Polysaccharide Bioshapes - Chemical design and Shaping into New Biomaterials Project title (PShapes)

FINAL REPORT

Title of the research project	**Polysaccharide Bioshapes- Chemical design and Shaping into New Biomaterials (PShapes)**
Coordinator of the project	Professor Pedro Fardim

BASIC PROJECT DATA

Project period	01.01.2014- 01.06.2017
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URL of the project	http://www.bioshapes.net/

FUNDING

Total budget in EUR	**1 290 166** EUR
Public funding from WoodWisdom-Net Research Programme:	Total funding granted in EUR by source:
<u>Austria</u> Federal Ministry of Agriculture, Forestry, Environment & Water Management (BMLFUW)	175.586 EUR
<u>Germany</u> Agency for Renewable Resources (FNR)	TITK: 241.299,00 EUR FSU: 244688 EUR

Slovenia
Ministry of Education, Science and Sport (MIZS) 208 924,32 EUR

Other public funding:

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Other funding:

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PROJECT TEAM (main participants)

Pedro Fardim, Ph.D, Professor	Sex (M)	Åbo Akademi University	Finland
Thomas Heinze, Ph.D, Professor	Sex (M)	Friedrich Schiller University of Jena	Germany
Frank Meister, Ph.D, Deputy Managing Director	Sex (M)	Thuringian Institute of Textile and Plastics Research	Germany
Silvio Hribernik, Ph.D, Principal investigator	Sex (M)	University of Maribor	Slovenia
Volker Ribitsch, Ph.D, Professor	Sex (M)	University of Graz	Austria

DEGREES (if relevant)
Degrees earned or to be earned within this project.

2018	Ph.D	Sex (F)	Poonam Trivedi, YOB 1984, MSc. Earned in 2006.	Åbo Akademi University	Pedro Fardim, Åbo Akademi University
2016	Ph.D	M	L. Amornkitbamrung, 1986, MSc. Earned in 2012	University of Graz	Volker Ribitsch University of Graz
2018	M.Sc	F	Mattea-Coco Marnul, 1987,	University of Graz	Volker Ribitsch,
2016	Master	M	Lars Gabriel, born 1989 M.Sc. earned in 2016, D.Sc., etc. Degree	University of Jena	Thomas Heinze, University of Jena

PROJECT SUMMARY REPORT

The goal of this project was to create new value-added bio-based products based on polysaccharides that are suitable for different value chains. These bio-based materials with multifunctional capabilities can create new concepts for utilization of forest resources in new high added value chains. Therefore, different bottom-up techniques were developed to prepare polysaccharide-based particles from solutions of the corresponding polymers. The different approaches available allow tailoring the size of the particles over two orders of magnitude (Figure 1). For example, two main concepts were adapted for the preparation of nanoparticles from hydrophobic cellulose esters: The emulsification-evaporation process from a lipophilic solution of the polymer and the solvent-displacement processes from a hydrophilic solution. These techniques yield, depending on the conditions, particles of a size 50 nm – 1 µm with narrow (even monomodal) particle size distribution. Alternatively, cellulose and hemicellulose particles can be prepared from polymer solutions by the formation of droplets using filament cutting or spinning disk atomization and the subsequent coagulation of the droplets in a non-solvent bath. The particle diameters obtained in this case is dependent on the size of droplets as well as on the coagulation conditions and can be tuned from 50 µm and a few millimetres(microparticles). Particles prepared by the dropping-coagulation method are termed as cellulose beads and feature outstanding high surface areas. The Polysaccharide-based nanoparticles and beads were successfully applied in the biomedical field (e.g. pH sensing in living cells, controlled delivery of active principles) and separation science (e.g. chromatographic systems, chelating sorbents, filter materials). However, the research activities in Europe were scattered to a few research groups and were limited to incremental development so far. This consortium has covered the whole research and development field including the chemical- and physical design of the particles, biorefinery aspects, biomedical applications, and industrial products. Beyond the state-of-the art, the synergies that arise has allowed to establish a clear structure property relationship for the particles with respect to the way of preparation and the starting material. Within the consortium, the fundamental knowledge gained was directly transferred in order to solve the questions related to consumer oriented applications. Due to this close interaction of the partners with different expertise and the positive feedback, the synthesis of multifunctional cellulose beads, nanoparticles were achieved and their application in the immunodiagnostics, drug delivery, tissue engineering can be foreseen.

1.1 Introduction

1.1.1 Background

The PShapes project is an International research consortium of the leading European groups to promote innovative research in the field of polysaccharide-based nanoparticles and beads to be utilised in high added value chains such as immunoassays, bio chromatography, wound care dressings and tissue engineering. The novel products obtained will provide a foundation to the forest-based industries to invest in this area and strengthen the bio economy

1.1.2 Objectives

Overall objectives

1. Create new value-added products based on polysaccharides that are suitable for different value chains.

2. Develop new bio-based materials with multifunctional capabilities.

3. Create new concepts for utilization of forest resources in new high added value chains

Scientific and technological objective.

1. Develop new and efficient methods to prepare tailored functional particles and beads combining advanced polysaccharide chemistry, physical chemistry and chemical engineering.

2. Develop new methods to use particles (nano and micro) as functionalization platform for functional materials, tissue healing materials, textiles and packaging.

3. Develop new and efficient methods to use functional beads in controlled drug delivery and separation/fractionation technology for biorefinery applications.

4. Create new technologies for production of high value-added biomaterials, fractionation of biomass components, functionalization of polysaccharides, and upgrade of biomass residues to new products

1.2 Results and discussion

We have achieved synthesis of multifunctional polysaccharide based nanoparticles for immunoassays applications with optimization of innovative methods for the nanoparticle synthesis. Incorporation of functional nanoparticles into fibers to design films with flame retardant and hydrophobic properties for packaging and biomedical applications was also investigated. We have also designed multifunctional cellulose based beads of micro to millimeter sizes and evaluated their applicability for tissue engineering applications.

The role of polysaccharide based functional Nano Micro and millimeter sized particles will open up new opportunities for the value-added applications in the fields of immunoassays, pharmaceuticals, Biomedical and Tissue Engineering and collaboration with material scientists, engineers and biomedical scientists

1.3 Conclusions

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1. Successful preparation of colored composite nanoparticles from hydrophobic cellulose esters and covalent coupling of antibodies for lateral flow immunoassays. Synthesis of xylan carbonates and nanoparticles preparation was achieved. Tosylation of cellulose beads was not successful. New method of activation by introducing carbonate moieties will be tried. New particles for applications in diagnostics, including Ebola virus.

2. Successful preparation of nanoparticles from the following cellulose esters.Cellulose beads prepared from cellulose acetate beads by deacetylation were tested for diclofenac (analgesic) drug loading and release at pH 2 and 7. Pre-osteoblast cells viability and adhesion studies with drug coated cellulose beads was performed. Completely deacetylated beads showed higher cell adhesion compared to acetylated cellulose beads. A comparative study between cellulose beads and periodate oxidized beads showed higher interaction of osteoblast cells with oxidized cellulose beads than deacetylated cellulose beads.

3. Sulphonated cellulose beads were designed and characterized. Beads with higher solvent content displayed higher core porosity than the conventional beads. Antibacterial tests were performed (gram negative bacteria E. Coli) with cellulose sulphonate beads. These beads did not show any bacteriostatic or bacteriocidal effect. Chitosan-cellulose porous hydrogel beads were designed and characterized for cell adhesion and viability studies. Cellulose aldehyde beads and aldehyde xylan were syntheised by periodate oxidation.

4. LDH-Cellulose films of 100µm thickness were prepared and characterized via SEM analysis. The films were tested for flame retardancy. Best flame retardancy was observed with 33% LDH concentration in films. Water vapour permeability tests were performed, and swelling behaviour was observed. Films broke during the tests.

5. Surfactant-based dissolution of poorly water-soluble drugs (Hydrophobic) and their loading into cellulose beads was studied. Preparation of micro and nano sized ethyl cellulose capsules via emulsification technique using biologically approved stabilisers. Synthesis and characterization of ethyl cellulose microcapsules for vitamin E encapsulation and their efficiency in vitamin E release and anti-oxidant activity. Fibre pre-treatment for efficient particle attachment formation of silica layers on cellulose fibres as possible fire-retardant coatings. Fibroblast proliferation on different polymer fibrous surfaces Polyethyleneterephthalate (PET), Alginate (ALG) and viscose (VIS) loaded with analgesic diclofenac sodium was studied.

6. The feasibility of functional cellulose beads for the separation/fractionation of biorefinery compounds has been diverted towards bio-chromatography and interaction with blood

components such as neutrophils and proteins. The designed conventional, oxidised and zwitterionic cellulose beads will be tested.

1.4a Capabilities generated by the project

The overall outcome of the project encompasses a clear vision to design polysaccharide based entities with varying functionalities/chemistry. The role of polysaccharide based functional Nano Micro and millimeter sized particles will open up new dimensions for the value added applications in the fields of immunoassays, pharmaceuticals, Biomedical and Tissue Engineering. New opportunities for valorization of polysaccharide and wood based materials.
The project has provided an opportunity to the PhD researchers to gain knowledge related to various aspects of the polysaccharide based materials chemistry, design and application. The results from the project will help in developing the bio-based immunodiagnostic products.

1.4b Utilisation of results

Give a brief description of how the results of the research and development have been used and/or what is the exploitation plan or plans for transferring the results into practice.

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1.5 Publications and communication

Articles in international scientific journals with peer review

1. ***Trivedi.P, Trygg.J, Saloranta. T, Fardim. P (2016). Synthesis of novel zwitterionic cellulose beads by oxidation and coupling chemistry in water. Cellulose 23: 1751-1761.
2. *Schulze.P, Gericke. M, Scholz. F, Wondraczek.H, Miethe.P, Heinze.T (2016). Incorporation of hydrophobic dyes within cellulose acetate and acetate phthalate based Nanoparticles, Macromolecular Chemistry and Physics, 217: 1823–1833.
3. * Obst. M, Heinze.T (2016). Simple Synthesis of Reactive and Nanostructure Forming Hydrophobic Amino Cellulose Derivatives. Macromolecular Materials and Engineering 301: 65–70.
4. *Trygg. J, Trivedi. P and Fardim. P (2016) CONTROLLED DEPOLYMERISATION OF CELLULOSE TO A GIVEN DEGREE OF POLYMERISATION. Cellulose Chemistry and Technology, 50: 557-567.
5. *Mohan. T, Rathner. R, Reishofer. D, Koller. M, Elschner. T, Spirk. S, et al.(2015) Designing Hydrophobically modified Polysaccharide derivatives for highly efficient enzyme Immobilization, Biomacromolecules.16 : 2403–2411.
6. Trivedi, P., Schaller, J., Gustafsson, J. and Fardim, P. (2017). Supramolecular Design of Cellulose Hydrogel Beads. *Journal of Renewable Materials*.

7. Trygg. J, Yildir. E, Kolakovic. R ,Sandler. N, Fardim. P (2015) Solid-State Properties and Controlled Release of Ranitidine Hydrochloride from Tailored Oxidised Cellulose Beads Macromolecular Materials and Engineering 300 : 210-217.
8. Trygg. J, Yildir. E, Kolakovic. R ,Sandler. N, Fardim. P (2014) Anionic cellulose beads for drug encapsulation and release Cellulose 21 :1945-1955.

9. Blachechen .LS, Fardim. P, Petri. DFS (2014). Multifunctional cellulose beads and their interaction with gram positive bacteria Biomacromolecules 15 : 3440-3448.

1.6 National and international cooperation

The project was coordinated by Åbo Akademi University, maintaining the trend of biannual steering- research group and online research meetings every two-three month. The kick-off meeting was organized in Turku, Finland on September 3rd, 2014. Steering – Research group meetings were held at: Abo Akademi University, Finland (9^{th}-10^{th} Dec 2014), University of Maribor, Slovenia (22-23 June 2015), University of Graz, Austria (9^{th}-10^{th} Dec 2015), Stora Enso Stockholm, Sweden (7^{th} -8^{th} June 2016), Academy of Finland, Finland (14^{th} Dec 2016). The final meeting was held at University of Jena, Germany (14-15^{th} June 2017). To follow up the research and progress in between biannual meetings, four online research meetings (14^{th} Nov 2014, 29^{th} Sep, 17^{th} Nov 2015 and 7^{th} Mar, 9^{th} May 2016) were also organized and coordinated by the Åbo Akademi University.

In order to create synergy between partners the coordination created and monitored a flow for exchange of materials that were further incorporated in design and preparation of nanoparticles, beads, fibre and films. The systematic exchange was very useful in promoting collaboration and in combination with periodic research meeting, in promoting collaboration.
The PShapes partners have been very enthusiastic about the exchange of ideas and improving the research to achieve the project goals. The individual work packages lead by the research groups has provided a base to form a network of materials demand and supply with the other partners. All the partners have done a press release in their respective countries.

We have been collaborating with partners of European Polysaccharide Network of Excellence (EPNOE), Cellulose and Renewable Material Division of the American Chemical Society (ACS) and several national non-partners. In Finland collaboration with Turku Biocity and University of Turku has been essential to advance the application of cellulose beads in biomaterials and biosciences.

We have been able to have a steering group that was hosted by our project observer in December 2015 at Academy of Finland in Helsinki. This occasion was very good to hear about the impressions about our project findings and potential opportunities to continue collaboration in science and innovation.