Bioencapsulation Research Group

Bioencapsulation Innovations

October 2014

CONTENTS

EDITORIAL

XXII INTERNATIONAL CONFERENCE ON BIOENCAPSULATION

Bratislava, Slovakia - September 17-19, 2014

The 22th International Conference on Bioencapsulation was very efficiently organized at Holiday Inn Hotel of Bratislava by Professor Igor Lacik and Mrs Silvia Podhradska from the Polymer Institute of the Slovak Academy.
The conference was a real success with regard to the quality of the oral and poster presentations, but also the services offered by the hotel team. The conference dinner took place on boat on the Danube under jazz music atmosphere provided by a jazz band and was the opportunity for a lot of informal exchanges.

One hundred and twenty researchers coming from 27 different countries, including 17 industrials and exhibitors attended the conference. Forty oral contributions and forty-three posters were presented during the conference. Their associated texts will be available soon on the BRG web site. One special session on regenerative medicine was supported by the JRDF.

The scientific committee selected the student 5 best oral and 5 best poster presentations and 10 students received one best contribution prize. The participants were invited to nominate a candidate for the Poncelet Award, sponsored by Procter and Gamble, and rewarding a person having strongly contributed to the innovation and development of the Bioencapsulation. This year the award was given to Professor Paul De Vos from Groningen University in Netherlands for his scientific work in Islet encapsulation but also his strong involvement in BRG activities.

The minutes of the General Assembly of the BRG association, held during the conference, are included in page 31. A call is open to volunteers to help in different tasks such as developing the BRG address-book, collecting industrials and diversified news ... The volunteers will be listed in the steering committee and will have priority access to BRG grants. For more information, mail to contact@bioencapsulation.net

Prof. Denis Poncelet
BRG President

CALENDAR

PROGRAM 2014

November

Improving Oral Bioavailability with Lipid-Based Delivery Approaches
November 1-2, 2014
San Diego, USA
www.aaps.org/Lipids.

December

Annual meeting
December 9 - 12, 2014
Nancy, France
http://www.sfnano.fr/?page_id=6121

16èmes Journées de la formulation
December 9-10, 2014
Villeneuve d'Ascq, France
http://www.gfp.asso.fr/events/16emes-journees-de-formulation

Mini conference PowTech: Properties, production and handling
December 11-12, 2014
Brussels, Belgium
http://powtech.sik.se/

Innovations in Encapsulation
December 12, 2014
London, UK
http://www.formulation.org.uk/inen14.html
http://www.rsc.org/events/detail/16678/innovations-in-encapsulation

PROGRAM 2015

January

International Workshop Polyelectrolytes in Chemistry, Biology and Technology
January 26-28, 2015
Singapore, Singapore
http://www.ntu.edu.sg/ias/upcomingevents/Polyelectrolytes15/Pages/default.aspx

February

6th AnnualGlobal Drug Delivery and Formulation 2015
February 9 - 11, 2015
Dusseldorf, Germany
http://www.ddfevent.com

6th training School on Bioencapsulation
February 23-27, 2015
Strasbourg, France
http://bioencapsulation.net/2015_Strasbourg/

April

8th International Conference on Bio-based Materials
April 3 - 15, 2015
Maternushaus, Cologne, Germany
http://www.biowerkstoff-kongress.de

1st European Conference on Pharmaceutics: Drug Delivery
April 13-14, 2015
Paris, France
http://apgi.org/Reims2015.htm

April

18th Microencapsulation Industrial Convention
April 22-24, 2015
Eindhoven, Netherlands
http://bioencapsulation.net/2015_Eindhoven/

June

7th International Symposium on Food Rheology and Structure (ISFRS 2015)
June 7 - 11, 2015
Zurich/Switzerland
http://www.isfrs.ethz.ch

Septembre

23th International Conference on Bioencapsulation
September 2-4, 2015
Delft, Netherlands
http://bioencapsulation.net/2015_Delft/

PROGRAM 2016

April

10th Workd Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology
April 4-7, 2016
Glasgow, UK
http://www.worldmeeting.org/home/

Include your conference, workshop, training school related to Bioencapsulation by sending information to

BI@bioencapsulation.net

We are looking for volunteers to help collecting information regarding conferences, exhibitions, symposia ... related to domains where encapsulation could be involved. If you wish to join the team,

contact@bioencapsulation.net

AWARDS

BRG Life-time Achievement Award

Dr Jean Paul Simon was one of the first BRG members to join the group in 1991. Over the years, his involvement in the BRG contributed to the success of the association both in accomplishing tasks and by promoting a friendly relationship between members of the group. Organizer of the third international conference on Bioencapsulation in 1993 and the 17th Industrial convention on Microencapsulation in 2013 in Brussels, Belgium, he and his research group at Institut Meurice presented more than 30 orals and posters to BRG meetings. He was one of the initiators and co-chairmen of the COST actions 840 and 865, European network fundings that really helped to develop the association.

Widely recognized both by the scientific and the industrial communities for his high competence, retired now, he still remains active as an expert and also as a support the BRG.

The association thanks him by attributing him the BRG Life-time Achievement Award.

2014 Poncelet Award

Prof Paul de Vos joined the BRG in 1993 as a young researcher and quickly became a leader in the field of islet encapsulation, working in particular on the immunological responses to capsules.

He strongly involves himself in the development of the BRG, organized in 2009 the XVIIth International Conference on Bioencapsulation in Groningen, The Netherlands, presented several excellent lectures at training schools and industrial conventions on micro encapsulation. He and his group already presented 23 oral and poster contributions during BRG events.

In 2013, he accepted the vice-presidency of the BRG and he performs the function of chief editor of the BRG newsletters.

The association recognizes his involvement in the BRG by attributing him the 2014 Poncelet Award

ARTICLE

TOWARDS ENCAPSULATION POLYMERS WITHOUT IMMUNE RESPONSES

Paul de Vos, Genaro A. Paredes-Juarez, University of Groningen, Pathology and Medical Biology, 9713 GZ Groningen, The Netherlands

INTRODUCTION AND OBJECTIVES

There are many diseases in which pharmaceutical intervention cannot prevent undesirable fluctuations in hormones and metabolites. Examples of these diseases are hemophilia B, anemia, dwarfism, kidney, and liver failure, pituitary disorders, central nervous system insufficiency, and diabetes mellitus. Long-term pharmaceutical intervention in these diseases may lead to side effects and complications. These side effects can be prevented by offering the patients a cell-transplant that regulates the hormone and metabolites on a minute-to-minute basis. Immunoisolation has been proposed to be a technology to enable such a therapy by protecting the cells for harmful effects of the host immune system.

Cellular transplants are normally rejected within hours after transplantation due to immune responses. By enveloping the islets in a membrane that is impermeable for the effector-side of the immune system (such as immunoglobulines and complement factors) this rejection can be avoided. One of the most commonly applied procedures for immunoisolation is encapsulation of tissues in alginate-based capsules. Alginate, the main component of the capsule, is a polysaccharide composed of different amounts of mannuronic acid (M-chains) and guluronic acid (G-chains) linked in blocks (MM-blocks, GG-blocks and MG-blocks). Binding the molecules with divalent cations such as Ca^{2+} or Ba^{2+} result in the formation of alginate beads. These beads can subsequently be coated with polyaminoacids to form capsules with a specific permeability. Capsules with specific physical and chemical properties can be obtained by varying the type and concentration of the cations and the polyaminoacids. During recent years, important advances have been made with this technology. New capsule types have been developed and tested. Human trials have been started and have demonstrated the principle

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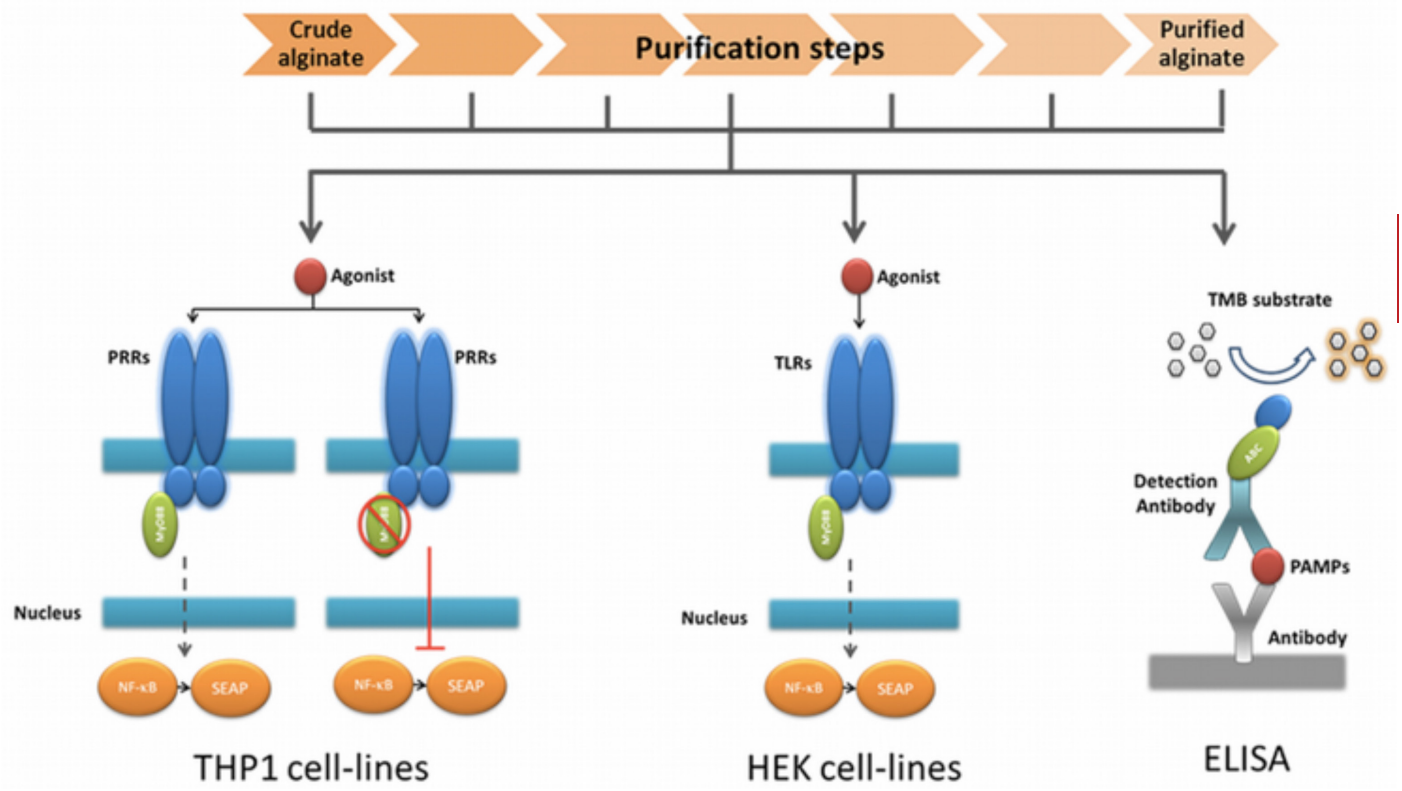

Figure 1 : Technology to assess the immunostimulatory capacity of alginate, to determine where in the purification process the pathogen associated molecular patterns (PAMPs) are removed, and which Toll-like receptors (TLRs) and ligands are being involved. We applied a THP1 monocytic cell-line in the first step to determine the immunostimulatory capacity of the alginate. Next, we applied a THP1 with a non-functional MyD88 coupling protein. This allowed us to confirm that the immune stimulation is PRR dependent. In the next step, HEK cell-lines that possess the specific receptors were applied. This approach allows for scaling down the number of candidate PAMPs that might be responsible for the response. The last step was application of an enzyme-linked immunosorbent assays to identify the specific PAMPs. Also published in (Paredes Juarez, 2014).

applicability of microencapsulation for the treatment of thyroid disorders and diabetes type 1. Although successful, these studies have also shown a major hurdle that has to be overcome, the large variation in success rates with capsules. Some laboratories will find capsules back without any signs of a tissues response while others retrieve capsules from their recipients that are totally overgrown with fibroblasts with death of the enclosed cells as a consequence. A poor standardization of the technology is causing these responses but also application of polymers that contain proinflammatory impurities is a major issue (de Vos, 2014).

Recently, we demonstrate that pathogen-associated molecular patterns (PAMPs) in alginates (Paredes Juarez, 2013, 2014) are one of the dominant molecules responsible for tissue responses after implantation of encapsulated tissues or cells. PAMPs are small molecular motifs found on groups of microorganisms and can be recognized by Toll-like receptors (TLRs) and other pattern recognition receptors (PRRs) on cells of the innate immune system. In the present study, we present a technology platform that can be used to evaluate the efficacy of purification procedures for alginate and other polymers applied in capsules. The platform is composed of several fast and cost-effective procedures designed to (i) stepwise assessment of the immunostimulatory capacity of alginate, (ii) wherein the purification process the PAMPs are removed, and (iii) which TLRs and their ligands are being involved and might result in a smaller sample size required for biocompatibility and animal testing.

EXPERIMENTAL

Intermediate-G alginate (ISP Alginates Ltd., City, UK) has been used (42% G-chains, 58% M-chains, 23% GG-chains, 19% GM-chains, 38% MM-chains, Mn = 428 kDa) for studies on efficacy of purification. The composition of these alginate samples was studied by proton nuclear magnetic resonance (^{1}H-NMR). Commercially available purified alginates from Les Laboratoires Brothier (Paris, France) and Pronova™ (FMC BioPolymer, Sandvika, Norway) were applied to compare the degree of purity and content of pathogen-associated molecular patterns.

QUANTI-Blue™ (InvivoGen, Toulouse, France) is a medium with a colorimetric enzyme used to detect activity of any alkaline phosphatase. QUANTI-Blue™ medium turns purple-blue in the presence of alkaline phosphatase (SEAP) and can be quantified using a spectrophotometer at 620–655 nm.

RESULTS & DISCUSSION

The first step in the platform is a fast screening for NF-ĐB based immunostimulation of THP1 monocytes by the polymers. If PAMPs are present in the polymer mix the cells will be activated, even when they are present in the range of picograms [28,32]. Next, to determine whether the PAMPs are

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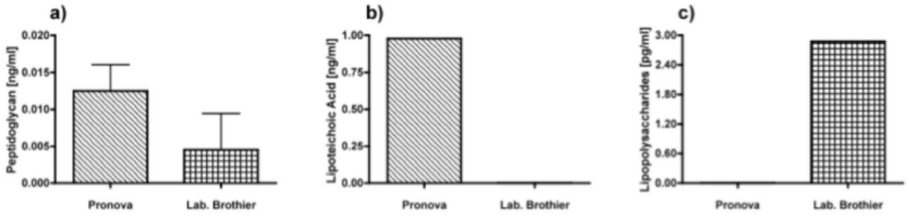

Figure 2 : Presence of (a) peptidoglycan (PG; TLR2 ligand); (b) lipoteichoic acid (LTA; TLR2 ligand); (c) lipopolysaccharides (LPS, TLR4 ligand) in commercially available ultrapure alginate of Pronova and Laboratoires Brothier. Also published in (Paredes Juarez, 2014).

ligands for Toll-like receptors (TLRs) or other pattern recognition receptors (PRRs) the polymer is incubated with THP1-cells with a deficiency in the MyD88-signalling. All TLRs with the exception of TLR3 require MyD88 for NF-κB activation. If the cells give no signal which in most cases happened we continued to the next step.

In order to identify the exact type and amount of PAMP in a stepwise fashion in the polymers, we apply a relatively inexpensive test involving transgenic human embryonic kidney (HEK) cells with one specific TLR. Our group have them for all TLRs as well as for many c-type lectines and other pattern recognition receptors. Also, these cells carry a NF-κB reporter allowing fast identification of the TLR involved. Knowing which receptors are involved provides insight in which contaminations may be present in the polymers. The last step in the platform is application of ELISAs to identify the specific PAMP in the alginate (Figure 1). This will allow for the design of methods to remove the PAMPs.

The advantage of the above described work flow is that it avoids a time consuming and expensive screening for non-specific PAMPs by ELISAs only. Another advantage is that it provides data on immunostimulatory capacity of the polymer preparations.

Our toolbox was applied to test the immunogenicity and presence of PAMPs in two commercially available alginates that are marketed as purified alginate. These were alginates purchased from Pronova (Pronova Ultra-Pure MVG G/M ratio ≥ 1.5, G content ≥ 60%, approximate Mw > 200 kDa, endotoxins ≤ 100 EU/g, viscosity > 200 mPa·s) and Les Laboratoires Brothier (now Kimica Algin, High G I-3G, viscosity 300–400 mPa·s). Both alginates had immunostimulatory capacity on THP-1 cells. Alginate from Pronova contained peptidoglycan and lipoteichoic acid (TLR2 ligands). The alginate from Les Laboratoires Brothier contained peptidoglycan (TLR2 ligand) and lipopolysaccharides (TLR4 ligand) (Figure 2). This demonstrated the need of a platform as presented here to test also commercially obtained alginates.

Up to now the LAL assay was applied to test for presence of endotoxins in polymers for encapsulation. LAL is an extract of blood cells from the horseshoe crab, *Limulus polyphemus*. We recommend using more specific measures such as the ELISA approach instead of LAL. LAL mainly reacts with bacterial lipopolysaccharide (LPS). Although it has recently been shown that also the presence of lipoteichoic acid (LTA) and peptidoglycan (PG) may result in positive LAL assays, it is unknown how sensitive the assay for other endotoxins or PAMPs is. In addition, our platform provides insight in which PAMPs are still present in the polymers, which gives researchers the opportunity to design specific means to remove the molecules.

CONCLUSIONS

All commercially available polymers for encapsulation that we have tested thus far do contain immunogenic components. We present here a technology platform to assess the immunogenicity of the polymer or its contaminants and which pattern recognition receptor and ligands are involved (Paredes Juarez, 2014). Our toolbox provides insight in the mechanisms by which encapsulation polymers provoke an immune response and it allows for identification of the specific components that the initiate response. The latter might be helpful in designing methods to remove the contaminating components.

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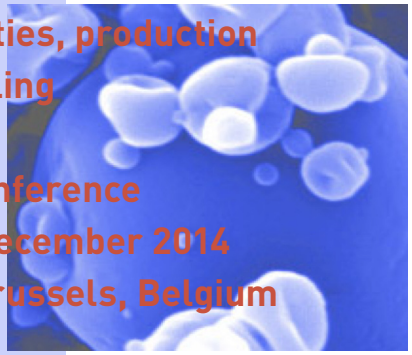
Paul de Vos received his M.Sc. in Immunology and physiology at the University of Groningen. In 1996 he received his PhD (Cum Laude) at the university Medical center in Groningen. Since that time Paul has been active in the field of encapsulation in the medical area. His accepted expertise is on surface characterization and host responses to microcapsules for immunoprotection of cells. However, in the past 5 years he has also worked on delivery systems for modulating the mucosal immune response. Paul has (co-) authored more than 150 scientific publications.

FUTURE CONFERENCES AND EVENTS SUPPORTED BY THE BRG

PowTech

Properties, production & handling



Mini conference
11-12 December 2014
UCB, Brussels, Belgium

http://powtech.sik.se/

Funded by the EU's 7th Framework Programme Marie Curie Actions – Initial Training Networks (ITN)

7TH TRAINING SCHOOL ON BIOENCAPSULATION

Strasbourg, France
February 23-27, 2015
11 lectures and
3 half days of practical demonstrations
http://bioencapsulation.net/2015_Strasbourg/

18TH MICROENCAPSULATION INDUSTRIAL CONVENTION

Eindhoven, Netherlands
April 22-24, 2015
12 lectures, one exhibition
and hundreds of
one-to-one meetings
http://bioencapsulation.net/2015_Eindhoven/

23TH INTERNATIONAL CONFERENCE ON BIOENCAPSULATION

Delft, Netherlands
September 2-4, 2015
40 oral presentations and
tens of posters
http://bioencapsulation.net/2015_Delft/

ARTICLE

SYNTHETIC POLYCATIONS FOR USE WITH CALCIUM ALGINATE-BASED CAPSULES: STRUCTURE & BIOCOMPATIBILITY

Kleinberger R.M., Burke N.A.D and Stöver H.D.H.

INTRODUCTION AND OBJECTIVE

Encapsulation of therapeutic cells in a matrix suitable for implantation has been proposed as a treatment for endocrine disorders. The most common way to encapsulate cells involves APA capsules, consisting of a calcium alginate gel core containing the cells, coated in sequence with poly-L-lysine (PLL) and alginate (Thu 1996). These capsules often show some fibrotic overgrowth attributed to the high charge density of PLL (Tam 2011). As well, differences in coating protocols often leads to different capsule morphologies, strengths and biocompatibilities.

This project explores a series of synthetic polycations with lower charge densities and different molecular weights, in a search for more cytocompatible polycation/alginate shells with sufficient mechanical integrity. These polycations are based on copolymers of 3-aminopropyl methacrylamide hydrochloride (APM) and 2-hydroxypropylmethacrylamide (HPM) prepared using reversible addition-fragmentation chain transfer (RAFT) polymerization.

MATERIALS AND METHODS

Na alginate (Pronova UP MVG) from Novamatrix; poly-L-lysine HBr (PLL, Mn 15-30 kDa), rhodamine B isothiocyanate (RbITC), 4-cyano-pentanoic acid dithiobenzoate (CTP), 4,4'-azobis(4-cyanopentanoic acid) (V-501), and aminopropyl triethoxy-silane (APTES), all Sigma Aldrich; and APM and HPM from Polysciences, were used as purchased.

RAFT co-polymerizations of APM and HPM with CTP as RAFT agent and V-501 as initiator were carried out in 2:1 water:dioxane. [M]o:[CTP]o was 130:1 or 330:1, and [CTP]o:[V-501]o was 1:0.33. The reaction mixture was purged with N2 for 45 min at RT and immersed in an oil bath pre-heated to 70oC, under N2 (Li 2006). MW, PDI and conversion were tracked by GPC and NMR. Polymers were isolated by precipitation in acetone and dried under vacuum. Polymers were fluorescently labelled with RbITC in 0.2M NaHCO3 buffer solution at pH 9.

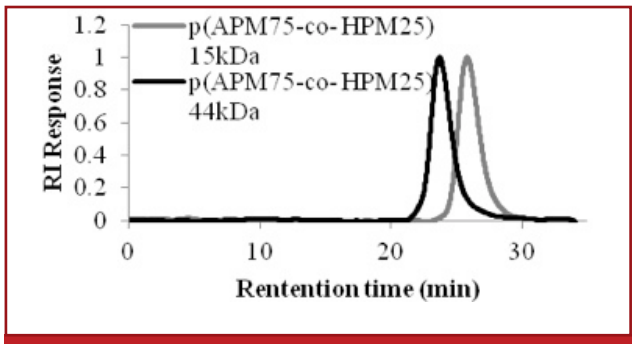


Figure 1: SEC chromatograms of p(APM75-co-HPM25) with Mn of 15kDa and 44kDa.

CaAlg beads were formed by extruding 1 wt% Na alginate in saline into 1.1 wt% CaCl2 + 0.45 wt% NaCl, using a syringe pump and a flat-tipped 27 G needle fitted coaxially inside an airflow tube. CaAlg beads with mean diameters of 500 ± 50µm were coated with 0.1 wt/v% p(APM-co-HPM) in saline (pH 7.0-7.5) for 6 min, followed by a 2 min saline wash.

C2C12 myoblasts were plated into plasma treated polystyrene 24 well plates at 50 000 cells/well and allowed to attach overnight. The media was removed and cells were washed with PBS. Cells were incubated in 0.1mg/ml polymer solutions in 1:0.8 ratio of serum free media to PBS (pH=7.4) for 20 hrs at 37oC. Alamar Blue was added to each well and incubated for 3 h before measuring at 530/590nm.

Glass bottom 96 well plates were coated with a 2 v/v% solution of APTES in 95% ethanol at pH 4.5 for 2 min, washed with 95% ethanol, dried over air and cured overnight. The wells were coated with 0.1 wt/v% polymethylvinylether-alt-maleic anhydride (PMM) in acetonitrile (ACN) for 5 mins and washed with ACN. Polycation solution in de-ionized water (pH 8-9) was added to each well and allowed to react overnight before washing with de-ionized water and drying. The plates were soaked in PBS and stored in fridge. Prior to use, the plates were sterilized with 70% ethanol for 30 min and washed with PBS. NIH/3T3 fibroblasts were seeded with 2500 cells/well in complete growth medium for 3 days before observing morphology with optical microscopy.

RESULTS AND DISCUSSION

Polycations were formed containing 10, 25, 50 and 75 mol% APM, each with Mn of 15 and 40 kDa and PDI of 1.1 – 1.4. These two Mn values give decent separation in SEC chromatograms (Fig. 1), suggesting such polymers should show different diffusion properties in CaAlg beads.

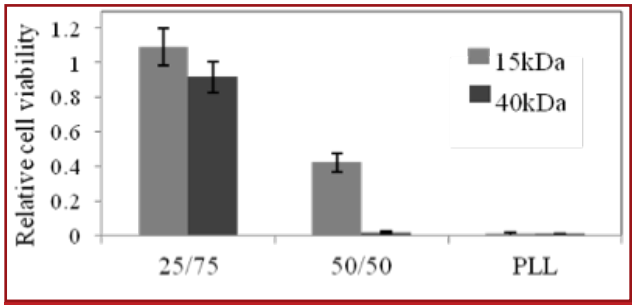


Figure 2: Cell viability by Alamar blue normalized to control after 20hrs exposure to polycation solutions of different APM/HPM ratios.

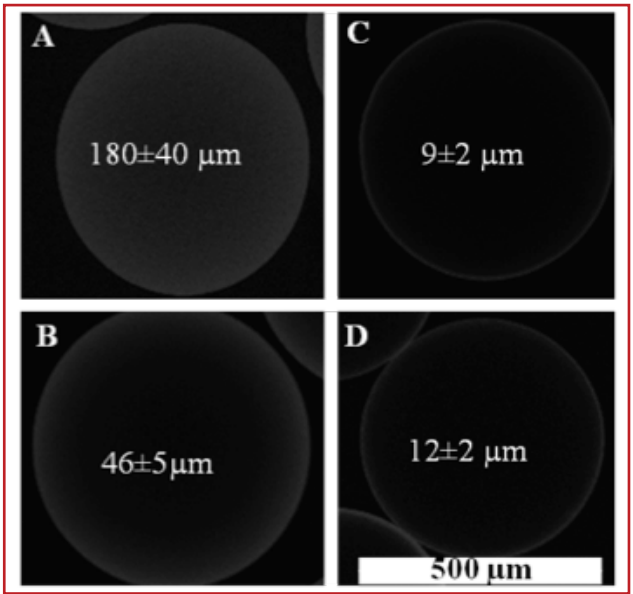


Figure 3: Confocal microscopy images A) p(APM25-co-HPM75) 15kDa; B) p(APM25-co-HPM75) 36kDa; C) p(APM75-co-HPM25) 15kDa; D) p(APM75-co-HPM25) 44kDa.

These polymers were tested for cytotoxicity against C2C12 myoblasts, using an Alamar blue assay to determine cell viability. Increase in charge density and Mn cause greater toxicity to cells as they are more capable of binding to the cells and disrupting their membranes (Fig. 2). P(APM50-co-HPM50) at 15kDa starts to show reduced toxicity compared to PLL.

Distribution of fluorescently labelled polycations within the alginate beads was assessed by confocal microscopy. The membrane thickness decreases with increasing charge density and molecular weight. Higher charge density and higher molecular weight polycations, bind more strongly to alginate and mobility into the core is more restricted (Fig. 3).

NIH/3T3 cell attachment was assessed by morphology after 3 days incubation on polycation modified glass. Polymers with higher charge density show greater cell attachment with spread cells as compared to lower charge density polycations. P(APM50-co-HPM50) 15kDa copolymers show a transition, where cell morphology is more spherical and less attached on surfaces of 15kDa polymer than cells attached to the 40kDa polymer. The higher Mn polycation may produce more loopy polymer surfaces and may expose more cationic charge suitable for cell attachment (Fig. 4).

CONCLUSIONS

Study of these polycations in solution shows reduced toxicity with lower charge density and molecular weight. Lower charge density and molecular weight polymers also show weaker binding to calcium alginate beads, and a thicker polyelectrolyte complex shell. Lower charge density polymers also reduce cell adhesion of NIH/3T3 fibroblasts.

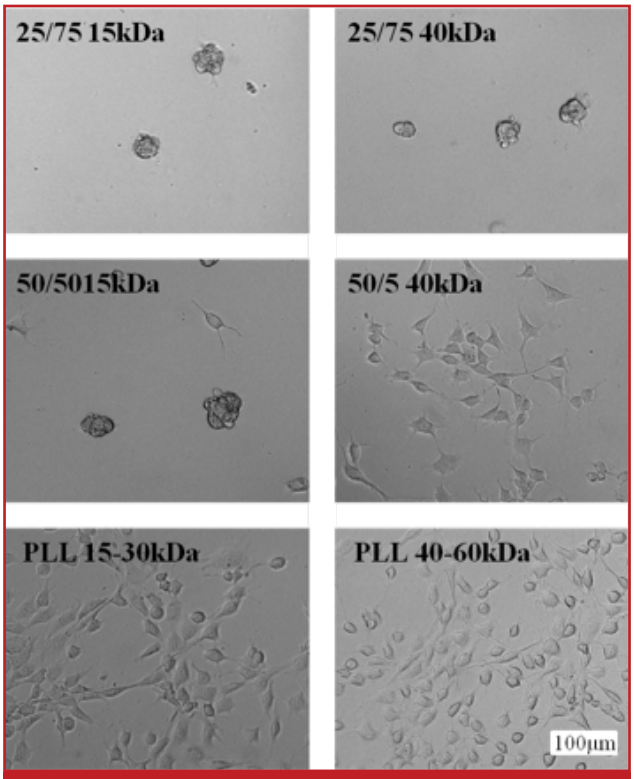


Figure 4: NIH/3T3 morphology after 3 days of incubation in complete growth medium on glass bottom wells modified with different APM/HPM ratios and Mn.

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McMaster University

ACKNOWLEDGEMENT

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Rachelle Kleinberger has completed her BSc. at McMaster University with a major in Chemistry and a minor in Psychology. She currently is a Ph.D candidate at McMaster University under the supervision of Dr. Stöver. Her research interests involve synthesizing and characterizing polymers using controlled polymerizations and studying their physical and biological properties.

ARTICLE

TOWARDS THE ENTRAPMENT OF MICROALGAE FOR BIOLOGICAL HYDROGEN PRODUCTION

Homburg, S. V., Venkanna, D., Doebbe, A., Kruse, O., Patel, A.

INTRODUCTION AND OBJECTIVE

The immobilization of microalgae via entrapment is an advantage for their protection and for the development of a continuous cultivation process to gain high-value products. The unicellular algae *Chlamydomonas reinhardtii* was already entrapped successfully in biocompatible Ca-alginate systems (Kosourov & Seibert, 2009).

In contrast to biopolymer gels, silica gels show an improved chemical and mechanical stability. Besides they resist microbial attacks and are optically transparent, which is crucial for photosynthetically active biomaterials.

Today, sol-gel materials are mostly alkoxides or sodium silicate derived (Brinker & Scherer, 1990, Hench & West, 1990, Hench, 2007). Disadvantages are high concentrations of organic solvents or sodium salts and alkaline or acid pH values limiting their biocompatibility.

To overcome these disadvantages we explored the "low-sodium" aqueous route (Rooke et al., 2011) with different additives like chitosan and TRIS (tris(hydroxymethyl)aminomethane) to form a less brittle and abrasion resistant network suitable for the entrapment of a hydrogen producing C. reinhardtii strain for protection and continuous cultivation in a stirred tank reactor.

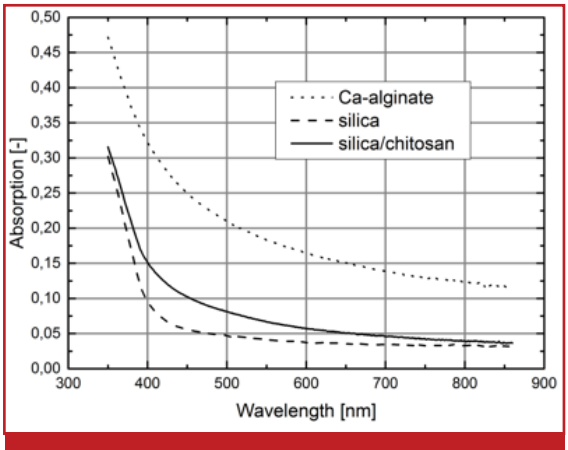

Figure 1 : Absorption spectra of the novel silica gel

MATERIALS AND METHODS

Gel synthesis

A sol consisting of 26 wt% sodium silicate precursor (Sigma-Aldrich) and the culture medium of C. reinhardtii was mixed with an acid ion exchange resin (Amberlite IR 120, Merck). The mixture was then stirred for 15 min at room temperature. After removing the resin via filtration, chitosan and distilled water were added to a resulting sol composition of 20 wt% silica and 0,026 wt% chitosan. The mixture was stirred for further 2 h. Subsequently the gelling was induced by adjusting the pH to 7,4 using a 2 mol/L TRIS buffer system.

Optical measurements

For transparency measurement, the hydrogel was formed in open cuvettes and analysed with UV-Vis spectroscopy. The absorption was measured against water over the range from 350 to 860 nm.

Preparation and abrasion test of lenses

For the preparation of lens-shaped biocatalysts, 1 mL of a C. reinhardtii cell culture (1,8 x 10^6 cells/mL) was added to 3 mL of the sol. Afterwards lenses of 50 µL were prepared, which were then partially desiccated in a constant airflow of 300 L/min at 25 °C for 1 h. For the abrasion test, the lenses were stirred at 250 rpm in 3 bioreactors (110 mL, diameter 46 mm) with a 20 mm cross-shaped magnetic stir bar. The diameter (d) of 10 lenses each was measured daily.

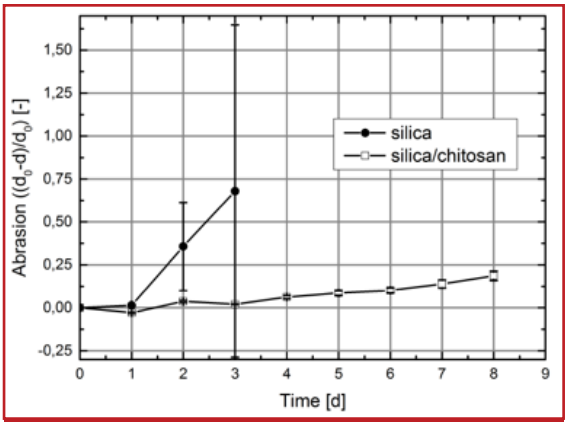

Figure 2 : On-going experiment on abrasion of the silica/chitosan lenses compared to silica lenses.

Cell viability and hydrogen production

To investigate the cell viability, cell morphology was studied via light microscopy, while the PSII quantum yield – a value for the photosynthetic activity – was measured with a Photosynthesis Yield Analyzer (Maxwell & Johnson, 2000). For hydrogen production cells were cultivated in the described bioreactors at 220 rpm and 350 µE.

RESULTS AND DISCUSSION

Gelation time and pH value

The pH of the sol was adjusted to pH 7,35 ± 0,05 using 50 µL of the TRIS buffer per 3 mL sol. Gelation time reduced to 1-2 min.

Transparency

For the application as photosynthetically active biomaterials, the gels

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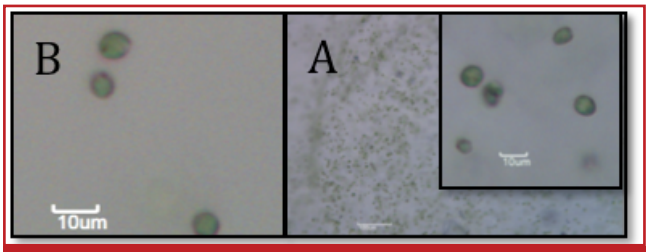

Fig 3 : Free (A) & entrapped (B) *C. reinhardtii* cells

should be as transparent as possible for visible light. Compared to a silica hydrogel without chitosan, the absorption spectrum of the silica/chitosan hydrogel was slightly increased. In contrast, a Ca-alginate gel showed a higher absorption (Figure 1).

For the use e.g. in a stirred tank bioreactor the lenses should be abrasion resistant and non-brittle. After 8 days the abrasion of the silica/chitosan lenses was below 0,25 (Figure 2). Without chitosan the lenses were more fragile and tended to brake or dissolve after 2-3 days. The mechanical properties of different gels such as brittleness and elasticity will also be studied with a texturometer.

Immobilization of *C. reinhardtii cells*

No morphological differences could be detected between free and immobilized cells, as shown by light microscopy pictures (Figure 3).

It was observed that after immobilization of the cells a regeneration phase was crucial for the photosynthetic activity (Figure 4).

The PSII quantum yield of free cells showed a value of 0,79 ± 0,04, whereas the value dropped to 0,51 ± 0,02 directly after the immobilization process. After 100 h, the PSII quantum yield of the entrapped cells increased to 0,71 ± 0,09 and was similar to the value of the free cells for the next 80 h.

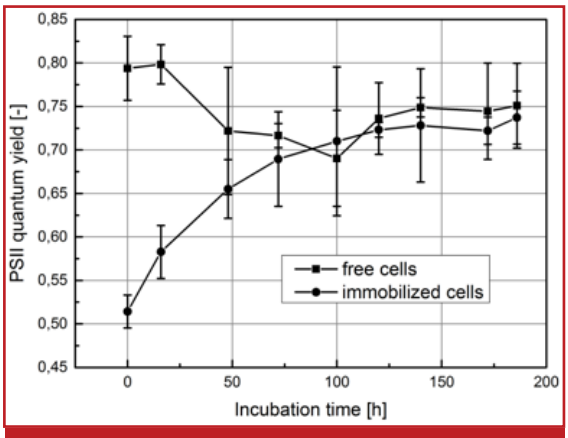

Figure 4 : Photosynthetic activity of free and entrapped C. reinhardtii cells

CONCLUSION

We reported that the sol-gel synthesis using TRIS-buffer with an addition of chitosan provided an easy procedure at a mild temperature of 25 °C and a short gelation time. Besides, it led to highly transparent hydrogels and more abrasion resistant lens-shaped materials suitable for the entrapment of C. reinhardtii cells in an aqueous solution. For the use in stirred tank reactors, the abrasion resistance has to be further increased.

Nevertheless, C. reinhardtii showed photosynthetic activity when entrapped in the presented silica/chitosan based network. In an on-going experiment hydrogen production of the immobilized algae strain is being investigated. Additionally, algae strains will be genetically modified for optimization of the biological hydrogen process.

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Currently she is working on her PhD thesis at the Bielefeld University of Applied Sciences under the supervision of Anant Patel in cooperation with Olaf Kruse (Bielefeld University). Her research focusses on the entrapment of microalgae in silica gels for the production of high-value products.

ARTICLE

MODIFICATION OF ALGINATE-BASED HYDROGEL MICROSPHERES FOR CELL MICROENCAPSULATION: COMPARISON OF ONE- AND TWO-COMPONENT HYDROGEL MICROSPHERES

Noverraz F., Passemard S., Crivelli V., Mahou R., Borcard F., Gerber-Lemaire S. and Wandrey C.*

INTRODUCTION & OBJECTIVE

Microencapsulation of living cells is a promising technology. It has a high potential for biomedical and biotechnological applications including 3D cell culturing, external organ systems in bioreactors and cell-based therapies. Common for all these applications is the entrapment of cells or cell clusters in a hydrogel microsphere. However, the specific requirements in terms of the encapsulation material and technology are governed by the cell type as well as the intended application and can be different. Biocompatibility remains the primary material requirement. Nevertheless, the physical properties of the hydrogel such as the hydrogel network density, stiffness, and hydrophilicity can have a crucial impact on cell proliferation, differentiation, and function. The most demanding applications are allo- and xenotransplantation into human bodies. The success of cell microencapsulation also depends on the encapsulation technology and will benefit from a simple production process, preferably a one-step production process.

Alginate-based hydrogels are the most frequently reported materials for cell microencapsulation. Despite known physical limitations, there is no doubt about the advantageous biological acceptance of sodium alginate (Na-alg) upon ionotropic gelation with divalent cations. There are many attempts to reinforce initially formed alginate beads by subsequent coating with polycations or other treatments. However, such modification either adds less biocompatible components or complicates the technology.

Considering materials and technological aspects, we produced one- and two-component hydrogel microspheres (MS) by combining ionotropic gelation of Na-alg using calcium ions with covalent crosslinking of poly(ethylene glycol) derivatives (PEG) or functionalized Na-alg. Here, we compare the physical properties of four types of MS (Figure 1), all produced by a one-step process, and we evaluate their application potential for cell microencapsulation.

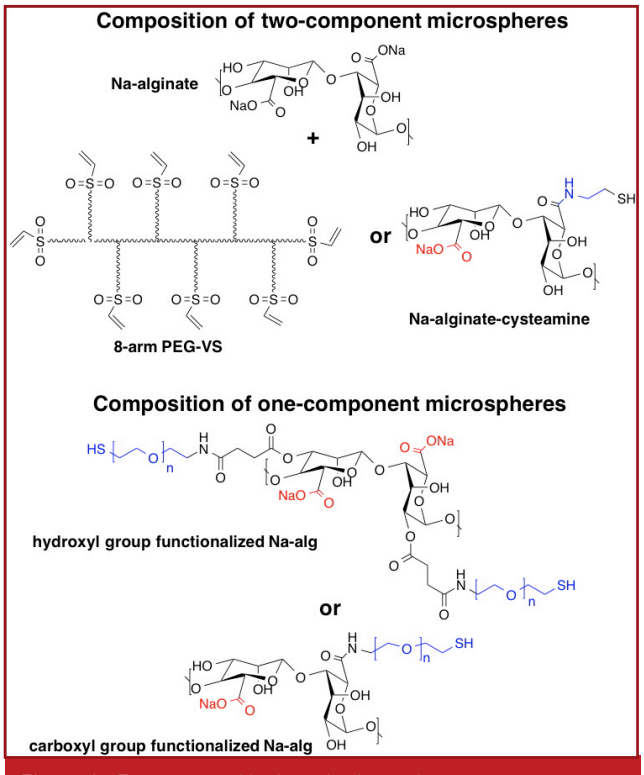

Figure 1 : Four types of hydrogel microspheres.

Two-component MS

Interpenetrating polymer network composed of either Ca-alg mixed with end group-functionalized multi-arm PEG or Ca-alg mixed with cysteamine grafted onto the carboxyl groups of Na-alg.

One-component MS

Combined electrostatic-covalent polymer network prepared from either carboxyl group- or hydroxyl group-PEGylated Na-alg.

MATERIALS & METHODS

Modification of Na-alginate

Na-alg was modified by grafting α-amine-ω-thiol PEG (2000 g/mol) either to the carboxyl or hydroxyl groups, or by grafting cystamine dihydrochloride followed by disulfide bond reduction to the carboxyl groups. The degree of grafting was adjusted in the range of 3% to 25% grafted saccharide units.

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Microsphere formation

A BÜCHI Encapsulator B-395 Pro (BÜCHI Labortechnik AG, Switzerland) was used to extrude the polymer solutions into the gelation bath containing CaCl2. For the covalent crosslinking of multi-arm PEG-VS inside the Ca-alg matrix, thiolated molecules (DTT) were added to the gelation bath. Na-alg (UP LVM PRONOVA, FMC BioPolymer, Novamatrix, Norway) was added to the multi-arm PEG-VS or to the Na-alg-cysteamine (Na-alg-cys) to form the two-component MS.

Characterization of the polymer solutions

All polymer solutions were prepared at defined concentrations and characterized by their dynamic viscosity. The osmolality and the pH were adjusted to physiological conditions.

Physical characterization of microspheres

The size of the MS, swelling in different media, and stability were observed by optical microscopy. Water uptake was

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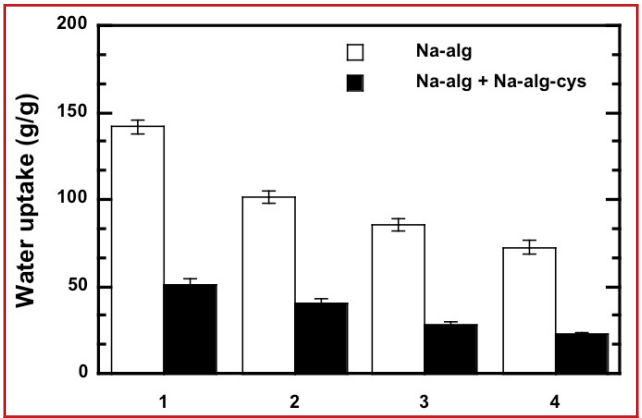

Figure 2 : Water uptake of MS prepared from only Na-alg or a mixture of Na-alg and cysteamine grafted Na-alg. The numbers on the x-axis refer to the following compositions (n=3±SD):
1: 1.5% Na-alg vs 1% Na-alg + 0.5% Na-alg-cys
2: 2% Na-alg vs 1% Na-alg + 1% Na-alg-cys
3: 2.5% Na-alg vs 1% Na-alg + 1.5% Na-alg-cys
4: 3% Na-alg vs 1% Na-alg + 2% Na-alg-cys

gravimetrically determined. Mechanical stability, deformability and mechanical resistance to compression were analysed using a TA-XT2i (Stable Micro Systems, Godalming, UK). Ingress diffusion experiments using fluorescence labeled dextran standards (40, 70, 150 kg/mol) served to estimate the permeability.

RESULTS & DISCUSSION

The polymer solution viscosity was identified as a key correlation parameter to adjust the MS diameter and ensure sphericity by appropriate Encapsulator settings. For all four MS types, swelling in water and water uptake could be significantly reduced compared to Ca-alg MS. Figure 2 shows typical examples.

The mechanical resistance to compression, the deformability, and the shape recovery could be improved for both two- and one-component MS. Figure 3 shows a typical example.

Varying the chemical composition of the alginate-based MS and the concentrations of the polymer components is suitable to modify the polymer network density and consequently the permeability of the MS. Another influencing parameter is the degree of grafting. For example, MS prepared from 5% hydroxyl group-functionalized Na-alg, degree of grafting 5% saccharide units, completely excluded dextran of 70 kg/mol, but were permeable for dextran 40 kg/mol. Decreasing the polymer concentration shifted the MWCO to 150 kg/mol.

From the multitude of results, only very few could be shown here.

CONCLUSIONS

The combination of electrostatic and covalent crosslinking offers a variety of possibilities to modify the physical properties of alginate-based MS and to adapt these to the microencapsulation of specific cell types. For the here presented two-component MS, the fast electrostatic crosslinking of Na-alg with calcium ions was used to provide a spherical matrix for the simultaneously but slowly occurring covalent crosslinking of an imbedded second polymer component. Grafting heterobifunctional PEG on Na-alg in such a way that sufficient carboxyl groups remain for fast electrostatic crosslinking also allows simultaneously reinforcing the MS by spontaneous covalent crosslinking of the PEG side chains. The overall advantage of all modifications presented here is the applicability of a one-step MS formation process. Biocompatibility was confirmed for all MS types by in vitro and/or in vivo experiments including the microencapsulation of different cell types.

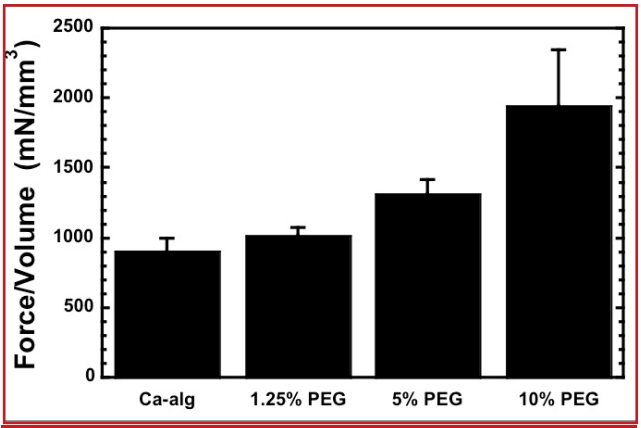

Figure 3 : Mechanical resistance to compression up to 90% of the MS diameter of MS prepared from Na-alg with 0% to 10% 8-arm PEG-VS (n=30±SD)

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After having completed his Master in Molecular & Biological Chemistry at the EPFL in April 2014, F. Noverraz started a PhD project at the EPFL in June 2014. He and Dr. S. Passemard, who obtained her PhD in Chemistry and Chemical Engineering at the EPFL in August 2014, will continue the research of Dr. R. Mahou, Dr. F. Borcard and V. Crivelli which was presented at the BRG 2014 in Bratislava.

ARTICLE

EMULSION-BASED ISLET ENCAPSULATION: PREDICTING AND OVERCOMING ISLET HYPOXIA

Fernandez SA, Bégin-Drolet A, Ruel J, Leask RL, Piret JM and Hoesli CA

INTRODUCTION & OBJECTIVE

Pancreatic islet transplantation offers a method to treat type I diabetes that allows as many as 80% of patients to avoid insulin injections over the following year (Ryan 2005). The two major constraints associated with this approach are the limited availability of donor tissue, and the need to administer immunosuppressant drugs to prevent rejection of the transplant. The first issue may be resolved through the use of pluripotent stem cells, which can be differentiated to generate beta cells. The second issue can be resolved by encapsulating islets in a material such as alginate in order to isolate them from the immune system.

Although islets make up 1% of the pancreatic mass, they receive 10% of the blood flow to the organ (Zanone 2008). This high amount of vascularisation is required for sufficient oxygenation, and plays a major role in proper islet development and function. The oxygen partial pressure around the islets is 31-37 mmHg. If this oxygen tension is not met, the environment may become hypoxic, leading to impaired beta cell differentiation (Heinis 2010) and function (Dionne 1993). Non-encapsulated islets cultured in vitro experience oxygen gradients (Kauri 2003), leading to the formation of a necrotic core within larger islets (Olsson 2006). Encapsulated islets are more difficult to adequately oxygenate because of the increased distance between the cells and the vasculature. Consequently, necrotic cores are more problematic when compared with non-encapsulated cells. It is therefore important to study encapsulated islet hypoxia and resulting changes in islet function in order to develop methods of overcoming diffusion limitations and controlling oxygen distribution.

The objective of this work was to confirm that hypoxic cell necrosis occurs in encapsulated islet-like cell clusters. The second objective was to devise a strategy to maximize oxygen distribution to encapsulated islets.

MATERIALS & METHODS

A simplified mass transfer and oxygen consumption model was used to calculate oxygen concentration profiles of encapsulated islets. At steady-state, the oxygen tension in a sphere with homogeneous zero-order oxygen consumption can be calculated from:

$$D_{O2}\,[1/r^2 \,.\, \partial/\partial r\,(r^2 \,.\, \partial C/\partial r)] - Q_{O2} = 0$$

(Equation 1)

where C is the oxygen concentration at radius r, Q_{O2} is the oxygen consumption rate (assumed to be constant if oxygen is >7 mmHg) and D_{O2} is the oxygen diffusivity. The values of Q_{O2} and D_{O2} were respectively 3.54 x 10^{-2} mol/m^3/s and 1.1 x 10^{-9} m^2/s within the islets (Dulong 2007). In the beads, the value of Q_{O2} was multiplied by the islet packing density and the value of D_{O2} was the weighted average of the diffusivity through islets and through alginate (2 x 10^{-9} m^2/s).

Mouse insulinoma 6 (MIN6) cells were cultured in DMEM medium supplemented with 10% serum, glutamine and antibiotics. The cells were encapsulated using the emulsion and internal gelation method (Hoesli 2011) originally developed by Poncelet et al. (Poncelet 1992). Briefly, a 10.5 mL mixture containing 2% alginate, 24 mM $CaCO_3$ and 10^6 to 2.5 x 10^6 cells/mL was emulsified in 20 mL light mineral oil in a 100 mL spinner flask at 500 rpm. After 12 min of emulsification, 10 mL of mineral oil containing 40 µL glacial acetic acid were added, leading to acidification, calcium release and internal gelation. The beads were recovered by repeated washes in HEPES buffer with 10% medium, centrifugation and oil aspiration, followed by filtration on a 40 µm nylon sieve. The cells were then cultured at 1 mL beads/5 mL total volume in complete medium in a polystyrene flask at 37° C and 5% CO_2 for two weeks. Necrotic cell cores present in the encapsulated MIN6 cells were visualised after cell expansion into islet-sized cell aggregates. The encapsulated cells were stained for 20 min in HEPES-buffered saline solution containing 8 µM each of ethidium homodimer and calcein AM. Fluorescence was visualized by confocal imaging on a Zeiss LSM 510 Meta microscope.

RESULTS & DISCUSSION

We applied a highly simplified model to calculate the oxygen tension required at the bead surface to avoid oxygen limitations in the islet core. A critical minimum oxygen partial pressure of 7 mmHg was set for the core of a hypothetical islet located at the centre of the bead. Figure 1 shows the effects of the islet and bead diameter on the bulk oxygen concentration required for different islet packing densities in the beads based on this simplified model. At saturated air oxygen partial

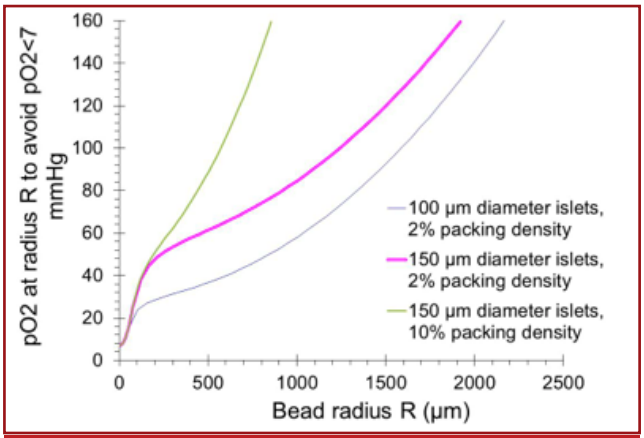

Figure 1. Bulk oxygen concentration required to avoid oxygen limitations (p<7 mmHg) for an islet located at the centre of the bead.

pressures (pO_2 = 160 mmHg), oxygen limitations are expected to occur in beads larger than ~900 µm for 150 µm diameter islets at 10% packing density in the beads. For MIN6 cells that have higher oxygen consumption rates, oxygen limitations are expected to occur even in smaller beads in vitro.

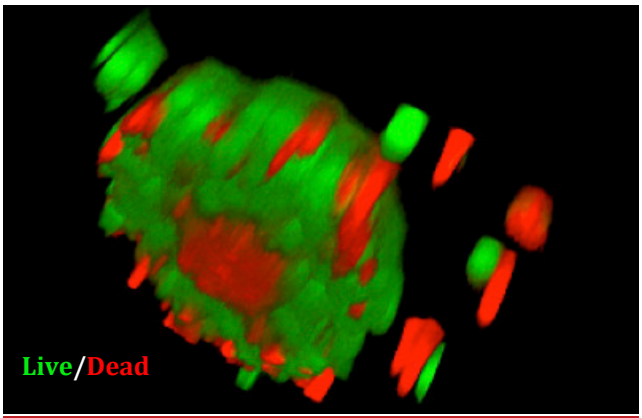

Figure 2. Rotated 3D projection of a slice of a ~140 µm diameter MIN6 cell cluster after 2-week expansion in emulsion-generated beads.

We had previously shown that beta cell lines encapsulated by the emulsion and internal gelation process can correct hyperglycemia in chemically-induced allogeneic diabetic mice. To determine whether the transplanted cells were likely hypoxic, we examined the formation of necrotic cores in encapsulated cells during in vitro culture, where the bulk oxygen tension is higher than in vivo.

Necrotic cores were observed in <150 µm diameter islet-like MIN6 cell aggregates in 2% alginate beads (Figure 2). Although hypoxia was not expected for primary islets of this size at similar ~10% cell cluster packing density, the MIN6 cells were expected to have higher oxygen consumption rates. These results indicate that the encapsulated beta cells in our transplantation experiments also likely suffered from hypoxia. These observations concur with the body of literature underlining the role of hypoxia in encapsulated islet cell death, both in vitro and in vivo.

CONCLUSIONS

This work demonstrates that central necrosis is observed in encapsulated islets in vitro. Based on simple mathematical modelling, this necrosis is likely due to hypoxia, which is also a key cause of islet transplant failure in vivo. This underlines the importance of developing devices that allow for adequate oxygenation of islet transplants. Future work to be undertaken involves developing a strategy for constructing a perfusable artificial pancreas via 3D printing. A sacrificial lattice made of carbohydrate glass would be 3D printed, and a cross-linkable matrix such as fibrin containing encapsulated islets would be formed around it. The lattice would then be dissolved, leaving perfusable channels within the tissue construct. This would allow for the study of islet survival and function, including insulin secretion, in a 3D culture. This system could be used to compare different lattice densities and study their effects on mature beta cell function and survival, as well as progenitor differentiation, in order to optimise encapsulated islet oxygenation and function.

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ARTICLE

SYNTHESIS OF ALGINATE MICROPARTICLES USING MICRO-FLUIDIC DEVICE

Pittermannová A., Bremond N., Bibette J. , Štěpánek F.

INTRODUCTION & OBJECTIVE

Nowadays the development of composite nano- and microparticles is an extensively studied area of research. This interest is growing especially for the potential use of such particles in drug delivery systems. Recent research has resulted in the first templates of alginate composite microparticles, which are able to release encapsulated active content upon an external stimulus (remote control). These composite alginate microparticles were prepared by the Ink-jet method with the smallest achievable size around 40 μm (Hanus et al., 2013). For further use of these particles in the field of biomedicine, their adhesion properties in living tissues will be need to be investigated. It is therefore desirable to decrease the size of the alginate microparticles to less than 6 μm - the typical size of the red blood cell.

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Microfluidic technique is one of the ways to produce such small microparticles. Two methods were investigated. First, synthesis of the particles was reached directly on the chip by a flow focusing device, where the continuous phase was 1 undecanol with Ca^{2+} ions and dispersed phase was aqueous solution of alginate (Zhang 2006). W/o emulsion was produced and gelation was achieved by diffusion of Ca^{2+} ions into the droplets. Second, the particles were synthesized in a two-step process. W/o emulsions with the required sized were collected and gelled in the bulk after the introduction of calcium ions.

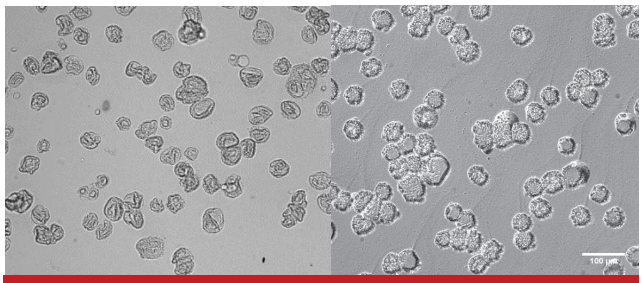

Figure 1: alginate microparticles (A) 0.15wt% CaI_2; (B) 0.75wt% CaI_2

MATERIALS AND METHODS

Chemicals

Polydimethylsiloxane (PDMS, Sylgard 184, Aldrich), Silicone rubber RTV 615 (RTV, Momentive Performance Materials), 1 undecanol (99%, Aldrich), Calcium iodide hydrate (98%, Aldrich), Abil (Abil EM 90, Prospector), Alginate Protanal LF 10/60 (FMC Biopolymer), Methanol (99%), 2-propanol (99%, Aldrich), Trichloro(octadecyl)silane (Aldrich).

Preparation of flow focusing and membrane chip

The chip was made by standard soft lithography techniques. It is composed of silicon rubber (RTV) and finally bonded to a cover glass coated with the same material. For the RTV device, the polymer and the curing agent were mixed together in a ratio 1:5, for the modification of the cover glass the ratio was 1:14. The RTV device was left in an oven at 70 °C for 22 minutes and the cover glass with a thin layer of RTV for 26 minutes. These two parts were then assembled together and left in an oven at 70 °C overnight. It is not possible to make extremely thin channels by this method, therefore the membrane chip with the thinnest channels of 1.5 μm was prepared from PDMS with standardized protocols based on bonding PDMS to a glass slide using vacuum oxygen plasma.

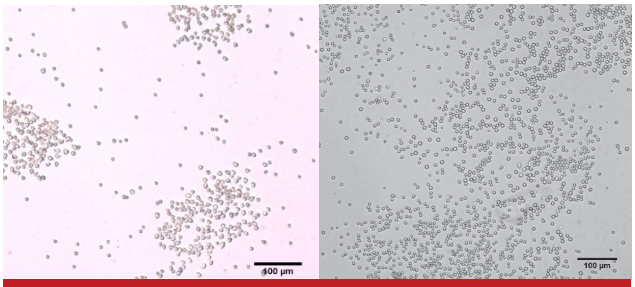

Figure 2: Alginate microparticles in A) 1-undecanol; B) 1-undecanol saturated by water

RESULTS & DISCUSSION

Flow focusing chip

For preparing w/o emulsions it is desirable to have a highly hydrophobic surface inside the channels. This hydrophobicity was reached by the first bonding method as described above. Calcium ions were introduced to the system by dissolving CaI_2 in 1-undecanol. As a surfactant we have used Abil. The dispersed phase was 1wt% alginate and the continuous phase was 3wt% Abil with 0.15wt% or 0.75wt% CaI_2 in 1 undecanol.Chemicals

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The coalescence and insufficient gelation of the particles (Figure 1) was caused by lower concentration of alginate and surfactant. Therefore the concentration of the alginate was increased from 1wt% to 2wt% and the concentration of surfactant was increased from 3 to 6wt%. These particles were stable and did not gel together (Figure 2). Their mean size was in the range from 4 to 6 μm, inside the flow focusing chip the particles were produced in a size range from 15 to 20 μm, though. The shrinking of the microparticles can be invoked by the extraction of water, therefore we prepared the particles from saturated

1 undecanol. The particles prepared with saturated 1 undecanol (Figure 2.B) were larger, but highly polydisperse (from 15 to 25 µm).

An important variable when producing monodisperse particles was the ratio between the flow rates of the continuous and the dispersed phase. For the preparation of monodisperse droplets it is recommended to use the stable dripping regime instead of the jetting regime. The dripping regime is usually producing droplets with the desirable size but accompanied by smaller satellite droplets. In a very narrow working window of flow rates, it is possible to find a stable dripping regime without the presence of satellites. For instance, when the ratio α between the flow rate of the continuous phase (Qc = 170 µl/h) and the disperse phase (Qd = 50 µl/h) was α = 3.4, satellite droplets were not observed in the system. Satellite droplets started to appear when increasing the flow rate of the continuous phase to Qc = 200 µl/h (α = 4) or decreasing Qc to 150 µl/h (α =3) (Figure 3).

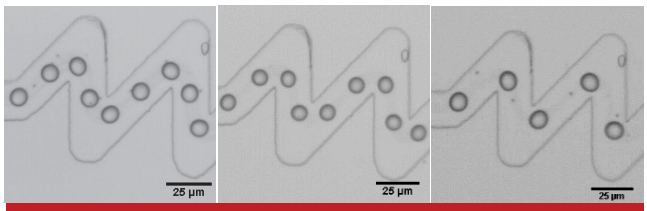

Figure 3: Flow rates ratio: A) α=3 B) α=3.4 C) α=4

Membrane chip

The membrane chip was producing emulsion by a step mechanism, when the height of the small channels was 1.5 µm and the step was 38 µm (Mittal 2014). With such a small size of channels and such an emulsification regime it is very difficult to avoid wetting of the dispersed phase onto channel walls. Therefore, a critical point to obtain monodisperse production is a suitable surface modification. The best modification was reached by 1wt% of trichloro(octadecyl)silane, the optimal protocol when all impurities and crystallizing phenomena in the channels were minimized was the following: plasma bonding followed by injection of 1wt% of trichloro(octadecyl)silane in mineral oil to the membrane chip through the exit channel. After the channel for continuous phase was filled, the reaction mixture was left for 15 minutes. The modified channel was washed with 3wt% Abil in mineral oil injected through the inlet of the disperse phase for 30 minutes. Afterwards 3wt% Abil in mineral oil was injected as the continuous phase and 1wt% alginate solution was slowly introduced as the dispersed phase. The prepared emulsion was stable and did not coalesce. The particle diameter ranged from 6 to 12 µm.

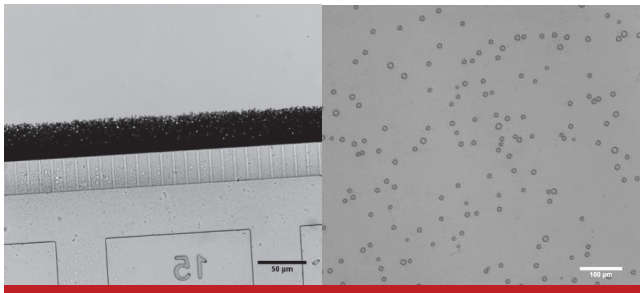

Figure 4: (A) step emulsification; (B) w/o emulsion

CONCLUSIONS

Alginate microparticles were prepared by flow focusing chip where the continuous phase was 1 undecanol with dissolved CaI_2 and surfactant (Abil), the dispersed phase was alginate solution. Prepared particles were stable and no coalescence was observed, with the size ranging from 15 to 25 µm. The influence of different flow rates for increasing monodispersity of alginate microparticles still has to be rigorously investigated. Another approach to prepare alginate microparticles was based on the preparation of w/o emulsion by step membrane emulsification and external gelation of this emulsion in bulk. The contribution of this method is the easier parallelization of the step channels therefore with the membrane chip it is possible to produce a larger amount of droplets compared to the flow focusing device. Hydrophobic modification of the channels was reached by trichloro(octadecyl) silane. The continuous phase was mineral oil with surfactant (Abil) and the dispersed phase was alginate aqueous solution. The emulsion was stable and did not coalesce, the particle size was 6 to 12 µm. The protocol to reach the best way of external gelation still has to be optimized.

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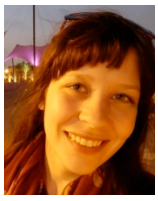

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ARTICLE

SKELETAL MUSCLE TISSUE AS A POTENTIAL TRANSPLANTATION SITE OF MICROENCAPSULATED ISLETS

Bochenek F. Qi J. Marchese J. Davis J. Wang J. Lacik J. Strand J. Oberholzer J.

INTRODUCTION

Human pancreatic islet transplantation into the liver of Type I diabetic patients can restore physiological blood glucose control and eliminate the need for insulin therapy. However, to prevent islet graft rejection recipient patients must be on a lifelong immune suppression regimen, which is associated with negative health risks and excludes children from receiving this therapy. Islet microencapsulation can potentially eliminate the need for these drugs by surrounding the islets in semi-permeable gel microbeads to isolate the islets from immune destruction. Commonly, microencapsulated islets are transplanted into the intraperitoneal (IP) cavity due to the large volume available for the islet graft. However, the IP is a hypoxic environment with substantial diffusion distances and may not be ideal for encapsulated islet function (De Vos 1996). Skeletal muscle tissue, on the other hand, has rich capillary beds with higher oxygen tensions and shorter diffusion distances (Svensson 2011). Muscle tissue, therefore, may be a superior transplantation site for encapsulated islets in terms of islet function and response time.

Winner of the Best Contributions
XXII International Conference on Bioencapsulation
Bratislava, Slovakia – September 17-19, 2014

The purpose of this study was to implant alginate microbeads into the muscle tissue to observe the host reaction to microbeads with and without islets and the ability of muscle tissue to support encapsulated islets for an extended period of time.

MATERIALS & METHODS

Study Design

In this study, naïve Lewis rats were transplanted with Ca^{2+}/Ba^{2+}-alginate microbeads in the skeletal leg muscle (gracilis major). Three groups were used:

1) control empty microbeads (n=9)

2) encapsulated isogeneic islets (n=9)

3) encapsulated allogeneic islets (SD islets, n=6).

UIC University of Illinois at Chicago

The microbeads were retrieved from all recipients at 8 weeks post transplantation and analyzed for signs of islet functionality (viability, morphology, positive insulin staining) and host cellular reaction to the presence of microbeads (weighted fibrotic overgrowth score and histology staining).

Encapsulation in Ca^{2+}/Ba^{2+} Alginate Microbeads

Briefly, islets were harvested from Sprague Dawley (SD) and Lewis rats and cultured for 24 hrs. The islets were suspended in a small volume of HBSS, and mixed with 2.0% (w/v) sterile-filtered, ultra pure low viscosity high guluronic (UPLVG) alginate (NovaMatrix, Norway) to a final islet suspension of 1.8% (w/v) alginate. An electrostatic microbead generator deposited droplets into a gelling solution of 50 mM $CaCl_2$ and 1 mM $BaCl_2$ to form gel microbeads of 480 – 550 µm in diameter. The encapsulated islets were cultured overnight (Qi M 2008).

Intramuscular Transplantation of Microbeads

Microbeads with or without islets (.2cc) were loaded with HBSS (.3cc total volume) into a 1 cc syringe. The microbeads were injected into the gracilis major muscle using a 17 gauge needle while simultaneously retracting the needle for even distribution along the needle tract.

Viability Assessment of Encapsulated Islets

The inclusion/exclusion dyes FDA/PI were used to stain for viability. FDA stains live cells green and PI stains dead cells red. Percentages of total viable cells of 50 whole islets were assessed pre-encapsulation, pre-transplant and post-transplant.

Retrieval of Alginate Microbeads

Microbeads were retrieved after 8 weeks. The entire leg was removed for histology processing. During some retrievals, the muscle tissue was further dissected to expose the site of transplantation. Free microbeads were collected and samples were tested for islet viability and overgrowth before being processed for histology.

Weighted Fibrotic Tissue Overgrowth Score

Retrieved microbeads (~200 per rat) were categorized under brightfield microscopy (25x) by the percentage of the total microbead surface area that was covered by overgrowth, 0%, <25%, 25-50%, 50-75%, >75%. The percent of microbeads in each category were then multiplied by a weighted value (0-4) to give a single numerical value for each rat that corresponded to the severity of tissue overgrowth. These weighted values for each group could then be statistically analyzed.

$$= \frac{\sum_{i=0}^{4} [\%\ \text{of retrieved microbeads in category i}](i)}{(100\%)}$$

RESULTS & DISCUSSION

Figure 1: A) No inflammation at site of injection. B) Freely floating microbeads with very little overgrowth. C) HE (within muscle tissue) showing little cellular reaction between the interface of muscle tissue and microbead surfaces.

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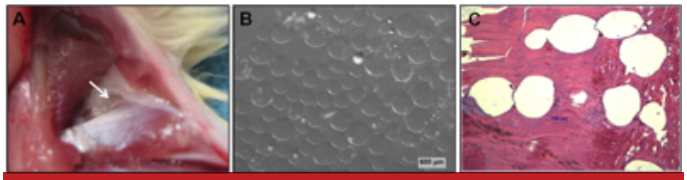

Figure 1: No immune response at 8 week retrieval of empty microbeadsnanocapsules

Figure 2: A) No inflammation at site of injection. B) DTZ staining of retrieved encapsulated isogeneic islet. C) HE (within muscle tissue) showing little cellular reaction.

Figure 2: No immune response at 8 week retrieval of encapsulated isogeneic islets

Figure 3: A) Viability of retrieved isogeneic islets was comparable to pre-transplant levels (95 ± 1.7%vs. 94.3±3.8%, respectively) (p=.24). B) HE staining of encapsulated isogeneic islet showing nice plump morphology. C) Positive insulin staining of encapsulated isogeneic islet.

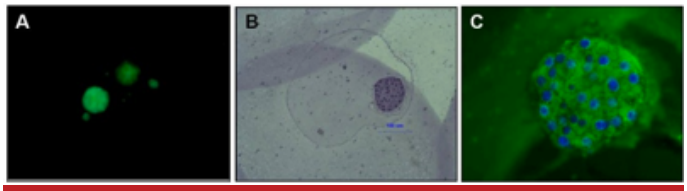

Figure 3: Encapsulated isogeneic islets can survive intramuscularly for 8 weeks

Figure 4: A) Large inflammation at site of injection. B) Neovascularized fibrotic clump of encapsulated allogeneic islets. C) HE staining of allo-clump showing heavy overgrowth 10-20 cell layers thick.

Figure 4: Heavy clumping and large immune response at 8 week retrieval of encapsulated allogeneic islets

Although there was no statistical difference between the weighted fibrotic tissue overgrowth scores of empty and iso-encapsulated islets (0.083 ± 0.017 and 0.130 ± 0.031, respectively, p=.11). Encapsulated allogeneic islets had a very high average weighted fibrotic tissue overgrowth score of 3.54 ± 0.79, which was significantly higher than empty and isogeneic microbeads, p=0.0016 and p=0.0017, respectively.

Figure 5: A) HE staining showing overview of allo-clump and indicated areas where α-SMA, CD 68+ and CD 3+ staining occurred. B) α-SMA staining consistent with myofibroblasts showed heavy concentration on the immediate surface of the encapsulated allogeneic microbeads. HE and α-SMA staining also showed a central path of elongated (migrating) cells. This is suggested to be a "myofibroblast highway" where activated myofibroblasts were migrating to a designated location on the surface of an encapsulated islet. C) CD 68+ staining for tissue macrophages and D) CD 3+ T-lymphocytes tended to stain positive in the interstitial tissue at the apex of overgrown microbeads that seemed to act like cellular "crossing guards," secreting cytokines and chemokines to drive the chemotaxis of myofibroblasts to the desired location on the surface of the encapsulated islets. The rather complex clump structure resulted in the death of encapsulated islets by allorejection.

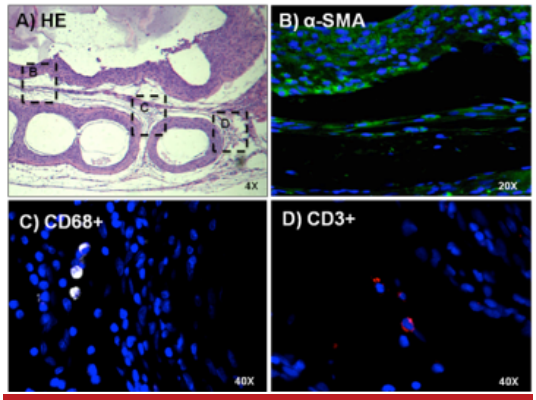

Figure 5: Immune cell infiltration of allo-clump

CONCLUSION

Skeletal muscle tissue may represent a potential site for encapsulated islet transplantation (8 week positive viability, morphology, insulin staining, no overgrowth); however, the properties of the present microbeads such as porosity may have to be modified to protect allogeneic tissue from rejection.

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ARTICLE

POLYMER NANOPARTICLES FOR THE TARGETING OF PODOCYTES IN KIDNEY GLOMERULUS

Colombo C., Min L., Rastaldi M.P., Cellesi F., Moscatelli D.

INTRODUCTION AND OBJECTIVE

Chronic kidney diseases (CKD) are recognized as a major health threat worldwide, with frequent progression to terminal renal failure. The majority of CKD are characterized by defects of the glomerular filtration barrier, where podocytes guarantee selective filter permeability (Patrakka 2009). Therapies to treat or slow the progression of kidney damage are still limited and are charged by a number of severe side effects. When they fail, the disease inevitably progresses to end-stage renal failure, with the need of renal replacement therapies. Due to these reasons there is a need to provide novel therapies aimed at the treatment of podocytes.

In this work fluorescent polymer nanoparticles (NPs) based on poly(ε-caprolactone) with different dimension (from 30 to 120 nm) and surface charge (PEGylated, positive and negative) have been synthesized and studied in in vitro preclinical studies on murine podocytes. The cellular uptake and cytotoxicity of the produced NPs were investigated to test how dimension and surface charge influence their behavior in vitro. Finally, the least cytotoxic NPs were loaded with the hydrophobic drug dexamethasone and were used on podocytes which were previously treated with adriamycin to mimic CKD damage to test whether drug-loaded NPs were able to treat damaged cells.

MATERIALS & METHODS

NP synthesis

NPs were produced through a two steps process. In the first step functionalized oligomers are produced through a bulk Ring Opening Polymerization (ROP) of ε-caprolactone initiated by 2-hydroxyehtil methacrylate (HEMA). The final product of this procedure is a macromonomer composed of three caprolactone units functionalized with the HEMA molecule (Ferrari 2011); the process is schematized in Figure 1.

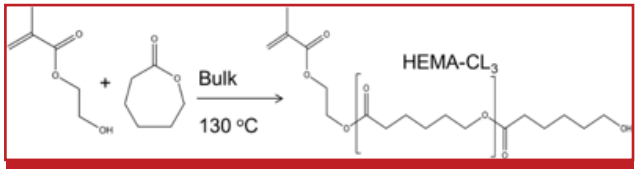

Figure 1: Synthesis of the HEMA-CL3 macromonomer

The produced HEMA-CL_3 macromonomer has been polymerized in a surfactant-free emulsion polymerization together with commercially available surfmers in order to produce NPs of different surface charge. Moreover a fluorescent macromonomer was synthetized to make NPs detectable in vitro through dicyclohexylcarbodiimide (DCC) coupling of Rhodamine B with HEMA (Dossi 2012). The structure of the adopted surfmer and the fluorescent macromonomer is reported in Figure 2.

From Figure 2 it is possible to see that the use of HEMA-SO^{3-} would lead to negatively-charged NPs, HEMA-Ch^{+} to positively charged ones while PEGylated NPs are neutrally charged.

All the NPs have been produced through a surfactant free emulsion polymerization process, in which a selected amount of hydro-soluble surfmer is loaded into the reactor while the hydrophobic HEMA-CL_3 and HEMA-RhB are fed through a syringe pump over one hour. By using different ratios between HEMA-CL_3 and the chosen surfmer it is possible to obtain NPs with different dimensions and a tunable surface charge.

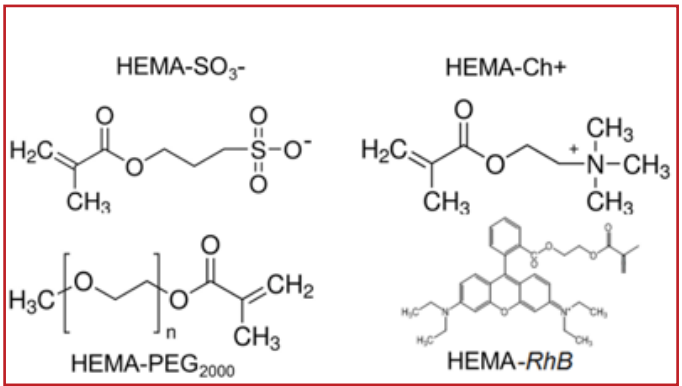

Figure 2: The different surfmers employed and the HEMA-RhB macromonomer.

In vitro studies and drug loading procedure

SV1 line murine podocytes were used for the determination of cytotoxicity and cellular uptake of the produced NPs. Cells were incubated with different NP concentration and after 24h their toxicity was evaluated through LDH assay as well as microscopy analysis to verify their morphology. By exploiting the signal of RhB covalently bound to the NPs it was possible to determine the cellular uptake by fluorescence spectroscopy. Through these experiments it was possible to determine the least cytotoxic of all the NPs produced, which were employed in further studies *in vitro*. Specifically, podocytes were treated with adriamycin (0.8 μM for 24h) to simulate CKD damage, afterwards they were washed with fresh medium and were incubated with NPs loaded with dexamethasone. The drug loading step is carried out after the NP synthesis and it involves the contact of the produce NP latex with the drug dissolved in DMSO in a mixing device.

RESULTS & DISCUSSION

NP synthesis

The adopted emulsion polymerization of the caprolactone-based macromonomer together with the surfmers allows to obtain NPs with different dimension and surface charge, as reported in Table 1.

Table 1 Library of produced NPs

Sample	Surfmer	Diameter [nm]	Surface Charge
1	HEMA-PEG	37	Neutral
2	HEMA-PEG	71	Neutral
3	HEMA-PEG	118	Neutral
4	HEMA-Ch^{+}	33	Positive
5	HEMA-Ch^{+}	75	Positive
6	HEMA-Ch^{+}	119	Positive
7	HEMA-SO^{3-}	33	Negative
8	HEMA-SO^{3-}	68	Negative
9	HEMA-SO^{3-}	107	Negative

In vitro results

In Figure 3 the LDH assay results are reported for different NPs as a function of their concentration.

It is possible to clearly see from Figure 3 that NPs #3 are the least cytotoxic even for high concentration, while positively-charged NPs exhibit high toxicity (possibly due to higher uptake levels); therefore NPs #3 were chosen as drug delivery carriers for dexamethasone for the tests on adriamycin-treated podocytes.

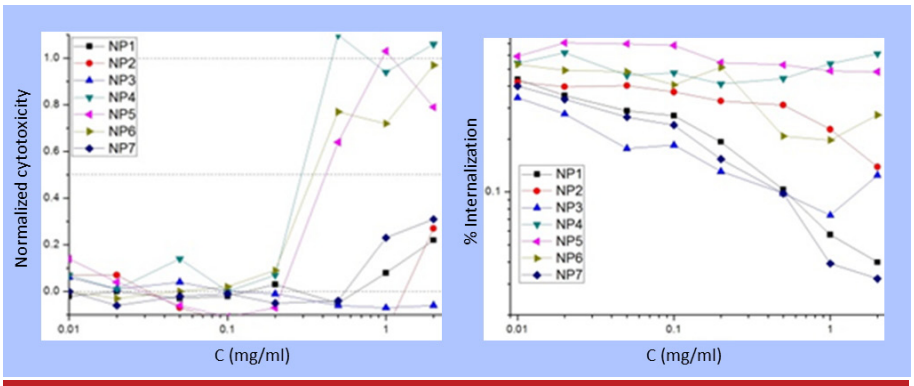

Figure 3: Panel A: LDH assay for the produced NPs; Panel B: cellular uptake studies

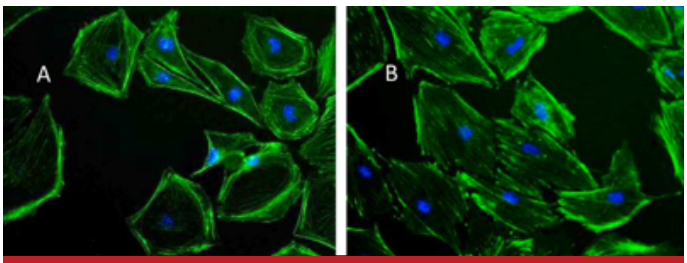

Figure 4: Panel A: microscopy image of adriamycin-treated podocytes; Panel B: the same cells after treatment with drug-loaded NPs.

Figure 4 shows that, after the treatment with drug-loaded NPs, podocytes partially re-adjusted the orientation of actin fibers (green), showing recovery from the induced injury. Since PEGylated NPs are widely studied as carriers suitable for intravenous delivery, the produced drug-loaded NPs could represent an effective way to efficiently deliver therapeutics to the podocytes.

CONCLUSION

A wide library of polyester based NPs with different surface charge and dimension obtained through emulsion polymerization have been set up. These NPs have been tested in vitro against murine podocytes where their cytotoxicity and cellular uptake have been established. The least cytotoxic NPs have been loaded with dexamethasone and employed on adriamycin-damaged podocytes: cells showed recovery from the damaged based on the orientation of actin fibers. The use of the produced drug-loaded NPs may represent a promising approach to reduce the high percentage of failure of current CKD therapies, minimizing dose regime and systemic side effects.

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ARTICLE

SYNTHESIS OF POSITIVELY CHARGED POLYMER NANO-PARTICLES FOR SIRNA DELIVERY

Dragoni L.*, Ferrari R., Moscatelli D.

INTRODUCTION & OBJECTIVE

Small interfering RNA (siRNA) is receiving increasing attention for the treatment of many genetic diseases, both acquired and hereditary, such as cancer and diabetes. Since siRNA is a high molecular weight polyanion, it is not able to cross cell membrane; moreover it is also unstable in physiological conditions. Accordingly, a biocompatible carrier able to deliver siRNA into the cells is required. In this work, we synthesized positively charged nanoparticles (NPs) based on both biocompatible poly(methyl methacrylate) (PMMA) and biodegradable polycaprolactone (PCL), suitable for the delivery of siRNAs. NPs were produced through batch emulsion polymerization (BEP) and monomer-starved semi-batch emulsion polymerization (MSSEP) with [2-(methacryloyloxy) ethyl]trimethyl-ammonium chloride (HEMA-Ch) as a surfmer. Different particle sizes have been obtained using different surfmer-to-monomer percentage weight ratios obtaining a simple relationship which can be used to tune the final particle size down to 15 nm. Cellular uptake and toxicity of both PMMA- and PCL-based NPs were evaluated in a tumor cell line, setting the suitable concentrations for the NP exposure. Here we demonstrate that both PMMA and PCL-based NPs are effective carriers of siRNAs into cells. In addition we prove that by changing the degradation rate of the PCL-based NPs it is possible to tune the siRNA delivery into cells.

MATERIALS & METHODS

NP synthesis

In this work both biocompatible PMMA-based NPs and biodegradable PCLn-based NPs have been obtained through a two steps process. At first, HEMA-CLn macromonomers (with n equal to 2 and 3) were produced through a ring opening polymerization reaction of ε-caprolactone (ε-CL)

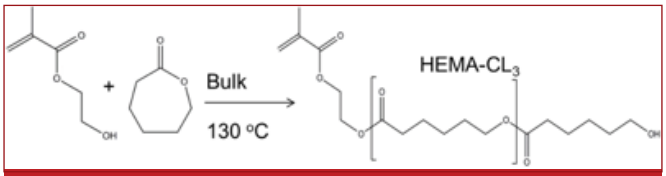

Figure 1: Synthesis of HEMA-CLn macromonomers.

using 2-hydroxy ethyl methacrylate (HEMA) as catalyst and Tin(II) 2-ethyl-hexanoate (Sn(Oct)2) as co-catalyst.

Then, the co-polymerization of the latter macromonomers or the MMA with [2-(methacryloyloxy)ethyl] trimethyl-ammonium chloride (HEMA-Ch) allows to obtain positively charged NPs, excellent carriers for the adsorption of negatively charged compound over their surface. In particular, HEMA-Ch can be considered as a surfmer; a compound which is able to stabilize NPs as a surfactant and, at the same time, can be incorporated into the polymer chains constituting the NPs due to the presence of a reactive double bond into its structure.

In order to make NPs suitable to conduct imaging studies, they have been functionalized with a fluorescence dye: Rhodamine B (RhB). In particular RhB has been previously functionalized through an esterification reaction with ed HEMA so as to be polymerized via free radical polymerization. Then, performing a co-polymerization between HEMA-RhB and the

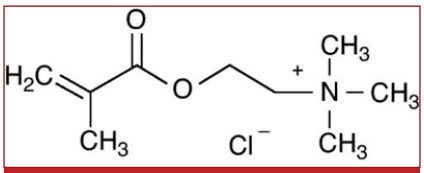

Figure 2: Structure of [2-(methacryloyloxy)ethyl]trimethyl-ammonium chloride (HEMA-Ch) used as surfmer.

selected monomer (MMA or HEMA-CLn), we obtained a fluorescent NPs suspension amenable to imaging studies. In this way RhB is covalently bonded to the polymer chains constituting NPs, avoiding all the problems which are related to the dye desorption from the NP surface.

PMMA-based and PCL-based NPs were produced through batch emulsion polymerization (BEP) and monomer-starved semi-batch emulsion polymerization (MSSEP). The solid content of all the latexes is 2% (w/w). NP size, polidispersity index (PDI) and ζ-potential have been determined by dynamic laser light scattering analysis (DLLS). Finally, as a confirmation of the NP average diameters obtained by DLLS measurements, TEM analysis has been performed.

Table 1. Data referring to fluorescent PMMA-based NPs

S/M [% w/w]	Dp [nm]	PDI	ζ –Potential [mv]
1	93	0.028	54
2	68	0.015	53
5	39	0.033	56
10	24	0.082	54
16.7	17	0.175	55
20	15	0.148	55

In vitro studies and siRNA loading procedure

4T1 cell line derived from a mouse mammary tumor were selected to conduct in vitro studies in order to assess cytotoxicity and cellular uptake of the produced NPs. 4T1 cells were seeded in 6-well plates at the concentration of 10,000 cell/ml. 24h after seeding the cells were incubated with

ARTICLE

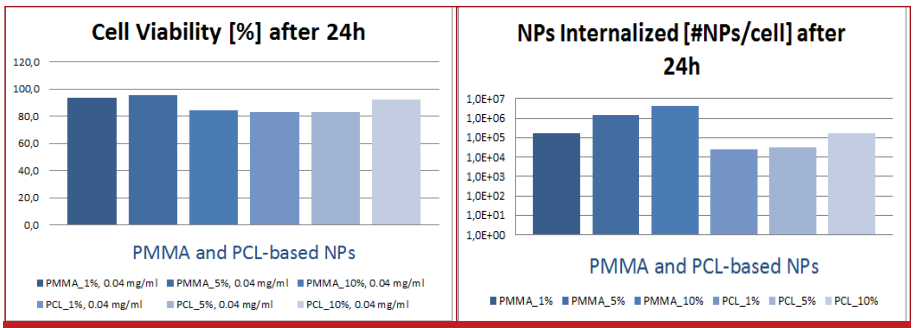

Figure 3 Cytotoxicity and cellular uptake results for different NPs as a function of their size

two different weight concentrations of NPs (0.04 mg/ml and 0.2 mg/ml). After 24h of exposure to NPs, cells were counted using a Coulter Counter ZM. The detection of cell fluorescence and the quantification of internalized RhB-NPs was done using a multimode microplate reader and a FACS Calibur flow cytometer. We analyzed 5000 cells for three replicated samples for each concentration and for each batch of NPs. Through these experiments it was possible to determine the cytoxicity of the produced NPs, and therefore select the best concentration for further in vitro studies. Moreover, it was possible determine the number of NPs internalized per cell and study the effect of the NP size on the cellular uptake.

The siRNA loading step is carried out after the NP synthesis. This step involves the mixing of the produced NP latexes with a solution of siRNA in a mixing device. Then, the loading efficiency has been determined through electrophoretic mobility shift assay tests.

RESULTS & DISCUSSION

NP synthesis

Table 2. Data referring to fluorescent PMMA-based NPs

S/M [% w/w]	Dp [nm]	PDI	ζ –Potential [mv]
1	188	0.016	52
5	106	0.076	49
10	68	0.130	69

At first, fluorescent biocompatible PMMA-based NPs have been produced through free radical emulsion polymerization (FREP). These NPs are suitable to conduct in vitro studies without the problems which are related to by-products formation derived from NP degradation.

For further in vivo applications PMMA-based NPs have to be substituted with biodegradable ones. Therefore biodegradable PCLn-based NPs have been selected.

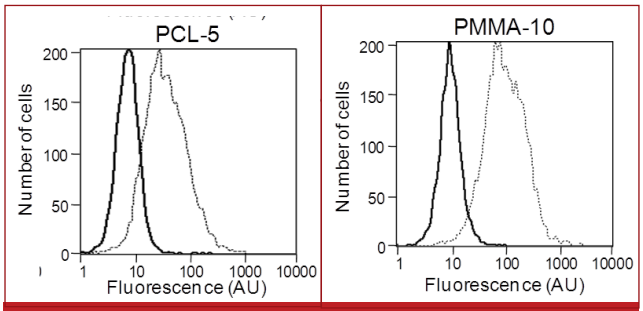

Figure 4: Fluorescence distribution of cells exposed to PMMA- and PCL-based NPs with siRNA.

As it is possible to observe from data collected in Table 1 and Table 2, all the produced NPs are positively charged and have sizes suitable for intravenous delivery application.

In vitro results

In Figure 3 cytotoxicity and cellular uptake results are reported for different NPs as a function of their size.

From Figure 3 it is possible to observe that all the produce NPs are able to be internalized into the intracellular region and that the cell viability is always higher than 80%.

Then, fluorescent measurements have been performed to demonstrate that the produced NPs are also able to carry siRNA into the intracellular region.

As shown in Figure 4, in cells incubated with siRNA-NPs, the fluorescence signal increased up to 100 times the control level (solid line), confirming the incorporation of fluorescent siRNA carried by NPs

CONCLUSIONS

The effect of the process parameters of the synthesis step on the final NP size has been investigated. Then, survival tests on 4T1 cells derived from a mouse mammary tumor indicate that the produced NPs are suitable for further biomedical application and finally, fluorescence measurements and gel retardation assay test demonstrate that these NPs are effective carriers for siRNA.

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Luca Dragoni completed his Master of Science in Chemical Engineering in Politecnico di Milano in 2012. He currently is a PhD candidate in the same university under the supervision of Prof. Davide Moscatelli. His research is focused on the synthesis of biodegradable polymer nanoparticles for drug delivery applications.

ARTICLE

COMPARISON OF THE ENCAPSULATION OF OIL PHASE WITH TWO KINDS OF EMULSIONS

Butstraen C., Devaux E. , Salaun F. , Gérardin K.

INTRODUCTION & OBJECTIVE

Encapsulation of active substances allows durable and targeted functionalization of textiles. Capsules as melamine-formol from in-situ polymerization with a size range from 1 to 10 µm are usually inserted on textile by conventional finishing process as coating or padding. In this work, the aim is to use Fibroline process, based on powder dispersion by alternative electric field which requires capsules size from 10 to 100 µm. To satisfy these requirements, two kinds of emulsion are compared before silica encapsulation of fire retardant compounds obtained by sol-gel process. Indeed, silica capsules exhibit enhanced fire resistance and improved thermal stability into polyethylene therephtalate matrix (Salaün, 2013). Castor oil as inert and a FR substance have been encapsulated. Two kinds of emulsifiers have been studied. The first one is Tween®20, a non-ionic hydrophilic surfactant (hydrophilic-lipophilic balance=16.7) known for its non toxicity and widely used for food, medical and pharmaceutical applications (Salaün 2009). The second one is based on the utilisation of nano solid silica particles allowing obstruction of the interface between the two phases, named Pickering emulsion (Salari, 2011).

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MATERIALS & METHODS

Materials

Bisphenol-A bis(diphenyl phosphate) (Devan Chemicals) and castor oil (Sigma Aldrich) are used respectively as FR and inert core. Tetraethylorthosilicate (Aldrich) is used as shell material. Tween®20 (Aldrich) and Evonik Aerosil R816 (Safic Alcan) combined with Hexadecyltrimethylammonium bromide (CTAB) (Aldrich) are employed as emulsifiers. Formic acid and sodium hydroxide from Aldrich are used as pH control agents.

Microencapsulation process

10 g of castor oil or FR are first of all dispersed at 2000 rpm into 100 mL of an aqueous phase containing 1 g of Tween® 20 or 0.1 g of Aerosil R816. 0.5 g of CTAB is added after the emulsion in the case of silica particle to promote hydrolysed silane migration at the droplets surface (EP2080552B1, 2009). 100 mL of tetraethoxysilane (TEOS) solution, previously hydrolised at pH 2.8, is then added dropwise to the emulsion. The mixture is kept under stirring condition at 45°C during 24 hours to initiate silane condensation. Sodium hydroxide 10% w/v is gradually added until pH neutralisation to speed up this condensation and shape a thick shell around the active substance. Capsules are then aged for 1h, filtered and dried 24 hours at room temperature. A thin powder is obtained.

Methods

Capsules diameters were obtained with a laser-lightblocking technique (AccusizerTM, model770, Santa Barbara, CA). Chemical structure of the shell was studied by Fourier transform infrared spectroscopy (FTIR) performed in the absorbance mode on KBr pellets using Nicolet Nexus. Thermogravimetric analyses (TGA) have been performed on TA 2050 instrument under nitrogen atmosphere at purge rate of 50ml/min with a heating rate of 10°C/min.

RESULTS & DISCUSSION

Morphological properties and size

Whatever the encapsulated oil phase and the kind of emulsion (based on non-ionic surfactant or silica particles), the particle size ranged from 0.5 to 100 µm and the mean size is closed to 30 µm (+/- 18). 90 to 95% of the capsules have a diameter included between 10 and 100 µm. Moreover, with Tween® 20, capsules are stickier than capsules with particles. This drives to assume, as expected, the blockage of leaching effect with particles, for the two kinds of oil which is convenient for textile applications.

Encapsulation validation by FTIR study

Figure 1 shows FTIR spectrum of capsules with FR (a) and inert (b) core with

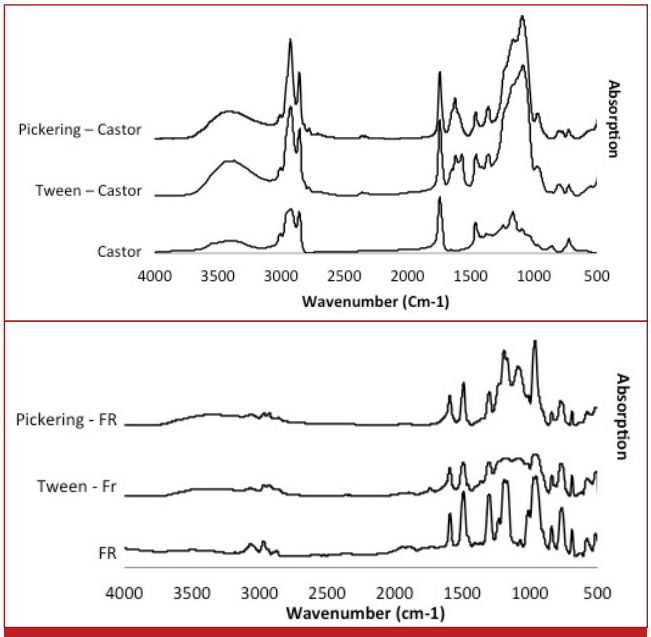

Figure 1 : FTIR spectrum row and encapsulated core : castor oil (a) and FR (b)

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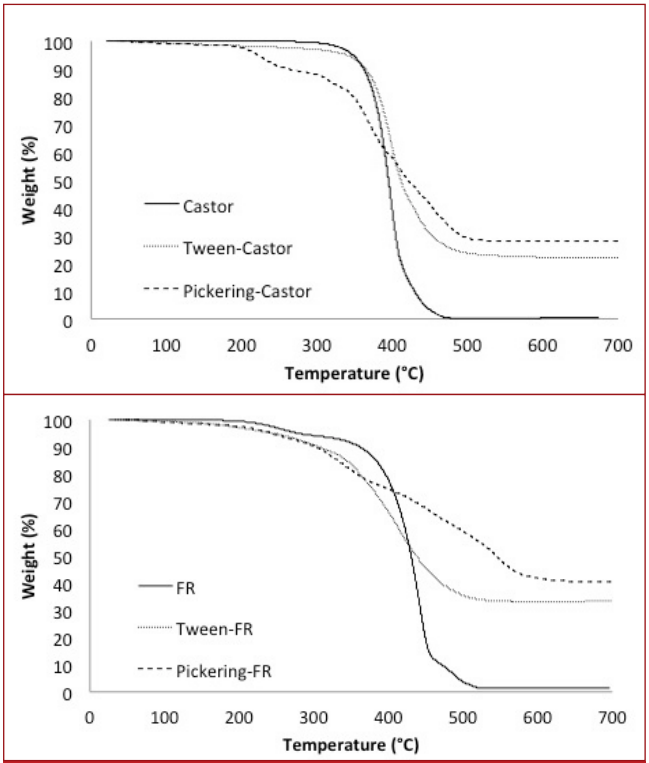

Figure 2 : Thermogravimetric analysis of row and encapsulated core : castor oil (a) and FR (b) with the two emulsifiers under nitrogen atmosphere

Tween®20 and Pickering emulsion. In figure 2 (a), spectrum of pure and encapsulated castor oil displays at the same time characteristics absorption bands of the core oil and of the shell. Indeed, two intense absorption bands at 2922 and 2854 cm-1 are attributed to C-H stretching vibrations and the bands around 3200-3500 cm-1 and at 1748 cm-1 are attributed to O-H stretching vibrations and C-O vibrations of castor oil. Moreover, capsules spectrum, for emulsifiers, present symmetric and asymmetric Si-O-Si stretching vibrations at 1088 and 789 cm-1 respectively. Likewise, bands of FR and polysiloxane shell can either be observed in figure 2 (b). FR fingerprint can be distinguished in all spectrums: 3000-3100 cm-1 band is attributed to aromatic C-H stretching vibration, and between 400 and 1600 cm-1, bands attributed to C=C stretching, C-C stretching and C-H bending in aromatics groups, to P=O stretching, to aromatic-O and P-O stretching are noticed. This confirms the encapsulation of castor oil and FR by polysilixane shell for the two emulsifiers.

Thermal stability

Thermogravimetric analysis before and after encapsulation are displayed in figure 2 (a) for castor oil and in figure 2 (b) for FR. With Pickering emulsion, for both cores, degradation of capsules begins at lower temperature than degradation of row core. This can be attributed to the deshydroxylation of hydroxyls groups and to the CTAB degradation. Yet, thermal stability is improved for higher temperatures: from 350°C for FR and from 420°C for castor oil. With Tween®20, thermal stability of castor oil is relatively unaffected by the emulsifier whereas degradation of FR occurs 100°C earlier. Nevertheless for all capsules, with the two cores and two kinds of emulsion, an amount of residue remains important, this corresponds to condensed silica from the shell.

CONCLUSIONS

For both inert and FR core and for the two kinds of emulsion, encapsulation has been successfully performed with suitable sizes for using Fibroline process. Thermal stability is affected by the emulsion formulation and depends on the oil chemistry but remains satisfying for fire retardant textile applications.

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Chloé Butstraen achieved her degree in Technical Textiles and Advanced materials – Chemistry Engineering from the ENSAIT (Superior National School of Arts and Textile Industries) in France in 2012. She had worked on the encapsulation of liposoluble substances by complex coacertion during her master degree. She is currently PhD student in the ENSAIT/GEMTEX laboratory. Her thesis deals with the encapsulation of fire retardants by thermomelted shell. The aim is to functionalize textiles without using latex processes.

New biofibre gum (BFG) is launched by Z Trim Holdings Inc.

The food-grade, soluble dietary fibre ingredient is proposed as an alternative gum emulsifier with microencapsulation properties and health benefits in the area of weight gain and cholesterol reduction.

More information
http://prn.to/1shZgoW

Microencapsulation of a putative probiotic Enterobacter species to protect rainbow trout against bacterial coldwater disease (BCWD).

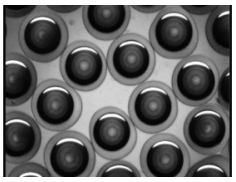
An Enterobacter species, C6-6, isolated from the gut of trout has been identified as a potential probiotic species providing protection against BCWD. A recent study by researchers at the National Centre for Marine Conservation and Resource Sustainability, University of Tasmania, Australia, examined the effects of alginate microencapsulation on the protective efficacy of C6-6 against BCWD in vivo when administered to rainbow trout fry.

More information
http://bit.ly/1vjPC7F

Buildings of the future may incorporate microencapsulated phase change materials to aid climate control.

Appvion (formerly Appleton Papers Inc.) is working on encapsulated phase-change materials for use in the walls or ceilings of buildings to help regulate temperature. The aim is to develop materials that absorb heat during the day and releases it at night.

More information
http://post.cr/1o1VrVg



AnaBio Technologies Ltd claim to have developed a microencapsulated omega fatty acid product with extended shelf life.

Encapsulating krill oil using a proprietary technology the Irish company AnaBio Technologies Ltd claim to have reduced DHA oxidation by 76% after 22 months of storage at 35°C. The technology aimed at fish oil as well as krill oil and other omega-3 fatty acid compositions is available for licensing.

More information
http://bit.ly/1D5lWMF

Scientists at the Wuhan University of Technology, China, exploit silica microcapsules for novel phase change materials.

Capric–stearic eutectic was encapsulated within a silica shell material without the use of emulsifiers which would otherwise impair the performance of the phase change materials. Improved thermal stability makes the materials suitable for use in thermo-regulated textiles, aiding the climate control of buildings and for use in heat-transfer fluids.

More information
http://bit.ly/1tmu9ee

UNIVERSITÄT BASEL

Microencapsulated insecticides deliver long residual activity against pyrethroid-resistant malaria vectors in central Côte d'Ivoire

Capsule suspension formulations of pirimiphos-methyl have proved effective alternatives to conventional insecticidal residual spray applications used inside of homes where pyrethroid resistant malarial mosquitoes (Anopheles gambiae) are endemic.

More information
http://bit.ly/1vZwedf

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Postdoctoral Position
Institute of Chemical Process Fundamentals of the ASCR

We are seeking for a motivated Post Doctoral Fellow to develop organic–inorganic optical sensors. The candidate must hold a Ph.D. or equivalent qualification for less than 2 years at the date of application deadline. Candidates with a solid background and training in microbiology, biochemistry, biotechnology, chemical engineering, analytical chemistry or another relevant field are encouraged to apply. Knowledge in Czech language is not required but welcome. Applicants should be able to work independently.

Salary, pension and terms of employment will be in accordance with the internal rules of Academy of Sciences of the Czech Republic KAV-667/OPV/2013. Currently, the monthly salary is 30,637 Kč . The salary might be increased up to 40,500 Kč.

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2. A signed copy of a) PhD certificate and b) Master of Science certificate.
3. List of publications.
4. A signed copy of declaration of the candidate agreement with the salary conditions and working programme.
5. Motivated letter of application (max. one page).

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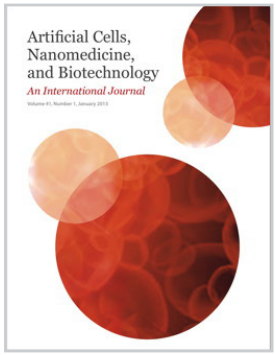

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- ***In vitro and in vivo inhibitory effects of some fungicides on catalase produced and purified from white-rot fungus Phanerochaete chrysosporium***
Berna Kavakçıoğlu , Leman Tarhan
Artificial Cells, Nanomedicine, and Biotechnology Oct 2014, Vol. 42, No. 5: 356–364.

BRG GENERAL ASSEMBLY

INTRODUCTION

Each participant was invited to attend the 2014 BRG General Assembly, held at the Annual Conference in Bratislava, Slovakia on September 18, 2014. .

2013 ACTIVITY REPORT

Four issues of the BRG newsletter were published in 2013 under the supervision of Paul de Vos from Groningen University (Netherlands) and edited by Brigitte Poncelet from Impascience (France). The newsletter is sent by email to 7000 persons.

- The march 2013 special issue on Encapsulation in by chemical methods was edited by B. Boh from Lujbjana University (Slovenia) and Yves Frères from Institut Charles Sadron (France).
- The June 2013 special issue on Microfluidics and micro encapsulation was edited by Raul Gomez and Johan Smet from P&G (Belgium).
- September 2013 was devoted to the XXI ICB held in Berlin, Germany, the 2013 Poncelet Award: Ronald Neufeld, and the best student contributions.
- Last issue, in december, was dedicated to enzyme encapsulation and edited by Luis Fonseca from IPT (Portugal) and Gabrie Meester from DSM (Netherlands).

Three events were organized in 2013

- 4th training school on microencapsulation organized in Nantes, France on April 9-12.
- 16th Industrial Convention on Microencapsulation, held in Madison, USA on June 25-17 and co-organized by Encapsys.
- 21th International Conference on Bioencapsulation, held in Berlin, Germany on august 28-30, co-organized by Prof. Stephan Drusch from TU Berlin.

The 3 events were very successful both in term of attendance and quality of the contributions (See table 1)

2013 FINANCIAL REPORT

The 2013 accounting was externally audited by HLP audit, Nantes, France.

A summary of the incomes and ex-

penses for 2013 events is presented in Table 2 while table 3 gives the evolution of the cash flow over 2013. The following general observations may be done:

- Despite a large number of grants/ Scolarships (see Table 1), the Nantes training school was beneficiary due to a large industrial participation.
- Madison industrial convention was largely beneficiary. One problem of organizing a meeting outside of the European area is the important bank exchange cost.
- The Berlin conference was globally overdrawn but beneficiary if we do-not take in account the large budget devoted to grants/scholarships.
- Overall the 2013 meetings were beneficiary of 10 700 euros despite the large support to participants (30 655 euros as grant + 49 400 euros as free registrations) and despite the low registration fees comparing to most other conferences.
- Taking into account some invoices related to 2012 or 2014 meetings, the cash flow in 2013 was slightly positive (3000 euros)

The General Assembly approved unanimously the 2013 financial report.

STEERING COMMITTEE

The General Assembly unanimously elected the following Steering Committee, in function until the next General Assembly to be held in September 2015:

- Denis Poncelet as President
- Paul De Vos as co-president and newsletter chief-editor, with support from Michael Whelehan and Brigitte Poncelet
- Thierry Vandamme as Secretary
- Ronald Neufeld as Treasurer

The Steering Committee will be completed with people who accept to take some responsabilities:

- Nicole Papen-Botterhuis (2015 Convention)
- Gabrie Meester (2015 Conference)
- Gorka Orive and Lena Markvicheva (Workshop)
- Amos NUssinovitch (Newsletter)
- Harald Stover and Laura Hermida (Addressbook)
- Luis Roque and Silva Catarina (Networking)

2013-2014 ACTIVITIES

Four events were and will be organized in 2014:

- 6th Training School on Bioencapsulation, held in Nha Trang, Vietnam, March 4-8
- 17th Industrial Microencapsulation Convention held in Brussels, Belgium, in May, and organized by Dr Jean Paul Simon
- 22th International Conference on Bioencapsulation, held in Bratislava, Slovakia, in September, and organized by Prof. Igor Lacik.
- 2nd South America Workshop on Microencapsulation, to be held in Joa Passao, Brasil, in November, mainly organized by Prof. Ana Luisa Braga

Three events are already scheduled for 2015:

- 7th Training School on Bioencapsulation, to be held in Strasbourg, France, February 23-27, and organized by Prog. Thierry Vandamme
- 18th Industrial Microencapsulation Convention to be held in Eindhoven, Netherlands in April 22-24, organized by Nicole Papen-Botterhuis from TNO
- 23th International Conference on Bioencapsulation, to be held in Delft, Netherlands, in September 2-4, organized by Dr Gabrie Meesters from DSM

Four issues of the BRG newsletter are scheduled in 2014

- Probiotic encapsulation, edited by Andre Brodkorb
- A special issue related to the presentations done during the 17th Industrial Convention in Brussels
- Best student contributions, prices awarded at the 21Ith International conference held in Bratislava.
- Controlled delivery system in December, edited by Thierry Vandamme, from Strasbourg

CLOSING

As no question were raised by the participants, the General Assembly was closed by the President.

Table 1	Participants					Contributions		Grants
	Industrials	Researchers	Students	Exhibitors	Total	Orals	Posters	
2013_Nantes	27	24	40		91	26	50	54
2013_Madison	57	14		19	90	12		12
2013_Berlin	24	63	59	18	164	40	76	42

Table 2 : Conference budget 2013					
	2013_Berlin	2013_Madison	2013_Nantes	BRG	TotaL
registration	68 033 €	78 848 €	38 550 €	3 211 €	188 642 €
Receptions	40 702 €	26 850 €	15 647 €	1 923 €	85 122 €
Printing/mailling	5 143 €	10 534 €	1 782 €	18 €	17 477 €
Administration	13 395 €	13 724 €	8 791 €	1 972 €	37 882 €
Grants	17 081 €	3 703 €	8 238 €	1 633 €	30 655 €
Bank costs	1 484 €	4 906 €	603 €	630 €	7 623 €
Divers			493 €	493 €	-6 499 €
Balance	-9 772 €	19 131 €	3 489 €	-3 458 €	9 390 €
Free registration **	15 400 €	13 200 €	20 800 €		49 400 €
* Grant/scholarship provided by BRG for travel and accommodation **equivalent to registration fees					

Table 3 : cash flow 2013	
Solde end of 2012	76 214 €
2012 Archamps	-3 919 €
2013 Berlin	3 545 €
2013 Madison	15 468 €
2013 Nantes	5 925 €
2013 BRG	-2 148 €
2014 Nha Trang	-5 402 €
2014 Brussels	-9 712 €
2014 Bratislava	-650 €
Solde end of 2013	79 321 €

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Bioencapsulation Research Group is a non-profit association promoting networking and research in the encapsulation technology of bioactives. It organises academic conferences and industrial symposiums, publishes newsletters and manages a website.

More information : http://bioencapsulation.net

KEEP CONTACT BY REGISTERING ...

Registration is based on a voluntary annual fee. If you wish to simply receive the newsletter and be advised about future events, register online at: **http://bioencapsulation.net**

Be an active member pay the registration fee and get more services

- Reduced registration fees to BRG events
- Full access to conference proceedings (› 1700)
- Access to the forum and internal mailling
- Possibility to contribute to the newsletter
- Reduction for the conference registration
- Priority for awarding of conference grants

Class	Annual fees
Industry members	100 €
Researchers [1]	60 €
Students [2]	30 €
Honorary member and corporate registration [3]	1000 €

[1] public and non-profit organizations, contact us for group registration
[2] registered for a master or PhD program, less than 30 years old.
[3] Open access to 1 full page in 1 issues (1/2 page in 2 issues ...) in the newsletter
Registration fees may be paid by credit card (preferably), bank transfer or cheque.

For more information or an invoice, see the registration page on **http://bioencapsulation.net**

Thanks to **Agence I** (**http://www.agence-i.eu/**) for designing the newletter. **Geraldine Brodkorb** (**gbrodkorb@eircom.net**) for English corrections,Brigitte Poncelet (**http://impascience.eu**) editing corrections and the editorial board for their help.

REGISTRATION DATA

- Title: .....
- First name: ..... Last name: .....
- Affiliation: ..... Department: .....
- Address: ..... Address (cont.): .....
- Zipcode: ..... City: .....
- State: ..... Country: .....
- Phone: ..... Fax: .....
- Email: ..... Website: .....
- Password: ..... Repeat password: .....
- Registration class: ..... Registration fees: ..... €

Send your registration to : Bioencapsulation Research Group 5 rue de la maison blanche44240 Sucé sur Erdre France