
REVIEW

Polysaccharides as Cell Carriers for Tissue Engineering: the Use of Cellulose in Vascular Wall Reconstruction

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Summary

Polysaccharides are long carbohydrate molecules of monosaccharide units joined together by glycosidic bonds. These biological polymers have emerged as promising materials for tissue engineering due to their biocompatibility, mostly good availability and tailorable properties. This complex group of biomolecules can be classified using several criteria, such as chemical composition (homo- and heteropolysaccharides), structure (linear and branched), function in the organism (structural, storage and secreted polysaccharides), or source (animals, plants, microorganisms). Polysaccharides most widely used in tissue engineering include starch, cellulose, chitosan, pectins, alginate, agar, dextran, pullulan, gellan, xanthan and glycosaminoglycans. Polysaccharides have been applied for engineering and regeneration of practically all tissues, though mostly at the experimental level. Polysaccharides have been tested for engineering of blood vessels, myocardium, heart valves, bone, articular and tracheal cartilage, intervertebral discs, menisci, skin, liver, skeletal muscle, neural tissue, urinary bladder, and also for encapsulation and delivery of pancreatic islets and ovarian follicles. For these purposes, polysaccharides have been applied in various forms, such as injectable hydrogels or porous and fibrous scaffolds, and often in combination with other natural or synthetic polymers or inorganic nanoparticles. The immune response evoked by polysaccharides is usually mild, and can be reduced by purifying the material or by choosing appropriate crosslinking agents.

Key words

Natural polymers • Bioartificial tissue replacements • Cell carriers
• Cell therapy • Regenerative medicine

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Introduction

Tissue engineering is an advanced biotechnology, defined as “an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function” (Langer and Vacanti 1993). These substitutes consist of two main components, i.e. cells and their carriers. The cell carrier, often referred to as a “scaffold”, can be made of purely biological molecules, such as collagen, elastin, fibronectin, laminin, hyaluronic acid and other extracellular matrix (ECM) molecules, of synthetic and inorganic molecules, e.g. synthetic polymers, carbon-based materials, ceramics, metal-based materials, or of various combinations of these materials. The materials should be biocompatible, i.e. non-toxic, non-mutagenic, non-immunogenic and matching the mechanical properties of the replaced tissue. However, in advanced tissue engineering, these materials should not just be passively tolerated by cells, but they should act as analogues of the native ECM, i.e. they should control the extent and the strength of cell adhesion, cell proliferation, cell differentiation and maturation to the desired phenotype, and to proper cell functioning.

Among the biological polymers, polysaccharides have emerged as promising materials for tissue engineering purposes. Polysaccharides are long carbohydrate molecules of monosaccharide units joined together by glycosidic bonds. Polysaccharides can be classified from several points of view. Polysaccharides composed of only one type of monosaccharide are referred to as homopolysaccharides (homoglycans, e.g. starch, glycogen, cellulose, chitin), and if two or more different types of monomeric unit are present, they are referred to as heteropolysaccharides (heteroglycans, e.g. glycosaminoglycans). Other classification criteria consider the morphology of polysaccharide molecules, which can range from linear to highly branched, or the function of polysaccharides in organisms, which is usually either structure-related or storage-related. The main storage polysaccharides are starch and glycogen, while structural polysaccharides include cellulose, chitin, agar, arabinoxylans and pectins. Some polysaccharides are secreted by bacteria, fungi and algae as an evolutionary adaptation to help them adhere to surfaces and to prevent them drying out. Humans have developed some of these polysaccharides into useful products, e.g. dextran, pullulan, gellan gum and xanthan gum. Polysaccharides can also be sorted by their source (animals, plants, microorganisms), by their electrical charge (neutral, cationic, anionic), by modifications of their molecules (pristine molecules and their derivatives), and particularly by their degradability, which is important in tissue engineering. Ideal scaffolds used in advanced tissue engineering should be constructed from resorbable materials that degrade in the organism and are gradually replaced by natural ECM. However, polysaccharides of non-mammalian origin are often non-degradable or very slowly degradable, and in order to adjust their proper degradability, they need to be physically or chemically modified. The criteria for classifying polysaccharides, with examples for each polysaccharide group, are summarized in Table 1. They are based on the studies by Lee *et al.* (2009), Baldwin and Kiick (2010), Oliveira and Reis (2011), Ulery *et al.* (2011), and also on the information available at www.cfs.purdue.edu/class/f&n630/pdf_full/yao-polysaccharide-2011.pdf.

Polysaccharides are widely applied in many fields, particularly in the food, pharmaceutical and cosmetic industries, in medicine and in various biotechnologies, such as drug and gene delivery or bioimaging. In tissue engineering, they have been used mainly for experimental purposes, though some of them,

e.g. hyaluronan, heparin or alginate, are also approved for clinical application.

The first, more general part of this review is dedicated to the use of polysaccharides in various areas of tissue engineering, and the second more specialized part is focused on the use of cellulose in vascular tissue engineering, including the collective experience of the authors of this article.

Polysaccharides relevant for tissue engineering

Not all polysaccharides are currently used in tissue engineering, or are suitable for this biotechnology, mainly due to their jelly-like consistency and insufficient mechanical properties. Even polysaccharides applicable in tissue engineering are usually combined with other natural or synthetic polymers, or are reinforced with inorganic particles. In addition, these combinations, together with functionalization and loading with various bioactive molecules, such as adhesion oligopeptides, growth factors and drugs, increase the bioactivity of the scaffolds.

The most relevant polysaccharides for application in tissue engineering, which will be discussed in the following text, are *starch, cellulose, chitin and chitosan, pectins, alginate, agar, dextran, pullulan, gellan, xanthan* and *glycosaminoglycans*. Other polysaccharides, namely glycogen, are promising as carriers for delivery of drugs or imaging agents (Filippov *et al.* 2012). Arabinoxylans are also potential candidates for the controlled release of biomolecules, e.g. insulin (Berlanga-Reyes *et al.* 2009) or lycopene, i.e. a carotenoid important in the prevention of chronic diseases such as atherosclerosis, skin cancer, prostate cancer and colon cancer (Hernández-Espinoza *et al.* 2012). In addition, arabinoxylans, owing to their bound phenolic acids, have been shown to have antioxidant activity (Hromádková *et al.* 2013, Rivas *et al.* 2013).

Starch

Starch is a polymer of glucose which is used as a storage polysaccharide in plants, being found in the form of linear amylose and branched amylopectin. Pure starch microfibrils, which could serve as scaffolds for cells in tissue engineering applications, were prepared recently by an electrospinning technique (Kong and Ziegler 2012). However, for these applications, starch has been usually combined with other natural or synthetic polymers in

order to achieve better mechanical properties and bioactivity of the scaffolds. For example, starch microfibrils were combined with collagen I nanofibers in order to construct an ECM equivalent for bone tissue engineering (Tuzlakoglu *et al.* 2011). As for combinations with synthetic polymers, starch has been blended with polycaprolactone or polylactide for bone tissue engineering (Gomes *et al.* 2008). Combination with starch enhanced the growth and differentiation of articular chondrocytes on polycaprolactone scaffolds

(Oliveira *et al.* 2007). Starch/polycaprolactone scaffolds also provided a suitable growth support for macro- and microvascular endothelial cells *in vitro* (Santos *et al.* 2007). When loaded with vascular endothelial growth factor (VEGF) or fibroblast growth factor-2 (FGF-2), these scaffolds also promoted the expression of neovascularization mediators such as receptors for VEGF (VEGFR1, VEGFR2) and VEGF itself, in cells colonizing the scaffolds after implantation *in vivo* into mice (Santos *et al.* 2013).

Table 1. Classification of polysaccharides.

Classification criteria	Examples
Function	
Storage	starch, glycogen
Structural	cellulose, chitin, alginate, agar
Secreted	dextran, pullulan, gellan gum, xanthan gum, welan gum, diutan gum
Chemical composition	
Homoglycans	starch, glycogen, cellulose, chitin
Heteroglycans	agars, alginate, carrageenans, xanthan, gellan, arabinoxylans, glycosaminoglycans
Structure	
Linear	glycosaminoglycans, amylose, cellulose, pectin, alginates, agarose
Branched	glykogen, amylopectin, xanthan gum, arabic gum, arabinoxylan
Electrical charge	
Neutral	amylose, amylopectin, cellulose
Anionic	alginates, carrageenans, gellan, gum arabic, xanthan
Cationic	chitosan
Source	
<i>Animals</i>	
Mammals	glycosaminoglycans (hyaluronan, heparin, chondroitin sulfate, keratan sulfate, dermatan sulfate, heparan sulfate)
Non-mammals	chitin
<i>Plants</i>	
Higher plants	cellulose, starch
Algae	agars, alginates, carrageenans
<i>Microorganisms</i>	gellan gum, xanthan gum
Modification	
Pristine	cellulose, alginate, chitin
Derivative	carboxymethyl cellulose, propylene glycol alginate, chitosan
Degradability in humans	
Degradable	dextran, glycogen, glycosaminoglycans
Non-degradable, slowly degradable	cellulose, chitosan, alginate, agar

Cellulose

Cellulose is a structural polysaccharide consisting of a linear chain of several hundred to over ten thousand $\beta(1\rightarrow4)$ linked D-glucose units. It was discovered and isolated from green plants by Payen (1838) (for a review, see O'Sullivan 1997). It is the most abundant biopolymer on Earth, synthesized by herbs, woody plants, many forms of algae, fungi and some species of bacteria, namely *Acetobacter xylinum*. Bacterial cellulose is identical to plant cellulose in chemical structure, but it can be produced without contaminant molecules, such as lignin and hemicelluloses, and does not require intensive purification processes. In addition, it is remarkable for its mechanical strength and biocompatibility, and it has therefore often been applied in tissue engineering (for a review, see Helenius *et al.* 2006, Petersen and Gatenholm 2011). In addition to vascular tissue engineering, which is discussed below, cellulose has also been used for engineering a variety of other tissues, such as bone (Shi *et al.* 2012a), cartilage (Andersson *et al.* 2010), skin (Kingkaew *et al.* 2010), skeletal muscle (Dugan *et al.* 2013), cardiac muscle (Entcheva *et al.* 2004) and heart valves (Mohammadi 2011). Cellulose has also been used for constructing nanofibrous three-dimensional carriers for liver cells (Bhattacharya *et al.* 2012), for encapsulating and immunoisolating Langerhans islets (Risbud *et al.* 2003), for creating tubes for regenerating damaged peripheral nerves (Kowalska-Ludwicka *et al.* 2013), and also for creating carriers for delivery and differentiation of mesenchymal stem cells (Gu *et al.* 2010) and neural stem cells (Mothe *et al.* 2013) for neural tissue regeneration. Even lignin, a macromolecule crosslinking different plant polysaccharides including cellulose, induced differentiation of embryonic stem cells into neuroectodermal cells, namely ocular cells and neural cells (Inoue *et al.* 2013). Cellulose acetate in the form of porous membranes has been applied for constructing a bioartificial renal tubule system (Sato *et al.* 2005), and in the form of electrospun porous microfibrillar three-dimensional scaffolds, for potential urinary bladder reconstruction (Han and Gouma 2006). Microporous scaffolds made of bacterial cellulose and seeded with human urine-derived stem cells supported the formation of a multilayered urothelium, and thus these constructs hold promise for creating tissue-engineered urinary conduits for urinary reconstruction and diversion (Bodin *et al.* 2010).

Chitin and chitosan

Chitin is a long-chain polymer of N-acetylglucosamine. It is the main component of the cell walls of fungi, the exoskeletons of arthropods such as insects and crustaceans (e.g. crabs, lobsters and shrimps), the radulas of mollusks, and the beaks and internal shells of cephalopods, including squid and octopus. Chitin itself has been used only relatively rarely for tissue engineering. For example, composite scaffolds containing chitin, pectin and CaCO_3 nanoparticles were tested with fibroblasts for potential use in tissue engineering and controlled drug delivery (Kumar *et al.* 2013). Biodegradable porous glycol chitin-based thermoresponsive hydrogel scaffolds promoted odontogenic differentiation of human dental pulp cells, and thus they proved to be promising materials for dentin regeneration (Park *et al.* 2013).

However, an important derivative of chitin, used for engineering a wide range of tissues and organs, is chitosan. To date, about 1400 papers concerning chitosan and tissue engineering can be found in the PubMed database. Chitosan is a linear polysaccharide composed of randomly distributed $\beta(1-4)$ -linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine. Chitosan, particularly in the form of nanofibrous scaffolds, in combination with other polymers, ceramic or carbon nanoparticles, growth factors and other bioactive molecules, has been applied for reconstructing almost all tissues, such as bone (Frohbergh *et al.* 2012), blood vessels (Du *et al.* 2012), heart valves (Hong *et al.* 2009), myocardium (Hussain *et al.* 2013), liver (Wang *et al.* 2005, Mareková *et al.* 2013), pancreatic islets (Deng *et al.* 2011), kidney (Gao *et al.* 2012), urinary bladder (Drewa *et al.* 2008), skin (Lin *et al.* 2013) or the central and peripheral nervous system (Shokrgozar *et al.* 2011, Hu *et al.* 2013).

Pectins

Pectins are a family of complex polysaccharides that contain 1,4-linked α -D-galactosyluronic acid residues. They are present in most primary cell walls and in the non-woody parts of terrestrial plants. Due to their simple and cytocompatible gelling mechanism, pectins have recently been exploited for various biomedical applications, including drug and gene delivery, wound healing and tissue engineering (for a review, see Munarin *et al.* 2012). Nanostructured pectin films deposited on titanium, glass and polystyrene substrates promoted the adhesion, growth and osteogenic differentiation of murine

preosteoblastic MC3T3-E1 cells and primary rat osteoblasts (Kokkonen *et al.* 2007, 2008, 2012). Pectin gels, particularly those functionalized with RGD-containing oligopeptides, which serve as ligands for integrin adhesion receptors, have been proposed as injectable carriers for osteoblasts for bone tissue regeneration, due to their ability to keep immobilized cells viable and differentiating (Munarin *et al.* 2011). For potential bone tissue engineering, pectins have also been used in combination with other natural and synthetic polymers, such as chitosan (Coimbra *et al.* 2011) or polylactide (Liu *et al.* 2004).

Alginate

Alginate, also called alginic acid, is an anionic polysaccharide distributed widely in the cell walls of brown algae, where through binding with water it forms a viscous gum. Alginate is a linear copolymer with homopolymeric blocks of (1-4)-linked β -D-mannuronate and its C-5 epimer α -L-guluronate residues.

Alginate has been used for engineering and regeneration of almost all tissues in the human organism. It has been widely applied in the form of injectable hydrogels, e.g. for encapsulation and delivery of Langerhans islets (Johnson *et al.* 2011), ovarian follicles (Tagler *et al.* 2012) and stem cells for neural tissue engineering (Banerjee *et al.* 2009), bone tissue engineering (Zhou and Xu 2011) and skeletal muscle regeneration (Liu *et al.* 2013). Alginate hydrogel-based scaffolds were also tested for engineering of cartilage (Wan *et al.* 2011) and intervertebral discs (Renani *et al.* 2012). In combination with hydroxyapatite, alginate hydrogels were applied for regenerating the osteochondral interface (Khanarian *et al.* 2012). Alginate hydrogels incorporated with poly(ethylene glycol) molecules and antibodies served for capture of endothelial progenitor cells from human blood (Hatch *et al.* 2011).

In the form of porous scaffolds, alginate has been used for creating a capillary bed in newly reconstructed tissues (Yamamoto *et al.* 2010), and in the form of electrospun nanofibrous scaffolds, for constructing vascular replacements containing endothelial cells and smooth muscle cells (SMC) (Hajiali *et al.* 2011), and for skin tissue engineering (Jeong *et al.* 2012). Alginate was also a component of scaffolds for heart valve engineering (Hockaday *et al.* 2012), and in combination with gold nanowires, for cardiac tissue engineering (Dvir *et al.* 2011).

Agar

Agar is a polysaccharide in red algae, serving as the primary structural support for their cell walls. Agar is a mixture of two components: the linear polysaccharide agarose, and a heterogeneous mixture of smaller molecules called agaropectin. Agarose, the predominant component of agar, is a linear polymer, consisting of a repeating monomeric unit of agarobiose. Agarobiose is a disaccharide containing D-galactose and 3,6-anhydro-L-galactopyranose. Agaropectin is a heterogeneous mixture of smaller molecules that occur in lesser amounts, and is made up of alternating units of D-galactose and L-galactose, heavily modified with acidic side-groups, such as sulfate and pyruvate (for a review, see McHugh 1987).

Agar is well-known as growth substrate for bacteria and other microbes, though it has also been used for cultivating cells of higher plants in order to develop effective methods for large-scale production of artificial seeds (Al-Hajry *et al.* 1999). Another well-known application is in testing the migratory potential and invasiveness of various cell types, including cells for tissue engineering (Ramaswamy *et al.* 2012), stem cells for cell therapies (Sabapathy *et al.* 2012) and for identifying cancer cells (for a review, see Discher *et al.* 2005).

Other current applications of agar-based materials are in testing the cytotoxicity of various drugs, chemicals and also artificial materials for tissue engineering (Korkmaz *et al.* 2007, Verma *et al.* 2009), and in testing the effects of mechanical loading on cell behavior (Shelton *et al.* 2003). Foils made of soy agar and collagen gel served as substrates for the deposition of cells by thermal inkjet printing, i.e. an advanced technology developed for tissue engineering (Xu *et al.* 2005). Similarly as alginate, agar can be used for cell encapsulation in tissue engineering applications. Due to its chondrogenic potential, agar was selected to entrap chondrocytes within poly-L-lactide scaffolds (Gong *et al.* 2007). In combination with gelatin, agar has been used for engineering nucleus pulposus (Strange and Oyen 2012), and in combination with hydroxyapatite and hyaluronic acid, for bone tissue engineering (Wagner *et al.* 2007). Composite membranes made of agar and type I collagen proved to be promising wound dressings for healing burns or ulcers (Bao *et al.* 2008). Agar gel, attached to the luminal surface of a microporous acrylate tube, supported the formation of *in vivo* tissue engineered autologous vascular prosthetic tissues, called “biotubes”

(Nakayama and Tsujinaka 2014). Agarose was used for neural tissue engineering (Bellamkonda *et al.* 1995) and as a vocal fold substitute (Choo *et al.* 2010). Together with hyaluronan and water, agar was used for creating an artificial vitreous humor (Kummer *et al.* 2007).

Dextran and pullulan

Dextran is a branched glucan (i.e. a polysaccharide composed of many glucose units) containing chains of varying lengths (from 3 to 2000 kDa). Dextran is synthesized from sucrose by certain lactic-acid bacteria (*Leuconostoc mesenteroides*, *Streptococcus mutans*, *Lactobacillus brevis*). In medical practice, it is used particularly for its antithrombotic properties. For tissue engineering applications, it has been used in combination with pullulan, another polysaccharide consisting of maltotriose units and produced from starch by the fungus *Aureobasidium pullulans*. Dextran and pullulan have been applied particularly in vascular and bone tissue engineering. In the form of electrospun nanofibrous scaffolds, dextran and pullulan promoted the development of a stable confluent monolayer of endothelial cells, and also the transition of vascular SMC from proliferative phenotype to quiescent contractile phenotype *in vitro* (Shi *et al.* 2012b). In the form of porous scaffolds, dextran and pullulan supported the viability, proliferation, differentiation and function of human endothelial progenitor cells isolated from cord blood (Lavergne *et al.* 2012). In bone tissue engineering, porous dextran/pullulan scaffolds, pure or supplemented with nanocrystalline hydroxyapatite, induced osteogenic cell differentiation *in vitro* and the formation of mineralized bone tissue *in vivo* (Fricain *et al.* 2013).

Gellan and xanthan

Gellan and xanthan have been shown to be excellent carriers for growth factors and matrices for several tissue engineering applications. These materials are able to gelify *in situ* within seconds, to retain large quantities of water, and thus to provide a similar environment to that of natural ECM (Khang *et al.* 2012, Dyondi *et al.* 2013). In addition, their mechanical properties can be fine-tuned to mimic the replaced natural tissues. Gellan/xanthan gels loaded with chitosan nanoparticles, basic fibroblast growth factor (bFGF), and bone morphogenetic protein 7 (BMP-7) promoted the differentiation of human fetal osteoblasts. At the same time, these gels showed antibacterial effects against

Pseudomonas aeruginosa, *Staphylococcus aureus* and *Staphylococcus epidermidis*, i.e. major pathogens causing the failure of bone implants (Dyondi *et al.* 2013). Gellan gum was loaded with alkaline phosphatase in order to support enzymatic mineralization of scaffolds for bone tissue engineering (Douglas *et al.* 2012). Gellan gum also supported the viability of encapsulated cells of the nucleus pulposus, and thus it holds promise in the construction of intervertebral disc replacements (Khang *et al.* 2012). A xanthan gum derivative was successfully used for encapsulation and delivery of chondrocytes for potential cartilage tissue engineering (Mendes *et al.* 2012).

Glycosaminoglycans

Glycosaminoglycans (GAGs) are long unbranched polysaccharides consisting of a repeating disaccharide unit. The general disaccharide unit consists of an N-acetyl-hexosamine and a hexose or hexuronic acid, either or both of which may be sulfated. The only GAG that is exclusively non-sulfated is hyaluronan, and sulfated GAGs include heparin, chondroitin sulfate, dermatan sulfate, keratan sulfate and heparan sulfate. In addition to sulfating, the members of the glycosaminoglycan family vary in the type of hexosamine, hexose or hexuronic acid unit that they contain (e.g. glucuronic acid, iduronic acid, galactose, galactosamine, glucosamine). They also vary in the geometry of the glycosidic linkage (for a review, see Collins and Birkinshaw 2013, Schnabelrauch *et al.* 2013).

Hyaluronan (also called hyaluronic acid or hyaluronate) is an anionic, non-sulfated GAG, which is an important component of ECM and synovial fluid. As hyaluronan is an important component of cartilage, it has been widely used for cartilage tissue engineering. To date, there are almost 3000 papers in the PubMed database dealing with the role of hyaluronan in cartilage tissue engineering, reconstruction and regeneration. Hyaluronan has been used mainly for engineering articular cartilage (Erickson *et al.* 2012, Kim *et al.* 2013), but also for meniscus reconstruction (Zellner *et al.* 2010), osteochondral defects (Filová *et al.* 2008, Galperin *et al.* 2013), tracheal defects (Hong *et al.* 2012) and intervertebral discs (Park *et al.* 2012). For these purposes, hyaluronan has been applied in the form of a hydrogel with encapsulated chondrocytes (Filová *et al.* 2008, Park *et al.* 2012) or mesenchymal stem cells (Zellner *et al.* 2010, Erickson *et al.* 2012, Kim *et al.* 2013). It is also possible to create hyaluronan fibers of submicron- and

nano-size by an electrospinning technique (Kim *et al.* 2013) or hyaluronan-based porous scaffolds (Yan *et al.* 2013). For constructing these scaffolds, hyaluronan has often been combined with other natural and synthetic polymers, such as collagen I and fibrin (Filová *et al.* 2008, Hong *et al.* 2012), silk (Park *et al.* 2012), chitosan (Chen *et al.* 2013) or methacrylates (Galperin *et al.* 2013), and for bone tissue engineering, also with a mineral component, e.g. calcium phosphates (Chen *et al.* 2013, Galperin *et al.* 2013). Hyaluronan is also a major component of skin, and it has therefore been applied in dermal tissue engineering (Mineo *et al.* 2013, Yan *et al.* 2013). Other tissue engineering applications of hyaluronan include reconstruction of heart valves (Duan *et al.* 2013), myocardial tissue (Dahlmann *et al.* 2013), skeletal muscle tissue (Desiderio *et al.* 2013), and also small-caliber arteries (diameter 2–4 mm). Hyaluronan-based biodegradable vascular grafts implanted into pigs supported spontaneous development of a neoartery segment composed of mature smooth muscle cells (SMC), collagen, and elastin fibers organized in layers and completely covered by endothelial cells on the luminal surface (Zavan *et al.* 2008). Hyaluronan has also been reported to play an important role in the onset and development of atherosclerosis and other vascular diseases, namely by its stimulatory effects on migration and proliferation of vascular SMC (Vigetti *et al.* 2008). This behavior of SMC can also lead to stenosis and failure of vascular replacements. However, these negative effects are typical for hyaluronan with low molecular weight (less than 500 kDa), while native high molecular weight hyaluronan (more than 1000 kDa) has been reported to have antimigratory and antiproliferative effects on SMC (Kothapalli *et al.* 2010). Antiproliferative activity of hyaluronan can also be induced by O-sulfonation of hyaluronan molecules (Garg *et al.* 1999).

The presence of sulfur in the molecules of GAGs is generally associated with antiproliferative effects on SMC. In adult healthy vascular wall, sulfated GAGs keep SMC in quiescent differentiated contractile phenotype (Glukhova and Kotliansky 1995). These effects can also be exploited in vascular tissue engineering. For example, SMC in hybrid vascular grafts, constructed on knitted Dacron grafts using endothelial cells, SMC, fibroblasts and artificial ECM consisting of type I collagen and dermatan sulfate, were predominantly of contractile phenotype after 12-week-implantation into dogs (Ishibashi and Matsuda 1994). Sulfated GAGs also

supported endothelialization of vascular grafts and their antithrombogenic properties. Expanded polytetrafluoroethylene (ePTFE) vascular grafts coated with perlecan, i.e. the major heparan sulfate proteoglycan in cell basement membranes, stimulated the growth of endothelial cells and suppressed the adhesion of platelets (Lord *et al.* 2009). The retention of endothelial cells on poly(carbonate-urea)urethane vascular grafts under shear stress was enhanced by modifying these grafts with a matrix consisting of collagen type IV and dermatan sulfate (Salacinski *et al.* 2001). Heparin in vascular grafts improved not only their antithrombogenicity, but also the retention of growth factors for endothelial cells, such as vascular endothelial growth factor (VEGF) (Ye *et al.* 2012) and basic fibroblast growth factor (bFGF) (Pitarresi *et al.* 2013). In addition, heparin enabled stable attachment of stromal cell-derived factor-1 α (SDF-1 α) to vascular prostheses, which served as a capture molecule for endothelial and smooth muscle progenitor cells from blood (Yu *et al.* 2012).

Heparin and other sulfated GAGs have also been applied in many other areas of tissue engineering. For example, heparin was used for bioactivation of electroconductive polypyrrole-based scaffolds for bone tissue engineering (Meng *et al.* 2013) and for synthesis of carbonated apatites, which proved to be more favorable to the proliferation and differentiation of MC3T3-E1 pre-osteoblasts than apatites prepared by a traditional method (Deng *et al.* 2013). Heparin was also a component of polymeric scaffolds for neural tissue engineering (Kuo and Wang 2012). In the form of hydrogels, heparin has been used for efficient seeding of chondrocytes on polymeric scaffolds for cartilage tissue engineering (Kim *et al.* 2012), for modifying scaffolds for liver tissue engineering (Bao *et al.* 2011) and for encapsulating dermal fibroblasts for skin tissue engineering (Choi and Yoo 2013). Keratan sulfate took part in cornea tissue engineering (Wu *et al.* 2013), and chondroitin and dermatan sulfates in the reconstruction of bone, cartilage and skin (Vandrovcova *et al.* 2011, for a review, see Schnabelrauch *et al.* 2013).

Polysaccharides and inflammatory reactions

Like other biological and artificial materials used in tissue engineering, polysaccharides are associated with a risk of undesirable immune activation of cells and inflammatory reactions. The reaction is usually manifested by expression of immunoglobulin and selectin

molecules on cells contacting the material. These molecules are e.g. intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and endothelial-leukocyte adhesion molecule-1 (ELAM-1). Other inflammatory markers include the production of cytokines (e.g. tumor necrosis factors and interleukins), infiltration with leucocytes, lymphocytes and macrophages, foreign body reaction and fibrous encapsulation of the implant. Some polysaccharide-based materials evoke a relatively severe immune reaction. For example, hyaluronan-based scaffolds (Hyalograft C) for engineering cartilage for laryngotracheal reconstruction implanted into rabbits induced a foreign body reaction leading to complete degradation of the neocartilage and failure of the implant (Weidenbecher *et al.* 2007). Intra-articular injections of Hylan G-F 20, a hyaluronan-based synovial fluid replacement, caused an acute inflammatory reaction with the activation of complement and local invasion of pro-inflammatory cells in a human patient with osteoarthritis (Dragomir *et al.* 2012). The immunogenicity of hyaluronan has been attributed to the presence of low-molecular weight fragments in this material, but it seems to be related rather to the presence of contaminating DNA in hyaluronan preparation (Filion and Phillips 2001). Paradoxically, alginate, which is often used for encapsulation and immunoprotection of cells, can also evoke an immune reaction. This is mainly due to impurities in this material, e.g. contamination with mitogens and endotoxins (Zimmermann *et al.* 1992, Breger *et al.* 2009). Other factors inducing immunogenicity of polysaccharides can be the agents used for their crosslinking, particularly glutaraldehyde (Lai 2012).

Nevertheless, in other polysaccharides, such as chitosan (VandeVord *et al.* 2002), gellan gum (Silva-Correia *et al.* 2013), bacterial cellulose (Helenius *et al.* 2006, Mendes *et al.* 2009, Esguerra *et al.* 2010) and dextran (Ferreira *et al.* 2005), the immunogenicity was classified as mild. Adding pullulan to dextran increased its immunogenic activity (Abed *et al.* 2011). The immune response to biodegradable starch-based materials was even slighter than the reaction to other currently used synthetic and natural polymeric materials (Marques *et al.* 2005). Similarly, titanium coated with heparin/fibronectin complex revealed a weaker inflammatory response than pristine Ti (Li *et al.* 2011).

Cellulose in vascular tissue engineering

The specialized part of this review is focused on the potential use of cellulose in blood vessel reconstruction. One of the first attempts at vascular tissue engineering was carried out with microfibers made of regenerated cellulose, i.e. purified plant cellulose chemically converted from short fibers (coming e.g. from trees) into long fibers used in textiles and nonwovens. These fibers were applied for constructing three-dimensional vascularized tissue *in vitro*. They were immobilized with fibronectin in order to improve cell adhesion, and were seeded with bovine coronary artery SMC. These cells proliferated on the scaffolds and, after they formed multilayers on the fibers, the fibers were removed by enzymatic digestion using cellulase. The remaining SMC aggregates maintained lumens after this procedure, and thus mimicked newly-formed blood vessels (Ko and Iwata 2001). Further experiments were performed on three-dimensional nanofibrous scaffolds with micropores made of bacterial cellulose. These scaffolds allowed the attachment and proliferation of human saphenous vein SMC on the surface and also in the inside of the scaffolds (Backdahl *et al.* 2008). In addition, the mechanical properties of nanofibrous bacterial cellulose scaffolds, evaluated by the shape of the stress-strain response, were reminiscent of the properties of the carotid artery, most probably due to the similarity in architecture of the nanofibril network (Backdahl *et al.* 2006).

Cellulose-based scaffolds were attractive not only for vascular SMC, but also for vascular endothelial cells. The adhesion, spreading, formation of an actin cytoskeleton, proliferation, metabolic activity and maturation of human saphenous vein cells was supported by nanofibrous bacterial cellulose scaffolds, particularly scaffolds functionalized with xyloglucan bearing RGD-containing oligopeptides, i.e. ligands for integrin adhesion receptors on cells (Bodin *et al.* 2007, Fink *et al.* 2011). Similarly, the population densities of endothelial cells and the spontaneous formation of capillary tube-like structures *in vitro* were improved on nano- and micro-fibrous cellulose acetate scaffolds after they were combined with chitosan (Rubenstein *et al.* 2007). The angiogenic response to cellulose was also observed under *in vivo* conditions, i.e. after implantation of bacterial cellulose scaffolds in the form of a dorsal skinfold chamber into Syrian golden hamsters (Esguerra *et al.* 2010).

Cellulose has also been used for creating tubular structures designed for replacing small-caliber vessels. The construction of functional small-caliber vascular replacements is relatively complicated, because these grafts are associated with the highest risk of restenosis and failure. Optimal prevention of restenosis is achieved by covering the inner surface of these grafts with endothelial cells. Wippermann *et al.* (2009) created hollow-shaped segments of bacterial cellulose 10 mm in length, 3.0–3.7 mm in inner diameter, and 0.6–1.0 mm in wall thickness. These grafts were used to replace the carotid arteries of eight pigs. After a follow-up period of 3 months, seven grafts (87.5 %) remained patent, whereas one graft was found to be occluded. All patent grafts developed not only a single inner layer of endothelium with a basement membrane and a thin layer of collagen, but also a concentric medial layer containing SMC and cellulose, and an outer layer of fibrous cells.

Similarly, bacterial cellulose grafts 4 cm in length and 4 mm in internal diameter were implanted bilaterally in the carotid arteries of eight sheep. Although 50 % of the grafts occluded within two weeks, all patent grafts developed a confluent inner layer of endothelial-like cells (Malm *et al.* 2012). In addition, the mechanical properties of tubular structures created from bacterial cellulose seemed to be advantageous for vascular tissue engineering. For example, these structures exhibited a compliance response similar to that of human saphenous vein, and even higher than in vascular grafts made of expanded polytetrafluoroethylene (ePTFE) and polyethylene terephthalate (PET; Dacron), i.e. in prostheses currently used in clinical practice. In addition, the surface of bacterial cellulose was less thrombogenic when compared with ePTFE and Dacron (Zahedmanesh *et al.* 2011).

In spite of all these encouraging findings, cellulose is not an ideal scaffold for tissue engineering, due to its low degradation ability. As mentioned above, ideal scaffolds used in advanced tissue engineering should be constructed from resorbable materials that degrade proportionally to the tissue regeneration and are gradually replaced by a newly-formed functional tissue (Collins and Birkinshaw 2013). However, cellulose in the human organism behaves as a non-degradable or very slowly degradable material. For example, the degradation time of viscose cellulose sponges implanted subcutaneously into rats was longer than 60 weeks (Märtson *et al.* 1999). This very slow degradability of cellulose is due to the absence of enzymes that attack the

$\beta(1\rightarrow4)$ linkage. These enzymes are present in microbial and fungal cells (for a review, see Märtson *et al.* 1999). In our experiments, we have therefore focused on inducing and controlling the cellulose degradability. An efficient method for inducing degradability of cellulose is oxidation. Oxidized cellulose can be generated by various techniques and by various oxidizing agents, such as the $\text{HNO}_3/\text{H}_3\text{PO}_4\text{-NANO}_2$ system, CCl_4 , periodate or 2,2,6,6-tetramethylpiperidiny-1-oxyl radical (TEMPO) combined with NaClO (Roychowdhury and Kumar 2006, Saito *et al.* 2009, for a review, see Novotná *et al.* 2013). Oxidized cellulose is degradable by hydrolysis, mediated by hydrolytic enzymes present in the serum supplement of cell culture media *in vitro* and in macrophages *in vivo* (Dimitrijevič *et al.* 1990a,b).

Cellulose oxidation induces conversion of the glucose residues to glucuronic acid residues containing $-\text{COOH}$ groups. The concentration of these groups modulates not only the degradation time of cellulose, but also its pH, its swelling in a water environment, its mechanical stability, its drug loading efficiency, and other behavior of the material (Zhu *et al.* 2011). In addition, the $-\text{COOH}$ groups, which are polar and negatively charged, can be used for functionalizing the oxidized cellulose with various biomolecules (Zimnitsky *et al.* 2005, 2006).

Oxidized cellulose has been widely used as a hemostatic and wound healing material. It is highly absorbable, able to initiate or accelerate blood coagulation, and displays antibacterial and antiviral effects. It is also a promising carrier for controlled drug delivery (for a review, see Novotná *et al.* 2013). Oxidized dialdehyde cellulose (DAC) has been applied as a biodegradable scaffold for tissue engineering. Microporous DAC membranes supported adhesion, proliferation and ECM formation in human neonatal skin fibroblast cells (Roychowdhury and Kumar 2006), and also proved to be suitable for engineering vocal fold lamina propria (Roychowdhury *et al.* 2009).

Our experiments were performed on cellulose-based materials modified by oxidation and/or functionalization with biomolecules. We have prepared fibrous scaffolds made of non-oxidized viscose, dialdehyde cellulose and 6-carboxycellulose with 2.1 wt. % or 6.6 wt. % of $-\text{COOH}$ groups. In addition, all these material types were functionalized with arginine, i.e. an amino acid with a basic side chain, or with chitosan, in order to balance the relatively acid character of oxidized cellulose molecules. Two groups of samples,

with and without functionalization, were then seeded with SMC derived from rat thoracic aorta by an explantation method (Novotná *et al.* 2009, 2013).

We found that oxidized cellulose with 2.1 wt. % of $-COOH$ groups was the most appropriate of all the tested materials for colonization with SMC. The cells on this material achieved an elongated shape, while they were rather spherical in shape on the other materials (Fig. 1). In addition, the number of cells found one week after seeding and the concentration of contractile proteins alpha-actin and SM1 and SM2 myosins, measured per mg of protein, were significantly higher on oxidized cellulose with 2.1 wt. % of $-COOH$ groups. Functionalization of

the material with arginine and chitosan further improved the phenotypic maturation of SMC, which was manifested by a further increase in the concentration of alpha-actin and SM1 and SM2 myosins in these cells. Chitosan also improved the adhesion and growth of SMC. However, in comparison with the control polystyrene dishes, the proliferation activity of the cells on our cellulose-based materials was relatively low. This suggests that these materials can be used in applications where high proliferation activity of cells is not desirable, e.g. proliferation of SMC on vascular prostheses, which can lead to restenosis of the graft.

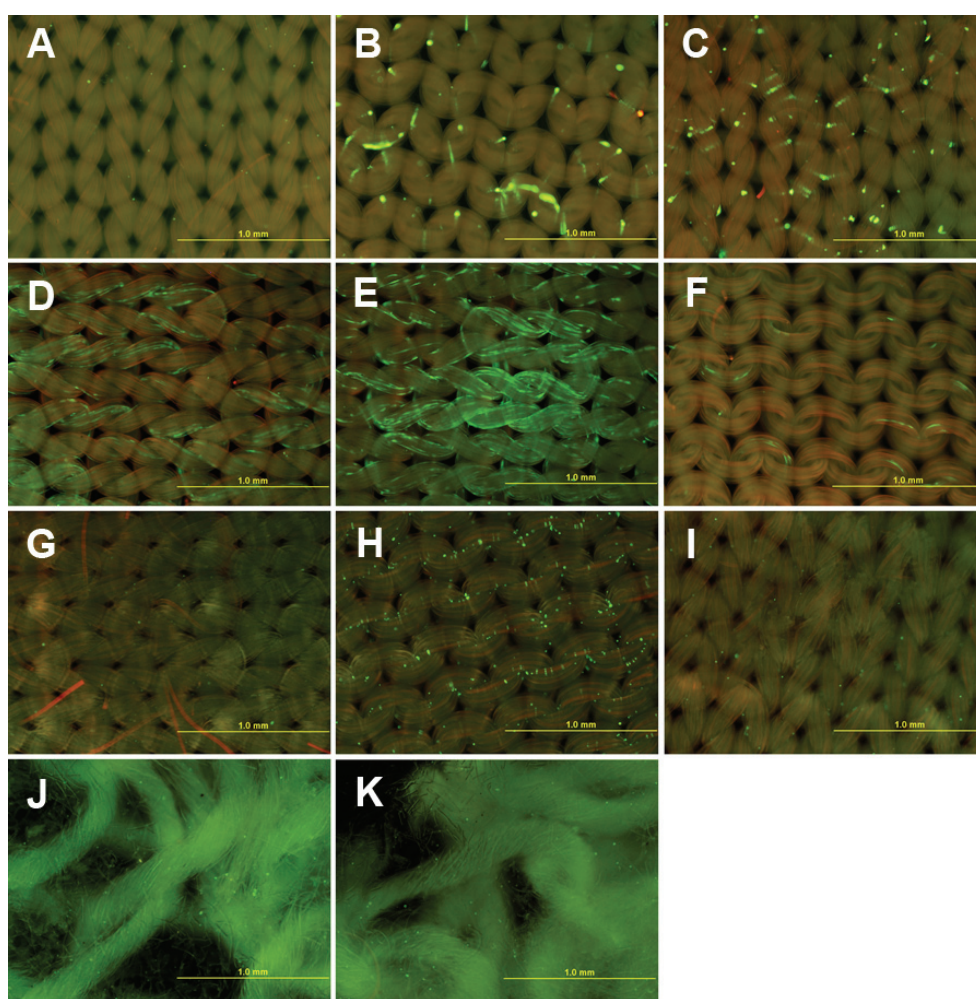


Fig. 1. Morphology and viability of vascular smooth muscle cells on day 7 after seeding on the following cellulose-based materials: viscose (**A**, **B**, **C**); oxidized cellulose with 2.1 wt. % of $-COOH$ groups (**D**, **E**, **F**); oxidized cellulose with 6.6 wt. % of $-COOH$ groups (**G**, **H**, **I**); dialdehyde cellulose (**J**, **K**). **A**, **D**, **G**, **J**: materials with no modification; **B**, **E**, **H**, **K**: materials functionalized with chitosan; **C**, **F**, **I**: materials functionalized with arginine. LIVE/DEAD staining, obj. 4x. Olympus IX 51 microscope, DP 70 digital camera, obj. 4x, bar = 1 mm.

On the other hand, 6-carboxycellulose with 2.1 wt. % of $-COOH$ groups, particularly if functionalized with arginine or chitosan, provided better support for SMC growth than corresponding groups of 6-carboxycellulose with 6.6 wt. % of $-COOH$ groups, viscose and dialdehyde cellulose. In the case of 6-carboxycellulose with 6.6 wt. % of $-COOH$, this was

probably due to the relatively high acidity of this material, and also due to its lower stability in the cell culture environment and during manipulation. In other words, the content of 6.6 wt. % $-COOH$ groups appeared to be too high for this material to be biocompatible and stable. In addition, the stability of dialdehyde cellulose proved to be very low, probably because of a specific

arrangement of the fibers in its fabric, which resembled a loose network, while in oxidized cellulose or viscose the fibers were densely packed in thick rope-like bundles (Fig. 1). The highest stability was observed in viscose materials, which had almost no tendency to degrade. The stability of oxidized cellulose with 2.1 wt. % of –COOH was sufficiently high, as it did not disintegrate while the samples were being handled, and its degradation in the cell culture system was relatively slow in comparison with oxycellulose with 6.6 wt. % of –COOH and dialdehyde cellulose (Novotná *et al.* 2009, 2013).

Similar results were obtained in our earlier study performed on polyethylene (PE) foils doped with various concentrations (0–20 wt. %) of calcium salt of oxidized cellulose (OKCEL Ca-L, Synthesia, Pardubice, Czech Republic). The surface of the samples was polished in order to enhance the exposure of oxidized cellulose to the cells. The samples were seeded with SMC derived from rat aorta. On all samples doped with oxidized cellulose, the cells proliferated better than on non-doped PE, but the highest cell numbers were found on samples with lower concentrations of oxidized cellulose, i.e. 1–5 wt. % (Mikulíková *et al.* 2005).

Conclusions

Polysaccharides have proved to be very important biological polymers indispensable in advanced tissue engineering, cell therapies and regenerative medicine. They are equally important as widely-used synthetic degradable and non-degradable polymers, such as polylactides, polyglycolides, polycaprolacton, PTFE and PET, though they often seem to be overshadowed by

these polymers. In the form of injectable hydrogels, polysaccharides, particularly alginate and hyaluronan, are essential for encapsulation and delivery of cells directly to the organism (pancreatic islets, ovarian follicles) or to three-dimensional scaffolds for tissue engineering. Polysaccharides can also be prepared in the form of porous or fibrous scaffolds, the latter often being created by an advanced electrospinning technique. Polysaccharides are also important biomolecules for further modification of various polymeric and other materials designed for tissue replacement, in order to increase their bioactivity, e.g. cell retention. For specific purposes, such as engineering of hard or conductive tissues, polysaccharides can also be combined with ceramic, carbon and metallic nanoparticles. Polysaccharides are also important carriers for the delivery of growth factors and drugs to cells. Thus, polysaccharides are promising materials for further development and for broader applications in clinical practice.

Conflict of Interest

There is no conflict of interest.

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