Polymers from Renewable Resources: Perspectives in Biomedical Applications

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ABSTRACT: Polymers, particularly those susceptible to undergoing biodegradation under physiological environments, can be considered the materials of choice for biomedical applications such as tissue engineering, regenerative medicine, and controlled and targeted drug delivery. The development of these relatively new fields of biomedical research represents the driving force towards the exploitation of renewable resources for the obtainment of biobased polymeric biomaterials.

This perspective article reports on the biomedical applications of three major categories of biobased polymeric materials obtained from renewable resources, namely, polysaccharides, proteins and polyesters of natural origins. Particular emphasis is given to biobased polymers that display only minor modification of their structure, thus maintaining most of their natural connotations. The advantages and major drawbacks related to their use for biomedical purposes are critically discussed.

KEYWORDS: Biodegradable polymers, renewable resources, biomedical applications, tissue engineering

1 INTRODUCTION

Biomedical sciences comprise the research and development of materials suitable for improving the lifestyle and life expectancy of diseased people. It encompasses several disciplines and requires the interdisciplinary efforts of researchers active in different scientific fields. The three main areas constituting biomedical sciences can be summarized as follows: (i) tissue engineering and regenerative medicine aimed at repairing, substituting or improving pathological tissues, (ii) delivery of low and high molecular weight therapeutic agents such as drugs, proteins, and genes to improve the beneficial effects of conventional therapies and to decrease their side effects, (iii) development of medical devices comprising, among others, medical textiles, sutures, screws, extracorporeal devices, biosensors and diagnostic tools. The materials conventionally used in the mentioned applications, named biomaterials, are usually ceramics, metals or polymers. Metallic and ceramic materials are conventionally applied in prostheses and composites and their use is not suitable for many biomedical applications. Indeed, polymers represent a versatile platform of biomaterials whose use has been widely investigated in all the aforementioned biomedical fields. According to recent statistics, polymers comprise a higher percentage of utilization as biomaterials [1] and their exploitation is expected to increase markedly in the future. The chemical and physical versatility of polymers allows for matching the requirements needed for the envisaged biomedical applications with an appropriate choice of the dedicated material. Tissue engineering involves the *in vitro* seeding and proliferation of cells in a support (scaffold) that should degrade consistently within the time, allowing new tissue formation. Accordingly, suitable scaffolds possessing biocompatibility and mechanical properties matching those of the damaged tissue and displaying the envisaged biodegradability, can be easily molded through the proper choice of the polymeric material. In drug delivery applications, the role of polymers covers multiple aspects, from the enhancement of the physical-chemical stability of the drug to the regulation of the drug-release profile and targeting. The biocompatibility and biodegradability of polymers often combine with the presence of functional reactive groups that allow for their biofunctionalization with targeting molecules such as antibodies or peptides, enabling the development of micro-nanostructured drug vehicles that are at the basis of the new and effective therapeutic regimes commonly included under the heading of nanomedicine. Many polymers displaying a well-assessed biocompatibility

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are also major components of medical devices such as sutures, screws, pins, etc. For these types of applications the possibility to tune the biodegradation profile represents the added value of polymers [2].

Despite the wide availability of known polymers, they are decisively the proper choice for successful use in biomedical applications. However, they must not induce adverse effects and must be able to be safely removed from the host organisms. For example, a molecular mass comprised between 30,000 and 40,000 Daltons is required for the renal clearance of polymers. If the administered polymer's size is larger than this threshold, then the polymer must undergo degradation into nontoxic fragments. To this end the polymers obtained from natural resources can fulfil strict requirements that are hardly matched by polymers of petrochemical origins. Moreover, nowadays there has been an upsurge of interest in polymeric materials obtained from natural and sustainable resources to limit the depletion of fossil resources. At the present time the demand for polymeric materials is continuously increasing. However, the substitution of petroleum-based polymers with biobased materials undoubtedly represents a challenge that will not be amenable to satisfy all the needs for commodity items that are increasing with the increase of world population [3]. The indiscriminate exploitation of renewable resources such as biomasses to fully replenish the current applications of petroleum-based polymers, although noteworthy, is hardly feasible and might deplete these resources irreversibly through unsustainable processes. Renewable resources would be, however, perfectly suitable for providing sufficient materials to match the biomedical demand as the relevant market represents only a niche of the global polymer market. The exploitation of polymers obtained from renewable resources is a must for biomedical purposes since they possess unique features (not found in many polymers from fossil fuel feedstocks) that perfectly match the strict requirements of biomedical applications. Biocompatibility, biodegradability and bioactivity inherently constitute the genome of these renewable materials since they are obtained from natural resources, and their structure is similar to that of the biopolymers that constitute living organisms. Although there are not yet universally recognized standards and guidelines for evaluating the sustainability of a product [4], the environmental impact resulting from their production, use, and disposal are generally taken into account when evaluating their sustainability [5]. Accordingly, the exploitation of renewable resources for the obtainment of polymeric materials suited for biomedical applications is much more sustainable than the exploitation of petrochemical resources. Indeed, their exploitation and disposal cause minor environmental concerns since most biopolymers are wholly biocompatible and biodegradable, and are purified and processed in water with limited use of toxic agents due to their consequent application in biomedical fields. The sustainability is even more accentuated if the biopolymers are obtained from waste materials whose exploitation allows converting low-value biomass, often harmful for the environment, to high-value biomaterials. Accordingly, the exploitation of food resources for the production of biomaterials should be limited if not sustainable and ethically unfair. Indeed, the exploitation of resources that do not compete with food and food chain productions should be ethically imperative. Sustainability is also decreased if biomaterials are obtained from animal resources, due to their limited availability and to the intensive treatments required for their purification in order to limit the risks of viral contamination and immunogenicity.

Although biopolymers can be obtained from renewable and sustainable resources, and have a less negative effect on our environment compared to petroleum-based materials, they show some minor limitations in terms of performance (thermal resistance, barrier and mechanical properties) as well as associated costs. Their mean compositions and molecular weights are not easily reproducible on a large-scale production since they depend strongly on the biological variability of the biomass resources [6]. These drawbacks have been overcome in the case of biopolymers obtained from bacterial resources. Indeed, these organisms are nowadays employed as renewable and sustainable bioreactors for the exploitation of polymers that are naturally produced for their life maintenance and development, as in the case of polyhydroxyalkanoates (PHAs) and bacterial cellulose (BC). Biotechnologies and genetic engineering allow optimization of the product yield and quality of the polymeric material, thus obtaining high reproducibility.

This review will summarize the biomedical applications of three major categories of biopolymers obtained from renewable resources, namely, polysaccharides, proteins and polyesters of natural origins, pointing out the advantages and major drawbacks related to their use. It is noteworthy to stress that these biopolymers are very often modified or functionalized with bioactive molecules to impart to them structural features specifically designed for biomedical applications. Indeed, the biopolymers usually employed as biomaterials are better defined as biobased materials. The biobased materials described in this review possess only a minor modification of their native structural features, thus maintaining most of their natural connotations. Polyurethanes have always found wide applications in biomedicine and nowadays there is

an increasing interest in the development of polyurethanes from renewable resources as reported in the literature [7]. Nevertheless, their description has been intentionally omitted in this review because the claimed renewability of this material is at least questionable due to heavy contamination of the petrochemical-based components usually constituting these polymers.

2 POLYSACCHARIDES

Polysaccharides undoubtedly represent the materials of choice for applications in the biomedical field mainly due to their widely ascertained biocompatibility and high chemical versatility given by the huge number of functional groups present in their structural units. Indeed, this last feature allows for a wide plethora of post-modification reactions to provide the material with the properties required for the envisaged applications. Biocompatibility, biodegradability and physical properties of the polysaccharide-based materials could be finely tuned by simply designing suitable chemical functionalizations. This opportunity cannot be found in most polymers of petrochemical origins often due to the overall lack of functional groups in their chemical structure. The renewability of polysaccharides strengthened by their huge abundance in nature provides the material with a high degree of sustainability.

2.1 Starch and Cellulose

Starch and cellulose represent, by far, the most appealing candidates in terms of availability. Starch is found mainly as short-term energy stores in plants, and cellulose is the most abundant organic molecule on earth since it represents the main component of plant cell walls. Both polymers constituted of D-glucose residues as monomeric units but differ in the configuration of their glycosidic bond (Fig. 1).

This subtle chemical difference deeply affects the properties of the aforementioned polysaccharides, whose biomedical applications would consequently differ. Indeed, their aptitude for biodegradation is markedly determined by the enzymatic recognition of the configuration of their glycosidic bond. In humans, starch is easily degraded by the host enzymes α-amylase, while the lack of specific enzymes for cellulose undoubtedly hampers its degradation *in vivo* [8]. The different configuration of the glycosidic bond, as well as the different reciprocal spatial arrangement of the polymeric chains, strongly determines a marked difference in the chemical stability and mechanical properties of the two materials. Accordingly, the superior strength of cellulose is mainly determined by its β-linked units that allow for the arrangement of the polymeric chains in a straight fashion, supported by the occurrence of many interchain hydrogen bonds. This highly ordered structure confers on cellulose high cristallinity and inertness towards water, thus further limiting its *in vivo* biodegradability, and it also determines unique mechanical properties that allow for its use as reinforcing material in tissue engineering applications [9–12].

The modern biotechnologies allow the exploitation of the natural work done by microorganisms for the production of polymeric materials through sophisticated biorefining techniques and genetic manipulations. To this end, bacteria-derived polysaccharides

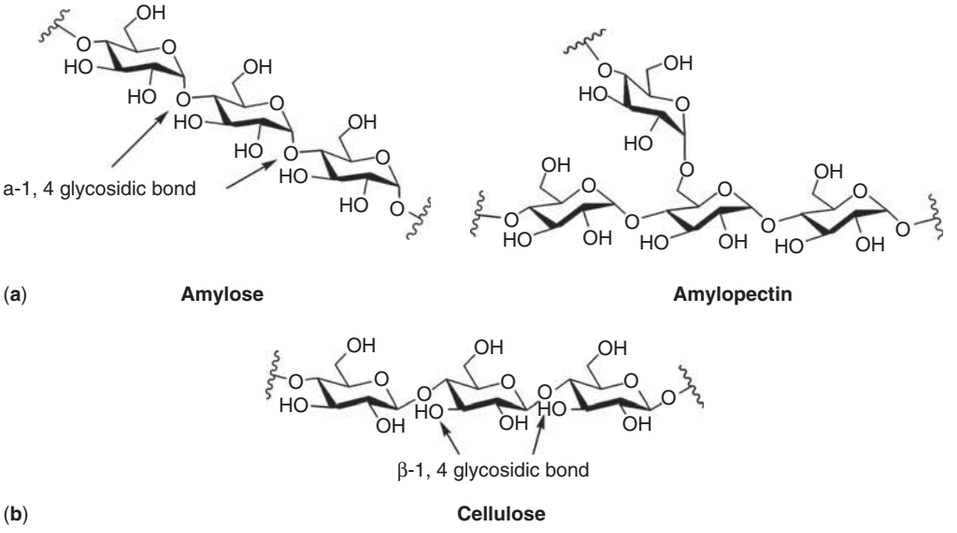

Figure 1 Chemical structure of **(a)** starch and **(b)** cellulose.

could meet the criteria required for being successfully interfaced with living host organisms. Indeed, their ease of purification and lack of biogenic contaminants typically found in polymers extracted from animal and vegetable biomasses [13], and the possibility of obtaining them without any seasonal constraint, make them amenable especially for biomedical issues. In such high-value applications, criteria regarding purity, homogeneity and quality consistency are very strict. For this purpose, cellulose obtained from bacteria represents a valuable candidate, especially as reinforcing material in composites for tissue engineering applications. Bacterial cellulose (BC) is synthesized by *Gluconacetobacter* bacterial strains such as *G. xylinus* (e.g., DSMZ 14666) in aqueous culture media during a time period of days or up to two weeks. These bacteria are found everywhere fermentation of sugars and plant carbohydrates takes place, such as on damaged fruits and flowers, and in unpasteurized or nonsterilized juice, beer, and wine, thus representing a wide renewable resource of cellulose-based biomaterials [14]. Although chemically indistinguishable from cellulose of vegetable origins, BC differs significantly from it as it is characterized by a typical nanoscale fiber network architecture. Fibril diameters are typically in the range of 30 nanometers, which are a hundred times thinner than the diameter of cellulose fibers found in common plants [14]. The main effect of the nanosized arrangement of the fibrils is an increase in surface area of the BC network, so that the structure stabilized by extensive hydrogen bonding confers high crystallinity, high water holding capacity and high tensile strength to the material [15]. BC purity is overwhelmingly higher compared to that of cellulose obtained from plants, thus resulting in its being more suitable for biomedical purposes. Indeed, it is free of functional groups (carbonyl, carboxyl), which are partially introduced in plant celluloses during their rigorous isolation and purification steps, and it is free of other polymers such as lignin, hemicelluloses, and pectin—typical components that commonly contaminate plant-derived cellulose. Since it has good biocompatibility, BC has been investigated for different biomedical applications, including wound healing [16] and engineering of various tissues, such as blood vessels [17], corneas [18], cartilage [19] and bone [20,21]. The most promising results were obtained by using BC membranes in wound dressing, and as composite materials in tissue engineering for inducing bone regeneration. Indeed, the gelatinous BC membranes (containing up to 99% of water) obtained directly from bacterial culture, other than displaying the aforementioned unique properties, mostly provided by their nanometric 3D structure, proved to be very effective as a barrier against microorganisms in wounds and burns, accelerating the healing process, providing pain relief and reducing scar formation [22–24]. Moreover, the results from the literature revealed that BC-based membranes promote effective bone formation at the site, besides being a low-cost treatment [25,26]. To this aim, BC-based nanocomposites have been developed by incorporating hydroxyapatite (Hap) nanoparticles into BC matrices in order to enhance their osteoconductivity and mechanical properties [21,27]. *In vitro* studies showed that the presence of Hap in the polymer phase favored the proliferation and differentiation of human bone marrow mesenchimal stem cells (MSCs) [21]. BC-Hap composite membranes composed of Hap nanocrystals with low crystallinity and having a Ca/P molar ratio similar to that of physiological bone, proved to be effective for bone regeneration in bone defects of rat tibiae, since the membranes accelerated new bone formation at the defect sites [28]. The addition of Hap to the bacteria culture medium during the formation of cellulose fibrils was also investigated as an alternative method for obtaining BC-Hap composites [29].

The major drawbacks related to the use of cellulose in biomedicine are given as poor processability and limited *in vivo* degradability. On the other hand, the exploitation of bacterial cellulose could partially overcome the problems related to the processing of the material since BC can be directly shaped in the culture medium. BC hydrogels adopt the dimension of the cultivation vessel, and the fiber network architecture can be controlled by modulating the experimental parameters in a very reproducible fashion [15].

In vivo degradability does not represent a limit in the case of starch-based materials, since starch can be enzymatically degraded mainly by α-amylase and phagocytozed by macrophages, and the degradation products (glucose or glucose derivatives) are reported to be safe for the host organisms [30–32]. Abundance and ascertained *in vivo* degradability represent the properties that prompt scientific attention towards the exploitation of starch for biomedical applications. Indeed, starch represents the major form of carbohydrate storage in plants and is ubiquitously found in nature. Its chemical structure consists of a mixture of linear poly(1,4-α-D-glucopyranose) (amylose) and branched poly(1,4-α-D-glucopyranose) with branches of (1,6-α-D-glucopyranose) (amylopectin) (Fig. 1b) occurring nearly every 25 glucosidic moieties. It is naturally produced in the form of semicrystalline granules of different sizes and compositions depending upon the source [33]. Amylose has a typical molecular weight of several hundred thousands, whereas the molecular weight of amylopectin is much higher and is in the order of tens of millions. Depending on the botanic origin of starch and on the relevant growth conditions, the ratio of amylase to amylopectin can vary considerably.

Disadvantages such as the relatively low tensile strength and high water absorbency of starch, and its poor solubility and processability, usually compel the use of starch in combination with additives. Plasticizers, such as water and low molecular weight alcohols, are thus often employed to enhance starch processability [33]. In addition, various starch-based materials developed by blending starch with thermoplastic polymers, showed improved thermal stability, mechanical properties and melt processability. In particular, blends of starch with ethylene vinyl alcohol (SEVA-C), cellulose acetate (SCA), poly(ε-caprolactone) (SPCL) and poly(lactic acid) (SPLA) have shown suitable properties for a wide range of biomedical applications. Indeed, the aforementioned starch-based materials are totally biodegradable, inexpensive, processable by different techniques and can be formed into diverse shapes; they have been proposed as engineered bone scaffolds, bone cements and microparticles or hydrogels for controlled drug delivery [34–37]. A number of studies by Reis and coworkers have investigated starch-based scaffolds for bone and cartilage tissue engineering, showing how their structure and functional properties can be tailored over a wide range through a proper choice of the blend component, material processing technique and possible additives or reinforcement fillers [38–43]. In addition, the incorporation of Hap in SEVA-C, SCA, and SPLC blends has been investigated for enhancing scaffold mechanical properties, as well as its bioactivity in influencing cell adhesion and behavior [41,44]. SPCL was also processed by melt spinning followed by fiber bonding to fabricate non-woven fibrous scaffolds for cartilage engineering that allowed colonization of bovine articular chondrocytes in the inner parts of the scaffold after 6 weeks of culture under dynamic conditions [45]. Starch-based products have also been successfully applied in vascular regeneration [46,47].

Starch has been extensively exploited as a carrier for drug delivery due to its biodegradability and long tradition as an excipient in drug formulations [48]. Micro- and nanoformulations based on starch alone [49–57], or blended with biodegradable polymeric materials such as poly(ε-polycaprolactone) (PCL) [58] or pectin [59], are widely reported by the literature, and the applications range from delivery of drugs such as dexamethasone [58], diclofenac sodium [49], flufenamic acid [53], doxorubicin [55] and folate [56], to growth factors [57], proteins [54] and cells [51]. Although the use of starch as material interfacing with living organisms is historically fascinating since it is popularly accepted as the safest biomaterial, and as such, is commercially appealing, some ethical and practical questions arise from its use. Indeed, the additives usually added to starch to improve its processability, mechanical properties and to decrease its hydrophilicity can compromise its safety profile. Moreover, the current global food crisis has raised serious questions about the use of agricultural land to grow crops for applications different from food purposes. Starch obtained from waste or non-edible resources could yet represent a valuable resource of biomaterials. Indeed, new and economically advantageous resources of non-edible starch, such as wasted and spoiled grain, are currently exploited for the preparation of biobased plastics [60].

2.2 Alginate and Ulvan

Algae undoubtedly represent an ideal renewable resource of biomaterials since they are very abundant and cheap, do not interfere with the food chain and are often involved in detrimental processes in the environment [61]. Indeed, their natural uncontrolled proliferation is responsible for the death of marine and aquatic organisms, and most of this huge biomass is left to decompose on the shore creating waste problems [62]. The exploitation of these aquatic organisms is still limited, but their potential as renewable and sustainable feedstock for energy and material production is gaining more and more attention. Indeed, microalgae are considered to be an excellent source for biodiesel production since they are characterized by high growth rates and high population densities, are ideal for intensive agriculture, and may contain huge lipid amounts needed for fuel production [63]. Macroalgae (seaweed) can produce a huge amount of carbohydrates per year [64] that when suitably processed through specific fermentation processes would provide renewable and sustainable biofuel. These materials are gaining particular attention due to their abundance, renewability and to their peculiar chemical composition not found in any other organisms. Moreover, they match perfectly the basic requirement of biocompatibility that materials should have to be interfaced with living organisms. Indeed, they do not require the accurate purification steps necessary for the exploitation of polysaccharides of animal origins due to their lower risks of immunogenicity and disease transmission [65].

Nowadays alginate represents the most representative polysaccharide material of algal origin investigated and used for biomedical purposes due to its biocompatibility, low toxicity and relatively low cost [66]. Commercial alginates are extracted primarily from three species of brown algae (i.e. *Laminaria hyperborea*, *Ascophyllum nodosum* and *Macrocystis pyrifera*), in which alginate comprises up to 40% of the dry weight [67]. Alginates are naturally-derived linear unbranched polysaccharides constituted of varying

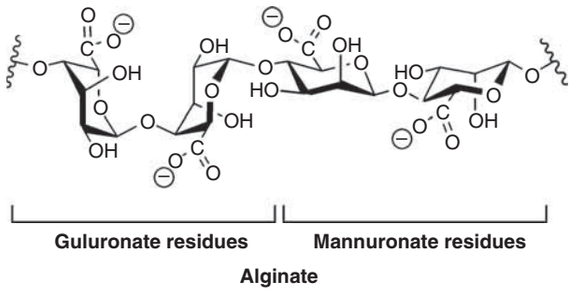

Figure 2 Representative chain portion of alginate.

amounts of (1-4)-linked β–D-mannuronic acid and α-L-guluronic acid (Fig. 2), whose composition and sequence is variable along the polymer chain.

Physical properties of alginates depend on the molecular weight, composition and extent of the sequences. The native alginates are mainly present as insoluble Ca^{2+} crosslinked gels, but they can form relatively stable hydrogels in the presence of other multivalent cations (i.e. Sr, Ba). These hydrogels are formed through ionotropic gelation given by interaction between carboxylic acid groups of the polymer and chelating cation [68]. Currently Ca^{2+} is preferred to crosslink alginate for biomedical applications because of the mild reaction conditions and the lack of cellular toxicity typical of both Ba^{2+} and Sr^{2+} [69]. Nevertheless, calcium crosslinked alginate materials suffer from a non-reproducible *in vivo* degradation and mechanical properties, due to the unpredictable dissolution rate of the individual chains in the fluids of the host organisms determined by the exchange of the binding cations with the monovalent cations present in the physiological environment. This uncontrollable disintegration presents significant limitations for the biomedical applications of this material [70]. Another major drawback related to the use of alginate in the biomedical field derives from the inherently non-degradability of this material in mammals due to the lack of specific enzymes (alginate lyases) [71]. Moreover, the average molecular weights of many commercially available alginates are higher than the renal clearance threshold of the kidneys, and likely will not be completely removed from the body [72]. An attractive approach to make alginate degradable in physiological conditions includes partial oxidation of alginate chains. Slightly oxidized alginate can degrade in aqueous media, and these materials have demonstrated potential as a delivery vehicle of drugs and cells for various applications [73].

Although the biocompatibility of alginate has been extensively evaluated *in vitro* as well as *in vivo*, there is still debate regarding its actual safety. Indeed, studies have been addressed to evaluate the impact of the alginate composition to the overall safety of the material [74,75], but the immunogenicity and inflammatory responses accidentally found during these studies were more likely attributed to impurities (heavy metals, endotoxins, proteins and polyphenolic compounds). Indeed, alginates purified according to very accurate protocols were not reported to induce any immunogenic response in animals [76,77]. Accordingly, alginate can be considered inherently biocompatible, but it requires accurate purification to be safely interfaced with living organisms.

The marked hydrophilic character of alginate mainly imparted by its carboxylic groups minimizes protein absorption, thus discouraging all the events mediated by this process such as immunogenicity and cell adhesion, therefore improving on one-side biocompatibility but also decreasing cell affinity [78]. Indeed, the chemical versatility of the carboxylic groups provides alginate with the possibility of covalently linking the oligopeptide containing the arginine-glycine-aspartic acid (RGD) sequences that are usually used in tissue engineering applications to promote cell adhesion [79,80]. This strategy, easily accomplished by straightforward chemical reactions mediated by carbodiimide-based reagents, has been widely utilized to make alginate suitable for cell interfacing applications [81–84]. Indeed, the incorporation of the RGD containing sequence into alginate proved to successfully affect the cell affinity towards this material [83, 85–88], and the amount [83] and chemical nature, such as spacer length and cyclic conformation [89] and spatial disposition [84, 90], of the RGD peptide proved to positively affect cell adhesion and spreading.

Covalent crosslinking of alginate is needed in those applications, such as scaffolding in tissue engineering, where mechanical and degradation stabilities of the materials require more reproducibility and improved performances in comparison with those typically obtained by ionic crosslinking. Although the use of low-molecular-weight crosslinkers might decrease the biocompatibility of the obtained materials unless thoroughly purified, their stiffness and mechanical stability results were highly improved and the biodegradation more easily controlled.

Alginate gels have been widely explored over the past several decades as vehicles for delivering proteins or cell populations that can direct the regeneration or engineering of various tissues and organs in the body. The various applications depended upon the gelling approach that ultimately defined the physical and biodegradation properties of the final materials. Indeed, the release of most proteins and cells would need the degradation of the supporting gel due to the unsuitable pore diameters of the original matrix [91,92].

Alginate gels have been successfully exploited in tissue engineering to induce neovascularization by the delivery of angiogenic molecules such as recombinant proteins or genes, or by transplantation of cells. A successful strategy was obtained by the sequential release of various growth factors involved in the early and late stages of angiogenesis in order to promote the formation of new vessels. Indeed, sequential delivery of the vascular endothelial growth factor (VEGF) followed by platelet-derived growth factor-BB (PDGF-BB) using alginate gels resulted in enhanced blood vessel formation, maturation and function when injected into ischemic hindlimbs of mice [93] and sites of myocardial infarction [94]. In general, VEGF plays an important role in initiating angiogenesis and forming new capillaries, while PDGF promotes the maturation of the resulting capillaries into larger functional vessels.

Transplantation of endothelial cells for the formation of new blood vessels was reported to be not effective in clinical trials unless integrated with the sustained release of specific growth factors such as VEGF and monocyte chemotactic protein-1 (MCP-1) [95,96].

The exploitation of alginate gels in bone regeneration has been investigated mainly due to the inherent advantages linked to their ability to be introduced into the body in a minimally invasive manner, and to fill irregularly shaped defects through physically induced gelling, combined with the ease of chemical modification with adhesion ligands (e.g., RGD) and controlled release of tissue induction factors (e.g., bone morphogenetic proteins [BMP]).

Several strategies have been successfully investigated using alginate hydrogels for bone regeneration, such as the delivery of suitable growth factors (BMP) either in combinations [97,98] or sequentially [99] for cell transplantation, especially using RGD-containing matrices [100, 101] and hybrid materials containing inorganic calcium compounds, such as calcium hydroxyapatites, to enhance bone tissue formation [102,103]. However, the low stiffness and inherent *in vivo* dissolution of alginate gels are representative of the severe limitations that might hamper their use for bone regeneration.

Considering the soft nature of alginate hydrogels, soft tissue engineering represents a more suitable field of application. To this end, alginate gels are also being actively investigated for their ability to mediate the regeneration and engineering of a variety of soft tissues and organs, including skeletal muscles, nerves, pancreas, and liver. As seen for vascular and bone regeneration, the two major strategies currently followed to induce soft tissue formations rely on the loading and release of specific growth factors [104, 105] and cell transplantation [106–110]. Alginate hydrogels had particularly promising results in the encapsulation of hepatocytes for the development of a bioartificial liver [106–109], and in the encapsulation and transplantation of encapsulated pancreatic islet allografts and xenografts for the treatment of diabetes type I in animal models [110,111].

Due to the historical role of alginate in medicine as a component in many pharmaceutical formulations as a thickening and stabilizing agent [112], its subsequent involvement in drug delivery applications naturally resulted. Indeed, many types of alginate hydrogels in the form of micro- and nanoformulations variously crosslinked by physical [113,114] and covalent [115] mechanisms have been investigated to impart sustained release of different drugs. To modulate the kinetics of drug release, alginate has been combined covalently with hydrophobic polymeric component (e.g., poly ε-caprolactone) [113], or by electrostatic interactions with cationic polyelectrolytes (chitosan) [116–118], or by covalent conjugation of the drug onto the polymeric matrix [73].

Alginate-based products, commercially available on the biomedical market, are mostly related to their use in wound dressing applications. Examples are Nu-Derm® commercialized by Johnson & Johnson in the USA, Curasorb® by Kendall or AlgiSite® by Smith & Nephew in the USA. Their main advantage is related to their role in keeping a moist environment around the wound, thus facilitating wound healing [119]. The improved healing properties are mostly related to the double functions of the dried calcium crosslinked alginate dressings to absorb fluid from the wound environment to re-gel and, as a swollen gel, to maintain a physiologically moist microenvironment, thus minimizing bacterial infection at the wound site [112]. Functional wound dressings containing bioactive compounds have also been investigated for wound healing promotion and prevention of bacterial infections [120–123], thus further improving the aforementioned performances.

Although nowadays alginate represents the algal polysaccharide most widely investigated in the field of biomedicine, a new class of polysaccharide is gaining growing scientific attention due to its unique chemical-physical and biological properties. Indeed, a relevant percentage of the polysaccharide fraction that composes most algal biomasses is constituted by sulphated components. These unique sulphated polysaccharides, not found in any other natural resources, are reported to possess beneficial biological activity whose exploitation could bring added value to the envisaged biomedical application. Indeed, the presence and the distribution of sulphate groups in these polysaccharides are reported to play an important role in the antiviral [124], anticoagulant [125], antioxidant

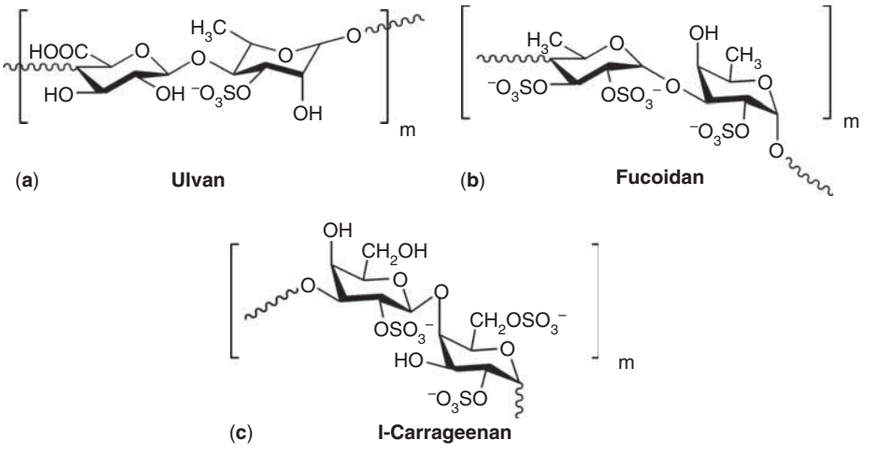

Figure 3 Representative chemical structure of three prominent sulphated polysaccharides obtained from different algal sources: **(a)** Ulvan from green algae, **(b)** Fucoidan from brown algae, **(c)** Carrageenan from red algae.

[126] and anticancer [127] activity of these materials. The chemical composition of sulphated polysaccharides, including the degree and the distribution of the sulphate groups, varies according to the species and the ecophysiological origin of the algal sources [128]. Anyhow, a structural differentiation depending on the different taxonomic classification of the algal origin has been found. According to the aforementioned classification, the major sulphated polysaccharides found in marine algae include fucoidan from brown algae, carrageenan from red algae and ulvan obtained from green algae (Fig. 3).

All the aforementioned polysaccharides are biomedically appealing since they share a common chemical structure very similar to those of extracellular glycosaminoglycan. In our research group we have been extensively investigating the sulphated polysaccharide Ulvan, a polymer that is abundant, easily obtainable from algal resources [129] and that displays numerous beneficial biological properties [130]. Ulvan is a complex sulphated polysaccharide extracted from the cell-walls of the green seaweeds belonging to *Ulvales* (*Ulva* and *Enteromorpha sp.*). These algae are the major components of the so-called Green Tides, a vast accumulation of unattached green macroalgae that are found on the shores of eutrophicated marine environments. Green tides represent a major concern worldwide from an ecological and economical point of view, thus their exploitation as a renewable resource for biomaterials is gaining increasing attention from the scientific community. Although Ulvan chains are shown to assemble in aqueous solutions into micro-domains whose organizations vary according to the different pH of the media [129], they are wholly soluble in water, and as such, not suitable for biomedical applications. To this purpose Ulvan can be gelled thermoreversibly in the presence of calcium ions and boric acid in slight basic conditions [131], but the obtained gels lack mechanical stability, especially in physiological conditions where the exchange of calcium ions with the surrounding monovalent cations unavoidably disrupt their integrity. Mechanical instability represents a major drawback commonly found in hydrogels made of polysaccharides due to their high water up-taking capability, and it is even more accentuated in the case of sulphated polysaccharides due to their enhanced hydrophilicity. Covalent crosslinking undoubtedly represents the optimum strategy for providing mechanical stability even to very hydrophilic polymeric supports by tightly joining their constituting chains. Research carried out in our group led to the preparation of stable Ulvan-based hydrogels by covalent means [132]. Covalent crosslinking was obtained through UV irradiation in the presence of a cytocompatible initiator after proper chemical functionalization of Ulvan with radical polymerizable groups. UV mediated crosslinking undoubtedly represents a smart technique perfectly suitable for biomedical purposes since it does not require the use of toxic additives and can be used easily *in situ* and in physiological conditions, thus improving the biocompatibility and reducing the costs of preparations [133]. Although ongoing research is, as of now, focused on identifying the optimum biomedical application of Ulvan whose inherent high hydrophilicity would likely limit its cell affinity, the reported preparation of Ulvan hydrogels undoubtedly represents a valuable example of sustainable process for the conversion of renewable and abundant waste material to high-added-value applications (Fig. 4).

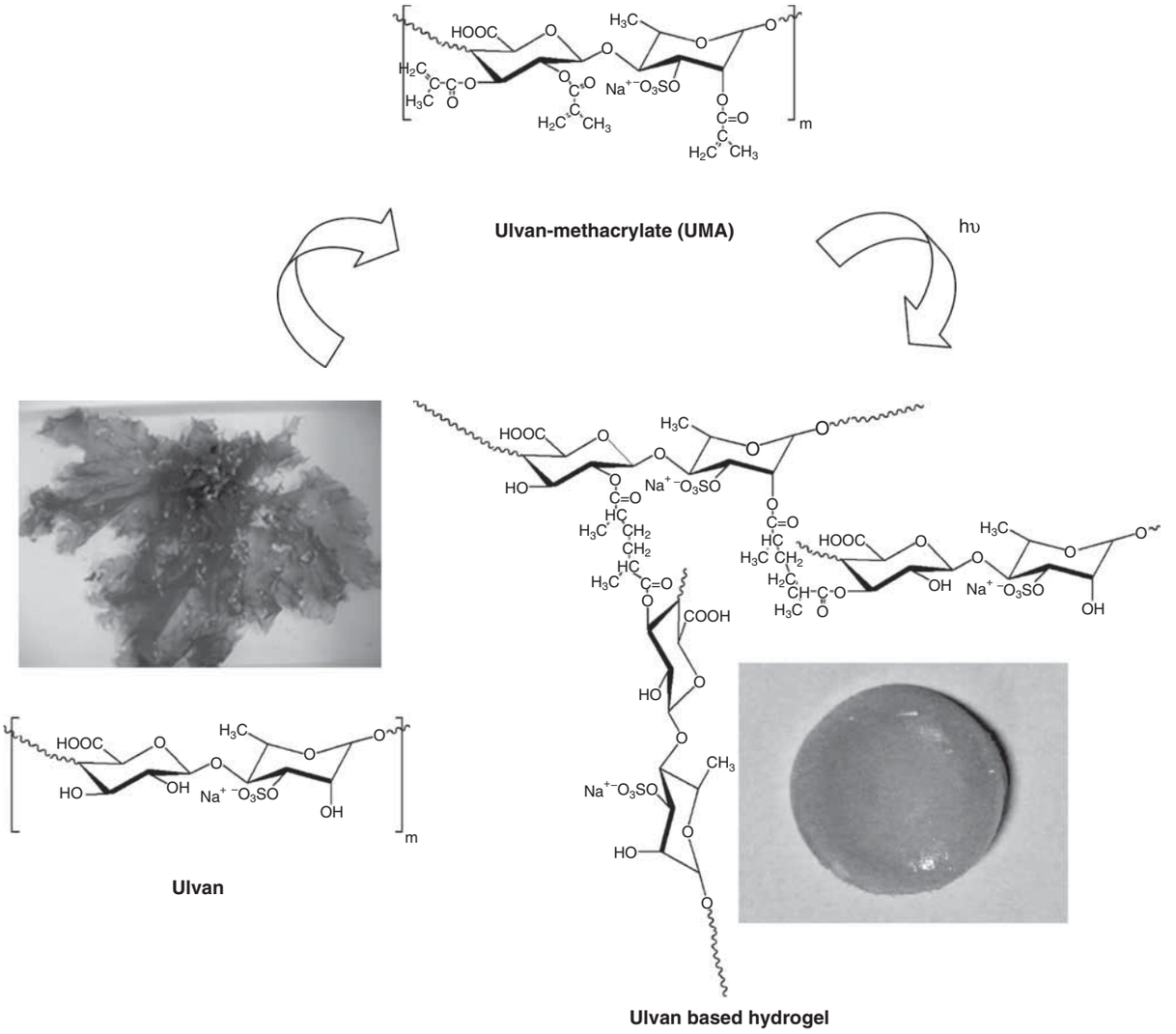

Figure 4 Scheme of the procedure employed for the obtainment of Ulvan hydrogels [132]: a sulfated cell-wall polysaccharide extracted from green seaweeds is functionalized by grafting methacryloyl moieties as a radical polymerizable group, and then photopolymerized under UV irradiation to provide suitable hydrogels.

2.3 Chitosan

When taking into account the global market of renewable materials mostly devoted to biomedical applications, chitosan is certainly one of the most important representatives. Chitosan is derived from deacetylation of chitin, a naturally occurring material that composes the exoskeleton of insects, fungi and crustaceans. The exoskeletons of crustaceans represent the most exploited resources of chitosan, since they are abundant and sustainable. When chitin is deacetylated to a certain degree (~ 60% deacetylation) it is referred to as chitosan. Chitosan is a linear copolymer of β-(1–4) linked 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glycopyranose (Fig. 5). The amine groups in chitosan are mostly found in the free form, although a 100% degree of deacetylation is not often practically achieved.

Its chemical structure makes chitosan unique among polysaccharides since it behaves as a polycationic base in solution due to the presence of many amino groups along its backbone. Almost all other renewable polysaccharides result neutral (starch) or acidic (alginate, ulvan, carrageenan, fucoidan) in solution due to the exclusive presence of hydroxyl and acid groups along their structure. The higher chemical reactivity of the amino groups usually allows chitosan to be more

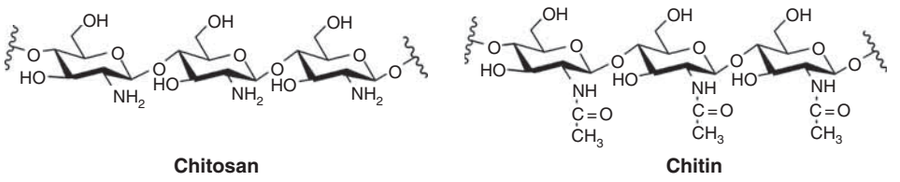

Figure 5 Representation of the major chemical structural units present in chitin and chitosan.

easily functionalized with respect to other polysaccharides, thus enhancing its versatility. The presence of amino groups also provides chitosan's antibacterial properties due to its positive charge character in aqueous solutions even at physiological conditions. A tremendous amount of literature supports the essential importance of polycationic structure in antimicrobial activity [134]. Chitosan is also widely reported to be highly biocompatible and to display low immunogenicity when interfaced with living organisms [135–139]. Moreover, it is susceptible to *in vivo* degradability since it has been shown to be degraded mainly by lysozyme (EC 3.2.1.17), which commonly exists in various human body fluids and tissues [140]. Many investigations have been reported on the degradation of chitosan by lysozyme [141–143]. All showed that the degree of deacetylation (DD) of chitosan is one of the key factors controlling its degradation [144]. Indeed, the DD is reported to affect both the chemical and physical properties of chitosan, such as reactivity, crystallinity, and viscosity, and the biological properties as well. Biocompatibility is reported to increase as the DD of the polymer increases and as the interactions between chitosan and the cells increase due to the presence of free amino groups [145].

Excellent features such as its biocompatibility, safe *in vivo* biodegradability (degradation products of chitosan are nontoxic, nonimmunogenic and noncarcinogenic), low toxicity and biological properties (antimicrobial activity and low immunogenicity), recommend this biopolymer as the perfect candidate for biomaterials applications. Indeed, a wide plethora of publications, most of which are summarized in a recent comprehensive review [146], have reported on the extensive investigations on the use of chitosan in almost every biomedical field of applications.

In virtue of its biocompatibility and biodegradability, chitosan was extensively used in developing drug delivery systems [147–152]. Different techniques have been employed for the preparation of the micro- and nanoformulations according to the needs of the envisaged applications [146]. In particular, chitosan particulate systems have been successfully prepared by emulsion crosslinking for intranasal systemic delivery of pentazocine [153], by coacervation/precipitation for gene delivery [154], by spray-drying for delivery of betamethasone disodium phosphate [155], by ionic gelation [156], by reverse micellar method for tumor targeted delivery of encapsulated dextran-doxorubicin conjugates [157] and by sieving method for the controlled release of clozapine [158].

Chitosan has been extensively investigated in tissue engineering applications as well. Chitosan was shown to be osteoconductive, enhancing bone formation both *in vitro* and *in vivo*, but its mechanical weakness and instability, together with its incapacity to maintain a predefined shape, narrow its applications [159]. Therefore, chitosan has been combined with a variety of materials, such as alginate, Hap, hyaluronic acid, calcium phosphates, poly(methylmethacrylate), and poly(L-lactic acid) (PLLA) for the development of osteogenic bone substitutes [160]. By incorporating either calcium phosphate [161] or natural coralline, the compression properties of chitosan implants were greatly improved. Hap inclusion into chitosan matrices has been shown to favor adhesion and proliferation of osteoblasts and osteoblast-like cells [162–164]. Chitosan/alginate hybrid scaffolds displayed improved mechanical strength and were shown to stimulate new bone formation and rapid vascularization during *in vivo* experiments [165]. Chitosan–Hap [166] and chitosan-PLLA/Hap [167] nanocomposites have also been proposed as tissue engineering scaffolds. A further successful strategy was developed by loading chitosan scaffolds with growth factors, such as PDGF [168] and BMP2 [169], in order to improve the ability of chitosan-based constructs to promote bone formation.

Chitosan has been widely exploited in cartilage tissue engineering mostly due to its chemical resemblance to cartilage-specific extracellular matrix (ECM) components such as type II collagen and glycosaminoglycans (GAGs). These structure similarities prompted scientists to investigate the aptitude of chitosan in mimicking these biological components in their native environment since they are known to play a critical role in regulating expression of the chondrocytic phenotype and in supporting chondrogenesis *in vitro* and *in vivo* [93]. Chitosan has been mostly employed in the form of hybrid materials in order to enhance

its physical and mechanical properties. Indeed, its use combined with alginate [170], poly (L-lactic acid) (PLLA) [171] and alginate-hyaluronan complexes [172] showed increased cell adhesion, proliferation and biosynthetic activity of seeded chondrocytes. The incorporation of specific growth factors, such as transforming growth factor (TGF)-β1, was revealed to be promising for promoting chondrocyte proliferation and matrix formation [173, 174] on chitosan supports. Chondrocyte cell encapsulation and transplantation have been successfully carried out on chitosan scaffolds as well. An injectable chitosan gel encapsulating primary chondrocytes resulted, which was able to support *in vitro* and *in vivo* accumulation of cartilage ECM [175]. Thermosensitive chitosan–pluronic hydrogels were developed as injectable cell delivery carriers, and *in vitro* experiments using bovine chondrocytes showed a substantial increase in cell proliferation and glycoaminoglycan synthesis during 28 days of cell culturing [176].

Exploitation of chitosan in soft tissue engineering is even more appealing due to the typical soft mechanical properties of the obtained hydrogels that usually match those required by the envisaged application. In particular, chitosan is selected as an appropriate scaffold material for hepatocytes culture mostly due to its chemical resemblance to GAGs, biological components of liver ECM that are very important for the maintenance of hepatocytes viability and differentiation functions [177].

Chitosan also proved to be suitable for nerve regeneration due to its biocompatibility and biodegradability. Indeed, neurons cultured on chitosan membranes were able to grow well and be active in promoting repairs of the peripheral nervous system [178]. Hybrid composites of chitosan with poly (L-lysine) [179] and gelatin [180] proved suitable for the regeneration of neural tissues as demonstrated by their beneficial cooperating effect in promoting cell adhesion and differentiation.

Chitosan-based materials, produced in varying formulations, have been used in a number of wound-healing applications. Many studies have reported on the use of chitosan as a wound-healing accelerator, and in fact, there is good evidence that chitosan can beneficially influence every separate stage of wound healing. Chitosan and its derivatives could accelerate wound healing by enhancing the functions of inflammatory cells, such as polymorphonuclear leukocytes (PMN), macrophages and fibroblasts, and increase the tensile strength of wounds [181]. Moreover, its antibacterial activity would prevent wound infections.

The numerous studies devoted to the biomedical applications of chitosan, exhaustively summarized in a dedicated review [146], confirm that chitosan would undoubtedly represent the perfect candidate to interface with living organisms. Other than its excellent biocompatibility and proven biodegradability, the possibility of obtaining chitosan from abundant and renewable waste materials also makes it suitable from an ecological and ethical point of view.

This section is intentionally dedicated to polysaccharides of plant and algal origins or obtainable from waste products, due to their abundance and ascertained biocompatibility and because their exploitation in biomedical fields does not require unsustainable processes. Indeed, many polysaccharides of animal origins cannot be included in this category since they cannot be obtained on a large scale given the limited animal tissue sources, and their recovery and purification are high cost due to the risk of viral contamination and immunogenicity.

3 PROTEINS

Proteins are important naturally occurring polymers produced by animals, plants, and bacteria [182]. Collagen, gelatin and silk fibroin represent relevant fibrous proteins that are finding an increased interest in the biomedical field due to their mechanical strength and biodegradability in physiological environments [7,33].

3.1 Collagen

Collagen is a protein found in the extra cellular matrix (ECM) of many biological tissues (skin, bone, cartilage, tendons, blood vessels, teeth) that provides structural and mechanical support. It is mainly composed of glycine (Gly) (nearly 33%) found in the polypeptide chain as every third residue forming a $(Gly\text{-}X\text{-}Y)_n$ repeating pattern, where X and Y are often proline (Pro) and hydroxyproline (Hyp). The polypeptide chain adopts a left-handed helical structure (α chain) longer than 1000 amino acids and with three residues per turn. Collagen fibrils are composed of three α chains wrapped around one another with a right-handed twist in a tightly packed triple helix, and are bonded by specific covalent crosslinks (Fig. 6) [183–185]. More than twenty-two different types of collagen have been identified so far in the human body, with the most common being Types I–IV, and Type I is the single most abundant protein present in mammals. Commercially available collagen for biomedical applications is obtained mainly from bovine or porcine skin, and bovine or equine Achilles tendons. However, these collagen-based biomaterials show mild immunogenicity, are high cost, have variable physical-chemical properties and present a risk of infectious disease transmission [186].

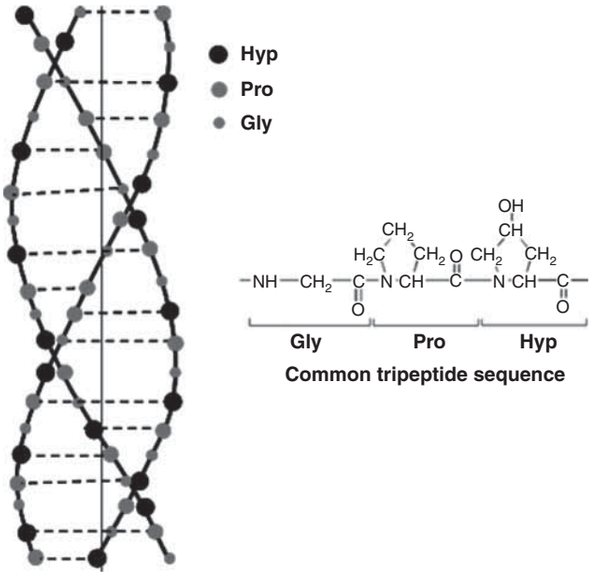

Figure 6 Representation of a triple chain structure of collagen fibrils and chemical structure of the most common tripeptide sequence found in collagen composed of glycine (Gly), proline (Pro) and hydroxyproline (Hyp) sequences.

For this reason, recombinant systems are currently under development to produce human sequence collagen [187].

Due to its biodegradability, relatively low antigenicity, cell-binding properties and tensile strength, collagen has been extensively investigated for different biomedical applications, especially in the engineering of several types of tissues (e.g., skin, blood vessels and bone) [185,188]. However, once implanted in the human body, collagen undergoes fast degradation via enzymes, such as collagenases and metalloproteinases, that leads to a rapid loss of its mechanical properties thereby compromising its employment in many applications [186,189]. Different studies proposed its combination with different materials, such as mineral crystals [190], natural polymers (e.g., elastin and glycoaminoglycans [191] or hyaluronic acid [192]) and synthetic polymers (e.g., methacrylate derivatives [193,194]) to enhance its degradation behavior. In addition, different crosslinking methods involving covalent bonding between amine side groups of different polypeptide chains have been explored [195]. However, the employed crosslinking agents (e.g., glutaraldehyde [196], 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide [197] and hexamethylene diisocyanate [198]) are not sufficiently cytocompatible [199]. Another strategy involves the formation of double bonds on collagen molecule side chains followed by crosslinking through free radical polymerization [200] or by enzymatic crosslinking [201].

A growing body of literature has investigated collagen as dermal matrices for the topical release of antibiotic and skin repair. This has led to different marketed products, such as gentamicin-loaded collagen sponges or membranes (e.g., Sulmycin®-Implant, Collatamp®-G, Septocoll [202]), and bi-layered living skin substitutes consisting of a dermis and a well-differentiated epidermis (APLIGRAF® [203–205]).

A number of studies have investigated electrospinning as a suitable technique for the development of collagen-based nanofibrous substitutes for the engineering of different tissues, such as skin and blood vessels [206–209]. Good adhesion and proliferation of human dermal fibroblasts were achieved on bilayered scaffolds coupling collagen and PCL nanofibrous matrices [210], as well as on PCL-collagen composite nanofiber meshes [211]. Powell *et al.* [212] developed electrospun collagen-based engineered skin with cellular organization similar to the clinically employed skin substitute models, achieving reduced wound contraction. An electrospun tubular scaffold made of a collagen/elastin blend mimicking the composition of natural blood vessel walls was shown to be suitable for vascular cells colonization [208]. Boland *et al*. [209] developed a collagen/elastin three-layered tissue engineered construct and seeded the outer layer with fibroblast, the medial layer with smooth muscle cells (SMCs) and the lumen with endothelial cells (ECs) to resemble the structure of native wall vessels. Collagen-coated PCL matrices by electrospinning were shown to be suitable for SMCs adhesion and migration inside the nanofibrous matrix [213], as well as for cardiomyocytes population [214]. An electrospun tubular scaffold, composed of a blend of type I collagen, elastin from ligamentum nuchae and poly(D-lactic acid) (PDLA), was seeded with ECs on the inner surface and SMCs on the outer surface showing after cell culture tissue composition and mechanical properties similar to native wall vessels [215]. Electrospun meshes of collagen type II from chicken sternae were investigated for cartilage tissue engineering, showing good adhesion and proliferation *in vitro* of human articular chondrocytes [216].

Due to the thrombogenicity of collagen and the role that it plays in the coagulation process, various collagen-based haemostats are currently on the market, including Sulzer-Spine® Tech, a sealant employed in cardiovascular and spinal surgical procedures consisting of bovine collagen and thrombin, CoStasis® Surgical Hemostat, composed of bovine microfibrillar collagen and bovine thrombin combined with autologous plasma, and Floseal®, a gel composed of collagen-derived particles and topical bovine-derived thrombin [186].

Collagen sponges have attracted interest for bone tissue engineering thanks to their ability to favor cell

attachment and growth [217, 218] and support cell differentiation into osteoblasts [219, 220]. In 1994, Wakitani *et al.* reported that mesenchymal stem cell (MSC)-seeded collagen gels implanted into rabbit osteochondral defects promoted the formation of both bone and hyaline cartilage with no evidence of tissue degeneration [221]. Bioactive glass nanofiber-collagen nanocomposites, in the form of either a thin membrane or a macroporous scaffold, were recently developed and investigated for their *in vitro* bioactivity. They showed rapid formation of bone-like apatite minerals on their surfaces when incubated in simulated body fluid, and good osteoblastic cell adhesion and proliferation [222].

3.2 Gelatin

Gelatin is a protein obtained through hydrolysis of the amide groups of collagen into carboxyl groups by applying either an alkaline process, yielding a high density of carboxyl groups, or an acid process, with limited conversion of amide groups [223]. This allows gelatin to be obtained with different isoelectric points, and is therefore either positively or negatively charged at physiological pH. For this reason crosslinked gelatin has been extensively investigated as a carrier of charged biomolecules, such as proteins and plasmid DNA, in various biomedical applications, including therapeutic angiogenesis, gene therapy, drug delivery and tissue engineering [224]. In order to make it insoluble in water and to improve its mechanical stability, different crosslinking methods have been developed showing that the crosslinking density influences its degradation by matrix metalloproteinases such as collagenase [225].

Gelatin is commonly employed for different pharmaceutical and medical applications because of its biocompatibility since the harsh acidic or basic conditions used for its preparation eliminate the antigenicity associated with collagen. Several gelatin-based biomedical products are currently on the market, such as Gelfoam®, a sponge for haemostatic applications, Gelfilm®, a film used in neurosurgery and thoracic and ocular surgery, and CultiSpher-G®, gelatine microcarriers for cell culture [226].

Thanks to its haemostatic properties, like collagen, gelatin has been investigated for the development of biological glues and topical haemostatic agents [227]. In addition, there is a large volume of published studies on gelatin as a carrier for the delivery of growth factors (e.g., basic fibroblast growth factor (bFGF), transforming growth factor-beta 1 (TGF-β1) and bone morphogenetic protein 2 (BMP-2)), either in the form of disks [228] or microparticles [229–231]. Gelatin carriers have also been studied for the encapsulation of different types of cells, including osteoblasts [232], chondrocytes [233,234], MSCs [235,236] and preadipocytes [237]. A few studies have recently reported the development of electrospun composite membranes coupling biodegradable polyesters giving mechanical stability and gelatin to promote cellular attachment and growth [238–240]. For instance, our group has recently developed novel polymeric micro-nanostructure meshes as a blood vessel substitute by combining ultra-fine fibers of a commercial elastomeric polyurethane (Tecoflex®) and gelatin by means of co-electrospinning techniques (Fig. 7) [241].

3.3 Silk Fibroin

Silks are fibrous proteins with remarkable tensile properties produced in fiber form by silkworms and spiders. Silks of different species and within a species present functional differences as a result of different structures due to a variety of primary amino acid sequences, processing and the impact of

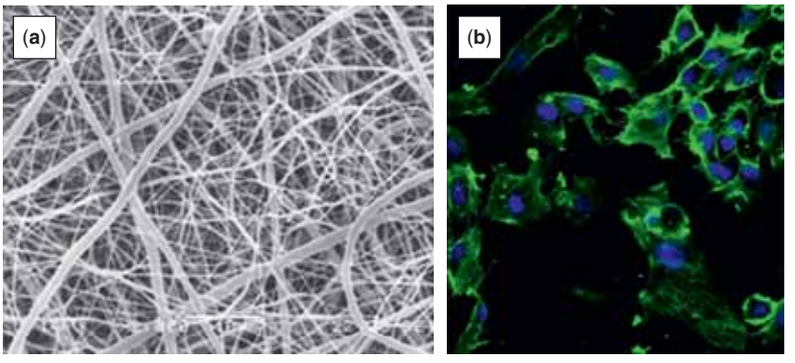

Figure 7 Tecoflex®/gelatin composite meshes [241]. **(a)** Representative scanning electron microscopy micrograph of composite mesh obtained by co-electrospinning; gelatin fibers (smaller) and Tecoflex fibers (larger) create a well-integrated network. **(b)** Confocal Laser Scanning Micrograph of ECs after 6 days of culture on composite mesh.

environmental factors [242,243]. Silk fibers from the filaments of native silkworm (*Bombyx mori*) cocoons have been successfully used as suture material for centuries [242,244,245]. They consist of a core structural protein fibroin, composed of repetitive protein sequences, coated with sericin, a family of glue-like proteins that hold fibroin fibers together [246]. Silk fibers contain at least two major structural fibroin proteins, light and heavy chains (25 and 325 kDa, respectively). Fibroin fibers consist of layers of antiparallel β sheets forming the crystalline region of polypeptide chains dominated by the hydrophobic sequence $GAGAGSGAAG[SG(AG)_2]_8Y$ [244,247].

The increased interest of the biomedical field in silk fibers is due to their slow degradability, high tensile strength and flexibility, genetically tailorable composition and sequence, and permeability to water and oxygen. Moreover, they can be processed in aqueous solutions into different forms (e.g., gel, sponge, powder, membrane and electrospun fibers), and can be easily modified because of the availability of amine and acid side chains [242]. However, their application as biomaterial requires complete removal of contaminating sericin, which can cause adverse immune reaction; but properly purified silk fibroin exhibits low immunogenicity and elicits *in vivo* foreign body response comparable to the most popular synthetic biomaterials [244,248–250].

Silk fibroin has found interest for many applications in the biomedical field, such as wound dressing [251], antithrombogenesis [252] and tissue engineering [242,244,253–260].

Sugihara *et al*. [251] tested silk films for healing full-thickness skin wounds in rats, observing that after 7 days of implantation there was faster healing and lower inflammatory response compared to traditional porcine-based wound dressings. Non-woven silk fibroin meshes produced from *Bombyx mori* were shown to support the growth of various human cell types, such as astrocyte, epithelial, fibroblast, keratinocyte and osteoblast cells [242,261,262], that colonized the fiber surface and spread across gaps in the net. In addition, it was shown that fibroin meshes support the growth and angiogenesis of human ECs [263], and microvessel-like structures were observed when meshes were cultured in combination with outgrowth ECs [264].

Silk fiber matrices have been investigated for ligament engineering [242,244], and different studies showed that silk sponges support osteogenesis or chondrogenesis of bone marrow-derived MSCs [254–257,265]. Compared to commercial poly(lactic-co-glycolic acid) (PLGA) slurry-gel, silk fibroin hydrogels showed improved *in vivo* bone remodelling and maturation when implanted in critical-size defects in trabecular bone of rabbits [260]. Moreover, an *in vivo* study showed that electrospun silk fibroin mats led to the complete healing of a calvarial defect with new bone at 12 weeks [266]. Electrospun fibroin meshes were also shown to support *in vitro* MSCs attachment, spreading and growth [267]. A recent study demonstrated that electrospinning aligned fibroin fibers could guide the morphology and orientation of human MSCs [268]. In addition, the loading of BMP-2 and Hap into electrospun fibroin scaffolds were shown to significantly enhance *in vitro* bone formation as shown by measures of mineralization and transcripts for genes involved in osteogenesis [269].

4 POLYESTERS

The possibility of exploiting renewable resources for the production of biodegradable poly(esters) for biomedical applications is nowadays considered a realistic strategy that can potentially replace their obtainment from petroleum derivates. In particular, this is true for polyhydroxyalkanoates and poly(lactic acid)-based polymers that are currently employed for different biomedical applications. In comparison to other classes of materials from renewable resources (i.e. polysaccharides and proteins), they are easily processable into desired shapes and sizes, and their physical, chemical, degradation and mechanical properties can be easily modified to meet the specific requirements of different applications [7,33].

4.1 Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHAs) are a class of aliphatic polyesters (Fig. 8) produced as intracellular carbon and energy storage compounds by many gram-positive and gram-negative bacteria under unbalanced growth conditions [245,270–273]. They comprise a large variety of homopolymers and copolymers (around 150 different types) offering a broad range of physical-chemical properties. In addition, a number of studies have shown the possibility to further vary the properties of PHAs by surface modification or by their combination with other polymers, enzymes or inorganic materials [271,274,275]. For instance, poly(3-hydroxybutyrate) (PHB) mechanical properties can be improved by the addition of plasticizer or by blending with other PHAs [272,276]. However, two of the major shortcomings of PHAs are their limited availability and the time consuming procedure for their extraction from bacterial cultures [276]. Therefore, the investigation of PHAs for biomedical applications has been mainly restricted to a few polymers, mainly PHB, copolymers of 3-hydroxybutyrate and

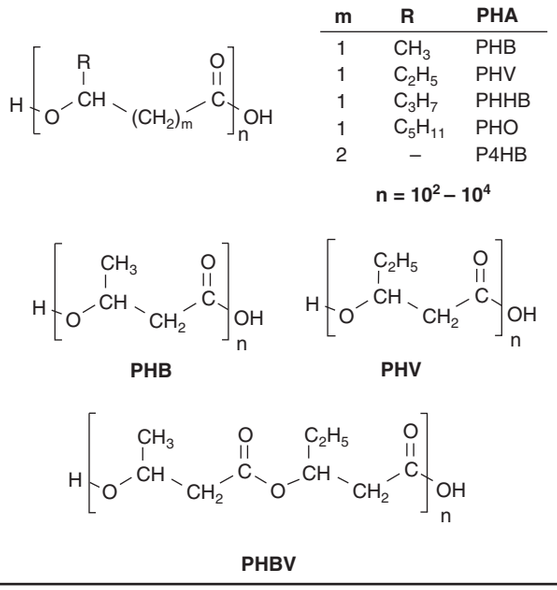

Figure 8 Chemical structure of polyhydroxyalkanoates (PHAs) commonly investigated for biomedical applications.

3-hydroxyvalerate (PHBV), poly(3-hydroxyoctanoate) (PHO) and copolymers of 3-hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx). Indeed, the operating cost of an industrial-scale extraction process might present a challenge for large-scale production of PHAs. However, the intensive research and development activity in both academic and industrial sectors on the production of PHAs from different renewable sources, and mainly wastes therefrom, is expected to overcome these shortcomings [7].

Due to their biodegradability, biocompatibility and tuneable mechanical properties, different PHAs, such as PHB and PHBV, have been largely investigated for biomedical applications. PHB has found interest as temporary stent, bone plate, patch, nails and screws [277,278], besides being widely investigated for drug release and tissue engineering applications [275,279,280]. PHB and PHBV were proposed in the form of matrix for localized delivery of antibiotics [281–283] and anticancer drugs [284]. Retinoic acid-loaded PHB nanoparticles were recently developed showing good cytocompatibility and prolonged release of the loaded agent [285]. Moreover, PHB and PHBHHX nanoparticles loaded with antineoplastic agents were conjugated with tumor-specific ligands for targeted delivery to cancer cells [286,287].

PHB biocompatibility to various cell lines, including osteoblasts, epithelial cell and ovine chondrocytes, has been reported by recent research articles [288,289]. Thanks to their piezoelectric properties, PHB and PHBV have been particularly investigated for the engineering of bone tissue. Materials based on PHB were shown to produce a consistently favorable *in vivo* bone tissue adaptation response with no evidence of an undesirable chronic inflammatory response, as well as no conclusive evidence of extensive structural breakdown after implantation periods up to 12 months [290]. Bone was rapidly formed close to the material and subsequently became highly organized, with up to 80% of the implant surface lying in direct apposition to new bone. PHBV foams implanted in defects created in rat femurs have shown regenerative potential eliciting minimal inflammation, as well as reduced fibrous tissue formation, throughout 6 weeks [291]. In addition, the incorporation of bioceramics, such as hydroxyapatite and bioactive glasses, into PHAs-matrices has been proven to enhance their mechanical strength and bioactivity performance [280,292,293]. Indeed, PHB and PHBV composite scaffolds loaded with hydroxyapatite particles exhibited compression mechanical strength of the same order of magnitude of several human bones, and *in vivo* studies showed that they can integrate well with the host tissue to promote bone growth [276,292,294].

Due to their good elasticity and mechanical strength in comparison to other PHAs, PHBHHx-based materials have been recently investigated for cartilage tissue engineering. *In vitro* studies showed improved proliferation and ECM synthesis of chondrocytes seeded on PHBHHx scaffolds in comparison with PHB scaffolds [295–297]. PHBHHx scaffolds seeded with chondrocytes and cultured *in vitro* for 10 days were implanted in rabbit articular cartilage defect, and after 16 weeks achieved successful full-thickness cartilage repair with white cartilaginous tissue showing histologically good subchondral bone connection [298]. As shown in Figure 9, a current research activity of our group is focused on the development of three-dimensional PHBHHx scaffolds for tissue engineering applications fabricated layer-upon-layer by means of a novel rapid prototyping technique based on the computer-controlled wet-spinning of polymeric solutions.

PHBHHx films were also shown to support the proliferation and differentiation of smooth muscle cells (SMCs) derived from rabbit aorta [299]. In addition, surface modification by silk-fibroin coating was shown to significantly improve the hydrophilicity of PHBHHx, and therefore, its ability to support the adhesion and proliferation of fibroblast and endothelial-like cells for vascular tissue engineering [300,301]. In addition, PHBHHx scaffolds implanted into the tarsal defects of rats were proven to be suitable candidates for eyelid engineering, leading to full defect repair after 8 weeks, although they elicited inflammation in the first 2 weeks [302].

A growing body of literature has investigated different PHAs for the repair of blood vessels. For instance,

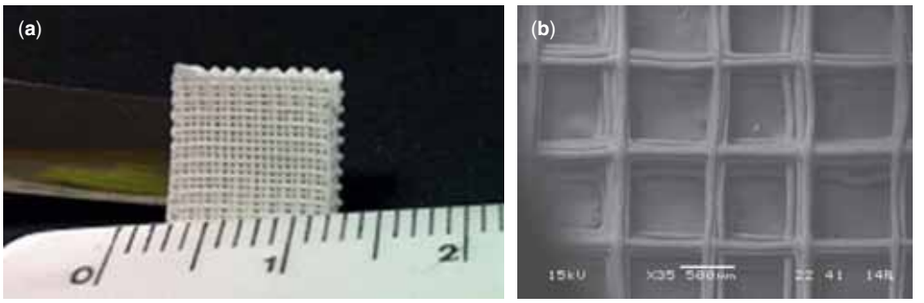

Figure 9 Picture **(a)** and scanning electron microscopy micrograph **(b)** of 3D PHBHHx microstructured scaffold by rapid prototyping.

tubular poly(4-hydroxybutyrate) (P4HB) scaffolds seeded with SMCs were dynamically cultured in a pulsatile flow bioreactor achieving confluent-layered tissue formation and mechanical properties comparable to those of native aorta [303]. After that, P4HB blood vessel scaffolds, after *in vitro* co-culture with SMCs and endothelial cells for 14 days, were implanted in the descending aorta of sheep resulting in full functionality for up to 3 months [304]. In addition, anatomically-shaped PH4B and PHO scaffolds fabricated by rapid prototyping techniques have been investigated both *in vivo* and *in vitro* for the engineering of heart valves [305–307].

4.2 Poly(lactic acid)

Poly(lactic acid) (PLA), together with poly(glycolic acid) (PGA) and their copolymers, are poly(α-hydroxy acids), a versatile class of biodegradable polyesters that have found great interest in the biomedical field due to their biocompatibility, good mechanical properties, convenient processing, and also their high and safe biodegradability since their degradation products can be resorbed through normal metabolic pathways [7]. They have been approved by the FDA for various applications, resulting in a great variety of biomedical products currently on the market, particularly in the orthopedic field, that include fixation screws, suture anchors, meniscal darts, suture reinforcements, skin replacement materials and duramater substitutes [186,308,309].

PLA is commonly synthesized on an industrial scale, with an efficient process in terms of yield and energy, by ring-opening polymerization of dilactide, the dimerization product of lactic acid. Considering that lactide is generally obtained by carbohydrates fermentation and that the macromolecule in turn degrades down easily back to lactic acid, PLA complies with the concept of renewable and sustainable development and is classified as an environmentally friendly material [310].

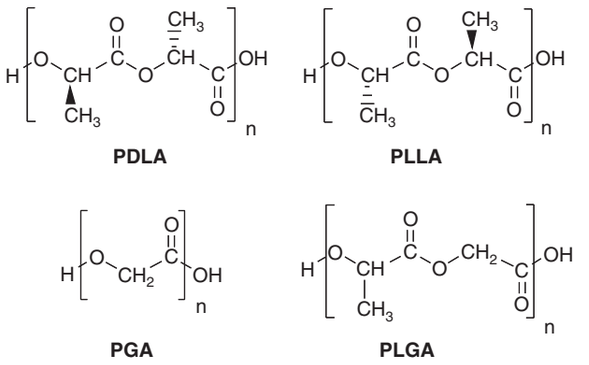

Figure 10 Chemical structure of short chain saturated aliphatic polyesters: poly(glycolic acid) (PGA); poly(lactic-co-glycolic acid) (PLGA); poly(D-lactic acid) (PDLA); poly(L-lactic acid) (PLLA).

Lactide is a chiral molecule existing in two optically active forms, D-lactide and the naturally occurring isomer L-lactide, which can be produced in bacterial systems, whereas mammalian organisms only produce the D isomer. Therefore, the stereochemical structure of related polymers can be varied by polymerizing a controlled mixture of L and/or D isomers [311]. Poly(L-lactic acid) (PLLA) is a semicrystalline polymer with good mechanical strength and toughness, while poly(D,L-lactic acid) (PDLLA) obtained from the polymerization of racemic lactide is a fully amorphous polymer (Fig 10).

Thanks to its good mechanical properties, PLLA has been widely investigated for load-bearing applications leading to a wide range of biodegradable products such as long-lasting sutures, as well as orthopedic fixation screws, suture anchors, meniscal darts and suture reinforcements [227]. PDLLA, on the contrary, displays much lower mechanical strength and thus finds application mainly in drug delivery systems [186]. PLA and the other poly(α-hydroxy acids) are degraded in the human body mainly through hydrolysis into monomeric acids and oligomers that are excreted via respiratory routes

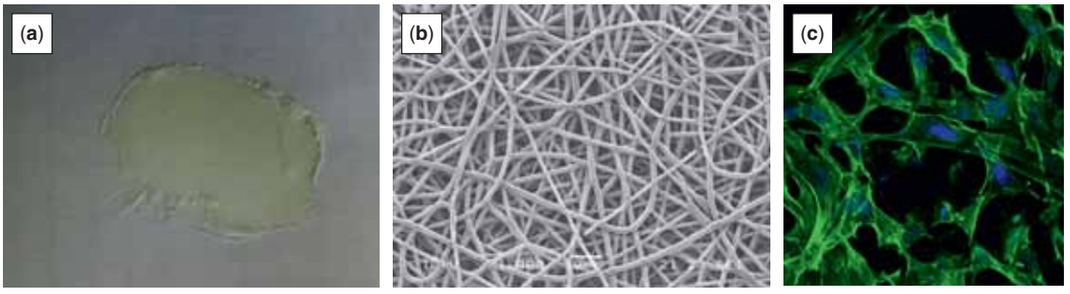

Figure 11 RA-loaded PLGA meshes by electrospinning [343]: **(a)** Picture of RA-loaded mesh; **(b)** scanning electron microscopy micrograph of RA-loaded mesh; **(c)** Confocal Laser Scanning Micrograph of MC3T3-E1 cells grown on RA-loaded PLGA mesh.

and renal filtration [312]. PLA, having a methyl-pending group in the repeating unit, is more hydrophobic than PGA (no pending group), showing, in general, a slower degradation. In addition, the hydrolysis of amorphous PDLLA is faster than that of PLLA due to the absence of crystalline domains [274]. The first degradation phase involves a nonspecific, bulk hydrolysis of ester bonds that can be catalyzed by the carboxylic acid end groups and degradation products leading in some cases to undesired effects, such as premature failure of the implant, adverse tissue reactions, denaturation of loaded agents and worsening of control over drug release kinetics [313,314]. Inclusion of bioceramics (e.g., hydroxyapatite nanopowder) into PLA matrices, besides enhancing material osteoconductivity [315,316], can represent a strategy to counteract the acidic degradation by stabilizing the pH of the environment surrounding the degrading polymer [317,318].

A number of studies have reported the employment of PLA for the development of tissue engineering scaffolds often in combination with stem cells, and different strategies have been explored to enhance its bioactivity [319–324]. For instance, Wei *et al*. [325] in a study demonstrated that *in vivo* release of BMP-7 from PLGA nanospheres immobilized onto PLLA scaffolds induced significant ectopic bone formation throughout the tissue-engineered construct. Due to PLLA's hydrophobic nature, surface modification techniques (e.g., plasma treatment) or its combination with other polymers such as collagen, chitosan or N-succinyl-chitosan were explored in order to enhance PLLA scaffolds wettability and improve cellular attachment [326]. In addition, PLLA nanofibrous structuring has been proven to significantly favor the adhesion and differentiation of different cell lines, such as MSCs [327], neural stem cells [328] and cardiomyocytes [329].

Thanks to the possibility of varying their biodegradation, mechanical and processing properties by adjusting the lactide/glycolide molar ratio or the lactide isomeric form (L- or D,L-), poly(lactic-*co*-glycolic acid) (PLGA) copolymers have been extensively investigated for their applicability in drug delivery [330-338] and tissue engineering [7,215,339-342] applications. This has led to marketed products, such as a PLGA drug delivery vehicle for prostate cancer and endometriosis (LUPRON DEPOT®), and a PLGA-collagen membrane for tissue regeneration (CYTOPLAST Resorb®) [186]. Our group has consistently investigated the possibility of incorporating therapeutic and bioactive agents into PLGA micro- and nanocarriers as reported in Figure 11 illustrating a study on retinoic acid (RA)-loaded PLGA ultra-fine fiber meshes that were able to maintain a sustained, controlled release for more than 3 months [343].

Several other copolymers starting from PLA or lactide dimer, such as PLLA-*co*-PCL [344,345], PLLA-*co*-trimethylene carbonate [346], poly(L-lactide-*co*-1,5dioxepan-2-one) [347], and PLLA-*co*-propylene glycol-*co*-PLLA [348], have been recently developed as biomaterials for tissue engineering applications. However, these materials are beyond the scope of this article since they cannot be wholly classified as renewable materials due to the heavy presence of petrochemical-origin components in their structure.

5 CONCLUSIONS

Biobased polymers, as attained from their natural counterparts often with no heavy chemical modification, represent an actual reality for biomedical applications, as witnessed by several biomedical products already present in the market. Polymers from renewable resources are characterized by important distinctive structural features that *impart susceptibility* to the biodegradability and biocompatibility attributes of the relevant items, which are of utmost significance in biomedical applications. It is clear, however, that in order to further strengthen the use of biobased polymeric materials in the biomedical field, research institutions should focus their attention on the development of biotechnological strategies aimed at increasing the materials batch-to-batch reproducibility in terms of composition and molecular mass, which

are determinant parameters in affecting the biocompatibility and biodegradability of the relevant items. Finally, another important aspect that should be taken into account when proposing biobased polymers for biomedical applications is the sustainability of the renewable resources exploited for their obtainment, which should be considered a compulsory stringent requirement.

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