4 Sophisticated Biocomposite Scaffolds from Renewable Biomaterials for Bone Tissue Engineering

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4.1 Introduction

Loss or the dysfunction of bone tissue may occur due to trauma, injury, disease, or aging [1]. Currently there are excessive amount of materials to be applied to bone regeneration [2]. In turn, the autograft-, allograft-, or xenograft-based bone regeneration techniques have their disadvantages such as the need for extra surgical procedures, infection, chronic pain, or tissue rejection, which in turn has increased the importance of tissue engineering and regenerative medicine [3]. The main goal of tissue engineering is to assemble isolated functional cells and biodegradable tissue scaffolds made from bioengineered materials with the aim of regenerating diseased or damaged tissue. Many scientists from this multidisciplinary field have focused on designing and generating appropriate scaffolds for various tissues, by primarily overcoming cell-dependent problems in addition to scrutinizing tissue engineering structures in vitro and in vivo [4].

This chapter aims at describing the importance of renewable materials which have great potential for use in bone tissue engineering. In this context, the chapter offers new approaches in the improvement of polymeric composite matrices with the aim of obtaining 3D tissue-engineered scaffolds from renewable biomaterials.

4.2 Biology of Bone Tissue: Structure and Function

Bone tissues are responsible for many crucial assignments, the most notable ones being structural support and protection against external forces in the vertebrates. Its ability to self-repair and rebuild by promoting mechanical requirements makes this tissue very unique in a structural sense. However, healthy bone functions can be influenced by many different pathological situations or diseases. On the other hand, the bone tissue has been established to have limited regenerative capacities depending on patient age, anatomical site, and fracture size since it is hard for the body to repair huge gaps by itself [5, 6]. Critical-sized fractures (~5 mm) do not have the ability to heal on their own and need surgical procedures to ensure the appropriate restoration. Typical fractures seldom give rise to the formation of a hole of critical size, whereas some trau-

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matic defects, cancer, infections of the bone, or age-related degenerations result in areas where the bone cannot renew by itself. Thus, bone tissue transplantation is the second most performed procedure after blood, with over 100 million operations a year, where patients only in the USA pay approximately $800 billion for treating bone diseases annually [6].

Bone, an enduring and extremely vascularized tissue, can keep reconstructing itself throughout a life span. Within its dynamics are different mechanical, biological, and chemical functions which act in controlled harmony. These include structural support, protection and regulation and storage of restorative cells and minerals, in addition to protection and regulation of Ca and P ions by arrangement of crucial electrolyte concentrations in the blood [7]. It actively contributes to the generation of various types of blood cells (known as hematopoiesis) by regulating homeostasis [8]. The bone structure has a complementary role in mobility, through the skeletal structure which has sufficient load-bearing capability and behaves as a protective cover for the sensitive interior organs of the body [9]. For a better understanding of the mechanical features of a compact bone tissue, it is significant in understanding the hierarchical constructional behavior they possess: (1) cancellous and cortical bone; (2) the microstructure (from 10 to 500 μm); Haversian systems, osteons, single trabeculae; (3) the sub-microstructure (1–10 μm); lamellae; (4) the nanostructure (from a few hundred nanometers to 1 micron): molecular structure of constituent elements like fibrillar collagen and embedded mineral; and (5) the sub-nanostructure (less than a few nanometers): molecular structure of component elements such as minerals, collagen, and non-collagenous organic proteins (Fig. 4.1). Thus, the components of bone material are both heterogeneous and anisotropic in nature [10].

The bone ultrastructure is composed of collagen and minerals such as tricalcium phosphate, and hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$. Synthetic HA is one of the most preferred bioc-

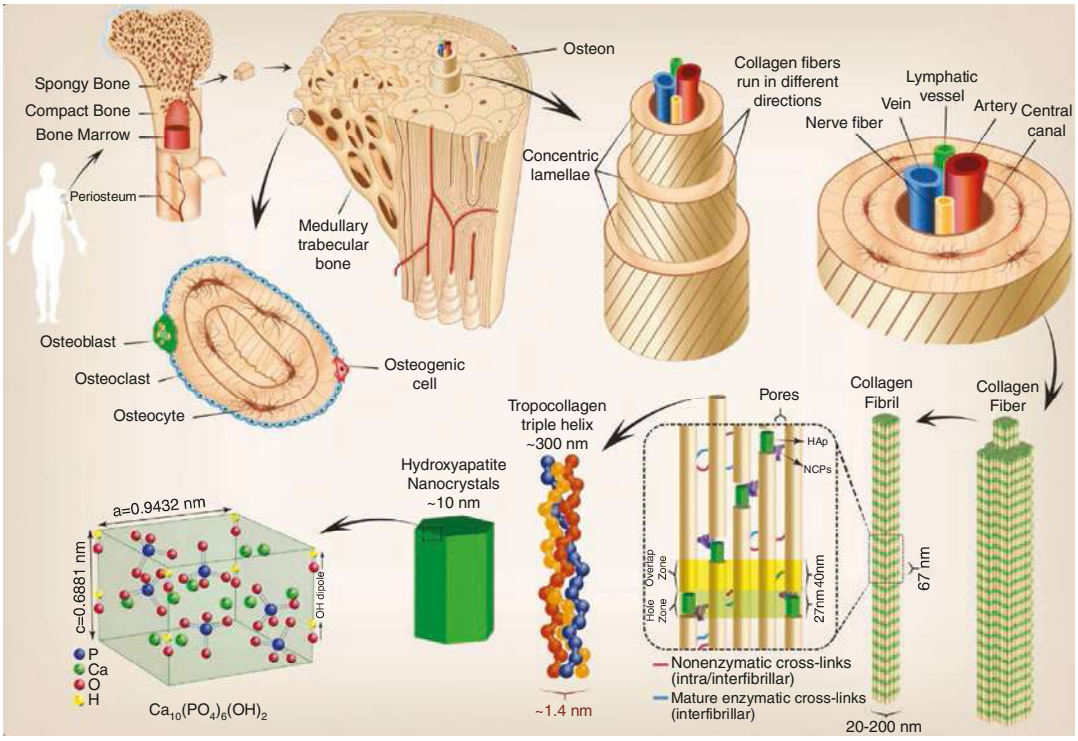

Fig. 4.1 Anatomy of bone tissue: The ultrastructure of compact bone [16]

eramic structures used in the construction of bone substitutes. When examined in detail, bone macromolecules are formed from collagen type I (90%) and over 200 different types of non-collagenous matrix proteins (i.e., osteocalcin, osteonectin, glycoproteins, proteoglycans, and sialoprotein) [11, 12]. These non-collagenous matrix proteins induce intermediate extracellular signals which tend to regulate the homeostasis of various cell types such as osteoblast, osteocyte, and osteoclast. The other crucial section of bone is the mineralized inorganic components (composed of 4-nm-thick plate-like carbonated apatite mineralities). Moreover, the compact structure composed of collagen and HA gives this tissue a unique compressive strength and high fracture toughness [12].

HA is a bioactive, biocompatible, osteoconductive, nontoxic, noninflammatory, and non-immunogenic ceramic for bone tissue engineering and one of the most widely used biomaterials due to its resemblance to the inorganic constituent of the vertebrae, bone and its ability to encourage cell-scaffold adaptation [13]. Hydroxyapatite nanoparticles (HAp) in collagen fibers reach for supporting assistants by activating the production of alkaline phosphatase in bone, resulting in its overwhelming endurance [14]. Nanoscale HAp ($50 \times 25 \times 3$ nm^3) is crucial for appropriate generation of osteocytes in the bone matrix. Naturally produced HAp has a Ca:P ratio of 1.67 which needs to be imitated in the production of HAp to acquire the necessary biological response, solubility, and mechanical sensitivity [15].

Autogenous bone implants are widely selected in bone replacement. Nevertheless, this treatment technique is limited due to insufficiency of donors, infection, veto of implant, etc., especially in wide fractures [17]. Various studies have been conducted since the discovery of the differentiation potential of human adipose-derived mesenchymal stem cells (hAMSCs) into osteogenic lineage, and hence these cells have been considered as an excellent source for bone tissue engineering applications. Even though first practices included the direct implementation of stem cells into fracture locations, nowadays scaffolds combined with stem cells, particularly MScs, are applied, so that they promote cell colonization, immigration, growth, and differentiation [18].

An optimal scaffold for bone tissue engineering practices should permit or enhance cell viability, attachment, proliferation, homing, osteogenic differentiation, vascularization, host integration, and high load-bearing capacity (Fig. 4.2). In addition, it should be simple to apply and susceptible to minimally invasive implant treatment. It should be reproducible on an industrial scale and at the same time be sterile. Eventually, all its features should be practical and meet the demands [19].

4.3 An Overview of Biomaterials in Tissue Engineering

The field of tissue engineering involves chemistry, biology, medicine, and engineering approaches, with the aim of repairing and/or replacing injured tissues and organs with the aid of bioartificial substitutes using biopolymers, cells, and biologically active agents such as growth factors and cytokines (Fig. 4.3). This is a thriving interdisciplinary field presenting new opportunities to scientists [7, 20]. The extracellular matrix comprises a complex combination of structural and functional proteins, glycoproteins and proteoglycans that are organized in a unique tissue-specific three-dimensional structure. They play a vital role in morphogenesis, composition, and function of tissues as well as organs [21].

Providing a suitable microenvironment, that is to say, fabricating scaffolds or decellularized extracellular matrices for cell growth, migration, and proliferation is crucial in tissue engineering (Fig. 4.4). This is due to the fact that scaffolds which include growth factors or other signaling molecules serve as a so-called niche for cells [7, 23, 24]. In essence, big progress in the fabrication of novel three-dimensional (3D) tissue-engineered scaffolds, using biodegradable polymers for the purpose of therapy, has been achieved. An extensive number of attempts at developing new scaffold technologies using both polymers and cells, including stem and/or

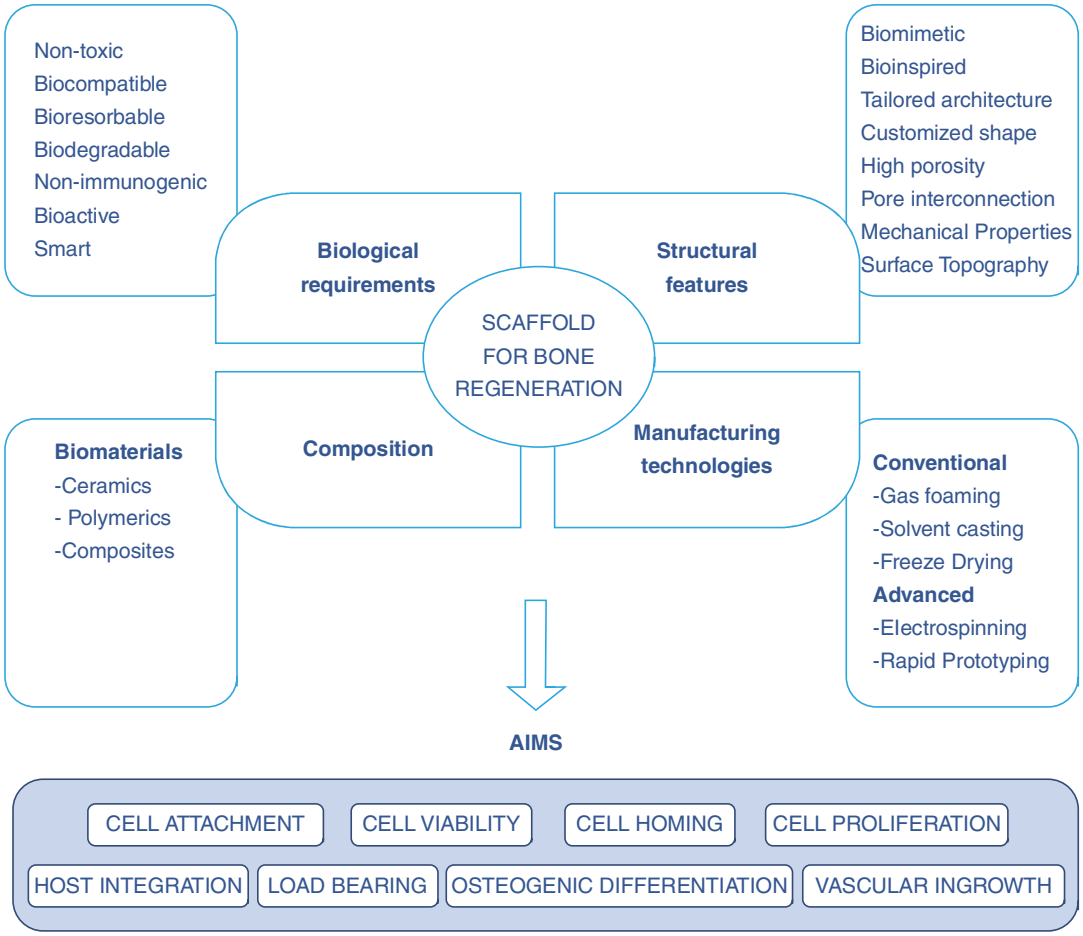

Fig. 4.2 General overview of scaffold construction for bone regeneration [19]

somatic cells, isolated from various tissues have been made. Polymers used in the fabrication of scaffolds in regenerative medicine can usually be categorized as synthetic or natural, where the commonly used polysaccharides (starch, alginate, chitosan, hyaluronic acid derivatives, etc.) and proteins (collagen, fibrin gels, silk, keratin, etc.) are examples for natural polymers (Table 4.1). On the other hand, synthetic polymers such as polylactic acid (PLA), poly(L-lactic acid) (PLLA), poly(D,L-lactic-co-glycolic acid) (PLGA), polyglycolic acid (PGA), and polycaprolactone (PCL), approved by U.S. Food & Drug Administration (FDA), can be easily processed and handled in contrast to natural polymers which is their superiority (Table 4.2) [25]. Major advances seen in biomaterials technology in recent years have led to the development of sophisticated materials [26]. Ideally, functionalized biomaterials like ceramics and natural/synthetic biodegradable polymers can be utilized for the production of 3D scaffolds which tend to supply not only mechanical support but also microscale architecture for neo-tissue construction allowing in vitro and in vivo cell growth, attachment, migration, and proliferation [24, 27, 28]. These biomaterials are seen to have a wide range of applications, including replacement of biological tissues and development of instruments for injury and surgical applications, and medical diagnosis has led to a revolution in biomaterial science [26].

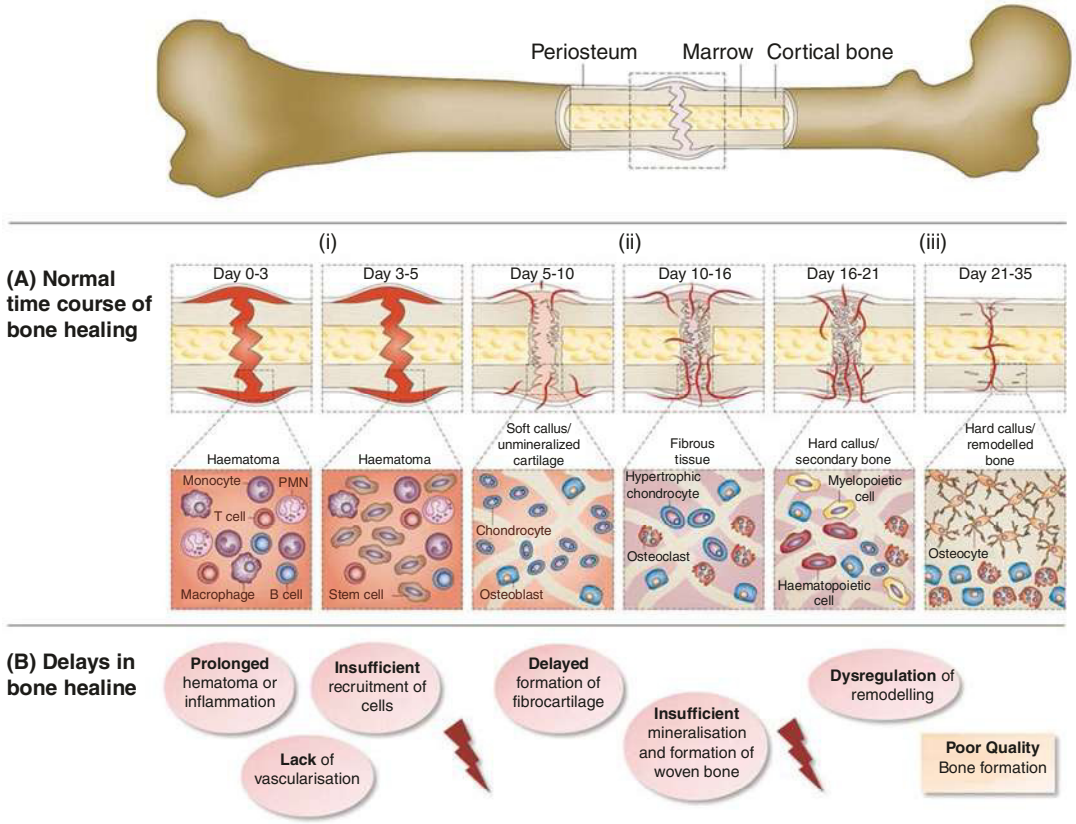

Fig. 4.3 The repairing mechanism of femur fractures and common complications that may occur [9]

4.4 The Importance of Popular Renewable Materials for Regenerative Medicine

The applicability of native materials containing polysaccharides and proteins in the structure of hydrogels has been well studied. These materials, including ECM proteins such as collagen, elastin, fibrin, keratin, hydroxyapatite, and hyaluronic acid, show significant bioactivity in biomedical applications [30].

Bone is a complicated material consisting of mostly collagen, proteins, with hydroxyapatite in organic component. Although HA is the essential inorganic constituent of bone, it does not have the ability to be applied as bone healing material alone because of its delicate and brittle nature. At present, many researchers have devoted themselves to the development of durable hybrid biomaterials of hydroxyapatite with proteins and alternative synthetic polymers [31–35]. For many years, HA ceramics that can improve bone mass and formation of the implant and the bone interface have become quite important as bone grafting material, due to their great mechanical properties, corrosion resistance, biocompatibility, bioactive properties, and perfect osteoconductive features [17, 36, 37]. Using an enhanced hygienic, nontoxic and in addition to an environmentally friendly approach, HA powders have been obtained utilizing bioproducts such as corals, cuttlefish shells, natural gypsum, natural calcite, bovine bone, sea urchin, starfish, and eggshell [38–41]. Chemical studies have demonstrated that these bio-wastes, contrary to popular opinion, are rich in calcium

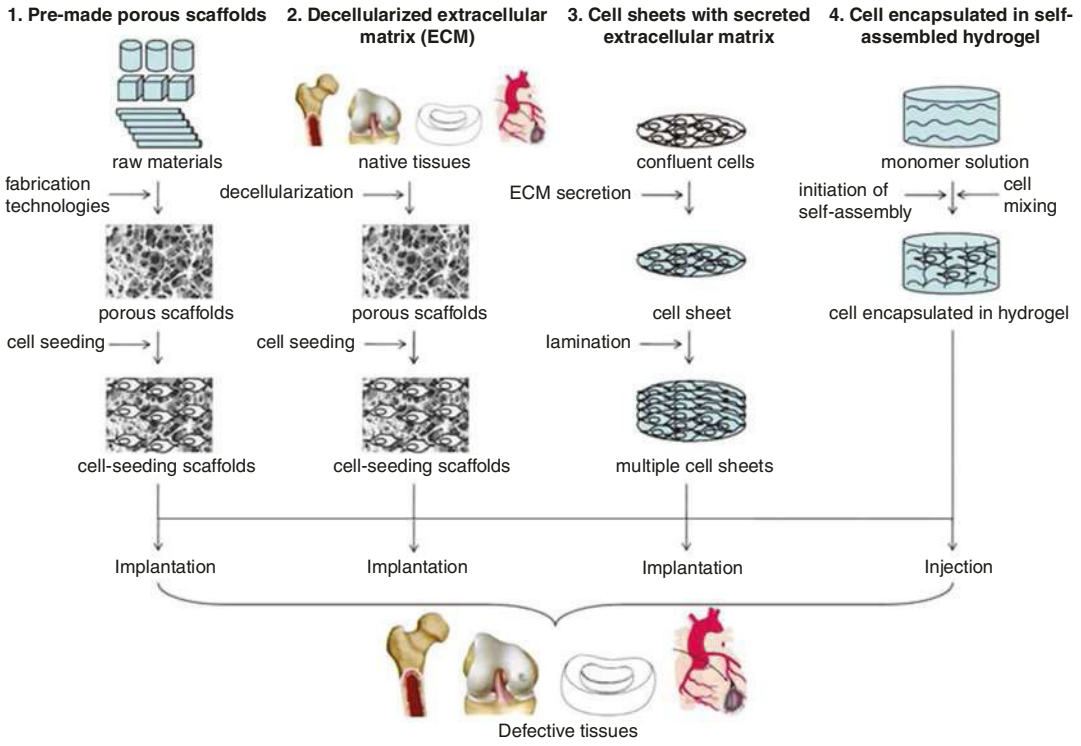

Fig. 4.4 Different scaffold fabrication techniques in tissue engineering and regenerative medicine [22]

in the form of carbonates and oxides. Eggshells are one of the best examples for bio-waste. Millions of tons of eggshells are produced by people as bio-waste on daily basis throughout the world. The eggshell constitutes ~11% of the whole weight of an egg and consisted of calcium carbonate (~94%), calcium phosphate (~1%), and organic matter (~4%) [42]. In addition, eggshells are inexpensive, abundant in nature, biocompatible, yet not osteoconductive. Therefore, transforming these powders in HA before implantation is favorable [43].

Keratins are structural proteins that display high mechanical resistance owing to numerous intra- and intermolecular disulfide bonds containing a fair amount of cysteine [44]. Keratin is mostly consisting of ß-sheets, a small number of α-helices, and loops [45, 46]. Waste keratins are generally obtained from human hair (Fig. 4.5), animal nails, horns, hoofs, wool, and feathers [47]. Additionally, about 300,000 tons of hair is wasted in hair salons, hospitals, and similar places each year [48]. Keratin obtained from renewable sources is highly biocompatible, possesses cellular interaction sites, and exhibits enhanced biodegradability. In contrast to alternative natural materials, human hair keratins have different benefits like being abundant, bioactive, having a powerful capacity to self-assemble inside hydrogels, and being an exact source of autologous proteins [49, 50]. Likewise, in addition to enhancing mechanical properties, this autologous protein has some signaling patterns like Leucine-Aspartic Acid-Valine (LDV) and Glutamic Acid-Aspartic Acid-Serine (EDS) peptide regions which increase the adhesion characteristics of cells [47, 51]. Nonetheless, new improvements have been made to obtain keratin easily from human hair which has resulted in good tissue engineering applications [52].

Collagen is the most widespread protein in the body and provides endurance and constructional stability to tissues containing skin, blood vessels, tendons, cartilage, and bone [27]. The characterizing property of the collagen is its molecular form that is defined by a unique

Table 4.1 Well-known naturally derived polymers used in tissue engineering and regenerative medicine [29]

Polymer	Biocompatibility	Disadvantage	Biodegradability	Application
Collagen	Minimal cytotoxicity, mild foreign body reaction, minimal inflammation	Proteolytic removal of small non-helical telopeptides	Bulk, controllable	Skin, cartilage, bone, tendons, ligaments, vessels, nerves, bladder, liver
Hyaluronic acid	Minimal foreign body reaction, no inflammation	Highly viscous solution, many purification steps after chemical modification	Bulk, 1 h to 1 month	Skin, cartilage, nerves ligaments, vessels, liver
Alginic acid	Minimal foreign body reaction, no inflammation	Uncontrollable dissolution of hydrogel	Bulk, 1 day to 3 months	Skin, cartilage, bone, nerves, muscle, pancreas
Chitosan	Minimal foreign body reaction, no inflammation	Uncontrollable deacetylation and molecular weight	Bulk, 3 days to 6 months	Skin, cartilage, bone, nerves, muscle, pancreas
Gelatin	Minimal cytotoxicity, mild foreign body reaction, minimal inflammation	Weak mechanical property	Bulk, controllable	Skin, bone, cartilage, breast ligaments
Fibrin	Minimal cytotoxicity, mild foreign body reaction, minimal inflammation	Weak mechanical property	Bulk, controllable	Skin, bone, cartilage, liver, tendons, vessels ligaments
Poly(hydroxyalkanoate)	Minimal cytotoxicity, mild foreign body reaction, minimal inflammation	Pyrogen removed	Bulk, controllable	Skin, bone, tendons, nerves cartilage, ligaments, heart; vessels, muscle
Silk	Minimal cytotoxicity, mild foreign body reaction, minimal inflammation	Inflammation of sericin	Bulk, controllable	Skin, ligaments, bone, cartilage, tympanic membrane, vessels, tendons

Table 4.2 Well-known synthetic polymers used in tissue engineering and regenerative medicine [29]

Polymer	Biocompatibility	Disadvantage	Biodegradability	Application
Poly(lactic acid)	Minimal cytotoxicity, mild foreign body reaction, minimal inflammation	Local inflammation, random chain hydrolysis	Bulk, 24 months	Skin, cartilage, bone ligaments, tendons, vessels, nerves, bladder, liver
Poly(glycolic acid)	Minimal cytotoxicity, mild foreign body reaction, minimal inflammation	Local inflammation, random chain hydrolysis	Bulk, 6–12 months	Skin, cartilage, bone ligaments, tendons, vessels nerves, bladder, liver
Poly(lactic-co-glycolic acid)	Minimal cytotoxicity, mild foreign body reaction, minimal inflammation	Local inflammation, random chain hydrolysis	Bulk, 1–6 months	Skin, cartilage, bone ligaments, tendons, vessels, nerves, bladder, liver
Poly(caprolactone)	Minimal cytotoxicity, mild foreign body reaction, minimal inflammation	Hydrophobic	Bulk, 3 years	Skin, cartilage, bone ligaments, tendons, vessels, nerves
Poly(ethylene oxide)	Mild foreign body reaction, no inflammation	Complex biodegradability	Bulk, 1 month-5 years	Skin, cartilage, bone, muscles
Polyanhydrides	Minimal foreign body reaction, minimal inflammation, minimal cytotoxicity	Limited mechanical property	Surface erosion, controllable	Bone
Poly(propylene fumarate)	Mild foreign body reaction, minimal inflammation	Weak mechanical property	Surface erosion, 1 week–16 months	Bone
Poly(orthoester)s	Mild inflammation, mild foreign body reaction	Weak mechanical property	Bulk ~ several months	Ear, bone, cartilage
Polyphosphazene	Minimal foreign body reaction, minimal inflammation	Wide molecular weight distribution	Surface erosion, 1 week–3 years	Skin, cartilage, bone, nerves, ligaments

conformation which is a three α-polypeptide chain of one or more spaces formed in a triple-helical structure of $[Gly–X–Y]_n$ arrangement in one of the main sorts of constructional ECM proteins [30, 53]. This design comprises a supercoiled triple helix that consists of three left-handed polyproline-like chains twisted together into a right-handed triple-helix. Hydroxyapatite and collagen, the most important structural protein present in bone, are two main constituents of bone. They compose 89% of the organic matrix and 32% of the volumetric constituent of bone. Therefore, it is a special protein that promises to produce bone from cultured cells [54]. Collagen is one of the most frequently used materials due to its superior biocompatibility, biodegradability, weak immunogenicity, and cell-adhesive properties in tissue engineering [55, 56]. Although collagen can be produced from different organisms, generally, bovine skin, tendon, and porcine skin-derived collagens for tissue engineering practices are preferred. Yet, collagen obtained from bovine sources includes the risk of infection with illnesses such as bovine sponge-like encephalopathy. Additionally, particularly porcine-derived mammalian collagens are refused for religious reasons [57]. Marine living creatures are also a native origin of collagen and, probably, are more secure source than

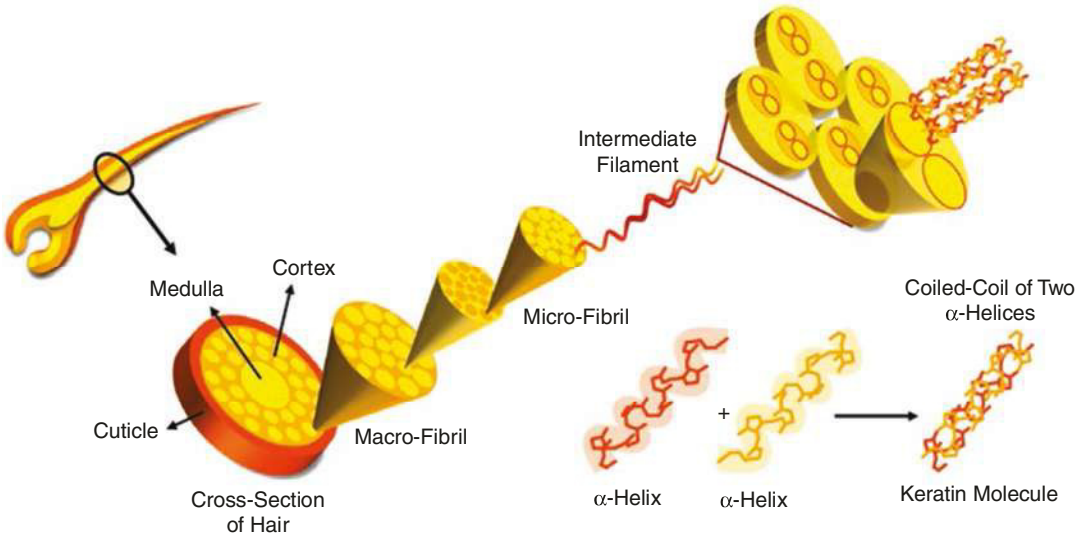

Fig. 4.5 The microscopic structure of hair [52]

mammals. Recent studies focus primarily on the extraction and characterization of collagen from various fish types like salmon, shark or deep-sea redfish and marine sponges. Jellyfish, which is also of marine origin, is another alternative charming source of collagen [58–61]. The worldwide growth of the jellyfish population has caused great concern in the ecological environment. Their potential for utilization in tissue engineering, in addition to the food industry and medicine, we believe, will assist in the preservation of the jellyfish population. Jellyfish has more than 60% collagen, thus the potential to become a perfect source for in biomedical applications [62–64].

4.5 Fabrication of 3D Scaffolds from Keratin-Collagen-nHA for Bone Tissue Engineering

Keratin is insoluble in several prevalent solvents like dilute acids, alkalines, water, and organic solvents. Soluble hair keratins can directly be obtained from human hair utilizing reducing assistant solutions in alkaline or acidic media (Fig. 4.6) [49, 50]. A common way of obtaining keratin includes the utilization of reducing assistants because the natural structure is difficult to extract, owing to its extremely cross-linked status with disulfide bonds [65–67].

Hydroxyapatite is usually obtained through chemical methods by way of calcium hydroxide or nitrate as pioneers [69]. Recently the synthesis of nanostructures using native resources or waste like eggshell, fish scale, or bovine bone has become an outstanding issue. Eggshell, one of the main residual outputs of the food industry, is a great resource of calcium carbonate (95%) enabling its use in the synthesis of HA. There are many different studies related to the synthesis of HA utilizing eggshells [70, 71]. Nanostructured HA has been obtained via various techniques, like homogeneous precipitation, hydrothermal synthesis, combination of electrospinning and thermal treatment, and application of fibrous β-$Ca(PO_3)_2$ crystalline as pioneer [72–74]. Derkus et al. [31] have demonstrated a significantly novel method, the sonochemical synthesis technique, which is a more applicable, homogenous, and cheap method for the synthesis of nanostructured HA (nHA) utilizing various resources. This technique was implemented in the synthesis of nHA using eggshells as the resource (Fig. 4.7), for the design and application of an aptasensor, which has emerged as an interesting application in literature [31].

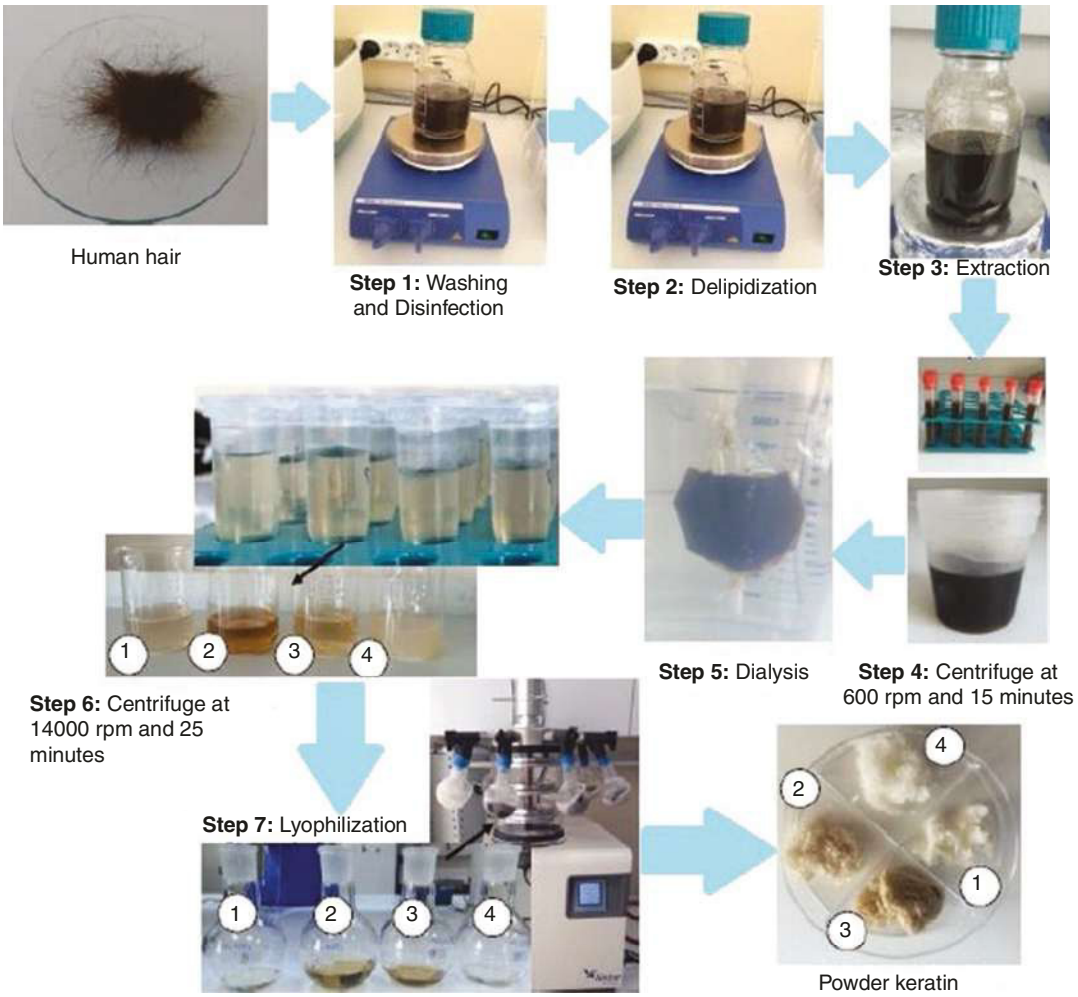

Fig. 4.6 Keratin extraction process from human hair [68]

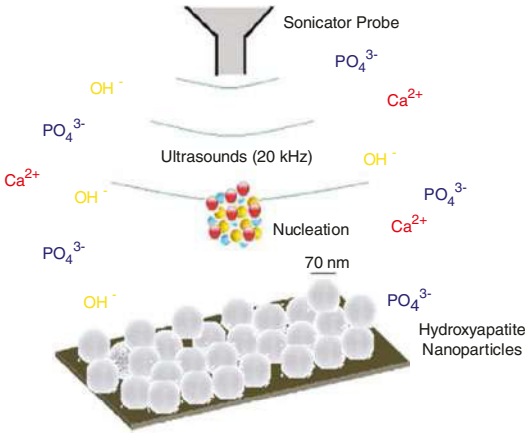

Fig. 4.7 nHA synthesis from eggshell by sonochemical method [77]

Collagen-originated biomaterials are actually based on three basic techniques and sub-techniques of these. The first one is to decellularize the collagen matrix protecting the primary tissue form and ECM architecture, whereas the second method is based on extraction, purification, and polymerization of collagen and its various constituents in order to create a handy scaffold and finally to obtain a collagen solution from different biomolecules. All methods could be applied to several cross-linking techniques and protocols that can be applicable to a large arena of tissue resources [75, 76].

The collagen matrix or ECM could be produced through decellularization methods. Gilbert

et al. [76] have discussed the three ways for tissue decellularization: physical, chemical, and enzymatic. Physical techniques include snap freezing, which disturbs cells by forming ice crystals, leading to high pressure that explodes cells and in turn agitates and stimulates cell lysis. The chemical processes of decellularization involve multiple reagents that remove the cellular ingredient of ECM. These materials range from acids to alkaline tests, which are as good as chelating agents like EDTA, ionic or non-ionic detergents and solutions of excessive osmolarity. Enzymatic therapies like trypsin, which particularly separates proteins and nucleases, evacuating DNA and RNA, are usually utilized to fabricate decellularized scaffolds as well. Nevertheless, all of these methods are unable to fabricate an ECM exactly free of cellular waste on their own; therefore a combination of different techniques is frequently necessary for this purpose [75].

The alternative source for collagen-originated biomaterials are actually marine resources as previously defined. Various ways were applied and enhanced to obtain collagen from jellyfish so as to be able to fabricate collagen-originated biomaterials (Fig. 4.8). Advanced isolation techniques were asserted on three major bases of solubility: in acid solutions, in inactive salt solutions, and in proteolytic solutions. Proteolytic extraction changes collagen molecular architecture by separating the terminal telopeptide areas resulting in the proportional decrease of tropocollagen self-assembled fibrils. In order to prevent this effect, endogenous proteases could be inhibited during acid solubilization. Nevertheless, acid ejection which utilizes light pepsin solubilization is the most efficient technique in terms of yield, although some telopeptides do separate or are partly denatured [77].

There are a limited number of studies concerning the application of bioengineered keratin, jellyfish collagen, and nHA scaffolds to bone tissue engineering. Arslan et al. [17] fabricated 3D tissue-engineered osteoinductive biocomposite scaffolds utilizing human hair keratin, jellyfish collagen, and eggshell-derived nanostructured spherical HA (Fig. 4.9). Two different osteoinductive scaffolds, collagen-nHA and collagen-keratin-nHA, were produced utilizing the freeze-drying method. hAMSCs were then seeded into these scaffolds and the early osteogenic differentiation markers were evaluated. The collagen-keratin-nHA osteoinductive biocomposite scaffolds were observed to have the potential of being used in bone tissue engineering.

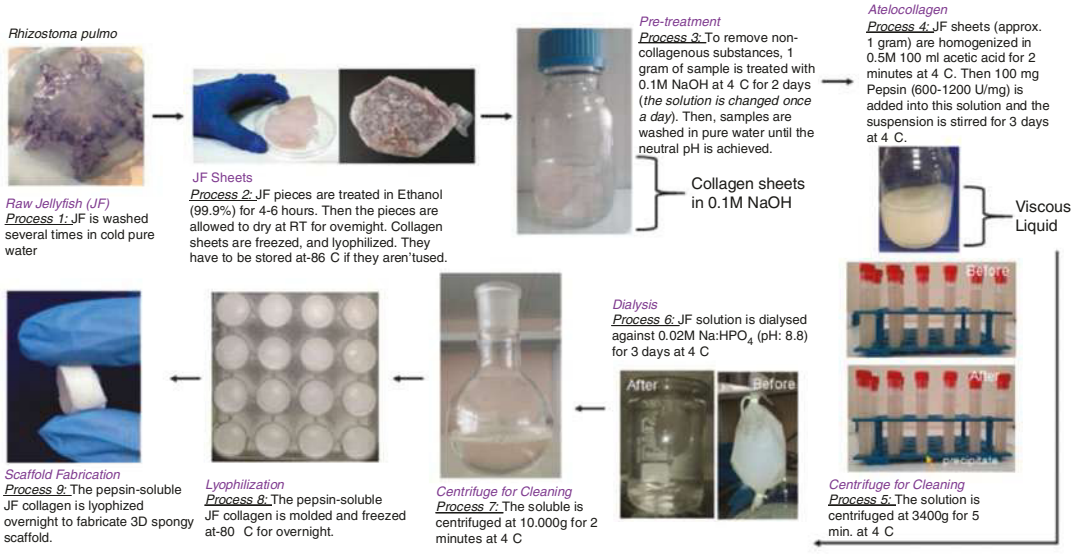

Fig. 4.8 Process steps of jellyfish collagen isolation [31]

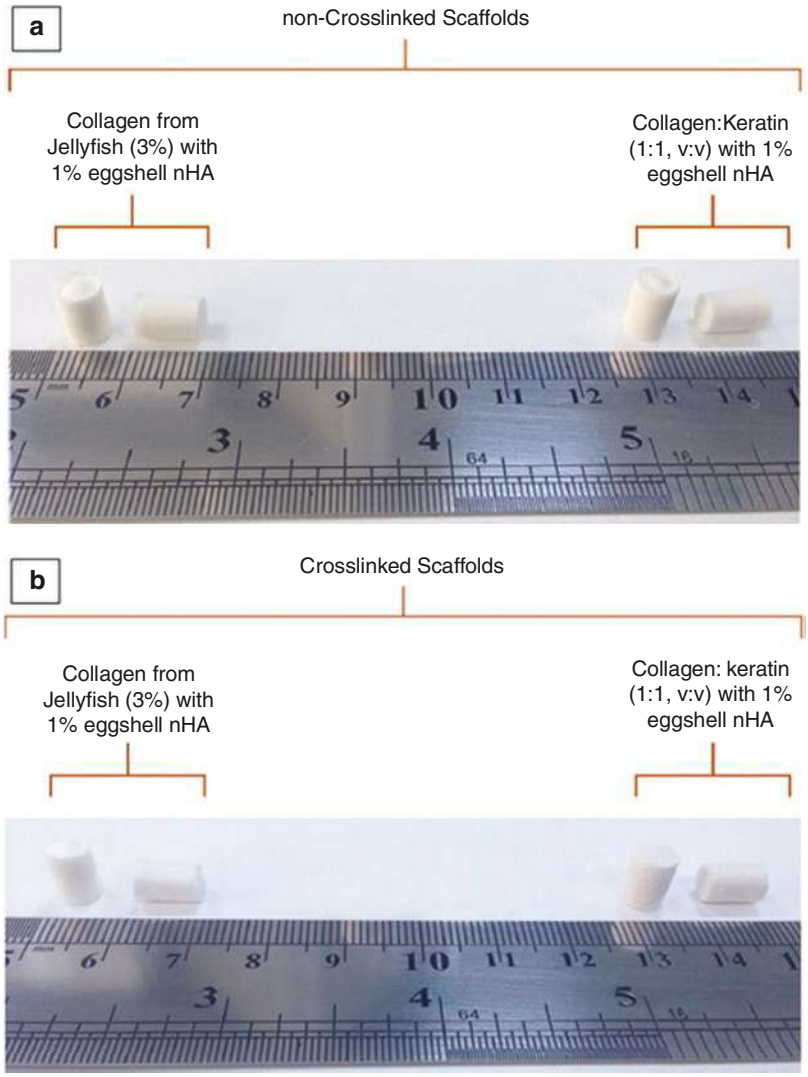

Fig. 4.9 Keratin-collagen-nHA 3D osteoinductive biocomposite scaffolds [17]

4.6 Conclusions

The field of tissue engineering and, in particular, bone tissue engineering has been studied extensively. Polymeric products, in combination with mineral based nanostructures, have been used by various research groups in order to trigger the osteogenic differentiation. Recently, natural resources have become popular due to their cost efficiency, nontoxic nature and easy-to-produce materials suitable for bone tissue engineering. Different research groups have focused on the synthesis of hydroxyapatite bioceramics, which constitute the inorganic phase of bone, using various waste material like mussel shells, flue gas desulfurization gypsum, fish bones, and eggshells. Likely, some research groups have been focused on the isolation of collagen with low immunogenicity and high purity from different kind of species such as jellyfish instead of the traditionally used skin or rodent tail. In our opinion, adaptation to this approach is like "killing two birds with one stone." Firstly, waste is evaluated as a renewable material resource of unlimited volume and chemical diversity. Secondly, it will have a posi-

tive effect on waste accumulation in the environment. Provided that biomaterials obtained from waste resources have low immunogenic response and toxicity, this technology can be expected to become available for clinical use in the next few years.

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