Carbohydrate Polymers 150 (2016) 408–418

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Hyaluronan delivery by polymer demixing in polysaccharide-based hydrogels and membranes for biomedical applications

Andrea Travan[a,*], Francesca Scognamiglio[a], Massimiliano Borgogna[a], Eleonora Marsich[b], Ivan Donati[a], Lorena Tarusha[a], Mario Grassi[c], Sergio Paoletti[a]

[a] Department of Life Sciences, University of Trieste, Via Licio Giorgieri 5, I-34127 Trieste, Italy
[b] Department of Medical, Surgical and Health Sciences, University of Trieste, Piazza dell'Ospitale 1, I-34129 Trieste, Italy
[c] Department of Engineering and Architecture, University of Trieste, Via Alfonso Valerio, 6/A, I-34127 Trieste, Italy

ARTICLE INFO

Article history:
Received 15 January 2016
Received in revised form 14 March 2016
Accepted 29 March 2016
Available online 31 March 2016

Keywords:
Polysaccharides
Hydrogels
Polymer demixing
Membranes
Wound healing activity

ABSTRACT

Alginate-based membranes containing hyaluronic acid (HA) were manufactured by freeze-drying calcium-reticulated hydrogels. The study of the distribution of the two macromolecules within the hydrogel enabled to highlight a polymer demixing mechanism that tends to segregate HA in the external parts of the constructs. Resistance and pliability of the membranes were tuned, while release and degradation studies enabled to quantify the diffusion of both polysaccharides in physiological solution and to measure the viable lifetime of the membranes. Biological studies *in vitro* proved that the liquid extracts from the HA-containing membranes stimulate wound healing and that fibroblasts are able to colonize the membranes. Overall, such novel alginate-HA membranes represent a promising solution for several medical needs, in particular for wound treatment, giving the possibility to provide an *in situ* administration of HA from a resorbable device.

1. Introduction

Novel polymer-based biomaterials in the form of surgical membranes, meshes and dressings are being developed for biomedical use in tissue engineering (Zhang, Zhang, Shi, & Miron, 2013), for the treatment of topical wounds and burns (Okamura et al., 2013; Plettig et al., 2014; Vasile, Pieptu, Dumitriu, Panzariu, & Profire, 2013; Xu et al., 2015), for the repair of hernias (Beale, Hoxworth, Livingston, & Trussler, 2012; Novitsky, 2013) and for the development of haemostatic devices (Granville-Chapman, Jacobs, & Midwinter, 2011; Smith, Laird, Porter, & Bloch, 2013; Gaharwar et al., 2014; Ohta et al., 2014). In the field of wound treatment, membranes based on both natural and synthetic polymers have been reported (Khil, Cha, Kim, Kim, & Bhattarai, 2003; Sionkowska, 2011). The composition of wound dressings can be optimized in order to tailor the physical-chemical properties of the biomaterial and to meet the needs of each wound stage (Fonder et al., 2008). For instance, Fleck and colleagues reported the possibility to modify the composition of membranes to tune their bioactive properties in terms of stimulating or accelerating the healing of the damaged tissue (Fleck & Chakravarthy, 2007). Several biopolymers from natural sources have been proposed as candidates to manufacture surgical membranes; the advantage of these materials is that their chemical structure can mimic the macromolecular environment of the extracellular matrix (ECM). Recently, membranes prepared from biopolymers like polysaccharides and collagen have been proposed for biomedical use (Fleck & Simman, 2010; Francesko & Tzanov, 2011; Cardoso, hado-Silva, Sabino, Santos, Jr., & Zavaglia, 2014; Lopes, Riegel-Vidotti, Grein, Tischer, & Faria-Tischer, 2014; Majumdar et al., 2015; Pandis et al., 2014; Zheng et al., 2014). Among natural polymers, polysaccharides like alginate, chitosan and hyaluronic acid (HA) offer several advantages in terms of biocompatibility, hydrophilicity and bioactive properties (Maggiori et al., 2010; Boateng, Matthews, Stevens, & Eccleston, 2008; Jayakumar, Prabaharan, Sudheesh Kumar, Nair, & Tamura, 2011; Powers, Morton, & Phillips, 2013). The use of 3D matrices based on the combination of several polysaccharides has been previously reported by some of the authors of this paper (Marsich et al., 2008; Marsich et al., 2013; Travan et al., 2009). HA and alginate are particularly appealing for the preparation of bioactive and biodegradable membranes for wound healing applications, as the former displays wound healing bioactivity (Dicker et al., 2014) while the latter is a biocompatible polysaccharide with

* Corresponding author.
E-mail address: atravan@units.it (A. Travan).

http://dx.doi.org/10.1016/j.carbpol.2016.03.088
0144-8617/

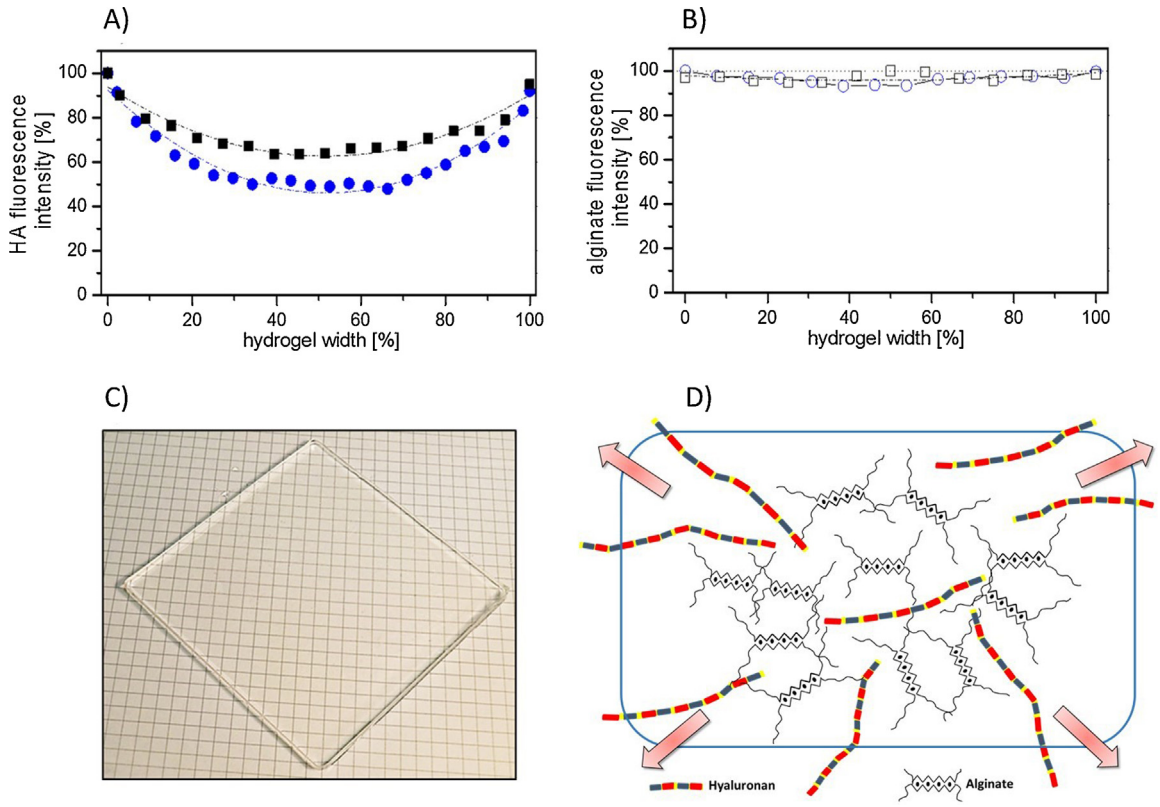

Fig. 1. Alginate-HA hydrogels characterization: (A) Profiles of HA localization in the cross-section (edge to edge) of hydrogels prepared with different $CaCO_3$ concentrations (black squares: 20 mM, Formulation D); blue circles (50 mM, Formulation E). The profiles reported were chosen as representative trends among 5 replicates. Dotted lines are drawn to guide the eye. (B) Profiles of alginate localization in the cross-section (edge to edge) of hydrogels prepared with different $CaCO_3$ concentrations (black squares: 20 mM, Formulation D; blue circles: 50 mM, Formulation E); (C) Macroscopic image of the transparent hydrogel (Formulation D); (D) Graphical representation of the process of hyaluronan demixing from the structure of the alginate hydrogel. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

considerable structural and mechanical properties (Lee & Mooney, 2012).

HA is a linear polysaccharide belonging to the family of glycosaminoglycans (GAGs), the main constituents of the ECM. At physiological pH values, it has a polyanionic structure that imparts excellent hydro-coordinating properties enabling the retention of large amounts of water. HA is involved in the regulation of several biological phenomena such as cell migration, differentiation, growth, adhesion, angiogenesis as well as the control of the immune response and the interaction with collagen fibres (Jiang, Liang, & Noble, 2007). In the human body, HA undergoes degradation through enzymatic catalysis (Markovsky et al., 2012). The wound-repairing properties of HA have been correlated to its effects on the inflammatory response, chemotaxis, cell migration, proliferation and angiogenesis (Jiang et al., 2007).

Alginate is a polysaccharide mainly isolated from brown algae. It is a linear copolymer whose comonomers are guluronic (G) and mannuronic (M) acids; it can form hydrogels in the presence of divalent cations such as calcium, which bind preferentially to the G-blocks (but to MG sequences as well, albeit to a lesser extent) in a highly cooperative manner. Alginates have long been known to possess haemostatic properties (Blair, Jarvis, Salmon, & McCollum, 1990; Sayag, Meaume, & Bohbot, 1996) and recently alginate-based dressings have been shown to reduce postoperative drainage volume in patients undergoing elective rectal resection for cancer (Maggiori et al., 2010). Alginate-based biomaterials can be manufactured into films (Esser & Tessmar, 2013; Rosellini, Cristallini, Barbani, Vozzi, & Giusti, 2009), fibres (Cuadros, Skurtys, & Aguilera, 2012; Kang et al., 2012) gels (Donati et al., 2005; Turco et al., 2011) and foams (Andersen, Markussen et al., 2014; Andersen, Melvik, Gåserød, Alsberg, & Christensen, 2014), according to their final applications. In the human body, this polysaccharide is bioeroded, partially degraded by macrophages and excreted through kidneys (Markovsky et al., 2012). Mixtures of alginate and HA have been previously investigated, aiming at combining the respective peculiar properties of those polysaccharides for various types of biomedical applications (Donati et al., 2011; Geremia et al., 2014; Oerther, Le et al., 1999; Oerther, Payan et al., 1999). Alginate hydrogels containing HA have been prepared and proposed for diverse applications, like cartilage transplant (Dausse et al., 2003; Gerard et al., 2005), articular surgery (Aulin et al., 2011; Chung et al., 2014) and wound healing (Catanzano et al., 2015).

Given these premises, the aim of this work was to develop novel biodegradable dressings in the form of pliable membranes based on HA and alginate starting from aqueous hydrogels obtained by freeze-casting from the mixture of these two polysaccharides. In essence, the construct design foresees a bi-component polymeric biomaterial which should release the bioactive polysaccharide, *i.e.* HA, from the 3D solid-like architecture of the calcium alginate structure. The investigation of the macromolecular distribution of the structural and mechanical features of the hydrogels was followed by a thorough characterization of the membranes, in terms of mechanical, functional (degradation, release), and biological (biocompatibility, wound healing capability) properties. From the fundamental macromolecular physical-chemical standpoint, particular attention was given to analyse the distribution profile of the two biopolymers arising from their demixing.