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Osteoblastic response to pectin nanocoating on titanium surfaces

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ABSTRACT

Osseointegration of titanium implants can be improved by organic and inorganic nanocoating of the surface. The aim of our study was to evaluate the effect of organic nanocoating of titanium surface with unmodified and modified pectin Rhamnogalacturonan-Is (RG-Is) isolated from potato and apple with respect to surface properties and osteogenic response in osteoblastic cells.

Nanocoatings on titanium surfaces were evaluated by scanning electron microscopy, contact angle measurements, atomic force microscopy, and X-ray photoelectron spectroscopy. The effect of coated RG-Is on cell adhesion, cell viability, bone matrix formation and mineralization was tested using SaOS-2 cells. Nanocoating with pectin RG-Is affected surface properties and in consequence changed the environment for cellular response. The cells cultured on surfaces coated with RG-Is from potato with high content of linear 1.4-linked galactose produced higher level of mineralized matrix compared with control surfaces and surfaces coated with RG-I with low content of linear 1.4-linked galactose. The study showed that the pectin RG-Is nanocoating not only changed chemical and physical titanium surface properties, but also specific coating with RG-Is containing high amount of galactan increased mineralized matrix formation of osteoblastic cells *in vitro*.

1. Introduction

One of the most important objectives in developing new implant surfaces is to understand the processes that take place at the bone–implant interface. This is essential in order to find the most appropriate implant surface for bone integration [1]. In order to improve osseointegration, titanium implant surfaces may be chemically and physically modified [2]. The physical modification can be done on the micro- and nanometer level. At the nanometer level, it has been demonstrated that an increased surface roughness changes the wettability of the surface and improves matrix protein adsorption, bone cell migration and proliferation [3]. It has also been demonstrated that an increased surface roughness at the micrometer level improves osseointegration [3].

By changing the physical and topographic properties of the surface, the chemical properties will be affected [2]. Thus, surface changes create a specific environment for the cascade of processes in the bone following the insertion of a titanium implants. Therefore, detailed knowledge about the titanium surface properties is important in order to control the bone healing process at the bone–implant interface.

Nanoscale modification of the implant surface by coating with organic molecules is one of the methods used to improve osseointegration [4]. Pectins, which are plant-derived polysaccharides, have been proposed as potential candidates for surface nanocoating of medical devices due to their effect on bone cells, and the possibility of controlling their structure [5–7]. The effect on bone cells has been explained by a direct adhesion of cells to pectins and an indirect mechanism through proteins bound to the pectins [8]. Pectins are large polysaccharide molecules found in the cell wall, and they control the porosity of the wall and the water-retaining capacity of the cell [9]. Their structure imitates the polysaccharides from the extracellular matrix of mammals, providing bio-specific cell adhesion [10]. It has also been reported that

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modified forms of pectins increased the surface hydrophilicity and had anti-inflammatory effects [5,11–19].

Studies by Inngjerdingen et al. showed that the Rhamnogalacturonan-I (RG-I) region of pectins isolated from *Glinus oppositifolius* might be responsible for B-cell proliferation and complement fixing activity, in addition to its stimulation of proliferation of bone marrow cells through the immune system [12]. RG-I isolated from the leaves of *Plantago major* L. has been reported to be responsible for wound healing [13]. Studies by Popov documented the anti-inflammatory effect of pectins explained by a binding mechanism between pectin RG-I, galacturonic acid (GalA) and β-integrins. This interaction prevents neutrophil adhesion to fibronectin, which represents a key step of the inflammatory response [15].

It is possible to control the structure of pectin RG-I (Fig. 1) by enzymatic modification. A variety of different pectin structures can be produced by changing the side chains (galactan and arabinan) or backbone (galacturonic acid and rhamnose) of the pectin molecule. Therefore a screening of different RG-Is is important for identifying the regions of the RG-I molecule most important for bone cells and mineralized matrix formation. The hypothesis of our studies is that cells cultured on specific RG-I nanocoated surfaces will produced higher levels of mineralized matrix than on control surfaces, without the nanocoating. The aim of the present study was to evaluate the effect of nanocoating titanium surfaces with unmodified and enzymatically modified pectin RG-I from potato (P) and unmodified pectin RG-I from apple (A) with respect to surface properties and osteogenic response in osteoblastic cells.

2. Experimental

2.1. Isolation, modification and analysis of RG-I

2.1.1. Isolation and modification of RG-I

RG-I from apple and potato pulps was isolated according to the procedure as previously published [20,21]. The pulp was destarched and the RG-I was extracted using polygalacturonase-I (PG-I, Novozymes, Denmark). The RG-I was further treated with polygalacturonase-III (PG-III, Novozymes, Denmark) together with pectin methylesterase (PME, Novozymes, Denmark). This was done to remove homogalacturonan and their methyl esters. Potato RG-I was dissolved in 0.2 M sodium-phosphate/citric acid buffer (1 mg RG-I/mL, pH 5.5). On separate aliquots of each, digestions were performed using enzymes from Megazyme at a rate of 0.05 U/mL; α-L-arabinofuranosidase and endo-arabinanase to remove arabinan side-chains; β-galactosidase and endo-β-1,4-galactanase to remove galactan side-chains. All four enzymes were added to debranch the RG-I. The solutions were incubated at 30 °C with shaking overnight, dialyzed extensively (cut-off: 12 kDa) against de-ionized water and lyophilized.

Only apple unmodified was used in this study (AU: apple unmodified). Four types of RG-I from potato was used in this study: PU: potato unmodified, PA: potato dearabinanated; PG: potato degalactanated; PAG: potato dearabinanated and degalactanated equal to potato debranched.

2.1.2. Monosaccharide composition

To RG-I (1 mg) was added 1 mL 2 M trifluoroacetic acid (Sigma-Aldrich, Brøndby, Denmark), the tube was sealed and the solution was heated to 121 °C for 1 h. The acid was evaporated under vacuum and the monosaccharides were resuspended in 2 mL milliQ water; analysis was performed using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (BioLC with a PA20 column, Dionex, California, U.S.A.).

2.1.3. Linkage analysis

Permethylation and linkage analysis were performed by partially methylated alditol acetates (PMAA) described by Hakomori [22] and Anumula & Taylor [23]. The finished PMAAs were dissolved in 15 drops of dichloromethane and 3 μL was injected into the gas chromatograph mass spectrometer (GCMS) (GC-2010 equipped with GCMS-QP2010 plus, Shimadzu, the column was SP-2330, 30 m × 0.20 mm, ID. 0.25 μm Supelco (USA)). The samples were run in splitless mode. Response factors were estimated using the effective carbon response approach [24].

2.2. Surface modification and characterization

2.2.1. Surface modification

Titanium discs (Ti) made of commercially pure titanium grade 2 with diameter 13 mm were aminated and thereafter pectin RG-Is coated. The amination procedure was performed using plasma polymerization with allylamine, in order to create covalent coupling of pectin RG-I [25]. The Ti discs were placed on a watercooled grounded electrode and deposition

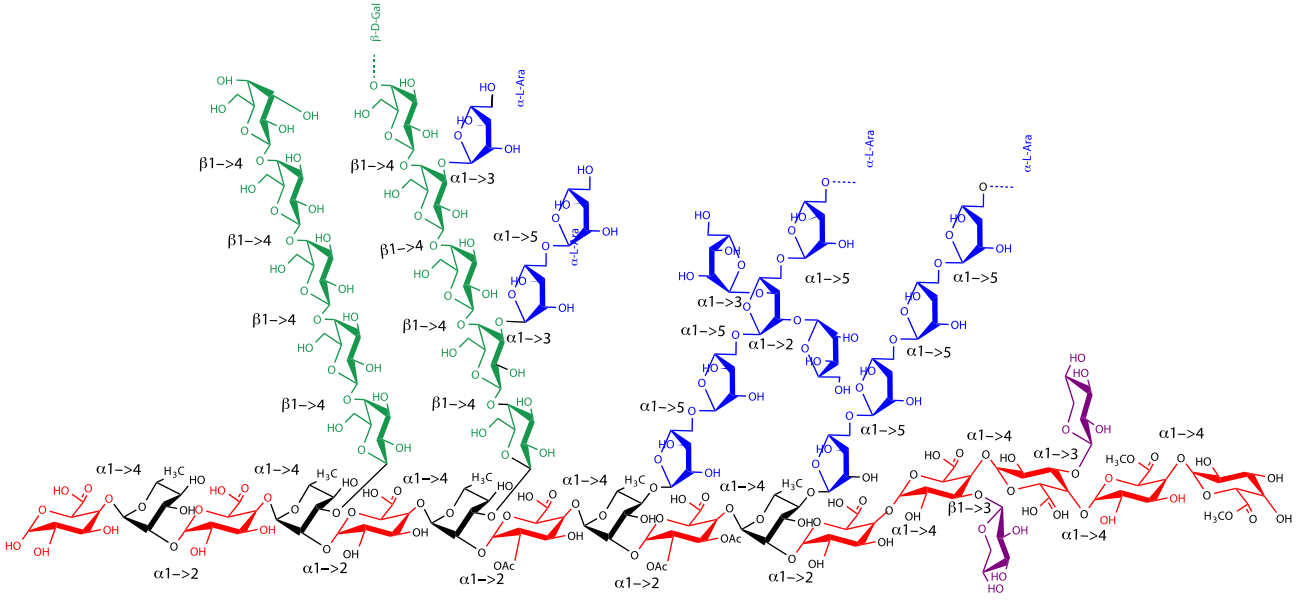

Fig. 1. Schematic illustration of Rhamnogalacturonan-I with a backbone consisting of alternating Rhamnose (black) and Galacturonic acid (red) residues. The Rhamnose can be substituted with either arabinan (blue) or galactan (green) side chains. The galacturonic acid can be substituted with Xylose (purple) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).