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Sulfonated chitosan and dopamine based coatings for metallic implants in contact with blood

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ABSTRACT

Thrombosis and calcification constitute the main clinical problems when blood-interacting devices are implanted in the body. Coatings with thin polymer layers represent an acknowledged strategy to modulate interactions between the material surface and the blood environment. To ensure the implant success, at short-term the coating should limit platelets adhesion and delay the clot formation, and at long-term it should delay the calcification process. Sulfonated chitosan, if compared to native chitosan, shows the unique ability to reduce proteins adsorption, decrease thrombogenic properties and limit calcification. In this work, stainless steel surfaces, commonly used for cardiovascular applications, were coated with sulfonated chitosan, by using dopamine and PEG as anchors, and the effect of these grafted surfaces on platelet adhesion, clot formation as well as on calcification were investigated. Surface characterization techniques evidenced that the coating formation was successful, and the sulfonated chitosan grafted sample exhibited a higher roughness and hydrophilicity, if compared to native chitosan one. Moreover, sulfonated surface limited platelet activation and the process of clot formation, thus confirming its high biological performances in blood. Calcium deposits were also lower on the sulfonated chitosan sample compared to the chitosan one, thus showing that calcification was minimal in presence of sulfonate groups. In conclusion, this sulfonated-modified surface has potential to be as blood-interacting material.

1. Introduction

When in contact with blood, at short-term, adsorption of plasma proteins, calcium and platelet adhesions are the main processes that will happen on the implant surface [1,2]. Adhered platelets could be thereafter activated, leading to the coagulation cascade and then thrombosis [1,3]. At long-term, calcification of implants (heart valves, breast implants, and stents) may also occur due to growing of calcium phosphates or other calcium salts deposits. Both problems, thrombosis and calcification, can compromise the functions of devices and reduce their lifetime and can lead to implant explantation [4].

Researches for blood-interaction biomaterials have motivated the development of a surface treatment to inhibit both thrombosis and calcification [5–7]. Thus, the combination of antithrombogenic and anticalcification properties is critical to determine the success of biomaterials when implanted. One way to ensure, or at least increase, the chances of clinical success of the implant, at short as well as long terms, is to coat the device with a more biocompatible substance playing the role of bio-interface [8]. Regarding, the interactions with blood components, these coated devices should present essential criteria such as: (i) limiting the adsorption of proteins which induce platelet adhesion (fibrinogen and thrombin), (ii) avoiding platelet adhesion/activation and formation of thrombin or/and fibrin, and (iii) preventing calcium deposits as well as hydroxyapatite (HA) formation. These different approaches and strategies to prevent the protein adsorption, platelet adhesion and calcification were summarized in the Table 1.

Among all the approaches described, chitosan emerges as a highly promising candidate due to its versatility and ability to act as key-factor for these different complications [5–7,13,18]. Moreover, it is a natural polymer, biocompatible, and in its structure, a linear chain of monomers of 2-amine-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose linked via glycoside bonds of β (1→4) [27], it presents amine and hydroxyl groups. These chemical functionalities along the chains are of particular interest, mainly because they can be used to graft chitosan on surfaces or to give to chitosan new properties through chemical modifications. Numerous examples of chitosan functionalization are reported, such as *O*-carboxymethyl-chitosan [27] (cell adhesion), *N*-carboxybutyl-chitosan [28] (healing), *O*-stearoyl-chitosan [29]

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Table 1Please check the layout of Table 2 if correct and amend as necessary
Approaches used in literature to avoid short and long-term complications for biomaterials in contact with blood. Biomaterials include numerous substrates such as polymers, metals, glass, nanoparticles and biological tissues.

Complication	Approaches	Reference
Short term		
Protein adsorption	Nonfouling surfaces	
	PEG	[9]
	Polysaccharides	
	Dextran	[10]
	Chitosan	[11]
	Kappa-carrageenan	[12]
	Sulfonated chitosan	[13]
	Phosphatidylcholine	[14]
	Poly(*N*,*N*-dimethyl-*N*-methacryloxyethyl-*N*-(3-sulfopropyl) (PDMMSA)	[15]
Platelet adhesion and activation	Use of dextran sulfate	[16]
	Use of poly(ethylene glycol) sulfonate	[17]
	Use of *N*-sulfofurfuryl chitosan	[18]
	Use of phosphorylcholine	[19]
	Heparinization	
	Immobilizing heparin	[20]
	Mimic heparin with sulfonated polysaccharides	[21]
	Plasma treatment of surfaces	[22]
Long term		
Calcification	Surface modification (use of Al^{+3} and Fe^{+3})	[4]
	Controlled release (use of protamine sulfate and levamisole)	[23]
	Nucleation delay	
	Use of heparin	[24]
	Use of sulfated poly(ethylene oxide)	[25]
	Use of chondroitin sulfate	[26]
	Surface topography	
	Lowering roughness with chitosan	[5]
	Lowering roughness with silk fibroin	[6]

(hemocompatibility), and methyl-pyrrolidinone-chitosan [30] (bone regeneration), but the most interesting modification regarding the application proposed for this work is the modification of chitosan by integrating sulfonate groups. Indeed, sulfonated chitosan can mimic the heparin structure, which is a sulfated polysaccharide, highly negatively charged [31], known for its hemocompatibility properties, by repulsing blood proteins and preventing platelet adhesion [13,32]. However, the cost of heparin purification step makes it an expensive product [33], while chitosan represents an affordable sub-product of fishing industry [34]. Moreover, sulfonate groups are also pointed as responsible to have an anticalcifying effect [35], as evidenced by Lee et al. [25] on bovine pericardium grafted with PEO-SO_3.

Thus, the aim of this work was to graft chitosan, both native and sulfonated, on metallic devices in contact with blood. Covalent grafting process allows to improve the coating stability under shear stress or blood flow when implanted [36]. In order to assess covalent bonds, dopamine was used first on the metallic substrate [37–39], then, poly(ethylene glycol) diacid, an antifouling molecule (Table 1), was chosen as a linking arm. The grafted surfaces were characterized by X-Ray Photoelectron Spectroscopy (XPS), Atomic Force Microscopy (AFM) and contact angle measurements. The biological properties, anti-thrombogenicity and anti-calcification, over native chitosan and sulfonated chitosan surfaces, were assessed by evaluating the surface behavior to induce calcification using Scanning Electron Microscopy and Electron Dispersive X-Ray Spectroscopy (SEM/EDS), by platelet adhesion and clotting time tests. The aim was to demonstrate the potential to obtain a sulfonated chitosan surface with enhanced anti-thrombogenic and anti-calcification properties when compared with native chitosan and stainless steel surfaces.

2. Materials and methods

2.1. Materials

All reagents used were analytical grade without further purification. Chitosan, dopamine, poly(ethylene glycol) bis (carboxymethyl) ether (PEGb) (Mn 600 Da), *N*-(3-dimethylaminepropyl)-*N*′-ethylcarbodiimide hydrochloride (EDAC) and 2-(N-morpholino) ethanesulfonic acid buffer (MES) were acquired from Sigma-Aldrich (USA). 5-Formyl-2-furansulfonic acid sodium salt (FFSA) was purchased from Alfa Aesar (USA). Methanol, acetone, glutaraldehyde and sodium borohydride were acquired from VETEC Química Fina (Brazil). Triethanolamine was acquired from Cinética Reagentes & Soluções (Brazil). AISI 316L stainless steel sheets (0.5 mm thickness) were obtained from Goodfellow Cambridge Limited (England). Hydrochloric acid, sodium chloride, potassium chloride, calcium chloride, magnesium chloride, sodium bicarbonate, dibasic potassium phosphate, sodium sulfate, tris (hydroxymethyl) aminemethane (Tris), acetic acid, sodium hydroxide, sulfuric acid, phosphoric acid, nitric acid and fluoridric acid were purchased from Laboratoire MAT (Canada). Vacuette® coagulation tubes (sodium citrate 0.109 M/3.2%) were purchased from Greiner Bio-One (Brazil).

2.2. Polymer solutions preparation

The detailed preparation of the solution is explained elsewhere [21]. Briefly, chitosan solution 2% (w/w) was prepared by dissolving 2 g of chitosan in 97 mL of distilled water followed by addition of 3 mL of acetic acid and magnetically stirred for 24 h at room temperature.

Sulfonated chitosan was obtained by mixing 50 mL of chitosan solution 2% (*w*/w) with 50 mL of methanol, containing 1 mL of triethanolamine, and 2 g de FFSA (sulfonating agent), and stirred for 12 h at room temperature to allow the reaction between the aldehyde group of FFSA and the amine group of chitosan. After this, 0.5 g of sodium borohydride were added to reduce the Schiff's base formed. The solution was precipitated in methanol and the precipitate was exhaustively washed with methanol:acetone 1:1. After dried, the mass obtained was milled to produce fine particles and used to prepare a sulfonated chitosan solution 2% (w/w), using the same methodology described for chitosan solution.