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Novel ethanol-induced pectin–xanthan aerogel coatings for orthopedic applications

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ABSTRACT

In this study, we developed a novel high methoxyl pectin–xanthan aerogel coating on medical-grade stainless steel, prepared by ethanol-induced gelation and subsequent supercritical drying. Two non-steroidal anti-inflammatory drugs, *i.e.* diclofenac sodium and indomethacin, were incorporated into the aerogel coating. Electrochemical analyses were performed on the coated samples using electrochemical impedance spectroscopy and cyclic polarization techniques. The results showed that all passivated samples were highly resistant to general corrosion. The release of both non-steroidal anti-inflammatory drugs was complete after 24 h, as confirmed by the plateau in the drug release profiles as well as by IR spectroscopy after the final release point. The potential of samples for use in orthopedic applications was evaluated on a human bone-derived osteoblast cell and all samples were shown to be biocompatible. The increased viability of some samples indicates the high potential of the developed approach for future evaluation of possible clinical use.

1. Introduction

Total hip arthroplasty (THA) is a common procedure in orthopaedics that is subject to constant innovation. In order to improve the quality of life of patients suffering from hip fractures, novel materials and techniques are continuously studied and some have also been introduced into clinical practice (Liu, Zi, Xiang, & Wang, 2015). The most commonly used materials in THA are titanium alloys, different ceramics, and stainless steel (Revell, 2014). The material used must be biocompatible with body tissues and fluids, be able to withstand wear and corrosion, have or ideally exhibit similar mechanical properties as bone, *etc.* (Silverwood et al., 2016) Therefore, many studies are now focused on developing coatings on base artificial hip materials in order to improve their functionality and durability. More recently, biomaterial-based coatings have been used as part of orthopedic implants in order to modulate the surrounding biological environment (Elyada, Garti, & Füredi-Milhofer, 2014; Finšgar, Uzunalić, Stergar, Gradišnik, & Maver, 2016; Goodman, Yao, Keeney, & Yang, 2013; Peterson, Möhwald, & Shchukin, 2012).

Amongst biomaterial-based coatings, polysaccharides were found to be especially promising as regards enhancing the respective implant integration (Travan et al., 2012). Pectin, a commonly found polysaccharide in nature, has previously been used to increase the hydrophilicity of surfaces (Gurzawska, Svava, Jørgensen, & Gotfredsen, 2012) and to enhance osteoblast-specific production (Kokkonen, Ilvesaro, Morra, Schols, & Tuukkanen, 2007). An important property of polysaccharides making them interesting for different applications related to materials science is their ability to form a gel, making them suitable for various gelation-related preparation procedures (the formation of hydrogels, the sol–gel method, *etc.*).

The sol–gel method is a rather simple process for obtaining various forms of materials with desired properties (*e.g.* porosity, mechanical strength, chemical reactivity, *etc.*). Wet gels can be dried by air-drying (evaporation), lyophilization, or above the supercritical point of the fluid, and the obtained dry gels are called xerogels, cryogels, and aerogels, respectively (Brinker & Scherer, 1990). The structure of a wet gel is defined right after the gelation point. The pores that are formed inside the gel during drying can vary from the nano- to the micro-range. Since there is only one

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phase present above the critical point, no surface tension is present and the possible collapse of the structure is avoided (Scherer, 1990). Therefore, supercritical drying is considered as the best method for preserving the structure of a gel. The resulting materials, aerogels, possess a wet-gel-like structure and have very low apparent densities, large specific surface areas, are nanostructured, and are in most cases present in an amorphous form (Pantić, Knez, & Novak, 2016; Tkalec, Knez, & Novak, 2015b). Polysaccharide-based aerogels have been successfully used as carriers for water-soluble (Veronovski, Tkalec, Knez, & Novak, 2014) and water insoluble (very low solubility) drugs (Tkalec, Knez, & Novak, 2015a). All of the above mentioned properties make polysaccharide-based aerogels interesting candidate materials for metal coatings in THA, especially considering the latest related research oriented towards drug-loaded coatings for local drug delivery (Finšgar et al., 2016; Neut et al., 2015; Taha et al., 2014).

The most commonly used drugs after the postoperative period are non-steroidal anti-inflammatory drugs (NSAIDs). They have a pronounced analgesic potency, an anti-inflammatory effect, and fewer side effects compared to opioids (Dhalla, Gomes, Mamdani, & Juurlink, 2012). NSAIDs and prophylactic radiotherapy can even prevent ectopic bone formation around the hip after total hip arthroplasty (Fijn, Koorevaar, & Brouwers, 2003). Therefore, two NSAID drugs, diclofenac sodium (DCF) and indomethacin (IND), commonly used drug to prevent heterotopic ossification (Bedi et al., 2012), were also used in this study.

2. Experimental part

2.1. Preparation of the medical-grade stainless steel

The same medical-grade stainless steel samples (disc shape 15 mm in diameter) and the same preparation procedure as reported previously (Finšgar et al., 2016) were employed in this study. However, in contrast to the previous study, the samples were additionally passivated by immersion for 1 h in a 30 wt.% HNO_3 solution to simulate real artificial hip material. Afterwards, the samples were thoroughly rinsed with ultra-pure water, dried under nitrogen of high purity (99.999 wt.%) and stored in a desiccator until further used.

2.2. Polysaccharide aerogel coating preparation

High methoxyl pectin (hmP) (Pectin Classic CU-L 069/13; degree of esterification 78%) was provided by Herbstreith & Fox (Germany), and xanthan (Xa) (800–1200 cps) was obtained from Sigma Aldrich (USA). All polysaccharide solutions were prepared with ultra-pure water.

Both polysaccharides, hmP and Xa, were chosen based on their good gelation ability and proven drug release performance (Tkalec et al., 2015a, 2015b). Various mass ratios of hmP and Xa solutions were prepared and finally the ratio 1:1 was found to provide the best adhesion on the medical grade stainless steel. HmP (0.5 wt.%) was slowly poured into the stirring water and mixed until homogenization at 400 rpm for 30 min. Then, Xa (0.5 wt.%) was added to the hmP solution and again mixed until homogenization for about another 30 min. It is essential for this process that both polysaccharides are added to the water/solution very slowly in order to avoid the formation of clumps.

The prepared hmP:Xa solution was sonicated for 30 min in order to remove any air bubbles formed during mixing. Then, 300 μL hmP:Xa solution was precisely transferred onto medical-grade stainless steel in order to cover the whole disc evenly. Absolute ethanol (Sigma Aldrich, ACS reagent, ≥99.5) was poured onto the polysaccharide coating. It was previously reported by our group that the gelation of both hmP and Xa can occur in the presence of ethanol (Tkalec et al., 2015b). This method was therefore used to produce hmP:Xa alcogels (ethanol in the pores of a gel) on the surface of medical-grade stainless steel. The outer layer of the polysaccharide solution gelled quickly after contact with absolute ethanol. For the drying process, it is essential that all water in the pores be replaced by absolute ethanol. Therefore, the coated samples were placed in absolute ethanol for 3 h before supercritical drying.

2.2.1. NSAID-containing polysaccharide coating

DCF (analytical grade, ≥99.0% purity) was provided by Chemos (Germany). HmP:Xa solution (1:1) was prepared as described above. Since DCF is a water-soluble drug (with a solubility of 50 mg/mL (Sigma Aldrich, 2016a)), this NSAID (1 wt.%) was added to the hmP:Xa solution while mixing. The hmP:Xa-DCF solution was sonicated in order to remove excess bubbles. Then, 300 μL of the homogenized solution was precisely transferred to the medical-grade stainless steel sample, followed by gelation in ethanol for 1 h. The ethanol solution was saturated with DCF in order to avoid the diffusion of this NSAID from the coating.

IND (analytical grade, ≥99% purity) was provided by Sigma Aldrich. IND has limited solubility in water as it belongs to BCS Class II (highly permeable with low solubility) (FDA, 2016). However, its solubility in ethanol is much higher than in water and it can reach up to 50 mg/mL (Sigma-Aldrich, 2016b) upon stirring. Therefore, loading of this model drug had to be different than for DCF. IND was loaded into the hmP:Xa gel coating on medical-grade stainless steel samples by diffusion through IND-saturated ethanol for 5 h.

2.2.2. Supercritical CO_2 drying

Drying was performed according to a method developed in our laboratory (Novak & Knez, 1997). The supercritical drying was conducted at 314 K and 12 MPa. The chosen temperature and pressure allow the formation of a homogeneous phase of CO_2 and ethanol inside the hmP:Xa alcogels in order to allow drying without the phase interface between the supercritical CO_2 and liquid phases and to preserve the adhesion of the coating. The drying was performed for 7 h at a CO_2 flow rate of approximately 200 L h^{-1}. After the supercritical drying, the system was depressurized at 0.2 MPa min^{-1}, after which white polysaccharide aerogel coatings on medical-grade stainless steel were obtained. The samples were left to cool down to room temperature and then stored in a desiccator.

2.3. HmP:Xa aerogel coating characterization

Specific surface area and porosity parameters were determined using the Brunauer-Emmet-Teller (BET) technique based on nitrogen gas adsorption (Micromeritics ASAP 2020) (Veronovski et al., 2014). Prior to that, the hmP:Xa, hmP:Xa-IND, and hmP:Xa-DCF aerogel coatings were removed from the medical-grade stainless steel and outgassed.

Micrographs of the prepared aerogels were obtained by field emission scanning electron microscope (FE-SEM) Sirion 400 NC. Aerogel coatings were scrapped from the medical-grade stainless steel surface, sputter-coated with gold particles and fixed to aluminum sample holders with a double-sided carbon tape. Then they were scanned at an accelerating voltage of 10 kV using a TLD detector.

2.4. Electrochemical analysis

Ultra-pure water (with a resistivity of 18.2 MΩ cm at 25 °C) produced by the Milli-Q® system was used to prepare a physiological body fluid of 0.9 wt.% NaCl used for the electrochemical analysis.