Bioencapsulation Research Group

Bioencapsulation Innovations

December 2012

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Internat.Conf. on Bioencapsulation

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EDITORIAL

XX INTERNATIONAL CONFERENCE ON BIOENCAPSULATION

Orillia, Ontario, Canada - September 21-24, 2012

For its 20th international conference, the Bioencapsulation Research Group was back In Canada, its mother country. The conference was held in Orillia a small city located between Lake Couchiching and Lake Simcoe, 200 km north of Toronto. At the end of September, the participants were able to enjoy the Canadian Indian summer while appreciating the very good service offered by the Geneva Park.

In a friendly atmosphere, 110 participants exchanged their experience through the presentation of more than 80 oral and poster contributions, number of discussions during coffee breaks, meals and evening.

The organizers wish to thank everyone who contributed to the success of the meeting, especially JRDF, P&G and Union Biometrica for their support.

CONTRIBUTING TO THE EXCELLENCE

During the recent years, the Bioencapsulation Research Group has developed new tools to promote the quality of its conferences and to acknowledge the innovation and the development of the members.

- Poncelet Award recognizes the achievements of one person in the area of bioencapsulation. In 2012, the prize was attributed to Professor Christophe Lacroix from ETH Zurich (see his contribution on page 4)

- Ten prizes were awarded to the best student contributions (both orals and posters) during the international conference 2012 held in Orillia, Ontario, Canada. This newsletter presents their contributions*.

** Paper from Swapnil Vilas Bhujbal (s.bhujbal@umcg.nl, CRP-Santé, Luxembourg. & University Medical Center Groningen, Netherlands) has been published in Ocotober 2012 newsletter*

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HISTORY

21 YEARS, MORE THAN 50 CONFERENCES, WORKSHOPS ...

THE BEGINNINGS

In 1990, the Bioencapsulation Research Group (BRG) was formed by a team of seven Canadian laboratory research leaders at McGill University (Montreal, Canada) to promote an exchange between laboratories working on microencapsulation. The name was chosen as those laboratories were working at that time mainly in the field of encapsulation of biological materials for diverse applications including lactic bacteria immobilisation, diabetes treatment, drug delivery or cheese ripening.

Initially, a meeting was organized, allowing each laboratory to present its activities. Through the presentations from the students and academic researchers, collaborations were anticipated.

A funding opportunity made it possible to invite ten researchers from Europe for a research workshop. The participants were so enthusiastic as a result of the scientific exchange, that it was decided to organize a subsequent meeting in France.

This international gathering of researchers involved in bioencapsulation technologies has now been repeated regularly over the years. During the past 22 years, the International Conferences on Bioencapsulation have attracted more than 4000 participants presenting over 2000 research contributions.

DEVELOPING INDUSTRIAL ACTIVITIES

During the initial years of the BRG, the activities involved one international scientific conference per year. The participants were mainly academic, but the conference was enriched through the participation of 10 to 20% from industry. An industrial symposium linked to a technology trade fair was organized in 1998, representing a new initiative, resulting in a boost to the BRG, both in terms of membership, but also in terms of the research based BRG becoming recognized for pioneering industrially focused events.

It subsequently became apparent that industrial events, symposia and thematic workshops, met a real need and as a result were very successful. These events also secured an important part of the financing of the Bioencapsulation Research Group. This unique research/industry partnership was of benefits every part of the network. Today, over 60% of the BRG membership is from ithe ndustry.

Figure 1 : List of BRG events, participants and contributions

BRG NETWORKING

In 1999, the activities of the association were extended by applying for funding from the European Science Foundation resulting in two COST actions (Program of Cooperation in Sciences and Technologies, COST 840 & 865). These actions provided financial support for organizing workshops, but also through support of short-term scientific missions. The actions enabled the establishment of strong, permanent links between the BRG members and promoted several collaborative initiatives leading to numerous scientific publications and European research projects.

Another network was also established with support of the European Space Agency leading to several publications and two collaborative projects.

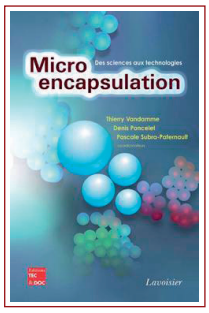
BRG PUBLICATIONS

More than 2000 contributions have been shared as oral presentations or posters during the conferences organized by the BRG. Most manuscript papers associated with the presentations are available on the BRG web site (http://bioencapsulation.net). Several collaborative papers supported through the COST actions have been published in international scientific journals. An important publication is an edited book written in French : Microencapsulation, des Sciences aux Technologies, Lavoisier 2007 (Figure 2). Forty-four authors contributed to the book, which has sold several hundred copies. One objective for the near future, is to renew this effort, but for

Figure 3 : Thanks to the organizers of the 20 International Conferences on Bioencapsulation

an updated and English version of the book.

During the first years of the BRG, a newsletter was published and mailed to members. However, due to the heavy workload, and cost of mailing newsletters to a growing membership, the newsletter was discontinued. In 2011, a new initiative resulted in the renewal of the newsletter, with the objective that it be a highly professional product, and vehicle for promoting exchanges, and the dissemination of innovation, research and information throughout the BRG network.



KEY TO SUCCESS

Starting from a group of seven Canadians, the BRG has grown to an international association with thousand of members representing more than 80 countries.

Several factors have contributed to the success of the Bioencapsulation Research Group:

- The BRG has always supported the participation of students, young researchers, and researchers from emerging countries, promoting a dynamism in the annual conference.
- The BRG has distributed more than 1,300 grants for a total of more than 1 million euros.
- The BRG is more than an association, promoting networking, exchanges and collaboration, while maintaining a collegial and friendly ambiance over the years in all of its events.
- The BRG supports a flexible administrative structure that encourages every member to be partner in its activities

The success would have not be possible without the contributions of a large number of people, illustrated in Figure 3 by the list of the international conference organizers.

FUTURE

Now well into the third decade of BRG operations, a new generation of members are actively involving themselves in the future development of the association. Moreover, while open to all countries, the BRG has concentrated activities within Europe. In the last two years, activities are more global and now extending to other parts of the world. Industrial Symposia are organized in the USA, several workshops have been succesfully organized in Chile and Brazil, and the BRG has received several requests for organizing workshops and training schools in Asia.

The new newsletter will be further developed and a microencapsulation encyclopedia both as book and as web site is now under construction. The BRG also plans to develop new exchange programs such as the European people research network.

This growth of the BRG implies also restructuring itself. The management cannot no longer be done by one or two people. For this reason, a steering committee was established, that will share the burden of the many different aspects of the organization. This committee will be what the BRG has been for more than two decades, an open forum that welcomes any members to join, share and participate, and to ensure that the next decades will be at least as successful as the past decades have been. .

Figure 4 : BRG conferences : a professional event but a friendly atmospher

ARTICLE

BIOENCAPSULATION AND MICROBIAL TRANSPLANT, WHAT'S THE USE?

Christophe Lacroix, A. Zihler, A. Dostal, S. Fehlbaum, C. Chassard, C.

OUR GUT MICROBIOME

The human large intestine is colonized by a dense and complex microbial community composed largely of anaerobic bacteria, whose cell numbers can exceed 10^{14} (100 trillions) microbes belonging to more than 1000 species, exceeding the total number of human cells by an order of magnitude. Presently, it is known that the metagenome of our intestinal microbes, also known as our microbiome, harbors over 3 million genes and vastly exceeds the coding capacity of our own ge-nome. Indeed the distal gut microbiota activities may be considered as a distinct human organ responsible for multiple physiologic functions related to nutrition and health of the human host. Consequently there is considerable interest in understanding the effect of diet and host genes on the gut microbiota composition and activity. In addition, the relation between intesti-nal microbiota and disease is actively studied, with over 25 diseases that have been associated with our intestinal microbiome. These include intestinal disease including inflammatory bowel syndrome (IBS) and disease (IBD), and also more systemic diseases such as metabolic syndrome, type 1 and type 2 diabetes, obesity, and autoimmune, asthma, and allergic diseas-es, many of which have reached epidemic proportions in recent years.

Resident commensal bacteria are further responsible for creating a powerful line of resistance to coloniza-tion by exogenous microbes. Adherent intestinal mi-crobes constantly compete with exogenous microbes for attachment sites in the brush border of intestinal epithelial cells, preventing pathogenic invasion and translocation into colonic tissue [1]. Colonization resistance requires a delicate ecological balance to avert the overgrowth of acquired opportunistic bacte-ria such as *Clostridium* difficile and prevent the path-ogenesis of these strains. Optimized nutrient con-sumption and metabolism by resident gut microbiota further attenuates pathogen proliferation as resident microbes are capable of adjusting their nutrient re-quirements and metabolic activities causing pathogen starvation [1].

C. DIFFICILE A HIGHEST THREAT TO OLD & YOUNG

C. difficile a highest threat to old and young is one of the best example of a disease resulting from major disruption of the gut microbiota by antibiotics is *C. difficile* infection (CDI). CDI has become a grow-ing public health problem in the last two decades, with a high prevalence rate in acute care facilities, and ac-companied by increasing rates of colectomy and death, with approximately 100,000 people dying an-nually in the U.S. with CDI. Older patients are partic-ularly susceptible to CDI, but no age group is spared, and the incidence of CDI-related hospitalizations has been rising even in the pediatric population [2].

Standard treatment of CDI is based on antibiotics, which have broad activity against the dominant phyla of colonic microbiota, but the risk of relapse following initial treatment of CDI is high approximately 20-25% [2-3]. Thus, a fraction of patients can develop chronic, recurrent form of CDI that can last indefinitely. It is now recognized that the presence of normal, healthy, intestinal microbiota offers protection against this serious infection.

Fecal microbiota transplantation (FMT), also com-monly known as "fecal bacteriotherapy" represents the one therapeutic protocol that allows the fastest reconstitution of a normal composition of colon mi-crobial communities [3]. This rather simple therapeu-tic solution aims to re-establish a normal intestinal flora, deprived of *C. difficile*, by administration of fecal material obtained from a healthy donor into the colon of patients with the disease. For many decades, FMT has been offered by select centers across the world, typically as an option of last resort for patients with recurrent CDI. Well over 200 cases have been reported with an approximately 90 % cumulative suc-cess rate in clearing recurrent CDI, without any noted adverse events. The lack of wider practice of FMT is due to multiple nontrivial practical barriers and not due to lack of efficacy. Such barriers include the do-nor screening, the production of a repeatable suitable material, safety concerns and aesthetic reasons [3].

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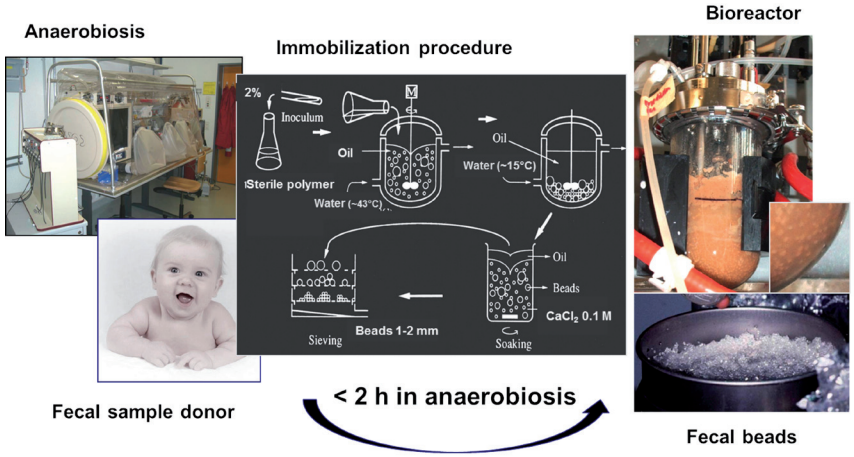

Figure 1. Preparation of fecal beads entrapping microbiota used to inoculate intestinal fermenta-tion models, exemplified with baby fecal donor.

IMMOBILIZATION A POWERFUL TOOL FOR GUT MICROBIOME STUDY

The design and complexity of in vitro gut fermenta-tion systems has broadened from simple batch cultures to single- or multistage continuous flow

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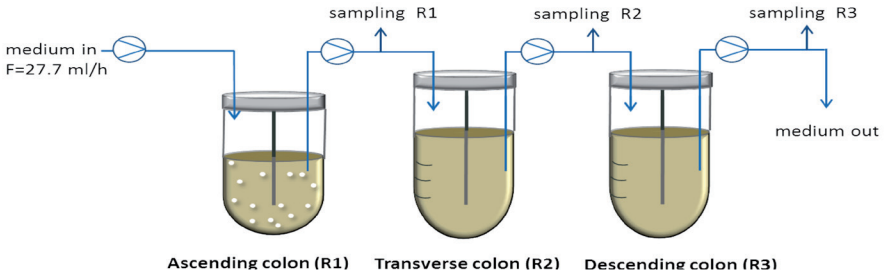

Figure 2. Three-stage colonic fermentation model, inoculated with immobilized fecal microbiota in gel beads in the a proximal colon reactor contain-ing beads.

models using a variety of different fecal inoculation techniques [1]. In vitro gut fermentation models enable the stable cultivation of a complete intestinal microbiota for a defined and model specific period of time. Such models have no ethical guidelines and are well-suited for mechanistic studies.

Perhaps the major discriminating factor between the different in vitro continuous fermentation systems is the technique used for fecal inoculation. Operation of most in vitro systems uses a liquid fecal suspension as inoculum, resulting in several limitations due to the free-cell state of the bacterial populations. In particu-lar continuous systems with liquid fecal inocula gen-erally experience a rapid washout of less competitive bacteria and are consequently limited in operational time to less than 4 weeks [1]. These systems also struggle in reproducing both the planktonic (free-cell) and sessile (biofilm-associated) states of bacterial populations in the colon.

To address problems associated with inoculum wash-out, we developed a mild process for the immobiliza-tion of very sensitive fecal microbiota (Figure 1) which was applied in a variety of fermentation designs to stably cultivate and study the gut microbiota of different hosts (from infant to elderly), diet and health conditions (e.g. infections), while preserving the ini-tial biodiversity of the donor fecal sample [4]. Here, fecal microbiota are entrapped within a highly porous polysaccharide matrix (2.5% (w/w) gellan gum, 0.25% xanthan gum and 0.2% sodium citrate) using a double phase dispersion process. The composition of the gel matrix was carefully selected to achieve me-chanical integrity of beads during long term fermenta-tion with complex microbiota. Fecal beads are transferred to the growth medium in R1 of a multistage continuous fermentation model (Figure 2). Limitations on substrate and toxic product diffusion within beads result in formation of a high-cell density peripheral layer, where cell release occurs spontaneously as a result of active cell growth. The released cells are transported to R2 and then R3, resulting in a self-contained continuous fermentation system of very high-cell density and population stability, close to the human GI tract (Figure 2).

The operational time of such systems using immobi-lized fecal microbiota has been functionally demon-strated for time frames of up to 120 days. We also showed using both cultivation and advanced molecu-lar methods for population profiling (quantitative real-time PCR, TGGE, HITChips, pyrosequencing) that the microbial community structure developed in the fermenter reflects the relative proportions and activi-ties of the major bacterial groups present in fecal sam-ples. Moreover, the biodiversity indices of fecal in-ocula and effluent samples from the distal colon reac-tor (most akin to feces) are very similar, exemplifying the preservation of the complex inoculum diversity. Inoculation and colonization of the system, however, results in a newly balanced

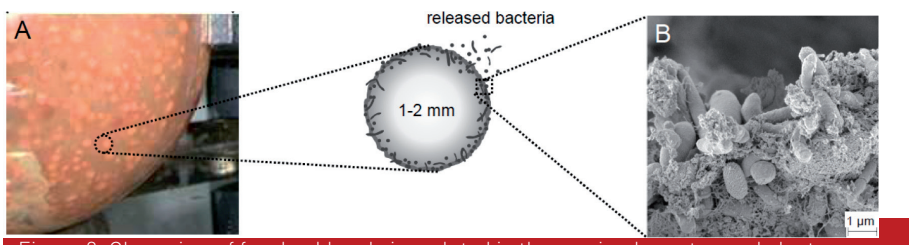

Figure 3. Close view of fecal gel beads inoculated in the proximal reactor, and electron microscope image of microbes embedded in a fecal bead [1].

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Christophe Lacroix has been Full Professor for Food Biotechnology at the Institute of Food Science, Nutrition and Health at the ETH Zurich since August 1, 2002. Born in 1958 in Dijon, France, he studied Food Technology at the Ecole Nationale Supérieure des Industries Agricoles et Alimentaires in Massy (now AgroParisTech), France, where received his food engineering degree in 1980. He then moved to Québec Canada where he completed a MSc and a PhD in Food Technology at Université Laval. From 1984 to 2002 he was Professor of Dairy Biotechnology in the Department of Food and Nutrition Sciences in Université Laval. , and remained Associated Professor. He also helped set-up and lead (from 1995 to 2002) the largest dairy research centre in North America (STELA). In 1997, he initiated the Canadian Network of Excellence on Lactic Acid Bacteria supported by the Natural Sciences and Engineering Research Council of Canada and industry partners, a network he was responsible for until the end of 2002. In 1990 he was a founding member of the Bioencapsulation Research Group.

2012 Winner of Poncelet Award
For his contribution to development and innovation into bioencapsulation domain
Sponsored by Procter & Gamble

His research addresses the fundamental and technological characterization of functional microbes and their roles in food, intestinal ecosystems and gut health, with multidisciplinary system- oriented aspects. This includes ecosystem study and microbe screening and characterization, functional studies and mechanisms, microbial technology (in particular high cell density biofilm based bioreactors with entrapped cells), and intestinal research (in vitro modeling, animal and human studies). Development of technology and products of well-characterized microbes is carried out in close collaboration with the food, biotech and pharma industries, with the goal of transferring research results to industrial processes and products for high quality, safe and healthy food, and for prevention and treatment of intestinal diseases in both developed and developing countries. In his teaching, he aims to bridge basic knowledge of natural sciences and engineering sciences in order to achieve the application of microorganisms, enzymes and metabolites for processing of high quality, safe and healthy food and functional ingredients. Christophe Lacroix has published over 220 scientific papers in peer-reviewed journals, and 10 patents, and has been supervising 47 Ph.D. theses.

ARTICLE

gut microbiota which is a result of both environmental factors and the initial qualitative diversity, but not initial quantitative bal-ance of the fecal inoculum. These changes in population ratios reflect applied fermentation conditions (e.g. retention time, culture medium, pH, etc.) which can be very well controlled but are never an absolute simula-tion of the conditions encountered in the host intestine and the inability to simulate major host functions. Because environmental factors can be manipulated in vitro, the enhancement of beneficial components of the gut microbiota can be envisaged, for example by supplying selective nutrients or changing the pH.

FECAL BEADS TO SAFE PROPAGATE OUR MICROBIOME, IS THIS THE SOLUTION FOR CDI?

In vitro fermentation models are an innovative techno-logical platform where the greatest advantages are exhibited by the virtually limitless experimental ca-pacity as experimentation is not restricted by ethical concerns. Based on the excellent characteristics of our intestinal fermentation models with immobilized fecal microbiota, we propose this technology to allow con-trolled propagation of healthy and functional gut mi-crobiota for safe application as inocula for FMT. Furthermore the downstream processing, including for-mulation of a protective phase, stabilization and stor-age conditions for complex anaerobic gut microbiota, must be designed.

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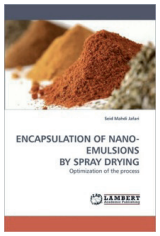

Encapsulation of nano-emulsions by spray drying

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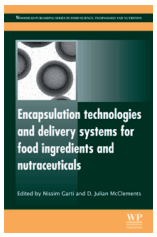
ISBN-10: 3838319478
ISBN-13: 978-3838319476.
http://www.amazon.com/ENCAPSULATION-NANO-EMULSIONS-SPRAY-DRYING-Optimization/dp/3838319478

From Seid Mahdi Jafari, Gorgan University of Agricultural, smjafari@gau.ac.ir



Encapsulation technologies and delivery systems for food ingredients and nutraceuticals.

Nissam Garti and Julian McClements (Ed.) Woodhead Publ.
ISBN-10: 0857091247
ISBN-13: 978-0857091246
http://www.woodheadpublishing.com/en/book.aspx?bookID=2460

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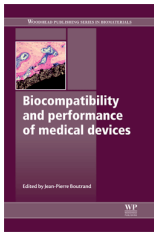
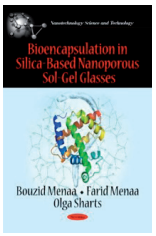
From James Oxley, SwRI, james.oxley@swri.org



Biocompatibility and performance of medical devices

J-P Boutrand (Ed.) Woodhead Publishing, Series in Biomaterials No. 50 (2012)
ISBN 0 85709 070 4
ISBN-13: 978 0 85709 070 6
http://www.woodheadpublishing.com/en/book.aspx?bookID=2352

From Jean-Pierre Boutrand, Namsa, jpboutrand@namsa.com



Bioencapsulation in Silica-Based Nanoporous Sol-Gel Glasses

Bouzid Menaa, Farid Menaa, Olga Sharts (Ed.) Nanotechnology Science and Technology Series, Nova Science Publishers, Inc., New York, USA (2011)
ISBN: 978-1-60876-989-6
https://www.novapublishers.com/catalog/product_info.php?products_id=12440&osCsid=b

From Bouzid Menaa, Fluorotronics, bouzid.menaa@gmail.com

Chitosan-Based Systems for Biopharmaceuticals: Delivery, Targeting and Polymer Therapeutics

Bruno Sarmento, José das Neves (Ed.) John Wiley & Sons, Oxford, UK
Print ISBN: 9780470978320
Online ISBN: 9781119962977
http://onlinelibrary.wiley.com/book/10.1002/9781119962977

From Bruno Sarmento, Universty of Porto, brunocsarmento@hotmail.com

Micrpincapsularea pentru sistem alimentare

T. Florea, St. Dima, G.M.Costin (Ed.)Editura Academica, Galati (2009)
ISBN 978-973-8937-54-3,
http://bjdb.ebibliophil.ro/en/detalii/microincapsularea-pentru-sisteme-alimentare

From Stefan Dima, University of Galati, dimastefan@yahoo.com

JOB REQUESTS

PhD in Physico-chemistry & Pharmacotechny looking for a job

After a Master in formulation and industrial chemistry, I did a PhD diploma in physico-chemistry and pharmacotechny. This pluridisciplinar formation gave me competences in various fields from the molecular biology to the process development, particularly in the pharmaceutical field.
I would like to work as engineer in R&D laboratory in the field of formulation.

Contact
Audrey Minost audrey.minost@gmail.com

Looking for a postdoctoral position on drug delivery

BA degree in Pharmacy with MSc degree on Pharmaceutical Sciences and double PhD degree (France/Brazil) on Pharmaceutical Technology. Large experience on the development of drug delivery systems, such as cross-linked and spray-dried polymeric microparticles for the colon delivery and lipid-based systems for the oral administration of lipophilic drugs for neglected diseases, and on physicochemical and in vitro characterization involving rheology, IR, TEM, HPLC, TG/DSC and cell culture.
PhD student on Pharmaceutical Technology at Université Paris Sud XI and UFRN (France/Brazil) (2009 – 2012) and lecturer on Medicinal Chemistry, Pharmacy Practice, Pharmacology and Quality Control on the majors of Pharmacy, Nursing and Physical therapy at public and private universities in Brazil (2009 – 2011).
Job or postdoctoral position in the field of research and development of drug delivery systems with emphasis on in vitro and in vivo studies.

Contact :
Acarilia Eduardo da Silva, acarilisilva@gmail.com

Looking for job or PhD

Bachelor degree in Pharmacy. I'm 24, graduated in October 2012 with mark 106/110 at university of Pavia (Italy). Experimental thesis written during the Erasmus period in Gandra (PT), title: SLN loaded with calcitonin: production, lyophilization and characterization with FTIR. License to practice achieved in December 2012. Great organisational skills, adaptability to multicultural environments gained during Erasmus and many travel experience. Several years of team working experience as volunteer. Excellent spoken and written English. Experience in production and characterization of SLN nanoparticles. Looking for a job or an internship in a chemical or pharmaceutical company.
Reference Bruno Sarmento (Bruno.sarmento@ff.up.pt), Cristina Bonferoni (cbonferoni@unipv.it)

Contact
Giulia Pilla, gpilla88@gmail.com

Pharmacist with PhD in Formulation and Pharmaceutical technology

I'm pharmacist and I'm currently finishing a PhD in the field of microencapsulation using supercritical CO2 (my defence will be held on September 2013). I have experience in supercritical processes with strong technical competence in innovative formulations and drug delivery. Excellent communication skills, problem solving, project management capabilities, organization and flexibility are my best qualities. I'm looking for an opportunity as a formulation scientist in the pharmaceutical or cosmetic industry in France. For more information please feel free

contact
Leila Hassani, Leila.hassani@gmail.com

JOB OPPORTUNITIES

Doctoral fellowships available

12 Doctorate fellowships will be offered in 2013 by the EMJD NanoFar consortium "European Doctorate in Nanomedicine and Pharmaceutical Innovation".

NanoFar is an Erasmus Mundus Joint Doctorate programme newly selected by EACEA involving the Universities of Angers and Nantes (France), Liège and Louvain (Belgium), Nottingham (United Kingdom) and Santiago de Compostela (Spain). NanoFar aims to train the best students in the field of nanomedicine at a doctoral level.

All information is available on the NanoFar website and selection process is open until 31th January 2013

More information
http://www.erasmusmundus-nanofar.eu/

From Frank Boury, University of Angers, frank.boury@univ-angers.fr

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ARTICLE

KINETIC EVALUATION OF NANOPARTICLE BIOCOMPATIBILITY FOR PREDICTING IN VIVO BEHAVIOUR

Jasper Huang and F.X. Gu.

INTRODUCTION

A wide range of nanocarriers has been used in the development of clinically available pharmaceutical products. Their applicability to systemic admini-stration largely depends upon complex interactions with biomolecules and immune cells present in the bloodstream. Given the widely varying surface characteristics, such as charge, hydrophilicity, curvature and density, of these nanocarriers, advances in the development of nanocarriers require a more fundamental understanding of how these material properties influence nanoparticle behaviour in the body.

Achieving a long circulatory lifetime is typically an important and highly desirable objective in the design of nanoparticles for intravenous administration. Specifically, the adsorption of blood proteins to the surfaces of nanoparticles in circulation has been noted as a key process in the recognition and clearance of nanocarriers from the bloodstream. As such, we have investigated the biocompatibility of a range of nanoparticles through the use of in vitro techniques assessing the extent of their interactions with blood components. This approach is given further depth through the incorporation of kinetic elements to an adapted complement activation assay, providing insight into the propensity of nanoparticles to interact with a particular class of immune proteins called complement proteins and facilitating a more in-depth evaluation of nanoparticle biocompatibility.

MATERIALS & METHODS

Materials

PLA and poly(lactic-co-glycolic acid)-poly(ethylene glycol) (PLGA-PEG) were purchased from Lakeshore Biomaterials (Birmingham, AL, USA). LUDOX colloidal silica and zymosan were obtained from Sigma-Aldrich (St. Louis, MO, USA). VBS2+ was obtained from Boston BioProducts (Ashland, MA, USA). Whole sheep blood and rabbit polyclonal antibody to sheep red blood cell stroma was purchased through Cedarlane Laboratories (Burlington, ON, Canada). Pooled human complement serum was obtained from Innovative Research (Novi, MI, USA).

Winner of the Best Contributions
XX International Conference on Bioencapsulation
Orillia, Ontario, Canada – September 21–24, 2012

Hemolysis assay

Polymeric NPs were purified and resuspended in VBS2+ at 40 mg/mL. Sheep erythrocytes were prepared at a concentration of 1x108 cells/mL. Varying amounts of the concentrated NP suspension were added to 200 µL of suspended sheep erythrocytes in volumes of VBS2+ necessary to obtain a total volume of 1 mL and final NP concentrations ranging from 1 to 20 mg/mL. After 60 minutes of incubation at 37 °C, absorbance measurements were recorded at 415 nm to determine the extent of hemolysis relative to negative and positive controls.

CH50 complement consumption assay

The CH50 complement consumption assay was performed as described elsewhere (Vonarbourg 2006). Briefly, NP suspensions were added to human blood serum in VBS2+ in volumes corresponding to a range of NP surface areas obtained through calculations and incubated for 60 minutes at 37 °C. The addition of different amounts of NP-serum mixture to sensitized sheep erythrocytes resulted in varying amounts of cell lysis, quantifiable using a microplate reader at 415 nm. The CH50 value is obtained as the amount of NP-serum mixture required to cause the lysis of 50% of the sensitized sheep erythrocytes.

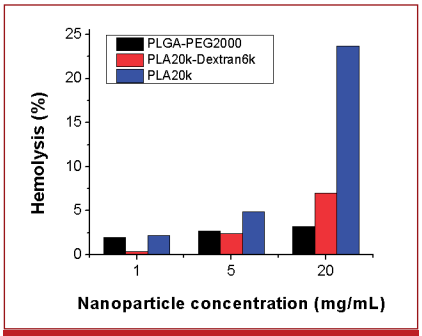

Figure 1 : Comparison of the extent of hemolysis (%) for three different types of NPs.

RESULTS & DISCUSSION

Hemolysis experiments were performed using NPs formulated from PLA-Dextran and PLGA-PEG block copolymers in addition to the uncoated PLA NPs. Fig. 1 shows the differences in hemolytic tendencies between NPs

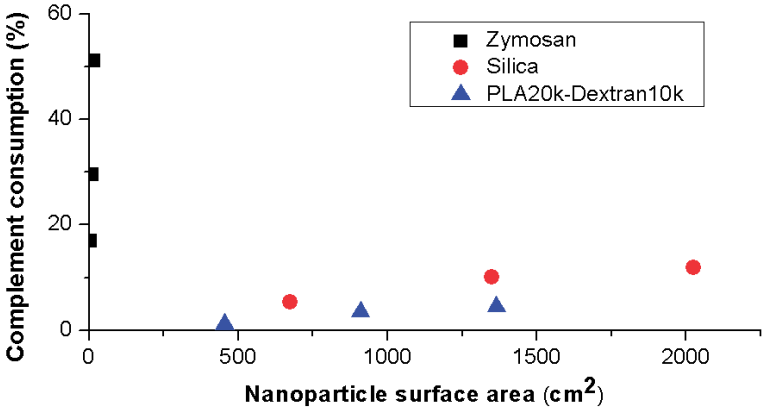

Figure 2 : Complement consumption of various materials.

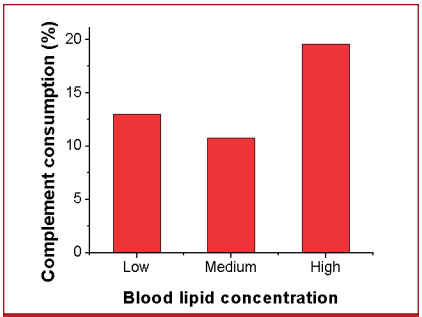

Figure 3 : Effect of blood lipid concentration on complement consumption by silica NPs.

formulated using the three different types of polymers. It is clear that the two block copolymer-based NP formulations outperform the uncoated PLA NPs, and the result supports the selection of hydrophilic polymeric blocks (PEG, dextran) to provide a more biocompatible surface.

The CH50 complement consumption assay was used to explore the biocompatibility of different NP formulations in greater depth. Zymosan microparticles, which are known to specifically activate complement, were used as a reference for measurements on the relatively hydrophobic silica NPs and hydrophilic PLA-dextran NPs. The results in Fig. 2 demonstrate the effectiveness of the assay in distinguishing between different types of materials based on the slopes of their consumption plots.

This methodology is also applicable for comparing samples with more subtle differences in composition or surface characteristics. Moreover, the technique can be utilized to explore more novel aspects of nanoparticle-blood interactions, such as the effect of blood lipid concentrations in human serum on NP complement consumption, as shown in Fig. 3.

Turbid pooled serum samples were centrifuged in order to remove excess lipids, resulting in clear samples with relatively low blood lipid concentrations (enzymatically determined lipid levels not shown). Initial indications are that lipid content in the blood may significantly influence complement consumption, and this result is noteworthy in light of recent reports that lipids comprise a major portion of adsorbed biomolecules on NP surfaces (Hellstrand, 2009).

Another key aspect of these interactions can be elucidated through kinetic assessment of complement activation. Silica particles were incubated with human serum for 30, 60, 90, and 120 minutes and evaluated using the CH50 complement assay, as in Fig. 4.

Based on this kinetic study, complement activation by silica NPs was seen to increase with moderate linearity over time. It is conceivable that less biocompatible nanoparticles with greater propensities to activate complement would more rapidly approach the 100% complement consumption limit, effectively saturating at some measurable time point. Future experiments will further explore this behaviour.

CONCLUSIONS

Increased interest in the development of nanoparticles for various applications, particularly in biomedical research, necessitates effective methods of assessing NP biocompatibility and predicting in vivo performance. We find that the CH50 complement consumption assay is highly applicable to studies varying from relatively straightforward comparisons between different NP formulations to more complex investigations of surface characteristics and factors such as blood lipid levels. Further work in this area will focus on developing a fundamental under-standing of how NP surface characteristics affect bio-compatibility, in addition to establishing correlation with in vivo studies.

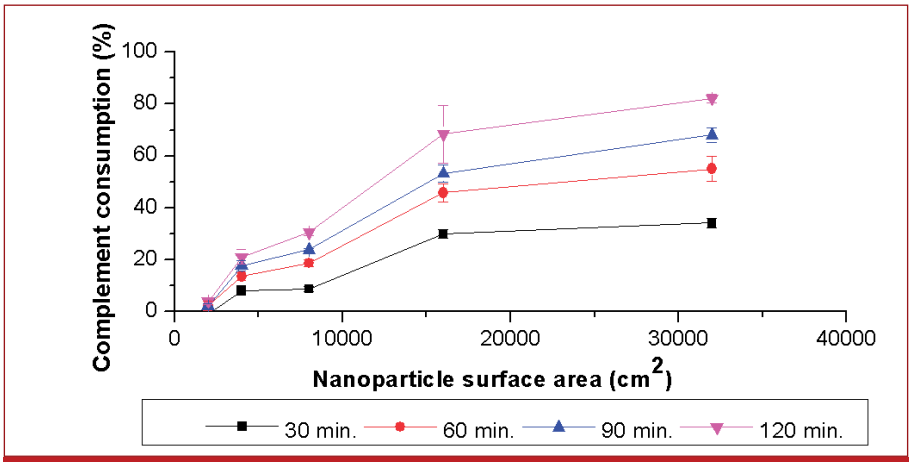

Figure 4 : Kinetic evaluation of complement consumption by silica from 30-120 min

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ARTICLE

SUPERCRITICAL CO2 AS HIGHLY EFFICIENT INNOVATIVE PROCESS FOR PROTEIN ENCAPSULATION

Leila Narimen Hassani, B. Calvignac, T. Beuvier, F. Hindre, A. Gibaud, F. Boury

INTRODUCTION

The challenge in protein drug delivery is the formation of microcarrier with well-defined characteristics: size, morphology, composition and density, these characteristics are important to achieve high bio-availability with a particular administration route. Traditional methods used to formulate microcarriers frequently cause protein aggregation and inactivation. In this point of view, supercritical fluid process presents an innovative route, which can evade most of the drawbacks of the traditional ones, especially Supercritical CO2 (SC-CO2).

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SC-CO2-based processes may be considered as environmentally friendly as CO2 is non-toxic, low-cost and may be recycled. Finally, its easy reachable critical point (T = 31.1 °C and P = 7.39 bar) makes SC-CO2 an especially adequate and mild process to manipulate sensitive compounds such as therapeutic proteins.

The aim of this work is to bring out a proof of the concept of bioencapsulation of therapeutic proteins using water in SC-CO2 emulsification within calcium carbonate CaCO3 particles as microcarriers. We tried to compare this process to protein-CaCO3 co-precipitation, in terms of protein loading, protein integrity and stability.

MATERIALS & METHODS

Calcium carbonate (CaCO3) microspheres containing lysozyme as model protein (1.0 g/L), were synthesized using an aqueous solution (25 mL) composed of calcium hydroxide (Ca(OH)2 108 mM), hyaluronic acid (0,1% m/v), glycine buffer at final pH of 10, which was emulsified in SC-CO2 at 40°C and 200 bar (Boury 2009).

Co-precipitation process was carried out by mixing the calcium solution (Ca(OH)2 216 mM, 12.5 mL) containing lysozyme (1.0 g/L) and carbonate containing solution (Na2CO3 216 mM, 12.5 mL).

X-Ray Diffraction analysis was obtained by X-pert diffractometer . The surface morphology and size of the microparticles were investigated by scanning electron microscopy (SEM) (JSM 6310F, JEOL). CLSM Confocal Laser Scanning microscopy (Olympus FV300) was used to evaluate the distribution of FITC-lysozyme in CaCO3 microspheres.

Lysozyme loading and encapsulation efficiency were obtained by measuring lysozyme activity using Micrococcus lysodeikticus bioassay. This assay is based on Micrococcus lysodeikticus membrane lysis under the action of active lysozyme, which results in a decreased suspension turbidity.

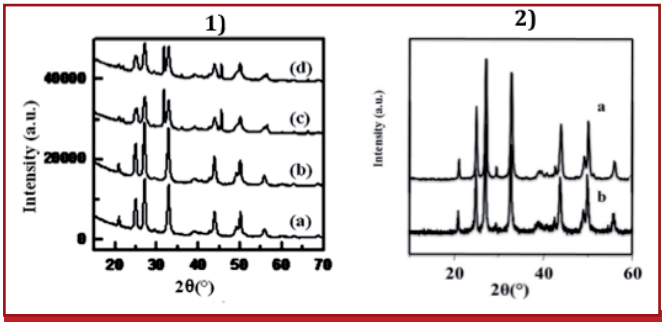

Figure I. XRD patterns of 1 a, b) unloaded and c, d) lysozyme loaded CaCO3 vaterite microspheres obtained by co-precipitation mode. 2 a) unloaded and b) lysozyme loaded CaCO3 vaterite microspheres obtained by SC-CO2.

RESULTS &DISCUSSION

The preparation and characterization of unloaded CaCO3 microparticles have been studied and reported in our previous work (Beuvier 2011).

The presence of hyaluronic acid (Dickinson and McGrath 2004) and glycine (Shivkumara 2006) may control the crystal growth of thermodynamically instable CaCO3 vaterite. Also CaCO3 crystallization is highly depending on the experimental parameters such as temperature, stirring, pressure and pH. All CaCO3 particles obtained by either supercritical process or co-precipitation; consist of almost pure vaterite as revealed by XRD analysis (Figure I). These results suggest that the presence of lysozyme and the used process have no effect on the polymorph of the obtained CaCO3.

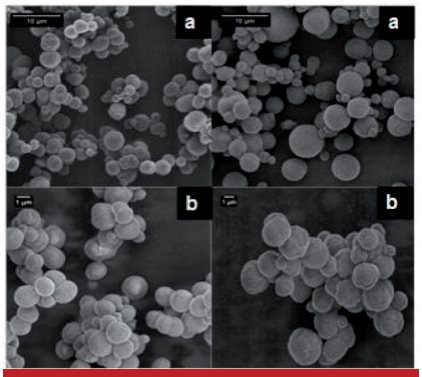

Figure 2. Morphologies of unloaded (1 a and 2 a) and lysozyme-loaded CaCO3 microspheres (1 b and 2 b) under SEM

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Morphologies of the CaCO3 particles prepared with two different methods are shown in SEM images (Figure II). Unloaded CaCO3 microspheres formulated by supercritical process (Fig. II-1-a and b) had spherical shapes with a size ranging from 1 to a maximum of 10 µm (average size of 4.9 ± 1.0 µm).

When lysozyme is encapsulated similar CaCO3 particles with spherical shapes 4.9 ± 0.3 µm in diameter, were obtained (Fig. II-1-b).

For those prepared by co-precipitation

process, the average size was 1.86 ± 0.4 µm (Fig. II-2-a and b).

Thus the presence of lysozyme has no effect on particles size; by contrast there is an important impact of the used process.

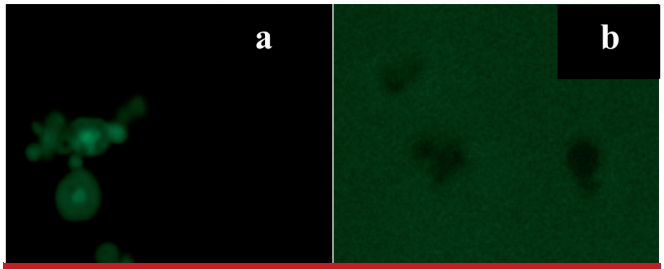

Figure 3. CLSM images of FITC-lysozyme loaded CaCO3 microspheres obtained by a) supercritical CO2 and b) chemical pathway

CLSM observations Fig.III-a show specific core shell-structure when supercritical CO2 is used. The interior circular cavity in each microsphere with a strong fluorescent signal corresponds to a high quantity of FITC-lysozyme in the core. In addition a homogeneous distribution of FITC-lysozyme was observed in the shell. For the microparticles obtained by chemical pathway Fig.III-b no fluorescence inside the microparticles was observed.

The highest encapsulation yield we could obtain was about 47.5% at the starting protein concentration of 1.0 g/L, which is much higher than results obtained by

interfacial reaction (Fujiwara 2008) or phase transition method (Fujiwara 2010). This result is expected to be enhanced by optimizing process parameters and experimental setup.

We successfully obtained CaCO3 microspheres in supercritical CO2 with high lysozyme encapsulation efficiency 47.5 ± 0.13 %, and lysozyme loading of 4.19 ± 1.13 % respectively (Table I). Lysozyme was scarcely encapsulated when co-precipitation process was used. We obtained a lysozyme loading of 0.12± 0.03 % and encapsulation efficiency of 2.26 ± 0.41 %. The first process allowed higher encapsulation efficiency and higher conservation of lysozyme activity.

CONCLUSION

In the present research, we compared two solvent free processes to obtain calcium carbonate microparticles encapsulating proteins. We showed that SC-CO2 plays an important role in encapsulating higher amount of protein. In addition SC-CO2 process offers optimal conditions (Winters 1996) in terms of protein stability compared to the adsorption loading mode described by (Ueno 2005) or co-precipitation.

Therefore, further efforts will be needed for process optimization, particle characterization, and encapsulation of therapeutic protein, in-vitro release, and particle surface modification as the necessary steps for the development of the final product. Results obtained from this work may be useful as well for other applications where protein encapsulation is required.

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Table I. Lysozyme encapsulation using supercritical process and CaCO3 co-precipitation

Encapsulation mode	SC-CO2	Co-precipitation
Lysozyme concentration (g/L)	1.0	1.0
Active lysozyme loading (%)	4.19 ± 1.13	0.12 ± 0.03
Encapsulation yield (%)	47.5 ± 0.13	2.26 ± 0.41
Average size (µm)	4.9 ± 0.30	1.86 ± 0.04

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Leila N. HASSANI is a pharmacist; she completed her masters degree in formulation and pharmacotechny from Châtenay Malabry Faculty of Pharmacy, Paris XI (Pr. E. Fattal). During her first research training, she worked on designing an innovative drug carrier based on chemically modified chitosane (Dr. K. Bouchemal). Currently she is working on a PhD program under the supervision of Pr. Frank Boury and Pr. Alain Gibaud, respectively, on the topic of growth factors encapsulation for tissue engineering. Her research focuses on protein encapsulation using supercritical carbon dioxide process into calcium carbonate microparticles. She possesses skills on both drug delivery and drug system design.

ARTICLE

CHARACTERIZATION OF ADHESION BETWEEN CHEMICAL ROBOTS AND BIOLOGICAL SUBSTRATES

Viola Tokarova, M. Ullrich, P.Haufova, A.Pittermannova, F. Stepanek

INTRODUCTION

Composite microparticles for targeted delivery of active substances are of both practical and fundamental interest. The purpose of such delivery vehicles is to store the active compound at a high concentration, transport it through an environment and eventually anchor to the target site and release the encapsulated payload either spontaneously or upon an external stimulus. For the design a targeted delivery system, not only the release kinetics of the encapsulated compound but also the specific interactions of the microcapsules with the target point of controlled release – their adhesion properties – are crucial.

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This work describes the synthesis, composition and characterization of two types of such composite particles – chemical robots – consisting of a hydrogel core and a mesoporous silica shell. The first particle type is prepared by the InkJet technology (Dohnal 2010) and consists of alginate hydrogel with immobilised phospholipid vesicles (liposomes) inside the gel. A silica coating of the alginate body is prepared by the sol-gel or layer-by-layer deposition processes (Haufova 2011). The second particle type consists of the thermoresponsive hydrogel PNIPAM and a silica nanoparticle shell prepared by the Pickering emulsion polymerization (Tokarova 2012).

The adhesion properties of the composite particles were investigated using a microfluidic flow cell which simulates flow conditions in the human body. Silica nanospheres which formed the outer shell of the chemical robots were used for specific adhesion based on antigen-antibody interactions. The surface of SiO2 is modified with antibody fragment (M75). Antibody used in this work specifically bind tumour-associated antigen (CAIX) which is a trans-membrane protein and gives us the possibility for target binding (Fig.1). The specific antigen-antibody interaction will be proved and adhesion properties will be compared to unspecific ones in the present work. The adhesion properties inside the microfluidic cell and the effect of volumetric flow rate on the overall particle adhesion will be also presented.

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MATERIALS & METHODS

Alginate/liposome/silica particles

The first step of particle preparation was the synthesis of liposomes by the Bangham method. Liposomes act as internal compartments for the storage or active components of their precursors and their role in the chemical robots is similar to that of vacuoles in a living cell. Liposomes can be selectively opened by the application of radiofrequency heating. Then, sodium alginate solution (usually 2% w/w) was mixed with a suspension of liposomes purified by gel chromatography and stirred for 15 minutes. The obtained mixture was then dripped into a solution of 2% (w/w) calcium chloride, where the alginate chains were cross linked due to divalent calcium ions and formed a gel (Dohnal 2010).

The core-shell capsules were synthesized as follows. First, the alginate gel cores (with or without liposomes) in the size range of 40 to 90 μm were prepared via the drop-on-demand InkJet technique. A silica shell has then been formed by a sol-gel process using alkoxysilane precursors (TMOS and APTrMOS). Since APTrMOS has positively charged groups, it can interact with the anionic alginate polymer through electrostatic force. Consequently the hydrolysis and polycondensation of methoxy functions of both precursors occurs and silica precipitates around the alginate core (Haufova 2011).

PNIPAM/SPION/silica particles

PNIPAM/SPION/SiO2 composite microcapsules were prepared by the Pickering emulsion polymerization. Dried SiO2 nanospheres prepared according to the Stober method (Stober 1968) was disperesed in toluene phase. The water phase consists of monomer NIPAM, crosslinker BIS, initiator of the polymerization reaction APS and superparamagnetic iron oxide nanoparticles (SPION). The o/w mixture was then heated to 70°C in order to initiate the polymerization reaction (Cejkova 2010).

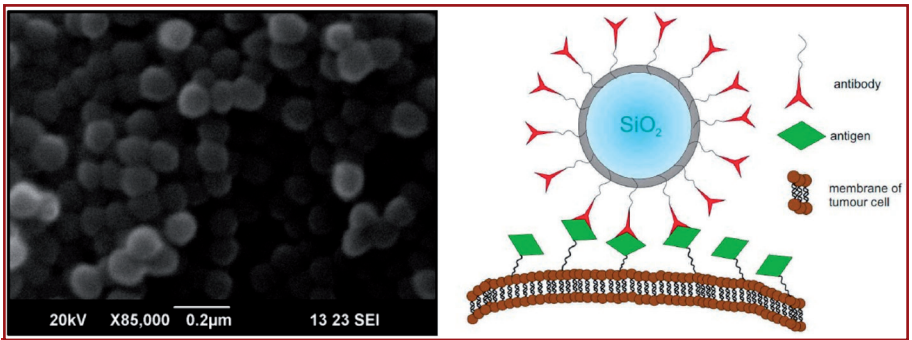

Figure 1: SiO2 nanoparticles SEM image and schematic picture of their specific adhesion

ADHESION STUDIES

The adhesion properties of the particles were studied in a custom-designed adhesion cell where it is possible to measure the ratio of adhered capsules with controlled volumetric flow rate, and which enables rapid exchange of the tested substrates (Tokarova 2012).

RESULTS & DISCUSSION

Adhesion experiments were carried out with the composite PNIPAM/SPION/SiO2 microcapsules using a range of substrate materials. Their selection was based so as to simulate various surfaces of both natural (cholesterol, palm oil, gelatin) and man-made (teflon, paraffin, polystyrene, rubber, glass) origin that could potentially be encountered during targeted delivery applications.

The fraction of adhered microcapsules decreased with increasing flow rate for all the investigated model surfaces. However, it can be seen that both the strength of the decrease and the actual fraction of adhered microcapsules for a given flow rate varied widely among the investigated materials.

Specific adhesion of antibody modified silica nanospheres (SiO2-IgG_M75) with tumour associated antigen CAIX were proved by the ELISA test (Fig. 2). The fluorescent responses of 3 types of particle in 4 different dilutions were compared. The difference between specific (SiO2-IgG_M75) interaction and unspecific (SiO2-IgG_irrelevant) or unmodified (SiO2) silica spheres is considerable. The test was repeated in time sequence and no antibody deactivation was observed.

CONCLUSION

This work described synthesis of two types of so called chemical robots. These structured microparticles are used as carriers of active substances for controlled delivery. The adhesion character of composite PNIPAM/SPION/silica particles was tested inside the microfluidic cell. The phase transition of the underlying PNIPAM, which is hydrophilic below its LCST and hydrophobic above its LCST, does not affect the adhesion force of the structured microcapsules. The surface character formed by the SiO2 nanoparticles directed the adhesion of microparticles which provides opportunities for their functionalization to tailor the adhesion towards specific substrates. Antibody modified silica was proved to specifically bind with antigen and is a suitable way to target delivery. Our future work will focus on adhesion experiments with biological substances like human cells and tissues to emphasize pharmaceutical application of chemical robots.

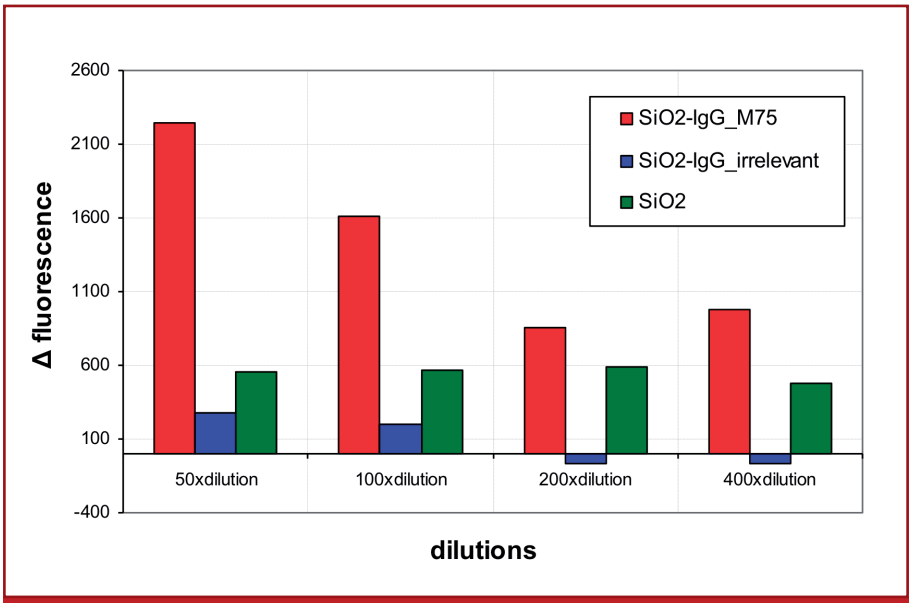

Figure 2: ELISA test of specific antibody-antigen interactions.

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Viola Tokarova completed her bachelor degree in physics and measurement (2008) and master degree on chemical engineering (2010) at ICT Prague. Currently she is working on her PhD thesis under supervision of Frantisek Stepanek. The research topic – Study of interaction of composite particles with complex substrates - is being carried out in the Chemical robotics laboratory at the Department of chemical engineering at ICT Prague. HerResearch interests are antibacterial silver nanoparticles, synthesis of composite hydrogel microparticles, adhesion studies of composite microparticles and specific adhesion of drug delivery systems

ARTICLE

ENCAPSULATION OF INSECT REPELLENT FOR VETERINARY APPLICATION

Audrey Minost, H. Fessi, J. Delaveau, M.A. Bolzinger, A. Elaissari

INTRODUCTION

Arthropod repellent formulations are needed for protection of Human as well as for animals to avoid the contact with pests and thus the carried diseases sometimes fatal. Canine species are targets for insect's blood meal that can lead to diseases such as Leishmaniasis transmitted by the bite of phlebotomine sand flies, making the veterinary industry a needy market of insect repellent products. The drawback of commercial products is their short time protection (few hours)(Debboun, 2007).

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The aim of this work is to develop a well-adapted formulation based on nanotechnology to extend the action duration. This study serves as proof of principle for the development of polymer-based carrier systems with inherent release regulator characteristics for insect repellent molecules.

Nanoparticle suspensions, widely studied for their ability to control the release of the active molecule, were the key element in our research works, with a particular attention on the ability to develop the fabrication process at industrial scale.

The surface interactions between nanoparticles and canine hair and skin were studied. Finally, we have proved the interest of nanoparticles on the long lasting pet protection against pests.

MATERIALS & METHODS

The nanoparticle suspensions containing 10% of active ingredient were prepared by the nanoprecipitation process (Fessi, 1989). The formulation was surfactant free; the process does require neither a solvent evaporation step nor a concentration step. Briefly, the nanoparticle suspensions were obtained by pouring an organic phase containing ethanol, active ingredient and Eudragi®RS100 as polymer matrix in deionized water. Picaridin®, an active ingredient developed by Bayer was formulated.

The particle size distribution and the zeta potential were obtained using a Zetasizer NanoZS (Malvern).

The surface interactions between nanoparticles and dog hair were investigated by electrokinetic study (Zetameter, Anton Paar) directly correlated to the zeta potential according to the Smoluchowski's equation (Hunter 1989).

Nanoparticle absorption on dog skin was characterized by static diffusion cells (Franz cells, Figure 1).

The repellence activity was evaluated using bioassays on fasted Drosophila. melanogaster as model insect (Ditzen, 2008).

Filter papers introduced in the truncated eppendorf tubes were treated with the repellent formulation on one side and a solution active molecule free on the other side. The repellency index was calculated using the following equation

$$RI=(N_1-N_2)/(N_1+N_2)$$

N1 and N2 are the number of drosophila in the side without repellent and with the formulation respectively. The repellence activity was demonstrated by a positive RI. Perfect repellent activity was reported by RI=1. One experimental result is the mean of at least 6 experimental boxes presenting non-significant variations.

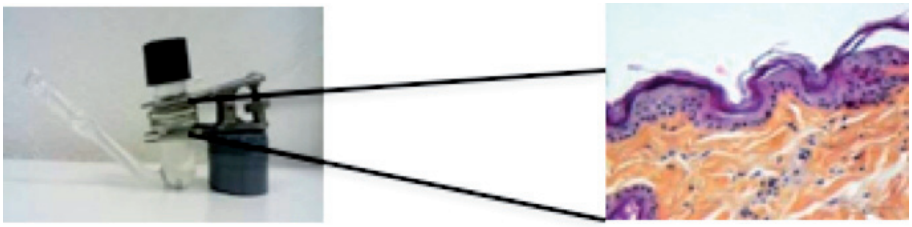

Figure 1: Franz cell containing dog skin and histological image of skin sample. SC: Stratum Corneum; VE: Vialble Epidermis; D: Dermis.

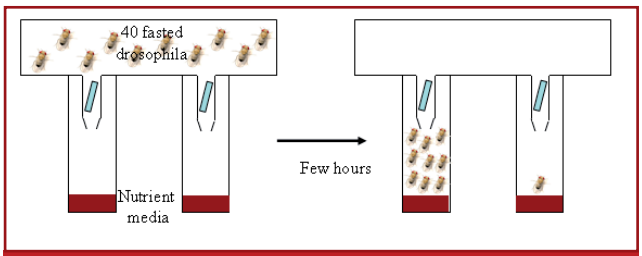

Figure 2: In vivo bioassay on Drosophila melanogaster. Experimental boxes at T0 and T+24hours with the left side treated with repellent formulation and right side treated with solvent.

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RESULTS & DISCUSSION

Nanoparticle formulation parameters were optimized to obtain uniform nanoparticle suspensions containing 10% of active molecule in the 150-300 nm range depending on the ratio active ingredient : polymer. The use of Eudragit®RS100 induced a positive zeta potential of +45mV ± 5mV at pH=6.5.

Interaction phenomena were reported by the electrokinetic study, as shown in Figure 3.

The analysis by the streaming poten-

tial method reveals the changes of the hair surface after hair treatment process with cationic nanoparticles. Nanoparticle adsorption is a fast phenomenon. The polycationic nature of Eudragit®RS100 led to the recovery of hair surface (and into the scales). The strong attach of nanoparticles guaranties the long term presence of nanoparticles containing the insect repellent molecules. Desorption study demonstrated the irreversible adsorption. This can be due to the possible high electrostatic interactions.

The distribution of the active ingredient on and in the dog skin was examined by static diffusion cell disposals comparing the solution and the suspension form.

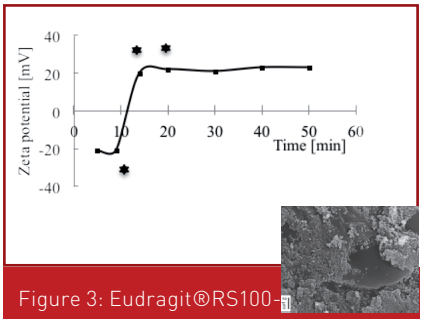

Figure 3: Eudragit®RS100-nanoparticle absorption. SEM micrograph of hair after nanoparticle adsorption.

The skin appendages act as nanoparticle reservoir. Contrariwise the formulation without polymer remains at the skin surface, thus the elimination by simple friction led to an insect repellent elimination.

The repellency indexes obtained on drosophila as a function of time for the Picaridin®-loaded nanoparticles are reported on Figure 5. Repellence was improved with nanoparticles, providing a long lasting effect. It is important to notice that these results were obtained with a fixed applied dose of 13µL/cm2, limited by the used equipment.

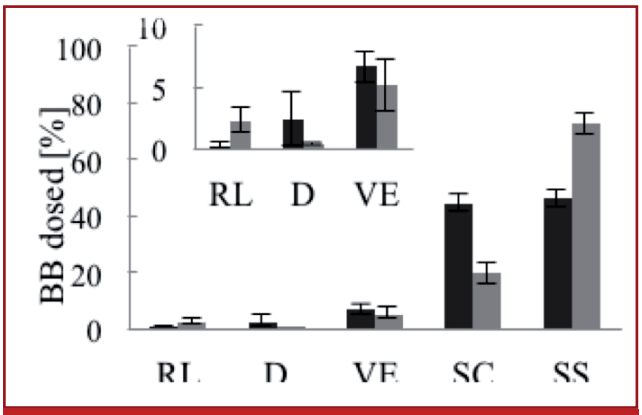

Figure 4: Distribution of insect repellent molecule in the skin layers from a solution (grey boxes) and from a suspension (black boxes). RL: Receptor Liquid; D: Dermis; VE: Viable Epidermis; SC: Stratum Corneum; SS: Skin Surface.

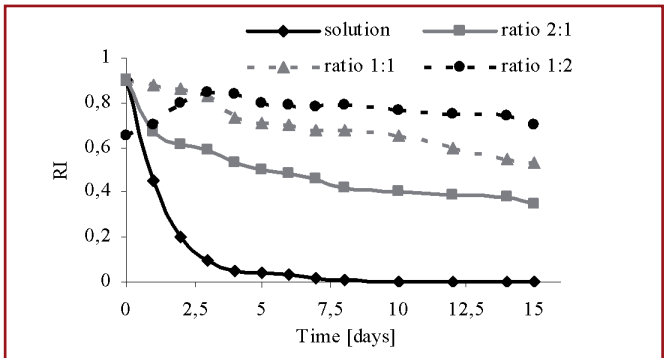

Figure 5: Variation of insect repellency (IR) as a function of time for 4 formulations: the solution as control and 3 nanoparticle formulations at different Picaridin®:Eudragit®RS100 ratio.

CONCLUSIONS

Surfactant free formulations of cationic nanoparticle suspensions were obtained by nanoprécipitation process. These suspensions present high active molecule content.

Nanoparticles spread on dog hair fur, a part of them penetrate in skin appendages and in the stratum corneum. Finally the repellence bioassay has proved the efficiency of nanoparticles in regulating the release of active and consequently the long lasting repellence activity.

It should be noted that the fabrication process of these innovative repellent products is simple, low costs, reproducible and does not require additional investment for a pharmaceutical (or veterinary) industry.

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Audrey MINOST is a Ph.D. graduate in Physico-Chemistry and Pharmacotechny. During her research trainings for the Master graduation, she elaborated a synthesis pathway of antiviral macromolecule for the treatment of Hepatitis C (Training of Master 1 Chemistry at Genosciences, Dr. J. Courcambeck) and she carried out a project on the formulation of nanoemulsions as vaccine adjuvant using specific processes (training of Master 2 Formulation and industrial Chemistry at Merial, Pr. N. Sintes, A. Parisot). She performed her thesis work at the University of Lyon at LAGEP laboratory (Pr. H. Fessi) with the collaboration of Merial (J. Delaveau). During this researcher position, she worked on designing innovative formulations based on the nanoparticles technology, on the topic of insect repellent encapsulation for a long lasting effect (active molecule protection, specific targeting and drug release regulation). She was rewarded during the conference Bioencapsulation 2012 (Orillia, Canada) for the presentation of her research work. Currently she is working on a post doctoral program under the supervision of Pr. H. Fessi. Her researches are focuses on the development of specific drugs for pediatric population. She possesses skills on project management, teamwork and autonomous work.

ARTICLE

SYNTHESIS OF NOVEL LOW MOLECULAR WEIGHT ALGINATE-POLY(METHYL METHACRYLATE) HYBRID MATERIAL USING LIVING RADICAL POLYMERIZATION FOR CONTROLLED DRUG DELIVERY

Vitaliy Kapishon, M. Cunningham, R. Whitney and R. Neufeld

INTRODUCTION

Alginate is a biocompatible anionic biopolymer, capable of crosslinking with divalent cations (Ca2+) and entrapping water thus forming a hydrogel. It has been employed in encapsulation and delivery of peptide based drugs, such as insulin, which are susceptible to gastric pH and enzymatic digestion. Native alginate hydrogel has high water solubility and permeability, causing excessive swelling and premature drug release.

In this study we chemically modify native alginate in order to improve the stability and release profile of the drug carriers. Our step-wise modification process is designed to produce small amphiphilic polymers capable of micellular self-assembly. The steps involved in the synthesis are alginate partial degradation (reduction in molecular weight), solubility modification, attachment of polymerization initiating sites and, finally, grafting synthetic polymers from alginate chains using living radical polymerization (LRP).

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MATERIALS & METHODS

Alginate degradation

1.5% solution of sodium alginate was treated with H2O2 at 80C for up to 3 hours. Molecular weight was determined by a Malvern ZetaSizer. Presence of LMWA was confirmed by H-NMR (Bruker 400Hz).

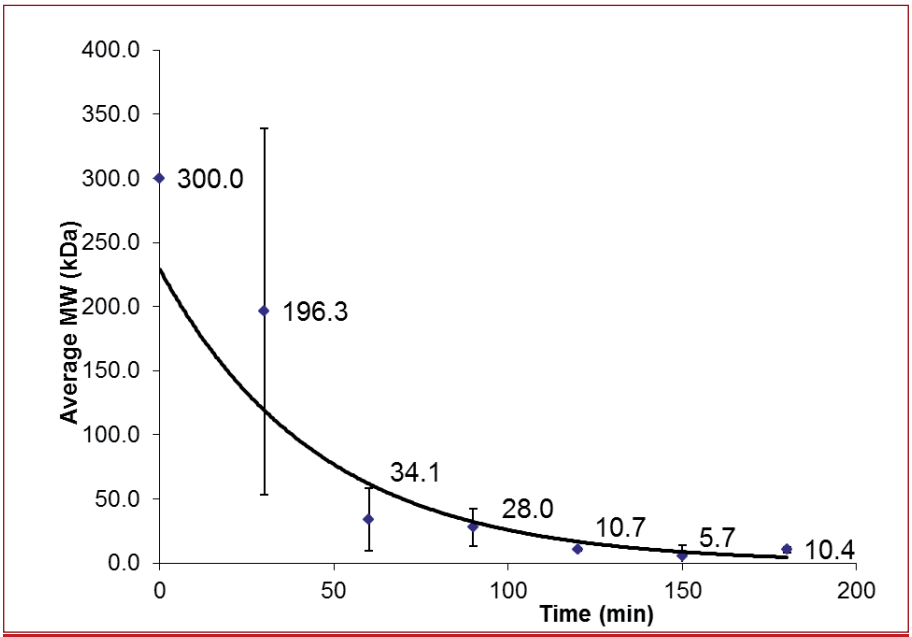

Figure 1. Partial degradation of sodium alginate

Solubility modification in organic solvents

Degraded alginate was acidified and neutralized with tetrabutylammonium hydroxide (TBAOH). Solution was lyophilized and LMWA-TBA was confirmed by H-NMR.

SYNTHESIS OF ALGINATE MACROINITIATOR

Bromoisobutyric acid was activated with various coupling agents and then added to alginate solution in DMF for a 12 hour esterification reaction. Structure of LMWA-Br was confirmed by H-NMR.

Living Radical Polymerization of methyl methacrylate (MMA) from alginate macroinitiator

Polymerizations were conducted in water or D2O. Monomer (MMA) amounts were varied. Copper wire was used as a LRP catalyst. Alginate-pMMA was confirmed by H-NMR.

RESULTS & DISCUSSION

Figure 1 shows an example of a kinetic degradation experiment. Over time, MW reduction of alginate from 300 kDa down to 10 kDa in the presence of H2O2 was confirmed by light scattering. Other degradation experiments involving higher oxidizer concentration and longer reaction time produced MW as low as 5 kDa.

Chemical modifications with alginate are limited due to its poor solubility in organic solvent systems. In our case, attachment of an initiating group onto alginate is a water sensitive reaction and had to be done in an organic polar aprotic solvent such as DMSO, DMF or THF. Therefore, tuning alginate's solu-

ARTICLE

bility for such reaction was a key step. Alginate-TBA salt was now soluble in DMSO and DMF.

Successful attachment of an initiating group onto alginate was confirmed by the H-NMR of the purified product from the esterification reaction (Figure 2A).

Grafting of methyl methacrylate from modified alginate was confirmed by H-NMR and increased viscosity over time (Figure 2 B and C). Having showed successful grafting technique from alginate we are now engineering a wide range of grafted alginates that will differ in alginate MW, and degree of substitution that will dictate the number of initiating sites per alginate molecule, and finally the length of the synthetic grafts. We are predicting that these grafted alginates will express different physiochemical properties and drug loading capabilities. Alginate molecules containing only few hydrophobic grafts might be able to self-assemble and entrap the drug within their hydrophobic nuclei; while more substituted alginates will be able to load the drug via Ca2+ cross-linking.

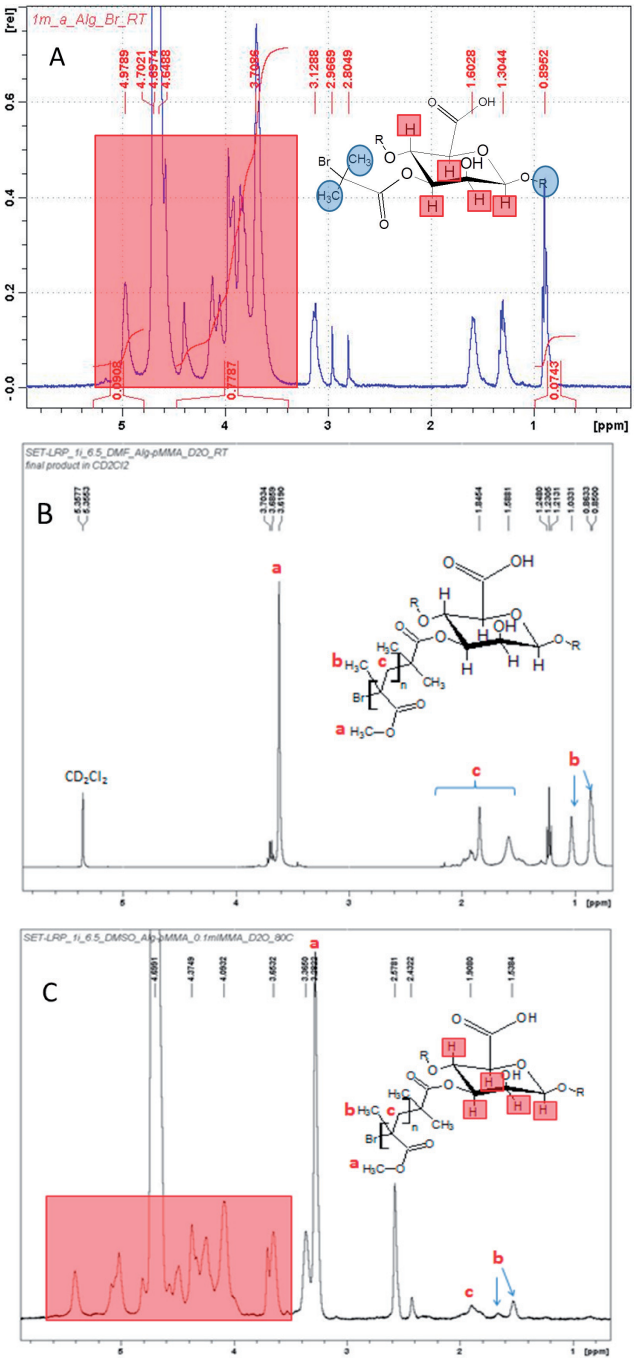

Figure 2. H-NMR spectrum of LMWA-Br macroinitiator (A) and LMWA-pMMA products with monomer to initiator ratio: 200:1(B) and 20:1(C)

CONCLUSION

A new type of alginate based biosynthetic material was prepared by first reducing the molecular weight of native alginate, functionalizing alginate backbone for living radical polymerization and finally grafting synthetic polymer to produce low molecular weight hybrid molecules with an increased hydrophobic character with a potential for improved controlled drug delivery applications.

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ARTICLE

PLURIPOTENT STEM CELL RECOVERY FROM POLYMER MICROCARRIERS PRODUCED BY MEMBRANE EMULSIFICATION.

Mariana-Petronela Vamanu and R.G. Holdich

INTRODUCTION

Embryonic stem cells (hESCs) hold a great potential for therapeutic applications. However, their use is hindered by the need for a large-scale culture system, as laboratory scale systems are inefficient, insufficient and labour intensive (Xu C., 2001). Such a system has to be capable of mass producing cells and preserving their capacities.

Our solution is developing a system in which cells are produced in a three dimensional culture by using microcarriers. Commercially available microcarriers are good at encouraging cell attachment and proliferation, but cell detachment is problematic as it requires the use of proteolytic enzymes which are damaging to critical cell adhesion proteins (Baumann H., 1979).

Loughborough University

The current innovative study is to engineer the microcarriers in terms of particle size, surface coating and properties, as well as triggered shrinkage or thermo-responsiveness for cell release. All these benefits are based on particle production by Membrane Emulsification (Holdich R.G., 2010). Membrane Emulsification is a relatively novel technique that has been first introduced in 1986 by Nakashima and Shimizu for the production of emulsions by employing a glass membrane (Nakashima T., 1986). Membrane emulsification has many advantages over conventional emulsification methods as it offers the possibility of producing very fine emulsions of controlled droplet sizes and with a narrow size distribution; allows the use of shear-sensitive compounds, such as proteins and has a simple design (Holdich R.G., 2010).

The polymers of choice are alginate and chitosan because of their biocompatibility and biodegradability and poly N-isopropylacrylamide (pNIPAM) because of the sharpness of its phase transition, biocompatibility and transition temperature at about 32°C, close to the physiological value. This paper will only focus on the work carried out with pNIPAM.

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pNIPAM structure consists of repetitive units of hydrophilic (amide) and hydrophobic (isopropyl) groups. pNIPAM in water demonstrated remarkable hydration-dehydration changes in response to changes in temperature. These characteristics make pNIPAM a very attractive material for Tissue Engineering applications. Cells are cultured on the hydrophobic surface at 37°C, above transition temperature and can be readily detached from the surface by lowering the temperature.

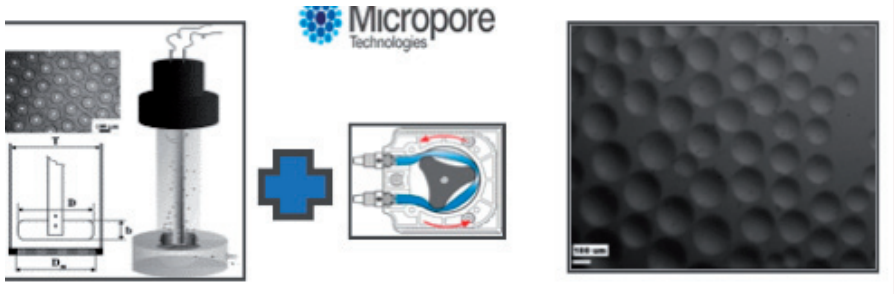


Figure 1: Set-up for pNIPAM droplet production by membrane emulsification

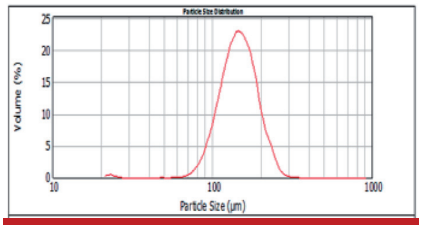


Figure 2: Size distribution for produced pNIPAM particles

The hydration and expansion of the polymer chains on the surface as a function of the temperature reduction are responsible for the cell detachment without the need for proteolytic enzymes (Canavan H.E., 2005).

MATERIALS & METHODS

All chemicals used for this work were acquired from Sigma Aldrich, UK unless otherwise stated. Particle production was performed in two stages: first, droplet production by Membrane Emulsification using the Dispersion Cell presented in Figure 1 and second, polymer cross-linking by free radical polymerization. The membranes used for this work are Nickel membranes with uniform pore sizes and inter-pore distances, also provided by MicroPore Technologies, UK.

The transition temperature of pNIPAM was measured as a turbidity variation with temperature increase at 500 nm by using a microplate reader (BMG Labtech).

The hESC line, H9 (WiCell Research Institute, USA) was cultured in feeder-free conditions on hESC-qualified MatrigelTM (BD Biosciences, UK) in fully defined medium, mTeSR1 (StemCell Technologies, France). The cells were dissociated using Accutase (Invitrogen, UK) and seeded onto the produced carriers and on a commercially available thermo-responsive surface provided by NUNC UpCell (Germany).

Prior to 3D culture, the hESCs were assessed for pluripotency by employing Flow Cytometry and Immuno-CytoChemistry (ICC). For ICC, the cells

were cultured on glass coverslips until 70% confluent, after which they were fixed, permeabilized and stained with DAPI (Invitrogen, UK), phalloidin (Sigma Aldrich, UK) and pluripotency markers (SSEA-1, SSEA-4, OCT 4) (R&D Systems, UK).

The 3T3 fibroblasts (ATCC, USA) were cultured using a high glucose DMEM supplemented with 10% foetal calf serum. Fibroblast dissociation was done by using TryPLE Select (Invitrogen, UK). Single cells were seeded on produced microcarriers and on the NUNC UpCell (Germany).

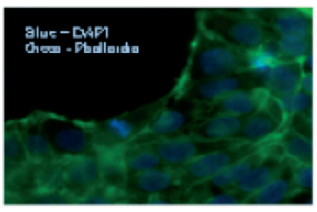
Cell viability and cytotoxicity was assessed by using Alamar Blue assay (Invitrogen, UK) and cell counts were done by Trypan Blue exclusion method using an automated system.-

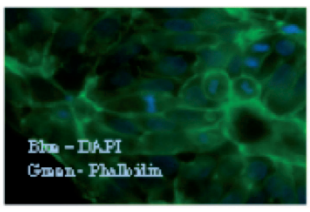
RESULTS & DISCUSSION

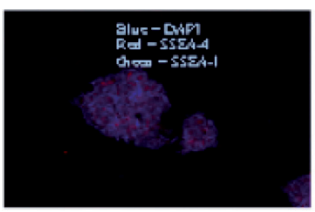
The Dispersion Cell platform technique allows a precise control over process parameters. By varying the shear stress provided by employing a mechanical stirrer and the injection rate of the dispersed phase, the particle size can be modified in a controlled manner.

The size and size distribution of the produced particles was measured by Malvern Instruments Mastersizer. We have produced pNIPAM particles with sizes between 100 and 200 μm with narrow size distribution (Figure 2).

Following characterization, the produced particles were sterilized by autoclaving and used with hESCs, H9 cell line and 3T3s fibroblasts. Prior to microcarrier culture, the H9 cells were assessed for pluripotency by Flow Cytometry and ICC (Figure 3).







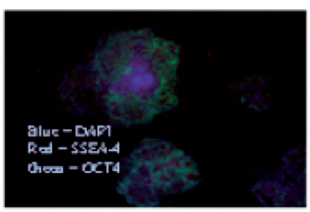


Figure 3: Immunocytochemistry images for H9 cells cultured on tissue culture plastic

Both cell types were also cultured on the UpCell surface for 48 h prior to the detachment study consisting of lowering the temperature from 37ºC to room temperature. Phase contrast pictures were taken at different time intervals and necessary time for complete cell detachment was recorded (Figure 4).

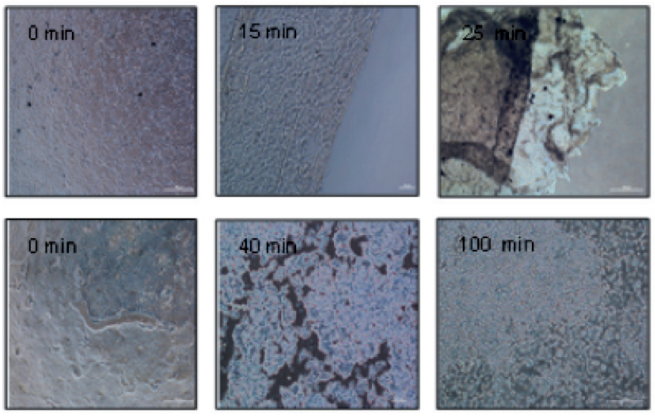


Figure 4: Cell detachment study performed on the NUNC UpCell (Top: 3T3 cells, Bottom: H9 cells).

CONCLUSIONS

We have successfully produced pNIPAM microcarriers with sizes within 100-200 μm with narrow size distribution by using the Dispersion Cell as a platform technology. The produced microcarriers were characterized and further used for 3T3 and hESCs expansion. The cell release properties of the prepared beads were compared to a commercially available product (NUNC UpCell).

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I have completed my bachelor degree in Biochemical Engineering at Babes-Bolyai University, Cluj-Napoca, Romania (2007). After that, I have worked as a Chemical Engineer for about 18 months, after which in 2009, I was awarded a full scholarship for starting a PhD degree in the Doctoral Training Centre in Regenerative Medicine scheme sponsored by the EPSRC group in the United Kingdom. Currently, I am in the final year of my PhD degree focusing on 'in-house' production of temperature responsive microcarriers by using the technique of membrane emulsification, for stem cell culture and release without the use of proteolytic enzymes. I possess skills in the field of chemical engineering, stem cell culture and characterization techniques, as well as experience in basic polymer chemistry and production of hydrogels.

ARTICLE

PATHOGEN-ASSOCIATED MOLECULAR PATTERNS AND IMMUNE RESPONSES AGAINST ALGINATE MICROCAPSULES

Genaro A. Paredes-Juarez G. A., B.J. de Haan and P. de Vos

INTRODUCTION

The World Health Organization (WHO) defines type 1 diabetes as an autoimmune disease characterized by the absence or low production of insulin by the pancreas, or the inability of the body to use it properly. For their daily demand patients have to take insulin injections. Long-term use of insulin is associated with complications and frequent episodes of hypoglycemia (Kendal Jr. et al., 2004).

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Transplantation of insulin producing pancreatic islets allows for a minute-to-minute regulation of the glucose levels and is not associated with complications (de Vos et al., 2010). Unfortunately, transplantation requires the application of immunosuppression which is at present not acceptable as alternative for insulin therapy. As a consequence islet-transplantation is not frequently applied. Encapsulation of islets may solve this issue (de Vos et al., 2007).

Encapsulation of pancreatic islets is a technique employed to protect the cells from the immune response of the host. The capsules can resist mechanical stress and allows appropriate diffusion of nutrients into the cells and release of metabolic products of interest (Antosiak IwaÐska et al., 2009).

One of the most widely materials used for encapsulation of islets is alginate. Alginate 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). The proportion and configuration of blocks and the binding with multi-valent cations (i.e. Ca2+ and Ba2+) when gelation occurs, give the alginate specific physical and chemical properties (Zimmermann et al., 2005). Alginates with varying MM-blocks, GG-blocks and MG-blocks have been applied. Reported success rates vary considerable (de Vos et al., 2010). Factors such as the presence of contaminations in the alginate have been hold responsible for these variations in success rates. The mechanisms of these responses have been poorly characterized but are essential if we wish to reproducibly make alginates with a predictable biocompatibility.

Immune responses are elicited after binding of undesired molecules to specialized receptors. These receptors are called pattern recognition receptors (PRRs). The PRRs bind the so-called pathogen-associated molecular patterns (PAMPs). PAMPs can be found on pathogens but it has been shown that more substances can contains molecules that bind to PAMPs. Toll-like receptors (TLRs) are the most commonly known PRRs. We hypothesize that alginates may contain PAMPs and elicit inflammatory responses via PRRs.

The present research is intended to study the role of PAMPs as triggering factors of the immune response against encapsulated islets in the first weeks after implantation.

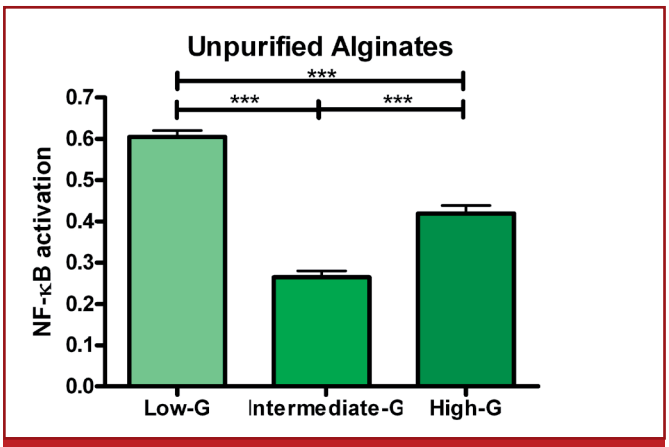

Figure 1 : Effects of alginate composition in activation of NF-kB. Values are presented as mean±SEM (n=20); p<0.001 (***)

MATERIALS & METHODS

Cell stimulation

Two cell-lines were stimulated using alginates containing different amounts of guluronic acid (G) chains (Low G alginate (Mannucol DM), intermediate G (Keltone LV), and high G (Manugel DJB) sodium alginates) in their unpurified and purified form to screen for PAMPs effects of the alginate. All alginate types were used at 0.3% (w/v).

university of groningen

Cell-lines from InvivoGen were used, such as Thp1-XBlueTM-MD2-CD14 (Thp1 MyD88 (+)), a human cell-line carrying all TLR's with a reporter plasmid under control of the NF-ÐB, expressing a secreted embryonic alkaline phosphatase (SEAP) gene that can be measured.

The second cell line is the Thp1-XBlueTM-defMyD (Thp1 MyD88 (-)) which has the same construction of Thp1-XBlueTM-MD2-CD14 but is deficient in MyD88 activity and it can be used to prove the activation via TLRs.

Alginate purification

For the purification of the alginates, the method by de Vos et al. (1997) was chosen, since it can reduce significantly the polyphenol in the alginate and maintain the levels of endotoxins

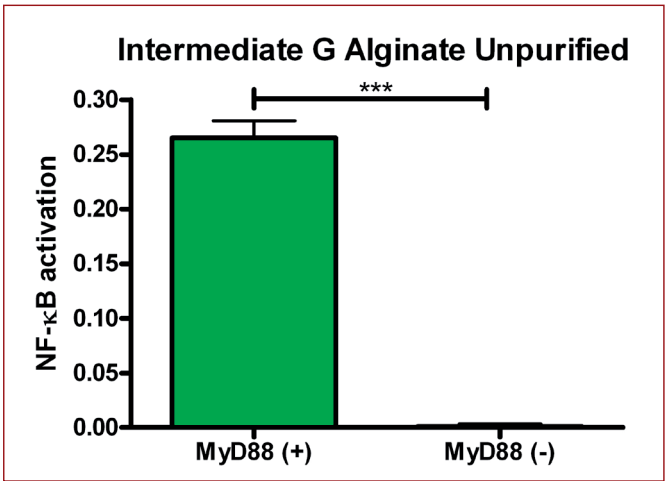

Figure 2 : NF-kB activation mediated via TLRs in intermediate G alginate unpurified. Values are presented as mean±SEM (n=20); p<0.001 (***)

below safety limits.

RESULTS & DISCUSSION

Researchers use different kinds of alginate when preparing capsules, with varying degrees of success. We hypothesized that different degrees of PAMPS are responsible for this. We first tested three different types of alginate on Thp1 MyD88 (+) cells expressing all TLRs and coupled via the intracellular messenger Myd88 to NF-kB As shown in Figure 1 all alginates activated NF-kB. The activation as higher however with low-G alginates than with intermediate-G and high-G alginates (Figure 1).

To determine whether this activation is TLRs dependent we also tested the alginates on THP1 cells with a knock out on Myd88, i.e. the intracellular messenger for TLRs. A reduction in the production of NF-kB would indicate that molecules bind via toll-like receptors. In figure 2 we show only the results for intermediate-G alginate, but we found that with all alginates the activation was gone in the absence of Myd88. This made us conclude that activation occurs via TLRs and not via other pattern recognition receptors that signal via other routes.

Next we investigated whether impurities or alginate itself activates TLRs. We did this experiment by comparing the responses against purified and unpurified intermediate alginates (Data shown only for intermediate G alginate unpurified, Figure 3).

These results demonstrate the TLR activating components are contaminating PAMPs in crude alginates. We show for the first time the mechanism behind responses against alginates..

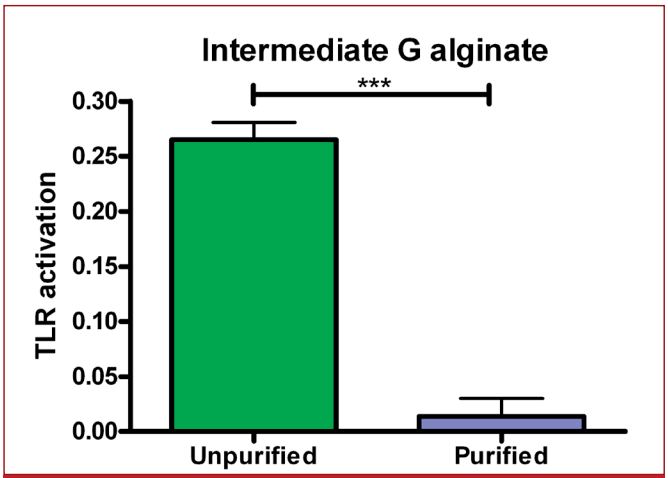

Figure 3 : Effects of purification of intermediate G alginate on NF-kB activation. Values are presented as mean±SEM (n=20); p<0.001 (***)

CONCLUSIONS

TLRs are involved in responses against alginates. The activation of TLRs is alginate dependent. Not alginate but contaminations present in crude alginate are activating TLRs The efficacy of purification procedures can be proved by testing the effect on TLRs.

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In my hometown I accomplish a Bachelor in Biology, and a Master in Biotechnology in 2008, both at the University of the Americas, Puebla (UDLAP). After that, I started working as a teacher at the UDLAP at the Department of Sciences. I did my master thesis in the area of human physiology, specially the alterations caused by diabetes, and I became interested in different aspects of this disease. Now I'm a PhD student at the UMCG at the Department of Pathology and Medical Biology, my research line focus on the development or improvement of new materials for immunoisolation and transplantation of Langerhans islets in patients with diabetes type 1 avoiding, both, immunosuppression and graft rejection.

ARTICLE

TRIPLE FORTIFICATION OF SALT USING SPRAY DRIED MICROCAPSULE

Elisa McGee and L. Diosady

INTRODUCTION

Micronutrient (i.e. vitamin and mineral) deficiencies directly affect an estimated 2 billion people worldwide causing death and impairment (World Health Organization, 2007). The most extensive problems arise in developing countries where people consume micronutrient-poor diets containing little iodine, iron, and folate. Food fortification is more effective than supplementation or dietary diversification to rectify this issue because it does not require active participation by the consumer and is more cost effective. Salt is the most effective food vehicle because it is consumed in constant daily amounts, is generally purchased (not harvested personally), has proven efficacy in the developing world, and has established distribution channels.

As a method to remedy iodine and iron deficiency simultaneously, double fortified salt was investigated. It was found that retaining iodine in salt fortified with iron stored at elevated temperatures and moisture levels required microencapsulation to form a physical barrier able to keep adsorbed water, iron, and iodine apart in the salt (Diosady et al., 2002). In 2011 Romita et al. developed spray dried ferrous fumarate microcapsules for use in coarse iodized salt (Romita et al., 2011). The particles produced through spray dry microencapsulation were too small to be visually detected (←20μm) and were small enough to adhere to salt crystals in the presence of moisture typical of unrefined commercial salt (Romita et al., 2011). Microcapsules that retained the most iodine were composed of 80% dextrin and 20% hydroxypropyl methylcellulose (HPMC) as the coating material (Romita et al., 2011).

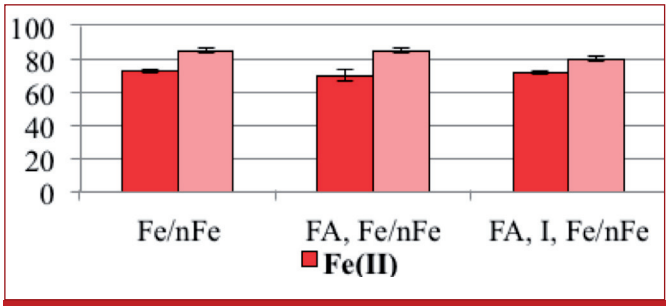

Figure 1: Percent retention of ferrous iron (1 year)

The objective of this project was to investigate micronutrient stability in salt fortified with iodine, iron, and folate using spray dried iron microcapsules and spray solutions containing iodine and folate.

Winner of the Best Contributions

XX International Conference on Bioencapsulation
Orillia, Ontario, Canada – September 21-24, 2012

MATERIALS & METHODS

Materials for fortification

Non-iodized refined Canadian salt was donated by Sifto Canada Corp. Folic acid (USP grade) was acquired from Bulk Pharmaceuticals Inc. Potassium iodate (ACS reagent grade) was purchased from Sigma-Aldrich Chemicals. Ferrous fumarate (food grade, mean diameter ~10μm) was donated by Dr. Paul Lohmann Chemicals. Hydroxypropyl methyl-cellulose (HPMC E15) was provided by Dow Chemicals Co., USA. Maltodextrin was donated by Cerestar, Indianapolis IN.

Table 1: Triple fortified salt formulations

Salt Sample #	Iodine (I) (ppm)	Folic Acid (FA) (ppm)	Iron (Fe) (ppm)	Encapsulated Iron (nFe) (ppm)
1	0	30	0	0
2	30	30	0	0
3	0	0	1000	0
4	0	0	0	1000
5	0	30	1000	0
6	0	30	0	1000
7	30	30	1000	0
8	30	30	0	1000

Iron microcapsule production

Microcapsules were prepared by Dan Romita by spray drying a suspension of ferrous fumarate in coating agents (Romita et al., 2011). The microcapsules contained 9% w/w iron and a coating consisting of 80% w/w maltodextrin and 20% w/w HPMC.

Fortification method

Salt was fortified in a bench-scale ribbon blender made by Les Industries All-Inox Inc., Montreal. Salt was added to the ribbon blender (250g-1000g) and blended for 2 minutes to break down any large salt clusters. Then folic acid and/or potassium iodate were added via solution sprayed through a spray bottle onto the salt. This added 3%

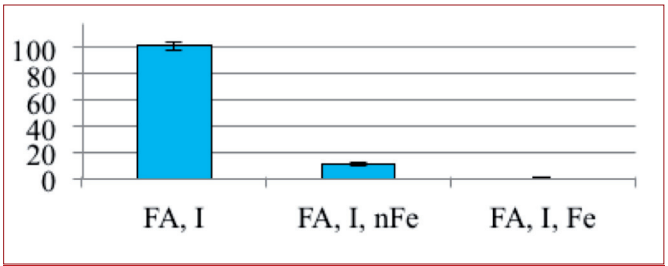

Figure 2: Percent retention of iodine (1 year)

moisture. Iron was added in powder form either as unencapsulated ferrous fumarate or as spray dried microcapsules. The salt was mixed for 15 minutes in the ribbon blender and then was taken out and put on sheets to dry overnight.

Stability testing

The salt was stored in Zip-LocTM polyethylene bags in the dark at 25ºC. The retention of the micronutrients in the different salt formulations was measured 1 year after production.

For iodine quantification iodate is reduced to iodine (I2) and titrated with sodium thiosulfate using a starch indicator (Method 33.149, Association of Official Analytical Chemists (AOAC)).

The total iron and ferrous iron content was determined by the complexation of ferrous iron with 1,10-phenanthroline followed by spectrophotometry at 512 nm. To measure total iron a reducing agent (hydroxylamine hydrochloride) was added to convert any ferric iron into ferrous iron before it was complexed with 1,10-phenanthroline.

The folic acid analytical method involved a series of three reactions that converted folic acid into a coloured product. Folic acid was reductively cleaved in hydrochloric acid by zinc. The product was diazotized and then coupled with 3-aminophenol. This was followed by spectrophotometry at 460 nm.

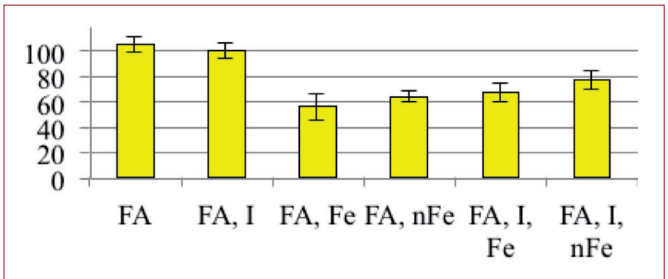

Figure 3: Percent retention of folic acid (1 year

RESULTS & DISCUSSION

It was confirmed that encapsulated ferrous fumarate retained 10% more ferrous iron than non-encapsulated ferrous fumarate. There was no significant change in ferrous iron retention due to folic acid or iodine in the salt (Figure 1). This may be due to the greater concentration of iron added to the salt compared to that of iodine and folic acid (1000 ppm vs. 30 ppm).

Iodine retention was greatly affected by iron. Without iron, iodine remained very stable in the salt (100% retention ± 3%). With the addition of encapsulated iron only a small amount was retained after 1 year of storage (11% ± 1%). When iron was added without being first encapsulated, iodine was completely lost during 1 year of storage (Figure 2). The loss of iodine is likely due to the redox reaction between iodate and ferrous iron (2IO3-(aq) + 12H+ + 10Fe2+ Đ I2(s) + 10Fe3+ + 6H2O).

The iodine subsequently sublimes from the salt. Unexpectedly, the microcapsules prevented only some of the iodine loss. This was likely due to the loss of capsule integrity. A large amount of water (3% w/w) was added to the salt when folic acid and iodine were sprayed on the salt. This partially solubilized the microcapsules releasing ferrous fumarate and allowing it to contact iodine.

Folic acid was fully retained in salt fortified with only folic acid (105% ± 6%) and in salt fortified with folic acid and iodine (101% ± 6%). Iron seemed to have a negative effect on folic acid, reducing the folic acid content by more than 20% in all cases. Iodine and iron encapsulation seemed to protect folic acid from degradation. In all salt formulations folic acid retention was greater than 50% and triple fortified salt containing encapsulated iron retained 76% ± 7%. Iodine and folic acid retention would be improved by increased iron capsule integrity. In the meantime adding an overage of folic acid could ensure sufficient folate content for the consumer.

The feasibility of iodine iron and folic acid fortification of salt has been demonstrated. The encapsulation of ferrous fumarate used in this trial was unsatisfactory resulting in a large loss of iodine and a lesser loss of folic acid. An increase in the concentration of folic acid and iodine in the spray solution would reduce the moisture added and thus help preserve the capsule integrity, preventing the reactions between iron and the other micronutrients Future work will concentrate on improving iron encapsulation, and reducing the amount of water used in fortifying the salt.

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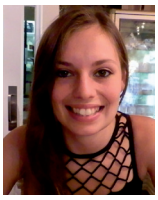

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I have obtained both my B.A.Sc. and M.A.Sc. in Chemical Engineering from the University of Toronto. I also obtained a Minor in Bioengineering during my undergraduate education and then further specialized in Food Engineering. I am currently continuing my studies at the University of Toronto to obtain a Ph.D. within the same specialty. In the future I hope to contribute my skills to the food industry

ARTICLE

POLYMER MICROCAPSULES AS MULTIFUNCTIONAL CARRIER SYSTEMS FOR SENSING AND DELIVERY

Markus Ochs, L.L. del Mercato, S. Carregal-R., A.Z. Abbasi, X. Yu, W.J. Parak

INTRODUCTION

In recent years, nano- and microsystems have become very interesting approaches for sensing and delivery in biological and medical applications. The use of nano- or microcontainers such as liposomes or polyelectrolyte microcapsules has reached great interest in this field. Functionalized polymer microcapsules fabricated via Layer-by-Layer (LbL) adsorption of polyelectrolyte materials on spherical templates (del Mercato 2010) are one promising approach to perform as multifunctional carrier system for various applications.

The described capsules serve as cages for the assembly or the separation of compounds that are entrapped in their inner cavities. This could facilitate for instance multiplexed measurements of various analytes (Abbasi 2011). Furthermore, ion-selective fluorophores embedded into these systems are creating an interesting tool for extra- and intracellular ion-sensing applications (del Mercato 2011*). The local ion concentration of various probes can be determined and different capsules can be combined to perform as multiplexed sensor tool (del Mercato 2011).

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In addition to the local sensing, the delivery of biological active substances or sensitive dyes to living cells can combine the advantages of delivery and sensor option in one multifunctional tool.

Capsules responsive to external stimuli can act in vitro as delivery vehicles (del Mercato 2010). Embedding nanoparticles with energy conversion properties into the polymer shell of such microcapsules enables for light induced opening of the containers and subsequent intracellular release of embedded cargo materials to the cytoplasm (Muñoz-Javier 2008). This technique is also suitable for multiple cargo release such as sequential release of different materials. Furthermore, heat-fragile materials like proteins have been proven to be released from such capsules in vitro without serious loss of functionality (Carregal-Romero 2012).

All these techniques can be easily combined with a targeting approach based on magnetic nanoparticles embedded into polymer microcapsules (Zebli 2005).

MATERIALS & METHODS

Microcapsules consisting of polyelectrolyte multilayer shells deposed on spherical CaCO3 templates have been fabricated via Layer-by-Layer deposition method. Porous CaCO3 particles are suitable for co-

precipitation of cargo moleculeswithin the solid, porous core material. After removal of the calciumcarbonate via chelating agents at mild conditions, resulting polymer microcapsules are enriched with the cargo material. Mainly fluorophores conjugated to dextran molecules of high molecular weight and Proteins have been encapsulated using this method.Alternatively smaller cargo molecules have been successfully encapsulated via post-loading method utilizing irreversible heat-dependent conformation changes of the polyelectrolytes.

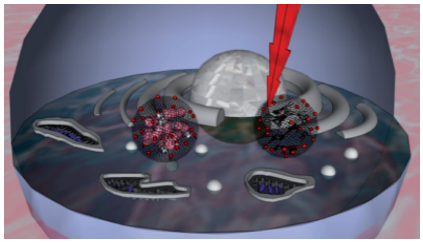

Fig 1: Release strategy of Au nanoparticle modified microcapsules via IR-laser treatment inside living cells

Materials for intended intracellular release have been encapsulated as well as functional fluorophores for ion sensing. With these materials ion concentrations of protons, potassium and sodium ions were successfully calculated by ratiometric fluorescence analysis. Multiplexed measurements with all types of sensor capsules in one pot have been performed utilizing fluorescent quantum dots embedded in the polymer shell as bar-code tagging.

Gold nanoparticles possessing energy conversion properties (ability to produce heat upon laser irradiation) were embedded into the polyelectrolyte layers of the shell. Near infrared laser light (830nm) located in the biological window of the electromagnetic spectrum was used for remote controlled opening of so-fabricated capsules. This ultimately led to the release of cargo material to the cytosol of the infiltrated cell culture. Delivery and sensing applications were combined to a multifunctional tool by delivering ion-sensitive probes to the cytosol and determining intracellular pH as well as endosomal proton concentration simultaneously. Furthermore controlled internalization of functional microcapsules via magnetic targeting of capsules modified with magnetic nanoparticles has been proven.

RESULTS & DISCUSSION

Polyelectrolyte microcapsules have successfully been modified to perform as carrier vehicles for various probes and molecules like fluorophores and proteins. Magnetic targeting was performed utilizing magnetic nanopar-

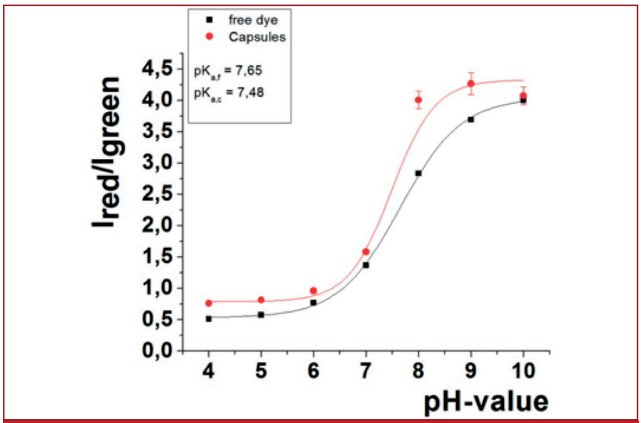

Figure 2: Calibration curve of endosomal and cytosolic proton concentration via ion selective fluorophores analysedinside internalized capsules and after triggered release to the cytosol.

ticles embedded into the polymer shell of the particles. Different fluorescent probes have been delivered into living cells by transporting them within microcapsules and releasing them into the cytosol. Infrared laser light has been proven to act as efficient energy source without harming the cell culture or showing significant energy loss in biological tissue. Though light controlled opening of Au nanoparticle modified capsules has been proven to be an efficient release strategy forin vitro experiments. Furthermore sequential release of various probes from different capsules in one single cell has been demonstrated. Intracellular release of proteins showed no significant loss in their functionality (fluorescent signal).

Polyelectrolyte capsules have been filled with ion-selective probes to perform as ion-sensors for a variety of possible applications. By embedding fluorescent quantum dots acting as bar-code tagging into the polymer shell such sensor capsules turned out to be a powerful mutliplexing sensor system.

Furthermore, by combining the sensor properties and the release mechanism of polymer capsules a highly sophisticated tool was developed which is capable for analysing intracellular probe concentrations. Releasing further, intracellular active compounds can actively manipulate these simultaneously determined values.

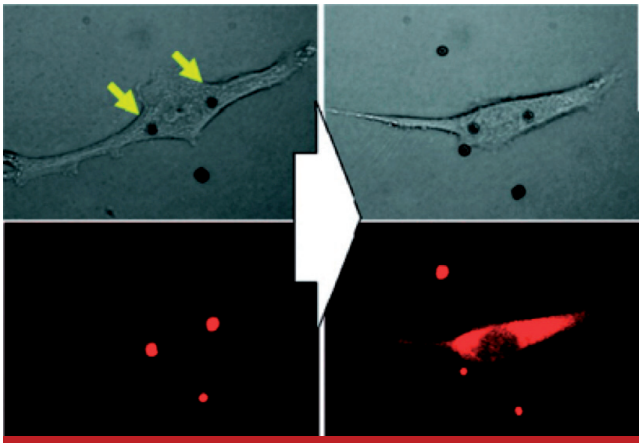

Figure 3 :Release of fluorescently labelled dextran from gold nanoparticle modified polymer microcapsules via laser induced heating.

CONCLUSIONS

Experimental data proof that the presented polymer microcapsules act as very versatile tool for in vitro sensing and drug delivery.

Recent investigations indicate for new and exciting possibilities concerning intracellular release of reactive compounds as well as biologically active substances. Further improvements of targeted delivery as well as intracellular reactions triggered by light controlled release of material in living cells are currently in progress.

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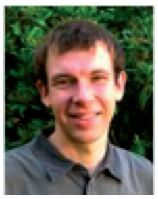

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Markus Ochs is a physicist; he completed his diploma in bio-nano-physics at the Philipps-University Marburg, (Prof. Dr. W. J. Parak). During this diploma thesis, he worked on the development and assembly of calciumcarbonate based polyelectrolyte multilayer capsules and the effective filling of the cavities with fluorescent probes. Currently he is working on a PhD program under the supervision of Prof. Dr. Wolfgang Parak (University of Marburg) on the development of the microcapsules towards drug delivery (intracellular release) and sensing applications. His research focuses on the implementation of nanoparticles into the polymer shell to enable remote-controlled release and targeted delivery of the capsules. Chemical assembly and microscopic characterization of the capsules are part of his skills as well as designing and developing the systems to fullfill the deverse requirements of biological applications.

CALENDAR

PROGRAM 2013

March	**Fluid bed processing_** March 19-21, 2013 - Binzen, Germany
	Encapsulation – an industrial approach March 25, 2013 - Copenhagen, Denmark
April	**drying in industrial applications** April 5, 2013 - Castre, France
	5th Training School on Microencapsulation April 9-12, 2013 - Nantes, France
	2nd Coating Workshop April 17, 2013 - Lille, France
	Granulation & Tabletting April 16-18, 2013 - Binzen, Germany
May	**EUCHIS 2013** 5-8 May 2013, Porto, Portugal
	Drying, Coating and Agglomeration May 22-24 2013 - Copenhagen, Denmark
June	**16th Industrial Symp. and 6th Trade Fair on Microencapsulation** June 25-27, 2013 - Madison, USA
	17th Gums & Stabilisers for the Food Industry Conference June 25th -28th 2013 - Glyndwr University, Wrexham, UK
JuLy	**Powders & Grains 2013** July 8-12, 2013 - Sydney, Australia
August	**21st International Conference on Bioencapsulation** August 28-30, 2013 - Berlin, Germany
September	**19th International Symposium on Microencapsulation** September 09-11, 2013 Pamplona, Spain
	3rd Conference on Innovation in Drug Delivery September 22-25, 2013 - Pisa, Italy
	Powder handling, quality control, and applied powder technology September 26-27 2013 - Copenhagen, Denmark
	Delivery of Functionality in complex food systems Sept 30-Oct 3 2013 - Haifa, Israel

Bioencapsulation Research Group

5th Training School on Microencapsulation
April 9-12, 2013 - Nantes, France
http://bioencapsulation.net/2013_Nantes (see page 29)

16th Industrial Symposium and 6th Trade Fair on Microencapsulation
June 25-27, 2013 - Madison, WI, USA
http://bioencapsulation.net/2013_Madison (see page 29)

21st International Conference on Bioencapsulation
August 28-30, 2013 - Berlin, Germany
http://bioencapsulation.net/2013_Berlin (see page 30)

TTC TECHNOLOGY TRAINING CENTER

Fluid bed processing_
March 19-21, 2013 - Binzen, Germany
http://www.ttc-binzen.de/cm/index.php?id=467

Granulation & Tabletting
April 16-18, 2013 - Binzen, Germany
http://www.ttc-binzen.de/cm/index.php?id=472

apgi

Drying in industrial applications
April 5, 2013 - Castre, France
http://www.apgi.org (French)

2nd Coating Workshop
April 17, 2013 - Lille, France
http://www.apgi.org/coating_WS

3rd Conference on Innovation in Drug Delivery
September 22-25, 2013 - Pisa, Italy
http://www.apgi.org

SEYDLITZ Engineering Courses

Drying, Coating and Agglomeration
May 22-24 2013 - Copenhagen, Denmark
http://powderinfonews.com/wp-content/uploads/2012/06/Fluid-Bed-Technology-20132.pdf

Powder handling, quality control, and applied powder technology
September 26-27 2013 - Copenhagen, Denmark
http://powderinfonews.com/wp-content/uploads/2012/10/Course-flyer-20131.pdf

From : Peter Dybdahl Hede, Novozyme PTHD@novozymes.com

19th International Symposium on Microencapsulation
September 09-11, 2013 Pamplona, Spain
http://www.symposiummicroencapsulation2013pamplona.com/ (see page 30)

From Juan Manuel Irache, Univ. Navara, jmirache@unav.es

EUCHIS 2013
International Conference of the European Chitin Society
5-8 May 2013, Porto, Portugal
http://www.skyros-congressos.pt/euchis2013

From Bruno Sarmento, Univ. Porto, brunocsarmento@hotmail.com

17th Gums & Stabilisers for the Food Industry Conference
June 25th -28th 2013 - Glyndwr University, Wrexham, UK
Call for Abstracts - deadline Dec. 31st 2012
http://www.gumsandstabilisers.org

From Peter A. Williams, Glyndwr University, williamspa@glyndwr.ac.uk

Delivery of Functionality in complex food systems
Sept 30-Oct 3 2013 - Haifa, Israel
http://DOF2013.org

From Yoav D. Livney, Technion, livney@tx.technion.ac.il

Nano2013.pt
October 11, 2013 - Lisbon, Portugal
http://fcts.ulusofona.pt/index.php/eventos/simposios/details/64-Nano%202013

From C. Pinto Reis, Univ. Lusófona catarinapintoreis@gmail.com

DANISH TECHNOLOGICAL INSTITUTE

Encapsulation, an industrial approach
March 25, 2013 - Copenhagen, Denmark
http://www.dti.dk/encapsulation-8211-an-industrial-approach/programme/32784.1

From Anne Louise Nielsen, Teknologisk Inst aln@teknologisk.dk

5TH TRAINING SCHOOL ON BIOENCAPSULATION

April 9-12, 2013
Nantes, France

http://Bioencapsulation.net/2013_Nantes

PROGRAM

Introduction and overview of Microencapsulation technologie
R. Neufeld, Queens' University - Canada

Food & Feed applications - Spray drying
S. Drusch, TU Berlin - Germany

Chemical methods of encapsulation
Y. Frères, Strasbourg University - France

Cosmetics and sensometry
P. Bellon, Symrise - France

EU ISEKI Food 4 network
P. Mitchell, ISEKI - UK

Technical evaluation of top-, bottom- and tangential spray particle coating
K. Eichler, Glatt - Germany

Cell Immobilization
A. Brodkorb, Teagasc - Ireland

Biomedical applications
P. De Vos, Groningen University - Netherlands

Pilote plant demonstrations

- Fluid bed coating
- Spray drying
- Continuous emulsification
- Spinning technologies

Microencapsulation in Pharmacy
A. Lamprecht, Franche Comté University - France

Laboratory demonstrations

- Dripping methods
- Interfacial Polymerisation)
- Microfludique and gelation
- Pickering and Emulsion)

Emulsion and/or Pickering and/or Liposomes
V. Schmidt, Bordeaux University - France

Scale up of microencapsulation process
D. Poncelet, Oniris - France

16TH INDUSTRIAL SYMPOSIUM AND 6TH TRADE FAIR

Organized by

Bioencapsulation Research Group

In collaboration With

ENCAPSYS MICROENCAPSULATION

JUNE 25-27, 2013
MADISON, WI, USA

http://Bioencapsulation.net/2013_Madison

Symposium program

11 lectures of 45 minutes from leading experts will cover a large scope of the microencapsulation field. The speaker selection mixes senior scientists with an understanding of encapsulation processes, with experienced business practitioners of well established practical applications.

Technology Trade Fair

Based on your own pre-selection among the list of participants, your optimized personal agenda may include up to 16 one-to-one 40 minute appointments. Coffee-breaks, exhibition, and lunch-times will give you additional networking opportunities to establish new contacts.

Exhibition

a broad state-of-the-art showcase presenting R & D services, Equipment & Tools, Material & Chemicals, Established Techniques in the realm of microencapsulation ...

CONEFRENCE

XXI INTERNATIONAL CONFERENCE ON BIOENCAPSULATION

August 28-30, 2013

Berlin, Germany

PROGRAM

SESSION 1. Agriculture and environmental issues
Chairperson A. M. Gimeno, GAT, Austria (to be confirmed)
Chairperson A. Nussinovitch - Hebrew Univ. of Jerusalem, Israel

SESSION 2. Bioactives in Food and in Feed
Chairperson G. Reineccius - Univ. Minnesotas, USA
Chairperson M.I. Re - Emac, France

SESSION 3. Engineering and innovative technologies
Chairperson Z. Zhang - Univ. Birmingham, UK
Chairperson L. Fonseca - Instituto Superior Técnico, Portugal

SESSION 4. Biomedical applications
Chairperson H. Stoover - McMaster Univ., Canada
Chairperson J. Irache - Univ. Pampelona, Spain

SESSION 5. Analytical & characterisation methods
Chairperson C. Sociacu - Prolanta, Romania
Chairperson G. Meester, DSM, Netherlands

More information :
http://Bioencapsulation.net/2013_Berlin

19th International Symposium on Microencapsulation

Discretization of Materials to Improve Added Value: Targeting - Controlled Release - Increased Availability - Shelf Life

Pamplona (Spain), September 09-11, 2013

The International Society on Microencapsulation is pleased to announce the 19th appointment of its symposium. The International Symposium on Microencapsulation has become a very well known scientific symposium related to the preparation, properties and uses of small particles; from conventional microcapsules to all other small particulate systems including micelles, polymers or self assembling structures that involve preparative manipulation. This time the meeting will try to focus on relevant uses of these devices for industrial, pharmaceutical, biotechnology, cosmetic and food applications. We believe that this important event will be a unique opportunity to share experiences and solve current problems and challenges in practice.

This 19th Symposium will take place at the Congress Auditorium of the University of Navarra in Pamplona, from the 9th to the 11th of September 2013. Please do not forget these dates and mark them clearly in your agenda.

http://www.symposiummicroencapsulation2013pamplona.com

LAMBSON created a microencapsulation division

Lambson Speciality Chemicals (www.lambson.com) has launched a microencapsulation division with the appointment of ex Ashland Inc (www.ashland.com) specialists, «... to support microencapsulation needs across a wide variety of application areas». David Palmer is the Technical Manager - Encapsulation Technologies, Sharon Martiny arrives as Technical Manager of a new Personal Care Division and Dr Andrew Mint is the new Director of Sales & Business Development, Personal Care and Encapsulation Technologies.

More information:
http://www.lambson.com/live/showscreen.php?site_id=103&screentype=site&screenid=103&newsaction=showitem&newsid=673&directcontent=%7bnews%7d&sn=News

CHREATHES opens its new laboratories

With 4 employees and a healthy €300k turnover Creathes (www.creathes.com), under the direction of Hervé Huilier, offer contract development services for microencapsulated ingredients. The new 300m2 facility in Belfort, France enables the company to provide support in the speciality chemicals sector, textiles, cosmetics and food ingredients.

More information:
http://www.industrie-techno.com/micro-encapsulation-creathes-ouvre-de-nouveaux-laboratoires.14028

Spanish consortium very active in microencapsulation

Nucaps® is the name of a consortium comprising the University of Navarre, CNTA - National Centre for Food Technology and Safety, Idifarma and Cinfa Laboratories. In the framework of the consortium, patents have been developed related to encapsulation of bioactive compounds and probiotic bacteria: WO2011104410 and WO2012007628 for Nanoparticles for encapsulation of compounds, the production and uses thereof. A third patents on encapsulation of probiotic has been recently applied.

More informations
www.cnta.es/nucaps

From Carolina González Ferrero, CNTA, Spain, cgferrero@cnta.es

TROY CORP. entrust encapsys for producing encapsulated biocide

Encapsys are also active in the biocides sector and in 2010 signed an agreement with Troy Corporation (www.troycorp.com) for the supply of microencapsulated biocides. We hope to hear more about their activity in this sector in the future.

More information:
www.encapsys.com/pdf/Encapsys-TroyAnnouncement.pdf

One day exchange on controlled release

IChemE and UKICRS held a one day microencapsulation meeting entitled "Controlled release at the interface between food, pharmaceuticals and agrochemicals 2012" hosted by Merck UK (MSD) on 14 November 2012. This brought together experts from industry and academia for cross sector discussions around adding value using encapsulation and controlled release technologies. There were excellent presentations from Syngenta and Merck and from the Universities of Reading, Birmingham, Nottingham and Leeds. PhD students presented their recent findings including talks on xanthan gum and membrane emulsions. Posters included contributions from Ashland, Colorcon and Micropore Technologies. It was a small but stimulating meeting, the kind that benefits the whole of the encapsulation sector.

More information:
http://www.ukicrs.org/ or http://www.icheme.org/

Innoavtive bioresorptive nanospheres

The invention provides the method for producing spherical particles with volume-mean particle diameter 10-40 nm on the base of amorphous magnesium and zinc-substituted calcium hydroxyapatite (HA) as material for wound healing. The clinical testing of HA particles on the flow of purulent traumatic process shows the wound healing effect on different stages.

More information
Patent priority № 2012150149 Bioresorptive nano-dispersed material on the base of amorphous hydroxyapatite and method for producing.

From Elena Krylova elenakrylova@mail.ru

FMC Corporation Signs Licensingwith GAT Microencapsulation AG

FMC Corporation announced that its Agricultural Products Group has signed a perpetual, global licensing agreement, along with distribution and services agreements with GAT Microencapsulation AG covering a range of advanced crop protection products and proprietary formulation technologies.

More information:
http://www.prnewswire.com/news-releases/fmc-corporation-signs-licensing-distribution-and-services-agreements-with-gat-microencapsulation-ag-182428791.html

DSM aquired FORTITECH to strenghen human nutrition business

DSM reports that they are to acquire Fortitech for around €495 million to strengthen their human nutrition business. DSM also announced that it had reached agreement with Cargill to acquire Cargill's cultures and enzymes business in a transaction valued at €85 million.

More information:
http://www.dsm.com/en_US/cworld/public/media/pages/press-releases/30-12-dsm-to-acquire-fortitech-to-strengthen-human-nutrition-portfolio.jsp

ENCAPSYS win P&G business partner award

Encapsys, the microencapsulation division of Appleton Papers Inc. USA, continued its successful relationship with P&G in 2012 and was recognised in September through P&G's business partner award. Encapsys supply P&G with sustained release fragrance products.

More information:
http://www.encapsys.com/pdf/2012%20Encapsys%20Earns%20TopSupplier%20Award.pdf

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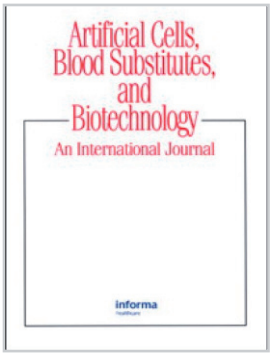

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