Naturally derived biomaterials for development of composite bone scaffold: A review

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Abstract. Over the last few decades, there has been an increasing demand of tissue engineered bone scaffolds as a substitute material for diseased or damaged bone segments. A variety of synthetic and natural biomaterials have been explored by researchers for development of composite scaffold. Naturally derived biomaterials have the advantages of immunomodulating, anti-toxic and biomimetic properties with the cellular environment in vivo, when compared to synthetic biomaterials. In this paper, different protein and polysaccharide based biomaterials used for developing composite bone scaffolds are reviewed. The properties of composite scaffolds developed from these biomaterials are highlighted. The study also includes different natural materials and biowastes which are used for deriving bioceramics for producing composite bone scaffolds. The overall study gives a brief idea on the importance of protein and polysaccharide based biomaterials and bioceramics for composite bone scaffold development and scope for future work in the field of naturally derived biomaterials.

1. Introduction
Efficient treatments of bone injuries and fractures have been a major area of concern for the orthopedic surgeons. Autogenous bone graft techniques are incapable of treating segmental bone defects, where a large fraction bone gets completely damaged [1,2]. Replacement of the fracture site with an artificially developed complete bone by tissue engineering, proved to be a promising alternative. “Tissue Engineering” is a term originally coined at a National Science Foundation workshop in 1988 and later defined by Vacanti as multidisciplinary scientific branch that combines cell biology, materials science and engineering, and regenerative medicine to repair a damaged tissue [3,4]. Bone tissue engineering via scaffolds involves development of a porous construct where cells are seeded in predefined biomechanical environment for cell growth and replacement of damaged tissues. A scaffold should essentially be porous (90% porosity) with well-connected pores of diameter 100-300 microns to allow diffusion of gases and transport of cellular fluids required to support cellular growth [5]. The porous architecture of the scaffold should withstand the mechanical loading to facilitate surgical handling during implantation. Compressive strength of 1-10 MPa is desired for the scaffold to function properly right from the stage of implantation to complete remodeling of the bone tissues [6]. Since scaffolds are a temporary support structure for cell growth, therefore they should be biodegradable with time to create space for the cells to produce their own extracellular matrix [7]. Most importantly, the scaffold must be biocompatible to allow cell attachment, proliferation and differentiation without causing cytotoxicity [8]. The current review paper focuses on different naturally derived biomaterials for producing composite bone scaffolds.
Development of scaffolds with adequate mechanical strength while retaining the porous architecture of the construct is the greatest challenge in the field of bone scaffolds and tissue engineering. Composite scaffolds are 3D porous constructs developed by combining two or more different biomaterials to enhance the overall properties of the scaffolds. Plenty of biomaterials have been developed with good mechanical and biocompatible properties but at the cost of adequate porosity or vice versa. Therefore a proper balance between mechanical strength and porosity is desired for successful development of functional scaffolds [4]. The biomaterial selected for developing scaffolds together with the scaffold synthesis technique plays the key role in fulfilling all the requirements of a scaffold. Biomaterial is a combination of one or more substances that are derived from synthetic or natural sources, which helps in treatment or replacement of any tissue, organ or function inside the body [9]. Polymers, ceramics, metals and composites are primarily the most explored synthetic biomaterials for scaffold synthesis [10]. However synthetic biomaterials, particularly synthetic polymers, have been reported to show insufficient cell growth and cell adhesion capabilities [11,12] as well as lack of functional groups required for surface modifications [13]. Metallic scaffolds lack biological recognition on material surface and possibility of releasing toxic metallic ions through corrosion or wear that might lead to inflammatory cascades and allergic reactions [14]. Ceramic based scaffolds are found to be brittle due to which they are difficult to shape for implantation [15].

Natural based biomaterials on the contrary have several advantages compared to synthetic biomaterials. Apart from the biomimetic properties and being ecologically safe, they are available from plenty of replenishable agricultural food resources and various biodegradable waste materials which reduce their synthesis and processing cost. Natural biomaterials show good biocompatibility and biodegradability [16] with possibility of producing various enzymatically modified derivatives that stimulates cell growth and attachment with minimal risk of tissue rejection [17,18]. Biomaterials derived from natural sources include polysaccharides, proteins, bioceramics, etc. and they have been explored for synthesis of porous composite bone scaffolds. This review article focuses on study of protein and polysaccharide derived natural biomaterials and naturally derived bioceramics for bone tissue engineering applications, with special emphasis on composite scaffold development. Properties of biomaterial derived from protein like Silk, Collagen and Fibrin and polysaccharide like Chitosan, Alginate, Hyaluronan, Agarose and some algae based sulphated polysaccharides are highlighted. A comprehensive study on the composite bone scaffolds developed from these biomaterials is carried out. In the subsequent sections, bioceramics derived from natural sources like Corals, Algae, Shells, Bones and Scales are listed and their applications in composite bone scaffold development are studied.

2. Natural biomaterials

Natural biomaterials include substances which are natural in origin or certain chemical modifications of the substances to be used for development of scaffolds or any other implants. Based on the nature of their origin, natural biomaterials can be broadly classified under two main categories, protein based and polysaccharide based natural biomaterials.

2.1. Protein based natural biomaterials

Protein based biomaterials consists of certain recombinant proteins with amino acids as the major constituent [19]. They involve certain mechanical and biological motifs derived from natural proteins that provide structural support, as well as guide to cell and tissue behavior. Silk, collagen and fibrin are the most explored protein based natural biomaterial.

2.1.1. Silk. Silk is a protein based polymer that is spun into fibers by some lepidoptera larvae such as silkworms, spider, scorpions, mites and flies [20]. The most widely used silks are from the domesticated silkworm-Bombyx mori, and from spiders-Nephila clavipes and Araneus diadematus [21]. Silk proteins have proved to be a promising biomaterial due to their unique combination of biocompatibility, biodegradability, self-assembly, mechanical stability and ability to tailor its structure and morphology. Silk protein in combination with some organic/in-organic solvents and water soluble gelatin materials have been extensively used for fabrication of 3D porous composite scaffolds for regeneration of bone, ligament and skin tissues [22–24].
2.1.2. **Collagen.** Collagen is the most abundant protein in the body that provides strength and structural stability to tissues including skin, blood vessels, tendons, cartilage and bone. Apart from its favorable mechanical strength and cell-binding properties, collagen has good biocompatible and biodegradable features [25]. 89% of the organic matrix of bone is composed of collagen-I combined with hydroxyapatite (HAp). Collagen crosslinked with HAp, chitosan fibres and mesenchymal stem cells have been used to generate porous scaffolds for bone tissue engineering [26–29]. Cross-linked collagen based composite scaffolds have been found to exhibit minimal inflammatory and non-fibrotic cellular growth [30].

2.1.3. **Fibrin.** Fibrin is a protein based natural polymer produced from fibrinogen that supports numerous living tissues and wound healing by inducing angiogenesis and promoting cell attachment and proliferation [31–33]. Due to the strong adhesive properties of fibrin to biological surfaces, it is often used as a biological sealant to fill sub-critical defects in bone with clot and generation of new bone tissues [34]. Studies report addition of fibrin with cells seeded into biodegradable scaffolds which have poor cell adhesion properties for cell delivery and tissue engineering applications [35]. Fibrin coating on PLLA based scaffold have been found to improve scaffold adhesion properties thereby promoting early tissue regeneration [36]. Fibrin based hydrogel scaffolds have been widely used for tissue engineering applications of cartilage [37,38] and bone tissues [39,40].

2.2. **Polysaccharide based natural biomaterials**
Natural polymers mimic the natural macromolecular environment of cells and display excellent biocompatibility with favorable mechanical properties and ability to be loaded with growth factors necessary for bone formation [41]. Polysaccharide based biomaterials are natural polymers consisting of sugar monomers. Polysaccharides are derived from plant or animal sources and known to be highly bioactive with regards to tissue engineering applications. Chitosan, alginate, hyaluronan and agarose are some of the potential polysaccharide biomaterials used for developing scaffolds for tissue engineering.

2.2.1. **Chitosan.** Chitosan is a linear polysaccharide derived from partial deacetylation of chitin, which forms the structural component structural components in the exoskeleton of arthropods or in the cell walls of fungi and yeast [42]. Chitosan has proved to be a promising biomaterial for synthesis of porous composite scaffolds due to its excellent biological properties like biocompatibility, biodegradability, support for cell attachment and proliferation and antimicrobial activities [43]. Chitosan-HAp composite scaffolds for regeneration of bone tissues have gained wide attention in biomedical applications because of their improved bioactivity and bone bonding ability [44].

2.2.2. **Alginate.** Alginate or Alginic acids are biopolymers composed of non-repeating unbranched exopolysaccharides derived from certain seaweeds, bacteria and brown algae [45]. Alginate is highly hydrophilic, biocompatible, relatively economical and biodegradable under normal physiological conditions [46]. Owing to its unique physiological properties, composite scaffolds have been found successful with alginate combined with different other biomaterials like HAp [47], chitosan [48], collagen [49] and silk fibroin [50] for bone regeneration.

2.2.3. **Hyaluronan.** Hyaluronic acid or Hyaluronan is a polysaccharide which is a major glycosaminoglycan present in the extracellular matrix of tissues that promotes early inflammation critical for wound healing [51]. Hyaluronan has unique visco-elastic properties with good biocompatibility, biodegradability and ability to maintain a hydrated environment conducive for cell infiltration [51], because of which they are widely used in synthesis of composite bone scaffolds. Composite scaffolds based on hyaluronan combined with synthetic polymers [52,53], collagen [54] and HAp [55] have been developed bone and cartilage regeneration.
2.2.4. **Agarose.** Agarose is a marine algal polysaccharide obtained by isolation of red algae and seaweed [56]. Agarose has the ability to form into hydrogel thereby providing a three dimensional environment for cell proliferation and retention of their phenotype [57]. Moreover the physical structure of the gels can be altered by varying the agarose concentration which results in desired pore sizes. Therefore agarose based hydrogel scaffolds [58] and composite scaffolds made of agarose combined with HAp [59] finds its applications in bone tissue engineering.

2.2.5. **Other algae based polysaccharide:** Algae is a rich source of plenty of different varieties of sulphated polysaccharides, which have antioxidant, anti-allergic, anti-inflammatory and anti-coagulant properties ideal for tissue engineering applications [60]. Sulphated polysaccharides like Ulvan extracted from green algae Ulva, Carrageenans from red algae Rhodophyceae and Fucoidan from brown algae Phaeophycophyta [61] have been extensively utilized for synthesis of composite bone scaffolds. Figure 1 and 2 shows macroscopic appearance and SEM images of silk fibroin, gelatin and HAp based composite scaffolds and their pore structures. A list of different protein and polysaccharide based biomaterials and the properties of composite scaffolds developed from these biomaterials are shown in Table 1. It can be seen from Table 1 that protein and polysaccharide based composite scaffolds have been successfully developed with adequate porosity and mechanical strength required for growth of bone cells. Thus biomaterials derived from natural sources like protein and polysaccharides have significant effect on the porosity, strength and bioactivity of composite bone scaffolds.

![Figure 1](image1.png)  ![Figure 2](image2.png)

**Figure 1.** Macroscopic appearance of 3D porous (a) silk fibroin, (b) fibroin-gelatin composite, (c) fibroin-HAp composite and (d) fibroin-gelatin-HAp composite scaffold. **Figure 2.** SEM images of (a) silk fibroin, (b) fibroin-gelatin composite, (c) fibroin-HAp composite and (d) fibroin-gelatin-HAp composite scaffold showing their respective pores. Picture courtesy: Moisenovich M. M 2014 [62]

| **Table 1.** Maximum range of porosity and mechanical strength attained for composite bone scaffolds using protein and polysaccharide biomaterials. |
|---------------------------------------------------------------|
| Scaffold raw materials | Biomaterial type | Scaffold properties |
| Silk | Protein | 10-600 μm pore dia. [23] | Compressive strength: 13 MPa [23] |
| Collagen | Protein | 100-200 μm pore dia. [26] | Compressive strength: 1.7-4.1 MPa [26] |
3. Natural based Bioceramics

Bioceramics are ceramic material specially designed as a biomaterial for promoting regeneration of tissues and fixing damaged body parts like tooth or bones [68]. Due to their high tensile strength, inert nature and ability to form stable bonds with the host tissues, bioceramics are often reinforced with other biomaterials to develop composite scaffolds for bone tissue engineering [16]. Hydroxyapatite (HAp) because of their structural similarity with the mineral portion of bone and tooth is the most popular Ca/P based bioceramic material used in bone tissue engineering applications [69]. Apart from synthetic raw materials, plenty of calcium containing natural materials has been used for deriving HAp. Some of the natural sources of bioceramics are discussed in the following sub-sections.

### 3.1. Corals

Coral is a marine invertebrate, whose exoskeleton consists of CaCO₃ with large number of interconnected pores [70]. Due to their identical pore structure to cancellous bone and improved biocompatibility and osteoinductivity properties, corals and HAp derived from corals have been extensively used for developing porous bone scaffolds [70,71].

### 3.2. Algae

Structural composition of certain class of mineralized red and green algae have been reported to be identical to corals and are therefore a rich source of calcium [61]. HAp derived from red algae Corallina officinalis [72] and green algae Phymatolithon calcareum [73] have been used for development of composite bone scaffolds.

### 3.3. Shells

Shells of certain animals like cockle, oyster and mollusc and eggshells, by-product of chicken egg, are rich in CaCO₃, have been effectively utilized for producing bioceramic HAp for orthopaedic applications. HAp derived from cockle shells [74,75], oyster shells [76,77], mollusc shell [78] and eggshells [79] have been combined with different biomaterials to develop porous composite bone scaffolds.

### 3.4. HAp from bones and scales

HAp derived from high temperature sintering of fish scales [80,81], fish bone [82,83], bovine bone [84] and pig bone [85] have also been used for development of composite bone scaffolds. Thus it is seen that bioceramic HAp has been derived from a wide range of natural sources like corals, algae and shells and biowaste materials like eggshells, scales and bones. HAp derived from these sources has been effectively utilized for development of composite bone scaffolds with excellent mechanical properties.

4. Conclusions

Bone tissue engineering tactics offers a promising alternative to autograft and allograft techniques in treatment of segmental bone defects. Scaffold biomaterials and their synthesis techniques play a crucial role in bone tissue engineering. Composite bone scaffolds acquire the properties of different
biomaterials to turn into a more enhanced porous construct with improved properties ideal for osteogenesis. Owing to the biocompatible, biodegradable and anti-toxic properties of natural materials, naturally derived biomaterials have been beneficial in synthesis of composite bone scaffolds compared to synthetic biomaterials. Polysaccharides and protein are the two most important sources of natural biomaterials used for composite scaffold synthesis. Silk, collagen and fibrin are important protein based biomaterial. Composite bone scaffolds with a maximum compressive strength of 13 MPa with 600 μm pore diameter have been achieved using silk protein based biomaterial. Polysaccharides are natural polymers known for their bioactivity and immunomodulating properties. Chitosan, Alginate, Hyaluronan and Agarose are important polysaccharide based biomaterials used for development of composite bone scaffolds. Compressive modulus of about 18 MPa with 82% porosity and 150 μm pore diameter have been achieved for alginate polysaccharide based composite bone scaffolds. HAp being the most popular bioceramic material has been derived from natural sources like corals, algae, shells of different animals and biowaste materials like egg shells, fish scales and bovine and pig bones. Composite scaffolds for regeneration of bones and teeth have been developed using HAp derived from these natural sources. However, incorporation of a perfect balance between mechanical strength, porosity and growth factors in bone scaffolds is still a challenging task. Moreover, synthetic biomaterials are difficult to synthesize and their synthesis process is cost inducing. Therefore the review will pave the biomaterial research community to further explore different naturally derived biomaterials and tune them to design scaffolds with more enhanced properties.

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