PectiCoat

Project no.
517036

Project acronym
PectiCoat

Project title
Nanobiotechnology for the coating of medical devices

Instrument
STREP - Specific Targeted REsearch or innovation Project

Thematic Priority
3rd Pritority - nanotechnologies and nanosciences, knowledge-based multifunctional materials and new production processes and devices.

Periodic Activity Report
(Second reporting period)

Period covered: from **April 01, 2005** to **March 31, 2007**

Date of preparation: **07/04/23**

Start date of project: **April 01, 2005**

Duration: **3 years**

Project coordinator name: **Prof. Marie-Danielle Nagel**
Project coordinator organisation name: **Divergent SA**

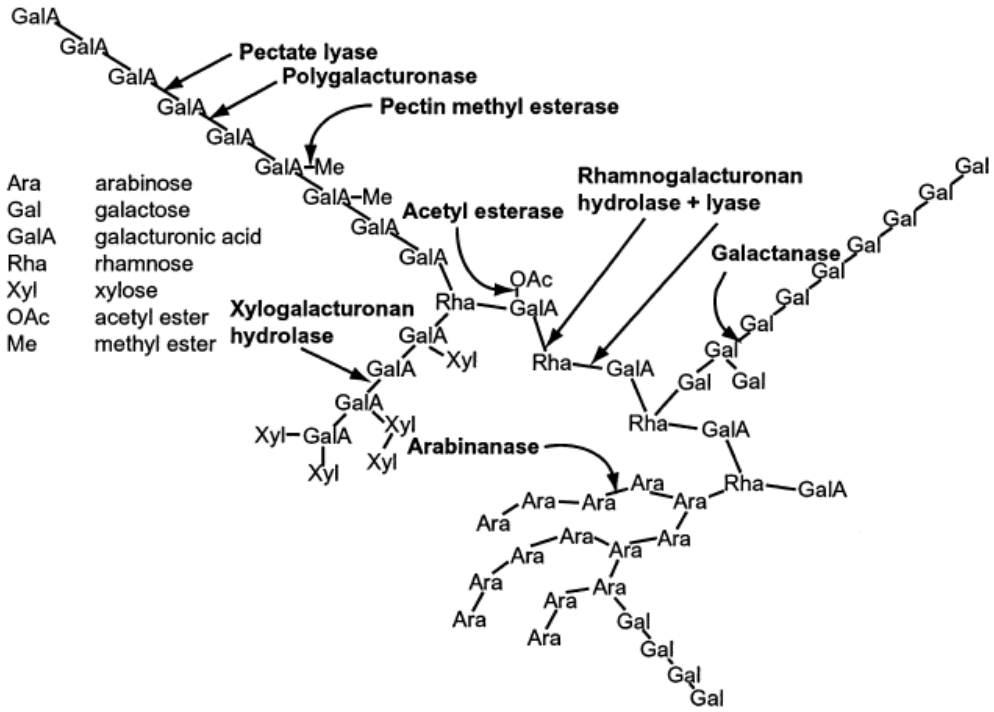
Version: [**draft-V1**]

Publishable executive summary

Description of project objectives

A cross-disciplinary nanotechnology approach is proposed that aims to impart material surfaces with novel and appropriate biological and bioactive properties using defined and tailored polysaccharides. The biomolecules that are to be grafted onto surfaces to function as nanocoatings have been selected from a class of complex polysaccharides, the pectic rhamnogalacturonans (RG-I), which are known to possess anti-inflammatory properties as well as desirable physical properties. Sugar molecular structure will be altered in a controlled manner so as to favourably influence living cell behaviour around the coated materials.

The goal of the programme is the fine-tuning of the surface properties of a range of biomaterials to optimize the interaction between the foreign materials and the host tissues in medical applications. Our cross-disciplinary research will promote a growth of the understanding of the molecular structure-function relationships of polysaccharide-modified surfaces and devices. With the realization of these goals a breakthrough in biomaterials production will be introduced to the biomedical industry.



Sites of attack elements to modify the homogalacturonan and rhamnogalacturonan structural elements of pectins. from Voragen, A. G. J.; *et al.* In *Food polysaccharides and their applications*; Stephen, A. M., Ed.; Marcel Dekker, Inc.: New York, 1995; pp 287-339.

Contractors involved

Current knowledge within our project includes:

-Technology to manufacture a range of tailored rhamnogalacturonan polysaccharides *developed through* ***WP2: "Tailor-made rhamnogalacturonans for coating",*** therefore creating flexible biomolecules that will be used to develop a range of innovative nanocoatings devoted to control cell behaviour.
WP2 contractors are: Partners #4 Wageningen University –NL- Laboratory of Plant Breeding (R. Visser) and Laboratory of Food Chemistry (H. Schols), and #5 Danish Institute of Agricultural Sciences –Dk- Biotechnology group (P. Ulvskov).

-Technology to attach rhamnogalacturonans covalently to relevant biomaterials and to characterize the grafted surfaces *developed through* ***WP3: "Interfacing biological and non biological components: function through design."***
WP3 contractors are: Partners #3 Nobil Bio Ricerche -I- (M. Morra), #7 University of Trento –I- Dipartimento di Ingegneria dei Materiali (C. Della Volpe), #9 University of Leeds –UK- Centre for Plant Sciences (J.P. Knox), #10 European Commission, Joint Research Centre, Institute for Health and Consumer Protection, International –I- (G. Ceccone).

-Technologies to assess nanocoated surface biocompatibility in vitro and in vivo, *developed through* ***WP4****: "Integration of biological and non biological systems: exploring interactions and control of cell behaviour."*
WP4 contractors are: Partners #2 CNRS-Technological University of Compiègne-F- UMR 6600 (M-D Nagel) and #6 University of Oulu-Fi- Department of Anatomy and Cell Biology, Medical Faculty (J. Tuukkanen).

-The purpose of ***WP1: "Industrial guidance" is to accelerate the process of innovation by optimizing the integration of scientific results in industrial product design.***
WP1 contractors are: Partners #3 Nobil Bio Ricerche -I- (M. Morra), #11 Statice santé –F- (S. Piranda), #12 Agliati –I- (G. Agliati).

-WP6: "Management and Dissemination" supports the co-ordination of the project as well as the diffusion of the results.
WP6 contractors are: Partners #1 (Co-ordinator) DIVERGENT S.A. -F- (Director-PDG, M. Cordonnier, michel.cordonnier @utc.fr) Centre de Transfert, Rond-point Guy Deniélou, 66 avenue de Landshut, 60200 Compiègne France - Tél: +33 344 234 530 / Fax: +33 344 234 560 contact@divergent.fr
#2 CNRS-UTC -F- Scientific management (M-D Nagel, marie-danielle.nagel@utc.fr) and #5 DIAS – Dk- Dissemination and exploitation of results (P. Ulvskov).

Work performed, results achieved so far and expected end results

1- Defined polysaccharide preparations for coating were delivered by WP2 partners.

-Rhamnogalacturonan preparations (called Modified Hairy Regions, MHR) differing in esterification, side chain composition and length from different plant materials as apple, potato (wild or transgenic) and carrot, were produced and supplied for surface coupling, characterization and in vitro cytocompatibility studies. Preparation of the different MHRs was performed as described by Schols et al. *Carbohydr. Res.* **1990**, *206*, 117-129. Samples have been delivered in the form of MHR-A, MHR-B, MHR-α, MHR-C and MHR-P or MHR-Ptr to the relevant partners. One apple sample (MHR-B) was treated with Rapidase liq+ enzyme, while the others, two apple (MHR-A and MHR-α), one carrot (MHR-C) and one potato (MHR-P) samples were treated with Rapidase C600 enzyme.
Structural characterisation has shown that with respect to the molecular weight distribution, sugar composition and the substituents present, the samples resemble the characteristics found for these vegetables and fruits before.
-Saponified or de-esterified samples MHR-Sα, MHR-CS and MHR-PS have been produced in gram quantities. Furthermore gram quantities of enzyme modified MHR-Sα has been made and delivered in the form of MHR-Sα-ara, MHR-Sα-gal, and MHR-Sα-ara-gal.

MHR-α → MHR-Sα → { MHR-Sα–ara; MHR-Sα–gal; MHR-Sα–ara/gal }

-MHR-B has been separated based on its size distribution. The different size pools of MHR-B have been isolated in gram scale to be analysed in WP4. Attempts to remove galacturonans from MHR-B

are ongoing, and β-elimination studies are currently performed to obtain more insight on the side chain architecture of the different MHRs samples.
Structure of the produced pectic molecules have been characterized using analytical techniques and identification of the bio-active structural epitopes of the produced pectic molecules has been performed with monoclonal antibodies (MAbs). 2 novel arabinan and at least 3 xylogalacturonan recognizing MAbs have been produced.
-New types of potato pectins are developed by making crossings between the available potato lines, thereby combining the action of two pectin modifying enzymes in a single plant.

2- A protocol for coupling of MHRs to materials surfaces has been succesfully established (deliverable 3.1.) and protocols for the characterization of coupled samples by a number of surface/interface sensitive techniques have been developed.

Coupling reaction was performed on polystyrene (PS) to graft MHRs from apple (B, A, α), carrot and wild or transgenic potato (C, P, Ptr), and derivatives of MHR-α and MHR-B on an underlying allylamine plasma polymerized film. XPS and ToFSIMS analyses clearly confirmed that all the MHRs were successfully grafted to the amminated surfaces and that the thickness of the coating was lower than 3-4nm. Results from surface characterization work confirmed the succesfull coupling of RG-I.
Interfacial characterization of pectin from apple (B, A, α) and carrot and potato (C, P) was performed together with analysis of the parent material, the polystyrene substrates and the allylamine deposited films. The activity then has mainly been focused on the fine-tuning and investigations on industrially relevant materials, that is titanium for bone contacting applications and silicone and polycarbonate in the field of prevention of adhesion. Samples of MHR coated Ti, Silicone, Polycarbonate have been prepared and supplied to WP3 partners for surface characterization. Moreover, and always in view of industrial applications, some sets of samples have been subjected to EtO sterilization to check sterilization effects by the different surface sensitive techniques available. Finally, the coupling strategy has been extended to whole pectins and transgenic MHRs.
Grafted-MHRs were also characterized by MAbs. All structural epitopes of RG-I and HG were generally maintained with minimal loss in the grafted pectin samples.

Existing protocols allow to support interactions with other WPs and the industrial application of the project (Ti/bone contacting devices)

3- Short and medium-term interactions between cells and MHR-nanocoatings have been analysed. Model cell lines were used for statistical evaluations, as well as primary cell and explant cultures. *In vivo* animal intrabony implantation tests have been performed.

Swiss 3T3 fibroblast morphology and adhesion, proliferation index, cell cycle and rate of cell death were established and statistical analysis performed. Results clearly demonstrate that grafted-engineered pectins induce specific cell responses. The influence of pectin coatings on fibroblast behaviour depends on the plant species and enzymatic treatment. Differences were also evidenced using primary bone cell population and MC 3T3 pre-osteoblasts.
Medium-term assays using SaOS-2 human osteosarcoma cells confirmed the lowest cell adhesion on MHR-α and C and the highest on MHR-B obtained from fibroblasts. All MHR-α modified surfaces (alpha and alpha derivatives) did not sustain cell growth. The original MHR-α sample was more cell resistant than its fractions.
Cultures of rat primary bone cells, chick embryo explant as well as bone-marrow derived human mesenchymal cells on coated Petri-dishes or Ti, clearly demonstrated that **MHR-B appears to be the most interesting pectin for bone cell applications.**

MHR-α on the contrary, as being resistant to protein adsorption, cell and bacteria adhesion, and non-inflammatory (it does not activate macrophages) is an **excellent candidate for anti-adhesive applications.**
The goal of the *in vivo* animal study was the evaluation of biomechanical integration and hystomorphometry evaluation at 4 weeks. Preliminary results indicate that absolute values agree with the expected trend.

4- Industrial guidance

Some bioincompatibility problems leading to adverse effects in patients implanted with various devices have been identified. Biological results have evidenced that grafted-B pectins were able to favour bone cell proliferation and/or differentiation *in vitro*, and on the contrary grafted-α pectins to show anti-adhesive properties for fibroblasts. A set of industrial criteria to monitor the activities of the project have been proposed and RG-I coupling protocols have been applied to materials relevant to industrial purposes. An application patent (PCT) has been filed by P#3 and P#12 for bone implant coating, P#11 is pending a patent for anti-adhesive polymer coating.

Plan for using and disseminating the knowledge

With regard to the progress of the project, the filing of a PCT patent application (by Partners #3 and 12) appeared to be appropriate. **The decision was made at the Mid-Term meeting that any dissemination action that** may compromise ongoing or future patenting efforts should be avoided.