Carbohydrate Polymers 152 (2016) 129–139

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Gelatin- and starch-based hydrogels. Part A: Hydrogel development, characterization and coating

Ine Van Nieuwenhove[a], Achim Salamon[b], Kirsten Peters[b], Geert-Jan Graulus[a], José C. Martins[c], Daniel Frankel[d], Ken Kersemans[e], Filip De Vos[e], Sandra Van Vlierberghe[a,f,*], Peter Dubruel[a,*]

[a] Polymer Chemistry & Biomaterials Group, Ghent University, Krijgslaan 281, Building S4-Bis, BE-9000 Ghent, Belgium
[b] Department of Cell Biology, Rostock University Medical Center, Schillingallee 69, D-18057 Rostock, Germany
[c] NMR and Structure Analysis Research Group, Ghent University, Krijgslaan 281, Building S4, BE-9000 Ghent, Belgium
[d] School of Chemical Engineering and Advanced Materials, University of Newcastle, Mertz Court, Claremont Road, NE1 7RU Newcastle Upon Tyne, United Kingdom
[e] Laboratory of Radiopharmacy, Ghent University, Ottergemsesteenweg 460, BE-9000 Ghent, Belgium
[f] Brussels Photonics Team, Vrije Universiteit Brussel, Pleinlaan 2, BE-1050 Brussels, Belgium

ARTICLE INFO

Article history:
Received 7 April 2016
Received in revised form 17 June 2016
Accepted 26 June 2016
Available online 27 June 2016

Keywords:
Gelatin
Starch
Biomaterials
Aggrecan
Tissue engineering

ABSTRACT

The present work aims at constructing the ideal scaffold matrix of which the physico-chemical properties can be altered according to the targeted tissue regeneration application. Ideally, this scaffold should resemble the natural extracellular matrix (ECM) as close as possible both in terms of chemical composition and mechanical properties. Therefore, hydrogel films were developed consisting of methacrylamide-modified gelatin and starch-pentenoate building blocks because the ECM can be considered as a crosslinked hydrogel network consisting of both polysaccharides and structural, signaling and cell-adhesive proteins. For the gelatin hydrogels, three different substitution degrees were evaluated including 31%, 72% and 95%. A substitution degree of 32% was applied for the starch-pentenoate building block. Pure gelatin hydrogels films as well as interpenetrating networks with gelatin and starch were developed. Subsequently, these films were characterized using gel fraction and swelling experiments, high resolution-magic angle spinning ^{1}H NMR spectroscopy, rheology, infrared mapping and atomic force microscopy. The results indicate that both the mechanical properties and the swelling extent of the developed hydrogel films can be controlled by varying the chemical composition and the degree of substitution of the methacrylamide-modified gelatin applied. The storage moduli of the developed materials ranged between 14 and 63 kPa. Phase separation was observed for the IPNs for which separated starch domains could be distinguished located in the surrounding gelatin matrix. Furthermore, we evaluated the affinity of aggrecan for gelatin by atomic force microscopy and radiolabeling experiments. We found that aggrecan can be applied as a bioactive coating for gelatin hydrogels by a straightforward physisorption procedure. Thus, we achieved distinct fine-tuning of the physico-chemical properties of these hydrogels which render them promising candidates for tissue engineering approaches.

1. Introduction

The lack of acutely available organs for transplantation is a worldwide issue which is even expected to worsen as the world population ages. Tissue engineering is an approach aiming at bridging this gap (Furth, Atala, & Van Dyke, 2007; Griffith & Naughton, 2002; Langer, 1997; Langer & Vacanti, 1993; Lemons, 2013). In this approach, cells are seeded onto scaffolds or implants to develop into functional tissues (Drury & Mooney, 2003; Gomillion & Burg, 2006; Liu, Xia, & Czernuszka, 2007; Lutolf & Hubbell, 2005; Peters et al., 2009). In addition, an increasing number of procedures can be found in literature which rely on the application of stem cells (Barry & Murphy, 2004; Gomillion & Burg, 2006; Griffith & Naughton, 2002; Gimble et al., 2007; Peters et al., 2009). Using mesenchymal stem cells (MSC), the present study aims at a scaffold guided strategy towards tissue regeneration. The constructed scaffold is

* Corresponding authors at: Polymer Chemistry & Biomaterials Group, Ghent University, Krijgslaan 281, Building S4-Bis, BE-9000 Ghent, Belgium.
E-mail addresses: sandra.vanvlierberghe@ugent.be (S. Van Vlierberghe), peter.dubruel@ugent.be (P. Dubruel).

http://dx.doi.org/10.1016/j.carbpol.2016.06.098
0144-8617/

a three-dimensional matrix serving as a surrogate extracellular matrix (ECM) enabling cell attachment and promoting cell proliferation as well as differentiation. The design of a scaffold resembling the natural ECM is preferred in order to mimic as closely as possible the natural aqueous environment that cells are experiencing (Chen, Wang, Wei, Mo, & Cui, 2010; Kim, Kim, & Salih, 2005; Kuo, Chen, Hsiao, & Chen, 2015). This natural ECM can be considered as a crosslinked hydrogel network consisting of polysaccharides as well as structural, signaling and cell-adhesive proteins. Taking this knowledge into consideration, it is of great interest to evaluate the potential of polymer networks mimicking this ECM composition. Therefore, gelatin and starch are applied as natural building blocks in the present work, representing both the protein and polysaccharide constituent of the natural ECM.

Gelatin is derived from collagen, which is the most abundant structural protein in mammals (Di Lullo, Sweeney, Korkko, Ala-Kokko, & San Antonio, 2002). In addition, it is generally non-immunogenic and retains informational signals including an arginine-glycine-aspartic acid (RGD) sequence which promotes cell adhesion, differentiation and proliferation (Gautam, Dinda, & Mishra, 2013). These properties and its unique gel-forming ability render gelatin an interesting biopolymer towards tissue engineering applications (Awad, Quinn Wickham, Leddy, Gimble, & Guilak, 2004; Dubruel et al., 2007; Li et al., 2005; Nichol et al., 2010). Starch, on the other hand, consists of a mixture of the polysaccharides amylose and amylopectin. The relative ratio of amylose to amylopectin strongly depends on the starch source considered. The application of starch offers several advantages including its biodegradability and ease of processing (Azevedo, Gama, & Reis, 2003; Puppi, Chiellini, Piras, & Chiellini, 2010). Starch-based polymers as well as blends have already been introduced as promising biomaterials for bone and cartilage tissue engineering applications due to these advantages. For instance, Mendes et al. (2001) showed the potential of starch/ethylene vinyl alcohol blends reinforced with hydroxyapatite for temporary bone replacement implants. Raafat, Eldin, Salama, and Ali (2013) developed a hydrogel series composed of starch/*N*-vinylpyrrolidone which were proven to exhibit *in vitro* bioactivity and blood compatiblity. Moreover, gelatin and starch are often combined for several food processing applications (Burey, Bhandari, Rutgers, Halley, & Torley, 2009; Firoozmand, Murray, & Dickinson, 2009; Marrs, 1982).

In this work, hydrogels were developed consisting of either a gelatin phase or the combination of both a starch and a gelatin phase. In the latter case, these hydrogels are so-called interpenetrating polymer networks (IPNs) if the appropriate crosslinking strategy is applied ensuring both building blocks to be covalently crosslinked but not bonded to each other (Alemán et al., 2007). The potential of gelatin hydrogels in contact with adipose tissue derived mesenchymal stem cells (adMSCs) was already demonstrated by Peters et al. (2009) towards the adhesion of these cells. Therefore, we selected the gelatin hydrogels as reference material for the IPNs of starch and gelatin. Pure starch hydrogels were not applied as these hydrogels were shown to be too brittle to process them in hydrogel films. To the best of our knowledge, we first reported on the combination of starch and gelatin in IPNs for the purpose of tissue engineering applications. Indeed, previous results reported by Van Nieuwenhove et al. (2015) on starch-based hydrogels were promising since the hydrogels developed in contact with adMSC were shown to be biocompatible.

IPNs have gained an increased attention the last decades mainly due to their high potential as hydrogels for biomedical applications (Dragan, 2014). However, most of the hybrid IPNs hydrogels, reported in literature, are obtained by either combining various polysaccharides or synthetic polymers and proteins with synthetic polymers (Dragan, 2014; La Gatta, Schiraldi, Esposito, D'Agostino, & De Rosa, 2009; Peng, Yu, Mi, & Shyu, 2006; Pescosolido et al., 2011). Only a few papers report on the combination of proteins and polysaccharides for the construction of (semi)-IPNs (Cui, Jia, Guo, Liu, & Zhu, 2014; Liu & Chan-Park, 2009; Picard, Doumèche, Panouillé, & Larreta-Garde, 2010; Turgeon & Beaulieu, 2001).

The present work focusses on the construction of the ideal scaffold matrix of which the physico-chemical properties can be altered according to the targeted tissue regeneration application. The latter is highly relevant as natural tissue is also characterized by different mechanical properties. Thus, altering the mechanical properties of the constructed hydrogel films is of great interest. For instance breast tissue, mainly composed of adipose tissue, is characterized by a storage modulus of 3.2 kPa (Abbas, Judit, & Donald, 2007), whereas the storage modulus of articular cartilage is in the range of 2–7 GPa (Silver, Bradica, & Tria, 2002). Due to their soft and rubbery consistence, hydrogels do not reveal such high storage moduli. However, these hydrogels can still be applicable as coating onto implants to target orthopedic applications.

For this reason, hydrogel films were prepared with varying chemical composition (*i.e.* ratio between gelatin and starch phase) and varying degree of substitution (DS) of the gelatin phase applied. First, gelatin and starch were chemically modified with photo-crosslinkable moieties. This modification enables their subsequent processing into hydrogel films and ensures sufficient stability of the materials upon *in vitro* application. In addition, the present work will evaluate whether a bioactive coating of aggrecan, the main articular cartilage constituent, can be deposited onto the materials *via* physisorption. More specifically, liquid atomic force microscopy and radiolabeling experiments will be performed to study this hydrogel coating.

2. Experimental section

2.1. Materials

For all the synthesis experiments, gelatin (type B), from bovine bone origine, was applied (Rousselot, Gent, Belgium). Furthermore, dimethyl sulfoxide (DMSO, 99.85%) was purchased from Acros (Geel, Belgium) and purified *via* distillation before use. Irgacure® 2959 was applied as photo-initiator (BASF, Kaisten, Switzerland) and dithiothreitol (Fisher Scientific, Erembodegem, Belgium) was used as a bifunctional thiol-based crosslinker agent. All other chemicals were purchased from Sigma Aldrich (Bornem, Belgium) and were used as received unless stated otherwise. The radiolabeling experiments were performed using Iodogen (1,3,4,6-tetrachloro-3a,6a-diphenyl-glycouril) obtained from Pierce (USA) and using a radioiodide solution (125I: Perkin Elmer, Massachusetts, USA).

2.2. Synthesis of hydrogel building blocks

Both the pentenoate-modified starch (SP) and the methacrylamide-modified gelatin (gel-MA) were synthesized as described earlier (Peters et al., 2009; Van Nieuwenhove et al., 2015). In brief, corn starch was dissolved in DMSO (5 w/v%, 70 °C), a catalytic amount of dimethylaminopyridine was added and the reaction mixture was stirred for 20 min. Subsequently, 4-pentenoic anhydride (37.5 equivalents with respect to the saccharide units) was added and reacted overnight. The purified product was obtained *via* precipitation in ethanol, followed by dialysis against double distilled water (MWCO: 12,000–14,000 Da) and freeze-drying by means of a Christ freeze-dryer alpha 2-4-LSC.

For the gelatin derivatives, the amount of crosslinkable side chains was adjusted by varying the amount of methacrylic anhydride added. Three different modifications were performed using 0.5, 1 and 2.5 equivalents methacrylic anhydride added with respect to the primary amines present along the gelatin backbone.