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.

Publishable Final Executive summary

Period covered: from **April 01, 2005** to **March 31, 2008**

Date of preparation:

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

Duration: **3 years**

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

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Overview of general project objectives

Surface modification of biomaterials and medical devices by polysaccharides is presently a topic of great interest, both at fundamental and applied levels. 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.
PectiCoat STREP was devoted to a **multi-disciplinary effort** aimed at **demonstrating the proof-of-concept for the use of engineered pectins (rhamnogalacturonans: RG-I) on the surface of medical devices** (e.g. titanium dental implants).
Pectic innovative flexible nanocoatings, that have the potential to endow medical devices with **antiinflammatory properties** and to be **tailored to stimulate desirable cell functions** (e.g. implant colonisation and living tissue healing and differentiation) can **solve problems of bioincompatibility** that currently lead to implant failure.
Selection among tailored pectins is made in accordance with biomaterial potential fields of application.
A special attention was given to molecular mechanisms through which cell activation is induced by RG-I: RG1-cell direct or indirect interaction via extracellular matrix proteins ("sugar language" understanding).
Tailoring RG-I molecules to control cell behaviour around nanocoated implants can lead to a **wide range of applications through industrial monitoring and engineering.** .Academic and Industrial involvement in the project covers the full production chain, from raw material to prototype design.

Contractors involved

Current knowledge within PectiCoat project includes:

1) technology to manufacture a range of tailored rhamnogalacturonan polysaccharides (WP2: P#5 University of Aarhus, DJF Dk, P#4 University of Wageningen, Food Chemistry and Plant Breeding, NL) to develop a range of innovative nanocoatings devoted to **control cell behaviour**,
2) technology to attach these macromolecules covalently to relevant **biomaterials** (e.g. titanium for dental implants), and to characterize the grafted surfaces interfacing biological and non biological components, (WP3: P#3 Nobil BioRicerche, P#7 University of Trento, I, P#9 University of Leeds, UK, and P#10 Joint Research Center EU),
3) technologies to assess nanocoated surface **biocompatibility** *in vitro* and *in vivo,* to explore interfaces and interactions and verify their impact on cell behaviour (WP4: P#2 Université de Technologie de Compiègne, UMR CNRS 6600 Fr, P#6 University of Oulu Fi),
4) Industrial monitoring and engineering (WP1: P#11 Statice Santé, Fr, P#12 Agliati, P#3 Nobil BioRicerche I) in order to allow the exploration of a wide range of applications of PectiCoated devices.

Main results

First, five structurally distinct pectic polysaccharides (modified hairy regions (MHRs)) were obtained by enzymatic liquefaction of apple (MHR-B, MHR-A, MHR-α), carrot (MHR-C) and potato (MHR-P) cells (Table I). Polystyrene (PS) Petri dishes, aminated by a plasma deposition process, were surface modified by the covalent linking of the MHRs. Results clearly demonstrated that MHR-B induces cell adhesion, proliferation and survival, in contrast to the other MHRs. Moreover, MHR-α causes cells to aggregate, decrease proliferation and enter into programmed cell death (apoptosis). The potential role of adsorbed proteins as mediators of pectin-cell interactions was addressed to identify the molecular mechanisms involved in the cell responses. Results indicated that adhesive

proteins are involved in the control of fibroblast behaviour by pectin MHRs [1].

Table 1: Sugar and substituent composition (in mol%, rhamnose/galacturonic acid ratio and total sugar in w/w%) of the different MHR samples.

MHR	Fuc	Rha	Ara	Gal	Glc	Xyl	GalA	GlcA	O-Me	O-Ac	Rha/ GalA	Total w/w%
-B	0	11	11	20	3	18	37	nd	34	11	0.30	78
-P	1	10	14	28	4	nd	42	nd	57	59	0.24	70
-C	2	22	8	32	2	2	30	2	16	46	0.75	82
-A	0	9	51	10	0	8	22	nd	42	60	0.41	86
-α	1	15	25	15	1	12	31	nd	37	45	0.49	86

O-Me : Moles MeOH per 100 moles GalA (max 100%)
O-Ac : Moles Ac per 100 moles GalA (max 200%)
nd : non detectable

Next studies were designed to learn more about the capability of engineered RG-I fractions of apple pectin to control bone cell and macrophage behaviour.
Thermanox® or polystyrene Petri dishes were surface modified by MHRs differing in relative amounts and lengths of their neutral side chains: (long-haired) MHR-α and (short-haired) MHR-B. Bone explants from 14-day-old chick embryos were cultured for 14 days on both pectic substrata. On MHR-B, cells showed proliferation, migration and differentiation. They did not on MHR-α [2].

Primary rat bone cells and murine preosteoblastic MC3T3-E1 cells were cultured on MHR-A and MHR-B –grafted PS or glass. Cell attachment, proliferation, and differentiation were investigated. Bone cells seem to prefer MHR-B coating to MHR-A coating. On MHR-A samples, the overall numbers as well as proportions of active osteoclasts were diminished compared to MHR-B, tissue culture PS, or bovine bone slices used as controls. Focal adhesions indicating attachment of the osteoblastic cells were detected on MHR-B and uncoated controls but not on MHR-A [3].

The proliferation and differentiation of osteoblastic line cells, primary osteoblasts and human mesenchymal cells on apple-derived MHRs (MHR-A and MHR-B) grafted on titanium discs was investigated . Results indicated that the pectin fragment type MHR-B allows osteoblast differentiation (ALP activity and calcium deposition) comparable to that on pure titanium, whereas MHR-A seems not to be supportive for cell differentiation [4].

MC3T3-E1 preosteoblasts were cultured on PS Petri-dishes grafted with potato wild type and transgenic pectins. Wild type potato pectin allowed cell spreading but no focal adhesions. ARA depletion reduced cell growth and spreading but GAL depletion stimulated spreading and induced clear focal adhesions. Thus, pectic material from potato transformants differing in sugar composition differently influences cell behaviour.

Then, studies were focused on inflammation

On MHR-α, J774.2 macrophages grew well, their percentage in G1 phase was decreased and in S phase increased, and they did not secrete either pro-inflammatory-cytokines or nitrites. Contrasting results were gained from macrophages on MHR-B, except for nitrite secretion [2].
The capability of MHRs to influence LPS-induced proinflammatory response of J774.2 macrophages was assessed through cell proliferation, cytokine and nitrite secretion. Experiments using grafted-MHR were performed. A suppressive effect was observed from MHR-α-grafted Petri dishes.

Thus, the engineering of plant-derived pectins can be a valuable tool to prepare novel and finely tuned polysaccharides (differing in charge, molecular weight, degree of branching and acetylation) to be used in the surface modification of medical devices and materials.

Coatings from tailored pectins show different biological activities *in vitro* and are potential innovative candidates for improving the biocompatibility of medical devices in various applications.

Taken as a whole, *in vitro* biocompatibility data were decisive to give the project directions relevant to the industrial needs expressed through industrial guidance: the success of the grafting process evidenced by surface characterization was confirmed by biological results. MHR-B could be seen as a good candidate for bone implant applications, in contrast with MHR-α, the most suitable MHR for anti-adhesive device purposes.

Thus, planning animal *in vivo* implantation was justified.

Osseointegration assays (intrabony grafted Ti screw implantation in rabbit) were subcontracted and sub-cutaneous tests in mice were performed using grafted-Ti disks. No significant adverse tissue response was detected in contact with MHR-B and histological results appear to have been reasonably well anticipated by the behaviour of bone cells in culture.

General Conclusion

Enzymatically-tailored and engineered pectins were produced, purified and analysed. They have been successfully grafted on different substrata and characterized. *In vitro* and *in vivo* biological studies demonstrated that some of these pectins are endowed with properties which are expected to improve the biocompatibility of dental implants and also other medical devices. Due to a real industrial participation through the project, guidance for defining and implementing applications has been fruitful.
Part of experimentation is still in progress, opening the way to new developments.

IP protection has been considered and 2 PCT application have been filed.

References

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