attachment and function is still in its initial stage, which needs innovative and technologically advanced therapeutic approaches [27]. However, not all is good with the use of polymer in biomedical research. The main drawback of biopolymers is their high degradability. They can be degraded very easily, releasing acidic products, which can trigger aseptic inflammation reactions and swelling [28]. The other limitation of polymeric scaffolds is their mechanical properties such as low tensile and compressive stresses, and inferior wire properties [29]. These disadvantages of polymer-based scaffolds, especially for load-bearing applications (dental and orthopedic surgery), could be overcome by utilizing biocompatible metallic materials (pure, alloy or composites). Standard surgical implant materials include stainless steel 316 L (ASTM F138), cobalt-based alloys (mainly ASTM F75, and ASTM F799) and titanium alloys; Ti–6Al–4V (ASTM F67 and F136) being the most utilized one. However, the main disadvantage of metallic biomaterials is their lack of surface biological recognition. The limitation could be overcome by implementing surface coatings or other surface modifications, preserving their mechanical properties. To improve intercellular communications, metallic biomaterials can also be organized inside porous scaffolds and suitable cellular ligands with signaling factors attached to the scaffold surface [10]. The biocompatibility of metal-based biomaterials has also been compromised due to the release of toxic ions and/or particles through their corrosion or wear, which might induce allergic reactions and inflammation of the target [11]. However, the problems can be avoided through appropriate surface treatment of the fabricated scaffolds or coating them by appropriate material.

Finally, hybrid or composite materials are another important class of materials, utilized for scaffold fabrication with ample success, especially as artificial joints and bone implants with the capability of stimulating specific growth factors and drug loading at molecular level. The most popular composites utilized for biomedical and therapeutic applications so far are made of polymer/bio-ceramics, and polymer (synthetic or natural)/metals. Novel metal/ceramic/polymer hybrid materials have also been proposed for the fabrication of load-bearing scaffolds [30]. In fact, in some critical clinical cases, tailored designed composite scaffolds are necessary for the reconstruction of structural diseases and bone defects [31]. Nevertheless, the mechanical property requirements for hard tissue repair are difficult to satisfy using porous polymer/ceramic composites [32].

2. Design, fabrication, and mechanical characterization of HAp scaffolds

Different techniques have been employed for manufacturing scaffolds for tissue engineering applications. Among them, the most common ones are computer-aided rapid prototyping (RP), injection or compression moulding, gel-casting, compacting, 3D printing, etc. [15,33–48] Computer-aided RP and 3D printings are the most advanced technologies used for the development of sophisticated scaffolds. Both the processes are controlled by high-speed computer processors and have been utilized for the development of different prototypes with robotic manipulation control over the device. In RP technique, the prototypes are generated by 3D printing machine inside the printer [49], to characterize further *in vitro* and *in vivo* to evaluate their suitability for biomedical applications. Scaffolds for tissue engineering must have specific geometrical shapes resembling to the damaged biological tissue/tissues, with adequate mechanical properties to provide structural support, and function in a defect during the growth of a tissue. They should also have sufficient biological affinities to stimulate and enhance the growth of damaged tissues, allowing the inclusion of seeded cells, proteins and/or genes to accelerate tissue regeneration. In

skeletal systems, depending on location, each bone contains distinct types of tissues. For example, cortical bone demands scaffolds with smaller empty spaces; spongy bone requires highly porous and durable scaffolds. Once inserted into the body, the scaffold provides a physical structure for directing the growth of new surrogate cells. Eventually, the inserted scaffolds are disintegrated inside the body, leaving only the body's natural tissues. In addition, the scaffolds offer the opportunity to introduce growth factors into the body, which stimulate the growth of new cells at damaged sites. There exist mainly three types of scaffolds used for tissue replacement.

1) Degradable scaffold: which disappears after implantation in the body system. Mostly bio-polymers are used as biodegradable scaffold material [biodegradable electrospun nanofibrous poly (β-caprolactone) for cardiovascular tissue engineering, polylactide-co-glycolide (PLGA) for urethra tissue engineering etc.] [50,51].
2) Non-degradable scaffold: which, after implantation in the body system, actively supports for the formation of new cells, serves as an active part and permanently associates with the body system. Metal and few ceramics are used as non-degradable scaffold such as titanium fiber mesh, alumina, zirconia, etc. [52,53].
3) Semi degradable scaffold: which degrade partially in the body system, providing full structural support to newly formed cells. Specifically, these are composite scaffold materials of polymers and bioceramics [non-degradable poly-vinyl alcohol (PVA) and degradable poly-lactic glycolic acid (PLGA) composites for articular cartilage replacement] [54].

The degradation of biomaterials (BM) is generally influenced by inorganic ions in physiological fluids through two pathways: (i) the presence of Cl^-, (the most abundant ions in physiological environment) is destructive to the BMs by breaking down the surface film of corrosion products; (ii) The presence of HPO_4^{2-}/PO_4^{3-}, HCO_3^-/CO_3^{2-} anions and Ca^{2+} cations which help to passivate different micro and macro elements like Mg and Fe etc. Fig. 1 illustrates schematically the proposed mechanism for the degradation of biomaterials in physiological condition [55].

Till date, a variety of techniques have been developed and employed to fabricate scaffolds for tissue engineering applications. These techniques can be divided broadly into two principal categories: (i) conventional fabrication techniques (Fig. 2), and (ii) solid freeform (SFF) or rapid prototyping (RP) techniques (Fig. 3). Each of these two broad, general techniques consists of several specific techniques, as has been presented in Table 2.

The rapid prototyping (RP) technique was first introduced in sophisticated scaffold manufacturing industry in early 1980s. This automated technology helps to fabricate products of different shapes, simply by modifying the computer-aided design (CAD) model, using 3D tomography data. The digital information is transferred to the RP machine, which builds customer-designed 3D objects by layered manufacturing strategy. Each layer represents the shape of the cross-section of the model at a specific coordinate. Current manufacturing technologies are very challenging to produce porous devices with complex structures, optimum pore size, and with adequate mechanical strength [30]. RP techniques, also known as solid free-form fabrication (SFF) or rapid manufacturing (RM) techniques, have been widely used for scaffold architecture, or critical shape formation [57]. RP methodologies were adopted for fabricating 3D porous polymers, ceramics, or metal scaffolds. Polymers and ceramics are very common materials used for RP modelling, although metal scaffolds have generated a strong attention in recent time.

2.1. Mechanical characterization

Although bioactive materials, whether composites or pure, improve the longevity of implanting devices such as "scaffolds", impose
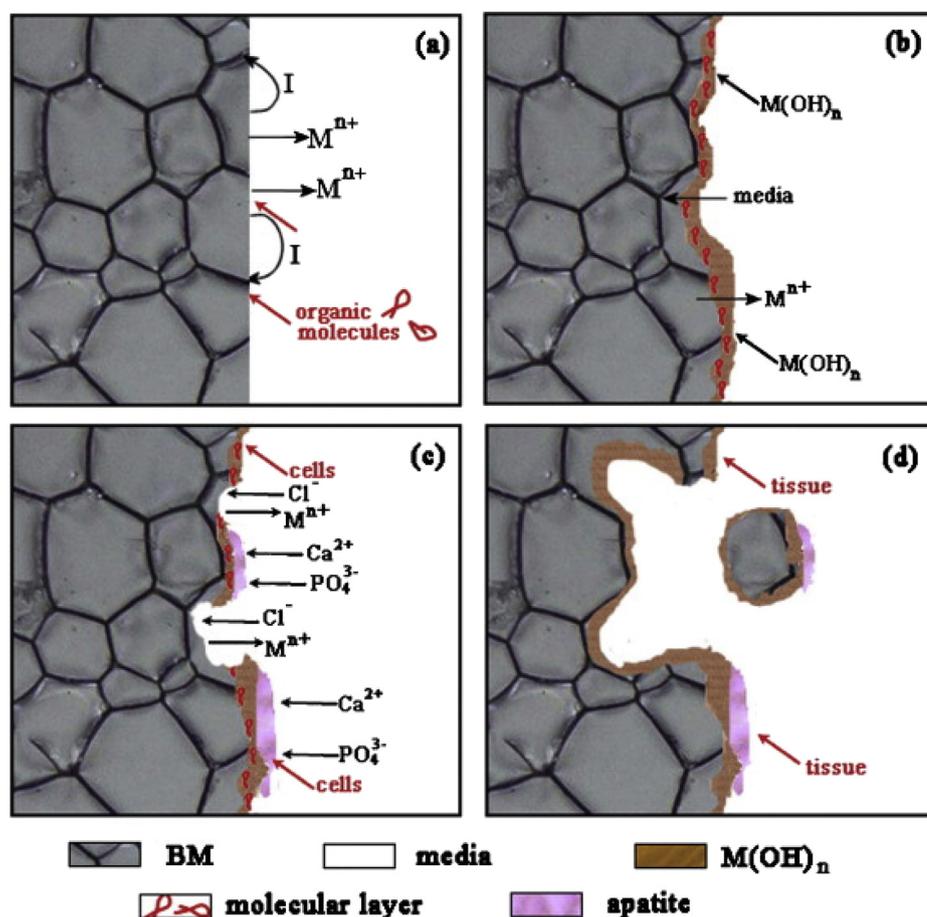
Fig. 1. Schematic illustration of the degradation mechanisms of biomaterials (BM: either metal, ceramics or composites) in a physiological environment, immediate after contacting the body fluid. (a) Organic molecules, such as proteins, amino acids, and lipids adsorb over the scaffold material surface, influencing the dissolution of BM; (b) Formation of corrosion products [M(OH)n] over BM surface; (c) Ions and calcium phosphate-based apatite deposits over the undissolved M(OH) n layer as the degradation proceeds. Cells are also observed to adhere to the surface of the biomaterial; (d) Depending on their size, the formed particles can be enclosed by fibrous tissues or macrophages, which helps to degrade the scaffold BM further. [Fig. 1 (Reprinted with permission from Zheng et al., Copyright 2014 Elsevier).] [55].

limitations on their applicability due to mechanical constraints. The performance and life expectancy of the fabricated scaffold depends on the quality of biomaterials and its biomechanical interaction [58]. The low compressive and tensile strengths of pure HAp bio-ceramics are the main constraints for their low load-bearing tissue engineering applications. Therefore, mechanical evaluation is one of the most important tasks for assessing the life span and functionality of scaffold materials. After implantation of a scaffold, a primary stage rejection occurs due to the immunogenic effect of the host body system. Though the metal based scaffold enhances the mechanical strength but for biomedical application the bioactivity is an important characteristic. HAp is a very well-known bioactive material which often used as a coating material on biomedical implant devices along with drug delivery applications [59]. The second major problem occurs due to the effect of mechanical failure. The mechanical failure of a composite scaffold can be avoided through a prior evaluation of frictional and wear properties of its constituting materials, as has been demonstrated by Bodhak et al. [60] for their novel high-density polyethylene (HDPE)–HAp–Al$_2$O$_3$ bio-composites in comparison with alumina counterpart [61]. Their studies clearly indicate that a significant improvement in the stiffness, hardness, as well as the biocompatibility of bio-inert HDPE can be possible by combining it with bio-inert and bioactive ceramic fillers. To give a few example, Mullen et al. manufactured optimized porous titanium structures for bone fabrication applications [62]. Selective laser melting (SLM) technology was employed to fabricate scaffold materials with < 45 μm titanium powder. All the prepared samples were sintered at 1400 °C temperature for 3 h. The mechanical study of the fabricated scaffold materials shows the compressive strength of ~60–64 MPa. Curodeau et al. developed porous scaffold molds by employing a 3D printing fabrication technology to cast Co–Cr alloys [63]. The authors reported the results in another article with a mechanical and *in vivo*

implant study in the dog model [64]. The *in vivo* study was performed for a time duration of 6, 12, and 26 weeks on 5 dogs with 24 experimental and 24 matched pair control sites (medial and lateral) on femur bones. The maximum interfacial shear stress of about 22.2 ± 7.9 MPa was calculated for 26 weeks' implant material. The histological study confirmed the formation of new bone callus and bone tissues on implant sites. On the other hand, Mondal et al. [15] have reported the use of fish scale derived HAp scaffold as potential bone tissue engineering material. The HAp biomaterial was synthesized by thermal decomposition of chemically treated *Labeo rohita* fish scales. The fabricated scaffold was successfully implanted in albino rabbit model, which mimic the cancellous/cortical bone system in terms of structure, porosity, mechanical strength, exhibiting excellent bioactive behavior. The developed scaffolds manifested good mechanical behaviors, with Vickers Hardness (HV) of ~0.78 GPa, 0.52 GPa compressive stress, 190 MPa tensile stress and ~35% porosity on sintering at 1200 °C (Fig. 4).

Murr et al. reported the fabrication of non-stochastic titanium (Ti–6Al–4V) structures by electron-beam melting (EBM) [14]. The Ti–6Al–4V alloy revealed excellent biocompatibility, light-weight with corrosion resistance, and balanced mechanical properties suitable for prosthetic implant. The ultimate tensile strength (UTS) of the specimen reaches ~1.18 GPa (with 16–25% elongations). The fully dense Ti–6Al–4V sample manifested ~5.0 GPa compressive strength, whereas the EBM fabricated porous scaffold revealed ~3.6–3.9 GPa hardness. Mondal et al. have reported the fabrication of HAp bioglass alumina composite scaffold with enhanced mechanical and biological performances for bone tissue engineering application. The developed composite scaffold exhibited good mechanical properties, with compressive strength of ~157 ± 2 MPa, tensile strength of ~83 ± 2 MPa, and porosity of ~20–25%. The enhanced mechanical properties were achieved through the formulation of composite with nanometric
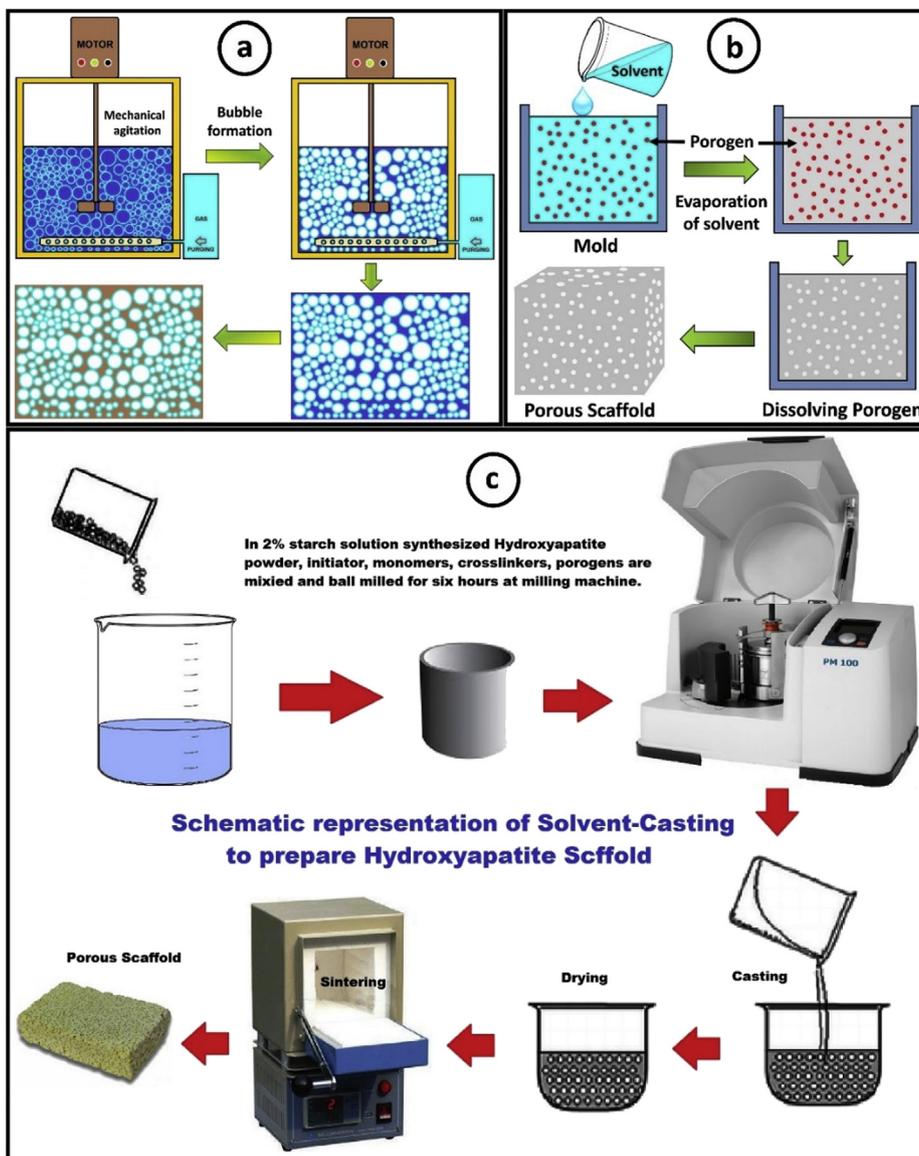
Fig. 2. Conventional scaffold fabrication techniques: (a) Gas foaming; (b) Particulate leaching/Freeze-drying; (c) Gel casting/solvent casting. [Fig. 2 (c) reprinted with permission from Mondal et al., Copyright 2016 Elsevier)] [15].
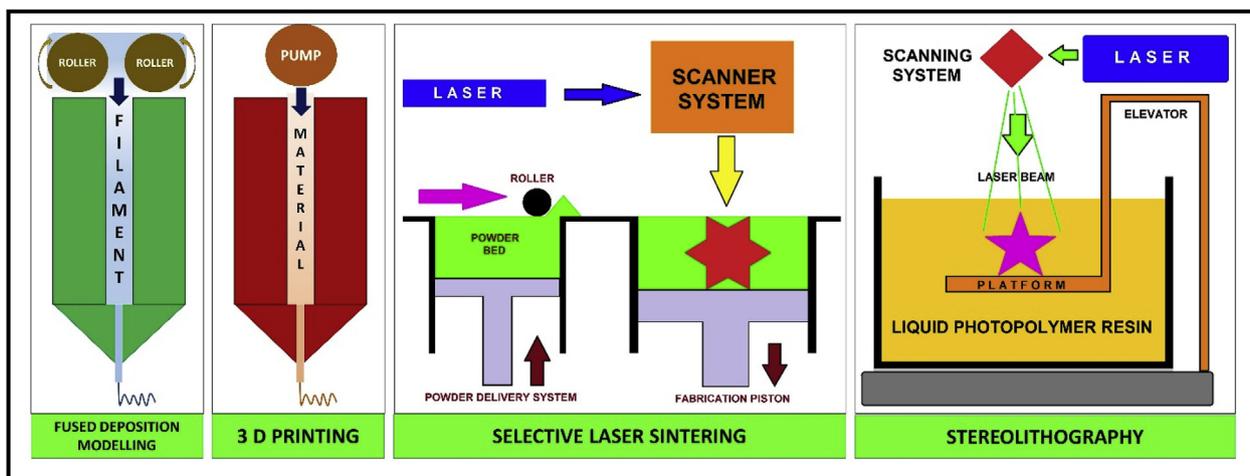

Fig. 3. Schematic presentation of different steps used in solid freeform scaffold fabrication (SFF) techniques.

Table 2
Different scaffold fabrication techniques.

| Conventional fabrication techniques		
Sl.	Technique	References
1	Particulate leaching	[33,34]
2	Freeze-drying	[35]
3	Gas foaming/supercritical fluid processing	[38]
4	Electrospinning	[39,40]
5	Powder-forming processes	[41,42]
6	Sol–gel techniques, Solvent casting	[15,41,43] [16,56]
Solid freeform fabrication (SFF) techniques/Rapid prototyping (RP)		
1	Fused deposition modeling (FDM)	[44,45]
2	Ink-jet printing technologies (3D printing)	[46]
3	Selective laser sintering (SLS)	[48]
4	Stereolithography (SLA)	[49]

alumina powder (Fig. 5) [65].

3. Clinical applications of HAp scaffolds: drug loading, drug release, *in vitro*, *in vivo* studies, medical application

Although HAp scaffolds fabricated using macroscopic HAp particles have been time-tested for their biomedical applications, recent progress on the fabrication of HAp nanostructures revolutionized this effort, resulting in the fabrication of nano HAp scaffolds with higher porosity, hardness and higher drug holding capabilities [66]. As in drug delivery systems a slow, controlled, local and sustained release of drug at the affected site is highly desirable, HAp scaffolds with high porosity, controlled pore size, and adequate hardness are most attractive. Utilization of nanostructured HAp in scaffolds provides most of these advantages. As the biocompatibility of nanostructured HAp has seen to be as good as macroscopic or bulk HAp counterpart due to compositional similarity to the mineral phase of bone and teeth, utilization of nanostructured HAp for scaffold fabrication has increased drastically in recent time. For example, a scaffold made of HAp nanoparticles have been fabricated through gel-casting and been tested for bone tissue engineering in the rabbit model, obtaining encouraging results [15]. While the recent progress on the development of nanostructured HAp such as nanoparticles, nano-/microfibers has opened up the possibility of scaffold fabrication with controlled pore size and pore volume [67], utilization of biopolymers (e.g. polycaprolactone PCL) in the fabrication of composite HAp scaffold also seen to be very effective for controlling pore size (open pores) and drug release rates [68]. Incorporation of polymeric additive in HAp scaffold as composite or just coating layer, not only improves their mechanical properties (such as compressive strength and elastic modulus), but also increases the % of drug holding, avoiding (to some extent) the initial burst release in aqueous biological media. The advantage of utilizing micrometric biopolymer vessels, such as poly (lactic-co-glycolic acid) (PLGA) for loading dexamethasone (DEX) through ionic immobilization on HAp scaffold has been demonstrated clearly by Son et al. [69]. As can be seen in Fig. 6, utilization of PLGA micro-vessels considerably reduces the release rate of DEX at the initial stage (Fig. 6 iii). Also, the utilization of ion-immobilized DEX-loaded PLGA microspheres enhances both the volume and quality of newly formed bone at defect sites, in comparison to defects filled with HAp scaffolds alone.

On the other hand, incorporation of selective bio-polymer, such as chitosan, can induce bactericide functionality in the fabricated HAp scaffolds, adding a new functionality very much useful for the protection of regenerated or new tissues from infection [67]. Fabricating composite scaffolds of chitosan-HAp nanorods and chitosan-HAp microtubes, Zhang and co-workers tested their drug loading and releasing profiles utilizing gentamicin sulfate (GS) as a test drug. The results obtained by them not only indicate a very high drug loading capacity (976.6 mg. g^{-1}) and sustained release profile for the scaffolds made of
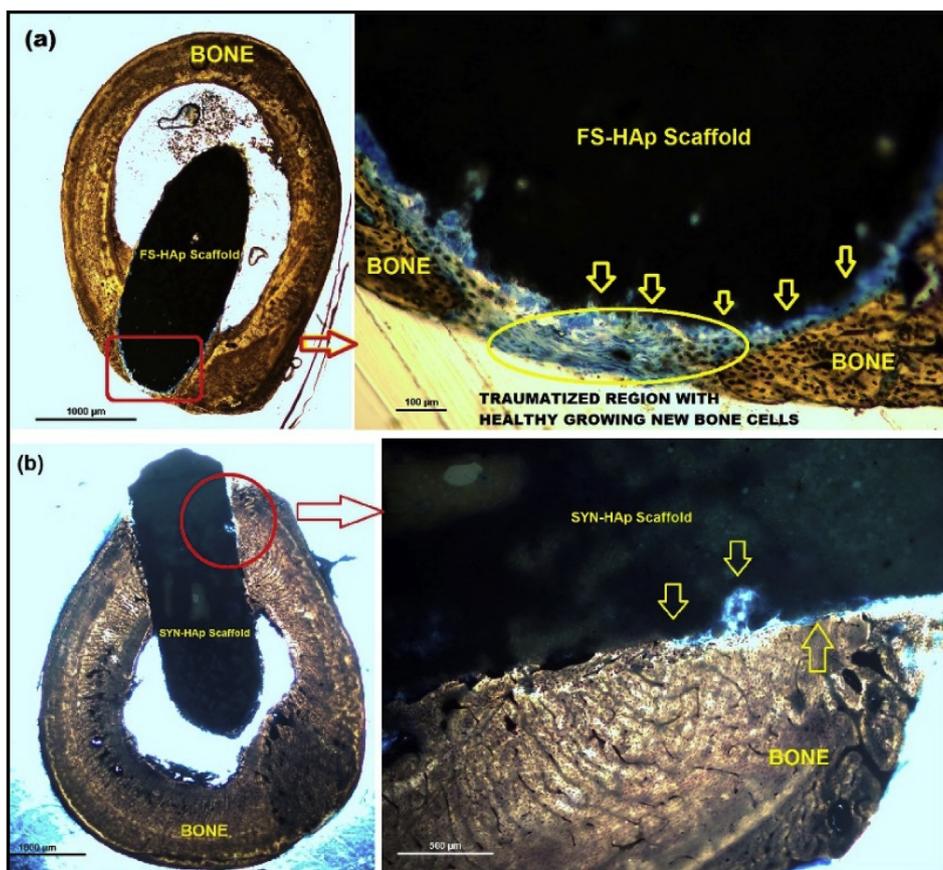
Fig. 4. Biological *in vivo* implantation study of fish scale derived HAp scaffolds [Reprinted with permission from Mondal et al., Copyright 2016 Elsevier]. [15].
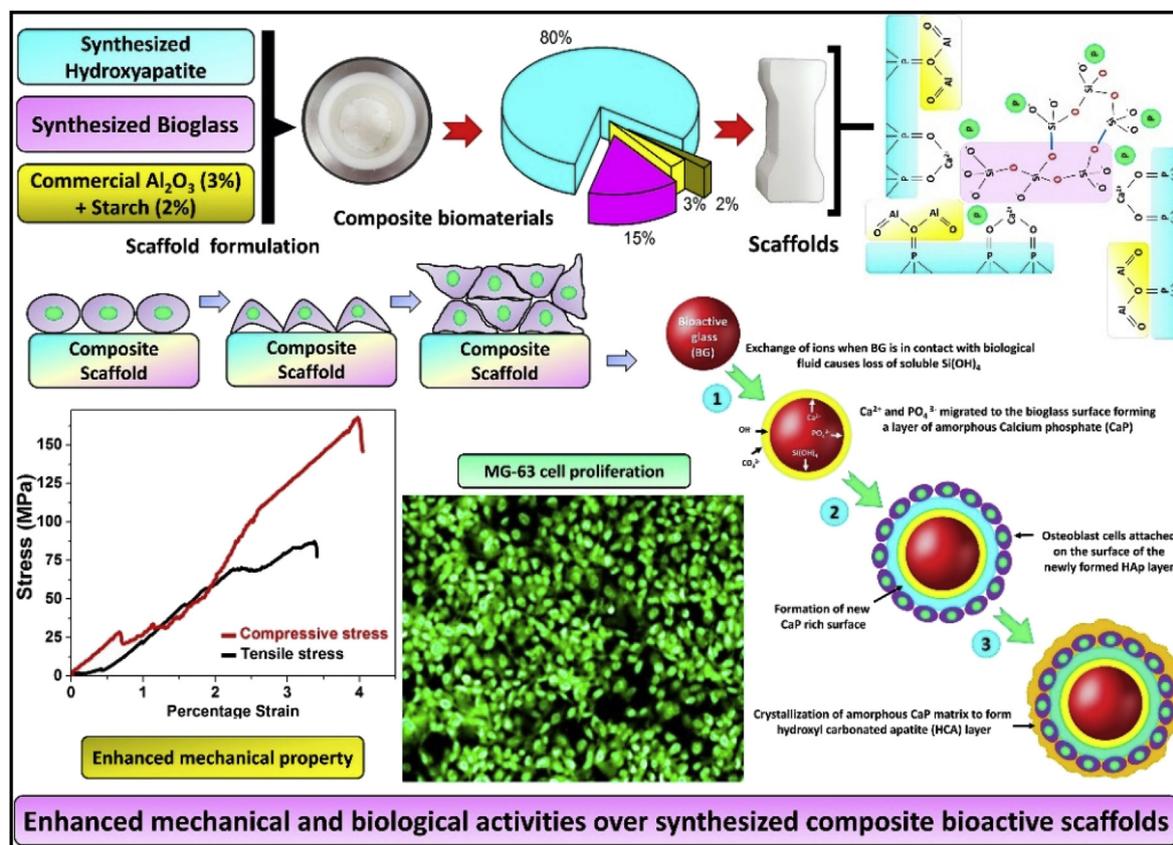
Fig. 5. HAp-based composite scaffold with enhanced mechanical properties and superior biological performance for tissue engineering application [Reprinted with permission from Mondal et al., Copyright 2018 Elsevier]. [65].

HAp microtubes, but also a substantial improvement in their mechanical properties (Fig. 7). Moreover, incorporation of the biopolymer chitosan enhanced the biocompatibility and cell adhesion properties of the fabricated scaffolds. In fact, utilization of polymeric reticulate such as a polycaprolactone (PCL) composite layer over HAp scaffold to improve their mechanical and drug holding properties has been demonstrated by Kim et al. [70] about two decades ago. While the biocompatibility of the utilized polymer layer (over the HAp scaffold) affects the biocompatibility of the finished scaffold, the solubility of the polymer also affects the release profile or sustainability of drug release.

Kim et al. [69] studied porous HAp scaffold coated with polymer for the delivery of vancomycin antibiotic towards wounded tissues and compared the drug release behaviors of HAp and Poly(ε-caprolactone) (PCL)-coated HAp materials. They observed an abrupt initial burst release of the loaded drug (~70–80%) in bare HAp, whereas, the coated sample manifested weaker initial burst (~44%) release of vancomycin. While about 90% of the drug was released within 24 h from scaffold made of bare HAp, the PCL-coated HAp scaffold revealed sustained release of vancomycin for 3 days [71]. On the other hand, Liu et al. developed biodegradable hydroxyapatite/polyurethane microsphere composite scaffold incorporated with ceftazidime model drug to generate antibiotic drug delivery system for bone regeneration. The reported quantity of released ceftazidime was ~29.19% at initial 4 h, 43.14% at the first day, and ~91% to the external medium at day 39. The initial burst release from the microspheres was ascribed to the diffusion of ceftazidime on their surfaces. A burst release of 62.20% was observed on day 1, which might be due to the direct incorporation of ceftazidime into the open pores of scaffold. At day 7 and day 30, about 81.10% and 95.50% of loaded drug was released to the external medium [72]. Very recently Kim et al. reported the optimization of zinc loading over HAp nanostructure [73]. The concentration (1, 2, 5 mol. %) of Zn loading is seen to affect the loading of a model drug

Doxorubicin (DOX). The HAp molecule consists of a central $Ca(OH)_2$ unit, surrounded by three $Ca_3(PO_4)_2$ groups. As Zn^{2+} ions were introduced into the HAp lattice substituting the central Ca atom, lattice distortion occurred due to the difference in size of the Zn^{2+} and Ca^{2+} ions. Incorporation of Zn caused the structural conformity of HAp to make it resembled to β-tri calcium phosphate, with enhanced c-axis bond length, providing additional space for the accommodation of added DOX molecules. The study revealed the excellent DOX loading (126.0 mg/g) and releasing behavior of Zn-HAp nanostructures with 1 mol % Zn (Fig. 8). Drug release behavior of the Zn-HAp nanostructures with optimum Zn content (1 mol %) was studied at different pH values of the Phosphate Buffered Saline (PBS) fluid medium. Maximum drug release was observed to occur at pH 4.5 (acidic condition), which is approximately 83.16 mg/g (66%) of the loaded drug [73]. A list of HAp and HAp composite scaffolds are reported in tabular form with their potential drug delivery application (Table 3).

3.1. Pros and cons of hap as scaffold material

The foremost limitation of HAp in the fabrication of scaffolds for biomedical application is the limited control over pore structures (size, shape, and distribution). In fact, the fabrication of patient-specific scaffold is a big challenge for researchers. Fabrication of HAp scaffold with critical biological shapes is very difficult for ceramics materials. To induce desired mechanical strength, frequently the fabricated HAp scaffolds require high-temperature sintering, as the ceramic scaffolds developed through low-temperature processing are very brittle, not suitable for bone tissue engineering applications. Use of ceramics such as HAp in scaffold application has two major disadvantages: lack of degradability in a biological system, and limited processing technique used for scaffold fabrication. The application of HAp bioceramics is also restricted primarily to bone tissue engineering.
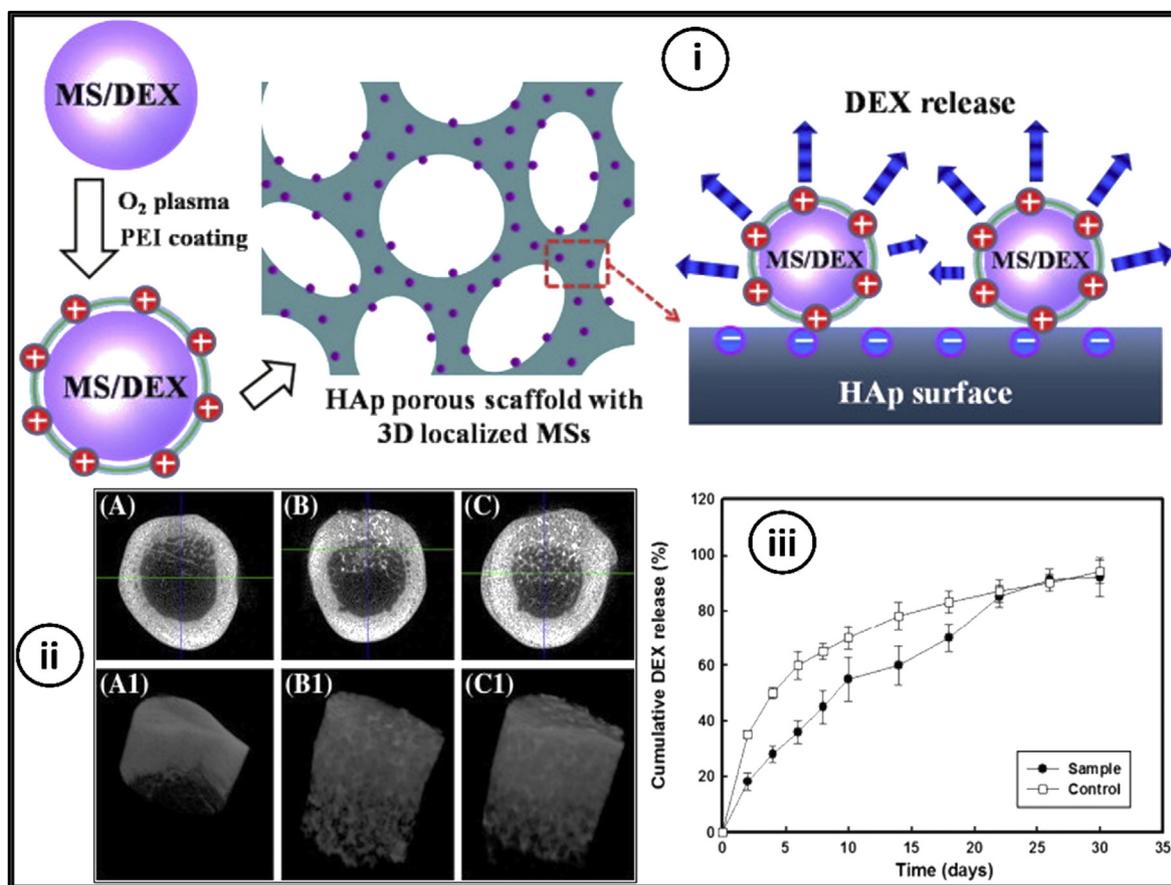
Fig. 6. (i) Schematic diagram of DEX-loaded PLGA macro-spheres and their immobilization at HAp surface; (ii) DEX-loaded PLGA microspheres immobilized onto HAp scaffold at 10 weeks' post-implantation in beagle femurs with 5 mm drill hole defects; (iii) The release profile of control and immobilized macro-sphere [Reprinted with permission from Son et al., Copyright 2011 Elsevier]. [69].

Overall, for biomedical applications, the benefits of ceramic materials are more than their limitations. There are many promising possibilities for HAp materials for utilization in biomedical applications. The most important characteristics of HAp is its biocompatibility and chemical structure similar to bone and teeth. HAp is a very stable biomaterial, which can tolerate high temperature up to 1260 °C. HAp bioceramics are non- or very low reactive material (in mild acidic conditions) and insoluble in most of the solvents including water, ethanol, acetone, methanol, hexane, isopropyl alcohol etc. On the other hand, HAp is a nontoxic bioactive material, easily accepted by the body system without any immunogenic reaction.

4. Prospects of HAp in biomedical applications

Although HAp biomaterials have been widely investigated, there remain several challenges to be consider before their clinical applications. The development of multifunctional properties with therapeutic ion release also has great potential for biomedical application. Doping of different metals such as Au, Zn, Ag, Mg, Mn, Sr, Cu, Fe, Eu, Gd, etc., might be useful to provide smart strategies for *in situ* therapeutic application. The HAp bio-ceramics could also be useful towards therapeutic as well as diagnostic purposes. HAp is a very promising material with a unique chemical structure, which allows cation substitution by other elements, providing a complex structure with multifunctional properties. HAp is a very promising material for theranostics (therapeutic and diagnosis) applications. On the other hand, possibilities of developing HAp based composites combining polymers, metals, and ceramics in appropriate proportion open up the possibility of tuning their physio-chemical characteristics favorable for biological

applications. In conclusion, we forecast a promising future of HAp based bio-ceramics for the support and improvement of human health.

5. Conclusions

Calcium phosphate-based bio-ceramics are the popular biomaterials due to their excellent biocompatibility and bioactivity driven by their compositional similarities with human bones and teeth. Porous scaffolds based on HAp have been widely used for hard tissue engineering due to its similar structure to the natural cancellous bone. Several techniques such as gel-casting, injection press moulding, solvent casting, freeze-drying, etc. have been utilized to fabricate HAp scaffolds of different shapes to control their porosity, hardness, drug holding and drug releasing characteristics as desired for their applications. However, the introduction of 3D printing and electrospinning has opened up new and exciting features in fabrication of HAp scaffolds, enhancing their applicability in the areas of tissue engineering and regenerative medicine. While bio-printing offers superior resolution, it also enables the precise spatial distribution of cells and biomaterials within complex 3D structures, 3D-printed structures are unable to provide suitable concurrent flexibility and high tensile strength, which are necessary for the engineering of ligaments and tendons. Along with major printing materials (such as HAp, or bio-glass), many bioactive molecules could be conjugated into scaffolds such as proteins, growth factors like BMPs, TGF-β, IGF, VEGF, or drugs like antimicrobial (vancomycin, penicillin, streptomycin), anticancer (Doxorubicin, 5-Fluorouracil), analgesic, anti-inflammatory etc. Mesoporous HAp nanostructures could be a promising drug delivery agent due to their large surface area and pore volume. New functionalities on HAp can be
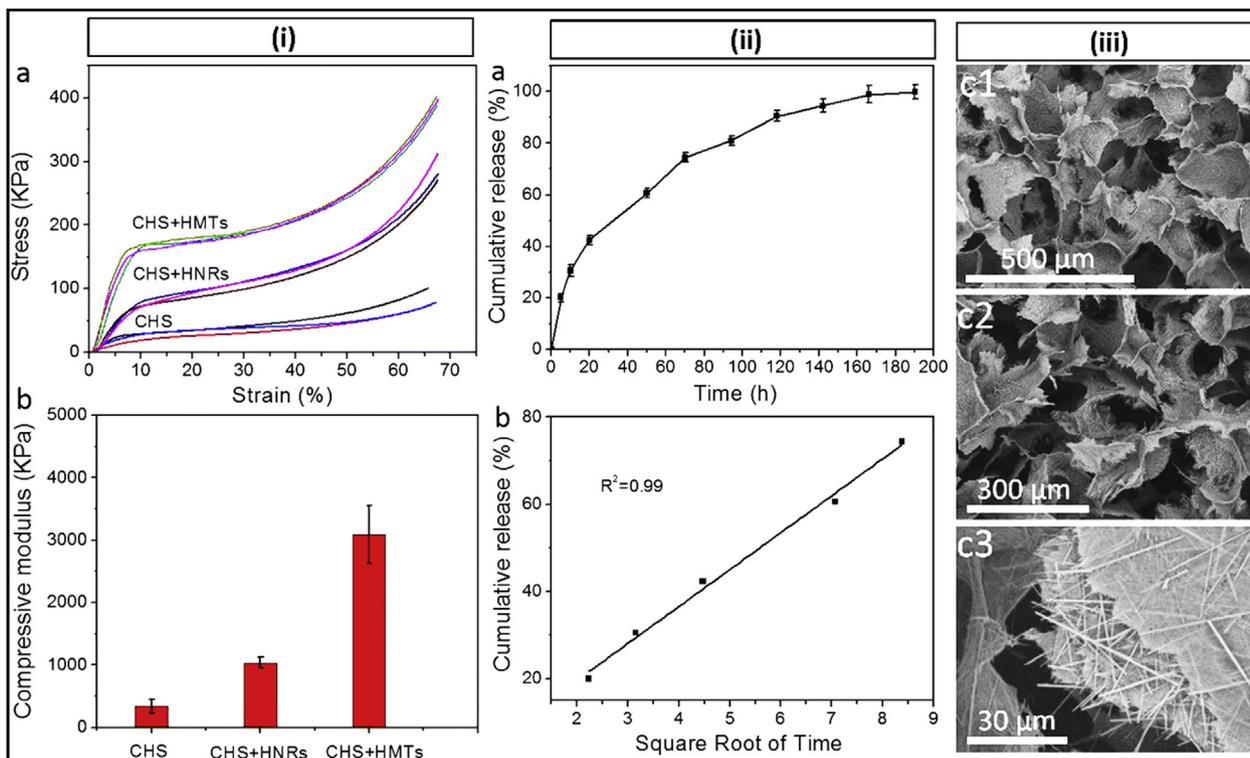
Fig. 7. (i) Mechanical properties of the scaffolds made of chitosan, chitosan-HAp nanorods and chitosan-HAp micro tubes; (ii) Drug release curve of the chitosan-HAp micro tube composite scaffold in PBS; the cumulative drug release percentage versus square root of release time for the chitosan-HAp micro tube scaffold; (iii) Typical scanning electron microscopic images of chitosan-HAp micro tube composite scaffold [Reprinted with permission from Zhang et al., Copyright 2014 RSC).] [67].

attained through doping with rare-earth ions, or by conjugation with magnetic materials. Although all the modifications deliver additional functionalities to HAp-based multifunctional nanostructures, further perfections, are essential to design optimum systems with controlled insistent drug release properties. Bone tissue engineering with composite scaffolds have provided promising ways to repair and replace damaged bones with enhanced drug delivery systems.

Conflicts of interest

The authors declare no conflict of interest.
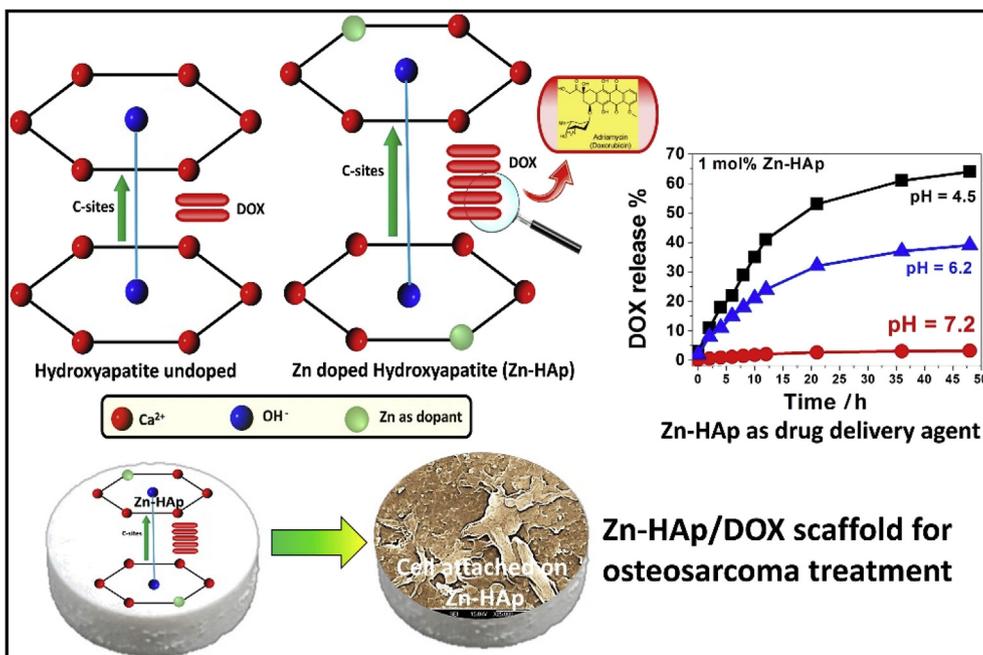
Acknowledgements

Fig. 8. Graphical representation of Zn doped hydroxyapatite (Zn-HAp) for potential application in drug delivery and bone tissue engineering application [Reprinted with permission from Kim et al., Copyright 2018 Elsevier]. [73].

Table 3

Hydroxyapatite scaffold mediated drug delivery.

Sl. No.	Scaffold Types	Loaded Drug	Ref.
1	Cylindrical cavity in calcium hydroxyapatite blocks	Gentamicin sulphate, Cefoperazone sodium, and Flomoxef sodium	[74]
2	Porous hydroxyapatite blocks (HA-b)	Arbekacin sulfate [1-N-(S)-4 amino-2-hydroxybutyryl dibekacin]	[75]
3	Hollow cylindrical hydroxyapatite implants	Pentoxifylline	[76]
4	Nano-crystalline hydroxyapatite and calcium sulphate as biodegradable composite carrier pellet material	Gentamicin and Vancomycin,	[77]
5	Cylindrical hydroxyapatitic grafts at two different degree of porosity (60% and 40%)	Ibuprofen-lysine and Hydrocortisone Na-succinate	[78]
6	Tablets containing hydroxyapatite and a pore forming agent (50% (w/w) Avicel PH 200/20, 37.5% and 50% corn starch/37.5% sorbitol)	Metoprolol tartrate, Riboflavin sodium phosphate	[79]
7	Inter porous hydroxyapatite blocks (2 cm^3 cubic block)	Aminoglycoside antibiotic (Isepamicin Sulfate; ISP)	[80]
8	Porous hydroxyapatite tablets	Ibuprofen	[81]
9	Antibacterial Chitosan Coating on Nano-hydroxyapatite/Polyamide66 Porous Bone Scaffold	Chitosan and Berberine	[82]
10	A collagen–hydroxyapatite scaffold	Recombinant human bone morphogenetic proteins (rhBMPs) and bisphosphonates (BPs)	[83]
11	Si-doped hydroxyapatite/gelatin scaffolds by rapid prototyping	Vancomycin	[84]
12	3D printed porous hydroxyapatite scaffold	rhBMP-2	[85]
13	Coralline hydroxyapatite poly lactic acid thin film platform	Gentamicin	[86]
14	1 mol% Zinc doped hydroxyapatite scaffold (1mol% Zn-HAp)	Doxorubicin (DOX)	[73]
15	Gold loaded collagen coated hydroxyapatite scaffold (Au–HAp–Col)	Doxorubicin (DOX)	[87]

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