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Research Paper

Ulvan-chitosan polyelectrolyte complexes as matrices for enzyme induced biomimetic mineralization

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ABSTRACT

Polyelectrolyte complexes (PEC) of chitosan and ulvan were fabricated to study alkaline phosphatase (ALP) mediated formation of apatitic minerals. Scaffolds of the PEC were subjected to ALP and successful mineral formation was studied using SEM, Raman and XRD techniques. Investigation of the morphology via SEM shows globular structures of the deposited minerals, which promoted cell attachment, proliferation and extracellular matrix formation. The PEC and their successful calcium phosphate based mineralization offers a greener route of scaffold fabrication towards developing resorbable materials for tissue engineering.

1. Introduction

Biomimetic mineralization is the process of preparing inorganic crystals in the presence of organic molecules. Biomimetic mineralization attracts interest since it usually leads to crystals with multiscale ordered structures, which are rare in natural minerals. Besides, it is also an easy way to synthesize novel organic/inorganic hybrids. $CaCO_3$ (Schuszter, Brau, & De Wit, 2016), $BaSO_4$ (Jin, Huang, Li, Yu, & Luo, 2015), $BaCO_3$, (Guo & Yu, 2007) and hydroxyapatite (HAP) (Coleman, Jack, Perrier, & Grøndahl, 2013) are the mostly investigated minerals synthesized via biomimetic mineralization. In the field of biomedical research, polymer scaffolds or hydrogels that mineralized apatite had improved osteoconductivity and osteoinductivity compared to unmineralized polymers (Habibovic & de Groot, 2007). Several polymers have been investigated for this application such as chitosan (Dash et al., 2015; Douglas et al., 2013; Lišková et al., 2015), ulvan (Dash et al., 2014), silk (Samal, Dash, Chiellini et al., 2014; Samal, Dash, Declercq, Gheysens, Dendooven, Voort, Cornelissen et al., 2014), gellan-gum (Douglas et al., 2014), poly(ethylene glycol) (Phadke, Zhang, Hwang, Vecchio, & Varghese, 2010), bacterial cellulose (Zimmermann, LeBlanc, Sheets, Fox, & Gatenholm, 2011), poly(L -lactic acid) (Ruhé et al., 2005) and polycaprolactone (Koupaei & Karkhaneh, 2016). In each case, some type of biological apatite was formed, such as calcium-deficient HAP (Zimmermann et al., 2011), dicalcium phosphate dihydrate (DCPD) (Costa, Dixon, & Rizkalla, 2012), carbonated apatite (Costa et al., 2012), or HAP (Phadke et al., 2010). In this study, polyelectrolyte complexes (PEC) of ulvan and chitosan are evaluated as matrices for biomimetic mineralization. PEC's are mixtures of positively and negatively charged polyelectrolytes blended at the molecular level. Ulvan is an anionic sulphated polysaccharide that is water soluble and semi-crystalline in nature (Dash et al., 2014), which can be obtained by extraction from the cell-walls of the green seaweeds belonging to Ulvales (Ulva and Enteromorpha sp.). (Morelli & Chiellini, 2010) The natural availability of ulvan represents a source of abundant and economic renewable resources (Morelli & Chiellini, 2010) with minimal concerns regarding toxicity towards host organisms. Sulfated rhamnose, xylose, glucuronic and iduronic acids are the main constituents of ulvan (Fig. 1) (Dash et al., 2014). Ulvan has been reported as anticoagulant, antioxidant, antitumor and immune modulator (Lahaye & Robic, 2007; Patel, 2012). Chitosan is a polysaccharide composed of ß-(1,4)-2-acetamido-2-deoxy-**d**-glucose and ß_-(1,4)-2-amino-2- deoxy-**d**-glucose units, is a deacetylated form of chitin (Dash, Chiellini, Fernandez, Piras, & Chiellini, 2011; Dash, Chiellini, Ottenbrite, & Chiellini, 2011; Dash, Piras, & Chiellini, 2009). This natural cationic polymer, offers unique

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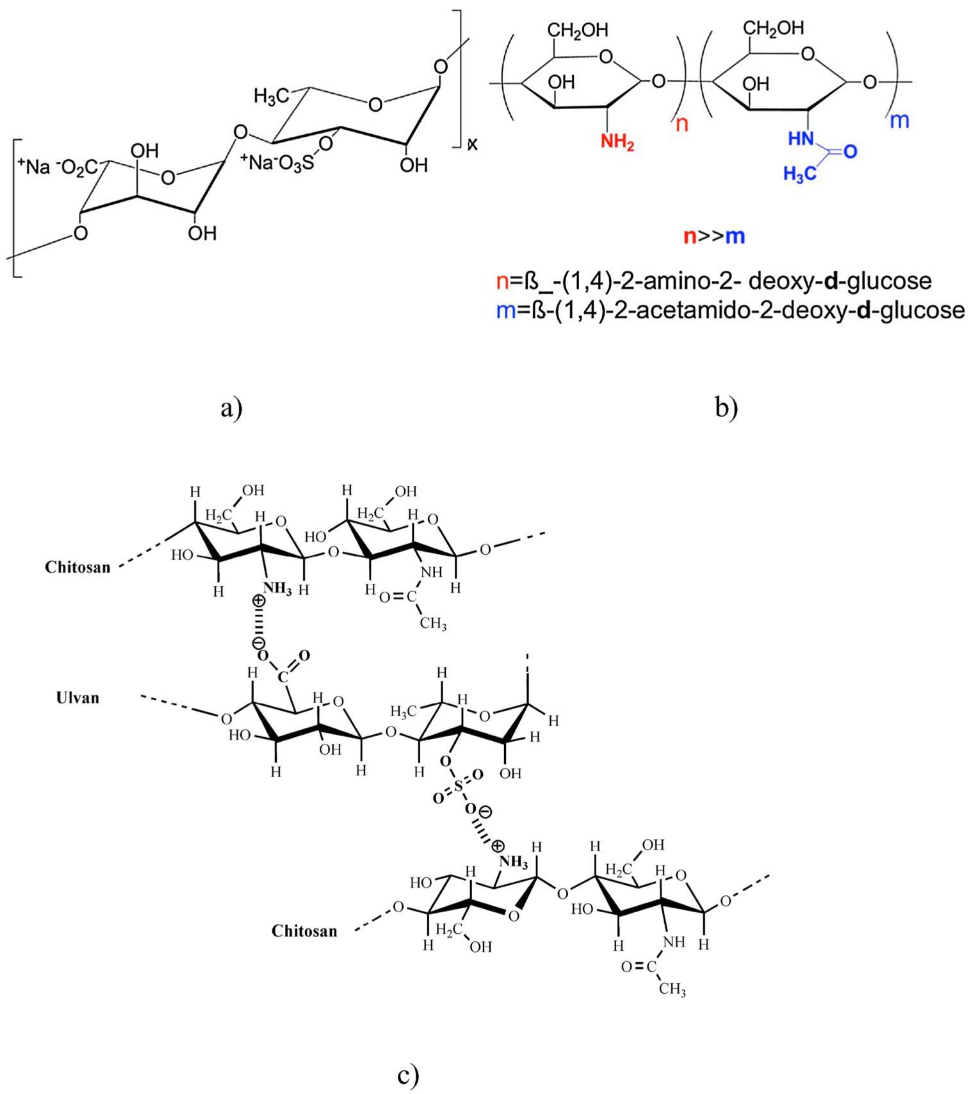

Fig. 1. The chemical structure representing (a) the main disaccharide repeating unit of ulvan, α-L-iduronic acid (1 → 4) α-L-Rha 3S → 1 (Dash et al., 2014) (b) chitosan, (c) the polyelectrolyte complexation between ulvan and chitosan.

properties; it is biologically renewable, biodegradable, biocompatible, non-antigenic, non-toxic, and biofunctional (Lupascu et al., 2015). Chitosan has been proven to accelerate wound healing, stimulate the macrophage activity, and inhibit the growth of tumor cells and posses antimicrobial properties (Muzzarelli et al., 1990; Muzzarelli, 2011). PEC's involve anionic and cationic side chain reactions on their macromolecular backbone (Schwarz, Richau, & Paul, 1991). Therefore, ulvan and chitosan as oppositely charged macromolecules can potentially form a polyelectrolyte assembly.

PEC's of ulvan and chitosan are biofunctionalized by employing the natural enzyme alkaline phosphatase (ALP) as mineralization inducer and the osteogenic cell activity of these scaffolds were evaluated. Mineralization and particularly enzymatic mineralization has been a topic of research interest over the last decade (Douglas, Gassling et al., 2012; Rauner, Meuris, Zoric, & Tiller, 2017; Saito, Fujii, Soshi, & Tanaka, 2006). A number of investigations have been reported on matrices like chitosan (Dash, Chiellini, Fernandez et al., 2011; Dash, Chiellini, Ottenbrite et al., 2011), fibrin (Douglas, Gassling et al., 2012; Gassling et al., 2013; Douglas, Messersmith et al., 2012), silk (Samal, Dash, Declercq, Gheysens, Dendooven, Voort and Kaplan, 2014) etc. In the present study, we have aimed to investigate the role of enzymatic mineralization on polymeric structures bearing both cationic and anionic groups. In some of our recent works with enzymatic biomineralization, we have studied the behaviour of both positively and negatively charged polymers individually. Herein, we performed a follow-up study, where the aim was to evaluate a combination of the two different types of charged polymers, whose presence could be beneficial to each other. This is a sequential study of the already published work on the mineralization of UV crosslinked ulvan methacrylate (UMA) scaffolds (Dash et al., 2014) wherein the rationale is to evaluate a greener material as compared to UMA since, exposure to UV radiation in some cases is known to cause significant degradation of materials. UV radiation might cause photooxidative degradation, which results in breaking of the polymer chains, produces free radical and reduces the molecular weight, leading to loss of mechanical properties. Mineralization includes advantages such as enhancing bioactivity post implantation (Dash et al., 2014, 2015), osteoblastic differentiation through increased stiffness (Olivares-Navarrete et al., 2017) and enhanced binding of growth factors which stimulate bone healing. (Vo, Kasper, & Mikos, 2012) The evaluation of such matrices could lead to tissue engineered bioactive bioresorbable scaffolds for bone tissue engineering.

2. Experimental section

2.1. Materials

Ulvan batch in powder as extracted from *Ulva armoricana* was kindly supplied by CEVA within the framework of the EU-founded project BIOPAL. The number average molecular weight of ulvan