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REVIEW ARTICLE

# Potential applications of natural origin polymer-based systems in soft tissue regeneration

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**Abstract**

Despite the many advances in tissue engineering approaches, scientists still face significant challenges in trying to repair and replace soft tissues. Nature-inspired routes involving the creation of polymer-based systems of natural origins constitute an interesting alternative route to produce novel materials. The interest in these materials comes from the possibility of constructing multi-component systems that can be manipulated by composition allowing one to mimic the tissue environment required for the cellular regeneration of soft tissues. For this purpose, factors such as the design, choice, and compatibility of the polymers are considered to be key factors for successful strategies in soft tissue regeneration. More recently, polysaccharide-protein based systems have being increasingly studied and proposed for the treatment of soft tissues. The characteristics, properties, and compatibility of the resulting materials investigated in the last 10 years, as well as commercially available matrices or those currently under investigation are the subject matter of this review.

**Keywords:** *Natural polymers, multicomponent systems, biotechnology, soft tissue, tissue engineering, polysaccharide-protein interactions*

## Introduction

Despite the many advances in tissue engineering (TE) approaches, scientists still face significant challenges in repairing or replacing soft tissues such as tendons, ligaments, skin, liver, nerve, and cartilage to improve the quality of life for people. The conventional therapeutic treatments targeted to reconstruct injured tissues or organs have some limitations such as donor limitations and graft rejections. Based on the principles of TE (Langer and Vacanti, 1993; Lanza et al., 2000), alternative therapeutic strategies have been developed as current treatments involving biodegradable constructs containing specific populations of living cells and growth factors. For example, skin can be regenerated using epidermal sheets, dermal replacements, and complex skin substitutes (Metcalfe and Ferguson, 2007; MacNeil, 2007; Barbul, 2001), while cartilage defects can be treated with cells seeded on three-dimensional (3D) matrices (Chung and Burdick, 2008; Temenoff and Mikos, 2000; Hutmacher, 2000). Nature-inspired routes involving the creation of polymer-based systems of natural origin (e.g. polysaccharide-protein) constitute an interesting alternative to produce novel materials that can fulfil all the necessary requirements for the success of these approaches. However, the properties of these systems will depend on the choice of the composition of the system, the intrinsic characteristics (e.g. molecular weight, charge) of each component, their degree of interaction, and their miscibility (McClements, 2006; Turgeon et al., 2003). Both polysaccharides and proteins exhibit the relevant characteristics such as their availability in nature, chemical diversity, biodegradability, and may be modified relatively easily (Damodaran, 1997; Lloyd et al., 1998; Gomes et al., 2008; Mano et al., 2007). These features

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when combined have proven to be a useful route to obtain bioengineered biomatrices with better mechanical and biological properties compared to their individual components. In this review, attention has been focused on polymer-based systems of natural origins composed mainly of chitin/chitosan, glycosaminoglycans, alginate, and cellulose combined with different proteins, which have been recently proposed for applications for soft tissue repair and regeneration.

## Natural origin polymers and their combinations

Most of the current researchers have headed towards studying the use of natural biodegradable polymers such as collagen (Gomes et al., 2008; Stark et al., 2006; Pieper et al., 2002), chitosan (Gomes et al., 2008; Kumar, 2000; Kim et al., 2008; Madihally and Matthew, 1999; Suh and Matthew, 2000), hyaluronic acid (Gomes et al., 2008; Yoo et al., 2005; Aigner et al., 1998; Kogan et al., 2007), cellulose (Gomes et al., 2008; Pulkkinen et al., 2006), starch (Malafaya et al., 2006; Gomes et al., 2008; Gomes et al., 2006; Oliveira et al., 2007; Santos et al., 2007; Silva et al., 2007a), soy protein (Gomes et al., 2008; Vaz et al., 2002; Vaz et al., 2003), gelatin (Gomes et al., 2008; Kang et al., 1999), silk fibroin (Gomes et al., 2008; Vepari and Kaplan, 2007; Altman et al., 2003; MacIntosh et al., 2008; Unger et al., 2004), and alginate (Gomes et al., 2008; Eiselt et al., 2000) separately, or combined them for TE applications (Gomes et al., 2008; Malafaya et al., 2007; Mano et al., 2007; Seal et al., 2001; Chang et al., 2003a; Funakoshi et al., 2005; Lee et al., 2005; Liao et al., 2007; Ma et al., 2003). The material choice for a particular application depends upon the type of material required, the nature of the tissues to be regenerated, and their regeneration time. Besides that, the wide variety of structures and the unique biological, chemical or physical functionalities of these polymers can be associated, allowing one to create interesting materials such as membranes, hydrogels, scaffolds, and micro/nanospheres. These natural origin polymer-based materials offer advantages such as the creation of new opportunities for mimicking the tissue microenvironment and can stimulate the appropriate physiological responses required for cellular regeneration. It seems that all these features associated with a controlled biodegradation rate and the biocompatibility of these naturally based-systems can be advantageous when compared to synthetic polymers. Figure 1 shows some devices organized according to their geometrical dimensions. The polymeric matrices can be produced using several techniques. For example, membranes can be obtained by solvent casting of polymeric solutions, while hydrogels can be processed by traditional synthesis, including cross-linking reactions and copolymerization reactions (Kopecek and Yang, 2007; Hennink and van Nostrum, 2002). However, some methods are limited with respect to the control of their resulting structures due to side reactions, unreacted pendant groups, and entanglements. Scaffolds are usually prepared by freeze-drying techniques, emulsion freeze-drying methods, salt leaching methods, rapid prototyping, fiber bonding, melt based methodologies, among many others (Gomes and Reis, 2004; Mano et al., 2007; Mikos and Temenoff, 2000; Correlo et al., 2007a; Chen et al., 2002; Lee et al., 2009). Among these, the freeze-drying technique is the most widely used method (Correlo et al., 2007a; Suh and Matthew, 2000) to produce scaffolds with different shapes, porosities, and pore size distributions by varying the parameters such as the polymer concentration, type of solvent, freezing temperature, and type of molds. Also, the electrospinning process has become a promising method for the preparation of 3D porous mats with large surface areas, and high porosity, which can mimic the extracellular matrix (ECM). Other advantages of this method are the possibility of large-scale production, easy processing, easy functionalization, and the availability of advanced modes of electrospinning (Agarwal et al., 2009; Lee et al., 2009). Recently, electrospun polyblend nanofibers have been prepared from a combination of polymers, which take advantage of the varying strengths, bioactivities, and degradation rates of all the components involved (Gunn and Zhang, 2010). Using this approach, the solubilization and electrospinning issues of some natural polymers have been overcome, allowing their production in nanofiber technology.

The selection of the design and processing techniques to create adequate scaffold architectures allows the preparation of porous structures with controlled porosity, pore size, and interconnectivity, as well as tissue matching mechanical properties (Gomes and Reis, 2004). However, these requirements will depend on the tissue to be regenerated (Lanza et al., 2000). For example, successful nerve regeneration requires tissue-engineered scaffolds that provide not only mechanical support for the growing neuritis, but also the biological signals to direct the axonal growth cone to the distal stump (Schmidt and Leach, 2003; Huang and Huang, 2006). This is particularly true for osteochondral defects, where the use of single scaffolds to regenerate cartilage may not be effective, and the employment of bilayered constructs has been proposed as an alternative solution (Mano and Reis, 2007; Malafaya et al., 2005; Oliveira et al., 2006; Gao et al., 2002). This approach consists of developing a 3D porous structure that combines a mechanical support resembling the subchondral bone, while also providing a chondrogenic support for cartilage repair.

In this review, the characteristics of the main biopolymers and their combinations are described in the following sections, and the tables (Tables 1 to 4) summarize the most frequently proposed blend systems (e.g. polysaccharide-protein) as applications for soft tissue repair. These tables also include the methods for production for blend systems, the matrix shape, the aimed TE application, the biologically

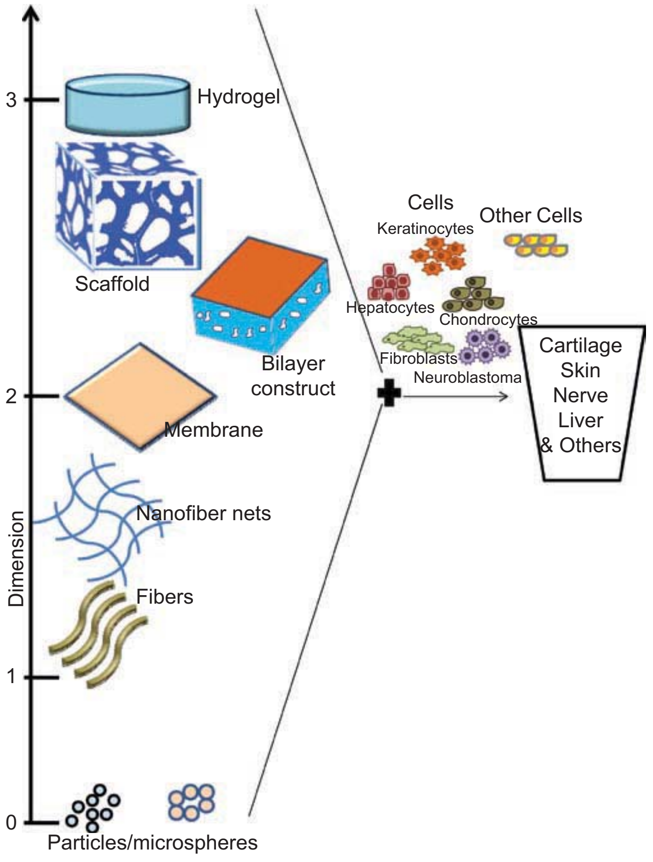

**Figure 1.** Scheme of different polymeric architectures proposed for soft tissue repair.

active biomolecule to be delivered, and the source of the cell used for the *in vitro* tests. When applicable the animal model used is also indicated.

### *Chitin/chitosan*

Chitin is the second most abundant natural polymer in nature, it is found in the shell of crustaceans, the cuticles of insects, and the cell walls of fungi (Kumar et al., 2004; Kurita, 2001; Kumar, 2000). Structurally, chitin is composed of β(1-4)-linked N-acetyl-D-glucosamine (Kumar, 2000). This polymer has bacteriostatic and fungistatic activities, which are favorable for promoting rapid dermal regeneration and for accelerating wound healing (Kumar, 2000; Peniche et al., 2008). However, the applications of chitin have been limited due to its insolubility in water and also in most of the common organic solvents (Kumar, 2000). Nevertheless, chitin is soluble in hexafluoroisopropanol, hexafluoroacetone and dimethylacetamide containing 5% lithium chloride (Kumar, 2000). Therefore, some researchers have investigated the use of some types of chitin-based materials (filaments, granules, sponges, and films) as components for wound management products (Koji et al., 1987; Fox and Allen, 1996). Despite the intrinsic properties of chitin, some studies have suggested that an improvement in the biocompatibility of chitin for use in appropriate dressings can be achieved by associating the chitin with other polysaccharides or proteins (Lee et al., 2004; Hirano et al., 2001), or by creating chitin derivatives (e.g. carboxymethyl chitin, water-soluble chitin, dibutyl chitin) (Pielka et al., 2003; Muzzarelli et al., 2005; Cho et al., 1999) using chemical modification.

Chitosan is a polysaccharide obtained by the alkaline deacetylation of chitin (Kumar, 2000; Kumar et al., 2004). This polymer has many properties such as its polyelectrolyte and cationic nature, mucoadhesion, hemostatic action, film-forming ability, biodegradability, bacteriostatic, and fungistatic activity (Kumar, 2000; Peniche et al., 2008). Besides, the presence of functional groups (hydroxyl, amine) it has been beneficial for chemical modification to introduce the desired properties into chitosan, which can be useful for specific uses in diversified fields (Kumar et al., 2004; Kim et al., 2008; Jayakumar et al., 2005; Mourya and Inamdar, 2008). Because of the stable crystalline structure, chitosan is normally insoluble in water, but soluble in dilute aqueous acidic solutions. These solutions have been used in the production of gels, membranes, microparticles, nanofibers, porous structures, and tubes, which combined with its pH sensitivity and high charge density (positive electrical charge) has allowed the development of drug delivery devices, composites, polyelectrolyte complexes, and implants for tissue engineering applications. Several chitosan-based matrices have been developed to be used as dermal substitutes, wound dressings, porous structures for cartilage repair, and other TE applications (see Table 1). Considering that chitosan promotes good protection of the wound, accelerated wound healing, and an antibacterial action (Khor and Lim, 2003; Kim et al., 2008), chitosan membranes have been proposed as simple wound coverings or as sophisticated artificial skin matrices, and hydrogels. Although the preparation, properties, and characterization of both chitosan films and bilayer membranes for use as wound dressings have been extensively investigated, (Aoyagi et al., 2007; Khan et al., 2000; Khan and Peh, 2003; Azad et al., 2004; Marreco et al., 2004; Azevedo et al., 2006; Mizuno et al., 2003; Queiroz et al., 2003; Mi et al., 2002b; Ma et al., 2001), a few reports concerned with clinical trials have been described in the literature (Azad et al., 2004).

With the rise of nanotechnology, chitosan together with other macromolecules has been fabricated into various bionanocomposites, providing alternative applications in regenerative medicine and drug delivery vesicles (Chen et al., 2008; Zhou et al., 2008; Lee et al., 2009). For example, composite nanofibrous membranes of collagen/chitosan/polyethylene oxide blends have demonstrated unique combinations of mechanical, biological, and structural properties suitable as wound dressings for skin regeneration (Chen et al., 2008). On the other hand, some studies have described the complexation of chitosan with

**Table 1.** Chitosan-based matrices proposed for applications in soft tissue repair. Targeted tissues and organs include cartilage, skin, liver, nerve, tendons, and ligaments.

| Composition | Processing Methodology | Matrix type | Active substance | Potential TE application | Cell type (source/line) | Animal model | Reference |
|---|---|---|---|---|---|---|---|
| Chitosan-alginate | Solvent casting | Membranes | – | Wound dressing | Fibroblast like cells | Sprague Dawley rats | (Wang et al., 2002) |
| | Interpolyelectrolyte complex method | Macroporous membranes | AgSD | Wound dressing | – | – | (Yu et al., 2005a ) |
| | Freeze-drying | Scaffolds | – | Cartilage | Chondrocyte like cells | – | (Li and Zhang, 2005) |
| | Coagulation bathspinning | Fibers | – | Cartilage | Chondrocytes (rabbit) | – | (Iwasaki et al., 2004) |
| | Freeze-drying | Scaffolds | – | Cartilage | ATDC5 cell line | | (Tigli and Gumusderelioglu, 2009) |
| | Wet spinning | Fibers | – | Tendons and ligaments | Fibroblast(rabbit) | – | (Majima et al., 2005) |
| | Spinning mandrel technology | Hydrogels | NGF | Nerve conduit | – | – | (Pfister et al., 2008) |
| Chitosan-alginate-fucoidan | – | Hydrogels | Mitomycin C | Wound dressing | Human dermal fibroblast/dermal micro-vascular endothelial | Sprague Dawley rats | (Murakami et al., 2010) |
| Galactosylated chitosan-alginate | Freeze-drying<br>Cross-linking ($CaCl_2$) | Sponges | – | Liver | Hepatocytes(mouse) | – | (Yang et al., 2001: Chung et al., 2002) |
| Galactosylated chitosan-alginate | Freeze-drying<br>Cross-linking($CaCl_2$) | Scaffolds | – | Bioartificial liver | Co-culture of hepatocytes (mice)<br>NIH 3T3 Fibroblasts | – | (Seo et al., 2006c) |
| Galactosylated chitosan-alginate-heparin | Freeze-drying<br>Cross-linking ($CaCl_2$) | Scaffolds | – | Bioartificial liver | Hepatocytes(mice)<br>NIH 3T3 Fibroblasts | – | (Seo et al., 2006a) |
| Chitosan-alginate-hyaluronan | Solvent casting<br>Freeze-drying | Membranes and scaffolds | – | Cartilage | Chondrocytes | – | (Hsu et al., 2004) |
| Chitosan-bacterial cellulose | Biosynthesis | Hydrogels | – | Wound dressing | – | – | (Ciechanska, 2004a) |
| Chitosan-chondroitin-6-sulfate-dermatan | Freeze-drying<br>Cross-linking | Scaffolds | – | Cartilage | Chondrocytes (Wistar rats) | – | (Chen et al., 2007b) |
| Chitosan-collagen | Freeze-gelation<br>Cross-linking (EDC) | Scaffolds | – | Skin | Embryonic Fibroblasts (human) | – | (Tsai et al., 2007) |
| | Quenching ethanol extraction | Porous membranes | – | Dermal regeneration template | – | – | Gao et al., 2003b) |
| | Freeze-drying DHT<br>Cross-linking (GA) | Scaffolds | – | Dermal equivalent | Human fibroblast | Rabbitear | (Ma et al., 2003a) |
| | Solvent casting | Films | HN-300 | Antimicrobial wound dressing | Fibroblast-like cells (mouse) | – | (Guan et al., 2007a) |
| | Solvent casting<br>Cross-linking (EDC) | Films | – | Artificial livers | Hepatocytes (rats) | – | (Wang et al., 2003) |

**Table 1.** continued on next page

**Table 1.** Continued.

| Composition | Processing Methodology | Matrix type | Active substance | Potential TE application | Cell type (source/line) | Animal model | Reference |
|---|---|---|---|---|---|---|---|
|  | Freeze-drying<br>Ammonia treatment | Scaffolds | _ | Liver | Hepatocytes (rats) | _ | (Wang et al., 2005) |
|  | Freeze-drying Steam-extrusion Cross-linking (GA) | Scaffolds | _ | Nerve regeneration | Retina cells | _ | (Wang et al., 2009b) |
| Carboxymethyl chitosan-collagen | Freeze-drying<br>Cross-linking (EDC) | Porous matrices | _ | Wound dressing | Fibroblasts (human) | Wistar rats | (Chen et al., 2006b) |
| Chitosan-collagen-chondroitin sulfate | EmulsionFreeze-drying<br>Cross-linking (EDC) | Microspheres encapsulated in scaffolds | TGF-β1 | Cartilage | Chondrocytes (rabbits ) | _ | (Lee et al., 2004a) |
|  | Freeze-drying<br>Cross linking (EDC) | Scaffolds | - | Cartilage | Chondrocytes (rabbits) | _ | (Lee et al., 2005) |
|  | Freeze-drying<br>Cross-linking (EDC) | Scaffolds | _ | Cartilage | Chondrocytes (rabbits) | Athymic male mice | (Yan et al., 2007a) |
|  | Freeze-drying | Scaffolds | _ | Dermal substitute | foreskin and adult dermal fibroblast | Rats | (Kellouche et al., 2007) |
| Chitosan-collagen-chondroitin sulfate | Freeze-drying | Sponges | - | Skin equivalent | Co-cultures of keratinocytes, fibroblasts, and HUVEC (human) | _ | (Black et al., 1998) |
| Chitosan-collagen-heparin | Freeze-drying<br>Solvent casting<br>Ammonia treatment | Scaffolds<br>Membranes | _ | Liver | Hepatocytes(rats) | _ | (Wang et al., 2005) |
|  | Freeze-drying<br>Cross-linking (EDC) | Scaffolds | _ | Liver | Hepatocytes (rats) | _ | (Yu et al., 2005b) |
| Chitosan-collagen-hyaluronan | Freeze-drying<br>Cross-linking (EDC) | Scaffolds | _ | Cartilage | Chondrocytes (rabbits) | _ | (Yan et al., 2006) |
| Chitosan-collagen-PEO | Electro spinning<br>Cross-linking (GA vapour) | Nanofibres membranes | _ | Wound dressing | Fibroblasts-like cells | SD rats | (Chen et al., 2007a) |
| Chitosan-collagen-silicone | Freeze-drying<br>Cross-linking (GA) | Bilayers (scaffold/membrane) | _ | Dermal equivalent | Fibroblasts (human) | _ | (Shi et al, 2005) |
|  | Freeze-drying<br>Cross-linking (GA) | Bilayers (scaffold/membrane) | _ | Dermal equivalent | Fibroblasts (human) | Pigs | (Ma et al., 2007a) |
| Chitosan-gelatin | Freeze-drying<br>Cross-linking (GA) | Scaffolds | _ | Artificial bilayer skin *in vitro* | Co-cultures of keratinocytes and fibroblasts | _ | (Mao et al., 2003) |
|  | Freeze-drying | Scaffolds | Plasmid DNA | Cartilage | Chondrocytes (rabbit) | _ | (Guo et al., 2006) |
| Chitosan-gelatin | Solvent casting<br>Cross-linking (EDC) | Films | Dexamethasone | Cartilage |  | _ | (Medrado et al., 2006b) |
|  | Freeze-drying | Scaffolds | _ | Cartilage | Autologous chondrocytes (pigs) | Pigs | (Xia et al., 2004) |
|  | Freeze-gelation | Scaffolds | _ | Cartilage | Chondrocytes (rabbit) | _ | (Wang et al., 2009a) |
|  | Solvent casting | Films | _ | Nerve regeneration | Rat PC12 cells | _ | (Cheng et al., 2003b) |

**Table 1.** continued on next page

**Table 1.** Continued.

| Composition | Processing Methodology | Matrix type | Active substance | Potential TE application | Cell type (source/line) | Animal model | Reference |
|---|---|---|---|---|---|---|---|
| | – | Gels | – | Liver | – | Rats | (Wang et al., 2007b) |
| | Freeze-drying, SFF Microreplication Cross-linking (GA) | Scaffolds | – | Liver | Hepatocytes (rats) | – | (Jiankang et al., 2007) |
| | 3D cell assembly technique Gelation Cross-linking (TPP and GA) | 3D structures | – | Bioartificial liver | Hepatocytes(rats) | | (Chen et al., 2005) |
| hitosan-gelatin | Solvent casting Cross-linking (G) | Membranes | – | Nerve repair | Fibroblast-like cells<br>Neuroblastoma-like cells | – | (Chiono et al., 2008b) |
| Chitosan-gelatin-hyaluronan | Freeze-drying Cross-linking (EDC) | Scaffolds | – | Artificial skin | Co-cultures of keratinocytes and fibroblasts | – | (Liu et al., 2007b, Liu et al., 2004b) |
| | Freeze-drying Cross-linking (EDC) | Scaffolds | bFGF | Cartilage | Chondrocytes (rabbits) | – | (Tan et al., 2007) |
| Chitosan-heparin | Solvent casting | Membranes | – | Wound healing | *In vitro* model | – | (Kratz et al., 1997a) |
| | - | Powder | – | Wound healing | - | Wistar rats | (Jin et al., 2007b) |
| | - | Powder ointment | – | Wound healing | – | Rats | (Kweon et al., 2003) |
| Chitosan-Hyaluronan | Solvent casting | Films | – | Wound dressing | Fibroblasts (human) | SD mice | (Xu et al., 2007) |
| | Wet spinning | Fibres | – | Cartilage | Chondrocytes (rabbits) | – | (Yamane et al., 2005) |
| | Wet spinning | Fiber scaffolds | – | Ligament | Fibroblasts (rabbits) | – | (Funakoshi et al., 2005a) |
| | Wet spinning | Fiber scaffolds | – | Ligament and tendons | Fibroblasts (rabbits) | Wistar-King rats | (Majima et al., 2007a) |
| | Freeze-drying | Sponges | AgSD | Wound dressing | – | Winstar rats | (Lee et al., 2003b) |
| Chitosan-galatosylated hyaluronan | Freeze-drying | Sponges | – | Bioartificial liver | Hepatocytes (wistar rats) | – | (Fan et al., 2010) |
| Chitosan-keratin | Solvent casting | Films | – | Wound dressing | Fibroblasts- like cells | – | (Tanabe et al., 2002a) |
| Chitosan-silkfibroin | Solvent casting | Membranes | | Wound dressing | – | – | (Kweon et al., 2001a) |
| | Cross-linking (G) | Sponges | – | Cartilage | Chondrocytes-like cells | – | (Silva et al., 2008) |
| | Freeze-drying | Scaffolds | – | Liver | Hepatocytes | – | (She et al., 2009) |
| Chitosan-silk fibroin-heparin | Freeze-drying | Scaffolds | – | Liver | Hepatocytes (mice) | – | (She et al., 2010) |
| Chitosan-soyprotein | Solvent casting Cross-linking (GA) | Membranes | – | Wound dressing | Fibroblasts-like cells | – | (Silva et al., 2005) |
| | Solvent casting | Membranes | – | Wound dressing | – | Sprague Dawley rats | (Santos et al., 2010b) |
| | Sol-gel process Freeze-drying | Scaffolds | – | Cartilage | – | – | (Silva et al., 2006b) |

AgSD: silver sulfadiazine; NGF: nerve growth factor; bFGF: basic fibroblast growth factor; CaCl2: calcium chloride; EDC: (N-(3-dimethylaminopropyl)-Nethylcarbodiimide; DHT: dehydrothermal treatment; GA: glutaraldehyde; PEO: polyethylene oxide; TPP: tripolyphosphate; 3D structures: three dimensional structures; TE: Tissue Engineering; SFF: solid free form: W/O: Water-in- oil emulsion; O/W emulsion: Oil/water emulsion; G- genipin.

**Table 2.** Glycosaminoglycans-based matrices proposed for applications in soft tissue repair. Targeted tissues and organs include cartilage, skin, liver, nerve, and ligaments.

| Composition | Processing methodology | Matrixtype | Activesubstance | Potential TE application | Cell type (source/line) | Animal model | Reference |
|---|---|---|---|---|---|---|---|
| Chondroitin- sulfate-collagen | Freeze-drying<br>Cross-linking (EDC) | Scaffolds | _ | Skin substitute | Co-cultures of keratinocytes and fibroblasts (human) | _ | (Powell and Boyce, 2006) |
| | Freeze-drying<br>Cross-linking (HT) | Scaffolds | TGF-01, PDGF-AB, EGF, or FGF-2* | Ligaments | Explants (human ACL) | _ | (Murray et al., 2003) |
| | Freeze-drying<br>Cross-linking (UV) | Scaffolds | _ | Cartilage | Chondrocytes (dogs) | _ | (Nehrer et al., 1997) |
| | Freeze-drying<br>Cross-linking (HT) | Scaffolds | _ | Cartilage | _ | Dogs | (Nehrer et al., 1998) |
| | Freeze-drying<br>Cross-linking (EDC, NHS) | Scaffolds | FGF-2, IGF-1 | Cartilage | Chondrocytes (dogs) | _ | (Veilleux and M. Spector, 2005) |
| Chondroitin- sulfate-collagen | Freeze-drying<br>Cross-linking (EDC, HT,GA, UV) | Scaffolds | _ | Cartilage | Chondrocytes (dogs) | _ | (Lee et al., 2001) |
| | Freeze-drying | Scaffolds | _ | Cartilage/bone | MSCs | _ | (Farrell et al., 2006) |
| | Freeze-drying | Porous matrices | _ | Nerve regeneration | _ | Rats | Chamberlain et al., 1998) Chamberlain et al., 2000) |
| Chondroitin-sulfate-collagen- chitosan | Freeze-drying | Scaffolds | _ | Skin equivalent model | Keratinocytes<br>Fibroblasts<br>HUVEC | _ | (Dambuyant et al., 2006) |
| Chondroitin-sulfate-collagen-heparin | Solvent casting<br>Cross-linking (DAH) | Films | _ | Liver | Hepatocytes (rats) | _ | (Kataropoulou et al., 2005) |
| Chondroitin sulfate -gelatin-hyaluronan | EmulsionFreeze-drying<br>Cross-linking (EDC) | Scaffolds containing microspheres | TGF-β1 | Cartilage | Mesenchymal stem cells (rabbits) | Rabbits | (Fan et al., 2006a) |
| | Freeze-drying<br>Cross-linking (EDC) | Scaffolds | _ | Cartilage | Chondrocytes (porcine knee joints) | Pigs | (Chang et al., 2003a, Chang et al., 2006) |
| | Solvent casting Freeze dryingcross-linking (EDC) | Porous- bilayered membrane | _ | Skin | Keratinocytes<br>Fibroblasts (human) | _ | (Wang et al., 2006a, Wang et al., 2007a) |
| Chondroitin sulfate-gelatin-hyaluronan | Freeze drying<br>cross-linking (EDC) | Bilayer scaffolds | _ | Skin | Keratinocytes<br>Fibroblasts (human) | Rats | (Wang et al., 2006b) |
| Chondroitin-hyaluronan-gelatin-PLGA | Low temperature-deposition<br>Cross-linking (EDC) Freeze-drying | Scaffolds | _ | Cartilage | Mesenchymal stem cells | Rabbits | (Fan et al., 2006b) |
| Hyaluronan-chitosan | Freeze-drying | Sponges | AgSD | Wound dressing | – | Wistar rats | (Lee et al., 2003b) |

**Table 2.** continued on next page

**Table 2.** Continued.

| Composition | Processing methodology | Matrixtype | Activesubstance | Potential TE application | Cell type (source/line) | Animal model | Reference |
|---|---|---|---|---|---|---|---|
| Hyluronan-chondroitin sulfate | Solvent casting<br>Cross-linking (PEG, propiondialdehyde) | Films | – | Bio-interactive dressing | – | Mouse | (Kirker et al., 2002a) |
|  | Freeze-drying<br>cross-linking (EDC) | Porous matrices | – | Dermal tissue regeneration | Fibroblasts (human) | Guinea pig | (Park et al., 2003b) |
|  | Freeze drying<br>cross-linking (EDC) | Porous matrices | bFGF, PDGF-BB, tobramycin, ciprofloxacin | Skin substitute | Fibroblasts (human) | Male Dunking-Hartley guinea pigs | (Park et al., 2004b) |
| Hyaluronan-collagen | Freeze-drying<br>Cross-linking (EDC) | Scaffolds | – | Cartilage | Chondrocytes(dogs) | – | (Tang et al., 2007a ) |
|  | Cross-linking (EDC) | Composite matrices | – | Cartilage | – | – | (Taguchi et al., 2002b) |
|  | Solvent casting<br>Cross-linking (HMDIC) | Membranes | – | Dermal substitutes | – | Wistar rats and IPR mice | (Koller et al., 2000b; Koller et al., 2001b) |
|  | Freeze-drying<br>Cross-linking (Ethylene glycol**) | Sponges | VEGF Fibronectin | Dermal substitutes | Fibroblasts | Sprague-Dawley rats | (Kubo and Kuroyanagi, 2003a; Kubo and Kuroyanagi, 2003b; Kubo and Kuroyanagi, 2004) |
|  | Freeze-drying | Sponge/ membrane | – | Cartilage | – | Chondrocytes (rabbit) | (Harvanova et al., 2009) |
| Hyaluronan-gelatin | Freeze-drying<br>Cross-linking (EDC) | Sponges | AgSD | Wound dressing | – | Wistar rats | (Choi et al., 1999a) |
|  | Freeze drying<br>Cross-linking (EDC) | Sponges | EGFAgSD | Wound dressing | – | Wistar rats | Hong et al., 2001) |
|  | Salt leaching | Sponges | TGF-β1 | Cartilage/bone | Bone marrow (rabbit) | – | (Angele et al., 1999) |
| Hyaluronan-gelatin-beta glucan | Freeze-drying<br>Cross-linking (EDC) | Scaffolds | – | Artificial dermis | – | – | (Lee et al., 2003a) |
| Hyaluronan-gelatin-chondroitin-6-sulfate | Freeze-drying<br>cross-linking (EDC) | Scaffolds | TGF-β1 | Cartilage | Chondrocytes (porcine knee joints) | – | (Chou et al., 2006) |
| Oxidized chondroitin sulfate-gelatin | Periodate oxidation | Hydrogels | – | Wound dressings | Fibroblast-like cells | – | (Dawlee et al., 2005) |

AgSD: silver sulfadiazine; HUVEC: human umbilical vein endothelial cells; Hep-2: human larynx carcinoma cells; PLGA: poly-(lactic-co-glycolic acid); TGF-β1: transforming growth factor- β1; VEGF: vascular endothelial growth factor; HMDIC:hexamethylenediisocyanate; EDC: 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide; SFF: solid free-form fabrication method; HT: hydrothermal treatment. TGF-01, PDGF-AB, EGF, or FGF-2*: The culture medium was supplemented with these growth factors; human ACL- human anterior cruciate ligament; MSCs: mesenchymal stem cells; DAH: 1,6-diaminohexane; Ethylene glycol*: Ethylene glycol diglycidylether.; NHS: N-hydroxysuccinimide.; UV – ultraviolet.; FGF-2: fibroblast growth factor; IGF-1: insulin-like growth factor; PEG: poly(ethylene glycol).

**Table 3.** Alginate-based matrices proposed for application in soft tissue repair. Targeted tissues and organs include cartilage, skin and liver.

| Composition | Processing methodology | Matrixtype | Activesubstance | Potential TE application | Cell type (source/line) | Animal model | Reference |
|---|---|---|---|---|---|---|---|
| Alginate-laminin derived peptide Alginate- elastin derived peptide | Peptide synthesis<br>Cross-linking (EDC) | Gels | – | Wound dressing | Fibroblasts (human) | Rabbit ear skin | (Hashimoto et al., 2004) |
| Alginate-gelatin | Freeze-drying<br>Cross-linking (EDC) | Sponges | AgSD Gentamicin sulfate | Wound dressing | – | Wistar rats | (Choi et al., 1999b; Choi et al., 2001) |
|  | Spinning Coagulation | Fibers | – | Wound dressing | – | – | (Fan et al., 2005) |
|  | Cell assembly<br>Cross-linking ($CaCl_2$; GA) | Gels /3D structure | – | Liver | Hepatocytes (rats) | – | (Yan et al., 2005b) |
| Alginate-hyaluronan | - | Beads | - | Cartilage | Chondrocytes (pigs) | - | (Lindenhayn et al., 1999) |
|  | Freeze-drying | Sponges | - | Cartilage | Chondrocytes (Winstar rats) | - | (Miralles et al., 2001) |
| Alginate-silk fibroin | Freeze-drying | Sponges | – | Wound dressing | – | – | (Lee et al., 2004b) |
|  | Freeze-drying | Sponges | – | Wound dressing | – | Male Sprague Dawley rats | (Roh et al., 2006b) |
| Oxidized alginate-gelatin | Periodate oxidation | Hydrogels | – | Wound dressing | – | Rat model | (Balakrishnan et al., 2005) |
|  | Periodate oxidation | Hydrogels | Dibutyryl cyclic adenosine monophosphate | Wound dressing | – | Rat model | (Balakrishnan et al., 2006) |

AgSD: silver sulfadiazine. EDC: 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide;$CaCl_2$: calcium chloride; GA: glutaraldehyde;

**Table 4.** Cellulose-based matrices proposed for application in soft tissue repair.

| Composition | Processing methodology | Matrixtype | Active substance | Potential TE application | Cell type (source/line) | Animal model | Reference |
|---|---|---|---|---|---|---|---|
| Cellulose-silk fibroin | Wet spinning | Fibers | _ | Wound dressing | _ | _ | (Strobin et al., 2006) |
| ORC-collagen | Freeze-drying<br>Cross-linking (dehydrothermal) | Scaffolds | _ | Clinical settings in wound repair | Fibroblasts (human) | Diabetic mouse | (Hart et al., 2002) |
| | Freeze-drying<br>Cross-linking (dehydrothermal) | Scaffolds | PDGF | Clinical setting in acute wounds | _ | Sprague-Dawley rats | (Jeschke et al., 2005a) |
| | Freeze-drying<br>Cross-linking (dehydrothermal) | Scaffolds | PDGF | Chronic wounds | _ | _ | (Cullen et al., 2002b) |

ORC: oxidised regenerated cellulose; GA: glutaraldehyde; PDGF: platelet-derived growth factor.

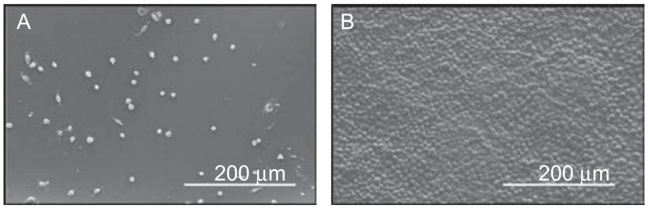

**Figure 2.** SEM micrographs of L929 cells cultured on chitosan membrane (a) and chitosan/soy protein blended membrane cross-linked with 0.1 M Ga (b). Culture time: 3 days. (Unpublished results).

selected negatively charged molecules (e.g. proteins, anionic polysaccharides, and nucleic acids) in the design of different polymeric matrices for tissue engineering applications (Liu et al., 2007b; Hamman, 2010; Yu et al., 2005). The chitosan-based complexes have an excellent ability to be processed into sponges and bilayer scaffolds for use as dermal equivalents, especially with collagen or alginate, (Ma et al., 2003; Ma et al., 2007) and also for skin regeneration. With regard to collagen/chitosan complexes, the presence of collagen resulted in the improvement of the cell compatibility of these matrices, where the additional use of cross-linking agents increased the biostability of the chitosan/collagen (CC) composite scaffolds (Ma et al, 2003). Interestingly studies have suggested that the polyelectrolyte complex (PEC) between alginate and chitosan should be mechanically stronger at a lower pH where chitosan dissolves (Hamman, 2010). Chitosan also interacts with gelatin to provide thermosensitive hydrogels for the controlled release of proteins (Chang et al., 2009) or in microspheres loaded with basic fibroblast growth factor (bFGF) to increase the production of laminin by human fibroblasts, which may be helpful for angiogenesis in skin regeneration (Liu et al., 2007a). Some examples of PEC systems formed by chitosan-alginate or chitosan-heparin, and others are shown in Table 1. Many papers have been concerned with chitosan/heparin complexes as nanoparticles (Liu et al., 2007b), and multilayer thin films (Lundin et al., 2010). Considering that heparin is well-known for its anti-coagulant activity and for having a high negative charge density due to its carboxyl and sulfonate groups (Salmivirta et al., 1996), the resulting matrices may have potential therapeutic uses for enhanced tissue regeneration. Some researchers (Kratz et al., 1997; Kweon et al., 2003; Jin et al., 2007) have suggested that the heparin-chitosan complex stimulates re-epithelialization of a full-thickness wound in human skin, in a heparin dose dependent effect (Kratz et al., 1997). Recent investigations have focused on the interactions between chitosan and other proteins, besides gelatin, as pathways to investigate matrices with suitable mechanical properties, biodegradability, and good biocompatibility for skin applications (Kweon et al., 2001; Silva et al., 2005; Tanabe et al., 2002). In our group, Silva and co-workers (Silva et al., 2005; Silva et al., 2007b) have explored the blending of chitosan with soy protein isolate, the major component of the soybean (Vaz et al., 2002), in the development of a series of blended membranes. The chitosan/soy protein (CSS) blended systems are not completely miscible and *in situ* chemical cross-linking with glutaraldehyde solutions has been used to enhance the degree of interaction between chitosan and the soy protein, and thus overcome the drawback of its immiscibility. As a consequence, the fibroblast-like cell attachment on cross-linked CSS membranes was found to be enhanced in comparison with chitosan membranes (Figure 2). Subsequent *in vivo* studies (Santos et al., 2010) demonstrated that the CSS membranes accelerated skin wound healing in rats after two weeks of dressing. All these findings support the suitability of CSS membranes as wound dressing materials.

Chitosan seems to be a good candidate particularly for cartilage tissue engineering applications, in that its structure and characteristics resemble those of glycosaminoglycans (GAGs), which are well known constituents of the cartilage extracellular matrix, and it also has a critical role in supporting chondrogenesis both *in vitro* and *in vivo* (Suh and Matthew, 2000). Even considering these characteristics, the development of an ideal chitosan scaffold for cartilage TE remains a challenging task. The most well known method to prepare chitosan scaffolds is freezing and then lyophilizing chitosan solutions in appropriate molds (Suh and Matthew, 2000; Oliveira et al., 2006; Silva et al., 2006; Madihally and Matthew,

1999) and several other methods such as melt processing (Correlo et al., 2007b; Correlo et al., 2009), sol-gel technique (Silva et al., 2006), and electrospinning (Lee et al., 2009) have all been used to produce chitosan scaffolds with different geometries and porosities, interconnectivity and so on. Promising results have also been obtained by associating chitosan with other biomacromolecules or bioactive agents in order to promote cartilage regeneration (Silva et al., 2008; Medrado et al., 2006; Lee et al., 2004). In addition, multi-component chitosan scaffolds, whose composition mimics the natural cartilage matrix, have been proposed to facilitate the formation of articular cartilaginous both *in vitro* and *in vivo* (Yan et al., 2007). It has also been reported that porous collagen/chitosan/GAG loaded with transforming growth factor-β1 (TGF-β1) provided the controlled release of TGF-β1 and promoted cartilage regeneration (Lee et al., 2004). Although the combination of chitosan with biomacromolecules, as well as technologies used in the creation of chitosan scaffolds have demonstrated some successful findings, other approaches using chitosan hydrogels/gels as injectable scaffolds for cartilage repair have also been widely studied (Hao et al., 2010; Nettles et al., 2002; Tan et al., 2009). For instance, some strategies involved the reconstruction of the tissue-engineered cartilage *in vitro* using injectable temperature-responsive hydrogel chitosan (Hao et al., 2010), while others have demonstrated that chitosan composite hydrogel matrices supported cell survival retaining the chondrocytic morphology (Tan et al., 2009).

Apart from the promising findings of chitosan-based matrices for skin and cartilage regeneration, their applications and benefits have also been expanded to liver regeneration. The role of a hepatocyte-specific 3D scaffold involved the creation of a microenvironment that mimicked the organized architecture of the native liver. Bearing this in mind, researchers suggested that collagen/chitosan (CC) matrices (Wang et al., 2003) can create an appropriate environment for the regeneration of liver cells. Nevertheless, the low mechanical strength and poor blood compatibility of these natural polymers have limited their further use in liver TE. Other researchers (Tai et al., 2010) have proposed the delivery of liver-differentiated human mesenchymal stem cells (hMSCs) from RGD-modified chitosan-alginate fibrous scaffolds as a potential therapy to aid in liver regeneration. In view of the complexity of the liver, some approaches have focused on the design of complex 3D architectures with predefined internal vascular channels to favor angiogenesis (Jiankang et al., 2007; Yan et al., 2005; He et al., 2009). Following these strategies, chitosan/gelatin scaffolds with well organized architectures and highly porous structures have been fabricated and combining rapid prototyping, microreplication, and freeze drying techniques. With respect to nerve regeneration, various studies have focused on nerve guidance conduits made from polyelectrolyte complexes between chitosan and alginate (Pfister et al., 2008) as well as the associations of chitosan with proteins (collagen, albumin, and gelatine) or poly-L-lysine (Cheng et al., 2003a; Cheng et al., 2003b) as promising alternatives to the conventional treatments. More details about these systems are shown in Table 1. Furthermore, applications of chitosan-based blended systems to tendon and ligament TE have been recently reported (Majima et al., 2007; Funakoshi et al., 2005).

### *Glycosaminoglycans (hyaluronic acid and chondroitin-sulfate)*

Glycosaminoglycans (GAGs), including hyaluronan (HA) and chondroitin sulfate (CS), are amino sugar containing polysaccharides that are present in the extracellular matrix (ECM) of all vertebrates (Kirker et al., 2002). Hyaluronic acid or hyaluronan (HA) is a linear polysaccharide with a repeating disaccharide structure composed of glucuronic acid and N-acetyl glucosamine residues (Kogan et al, 2007). This water soluble polysaccharide is widely distributed throughout the ECM of all connective tissues in humans and other animals (Kogan et al., 2007) and has various important biological functions. Based on its positive biological effects on cell behavior *in vitro*, HA have participated in many polymeric systems for different TE applications, as listed in Table 2. There have been several studies involving HA alone or mixed with collagen and gelatin for skin applications (see Table 2). In some cases, the collagen/HA mixture was cross-linked to stabilize the materials, and then their performance was improved (Tang et al., 2007; Taguchi et al., 2002; Koller et al., 2000; Koller et al., 2001; Bakos and Koniarova, 1999; Kubo and Kuroyanagi, 2003; Park et al., 2003). Park et al. (2003) showed that dermis treated with EDC-cross linked-collagen-HA matrix was thicker than the control (porous polyurethane matrix), and that epithelial regeneration was accelerated *in vivo*. The presence of growth factors into collagen-hyaluronan matrices significantly enhanced wound healing (Park et al., 2004). As commented previously, the matrices from combinations of HA and collagen have been actively developed for wound repair. However, these and other HA-based combinations have been suggested for both cartilage and neural repair (see examples in Table 2).

Chondroitin sulfate is quite water soluble, and for this reason it has been frequently combined with other polymers (see examples listed in Table 2). In fact, its anionic nature enables efficient interaction with cationic molecules to form interesting structures for soft tissue repair. For skin regeneration, Yannas and Burke (Yannas and Burke, 1980; Burke et al., 1981) developed a bilayer artificial skin from the association of chondroitin sulfate with collagen, which is known as Integra® (Integra Life Sciences Holding, New Jersey). Similarly, bi-layered gelatin-chondroitin sulfate-HA constructs with different pore sizes on either side were prepared to mimic the composition of skin and to create an appropriate microenvironment for cell growth, differentiation, and migration (Wang et al., 2006). As a component of cartilage ECM, chondroitin sulfate has a stimulatory potential on both cell-proliferation and matrix retention, in turn this polymer has been used as an interesting component in the production of multi-component scaffolds for use in cartilage TE. For instance, chondrocytes seeded on gelatin/chondroitin sulfate/hyaluronan scaffolds were evenly distributed in matrices, secreted new ECM, retained their phenotype, and secreted type II collagen (Chang et al., 2003a). Moreover, TGF-β1 was immobilized onto the surface of gelatin/hyaluronic acid/chondroitin-6-sulfate (GHCS) to suppress any undesired differentiation during cartilage growth *in vitro* (Chou et al., 2006). As proposed by Murray et al. (Murray et al., 2003), the addition of selected growth factors to medium for an implantable collagen-glycosaminoglycan (CGG) scaffold may enhance ligament cell behavior within the CG scaffold.

### *Alginate*

Alginate is a polymer derived from sea algae, formed by linear block copolymers of 1-4 linked β-D-mannuronic acid and L-guluronic acid. It is water soluble at room temperature and in the presence of certain divalent cations, such as calcium, barium, and strontium; it forms stable hydrogels that have been explored for a broad range of biomedical applications (Gomes et al., 2008; Eiselt et al., 2000; Hunt and Grover, 2010). One of the drawbacks of alginate hydrogels can be that degradation occurs via a slow and unpredictable dissolution process *in vivo* (Boontheekul et al., 2005; Bouhadir et al., 2001). Alginate has been used as a wound dressing (Qin, 2008; Suzuki et al., 1998; Chiu et al., 2008), delivery vehicles for drugs (Hunt et al., 2009), and cell encapsulation (Hunt and Grover, 2010). Furthermore, combinations of alginate with another polysaccharide have led to the formation of biopolymeric matrices for soft tissue repair (see Table 3). Most of these have been based on the addition of one or two components of ECM to alginate in order to create composites that mimic the properties of natural tissues and then to enhance the functionality of the engineered materials. For instance, a commercial collagen-alginate topical wound dressing (FIBRACOL PLUS Dressing, Johnson & Johnson Gateway®) has demonstrated its efficacy and safety in the treatment of diabetic foot ulcers (Donaghue et al., 1998). Although these strategies are of interest for TE, the inclusion of other biomacromolecules such as silk fibroin to alginate could also contribute useful properties for wound treatment (Roh et al., 2006). In cartilage studies (Gerard et al., 2005), beads composed of alginate-hyaluronan that combine the gel forming ability of alginate with the healing properties of hyaluronan have been proposed. However, studies involving alginate-based systems have also been expanded towards liver regeneration, where conjugations of alginate with galactosylated chitosan (Seo et al., 2006b), and also with heparin (Seo et al., 2006a) have been suggested to enhance their liver specific function for the design of bioartificial liver devices.

### *Cellulose*

Cellulose is the most abundant organic polymer in the world. It is insoluble in most solvents due to its strong intra or intermolecular hydrogen bonding (Klemm et al., 2005). Despite this, it is still used in the mass production of conventional dressing materials (Boateng et al., 2008). Cellulose derivatives can be associated with proteins (e.g. silk fibroin, collagen) with the formation of sponges for cartilage tissue engineering (Pulkkinen et al., 2006), and as scaffolds in clinical settings for wound repair (Hart et al., 2002; Jeschke et al., 2005; Cullen et al., 2002) (see Table 4). Oxidized regenerated cellulose and its blends have been applied as a wound dressing, this is because oxidized cellulose has proved be an effective hemostat and also has antibacterial activity (Martina et al., 2009). For instance, in the presence of chronic wound exudates, ORC/collagen forms a soft, conformable, and biodegradable gel that physically binds and inactivates the matrix metalloproteases (MMPs), stabilizing their levels and contributing to a positive effect on the wound healing process, since a high level of MMPs in chronic wounds may lead to the degradation of important proteins and inactivate growth factors (Hart et al., 2002; Cullen et al., 2002). Promogran® is an example of a spongy matrix containing oxidized regenerated cellulose (45% ORC) and collagen (55%), which has been introduced to both the USA and EU markets. Recently, room temperature ionic liquids have been proposed as possible new solvents for the derivatization of cellulose, opening new paths for the shaping of cellulose through its precipitation with an excess of polar solvents like water, acetone or a combination of these (Pinkert et al., 2009). Also, cellulose composites in different shapes (films, beads, scaffolds) can be obtained by changing the composition, the precipitation method and conditions, which have a potential use in tissue engineering. Cellulose can also be produced by *Gluconacetobacter xylinus* (*Acetobacter xylinum*) (Klemm et al., 2005; Svensson et al., 2005; Czaja et al., 2007). Although identical to the cellulose of plant origin in terms of molecular formula, bacterial cellulose is characterized by a crystalline nano and microfibril structure that determines its extraordinary physical and mechanical properties (Czaja et al., 2007; Klemm et al., 2005). Bacterial cellulose or microbial cellulose has unique properties, including high purity, high crystallinity, moldability in situ, biocompatibility, and high water holding ability. In addition to its cost efficient production, it has high mechanical strength in the wet state (Czaja et al., 2007; Svensson et al., 2005).

Due to its versatility, bacterial cellulose has been studied as a wound dressing, for tubular implants, and as scaffolds for cartilage repair, among other applications (Czaja et al., 2007; Svensson et al., 2005; Klemm et al., 2005). Bacterial cellulose/chitosan wound dressings have good antibacterial and barrier properties; they also have mechanical properties in the wet state and optimal moisture conditions for rapid wound healing without irritation (Ciechanska, 2004). In cartilage studies (Svensson et al., 2005), bacterial cellulose has been shown to be a potential scaffold for cartilage TE since the chondrocytes maintained their differentiated form and the scaffold supported cell ingrowth.

## Consideration of polysaccharide and protein interactions

In biological systems, proteins and polysaccharides have an important role in the organization of living cells, and the interactions between these polymers of natural origin leads to the formation of macromolecular structures through association. Basic information related to the phase behavior and the interactions between polysaccharides and proteins have been obtained during the last three decades, mainly in the field of food science (McClements, 2006; Turgeon et al., 2003; Ya and Tolstoguzov, 1997; Tolstoguzov, 2000; Doublier et al., 2000; Kruif and Tuinier, 2001). Mixed systems of globular proteins and polysaccharides have been widely used to control the structure, texture, and stability of food products (Musampa et al., 2007; Berthand and Turgeon, 2007), whereas polymer based systems of natural origin can also be used for biomedical applications. Different examples of the biomedical applications of these systems are shown in Tables 1 to 4. Besides the low cost and versatility of this strategy, the introduction of proteins into matrix materials may improve its cell behavior because they are able to interact favorably with cells through specific recognition domains present in their structure. Also, the interaction between natural polymers with different chemical structures through hydrogen bonding or electrostatic interactions in nature may reinforce the mechanical properties of the materials obtained from such mixtures. Nevertheless, most polymer blends are immiscible or only partially miscible. Depending on the polymer characteristics (molecular weight, polysaccharide/protein ratio, conformation, and charge density), and on the solution conditions (pH, ionic strength, total concentration, solvent quality, etc.), the association of biomacromolecules may result in the formation of a complex or a phase separation (Turgeon et al., 2003). When polysaccharides and proteins attract each other through electrostatic interactions, the polymers associate excluding the solvent from their vicinity (complex coacervation), thus allowing the formation of soluble complexes or an aggregative phase separation (precipitate) (Doublier et al., 2000; de Kruif et al., 2004). Sometimes the complex coacervates are highly unstable and a structural stabilization by chemical agents can become necessary (Sanchez and Renard, 2002). The formation of complexes and coacervates induced by electrostatic interactions is a fundamental physico-chemical phenomenon, which is relevant to a number of known biological processes such as protein transcription and antigen-antibody reactions (Turgeon et al., 2003). Additionally, protein-polysaccharide complexes are important in the design of multi-layered structures (Noel et al., 2007), encapsulation processes (Xing et al., 2005), and the formation and stabilization of food emulsions (Dickinson, 2006). On the other hand, the phase separation can occur due to a strong repulsion between the polymers caused by similar electrical charges or because one or both polymers are uncharged (McClements, 2006; Doublier et al., 2000). At low concentrations, the polymers can be intimately mixed and form a one phase solution. However, when the total concentration of the system increases, exceeding a certain critical value of about 4% for globular proteins and polysaccharide mixtures (Musampa et al., 2007), a phase separation occurs. As a result, the system exhibits one phase that is rich in protein and the other is rich in polysaccharide (Doublier et al., 2000; McClements, 2006). Miscibility in a polymer blend is associated with specific interactions between the polymeric components. The major forces responsible for the polymer interactions are electrostatic in nature but other common interactions such as hydrogen bonding or hydrophobic interactions may be significant in the stabilization of the interactions (McClements, 2006; Feldman, 2005). Several authors (Taravel and Domard, 1995; Yin et al., 2005; Yin et al., 1999; Silva et al., 2007b; Berthand and Turgeon, 2007; Malay et al., 2007; Palmiere et al., 1999; Naidu et al., 2005; Sionkowska et al., 2004) have studied the interactions between polymers of natural origin regarding their promising applications in food formulation, biotechnological, and biomedical areas. For example, studies performed by Taravel and Domard (Taravel and Domard, 1993; Taravel and Domard, 1995) and Sionskowsha et al. (2004) suggested that chitosan/collagen blends are miscible and that the interactions between them are electrostatic in nature with the formation of low complexes. These matrices have been proposed as films for dermal regeneration templates (Gao et al., 2003), as wound dressings (Guan et al., 2007), and as scaffolds for liver TE (Wang et al., 2003). Malay et al. (Malay et al., 2007) investigated the formation of pH-induced complexation of silk fibroin and hyaluronic acid, while Naidu et al. (Naidu et al., 2005) evaluated the compatibility of sodium alginate/hydroxyethylcellulose blends both in solution and as solid films. One study (Christopoulou et al., 2000) indicated that the compatibility between the polymers in solution would remain, even when the solvent is absent ("memory effect"). Besides the miscibility, the nature and strength of interactions involved between polymer based systems of natural origin can be studied using a wide variety of analytical techniques. For example, in polymer blend solutions, the existence of the thermodynamic interaction (attraction or repulsion) between polymers induces a non-ideal mixing, resulting in changes of viscosity. Therefore, viscosimetry is an effective, quick, and inexpensive method to determine the miscibility of polymers (Naidu et al., 2005). Also, the compatibility of a blend system can be studied by its glass transition temperature ($T_g$), which is usually determined by differential scanning calorimetry (DSC). An immiscible blend usually exhibits the $T_{gs}$ of the components, while miscible polymers involve thermodynamic solubility and should have one phase and only a single $T_g$. On the other hand, the type of morphology in a blend system is dependent on the nature and amount of the polymers in the mixture, viscosity, and also on their miscibility (Malay et al., 2007; Koning et al., 1998). Heterogeneous blends can appear as a dispersion of one polymer in the matrix of the other polymer, with the formation of co-continuous morphology.

## Strategies for compatibilization and surface modification on polymeric blends

### Compatibilizers and chemical cross-linking treatments

As most polymeric blends are immiscible, compatibilization could be required. The compatibilization of polymer blends is possible by adding to the system non reactive or reactive compatibilizers (Feldman, 2005; Koning et al., 1998). Reactive compatibilizers chemically react with the blend components, while non-reactive compatibilizers are block and graft copolymers, having chain segments that are identical or similar to the components to be mixed (Feldman, 2005; Koning et al., 1998). Graft copolymers work as emulsifiers reducing the interfacial tension of blends. This leads to a phase size small enough for the material to be considered as macroscopically homogeneous and consequently improving the mechanical properties of the system (Feldman, 2005). Besides the usual compatibilizers, cross-linking methods have been used to improve the structural stability and mechanical properties of binary systems. A particular cross-linker should be chosen based on its chemical reactivity, solubility, spacer length, and compatibility of the reaction with the application. Glutaraldehyde (GA), formaldehyde, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), polyepoxide, and polyglycidyl ether are commonly used as cross-linking agents (Hennink and van Nostrum, 2002; Silva et al., 2005; Park et al., 2003; Park et al., 2002; Harriger et al., 1997; Silva et al., 2004; Sung et al., 1996). Of these, glutaraldehyde and EDC are the most widely used for polymeric systems due to their high cross-linking efficiency. However, depending on the concentration used, extracts from the cross-linked materials can be released into the tissue, resulting in cytotoxicity and inflammation. In contrast to glutaraldehyde, water-soluble carbodiimide (EDC) does not remain as a part of the linkage but simply changes into water-soluble urea derivatives with low cytotoxicity. Park et al. (Park et al., 2002) reported that a collagen/HA matrix cross-linked with EDC had good resistance to enzymatic degradation with acceptable toxicity. Genipin has been considered as a natural cross-linking agent with a lower cytotoxicity when compared to the alternative cross-linkers like glutaraldehyde (Sung et al., 1998). Genipin is obtained from its parent compound geniposide, which is extracted from the fruits of *Gardenia jasminoides Ellis* (Koo et al., 2004). Genipin has been used to cross-link gelatin (Chang et al., 2003b; Liu et al., 2004), collagen (Sundararaghavan et al., 2008), kappa-carrageenan (Meena et al., 2007), chitosan (Mi et al., 2002a; Kuo and Lin, 2006; Chen et al., 2005; Muzzarelli, 2009), alginate/chitosan (Chen et al., 2006), chitosan/gelatin blends (Chiono et al., 2008), and the chitosan/silk fibroin system (Silva et al., 2008). One interesting characteristic of the reaction of genipin with amino acids lies in the formation of dark blue pigments, which are the result of oxygen radical induced polymerization of genipin (Sung et al., 1998). These blue pigments are used as a natural colorant in foods (Park, 2002), and can also aid in following the evolution of genipin cross-linking reactions (Chen et al., 2005). Typically, matrices cross-linked with genipin present good mechanical properties, reduced swelling, a slower degradation rate, and good biocompatibility (Chang et al., 2003b; Mi et al., 2002a). Proanthocyanidins (PAs) have also been indicated as non-toxic cross-linkers (Han et al., 2003). PAs are widespread in fruits and vegetables, and belong to the category known as condensed tannins, which consist of highly hydroxylated structures capable of forming insoluble complexes with carbohydrates and proteins (Han et al., 2003). Studies have indicated that PAs are less cytotoxic than glutaraldehyde, could efficiently cross-link collagen matrices (Han et al., 2003), and chitosan/gelatin membranes (Kim et al., 2005). Kim et al. (Kim et al., 2005) reported that proanthocyanidin cross-linked chitosan/gelatin membranes have good mechanical properties, thermal properties, and a slower degradation rate *in vivo* with no inflammatory reaction found in all the implants that were tested when compared to uncross-linked gelatin films and chitosan/gelatin membranes.

### Surface modification

A biomaterial with good bulk properties does not necessarily possess the surface characteristics suitable for a given biomedical application. Therefore, modification of the biomaterial surface is often needed (Oehr, 2003; Chu et al., 2002). Various methods have been employed for modifying polymer surfaces including chemical modification (Pashkuleva et al., 2005; Tangpasuthadol et al., 2003), ultra-violet (UV) (Olbrich et al., 2007; Welle et al., 2005; Gumpenberger et al., 2003), gamma irradiation (Yang et al., 2002; Mao et al., 2004), and plasma surface modification (Oehr, 2003; Pashkuleva et al., 2005; Silva et al., 2007c; Zhu et al., 2005; Pashkuleva and Reis; 2005; Huang et al., 2007; Lopez-Perez et al., 2007; Ratner, 1995). For instance, these modifications will determine the possible interactions of polymers with bioactive agents, namely drugs, growth factors as well as the possibility of allowing for their clinical use in the regeneration of hard/soft tissues (Pashkuleva and Reis, 2005; Goddard and Hotchkiss, 2007; Ratner, 1995). Depending of the chosen method and conditions, a surface can be modified to become hydrophilic or hydrophobic, be functionalized or only be activated for further reactions (Pashkuleva and Reis, 2005; Goddard and Hotchkiss, 2007). Chemical etching with a potassium permanganate-nitric acid system has been shown to enhance the surface energy and wettability of starch based blends (Pashkuleva et al., 2005). UV irradiation has been used to create patterned polystyrene substrates for tissue engineering applications, especially in neuroscience (Welle et al., 2005). Plasma surface modification is a method widely used to tailor surface functionality using different atmospheres (Chu et al., 2002; Pashkuleva and Reis, 2005). Usually, plasma treatment only affects the outermost layers (2.5–10 nm) of the material's surface, while the bulk properties of the polymer remain intact (Oehr, 2003). When a material is exposed to a partially ionized gas, its surface is bombarded with ions, electrons, and radicals from the plasma. This process results in the formation of radicals on the polymer surface. The highly reactive species so formed combine with the radicals from the working gas to modify the surface (Chu et al., 2002). Depending on the interaction between the plasma and the polymer, and on the operating conditions (gas, power, and exposure time), the reactions of modification and degradation can occur (Chu et al., 2002; Oehr, 2003; Khonsari et al., 2003). When degradation is prominent, etching will take place on the polymer surface. An etching reaction occurs when the polymers are exposed to plasma for a long period, and the exposed layers of the polymers are etched off (Chu et al., 2002). As a result, etching produces nano-roughness on the polymer surface, which can create the desirable features on biomaterials to meet the requirements of biocompatibility *in vivo* (Chu et al., 2002). Figure 3 shows that the prolonged exposure time on chitosan/soy protein membranes to argon plasma (Figure 3B and 3C) promoted an increase of the surface roughness when compared to the initial surface membrane (Figure 3A). On the other hand, plasma can also be used to create functionalized surfaces with direct binding of new chemical groups or their inclusion after surface activation by plasma treatment (Chu et al., 2002; Lopez-Perez et al., 2007). For example, oxygen (-OH, -C=O, -COOH groups) or nitrogen (-$NO_2$, -$NH_2$, -$CONH_2$ groups) plasma have been used to increase the material hydrophilicity (Inagaki, 1996). As a result,

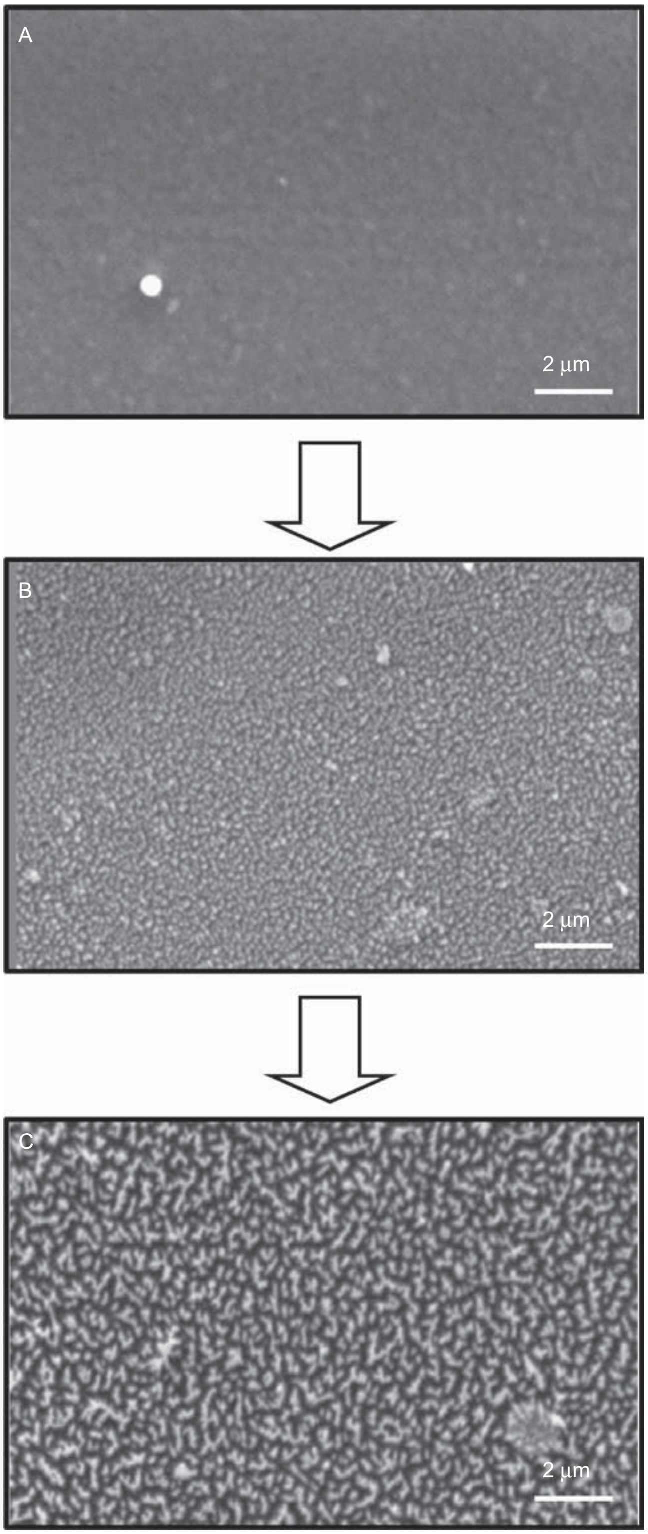

**Figure 3.** SEM micrographs showing the effect of the prolonged time exposure to argon plasma on surface of chitosan-soy protein (CSS) based membranes; (a) CSS membrane without treatment, (b) CSS after argon plasma (40 watts, 5 minutes) and (c) CSS after argon plasma (40 watts, 20 minutes). (Unpublished results)

improved adhesion strength, biocompatibility, and other relevant properties were observed (Inagaki, 1996; Chu et al., 2002). Similar results were obtained in our group by Silva et al. (Silva et al., 2007c) who investigated the surface modification of chitosan membranes using nitrogen and argon plasma to improve its fibroblast cell adhesion *in vitro*. The proposed modifications would facilitate the use of chitosan-based materials as wound dressings. In addition, plasma grafting polymerization can be used to modify inert surfaces. This includes activation of the surface by plasma followed by polymerization reactions, resulting from the contact between the activated surface with monomers in the liquid or gas phase (Chu et al., 2002; Pashkuleva and Reis, 2005; Lopez-Perez et al., 2007; Forch et al., 2005). Therefore, grafted copolymers are formed onto the surface. Using such procedures, smart surfaces may be produced, where the wettability can be responsive to the change of external variables, being useful for some biomedical applications (Ando et al., 2007; da Silva et al., 2007). Recently, Huang et al (Huang et al., 2007) suggested that oxygen plasma is a better method to incorporate laminin onto the surface of chitosan membrane, resulting in a significant increase of the attachment of Schwann cells and to help the affinity for directing peripheral nerve regeneration.

## Final remarks

Several polymers based on natural origins, either alone or in binary or ternary blend systems, are proposed for use in soft tissue repair. Among the various tissues that these systems can target, skin, and cartilage are probably the most prominent. The features of a particular blended system, in general, must be modulated and designed in an appropriate shape for a determined biomedical application. Most of the polymer combinations have been used as a means to overcome problems observed in simple systems with regard to the mechanical strength of the scaffolds, proliferation ability, and implantation difficulties. Although some successful findings have been reported, these systems still need some improvement in terms of structural stability due to water-solubility for some polysaccharides, which have been solved through their complexation or cross-linking reactions. In addition, the construction of adequate surface properties is also important for the tissue/cell interface, making it necessary to apply surface modification on the polymer matrix. With regard to tissue targets, interesting strategies have been proposed such as co-cultures of keratinocytes and fibroblasts on bilayer constructs (scaffold/membrane) as dermal equivalents, as well as the development of multi-component scaffolds with living cells for cartilage repair have shown promise. In some cases, a sustained release of a bioactive substance (drugs or growth factors) incorporated into these biomatrices enhanced the cell response, and thus tissue regeneration. Moreover, advanced processing techniques such as a solid-free form have been proposed to produce porous matrices with complex geometric shapes and suitable porosities tailored to the tissue target, for example in the design of bioartificial livers. Although the approaches described have demonstrated promising results both *in vitro* and *in vivo*, there are still many challenging issues to be addressed in order to obtain clinically successful materials as well as to create novel therapeutic approaches. Bearing this in mind, extensive research must be done to develop materials interacting with cells on healing tissue and other host processes. Also, further study is needed to understand the *in vivo* interactions among biomaterials, cells and growth factors at the molecular level. Current trends also suggest that the intensification of the development of hydrogel materials from blended systems with thermosensitive materials can be a powerful tool for the production of multifunctional materials that can provide complex biological signals and respond to environmental stimuli. For all these purposes, it is essential to have collaborative research between material researchers, biologists, and clinicians which could lead to advanced materials that fulfill all the needs of polymer combinations for soft tissue regeneration.

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