Chapter VIII

Promising biomolecules

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Abstract The osteochondral defect (OD) comprises the articular cartilage and its subchondral bone. The treatment of these lesions remains as one of the most problematic clinical issues, since these defects include different tissues, requiring distinct healing approaches. Among the growing applications of regenerative medicine, clinical articular cartilage repair has been used for two decades and it is an effective example of translational medicine; one of the most used cell based repair strategy include implantation of autologous cells in degradable scaffolds such as alginate, agarose, collagen, chitosan, chondroitin sulfate, cellulose, silk fibroin, hyaluronic acid, gelatin, among others. Concerning the repair of osteochondral defects, tissue engineering and regenerative medicine started to design single- or bi-phased scaffold constructs, often containing hydroxyapatite-collagen composites, usually used as a bone substitute. Biomolecules such as natural and synthetic have been explored to recreate the cartilage-bone interface through multilayered biomimetic scaffolds. In this chapter, is given a succinct description about the most relevant natural and synthetic biomolecules used on cartilage and bone repair, describing the procedures to obtain these biomolecules, their chemical structure, common modifications to improve its characteristics and also their application in the biomedical fields.

8.1 Introduction

Tissue engineering field has been responsible for promising applications development to regenerate and repair osteochondral defects. The biomolecules studied and applied for cartilage and bone repair, are selected considering the knowledge of the anatomical complexity of both structures. The increase of knowledge in the biomolecular area, with progress in new technology such as cellular, molecular biology and biochemistry, offer a good opportunity to create biomolecules with stimulating and precise properties [1, 2].

The main properties sought in a biomolecule to be applied for cartilage and bone regeneration are: biocompatibility, bioactivity, biomimetic skill, biodegradability, being bio-responsive, highly porous, suitable for cell attachment, proliferation and differentiation, osteoconductive, non-cytotoxic, flexible and elastic, and non-antigenic [3, 4]. Currently, several natural and synthetic polymers, with most of these characteristics, have been studied as therapy for cartilage repair. The natural polymers, more used to osteochondral defects, are collagen, fibrin, alginate, silk, and chitosan. These biopolymers have been investigated as bioactive scaffolds for bone engineering such as alginate, agarose, fibrin, hyaluronic acid (HA), collagen, gelatin, chitosan, chondroitin sulphate, and cellulose [5, 6]. Regarding synthetic polymers, they are often considered to be used for proteins and growth factors delivery with or without cells locally to enhance tissue repair and regeneration such as Poly(ethylene glycol) (PEG), Poly(lactic-co-glycolic acid) (PLGA), Poly-L-lactic acid (PLLA), Polycaprolactone (PCL), Poly(N-isopropylacrylamide) (PolyNiPAAm), and polycarbonate [7, 8]. At this point, it is important to clear up that both natural and synthetic materials can be considered biomolecules, since that they can be equally used to medical and surgical purposes.

In the following section, promising trends in the development of biomolecules for cartilage and bone repair are described.

8.2 Biomolecules

Bone and cartilage are two different tissues with specific structural and mechanical properties belonging to osteochondral interface [9].

The biodegradable scaffolds, with or without cells and/or growth factors, have been widely used for cartilage and bone repair. Cartilage tissue shows single conjunction of nonlinear tensile and compressive properties due to arranged collagen fibrils,

proteoglycans, and proteins. The structural and mechanical properties of natural tissues can be mimicked by engineered bio-nanocomposites through the use of polymers and nanoparticles [10].

It is important to highlight that classical scaffolds cannot be together for both chondrogenesis and osteogenesis. Scaffolds with two layers have more potential, being organized by different space structures and mechanical forces. One layer is responsible by the repair of the cartilage and the other layer supports the regeneration of the subchondral bone [11].

8.2.1 Natural materials

Nature has been using natural polymers long before the creation of plastics and other synthetic materials. For that reason, the natural biomolecules development is not truly a scientific area. Nonetheless this not turns it less important, in fact the use of this products is currently resurging in the biomedical field. Natural polymers remain attractive compared with synthetic polymers since they can avoid waste-disposal problems that are associated with traditional synthetic polymers. Moreover, these polymers present more advantages related to the possibility of several chemical modifications, potential to be degradable and biocompatible due to their natural origin [12].

Over the past few years, this area have gained special attention and a large number of biomolecules have been reported as a great option to apply on osteochondral defects repair and regeneration [13-20].

Silk fibroin

Silk fibroin (SF) is a natural polymeric biomolecule composed of two proteins: hydrophobic fibroin and hydrophilic sericin. This biopolymer shows several interesting characteristics for tissue engineering, such as mammalian cell compatibility, remarkable oxygen and water vapor permeability, biodegradability and suitable mechanical properties, with an unique combination of elasticity and strength [21]. Recently, the attention for silk biomolecules has increasing, since this biomolecule has shown to be a great option as a therapeutic material like biomimetic scaffolds for tissue engineering [22, 23].

Silk fibroin presents crystalline regions, with an amino acid sequence (**Table 1**) due to their heavy chains (350 kDa) and non-repetitive amorphous regions. Moreover, SF also presents three crystal structures denoted as silk I, silk II and silk III [24, 25].

Silk proteins, present in glands of silk, can be obtained from silkworms, spiders, scorpions, mites and bees and spun into fibers during their metamorphosis [26]. The cannibalistic nature of spiders represents a difficulty on the commercial production of spider silk. For that reason, silkworm's silk, an established fiber, has been the option for textile but also for medical research area. In fact, the yield of fiber from a single silk cocoon is 600–1500 m, compared to only about 137 m from the ampullate gland of a spider and about 12 m from the spider web [27]. Silk based biomaterials can be easily prepared from *Bombyx mori* silkworm silk. The raw silk fibers (cocoons) are degummed Na_2CO_3 solution at 100 °C and then rinsed with distilled water in order to extract the highly immunogenic sericin protein (other protein secreted by silk worm) and then air-dried. SF thus extracted is dissolved in a ternary solvent containing CaCl_2 , $\text{CH}_3\text{CH}_2\text{OH}$ and H_2O , at 70 °C for 1 h, with continuous stirring. The final regenerated SF solution is obtained following dialysis against distilled water and filtration at room temperature [28].

As refereed above, SF fibres have been extensively used in biomedical field, namely as surgical sutures, not presenting side effects for humans [24]. In fact, due to its excellent properties, SF is considered one of the best biomolecule for tissue engineering, more specifically for skeletal and osteoconal applications [26, 29]. Another add-value of SF is the fact that besides the ways it is processed (films, sponges or hydrogels), it is able to sustain cell adhesion, proliferation, even shown capable of ECF production *in vivo* and *in vitro* with a low inflammatory potential [30].

Several research studies have shown the potential of SF for osteochondral defect regeneration. In fact, silk fibroin scaffolds showed to be osteoinductive with good integration of stem cell-silk biomolecule, which is elementary in the bone or cartilage area [31].

Collagen

Collagen is the primary structural material of vertebrates and is the most abundant protein in mammalian, accounting for about 20–30% of the whole-body protein content [32]. In the human body, collagen is mostly found in fibrous tissues such as tendons, skin, ligaments and also in tissues (as cartilage, bone and intervertebral disc) and mostly of collagen in the body is of type I [33]. In fact, it is in the skin that collagen is more abundant, and about 70% of the material, besides water that are present in dermis of skin and tendon, is collagen [34]. Collagen is synthesized by fibroblasts, which usually originate from pluripotential adventitial cells or retic-

ulum cells [35]. There are different collagen types, differentiated by their complexity and diversity in their structure, their splice variants, the presence of additional, non-helical domains, their assembly and their function. Types I and V collagen fibrils are the main contribution for the structural backbone of bone while types II and XI collagens predominantly contribute to the fibrillar matrix of articular cartilage [36].

Collagen is gaining popularity not only for the easier way of fabrication since it can be processed, through chemical and biochemical modifications, into a variety of forms including cross-linked films, steps, sheets, beads, meshes, fibres, and sponges. Furthermore, it presents a unique structural, physical, chemical and immunological properties, being biodegradable, biocompatible and non-cytotoxic, supporting cellular growth [37, 38].

Collagen can be extracted from bovine skin, pig skin and chicken waste, but these kinds of sources could have religious and ethnic constraints. Taking this in account many reports have been showed to extract collagen from marine sources as alternative source and have used to screen their potential industrial applications [39, 40].

Collagen molecules are structural macromolecule of the extracellular matrix (ECM) comprising three polypeptide chains. These chains, aligned in a parallel manner and coiled in triple-helix, wrap around each other to form that is stabilized by inter-strand hydrogen bonds [38]. Collagen production is quite simple and a diversity of matrix systems, such as meshes, hydrogels, scaffolds, injectable solutions and dispersions, among others can be achieved [41]. Several studies showed that collagen-based scaffolds provide a suitable scaffold for cartilage and bone regeneration, as it supports the adhesion, migration and proliferation of cells *in vitro* [18, 42].

Chitosan

Chitosan polysaccharide is the only pseudo-natural cationic polymer and an important derivative of chitin. Chitin is a homopolymer comprised of 2-acetamido-2-deoxy-b-D-glucopyranose units [43], **Table 1**. It has application on the biomedical field and many others, due to its unique cationic character (protonation of the amino group) [44]. Extensive research has been conducted in order to improve chitosan characteristics for specific applications. This polysaccharide mostly finds its application in wound dressings, scaffolds (on physical cross-linking) and as antimicrobial agent [45, 46]. Moreover, although it has several interesting properties (such as

biodegradability and non-toxicity) [47] this biopolymer has poor mechanical properties and high swelling ability, getting easily deformed, which is generally improved by blending with other polymers [48].

Alginate

Alginate is a linear and anionic natural polysaccharide derived from brown seaweed such as *Laminaria hyperborea*, *Macrocystis pyrifera* and *Ascophyllum nodosum*. This biopolymer is composed of alternating blocks of α -1, 4-l-guluronic acid (G) and β -1,4-d-manuronic acid (M) units [49], **Table 1**. The monomer sequence can diverge depending on algal species and different tissues of the same species. The ratio between monomers and the block structure have a significant effect on the physicochemical and rheological properties of alginates. These biopolymers with more industrial significance usually show high G content [50, 51]. Although it can be produced by bacterial sources, it is commercially available from algae in form of salt, e.g. sodium alginate. This biomolecule presents excellent properties, namely their biodegradability, low toxicity and chemical versatility, but also its unique property to form stable gel in aqueous media and mild condition by addition of multivalent cations makes this biopolymer very useful for drug delivery and cell immobilization [52].

The main industrial applications of alginates are linked to their ability to crosslink with ions, retain water, gelling, viscosity booster and stabilizing properties [53].

Alginate's extraction procedure from brown algae have suffered an optimization to obtain more industrially and economically sustainable products, with controlled properties as to envisage different therapeutic applications [50, 54].

Alginate is widely used on biotechnological industry, due to its ability to efficiently bind divalent cations, leading to hydrogel formation, being also a perfect candidate for chemical functionalization. By forming alginate derivatives through chemical functionalization, the various properties such as solubility, hydrophobicity, and physicochemical and biological characteristics may be improved. Several studies reported the alginate chemical modification of hydroxyl groups through different techniques such as oxidation [55], reductive-amination of oxidized alginate [56], sulphation [57], copolymerization or cyclodextrin-linked alginate [58], and also chemical modification of carboxyl groups using other techniques as esterification [59], Ugi reaction [60] or amidation [61, 62]. Alginate has been extensively used for many biomedical applications. It could form scaffolds through the use of ionic cross-linking, allowing for encapsulation of cells.

Cellulose

Cellulose is the most widely spread natural raw material with total production of 10^{11} – 10^{12} tons/year. It is a cheap, biodegradable and renewable polymer, being fibrous and the main constituent of the cell wall of green plants. Cellulose has a versatile production, and exist in a wide range of forms and shapes, e.g. as membrane sponges, microspheres and non-woven, woven or knitted textiles [33]. Commercial sources of cellulose include mainly wood or cotton. However, cellulose can also be extracted from different parts of plants and other sources [33]. Usually, purification and isolation processes almost engender the degradation of cellulose and also permit the cellulose to undergo oxidation by reaction with both acids and bases [63].

Cellulose is one of the many beta-glucan compounds, it is a polycarbohydrate composed of a series of cellubiose units, formed by two anhydroglucose subunits, **Table 1**. This polysaccharide presents unique properties and cannot be synthesized or hydrolyzed due to its intricate hydrogen bond network. This complex is also responsible for the good mechanical properties of cellulose, important for its function in nature [63]. Purification and isolation of cellulose comprises several steps including a pulping process, partial hydrolysis, dissolution, re-precipitation, and extraction with organic solvents. Cellulose polymer allows the proliferation of chondrocytes and also have shown an interesting biocompatibility [64]. Moreover, it was found that a product based on hydroxypropylmethylcellulose hydrogel may be used for articular cartilage repair [16]. This versatile compound could also be modified for bone defects, due to its good mechanical properties.

Agarose

Agarose is a neutral linear polysaccharide extracted from red algae (*Rhodophyceae*), and is the major component of agar, being the other component agaropectin. It is composed of alternating β -d-galactopyranose and anhydro- α -l-galactopyranose. [16], **Table 1**. Agarose gel characteristics are determined by two parameters: temperature and concentration, being obtained through changing temperatures. Cross-linked alginate matrix can be formed via ionic bonding in the presence of Ca^{2+} [65]. Agarose hydrogels are at present well-characterized and have been studied in different biomedical fields. The hydrogels may be polymerized *in situ*, reducing invasiveness of the surgery and allowing the hydrogel to acquire the required shape [66]. These kind of hydrogels have been extensively studied for chondrocyte culture and cartilage tissue engineering, by its mechanical and cell-seeding density properties [67–69]. Moreover, agarose also demonstrated to be a great option for cell ther-

apy with chondrocytes or mesenchymal stem cells (chondrocyte- or MSC-laden hydrogels) in osteochondral defects. This approach ensure physiologically relevant mechanical properties and allows the formation of a repaired tissue containing collagens and proteoglycans [70].

Hyaluronic acid

Hyaluronic acid (HA) is a polysaccharide that can be found in all tissues and body fluids of vertebrates. It can also be found in some bacteria such as *Streptococcus* genus. This polysaccharide, a linear polymer composed of N-acetylglucosamine and glucuronic acid (**Table 1**), is especially abundant in loose connective tissue. HA is a polyanion with a pKa around 2.9. It is a pseudoplastic material, and in aqueous solutions it has shear-thinning properties, behaving like gelatin [71]. HA has a remarkable hydrodynamic characteristics, especially in terms of its viscosity and its ability to retain water; thus having an important role in tissue homeostasis and biomechanical integrity [72]. HA is produced through bacterial fermentation of *streptococcus* species or extracted from rooster combs, umbilical cords, synovial fluids, skin or vitreous humour for commercial purposes. The usual sources for its industrial production are bovine vitreous humour, bovine synovial liquid, and rooster crest, with an increasing interest to bacterial cultivations [73].

Although the HA extraction protocols have been developed and optimized over the years, but these protocols are still limited to low yields. The HA products are most from animal origins, which have several risks of proteins and viruses' contaminations. But the risks can be reduced if there is a special care in choosing tissues from healthy animals [74].

Alternatively, HA biomolecule could be functionalized in order to obtain a more rigid and stable, hydrophobic, and less susceptible by enzyme material. These functionalizations include sulphation, carbodiimide-mediated modification, esterification, hydrazide modification, cross-linking with polyfunctional epoxides, divinyl sulphone and glutaraldehyde, among others [71].

HA presents several advantages namely for adhesion of cells, extracellular matrix deposition, transport of gases and nutrients, metabolic product release. It offers a good interface of material-cell function mainly due to the HA-3D structure with a significant porosity, surface and space area. This polymer has been reported as an important role in joint lubrication, nutrition and preserving cartilage properties since it can help in the control of water balance [71, 75, 76]. All these evidences turn HA a good option for articular cartilage, besides the fact that it is also able to maintain

normal growth of cartilage cells and promote the integration of transplanted chondrocytes and damaged cartilage [77, 78].

Gellan Gum

Gellan gum, a bacterial polysaccharide, is a linear and anionic exopolysaccharide, with the repeating unit consisting of α -l-rhamnose, β -d-glucose, and β -d-glucuronate, in molar ratios of 1:2:1 (**Table 1**). Gellan is produced by the bacteria *Sphingomonas elodea* and the process efficiency is dependent on many factors such as media composition, pH, temperature, agitation rate and available oxygen. Native form of gellan contains two types of acyl substituents, namely l-glyceryl and acetyl. Usually, alkaline hydrolysis is used to remove both of the residues and gives deacetylated gellan, also called low-acetyl or low-acyl [79].

Native as low-acyl gellan form hydrogels in the presence of mono-, di- and trivalent cations [80]. With native gellan, it is possible to obtain a soft, easily deformable gels; while with deacetylated one, it results to rigid and brittle gels. The production of gellan gum is temperature dependent, at least 70 °C is needed, with subsequent cooling (to room temperature) to change the conformation of polymer chains [80-82]. Commonly, gellan gum is used as agar substitute since it can be clarified by filtration of hot deacetylated gellan gum [79]. In fact, due to the advantages of GG, and it has received both US FDA and EU approval, being widely used in many food, cosmetics, pharmacy and medicine applications [80, 83]. It has also been applied in tissue engineering, mostly as a material for cartilage reconstruction [80, 83]. Moreover, some researchers have been modified gellan gum with methacrilated groups to improve its physical and mechanical properties, without affecting its biocompatibility, being relevant in a widespread of tissue engineering applications [84]. Moreover, gellan gum gel also showed to be a promising material for cartilage tissue engineering [85].

Chondroitin sulfate

Chondroitin sulfate, member of the glycosaminoglycan family, is a complex sulfated polysaccharide containing repetitive units of glucuronic acid and galactosamine extensively distributed in human, other mammals and invertebrates, as well as some bacteria [86, 87] (**Table 1**). It is negatively charged and responsible for the water retention of the cartilage, which is essential for pressure resistance [88].

Moreover, chondroitin sulfate has a molecular mass of 20–80 kDa [89] depending with source, being always heterogeneous with respect to size. The sources for extraction can be bovine, chicken and porcine and also marine species such as bony fishes, whale, shark, squid and salmon [87, 90, 91]. This polysaccharide is largely used as a biomacromolecule in the treatment of osteoarthritis via oral administration and alone, or in combination with other active ingredients [91]. The isolation process of from cartilage has been defined for many years and generally include four steps: (1) chemical hydrolysis of cartilage; (2) breakdown of proteoglycan core; (3) elimination of proteins and recovery; (4) purification [87]. Recently, chondroitin sulfate was shown to enhance resistance to apoptosis in vascular cells [92].

8.2.2 Synthetic materials

Synthetic polymers are very attractive candidates as their material properties are typically more flexible than those of natural materials, being also possible to control the mechanical and chemical properties of synthetic polymers. Since it is possible to have a superior control on the production, synthetic materials can be non-toxic, available, inexpensive to produce and can be compatible with cells. For these reasons, synthetic polymers are being used for tissue engineering and regenerative medicine, however they do not have the inherent qualities that can promote desirable cell responses. The most common synthetic polymers are poly-lactic acid (PLA, which is present in both L and D forms), poly-glycolic acid (PGA), and their copolymer poly-lactic-co-glycolic acid (PLGA) [87, 91]. Some examples of synthetic biomolecules will be discussed in the following topics.

Polycaprolactone

Polycaprolactone (**Table 1**) is a hydrophobic and semi-crystalline polymer. Its crystallinity tends to decrease with increasing molecular weight. Extensive research on its application in the biomedical field has been performed due to its amazing properties, for instance good solubility, low melting point and exceptional blend-compatibility. This polymer can be prepared by either ring-opening polymerization of ϵ -caprolactone using a variety of anionic, cationic and co-ordination catalysts or also via free radical ring-opening polymerization of 2-methylene-1-3-dioxepane. Polycaprolactone has many advantages over other polymers such as tailorable degradation kinetics and mechanical properties (ease molding and manufacturing enabling suitable pore sizes conducive to tissue in-growth, and controlled delivery of

drugs). Furthermore, functional groups can be added to turn the polymer more hydrophilic, adhesive, or biocompatible that enabled favorable cell responses [91, 92]. Recent research demonstrated that the PCL-HA scaffolds loaded with bone marrow cells improved chondrogenesis and implantation of these scaffolds for osteochondral repair enhanced integration with host bone [93].

Poly(lactic-co-glycolic acid)

Poly(lactic-co-glycolic acid) (**Table 1**) is a copolymer of poly lactic acid and poly glycolic acid. This compound contains an asymmetric α -carbon which is typically described as the D or L form in classical stereochemical terms and sometimes as R and S form, respectively. The enantiomeric forms of the polymer poly lactic acid are poly D-lactic acid and poly L-lactic acid. This polymer is the best-defined material available for drug delivery regarding to shape and performance. In fact, poly(lactic-co-glycolic acid) can have any shape and size, and can encapsulate molecules of any size. It is soluble in wide range of common solvents including chlorinated solvents, tetrahydrofuran, acetone, ethyl acetate and water (by hydrolysis of its ester linkages). Due to the hydrolysis of this polymer, parameters of a solid formulation can change, such as the glass transition temperature, molecular weight and moisture content. The change in poly(lactic-co-glycolic acid) properties during polymer biodegradation influences the release and degradation rates of incorporated drug molecules. Mechanical strength of the poly(lactic-co-glycolic acid) is related to physical properties such as molecular weight, polydispersity index that can be affect the ability of drug delivery device and may control the device degradation rate and hydrolysis. Mechanical strength, swelling behavior, capacity to suffer hydrolysis and biodegradation rate of the polymer are influenced by the degree of crystallinity of poly(lactic-co-glycolic acid) [87, 94]. Several researches showed the use of this biomolecules to treat osteochondral defects [95, 96].

Poly(ethylene glycol)

Poly(ethylene glycol) (**Table 1**) is a linear or branched neutral polyether that has molecular weights less than 1000. This biomolecule is viscous and soluble in water and also in most organic solvents. The melting point of the solid is proportional to molecular weight, approaching 67 °C. Poly(ethylene glycol) is normally prepared by an anionic initiation process with few chain-transfer and terminal steps. This polymer has much interest in biomedical community because is nontoxic, soluble,

high mobile, and does not harm active proteins or cells. This biomolecule is weakly immunogenic and FDA approved for internal consumption. Furthermore, it interacts with cells membranes and partitioning controlled by making derivatives. If covalently linked, poly(ethylene glycol) will solubilize other molecules, render proteins nonimmunogenic, and toleragenic, change electroosmotic flow, change render surfaces protein-rejecting, move molecules across cell membranes and change pharmacokinetics [97, 98]. Hui and co-workers showed that the rehydrated freeze-dried oligo[poly(ethylene glycol)fumarate] hydrogel can enhance formation of hyaline-fibrocartilaginous mixed repair tissue of osteochondral defects in a small model [99].

Poly(N-isopropylacrylamide)

Poly(N-isopropylacrylamide) (**Table 1**) is a biocompatible and stimuli-responsive polymer with potential pharmaceutical applications, including controlled drug delivery, artificial muscles, cell adhesion mediators and precipitation of proteins. This polymer can be synthesized from N-isopropylacrylamide, which is commercially available and can be polymerized through different methods. This polymer, water-soluble at room temperature, is able to suffer transition above 32 °C (the low critical solution temperature, LCST). This temperature of such thermally sensitive poly(N-isopropylacrylamide) can be adjusted to a desired temperature range by copolymerization with a more hydrophilic comonomer, which raises the LCST, or a more hydrophobic comonomer, which lowers the LCST. When temperature is increased above its LCST (hydrophobic) the hydrophobic isopropyl groups are exposed to the water interface and so insoluble, suffering a collapse into gel form. Therefore researches reported the modifications of poly(N-isopropylacrylamide) which showed a great interest to tailor the LCST of poly(N-isopropylacrylamide) systems for drug delivery [100, 101]. Moreover, it has been shown that hydrogel obtained by covalently grafting poly(N-isopropylacrylamide) to hyaluronan is biocompatible and does not interfere with the intrinsic healing response of osteochondral defects in a rabbit model [102].

8.3 Final Remarks

The biomolecules discussed in this chapter covered some of the most current materials that can be used in osteochondral defects treatment. The biomolecule production procedures, their chemical structures, and also their modifications as well as their applications on bone and cartilage, was described for each one of them, as well

as its characteristic favorable and the unfavorable properties. To overcome these unfavorable characteristics, several methodologies have been employed and are under research nowadays. In fact, physical and chemical modifications could be performed to overcome these problems.

The field of cartilage tissue engineering has developed novel biological solutions; there is still a paucity of clinical options for treatment. Although the field has concentrated on finding therapies for local lesions, it has now developed sufficiently to begin considering the challenge of finding novel solutions for the extensive joint damage osteochondral defects. In fact, the development of novel biomolecules to apply on osteochondral defects is an extremely active and challenging area of research, due to the complexity of treating two different tissues. The best biomolecules should possess several properties and characteristics, which actively participate both on cartilage and bone repair and regeneration.

Table 1. Structures of the main biomolecules used in osteochondral tissue engineering.

OSTEOCHONDRAL DEFECTS	
Natural biomolecules	Synthetic biomolecules
<p>Collagen</p>	<p>Polycaprolactone</p>
<p>Silk fibroin</p>	<p>Poly(lactic-co-glycolic acid).</p>
<p>Chitosan</p>	<p>Poly(ethylene glycol)</p>
<p>Cellulose</p>	<p>Poly(N-isopropylacrylamide).</p>
<p>Agarose</p>
<p>Hyaluronic acid</p>
<p>High acyl gellan gum</p>
<p>Chondroitin sulfate.</p>	

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