Bioencapsulation Research Group

Bioencapsulation Innovations

June 2012

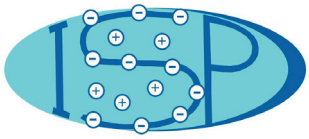
CONTENTS

EDITORIAL

POLYELECTROLYTES FOR MICRO- AND NANOENCAPSULATION

July 9-12, 2012, the «9th International Symposium on Polyelectrolytes - ISP 2012» will take place at the Ecole Polytechnique Fédérale de Lausanne (EPFL) in Switzerland, in the same rooms where the BRG organized in 2006 the «XIV International Workshop on Bioencapsulation & COST 865 meeting». What is the link between these two events?

Polyelectrolytes, macromolecules which have positively charged, negatively charged, or both, ionic groups along their polymer backbone, are an active area of research in fields such as chemistry, physics, biology, medicine, materials science, food science, and nanotechnology. PEL cover a wide range of compounds including biopolymers such as proteins and polysaccharides and many synthetic polymers industrially produced on a large scale. Due to their ionic charges, these compounds can interact by electrostatic Coulomb interaction and form polyelectrolyte complexes. The material properties of such complexes range from completely soluble complexes over hydrogels to solid materials.



HTTP://ISP2012.UNIGE.CH/

Applying specific technologies, such as microencapsulation or layer-by-layer deposition, PEL can be processed into high performance and even environmental responsive materials. Despite under intense study, the potential of such complexes for the encapsulation of actives or even cells for use as food additives, agricultural innovations, or for biomedicine, pharmaceuticals and biotechnology is far from being exhausted. Examples of already practically used PEL components for complex materials are, for example, alginate, carrageenan, xanthan, gum Arabic, modified starch, chitosan, poly-DADMAC, or cellulose sulfate.

PEL complexes will also be part of the ISP 2012 program. Basic research on PEL, their analysis, characterization, solution and complex formation behavior will definitely contribute to optimize encapsulation technologies, to invent novel application fields and/or improve the performance of already existing materials and applications. It is therefore not surprising that not only academics but also industrials participate in the ISP 2012. This Newsletter will present some recent results in the field of PEL complex formation.

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

Christine Wandrey

Professor at EPFL, Lausanne, Switzerlands, Co-organizer of the International Conference on Bioencapsulation 2006 and the International Symposium on Polyelectrolytes 2012

ARTICLE

POLYELECTROLYTE LAYER-BY-LAYER ASSEMBLY FOR DRUG DELIVERY

Cyril Moccand, Department of Botany and Plant Biology, University of Geneva, Switzerland

INTRODUCTION

Novel materials and technologies for drug delivery have evolved in the past decades, with the constant need of improving drug transport to a specific target by optimizing bioavailability and lowering drug toxicity. The field of drug delivery in particular is intimately related to new findings in nanotechnologies. Nanocapsules composed of tailored complexes provide novel opportunities for controlled released of a variety of therapeutics. An emergent class of carriers that appeared in the later 1990s, multilayered polyelectrolyte micro and nanocapsules, have undergone an exceptional evolution from physicochemical studies to promising drug delivery systems [1-3]. They have been fabricated and engineered to encapsulate a large variety of drugs, using biodegradable polymers that can respond to different well-defined stimuli for delivery. Hitherto, numerous nanocapsules-based drug formulations have been developed for the treatment of cancer, infections and diabetes among other diseases [4, 5].

Polyelectrolyte micro and nanocapsules are generally synthesized via a so-called layer-by-layer technique, with the use of a removable template of size ranging from 0.1 to 10 µm, resulting after its dissolution in a hollow capsule surrounded by a polyelectrolyte multilayer membrane and subsequently loaded with the drug, as shown in Figure 1 [6, 7]. Three main approaches have been developed for the synthesis of these capsules. The first and obvious one involves the coating of the drug itself, leading to the formation of a drug particle covered by a polyelectrolyte multilayer membrane [8]. The second approach is defined by the formation of capsules surrounding a soluble template that is removed after the formation of the polyelectrolyte multilayer. The membrane is then permeabilized by a pH shift or by changing solvent polarity, allowing the diffusion of macromolecular drug particles in the capsule [9, 10]. Finally, the third strategy combining the two first ones involves the utilization of porous inorganic templates preloaded with the therapeutics before being coated by the polyelectrolyte [11, 12]. Their dissolution results in low-molecular mass ions diffusing through the membrane while the macromolecular drug substances remain trapped in the capsule.

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LAYER-BY-LAYER ASSEMBLY OF POLYELECTROLYTE CAPSULES

The formation of polyelectrolyte multilayers is performed by the sequential adsorption of polycations and polyanions on the surface of a charged template [6]. Standard synthetic polyelectrolytes comprise polyanions such as poly(styrene sulfonate) (PSS), dextrane sulfate (DXS) or poly(vinyl sulfate) (PVS) and polycations, like poly(allylamine hydrochloride) (PAH), protamine dextrane (PRM) or poly(dimethyldiallylammonium chloride) (PDDA) [13]. In general, layer-by-layer assembly is made in three steps, as depicted in Figure 2. The first one involves the immersion of a charged substrate in a solution of oppositely-charged polyelectrolyte to adsorb the first monolayer, followed by a washing step enabling the removal of unbound material. The next step is the immersion of the coated substrate to deposit the second layer for the formation of a multilayered capsule [14]. This technique takes advantage of the electrostatic interactions between charged polymers and oppositely charged surfaces, allowing stepwise formation of a polyelectrolyte membrane by repetitive exposure of substrates with the next colloid.

ADVANTAGES OF NANOPARTICLE BASED DRUG DELIVERY

The use of nanotechnologies for targeted drug delivery enables to overcome a variety of limits imposed during the administration of free drugs. Nanocapsules offer the opportunity to functionalize their surface for specific targeting. They enable stabilization and protection of the encapsulated drugs to avoid their systemic clearance and offer the possibility of multiple drug

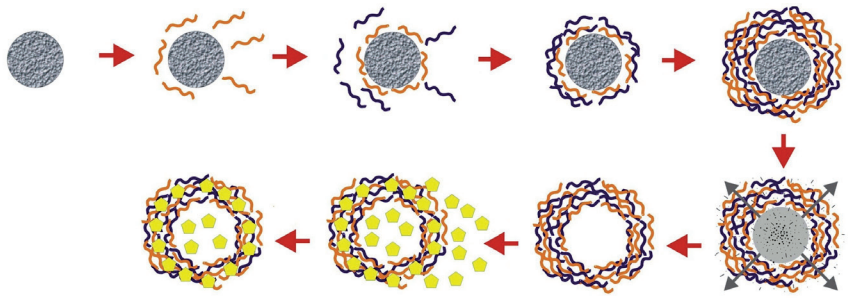

Fig. 1 Schematic illustration of the formation of a drug-loaded polyelectrolyte capsule. Consecutive adsorption of layer-by-layer assembled polyelectrolyte multilayers (orange and blue) onto template microspheres (gray) is followed by dissolution of the core and incubation with therapeutics for drug loading (yellow) in the capsule.

delivery in a single system. Moreover, they can be functionalized to respond to specific stimuli, making delivery fully controllable, all this at a nanometer range [15]. These features enable to reduce the lack of selectivity, specificity, the high degradation susceptibility and the difficult drug transport that is currently limiting drug administration, especially during chemotherapy for the treatment of metastasized cancers [15]. In addition, the concentration of therapeutics can be significantly reduced when encapsulated, leading to low dose-dependent side effects of the drugs as well as a reduction of the utilization of toxic adjuvants, used to improve the bioavailability of the active compounds [15].

The main advantages of polyelectrolyte capsules compared to other technologies in drug delivery are certainly their modularity and multifunctionality. The electrostatic driving force for multilayer formation as a basic principle for capsules fabrication enables the utilization of a wide variety of constituents such as synthetic polyelectrolytes, enzymes, lipids, nanoparticles, and so on [1]. Moreover, polyelectrolytes can be synthesized without the use of organic solvents, which is the case for many other particles used in drug delivery, such as poly(lactic-co-glycolic acid) microspheres and liposomes [16]. Considering the fact that polyelectrolyte assemblies are endocyted by cells, their potential for therapies using drug delivery is promising, but out of the numerous layer-by-layer polyelectrolyte microcapsules that have been engineered so far, none of them have been yet approved by the FDA.

STIMULI RESPONSIVE NANOCAPSULES

An interesting feature of multifunctional delivery systems such as polyelectrolyte multilayer microcapsules and nanocapsules is the possibility of external guidance via remote physical control. Responsiveness to temperature, pH, solvent polarity, CO_2 or glucose levels, light, ultrasounds, magnetic field as well as electrochemical stimuli has already been reported [16-22]. Here we review promising data on capsules possessing remote physical control for navigation and delivery of drugs.

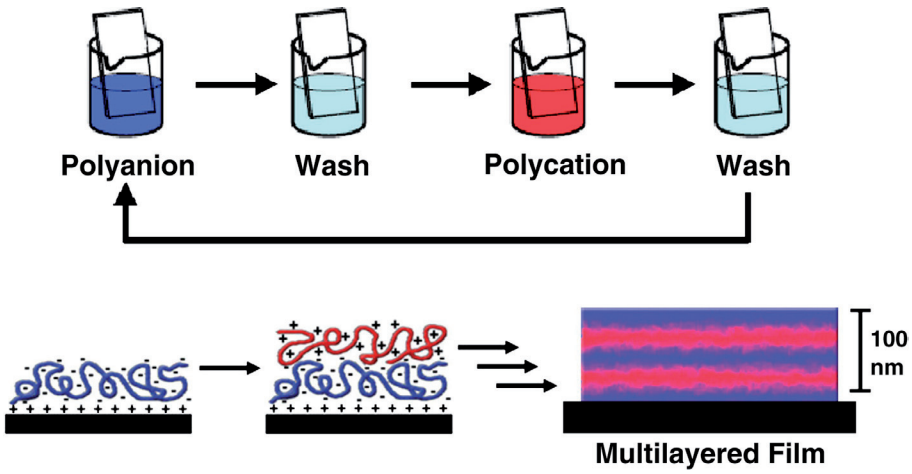

Fig. 2 A schematic representation of the alternate adsorption of the polyelectrolyte species to produce a multilayered structure

pH-responsive assemblies

Certain types of capsules have the ability to deliver their payload in response to a change in the medium pH. This change is attributed to a decrease of the charge density of these assemblies leading to an increase in permeability when the pH of the medium containing the polyelectrolytes is close to their apparent pKa [23]. Drug delivery by means of a shift in the pH can be utilized in cancer therapy, since it is known that inflammatory and tumor tissues display a mildly acidic environment [24]. Moreover, accumulation of nanocapsules in tumor tissue is facilitated by the Enhanced Permeability and Retention effect (EPR), due to the presence of fenestrations in tumor blood vessels as well as a lack of effective lymphatic drainage [25]. A second field of application relies on the acidification taking place in the gastrointestinal tract [26]. However, simpler therapies by oral administration have been developed and optimized, rendering controlled release obsolete in this case.

Nanocapsules with pH-responsive properties can also be exploited in cellular vesicles like lysosomes (pH 4.5-5) and endosomes (pH 5.5-6) [24]. As demonstrated by Gianotti *et al.*, a polyelectrolyte composed of trimethyl chitosan (TMC) and the lysosomal enzyme α-galactosidase A (α-GAL) was synthesized through self-assembly, with an ability to release the enzyme at acidic pH. This capsule showed a good potential as advanced protein delivery systems for the treatment of lysosomal storage disorders such as the Fabry disease, characterized by a deficiency of α-GAL [18]. However, such pH triggered release is not obvious under physiological conditions since it is difficult to predict the apparent pKa of polyelectrolytes within a multilayer assembly [23].

Capsules with optical response

Generally, release of capsule payload by optical response was related to the accumulation of light energy by a chromophore embedded in non-absorbing polyelectrolyte, thus heating the chromophore and leading to an increase in the permeability of the shell or its rupture [27]. One requirement for the use of light-responsive polyelectrolyte capsules is to avoid destruction of the surrounding environment and the encapsulated drug by means of the illumination. The best region in the electromagnetic spectrum is in between 780 and 900 nm, where no chief chromophore absorbance is observed [27].

The absorption properties of the capsule can be controlled by choosing the size and the substrates of the nanomembrane, thus regulating heat production and response. Organic dyes have been investigated as absorbers for light-responsive polyelectrolytes capsules by coating. A new and different type of optically addressable capsules has been reported by Wang *et al.* They used encapsulated photosensitizers to induce cell death for the

ARTICLE

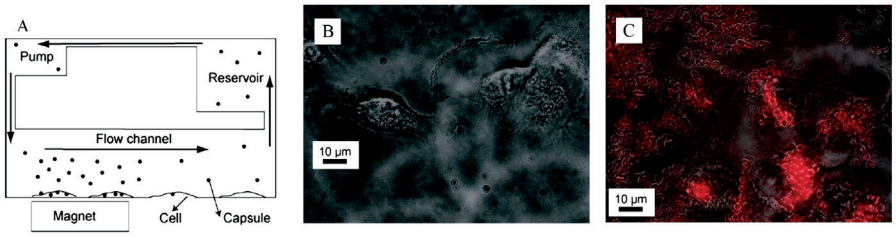

Fig. 3 Model for the magnetic delivery of polyelectrolyte capsules functionalized with magnetic nanoparticles (A). Living breast cancer cells attached to the bottom of the flow channel and located 11mm away (B) or just above the edge of the permanent magnet (C). Internalized capsules are recognized by their luminescence attributed to CdTe nanocrystals incorporated in the shells.

treatment of cancer and viral infection by generation of singlet oxygen upon exposure to light *in vitro* [22]. This experiment showed that light-responsive microcapsules are good candidates for targeted drug delivery of therapeutics that need to be protected from the environment before their release on the biological target.

Magnetic polyelectrolyte nanocarriers

Navigation of polyelectrolyte microcapsules by means of a magnetic field represents a promising technology for controlled release, since they can be remotely targeted to the tissue of interest. The introduction of magnetic iron oxides in these assemblies represents the most common way of production of these responsive layer by layer capsules. The first attempt to produce magnetic polyelectrolyte capsules was performed by Caruso *et al.*, where polystyrene latex beads were coated with stablized negatively charged Fe_3O_4 nanoparticles and polycation PAH, alternatively [28].

Many different strategies were developed for the synthesis of magnetic-responsive polyelectrolyte capsules, such as the formation of hollow magnetic nanoparticles after template removal [29], or by using the pH-dependent permeability of the polyelectrolyte membrane to selectively impregnate the magnetic nanoparticles [30]. In the latter example, the Fe_3O_4 nanoparticles could diffuse through the poly[allylamine hydrochloride]/poly[styrene sulfonate] polyelectrolyte membrane which is permeable to species smaller than 10 nm at pH 4.5 and therefore reach the interior of the shell.

An interesting feature of magnetic-responsive polyelectrolyte nanoparticles is the ability of these capsules to align or move driven by a magnetic field [28]. *In vitro* experiments using Fe_3O_4-impregnated poly[styrene sulfonate]/poly[allylamine hydrochloride] capsules targeted on breast cancer cells showed that a high level of cellular internalization was reached when the particles were placed under a magnetic field (Figure 3) [31].

Such functionalized polyelectrolyte capsules can also be used to remove their payload by means of a magnetic field. Hu *et al.* observed the rupture of Fe_3O_4/poly[allylamine hydrochloride] capsules leading to the release of fluorescein isothiocyanate–dextran upon exposure to high frequency magnetic field [20]. Moreover, fast uptake of cancerous cell line was observed, along with low cytotoxicity. The release mechanism is attributed to local heating as well as stress induced by the alignment of magnetic capsules exposed to the magnetic field, leading to a relaxation of the polyelectrolyte membrane [20].

It is noteworthy that these particles can be used in magnetic resonance imaging (MRI) for drug delivery tracking *in vivo* as well as visualization of contrast agents [32]. The main advantage of magnetic polyelectrolyte nanoparticles is their ability to respond to a specific stimulus for both navigation and payload release, making them an attractive delivery system *in vitro* with a possibility for *in vivo* applications.

Electrochemical delivery from polyelectrolyte multilayers

Another type of controlled delivery via remotely applied external physical stimulus was recently reported by Graf and co-workers. The utilization of an electrochemical stimulus enabled delivery to cells of the dye calcein that was previously loaded in liposomes embedded in a sandwich of polyelectrolyte multilayers (Figure 4) [19]. This type of controlled release could be used for surface mediated drug delivery or for realizing intelligent cell cultures.

Ultrasound addressable capsules

The low level of side effects on humans by use of ultrasounds enabled this technique to be widely used in therapy and diagnostics of several diseases. Therefore, the potential for controlled release of drugs by ultrasound-responsive polyelectrolyte capsules is of great interest. However, it remains challenging to produce polyelectrolyte carriers that are able to respond to ultrasounds at power and frequencies known to be not destructive for living organisms. Among the numerous studies performed in this regard, Mason *et al.* showed protein release from polymer and gold capsules under exposure to ultrasounds with frequencies close to biomedical applications (850 kHz and 1-3 W), thus avoiding any destruction of tissues [21].

POLYELECTROLYTE MICROCAPSULES FOR GENE THERAPY

The property of polyeletrolyte microcapsules to release their payload to a specific target is of particular interest for gene therapy, since DNA or siRNA can be used to directly supplement or alter genes within an individual's cells. The polyionic nature of these molecules can enable their incorporation into the polyelectrolyte multilayer, as described by Schüler and Caruso [33]. However, several obstacles impair fast development in gene therapy. First of all, the capsules need to leave the phagosome/endosome/lysosome and subsequently enter the nucleus after transiting by the cell cytoplasm. Observations by De Geest and co-workers showed evidence that the polyelectrolyte capsules enter the cell through lipid-raft mediated endocytosis and end up in phagosomal compartments without releasing their payload into the cytosol [34]. Therefore, optimization of capsule formation

for controlled release in the cytosol is a prerequisite for gene therapy using the technology of polyelectrolyte multilayer assemblies. However, some groups reported transgene expression after internalization and release of a capsule composed of the corresponding coding DNA and a polycation. Indeed, EGFP was observed in fibroblast-like cell lines (COS-7) 48h after

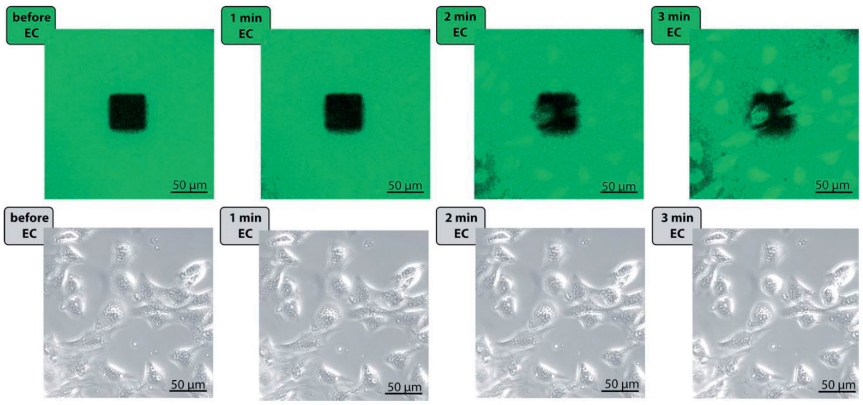

Fig. 4 Time series of a current application (50 µA.cm^{-1}) for 3 min. The upper row represents the evolution of fluorescence intensity, showing cellular uptake. The lower row is the corresponding phase contrast images of the cells. The bleach region is used to adjust the microscope settings.

their transfection by surface-mediated delivery of DNA *in vitro*, as showed by Jewell *et al.* [35].

Small-interfering RNA fragments have been shown to silence gene expression with a relative low cytotoxicity. They are composed of 19-21 base-paired double stranded RNA that suppress gene expression post-transcriptionally in a really specific way and at a very low concentration *in vivo*, rendering them of great interest for the treatment of several diseases including cancer [36]. Their rapid degradation rate in physiological environments prompted researchers to find a protective capsule for delivery. Several examples in the literature describe the use of polyelectrolyte-based siRNA delivery *in vitro* and *in vivo*. More recently, Cho and co-workers showed that a poly-L-arginine and dextrane sulfate-based polyelectrolyte complex encapsulating an epidermal growth factor receptor siRNA could silence this gene in different cancer cell lines and induced tumor growth inhibition in a mouse model [37].

CONCLUSIONS AND OUTLOOK

Polyelectrolyte micro and nanocapsules have emerged as promising assemblies for navigation and controlled release *in vitro* and *in vivo*. The simplicity to synthesize them by means of the layer-by-layer technique as well as the ability to tailor functional surfaces and the wide variety of substrates that can be encapsulated renders them extremely multifunctional. One of the main advantages of their modularity resides in their ability to be functionalized to make them responsive to different physical or chemical stimuli such as light or pH-shift.

The use of polyelectrolyte multilayers enables to stabilize encapsulated drugs while controlling their release as a function of time. Moreover, the biocompatibility is increased and drug concentration is lowered. The internalized capsules are shown to be functional for drug delivery *in vitro* and *in vivo* but applicability of certain assemblies such as stimuli-responsive capsules for clinical trials is of concern at the moment. The approval of polyelectrolyte-based delivery systems by the FDA remain problematic and moreover, the recent directive of the European Commission limiting the use of nanostructured materials is a new obstacle to overcome for these new technologies.

Another existing problem is linked with the long time needed to form the shell which could impair industrial production of polyelectrolyte capsules. Nevertheless, a German company, Capsulution NanoScience AG, is currently trying to produce capsules at an industrial level. However, fast development in the field suggests that polyelectrolyte multilayers are a promising technology for remotely guided drug delivery including gene therapy.

BIBLIOGRAPHY

1. De Geest, B.G., et al., Polyelectrolyte microcapsules for biomedical applications. Soft Matter, 2009. 5(2): p. 282-291.
2. Decher, G., J.D. Hong, and J. Schmitt, Buildup of ultrathin multilayer films by a self-assembly process: III. Consecutively alternating adsorption of anionic and cationic polyelectrolytes on charged surfaces. Thin Solid Films, 1992. 210–211, Part 2(0): p. 831-835.
3. Johnston, A.P.R., et al., Layer-by-layer engineered capsules and their applications. Current Opinion in Colloid & Interface Science, 2006. 11(4): p. 203-209.
4. Brannon-Peppas, L. and J.O. Blanchette, Nanoparticle and targeted systems for cancer therapy. Advanced Drug Delivery Reviews, 2004. 56(11): p. 1649-1659.
5. Farokhzad, O.C. and R. Langer, Nanomedicine: Developing smarter therapeutic and diagnostic modalities. Advanced Drug Delivery Reviews, 2006. 58(14): p. 1456-1459.
6. Decher, G., Fuzzy Nanoassemblies: Toward Layered Polymeric Multicomposites. Science, 1997. 277(5330): p. 1232-1237.
7. Sukhorukov, G.B., et al., Stepwise polyelectrolyte assembly on particle surfaces: a novel approach to colloid design. Polymers for Advanced Technologies, 1998. 9(10-11): p. 759-767.
8. Qiu, X., et al., Studies on the Drug Release Properties of Polysaccharide Multilayers Encapsulated Ibuprofen Microparticles. Langmuir, 2001. 17(17): p. 5375-5380.
9. Antipov, A.A. and G.B. Sukhorukov, Polyelectrolyte multilayer capsules as vehicles with tunable permeability. Advances in Colloid and Interface Science, 2004. 111(1–2): p. 49-61.
10. Antipov, A.A., G.B. Sukhorukov, and H. Möhwald, Influence of the Ionic

Strength on the Polyelectrolyte Multilayers' Permeability. Langmuir, 2003. 19(6): p. 2444-2448.

11. Sukhorukov, G.B., et al., Porous calcium carbonate microparticles as templates for encapsulation of bioactive compounds. Journal of Materials Chemistry, 2004. 14(14): p. 2073-2081.
12. Wang, Y., A.S. Angelatos, and F. Caruso, Template Synthesis of Nanostructured Materials via Layer-by-Layer Assembly†. Chemistry of Materials, 2007. 20(3): p. 848-858.
13. Bertrand, P., et al., Ultrathin polymer coatings by complexation of polyelectrolytes at interfaces: suitable materials, structure and properties. Macromolecular Rapid Communications, 2000. 21(7): p. 319-348.
14. Lvov, Y., et al., A careful examination of the adsorption step in the alternate layer-by-layer assembly of linear polyanion and polycation. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 1999. 146(1–3): p. 337-346.
15. Vergaro, V., et al., Drug-loaded polyelectrolyte microcapsules for sustained targeting of cancer cells. Advanced Drug Delivery Reviews, 2011. 63(9): p. 847-864.
16. De Geest, B.G., G.B. Sukhorukov, and H. Möhwald, The pros and cons of polyelectrolyte capsules in drug delivery. Expert Opinion on Drug Delivery, 2009. 6(6): p. 613-624.
17. Antipina, M.N. and G.B. Sukhorukov, Remote control over guidance and release properties of composite polyelectrolyte based capsules. Advanced Drug Delivery Reviews, 2011. 63(9): p. 716-729.
18. Giannotti, M.I., et al., pH-Responsive Polysaccharide-Based Polyelectrolyte Complexes As Nanocarriers for Lysosomal Delivery of Therapeutic Proteins. Biomacromolecules, 2011. 12(7): p. 2524-2533.
19. Graf, N., et al., Electrochemically driven delivery to cells from vesicles embedded in polyelectrolyte multilayers. Soft Matter, 2012. 8(13): p. 3641-3648.
20. Hu, S.-H., et al., Controlled Rupture of Magnetic Polyelectrolyte Microcapsules for Drug Delivery. Langmuir, 2008. 24(20): p. 11811-11818.
21. Pavlov, A.M., et al., Controlled protein release from microcapsules with composite shells using high frequency ultrasound-potential for in vivo medical use. Soft Matter, 2011. 7(9): p. 4341-4347.
22. Wang, K., et al., Encapsulated photosensitive drugs by biodegradable microcapsules to incapacitate cancer cells. Journal of Materials Chemistry, 2007. 17(38): p. 4018-4021.
23. Petrov, A.I., A.A. Antipov, and G.B. Sukhorukov, Base–Acid Equilibria in Polyelectrolyte Systems: From Weak Polyelectrolytes to Interpolyelectrolyte Complexes and Multilayered Polyelectrolyte Shells. Macromolecules, 2003. 36(26): p. 10079-10086.
24. Galvin, P., et al., Nanoparticle-based drug delivery: case studies for cancer and cardiovascular applications. Cellular and Molecular Life Sciences, 2012. 69(3): p. 389-404.
25. Maeda, H., The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting. Advances in Enzyme Regulation, 2001. 41(1): p. 189-207.
26. Hörter, D. and J.B. Dressman, Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. Advanced Drug Delivery Reviews, 1997. 25(1): p. 3-14.
27. Vogel, A. and V. Venugopalan, Mechanisms of Pulsed Laser Ablation of Biological Tissues. Chemical Reviews, 2003. 103(2): p. 577-644.
28. Caruso, F., et al., Magnetic Core–Shell Particles: Preparation of Magnetite Multilayers on Polymer Latex Microspheres. Advanced Materials, 1999. 11(11): p. 950-953.
29. Caruso, F., et al., Magnetic Nanocomposite Particles and Hollow Spheres Constructed by a Sequential Layering Approach. Chemistry of Materials, 2000. 13(1): p. 109-116.
30. Antipov, A.A., et al., Polyelectrolyte multilayer capsule permeability control. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2002. 198–200(0): p. 535-541.
31. Zebli, B., et al., Magnetic Targeting and Cellular Uptake of Polymer Microcapsules Simultaneously Functionalized with Magnetic and Luminescent Nanocrystals. Langmuir, 2005. 21(10): p. 4262-4265.
32. Ai, H., Layer-by-layer capsules for magnetic resonance imaging and drug delivery. Advanced Drug Delivery Reviews, 2011. 63(9): p. 772-788.
33. Schüler, C. and F. Caruso, Decomposable Hollow Biopolymer-Based Capsules. Biomacromolecules, 2001. 2(3): p. 921-926.
34. De Geest, B.G., et al., Intracellularly Degradable Polyelectrolyte Microcapsules. Advanced Materials, 2006. 18(8): p. 1005-1009.
35. Jewell, C.M., et al., Multilayered polyelectrolyte films promote the direct and localized delivery of DNA to cells. Journal of Controlled Release, 2005. 106(1–2): p. 214-223.
36. Elbashir, S.M., et al., Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature, 2001. 411(6836): p. 494-498.
37. Cho, H.-J., et al., Poly-L-arginine and Dextran Sulfate-Based Nanocomplex for Epidermal Growth Factor Receptor (EGFR) siRNA Delivery: Its Application for Head and Neck Cancer Treatment. Pharmaceutical Research, 2012. 29(4): p. 1007-1019

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After having completed his MSc in Molecular and Biological Chemistry at the EPFL in Switzerland and at the University of Adelaide in Australia, Cyril Moccand started a PhD in Biochemistry at the ETH in Zürich before moving to the University of Geneva in 2009.

His current work focuses on the biosynthesis and regulation of vitamins, combining techniques ranging from molecular biology to plant biochemistry, with an emphasis on protein science and enzymology. In order to fulfill his PhD degree requirements, he needed to write a review not directly related to his field of research. After having followed a course given by Prof. Jeffrey Hubbell at the EPFL, he became interested in combining his knowledge with new technologies in the field of drug delivery and decided to write a review article about polyelectrolyte capsules for drug delivery, using the layer-by-layer synthesis technique.

NEXT BRG CONFERENCE

XX INTERNATIONAL CONFERENCE ON BIOENCAPSULATION

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

Organized by

Professor Ronald Neufeld

Queen's University

Professor Frank Gu

University of Waterloo

Professor Corinne Hoesli

Université Laval

Our 20th annual international conference will take place in one of the most beautiful tourist area of Canada, the natural peninsula on Lake Couchiching. It is only 90 minutes from Toronto, Ontario, and offers a rare combination of extraordinary natural surroundings while being close to urban amenities.

Benefit from three days to talk with other bioencapsulation experts from all over the world in a professional but friendly atmosphere, a lifetime away from the stress of daily life... Join us ... and let's celebrate together the BRG 20th annual international conference, in the course of a 2 and ½ days residential conference, which we hope, will remain an unforgettable event for all.

PROGRAMME

September 21, 2012

Arrival & dinner

September 22, 2012

Session 1 : Islet encapsulation and diabetes (Sponsored by JRDF)

Chairperson: Prof. Paul De Vos, Univessity of Groningen, Netherlands

Coffee break and poster session

Session 3 : Tissue engineeering and regeneration

Chairperson: Prof. Harald Stover, McMaster University, Canada

Lunch and poster session

Session 4 : Nutrition, food and feed

Chairperson: Prof. Muriel Subirade, Laval University, Canada

Coffee break and poster session

Session 5 : Emerging nanomaterials

Chairperson: Dr. Arthur Carty, Waterloo University, Canada

September 23, 2012

Session 6 : Delivery of biopharmaceuticals

Chairperson: Prof. Frank Gu, Waterloo University, Canada

Coffee break and poster session

Session 7 : Biopolymers and biomaterials

Chairperson: Mary Ann Augustin, CSIRO, Australia

Lunch and poster session

Session 7 : Environment and agriculture

Chairperson: Luz de Bashan, CIBNOR, Mexico

Coffee break and poster session

Session 8 : Encapsulation technologies

Chairperson: André Bodkorb, Teagasc Food Research center, Ireland

September 24, 2012

Session 9 : Innovative technologies

Chairperson: James Oxley, SWRI, USA

Coffee break and poster session

Session 10 : Cell immobilization

Chairperson: Dr. Claude Champagne, Agr. Canada

Lunch and prize distribution

More information & registration
http://bioencapsulation.net/2012_Orillia

BIBLIOGRAPHY

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- **Formulation, optimization and evaluation of spray-dried mucoadhesive microspheres as intranasal carriers for Valsartan** Chandrakant V. Pardeshi, Pravin V. Rajput, Veena S. Belgamwar, Avinash R. Tekade - Journal of Microencapsulation 2012, Vol. 29, No. 2: 103–114.
- **Mixture designs in the optimisation of PLGA nanoparticles: influence of organic phase composition on Ð-aescin encapsulation** H Van de Ven, J Vandervoort, W Weyenberg, S Apers, A Ludwig - Journal of Microencapsulation 2012, Vol. 29, No. 2: 115–125.
- **Mucoadhesive bilayer buccal tablet of carvedilol-loaded chitosan microspheres: in vitro, pharmacokinetic and pharmacodynamic investigations** Pramod Yedurkar, Munish Kumar Dhiman, Kailash Petkar, Krutika Sawant - Journal of Microencapsulation 2012, Vol. 29, No. 2: 126–137.
- **Albumin nanoparticles with predictable size by desolvation procedure** B. von Storp, A. Engel, A. Boeker, M. Ploeger, K. Langer - Journal of Microencapsulation 2012, Vol. 29, No. 2: 138–146.
- **Sealing liquid-filled pectinate capsules with a shellac coating** Stefan Henning, Sabine Leick, Maureen Kott, Heinz Rehage, Dieter Sute - Journal of Microencapsulation 2012, Vol. 29, No. 2: 147–155.
- **Salmon calcitonin-loaded Eudragit® and Eudragit®-PLGA nanoparticles: in vitro and in vivo evaluation** Meltem Cetin, Mustafa Sinan Aktas, Imran Vural, Murat Ozturk - Journal of Microencapsulation 2012, Vol. 29, No. 2: 156–166.
- **Effects of an oral insulin nanoparticle administration on hepatic glucose metabolism assessed by 13C and 2H isotopomer analysis** Catarina Pinto Reis, Ronald Neufeld, Francisco Veiga, Isabel V Figueiredo, John Jones, Ana F Soares, Patrícia Nunes, Christiane Damgé, Rui A Carvalho - Journal of Microencapsulation 2012, Vol. 29, No. 2: 167–176.
- **Development and evaluation of lipid microbubbles targeted to alpha(v)beta(3)-integrin via biotin–avidin bridge** Wei Wang, Guang-Jian Liu, Xiao-Yan Xie, Zuo-Feng Xu, Li-Da Chen, Guang-Liang Huang, Lu-Yao Zhou, Ming-De Lu - Journal of Microencapsulation 2012, Vol. 29, No. 2: 177–184.
- **Encapsulation of a pressure-sensitive adhesive by spray-drying: microparticles preparation and evaluation of their crushing strength** Cécile Gavory, Robin Abderrahmen, Jean-Pierre Valour, Didier Chaussy, Mohamed Naceur Belgacem, Hatem Fessi, Stéphanie Briançon - Journal of Microencapsulation 2012, Vol. 29, No. 2: 185–193.
- **Studies on pharmacokinetics and tissue distribution of bifendate nanosuspensions for intravenous delivery** Yue Liu, Dianrui Zhang, Cunxian Duan, Lejiao Jia, Pengcheng Xie, Dandan Zheng, Feihu Wang, Guangpu Liu, Leilei Hao, Xueshun Zhang, Qiang Zhang - Journal of Microencapsulation 2012, Vol. 29, No. 2: 194–203

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http://informahealthcare.com/toc/mnc/29/3

- **Preparation, characterisation and viability of encapsulated Trichoderma harzianum UPM40 in alginate-montmorillonite clay** Fariz Adzmi, Sariah Meon, Mohamed Hanafi Musa, Nor Azah Yusuf - Journal of Microencapsulation 2012, Vol. 29, No. 3: 205–210.
- **The effect of treatment with a sustained-release prostacyclin analogue (ONO-1301-loaded PLGA microsphere) on short-term memory impairment in rats with transient global cerebral ischemia** Mai Hazekawa, Yoshiki Sakai, Miyako Yoshida, Tamami Haraguchi, Takahiro Uchida - Journal of Microencapsulation 2012, Vol. 29, No. 3: 211–218.
- **Preparation and evaluation of niosomes containing autoclaved Leishmania major: a preliminary study** Abbas Pardakhty, Mojtaba Shakibaie, Hamid Daneshvar, Ali Khamesipour, Tayebe Mohammadi-Khorsand, Hamid Forootanfar - Journal of Microencapsulation 2012, Vol. 29, No. 3: 219–224.
- **Preparation and in vitro evaluation of salbutamol-loaded lipid microparticles for sustained release pulmonary therapy** Santo Scalia, Rania Salama, Paul Young, Daniela Traini - Journal of Microencapsulation 2012, Vol. 29, No. 3: 225–233.
- **Enhanced percutaneous delivery of recombinant human epidermal growth factor employing nano-**

BIBLIOGRAPHY

liposome system Sang-Ok Jeon, Hee-Jin Hwang, Dong-Ho Oh, Jo-Eun Seo, Kyeung-Hwa Chun, Sun-Mi Hong, Min-Ju Kim, Won-Chul Kim, Min-Sun Park, Chae-Ha Yoon, Kyung-Hyun Min, Chang-Woo Suh, Sangkil Lee - Journal of Microencapsulation 2012, Vol. 29, No. 3: 234–241.

- **Hollow poly(MPC-g-PEG-b-PLA) graft copolymer microcapsule as a potential drug carrier** Chaoyong Liu, Lixia Long, Zhi Li, Bin He, Liuheng Wang, Jiapeng Wang, Xubo Yuan, Jing Sheng - Journal of Microencapsulation 2012, Vol. 29, No. 3: 242–249.
- **Novel alginate gel microspheres produced by impinging aerosols for oral delivery of proteins** Dewi Melani Hariyadi, Yiwei Wang, Sharon Chien-Yu Lin, Thor Bostrom, Bhesh Bhandari, Allan G. A. Coombes - Journal of Microencapsulation 2012, Vol. 29, No. 3: 250–261.
- **Trigger release liposome systems: local and remote controlled delivery?** Sagida Bibi, E. Lattmann, Afzal R. Mohammed, Yvonne Perrie - Journal of Microencapsulation 2012, Vol. 29, No. 3: 262–276.
- **Mechanical characterization of microspheres – capsules, cells and beads: a review** Ruben Mercadé-Prieto, Zhibing Zhang - Journal of Microencapsulation 2012, Vol. 29, No. 3: 277–285.
- **Microencapsulation of bioactives in cross-linked alginate matrices by spray drying** Monica Santa-Maria, Herbert Scher, Tina Jeoh - Journal of Microencapsulation 2012, Vol. 29, No. 3: 286–295.
- **Preparation of novel solid lipid microparticles loaded with gentamicin and its evaluation in vitro and in vivo** Emmanuel Chukwuebuka Umeyor, Franklin Chimaobi Kenechukwu, John Dike Ogbonna, Salome Amarachi Chime, Anthony Attama - Journal of Microencapsulation 2012, Vol. 29, No. 3: 296–307.

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http://informahealthcare.com/toc/mnc/29/4

- **Tamoxifen-loaded nanoparticles based on a novel mixture of biodegradable polyesters: characterization and in vitro evaluation as sustained release systems** Elena Pérez, Marta Benito, César Teijón, Rosa Olmo, José M. Teijón, M. Dolores Blanco - Journal of Microencapsulation 2012, Vol. 29, No. 4: 309–322.
- **Development of novel flurbiprofen-loaded solid self-microemulsifying drug delivery system using gelatin as solid carrier** Dong Wuk Kim, Jun Hyeok Kang, Dong Hoon Oh, Chul Soon Yong, Han-Gon Choi - Journal of Microencapsulation 2012, Vol. 29, No. 4: 323–330.
- **Ciprofloxacin hydrochloride-loaded glyceryl monostearate nanoparticle: factorial design of Lutrol F68 and Phospholipon 90G** Malay Shah, Yadvendra Agrawal - Journal of Microencapsulation 2012, Vol. 29, No. 4: 331–343.
- **Doxycycline delivery from PLGA microspheres prepared by a modified solvent removal method** Roshni S. Patel, Daniel Y. Cho, Cheng Tian, Amy Chang, Kenneth M. Estrellas, Danya Lavin, Stacia Furtado, Edith Mathiowitz - Journal of Microencapsulation 2012, Vol. 29, No. 4: 344–352.
- **Poly(methyl methacrylate) particulate carriers in drug delivery** Ana Bettencourt, António J. Almeida - Journal of Microencapsulation 2012, Vol. 29, No. 4: 353–367.
- **Swelling behaviour and controlled drug release from cross-linked Đ-carrageenan/NaCMC hydrogel by diffusion mechanism** Hadi Hezaveh, Ida Idayu Muhamad, Iman Noshadi, Lim Shu Fen, Norzita Ngadi - Journal of Microencapsulation 2012, Vol. 29, No. 4: 368–379.
- **Application of acid-catalyzed hydrolysis of dispersed organic solvent in developing new microencapsulation process technology** Honghwa Lee, Sunhwa Lee, Himanshu Bhattacharjee, Hongkee Sah - Journal of Microencapsulation 2012, Vol. 29, No. 4: 380–387.
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- **Preparation and characterization of amoxicillin mucoadhesive microparticles using solution-enhanced dispersion by supercritical CO2** Jayvadan Patel, Priyanka Patil - Journal of Microencapsulation 2012, Vol. 29, No. 4: 398–408.

ARTICLE

ALGINATE-BASED HYDROGELS FOR CELL MICRO-ENCAPSULATION: PHYSICAL, CHEMICAL OR HYBRID?

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CELL MICRO-ENCASULATION

Cell microencapsulation within three-dimensional (3D) biomaterials is a promising approach in biotechnology and medicine. Cell microencapsulation denotes the physical immobilization of cells within microspheres with diameters covering the range from 100 µm to 1 mm. The technology has been shown to be efficacious in mimicking natural environment of the cells. Thereby it improves the efficiency of the production of different metabolites, and it protects the encapsulated cells from both mechanical stress and the host immune system. Moreover, it provides a control over the passage of molecules across the biomaterials, as illustrated in Figure 1. Therefore, cell microencapsulation allows for further utilization of the entrapped cells to locally and continuously deliver therapeutic products, regenerate tissues, sustain organ functions, or develop bio-artificial organs.

However, and in spite of the promises and the broad field of application, cell microencapsulation nevertheless remains a challenging technology that requires the input from materials scientists, organic and physical chemists, biologists and physicians. This contribution discusses the technology of cell microencapsulation from the point of view of the materials utilized to entrap the cells. Only questions related to preparation and characterization of hydrogel microspheres will be briefly addressed.

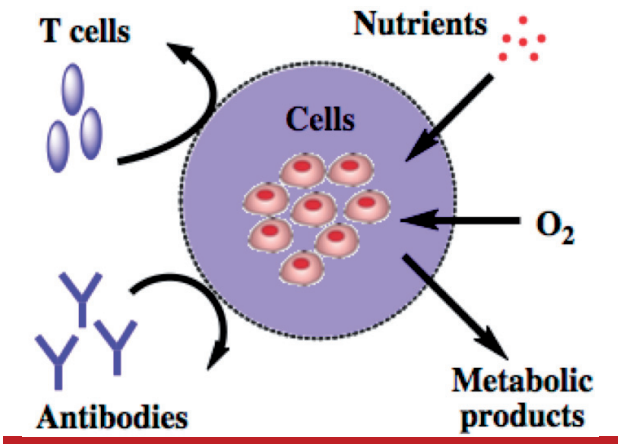

Figure 1. Schematic representation of the cell microencapsulation principle.

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HYDROGELS

Since the pioneering work of Wichterle and because of their hydrophilic character and potential to be biocompatible1, hydrogels have been of great interest among the materials potentially suitable to encapsulate cells. Hydrogels are soft three-dimensional networks prepared from polymers either physically or covalently cross-linked. Hydrogels contain a high percentage of water, more than 95 % in most cases. The encapsulation within hydrogels maintains cell viability and metabolic functionality, offers mechanical and potentially immune protection, which are major prerequisites to be satisfied when subsequent application in the biomedical field and targeted drug delivery is addressed.

HYDROGELS: PHYSICAL OR CHEMICAL?

Hydrogels are called "physical" or "reversible" if the networks are held together by molecular entanglements, or by secondary forces such as ionic interactions, hydrogen bonding, or hydrophobic interactions. Physical hydrogels are not always homogeneous, since clusters of molecular entanglements, hydrophobic or ionic domains can create inhomogeneity. Free chain ends or chain loops represent transient network defects in physical gels2.

When a polyelectrolyte is combined with a multivalent ion of the opposite charge, it may form a physical hydrogel known as "ionotropic" hydrogel. Further, when polyelectrolytes of opposite charges are mixed, they may gel depending on their constitution, concentrations, as well as the ionic strength and pH of the solution, as shown in Figure 2. The products of such gelation process are known as complex coacervates, polyelectrolyte complexes or simplexes.

Nowadays, hydrogels prepared from sodium alginate (Na-alg) remain the most reported physical hydrogels for cell microencapsulation due to the abundance of Na-alg in nature, its easy gelling properties and obvious biocompatibility. Although the suitability of other natural, modified natural and synthetic polymers and biomacromolecules is under investigation, none has reached the same level of performance as Na-alg. However, besides being relatively weak, all the associative forces involved in the formation of alg-based physical hydrogels can be disrupted by changing the physical conditions such as ionic strength, pH, temperature, or by application of mechanical stress.

A convenient approach to overcome these drawbacks is the preparation of chemically cross-linked hydrogels. For this purpose, a wide range of polymers having reactive groups such as acrylates or vinyl sulfone have been employed for the preparation of such 'permanent' or 'chemical' hydrogels. Other approaches using photosensitive starting material to prepare chemical hydrogels by radical polymerization were proposed. However, the

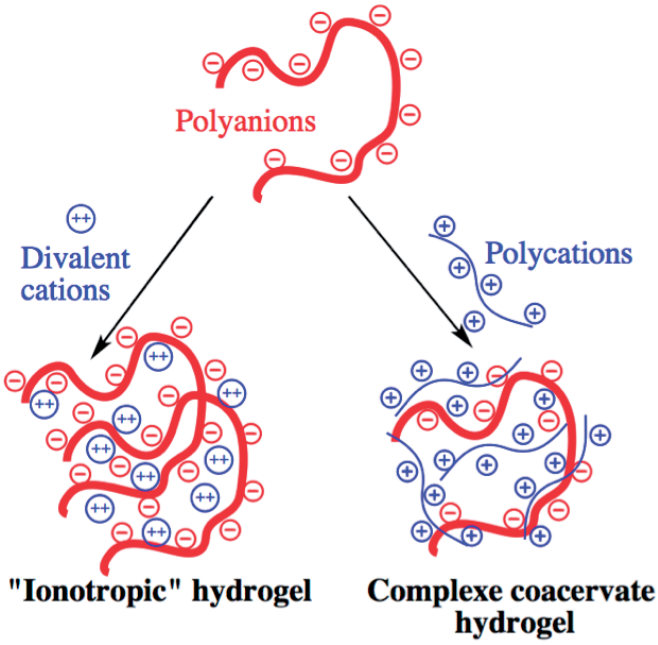

Figure 2. Schematic representation of two principles to form physical hydrogels.

presence of cells during the preparation of chemical hydrogels has significantly limited the number of adequate materials and preparation pathways. Indeed, the process of hydrogel formation has to be mild and must support cell integrity and viability.

Because cells are suspended in a liquid precursor solution prior to the encapsulation process, the choice of precursors is limited to water-soluble components. In addition, the aqueous solution must be buffered with appropriate osmolality to prevent cell lysis. Moreover, the rheological properties of the precursor solution are crucial to maintain cell viability and cell–cell adhesion during the encapsulation process. Mixing cells with highly viscous solutions can physically damage cell membranes because of the high shear stress.

DID YOU SAY HYBRID?

The preparation of hybrid hydrogels is emerging as a new and promising approach to prepare engineered hydrogels. The term "hybrid" refers to the mechanism by which the hydrogels are built, which combines the preparation of alginate-based physical hydrogel and simultaneous covalent cross-linking. The approach takes advantage of the fast ionotropic gelation of Na-alg in presence of divalent cations, which yields physical blending of polymers while prescribing the desired shape and dimension. Then the more time consuming –but still biocompatible and cell friendly- chemical cross-linking efficiently strengthens the microsphere and allows obtaining engineered hybrid hydrogels possessing well-defined physical characteristics. Two main systems have been investigated:

1. Multi-component systems

Multi-component systems denote hydrogels prepared from several polymers and biomacromolecules. Generally, aqueous solutions containing polymer precursors are mixed with Na-alg and finally extruded into a gelation bath. In this sense, a mixture of vinyl sulfone terminated poly(ethylene glycol) and Na-alg was utilized to prepare hybrid microspheres3. The combination yielded interpenetrating networks with well-controllable physical properties. Different preparation conditions were evaluated in terms of mechanical stability, swelling, and permeability of the microspheres.

Other technology entrapped synthetic methacrylate based polymers into physical microspheres, and subsequently allowed to form covalent network4. It has been demonstrated that either cross-linked shell or cross-linked core were obtainable by adjusting the molar mass of the cross-linker. Hybrid hydrogels were also

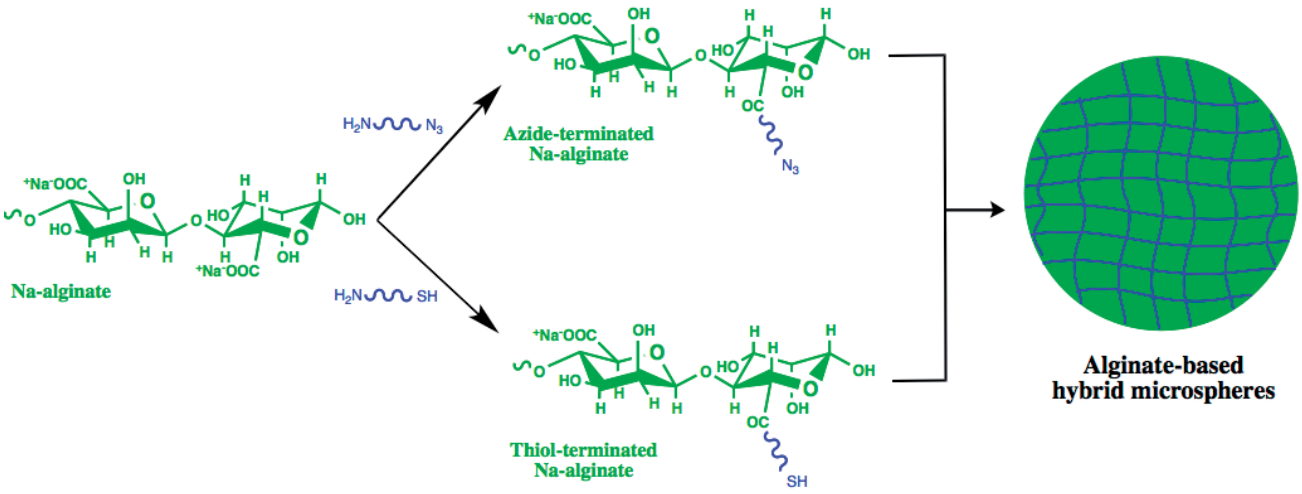

Figure 3. Functionalized Na-alg maintains the gelling capacity in presence of calcium ions and allows preparing chemical cross-linked networks.

prepared starting from fibrinogen and Na-alg. Primary human dermal fibroblasts were successfully encapsulated by using calcium ions and thrombin as cross-linkers. In comparison to fibrin gel, fibrin-alginate hybrid hydrogels have promoted higher cell proliferation and spreading.

Multi-component systems include also multi-step formation mechanism. For instance, genipin cross-linked alginate-chitosan hybrid microcapsules composed of an alginate core with a genipin cross-linked chitosan membrane were obtained in three steps: formation of Ca-alg beads, coating with chitosan, and finally cross-linked by incubation in an aqueous solution of genipin. It has been shown that the cross-linking by genipin substantially reduced swelling and physical disintegration of hybrid microcapsules induced by non-gelling ions and calcium chelating agents. Higher resistance to mechanical shear force and improved durability against enzymatic degradation were achieved.

2. One-component system

As the name suggests, one-component systems denote hybrid hydrogels prepared from a single macromolecule able to form both physical and chemical links. As native Na-alg unable to form chemical hydrogels, the research focused on equipping Na-alg with chemically reactive side chains, as shown in Figure 3. For instance, the functionalization of Na-alg with a side chain having azide end group has been successfully performed5. The presence of the azide end group allowed for subsequent chemical cross-linking via the chemoselective Staudinger gelation. This was achieved by incubation in a gelation bath containing phosphine-functionalized agents. Hybrid hydrogels of spherical shape exhibiting tailored characteristics, such as water uptake and mechanical resistance were obtained. Moreover, the nontoxic nature of the Staudinger ligation allowed for the microencapsulation of several cell types.

Similar approach has focused on the preparation of Na-alg with thiol end groups6-7. The modified Na-alg maintained the gelling capacity in presence of calcium ions, while thiol end groups allowed for preparing chemically cross-linked hydrogel via disulfide bond formation. Being biocompatible, spontaneous, and catalyst free, the formation of disulfide bond successfully allowed to form hybrid microspheres in a one-step extrusion process under physiological temperature, pH, and osmolality. Good survival rate and improved proliferation were obtained upon microencapsulation of liver-derived cells within hybrid microspheres. Moreover, albumin secretion confirmed the suitability of these hybrid microspheres for the microencapsulation of cells.

OUTLOOK

Although there is no doubt about the advantageous properties of Na-alg, alginate-based physical hydrogels frequently suffer from mechanical stability deficiency, durability issues, and permeability drawbacks. The most utilized approach to overcome these problems is the subsequent coating of the initially formed hydrogels with polycations. Despite known biocompatibility issues related to the presence of positively charged surfaces, the method is still used for many studies mainly because of the lack of engineered hydrogels and the systematic use of preexisting material with only limited adaptation to an intended application. Therefore, the preparation of hybrid hydrogels appears today as very attractive approach. The studies conducted in our lab are aiming to prepare engineered hybrid microspheres, establish fundamental composition-properties relationships, elaborate reproducible preparation conditions, and demonstrate the suitability of the material for cell microencapsulation for ultimate use in transplantation and development of bio-artificial organs. The results of the research are expected to contribute to progress in cell microencapsulation technologies.

REFERENCES

1. Wichterle, O., Lim, D. Hydrophilic gels for biological use, Nature, 185 (1960) 117-118.
2. Hoffman AS. Hydrogels in biomedical applications, Adv Drug Deliv Rev, 43 (2002) 3-12.
3. Mahou, R., Wandrey, C. Alginate–poly(ethylene glycol) hybrid microspheres with adjustable physical properties, Macromolecules, 43 (2010) 1371-1378.
4. Mazumder, M.A.J., Burke, N.A.D., Shen, F., Potter, M.A., Stover, H.D.H. Core crosslinked alginate microcapsules for cell encapsulation, Biomacromolecules, 10 (2009) 1365-1373.
5. Gattas-asfura, K., Stabler, C.R. Chemoselective cross-linking and functionalization of alginate via staudinger ligation, Biomacromolecules, 10 (2009) 3122-3129.
6. Mahou, R., Wandrey, C. Versatile route to synthesize heterobifunctional poly(ethylene glycol) of variable functionality for subsequent pegylation. Polymers, 4 (2012) 561-589.
7. Mahou, R., Tran, N.M., Dufresne, M., Legallais, C., Wandrey, C, Encapsulation of Huh-7 cells within alginate-poly(ethylene glycol) hybrid microspheres, J Mater Sci Mater Med., 23 (2012) 171-179.

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After obtaining a Master degree in chemistry at the university of Fribourg, Switzerland, Redouan Mahou earned his Ph.D. degree in Chemistry and Chemical Engineering from EPFL, with his thesis addressing the development of new hydrogels suitable for cell microencapsulation. Currently he is a postdoc fellow at the laboratory for regenerative medicine and pharmacobiology, EPFL. Current research is directed toward polymer chemistry and cell biology ultimately applied in tissue engineering, cell-based therapies, and drug delivery

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ARTICLE

CELL ENCAPSULATION FOR COMBINATORIAL STEM CELL BIOLOGY

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INTRODUCTION

Electrospray techniques have become established in the life sciences for uses from cell encapsulation (Chang, 1964) to directed cell placement in more recent times (Jayasinghe et al., 2006). One major field of work is the use of electrospraying to create cell encapsulations. During this process, a conducting fluid in a needle connected to a high voltage power supply is charged and then drawn towards a grounded electrode by an electric field resulting in fine droplets which are then chemically cross-linked creating encapsulations. Cells and other materials can be encapsulated by suspending them in an alginate solution and electrospraying directly into a solution containing of a crosslinking agent, most commonly calcium chloride. This technique can be used to directly process and encapsulate many different types of materials (Jayasinghe and Townsend-Nicholson, 2006, Jayasinghe, 2007, Patel et al., 2008).

This research has adapted this general approach further and progressed it for use in combinatorial stem cell culturing. Combinatorial Cell Culture is a system designed to significantly reduce the number of experiments and iterations as well as the time needed for cell culturing. In traditional cell culture, to observe the effect of a combination of certain growth factors and chemicals on the development of cells, a single experiment is done for each set of conditions. In contrast, a combinatorial technique uses different conditions concurrently to explore a large number of permutations simultaneously (Choo, 2008). CombiCult works by using a large matrix of conditions in a single experiment to explore a vast number of permutations simultaneously.

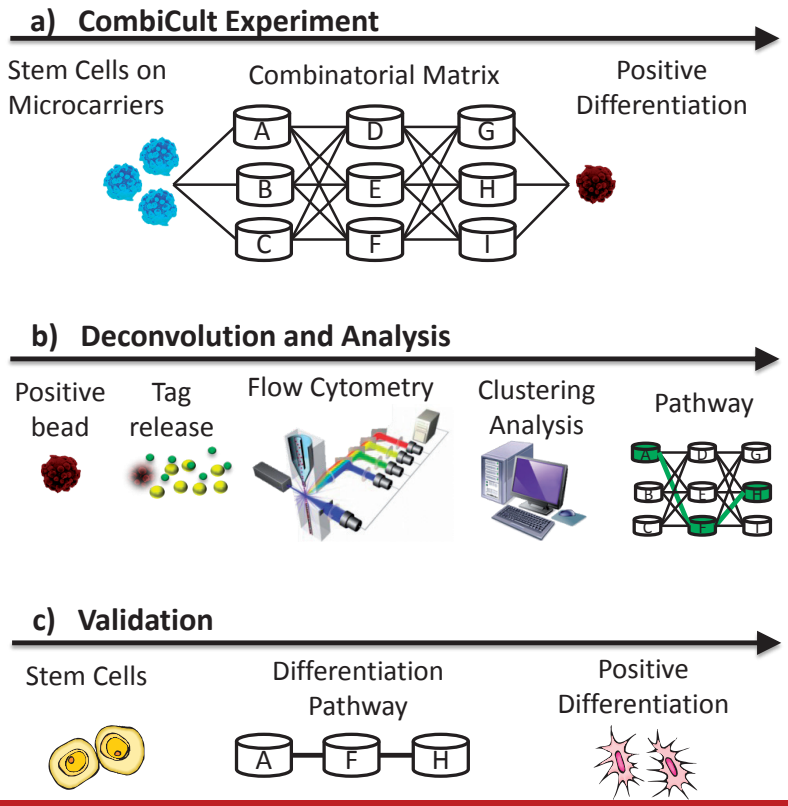


Figure 1: Diagram illustrating the CombiCult workflow. The first step (a) is to conduct a CombiCult experiment using a matrix of conditions aimed to direct differentiation. Any positive beads are then analysed (b) by first releasing the tags from the microcarriers. These are then measured and analysed using a flow cytometer and specialised software. The pathways discovered from the data are then validated manually in the final stage (c).

Microcarriers are used to put cells through the matrix and cover all permutations. Once the experiment is completed and the cells are fixed, genetic markers or antibody staining can reveal the beads which successfully differentiated to the cell type intended. These are sorted from the bulk of the sample by large particle flow cytometry. The set of conditions each successful bead was exposed to over the duration of the experiment is then read, again through flow cytometry, and the resultant data set analysed for correlations. From this analysis, differentiation pathways can then be selected and validated manually.

To be able to use a combinatorial approach, it is necessary to divide cells into separate units which can be encoded with information identifying their previous culturing conditions. Previously, this was done by seeding

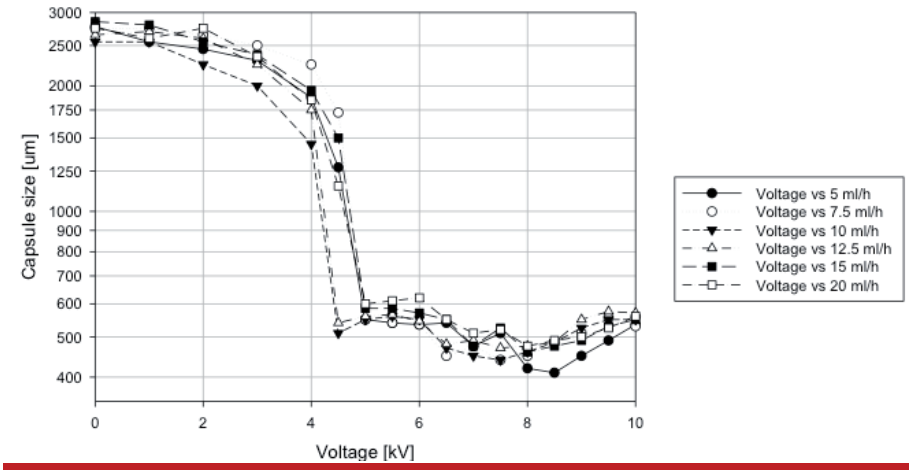


Figure 2: Nisco Encapsulator 2% alginate operational map, 0-10kV, using the standard dish electrode at 1cm distance to a 0.5mm flat ended spraying needle.

cells onto porous microcarriers. This however, limits the technology to using cell lines which readily adhere to the surface of these microcarriers. Electrospray encapsulations on the other hand do not suffer from this shortcoming as virtually any type of cell or material which can be suspended in an aqueous solution containing alginate can be encapsulated.

The encapsulator used in these experiments is a Nisco Var-V1. Cell suspensions were prepared by recovering the cells from their culture vessel and suspending them at a predetermined concentration in a solution of 2% alginate in PBS. This suspension is then transferred into a syringe which is driven by a Harvard PHD2200 syringe pump. Spraying was performed at a wide variety of different voltages and flowrates. The sprayed droplets are solidified using 200mM Calcium Chloride as a divalent cross-linking ion and collected directly after spraying.

Once the cell encapsulations have fully solidified and collected from the cross-linking solution, they can be washed in DPBS or standard medium and cultured according to standard cell culture protocols. Due to the nature of the cross-linking however, it is not advisable to culture or wash alginate encapsulations in calcium free buffers or media for prolonged periods of time as this may remove some of the Calcium ions from the cross-linked gel and liquefy the gel.

In order to encode information for Combinatorial Stem Cell biology, it is necessary to create structures with multiple layers over an extended period with fluorescent markers contained within the layers. These additional layers are created at each of the culturing/encoding stages through an electrostatic layer by layer adsorption process. The presence of these fluorescent tags allows the encoding of information which can then subsequently be recovered once the experiment is completed and beads with positive differentiation have been identified.

Previous work has established the viability of different types of electrosprayed cells ranging from primary cells to embryos and stem cells by means of FACS scans (fluorescence activated cell sorting), RT/PCR (real time polymerase chain reaction), and surface molecule studies. This has been shown on different scales from entire embryos (Clarke and Jayasinghe, 2008) and individual cells (Eagles et al., 2006) all the way to the genetic level (Barry et al., 2008, Hall et al., 2008). Viability was also found to be unperturbed for the alginate encapsulations in these experiments, results confirmed by other groups (Basta et al., 2004, Ma et al., 2003, Peirone et al., 1998). Encapsulations and Layer-by-layer tagging for CombiCult were performed on a number of cells, both mouse and human, and ranging from embryonic stem cells to mesenchymal and hematopoietic stem cells. The following micrographs illustrate the

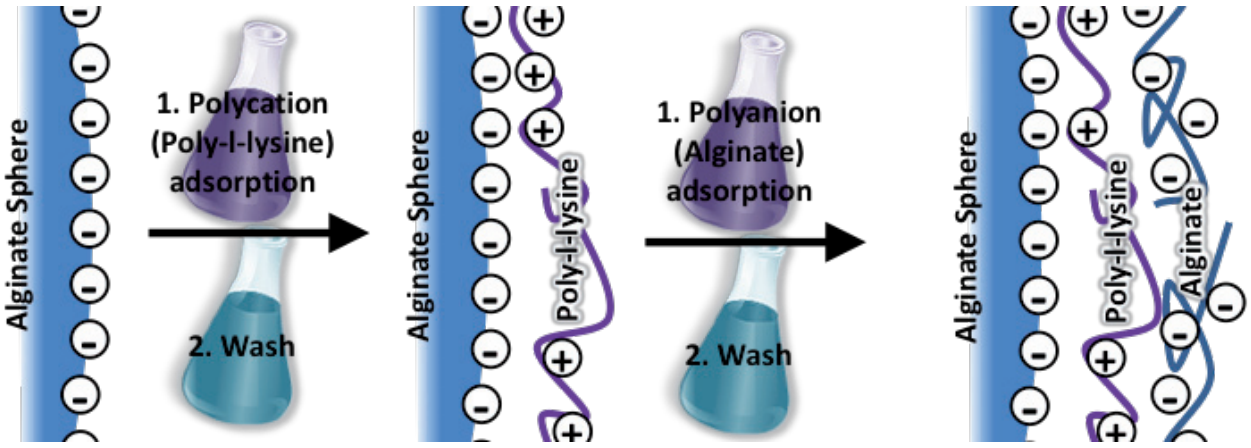


Figure 3: Polyelectrolyte adsorption, adapted from Peyratout and Dahne (2004). The initial alginate microsphere has a anionic surface upon which the polycationic PLL can form a layer through adsorption. After adsorption, excess PLL solution is washed off before the beads, which now have a cationic surface, are placed in a solution of polyanionic alginate which creates another layer through adsorption. These steps can be repeated to add additional layers. Where tagging is required, tags of either polarity can be included at the appropriate steps.

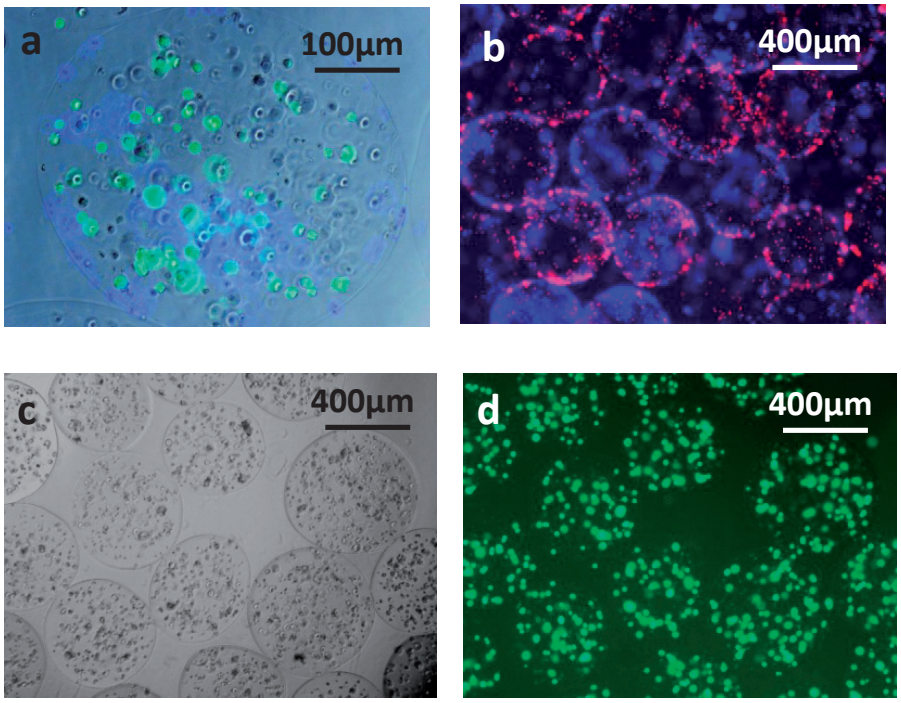


Figure 4: Micrographs of Alginate Encapsulations. Panel A shows an individual alginate bead containing mouse embryonic stem cells green fluorescent through Calcein AM and tagged with blue fluorescent microcarriers. Panel B shows a number a microbeads tagged with two different types of tags through Layer-by-layer adsorption. Panels C and D show the same culture of embryonic stem cells in bright field and Calcein AM fluorescence illustrating viability.

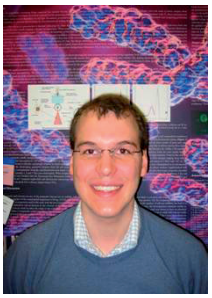
resultant cell encapsulations showing both stem cells and fluorescent tags.

The newly developed technique has both advantages and disadvantages over the current technology. One of the central advantages of using the alginate encapsulation and layering system is that it can handle different and more types of cells than the existing CombiCult technology. Alginate encapsulations can be prepared of virtually any cell type, both adherent and non-adherent, regardless of their natural preferred substrates or culturing surfaces.

The novel encapsulation and encoding technique outlined here has a number of advantages over the currently available technology and has been filed as patent PCT/EP2010/006459.

REFERENCES

1. Barry S. P., Jayasinghe, S. N., Pericleous, C., Hubank, M., Lactman, D. S. & Stephanou, A. (2008) Gene expression studies on bio-electrosprayed primary cardiac myocytes. Biotechnol J, 3, 530-5.
2. Chang,T.M.S.(1964)Semipermeable Microcapsules. 146, 524 - 525.
3. Choo, Y. (2008) Use of Combinatorial Screening to Discover Protocols That Effectively Direct the Differentiation of Stem Cells. IN Shi, Y. & Clegg, D. O. (Eds.) Stem Cell Research and Therapeutics. Springer Science + Business Media.
4. Clarke, J. D. & Jayaginghe, S. N. (2008) Bio-electrosprayed multicellular zebrafish embryos are viable and develop normally. Biomed Mater, 3, 11001.
5. Eagles, P. A., Qureshi, A. N. & Jayaginghe, S. N. (2006) Electrohydrodynamic jetting of mouse neuronal cells. Biochem J, 394, 375-8.
6. Hall, R. P., Ogilvie, C. M., Aarons, E. & Jayaginghe, S. N. (2008) Genetic, genomic and physiological state studies on single-needle bio-electrosprayed human cells. Analyst, 133, 1347-51.
7. Jayaginghe, S. N. (2007) Bio-electrosprays: the development of a promising tool for regenerative and therapeutic medicine. Biotechnol J, 2, 934-7.
8. Jayaginghe, S. N., Eagles, P. A. & QURESHI, A. N. (2006) Electric field driven jetting: an emerging approach for processing living cells. Biotechnol J, 1, 86-94.
9. Jayaginghe, S. N. & Townsend-Nicholson, A. (2006) Stable electric-field driven cone-jetting of concentrated biosuspensions. Lab Chip, 6, 1086-90.
10. Patel, P., Irivine, S., Mcewan, J. R. & Jayaginghe, S. N. (2008) Bio-protocols for directly forming active encapsulations containing living primary cells. Soft Matter, 4, 1219 - 1229.
11. Payraroute, C. S. & Dahne E, L. (2004) Tailor-made polyelectrolyte microcapsules: from multilayers to smart containers. Angew Chem Int Ed Engl, 43, 3762-83.



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ARTICLE

POLYELECTROLYTES ENCAPSULATION OF COMPLEX EMULSIONS FOR BIOMEDICAL APPLICATIONS

A. Elaissari, Lagep, Claude Bernard University, Villeurbane, France

INTRODUCTION

The encapsulation science is of a paramount importance in various domains particularly in foods, cosmetics, therapy, in vivo and in vitro biomedical diagnostic. In vivo bio-related applications, it is of necessity to encapsulate small organic molecules (e.g., fluorescent dyes), proteins for immunotherapy, nucleic acids for gene therapy, labels (inorganic nanocrystals) for molecular living cells imaging, or to design well defined particles for in vitro to extract biomolecules contained in a complex biological sample (blood, tissue, sputum, etc.) For these purposes, various encapsulation processes have been developed using chemistry (polymerization process), physical chemistry of polymers (polymer nature in a given conditions) and polymer at interfaces (adsorption).

Regarding to polymerization process, the encapsulation of oil in water emulsion containing inorganic nanoparticles, via seed polymerization or miniemulsion polymerization, remains a challenge in the research area [1]. Such processes lead to various particles morphologies e.g. anisotropic (Janus like or moon like), and in well-controlled reaction conditions to perfect core-shell hybrid particles [2].

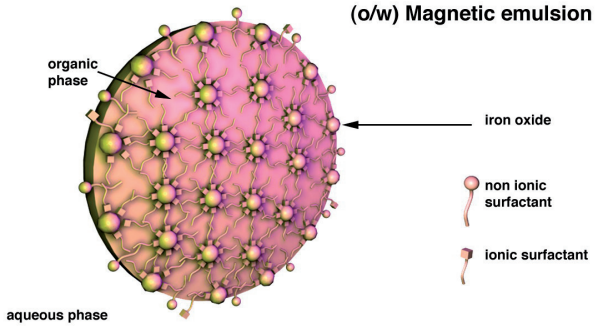

Figure 1 : Schematic illustration of oil in water magnetic emulsion

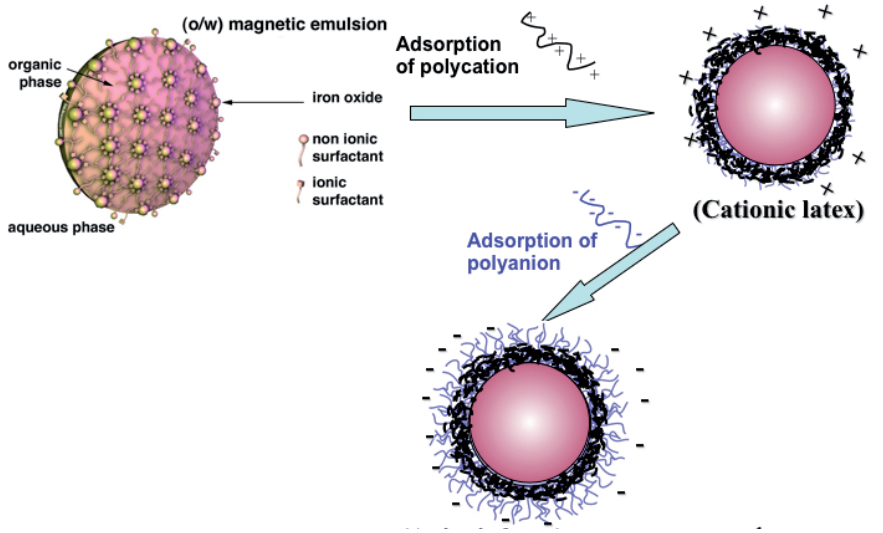

Figure 2 : Schematic illustration of sequential polyelectrolyte adsorption [4]

Consequently, the physico-chemical approach was used in order to induce surface modification and seed encapsulation of oil in water magnetic emulsions. The surface modification can be induced via various processes; the first and the commonly used method depends on polymers adsorption. Interestingly, using preformed polymers and mainly polyelectrolytes can be considered as a universal process leading to well encapsulation for charged seeds such as emulsions or complex emulsions (i.e. inorganic nanoparticles dispersed in organic phase dispersed in water) mainly oil in water systems.

The encapsulation of colloidal particles using sequential adsorption of oppositely charged polyelectrolytes is new methodology and promising process easy to conduct. In fact, this process is based on sequential adsorption of polyelectrolytes (one cationic and the second anionic) via attractive electrostatic interactions. The first polyelectrolyte should be oppositely charged compared to the used emulsion seed. After removal of the free polyelectrolyte molecules, the second polyelectrolyte was then adsorbed on the first layer. This layer-by-layer (Layer-by-layer) adsorption process [3] leads to polymer shell formation surrounding the used seed. For illustrating such behaviour, this encapsulation process has been recently used to encapsulate complex oil in water magnetic emulsion [4]

The surface charge density of the used seed was related to the used stabilizing agent. As a general tendency, magnetic emulsions are negatively charged, and in turn they need the use of cationic polyelectrolyte. In order to avoid aggregation phenomenon induced by bridging flocculation of the adsorbed oppositely charged polyelectrolyte, the magnetic emulsion was dropwisely added in high concentrated polyelectrolyte solution. The removal of free polyelectrolyte (unreacted) is of great importance, since the residual polyelectrolytes leads to nanoparticles formation during the addition of the second oppositely charged polymer.

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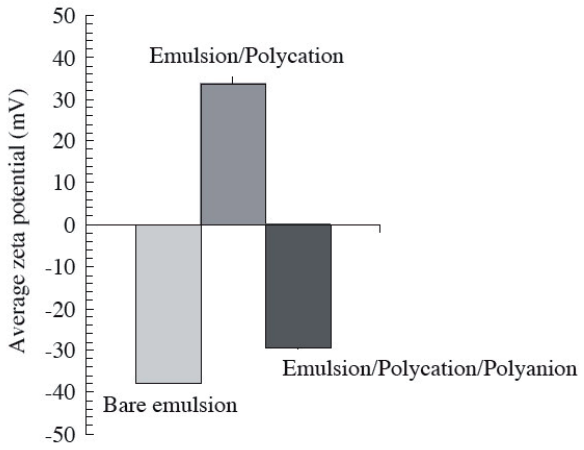

Figure 3: Zigzag like zeta potential profile of magnetic emulsion as a function of polyelectrolyte adsorbed layer nature [4]

POLYELECTROLYTE ADSORPTION

The adsorption of polymers onto colloidal particles was widely used in numerous applications in order to induce surface modification. In fact, because of their interest in various industrial domains, the interactions between polymer chains and colloidal dispersions were extensively investigated in terms of thermodynamic and kinetic aspects [5,6]. The effect of polymer adsorption on the stabilization/destabilization of the colloidal dispersion is the most studied subject. In this area, the most encountered problem was related to aggregation phenomena, which induced during the adsorption step. The adsorption can be governed by various interactions such as, hydrophobic forces, Van der Waals, hydrogen bonding and electrostatic attractive interactions. Since the colloidal stability of the dispersions bearing adsorbed polymer is of paramount importance, the conformation of interfacial polymer was examined as a function of various parameters such as, polymer molecular weight, polymer micro-structure, interactions type and physico-chemical properties of the adsorption medium (i.e. pH, salinity, temperature and dispersing medium nature).

In order to avoid aggregation during the adsorption process, two main criteria should be considered: (i) the polymer concentration in the adsorption medium should be largely above the polymer amount needed to reach particles surface saturation (i.e. above one adsorbed monolayer) and (ii) the adsorption should be performed by adding particles dispersion drop by drop into concentrated oppositely charged polymer solution.

The encapsulation process of emulsion droplets via layer-by-layer electrostatic assembly of polyelectrolytes was recently reported by Elaissari et al [4] to elaborate highly magnetic submicron colloidal particles (Figure 2). The methodology is based on sequential adsorption processes of oppositely charged polyelectrolytes onto charged seed particles.

Various works have been reported using numerous polyelectrolytes such as polystyrene sulfonate/poly(diallyldimethylammonium chloride) PSS/PDADMAC-coated polystyrene latex particles [3]. In such studies, the drastic point is to prevent aggregates formation during the adsorptions steps. Consequently, polymer concentration and mixing step of the colloidal particles and polymer solution are incontestably of paramount importance.

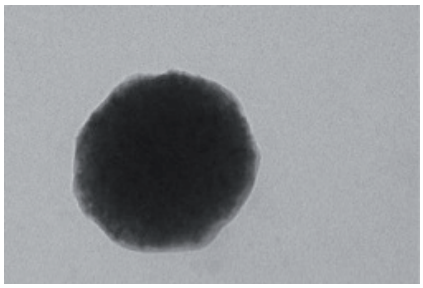

Figure 4: Transmission electron micrograph of layer-by-layer encapsulated magnetic emulsion. [4]

Based on the above-mentioned criteria, the surface modification of negatively charged magnetic emulsion for instance was investigated as a function of pH and polycation concentration. Using high polycation concentration surpassed the aggregation of magnetic nanodroplets. The adsorption of polycation onto negatively charged magnetic emulsion was evidence by measuring the zeta potential as a function of pH of the dispersing medium and as a function of polycation concentration.

As previously mentioned, the drop-by-drop addition process of colloidal dispersion leads to good colloidal stability and homogeneous functionalized particles. Then, the polyanion adsorption on the obtained cationic magnetic emulsion was performed by adding slowly the colloidal dispersion to the highly concentrated polyanion (i.e polyacrylic acid polymer). The driving forces involved in the adsorption process are the combination of attractive electrostatic interactions and hydrogen bonding. The surface charge inversion was easily examined by measuring the zeta potential at a given pH and as function of added polyelectrolyte layer as shown in Figure 3. The zigzag profile of the zeta potential versus number of adsorbed layers was found to be conserved at less until ten adsorbed layers, as reported [4] revealing the sequential polyelectrolytes adsorption..

The particle size was not drastically affected since the thickness of the adsorbed layer is only few nanometres in the dried state. The layer thickness can be easily investigated by transmission electron microscopy as below illustrated in Figure 4. Due to the bridging flocculation of adsorbed polyelectrolytes, the presence of some clusters was not excluded, which needs filtration step before any colloidal characterization.

CONCLUSION

The encapsulation of complex emulsions via layer-by-layer (Layer-by-layer) adsorption of polyelectrolytes revealed to be of paramount importance process leading to new colloids.

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In fact, the sequential adsorption of the oppositely charged polyelectrolytes onto the oil in water nanodroplets was found to induce encapsulation efficiency of complex colloidal systems such as oil in water dispersion containing inorganic nanoparticles or nanocrystals. In fact, the homogeneous and well-defined polymer shell was clearly viewed by transmission electron microscopy analysis and changes of surface charge density of the colloidal dispersions. Accordingly, such process can be extended to the encapsulation of more complex systems leading to fascinating colloidal dispersions.

The encapsulated oil in water magnetic emulsion was found to be interesting as support of biomolecules for in vitro biomedical diagnosis (i.e. ELOSA, ELISA), specific and non- specific nucleic acid capture, purification and extraction, viruses extraction and detection results in improved sensitivity. In addition, such submicron dispersions will be more convenient to solve various problems in microfluidic and automated systems for bionanotechnology applications.

REFERENCES

1. Rahman, M., M; Elaissari, A., Organic–Inorganic Hybrid Magnetic Latex. In HYBRID LATEX PARTICLES, van Herk, A.; Landfester, K., Eds. Springer: 2011; Vol. 233, pp 237-281.
2. F. Montagne, O. Mondain-Monval, C. Pichot, A. Elaissari, Journal of Polymer Science: Part A: Polymer Chemistry 44 2642-2656, 2006.
3. F. Caruso, Vol. 227, 2003) p. 145168.
4. R. Veyret, T. Delair, A. Elaissari, J.Magn.Magn.Mater. 293, 171-176, 2005
5. P.G. de Gennes, Cornell University Press, Ithaca and London (1979).
6. R. Varoqui, A. Johner and A. Elaissari, J.Chem.Phys. 94 (1991) 6873.
7. F. Caruso, R. A. Caruso, H. Möhwald Science Vol.282 - 6 November 1998

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Abdelhamid Elaissari is Director of research at CNRS, received his undergraduate education from Agadir University, Morocco in 1988. He moved to the Institute Charles Sadron, Louis Pasteur University, Strasbourg-France in which he received his Ph.D. degree in chemical physics of polymers and colloids in 1991. In the same year, he got a permanent position in CNRS and then he joint CNRS-bio-Mérieux laboratory (semi-academic laboratory in ENS-Lyon, France) until 2007. During this period, Dr. Elaissari has developed various aspect related to colloids from synthesis to in vitro biomedical diagnostic applications.

Now he is vice director of the Engineering Processes and Automatic Laboratory (LAGEP), which is three parts collaboration between CNRS, Claude Bernard University of Lyon and CPE-engineering School. In this famous Academic laboratory (CNRS-University of Lyon-1), Dr. Elaissari is conducting fundamental research with purposes applied on the elaboration of reactive and stimuli-responsive colloids for biomedical, environmental and bionanotechnological applications and also on the elaboration of nanoparticles and nanocapsules for drug delivery, cosmetics and veterinary field. Dr. Elaissari is also in charge of Direction of International Relations and Promotion of Doctoral Studies in Chemistry in University of Lyon-1, and he is also acting as European Editor for Journal of Biomedical Nanotechnology (JBN).

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POLYELECTROLYTE COMPLEXATION: IS IT ADVANTAGEOUS FOR PREPARING NANOPARTICLES?

Susana Rodrigues, Marita Dionísio, Ana Grenha - University of Algarve, Faro, Portugal

INTRODUCTION

Nanotechnologies are nowadays very popular in many different areas, from electronics, to more consumer-contact fields, including food industry, cosmetics and, generally, pharmaceutics. Such a small scale of work has led to an important outcome since the very beginning: the fact that it demanded collaborative work between disciplines that were typically segregated before, like engineering, physics, molecular biology and chemistry, thus initiating fruitful cooperation. For some of the considered applications, nanotechnologies are designed to confer protection to a determined molecule that is incorporated inside a nanodevice and, in many cases, it is further expected to permit a release of the referred molecule according to controlled or prolonged kinetic profiles.

Nanodevices might display different structures and compositions, and include several different systems like nanoparticles or liposomes, among others. The former may be composed of many different materials, from polymers and lipids, to inorganic compounds. Polymeric nanoparticles are those gathering the greatest deal of attention, being composed of polymeric materials, either of synthetic or natural origin. It is now very frequent to observe the application of nanoparticles to improve the properties of nutritional additives, to provide stronger or more stable flavours and colours, or to simply enhance the penetration of active substances to the deeper layers of the skin. However, in the strict pharmaceutical area, the list of commercially available products becomes very narrow, mainly because of regulatory hurdles to demonstrate their safety for human use. Nevertheless, the number of pharmaceutical applications proposed for nanoparticles is constantly increasing, being reflected in a very prominent number of papers and patents, as well as formulations undergoing clinical trials.

NANOSIZED POLYELECTROLYTE COMPLEXES

The great interest around nanoparticles relies on several advantages displayed by these systems (Table 1). Among the most important, it is worth mentioning the increased surface-to-volume ratio that tremendously increases the contact surface with the surrounding environments, the usually high loadings of encapsulated molecules, and the ability to enter intracellular compartments, although in a size-dependent manner [1]. As referred above, polymeric nanoparticles can have a multitude of compositions and, therefore, many techniques have been described that permit their production. In most cases, bottom-up fabrication processes are the leading techniques, which involve the assembly of molecules in solution to form the nanoparticles. In this context, techniques based on emulsification are very popular and, thus, frequently used. However, they necessarily involve the use of organic solvents and, very often, further include other aggressive preparation conditions like the application of ultrasound energy, thereby limiting their use.

These limitations have shifted the interest towards softer methodologies and, in this context, polyelectrolyte complexation is one of the methods gathering more popularity, presenting the great advantage of permitting the reaction to occur in a complete hydrophilic environment and, therefore, being classified as a very mild technique. As the name suggests, attractive interactions between two oppositely charged electrolytes induce the formation of interpolymer complexes, as depicted in Figure 1. As the interaction occurs, and provided that the concentration of the involved electrolytes is adequate, nanometric complexes are formed that comprise the nanoparticles [2]. The interaction established between the polymeric networks is mainly based on strong, but reversible, electrostatic links, hence resulting in non-permanent structures that are, therefore, more sensitive to changes in environmental conditions [3, 4]. Nevertheless, hydrogen bonding, hydrophobic interactions and van der Waals forces also complement the overall interaction taking place between the macromolecules [4].

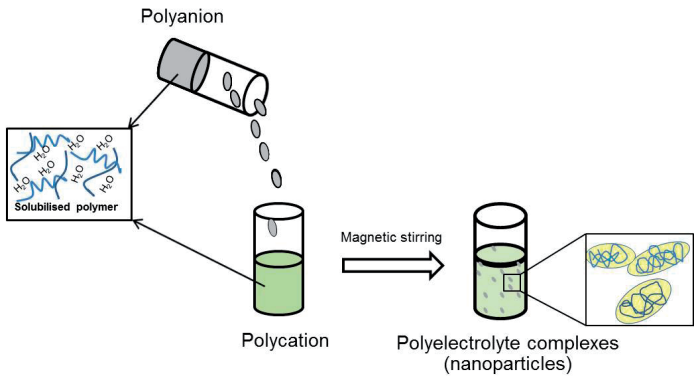

Figure 1. Schematic representation of the assembly of polyelectrolyte complexes.

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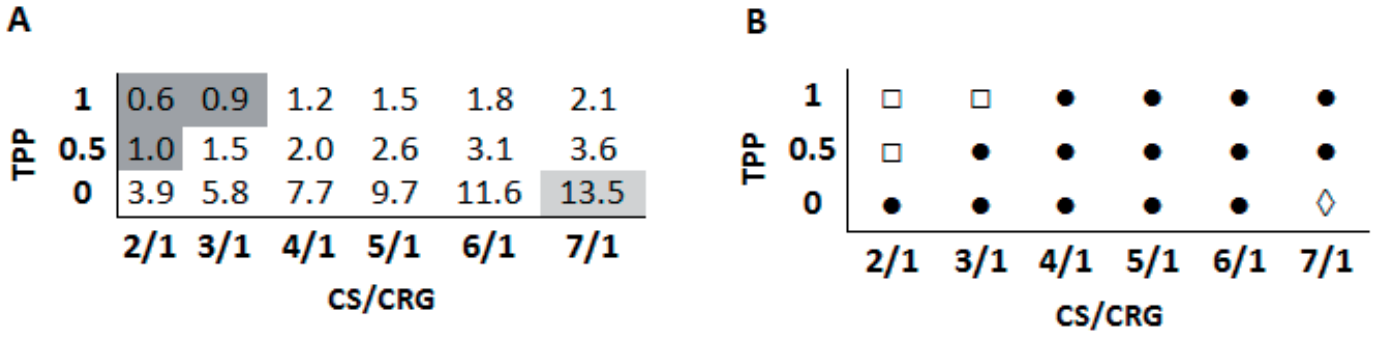

Figure 2. Representative scheme of (A) positive/negative charge ratio of different chitosan/carrageenan/tripolyphosphate (CS/CRG/TPP) nanoparticle formulations (white fill: formation of nanoparticles; dark grey: precipitation; light grey: inability to form nanoparticles); (B) the ability to form nanoparticles according to formulation composition: precipitation (□); nanoparticles (●); solution (◊). Adapted with permission from [5].

OPTIMISING NANOPARTICLE PRODUCTION

From our experience with this methodology, and also taking into account the literature reports on the subject, several aspects inherent to the preparation procedure should be mentioned that affect the final properties of the nanoparticles. The degree of ionization of the used polyelectrolytes in the moment of the formation of the complexes, obviously plays a major role. Therefore, the charge density of each polymer and its distribution along the polymeric chain, as well as the concentration of each polyelectrolyte that is used for the reaction, are extremely relevant. In addition, the relative polymeric mass ratio that is selected is a very pertinent issue, as this will directly affect the charge ratio and, necessarily, the final properties of the nanoparticles. In fact, the charge ratio will determine the range of conditions leading to the successful production of nanoparticles.

In a recent study by our group in which we produced chitosan/carrageenan/tripolyphosphate nanoparticles, the first material being positively charged and the other two evidencing negative charge, it was observed that a positive to negative (+/-) charge ratio around 1 resulted in precipitation rather than in the formation of nanoparticles, as is shown in Figure 2. On the contrary, very high charge ratios did not allow the formation of any structure. The precipitation is due to the fact that, at lower charge ratios, there is an excess of anionic charges, leading to neutralisation of chitosan positive charges and, therefore, reducing/eliminating electrostatic repulsion, which finally results in aggregation/precipitation [5]. Nevertheless, it should be recalled that a 1:1 +/- charge ratio does not imply necessarily a complete charge neutralisation, due to different charge spacing in the intervenient species and to steric constraints [6].

In turn, the absence of nanoparticle formation at high charge ratios should be attributed to an insufficient number of opposite charges to induce the interaction that leads to the formation of a defined structural entity [5]. Although this example is based on chitosan, undoubtedly one of the polymers that mostly represent the technique of polyelectrolyte complexation, especially concerning nanoparticle production, these observations can roughly be extended to all the polymers used in the formation of polyelectrolyte complexes.

Several other aspects should be mentioned that might affect the process of polyelectrolyte complexation for nanoparticle formation. These include the order followed for the polymer mixture, their molecular weight, the temperature at which the reaction takes place, the pH and ionic strength of the reaction medium and, occasionally, even the chain flexibility and the duration of the interaction. In addition, some parameters usually assumed to have irrelevant contributions, such as the stirring pattern and the conditions of centrifugation, are in this case known to influence the overall process and the corresponding final outcome.

Although the formation of the polyelectrolyte complexes is known to take place instantaneously when two oppositely charged electrolytes are mixed together [4], the stirring speed is an important parameter to control, as insufficient stirring will not permit a complete and even interaction of the involved materials, leading to a more reduced number of formed particles with less homogeneous size. A centrifugation step should be always performed to separate the formed nanoparticles from the reaction medium that most probably contains free polymeric materials that did not interact to provide the formation of nanoparticles. This leads to the concentration of nanoparticles in a pellet, demanding a subsequent step of re-suspension (in water, generally), so that the nanoparticles reassume their original properties. The speed and duration of the centrifugation cycle should be controlled in order to maximise the amount of nanoparticles that is deposited and, thus, recovered. In addition, most of the materials will not permit a successful recovery of nanoparticles if an adequate substrate layer is not used to allow nanoparticle deposition during centrifugation. To overcome this limitation, it is usual to perform the centrifugation of nanoparticles over a layer of glycerol, which will embed the nanoparticles and enable an easy and effective re-suspension.

Polyelectrolyte complexation has, therefore, many parameters demanding optimisation so that the resulting systems evidence the adequate properties for a determined application. Apart from some advantages mentioned before, a very important achievement of this technique is that it is scalable, potentially permitting an industrial application without a very laborious optimisation. In this sense, our group has recently observed the successful increase of scale of the production of chitosan/carrageenan/tripolyphosphate nanoparticles up to 120 times

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without observing expressive alterations in nanoparticle physicochemical properties considering their intended use (a size increase from 300 nm to 400 nm was observed; zeta potential remained unaltered).

Table 1 - Potential advantages of nanoparticles

High surface/volume ratio
Protection of encapsulated molecules
Ease of surface modification
Possibility to provide controlled release
Possibility of targeted delivery
High loading of molecules
Maximized contact with surrounding environment
Ability for cell internalisation

Many materials have been reported to be used for nanoparticle production by this method of polyelectrolyte complexation, including several categories such as polysaccharides (chitosan and derivatives, alginate, carrageenans, hyaluronic acid, arabic gum, carboxymethyl cellulose, chondroitin sulfate, cyclodextrins, dextran sulfate), proteins (gelatin and insulin), DNA and also synthetic materials (polyacrylic acid). Roughly, it can be said that any material evidencing a charge and holding attractive properties for a determined end, can be used in this context. As demonstrating the potential of the technique, a wide variety of applications has been described responding to demands from different areas, from biotechnology and biomedical related fields to environmental areas.

REFERENCES

1. Kumari, A.; Yadav, S. K.; Yadav, S. C., Biodegradable polymeric nanoparticles based drug delivery systems. Colloids and Surfaces B: Biointerfaces 2010, 75, 1-18.
2. Grenha, A., Chitosan nanoparticles: a survey of preparation methods. Journal of Drug Targeting 2012, 20, 291-300.
3. Hamman, J. H., Chitosan based polyelectrolyte complexes as potential carrier materials in drug delivery systems. Marine Drugs 2010, 8, 1305-1322.
4. Hartig, S.; Greene, R.; Dikov, M.; Prokop, A.; Davidson, J., Multifunctional nanoparticulate polyelectrolyte complexes. Pharmaceutical Research 2007, 24, 2353-2369.
5. Rodrigues, S.; Rosa da Costa, A.; Grenha, A., Chitosan/carrageenan nanoparticles: Effect of cross-linking with tripolyphosphate and charge ratios. Carbohydrate Polymers 2012, 89, 282-289.
6. Crouzier, T.; Picart, C., Ion pairing and hydration in polyelectrolyte multilayer films containing polysaccharides. Biomacromolecules 2009, 10, 433-442.

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OPEN POSITION

PHD POSITION: MICROENCAPSULATION OF ACTIVES FOR CONTROLLED POLYMER MATERIAL DEGRADATION

Background

Degradability of polymer is a real challenge as still a large part of the object could not be easily and efficiently recycled. To increase the degradability, some actives may be incorporated in the matrix but they need protection during thermomechanical processing of the polymer, as well as a controlled release to monitor the degradability.

Microencapsulation is one powerful system to reach this objective and optimise the dispersion of the actives in the matrix.

In industrial partnership (Carbios), Oniris (Nantes) and ISPA (Alençon), two French engineering schools are looking for a PhD candidate with some competence in the following area : organic chemistry (polymerization), analyticals metods (IRTF, RMN, DSC, ATG, DMTA) and thermomechanical treatment.

The candidate will be in charge of:

- developing innovative microencapsulation technologies
- analysing the incorporation of these capsules in polymer matrix, using industrial processing such as extrusion
- determining the degradability of resulting material
- transfering the technologies and knowledge to the industrial partner.

For applications, send CV to:

denis.poncelet@oniris-nantes.fr and Laurentcauret@ispa.asso.fr

Delft University of Technology

PHD POSITION: SIMULATING COATING AND AGGLOMERATION OF NANOPARTICLES

Background

Core-shell nanoparticles have much-highlighted potential applications in hetero¬geneous catalysis, energy storage, and medical applications. There is currently one big catch: we have no idea how to make large quantities and are essentially limited to lab-scale processes. You will change that. You will study the coating of nanoparticles in the gas phase with atomic layer deposition (ALD): a technique from the semi-conductor industry that can deposit a wide range of materials. Your challenge is to understand how nanoparticles agglomerate to loose dynamic clusters and understand the agglomeration process in gas flows during coating, such that uniform coatings can be made.

Delft University of Technology is a renowned research-university, located in the Netherlands. Its department of Chemical Engineering is one of the leading schools in Europe (ranked 22nd worldwide). Product and Process Engineering (PPE) and Transport Phenomena (TP) are two of the eight research groups of this department. This PhD position is funded by NanoNextNL, and will be carried out in the framework of a larger programme in our department funded by the prestigious ERC starting grant from the European Union.

The position

The PhD student will aim at developing numerical models for the gas dynamics in fluidized beds with agglomerates of nanoparticles, their interaction and coating.. Since the relevant length scales range from Å to cm, we will apply a multi-scale modelling approach. A comparison with flat substrate coating will be made. The student will do some minor experimental work; (s)he will mainly use experimental data from the other students. The PhD student will be co-supervised by dr. J.R. van Ommen and prof. C.R. Kleijn.

You

- have an MSc degree in a relevant field, such as chemical engineering, applied physics, physical chemistry, or fluid mechanics.
- combine creativity with a sound academic attitude.
- have excellent modelling and/or simulation skills, preferably supplemented with some experimental skills.
- have affinity and preferably experience in the development of large scale computer codes in Fortran and/or C.
- have excellent communication skills, and want to work in a multidisciplinary team.

Although the position will remain open until filled, applicants are encouraged to submit their applications before June 20th, 2012.

For more information, please see
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Oniris
École Nationale Vétérinaire, Agroalimentaire et de l'Alimentation Nantes Atlantique

POSTDOC POSITION: COMPUTATIONAL FLUID DYNAMIC OF FLUID BED COATING

Background

Fluid bed coating consists of suspending particles in air upstream and pulverizing a coating solution on the particles. One essential aspect of this process is the particle circulation in the fluid bed. It defines the residence time of the particles in the different part of the reactor, the frequency of passage prior the pulverisation, the concentration of particles in the pulverisation zone and so on. A good circulation may affect the quality of the coating and the efficiency of the process.

Circulation is affected by the particles themselves (density, size, surface characteristics), the quality and quantity of air flow but also and largely the design of the reactor.

To understand the impact of the different aspects, experimental studies will be performed. However, modelling may help to predict some behaviour and allow to optimise experimental design. This is especially the case for the reactor design, where numerical modelling may help to test different configurations to optimize the fluid bed coating process.

We are looking for PhD candidate with a background in computational fluid dynamic (the COMSOL® software package is preferable). Some experience in modelling of air flow, particle motion, fluid bed or similar problems will be an advantage.

We are applying for both a regional and a European funding. Deadline July 6th, 2012

For more information, please contact :
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ARTICLE

DID WE TAME WATER WITHIN ALGINATE MICROSPHERES?

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ELECTROMAGNETIC RADIATION AND MICROWAVE ABSORBING MATERIALS

Electromagnetic radiation contains electric and magnetic field components. Microwave radiation is electromagnetic radiation, which frequencies are between infrared and radio frequencies (GHz range). Cell phones, microwave ovens and wireless communication devices, for example, operate at microwave frequencies.

There are applications (black body calibration targets for ground based or meteorological satellites radiometers, electromagnetic shielding, 'invisible' objects in military, etc.) which use materials specially designed to absorb microwave radiation and to reflect it as less as possible. Mentioned materials are usually mixtures of low loss epoxy matrices and iron or steel spherical inclusions. Composite material can exhibit only dielectric or both dielectric and magnetic properties at microwave frequencies depending on the type of inclusions. When the material sample is exposed to electromagnetic radiation, there will be reflected field. The magnitude of the reflected radiation depends on the difference in the characteristic impedances of air and the material sample. Currently, the situation with commercial absorbing materials is that when their absorption increases, reflection also increases. In case of low reflection the material absorbs less radiation, which means a thicker sample is needed, so incident radiation will not 'see' metal backing where the material is placed. When the material is highly absorptive then the mismatch between characteristic impedances of air and the material is higher so producing higher reflection. On the other hand, because of good absorption, thinner samples than in the case of less absorptive material is needed to absorb the same amount of radiation. Ideal characteristics of material would be to be highly absorptive but to have as low reflection as possible.

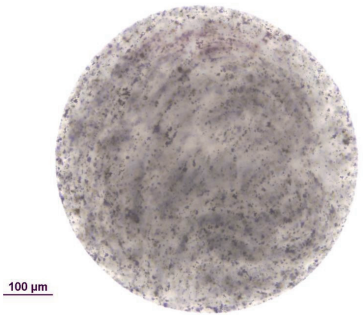

Figure 1. Microphotograph of a Ca-alginate hydrogel microsphere loaded with iron particles, before incorporation into epoxy W19.

WATER AS ABSORBER OF MICROWAVE RADIATION

Water exhibits extremely high absorption at microwave frequencies, but also high reflection. The loss mechanism of water is as follows. When exposed to electromagnetic radiation, the water dipole reorients itself. Depending on the frequency, the dipole may move in time to the field, lag behind it, or remain unaffected. When the dipole lags behind the field then interactions between the dipole and the field leads to an energy loss by heating. In free liquid water this movement occurs at GHz frequencies (microwaves) whereas in more restricted 'bound' water it occurs at MHz frequencies (short radiowaves), and in ice at kHz frequencies (long radiowaves). So far, there are many attempts to use the microwave absorption property of liquid water in building blackbody calibration targets or for electromagnetic shielding purposes. The results are robust and complicated geometries with limited uses. We propose a novel, two-component composite material that uses the good absorptive properties of water and, at the same time, exhibits very low reflection compared to commercial absorbers.

ALGINATE MICROSPHERES AND EPOXY COMPOSITE AS A CANDIDATE FOR MICROWAVE ABSORBER

Alginate can form hydrogels that contain over 95% of water. The new absorbing material that we propose consists of Ca-alginate microspheres incorporated into a low loss epoxy matrix. The procedure to synthesize our composite is as follows. 'Empty' spheres as well as spheres loaded with iron particles are formed. Figure 1 shows a microphotograph of a Ca-alginate microsphere loaded with spherical iron particles (diameter 3-5 microns).

Figure 2. Fabricated sample composed of Ca-alginate microspheres incorporated into W19 commercially available low loss epoxy material.

Figure 2 represents a photograph of a fabricated sample, which is a mixture of commercially

available low loss epoxy W19 and 22 wt% of alginate spheres. The fabricated samples were exposed to microwave radiation at frequencies from 70 to 110GHz and reflection and transmission properties were observed. We concluded from the measure-

ments that our material exhibits very low reflection and high absorption. As illustration, Figures 3 and 4 represent simulated reflection parameters of samples of different thicknesses. The simulated reflections shown are of W19 epoxy and alginate composite (Figure 3) and commercially available and widely used CR114 microwave absorber (Eccosorb CR type, Figure 4). From these figures becomes obvious that the composite that we propose has 6dB lower reflection than the commercial absorber. That means that the alginate composite reflects back 75% less of the incident radiation than the CR114 absorber for the same thickness of material samples!

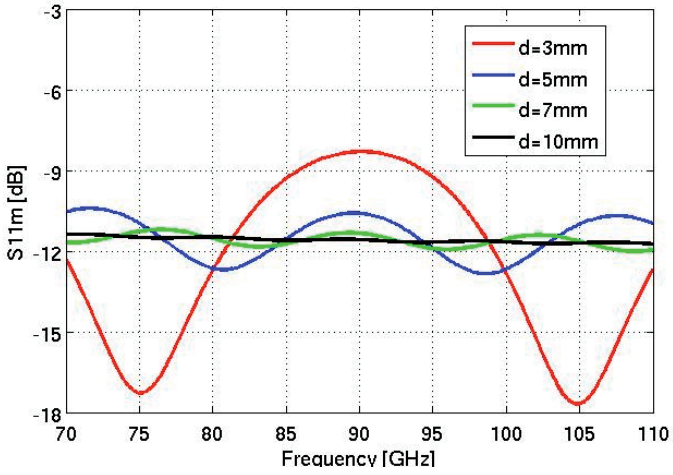

Figure 3. Simulated reflection coefficient of alginate composite material for different sample thicknesses.

CONCLUSION

This preliminary study is very promising and shows that alginate composites can be used

as absorbers of microwave radiation. Our solution represents an elegant way how to exploit the microwave absorptive properties of water. Relatively thin layers of the presented composite can be used for electromagnetic shielding purposes (ie. protection from the electromagnetic radiation exposure).

Future studies will focus on the characteristics of composite material containing variable volume fractions of Ca-alginate spheres of different sizes, 'empty' and loaded with iron particles, and incorporated into epoxy resin.

REFERENCES:

1. Yang, R. B. and W. F. Liang, Microwave properties of high aspect ratio carbonyl iron/epoxy absorbers, Journal of Applied Physics, Vol. 109, 07A311, 2011.
2. Gama, A. M. and M. C. Rezende, Complex permeability and permittivity variation of carbonyl iron rubber in the frequency range of 2 to 18 GHz, Journal of Aerospace Technology and Management, Vol. 2, No. 1, 2010.
3. Wollack, E. J., et al., Electromagnetic and thermal properties of a conductively loaded epoxy, International Journal of Infrared and Millimeter Waves, Vol. 29, 51-61, 2008.
4. Zivkovic, I., Wandrey, C. and B. Bogicevic, Alginate beads and epoxy resin composites as candidates for microwave absorbers, Progress In Electromagnetics Research C, Vol. 28, 127-142, 2012.

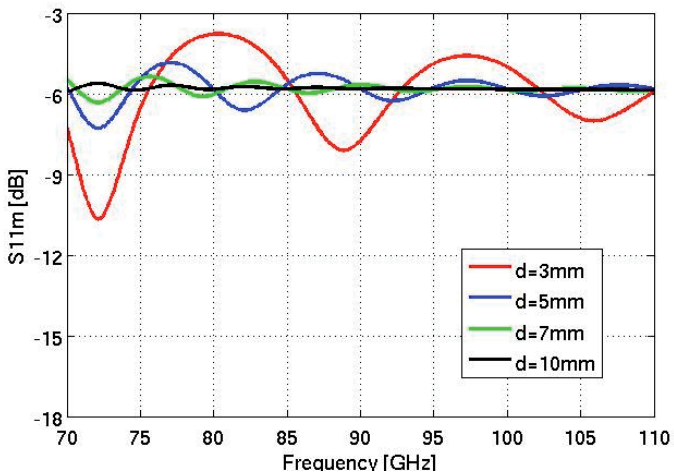

Figure 4. Simulated reflection coefficient of commercially available absorbing material Eccosorb CR114 from Emerson&Cuming Company.

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ARTICLE

POLYELECTROLYTE MICROCAPSULES IN CELLULAR UPTAKE - ACHIEVEMENTS AND PERSPECTIVES

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INTRODUCTION

Since the onset of the "magic bullet" concept by Paul Erlich (19th century) that researchers working in the medical and pharmaceutical fields intend to reach targets at cellular level. Many efforts have been made until now and, although there is still a lot to discover and understand, important developments were made.

Particularly in the areas of vaccination (antigen delivery) [1, 2], gene therapy [3] and cancer treatment [4] effective interactions between cells and drug delivery carriers are crucial and can be achieved mainly by surface modification, which can lead to specific actions and offer a great flexibility for the targeting of certain cells.

Actually, drug carriers can internalize themselves by means of natural cellular transport mechanisms. One of these new platforms is the polyelectrolyte microcapsules. Produced as multilayer charged particles, these systems show dense surface net charge, by adsorbing mainly through a layer-by-layer method, several coatings of polyelectrolyte polymers, which are easily controlled in the laboratory by sequential zeta potential measurements during the experiments [1, 4].

Briefly, layer-by-layer is an adsorption technique by which polycations and polyanions are adsorbed at the capsules' surface and form a stable film. Both crosslinked (intact core) and hollow (core removed with an EDTA solution) microcapsules can be obtained with this method [2, 4, 6].

These systems can be used whether for the entrapment of biomolecules (DNA, siRNA) or for antigen attachment to the outer wall. Polymers can be synthetic, partially synthetic and fully natural and may be mixed during the microcapsule formation.

NATURAL OR SYNTHETIC POLYMERS: IS THERE A DIFFERENCE IN CELLULAR UPTAKE?

Due to polyelectrolyte microcapsules great versatility, a large variety of materials have been used until now, but there is clearly a recent interest in developing these systems with natural polymers such as polysaccharides, polypeptides, polynucleotides and lipids [3, 7]. Cell penetrating peptides are a true example of natural cationic polymers which application is increasing in the medical field [5]. Because of their capability of cellular uptake, cell penetrating peptides are able to cross the biological membranes and reach the cellular organelles to improve a targeting action [5]. Cell penetrating peptides also have the capability to interact with the cells' surface and the lipid membrane, but the internalization mechanism of cell penetrating peptidess is still an unresolved issue to deal with [8].

In fact, polycations are known to show cytotoxicity at higher concentrations, due to the presence of protonated amino groups at the side chains. Several characteristics such as charge density, molecular weight and type of polyelectrolyte were suggested to affect the interaction between the particles and the cells [1]. Still, cationic polymers show bigger interest in this field since they demonstrate higher cellular internalization due to strong interactions [1].

Thus, An and co-workers [7] have studied the influence of cationic natural and synthetic materials in cell viability and proliferation. Results were clear: cell viability and proliferation did not depend on the type of polyelectrolytic polymer chosen, but instead on the concentrations used and on specific biochemical mechanisms of the cell, during the time period of the in vitro conducted studies. Polysaccharides such as dextran-sulfate and chitosan are also commonly elected as polyelectrolytic polymers; also, polyethylene glycol, a reference for coating drug delivery systems, show many properties in specific cellular uptake, as well known, and also excellent properties as a "stealth" polymer [7].

Besides, it can prolong blood circulation time and shows specific affinity for the endothelial cells of the blood brain barrier [9]. However, polyethylene glycol can hide the surface charge of these drug carriers decreasing cellular uptake [9].

ADVANTAGES OF POLYELECTROLYTE MICROCAPSULES OVER POLYMERIC PARTICLES

Polyelectrolyte microcapsules show, in fact, many advantages in comparison with polymeric particles. Firstly, this new generation of drug delivery systems can be fabricated in aqueous, mild and bio-friendly conditions [3, 10] and drug entrapment and release can be easily controlled by varying formulation parameters such as the number of layers of which the polyelectrolyte microcapsules are composed or through controlled enzymatic degradation [10].

Besides, polyelectrolytic microcapsules showed similar immunologic response compared with Poly(lactic-co-glycolic acid) microparticles [10]. In another study, poly(lactic-co-glyco-

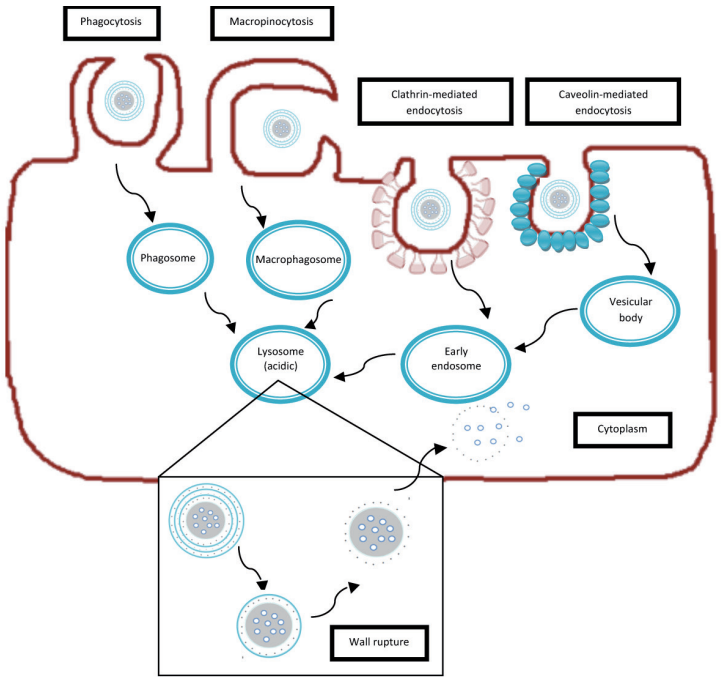

Figure 1 – Schematic representation of Polyeletrolyte microcapsules cellular uptake and degradation

lic acid) microparticles were entrapped through physical deposition by layer-by-layer of two opposite charge polyelectrolytes: polyethylenimine and dextran sulfate. The two polyelectrolytes formed a dense charged shell over the microparticles, stabilizing the system, which demonstrated to efficiently delivery antigens to the antigen-presenting cells and induce specific immunological responses in murine macrophages cellular lines [1].

In addition, when studied in the pulmonary environment, dextran sulfate polyarginine polyelectrolyte microcapsules were able to efficiently target the local pulmonary antigen presenting cells and lymph nodes, making these polyelectrolyte microcapsules a promising immune system for protection against intracellular pathogens, such as mycobacterium tuberculosis and HIV [6, 11].

In spite of all these advances, cell interactions aren't well known yet and many doubts arise: How is the effect of the microcapsules in the cell viability and proliferation? Are biodegradable and biocompatible materials better and safer? Can microcapsules interact by means of a unique mechanism or are several independent possible paths involved?

INTERNALIZATION OF POLYELECTROLYTE MICROCAPSULES INTO THE CELLS

Recently, it was stated that cell penetrating peptidess were internalized within cells by nonspecific endocytosis mechanisms [5]. In fact, the presence of a high density charge is preferable for cell membrane entrance since mechanisms like endocytosis mediate charged particles through non-specific receptors [9].

Thus, alternative nonespecific endocytosis mechanisms (via clarithin-independent endocytosis, macropinocytosis or caveolar-dependent mechanisms) are also important for this type of cellular transportation [9]. In terms of cellular uptake, most microparticles are described to enter the cells by phagocytosis processes [1] or by macropinocytosis [8]. Microcapsules with a size range between 0.1 and 10 μm can be easily phagocytosed by macrophages and dendritic cells.

Koker et al. [12] demonstrated that hollow polyelectrolyte microcapsules could be internalized within dendritic cells in a high amount with a cellular viability of 80%. Besides, it was also discovered that microcapsules deformation occurred inside the cells cytoplasm by a combination of enzymes and mechanical deformation.

Two years later, the same authors stated that polyelectrolyte microcapsules may enter the acidic compartments within the cell where the wall rupture happens by lysosomal proteases. Then, the polyelectrolyte microcapsules are able to leave these compartments and release the entrapped molecules in the cell cytoplasm. The transport mechanism is mainly by macropinocytosis [4].

Furthermore, Baldassare et al. showed that poly(styrene sulfonate)/poly(allylamine hydrochloride) and dextran sulfate/poly-l-lysine microcapsules can be internalized into living cells through lipid raft-mediated endocytosis (or caveolar mediated endocytosis).

DEGRADATION MECHANISMS OF POLYELECTROLYTE MICROCAPSULES

However, differences in degradation were observed according to the type of polymer used: Poly(styrene sulfonate)/Poly(allylamine hydrochloride), which are not degradable polyelectrolytes, were not degraded by proteases and their localization was perinuclear; dextran sulfate-poly-L-lysine, both biodegradable polymers, were instead degraded by these enzymes and were excluded from the nuclei. Duration of the multilayer wall integrity was also variable, as the Poly(styrene sulfonate)/Poly(allylamine hydrochloride) capsules were still intact after 3 hours, in comparison with dextran sulfate-poly-L-lysine capsules with intact core [4].

Thus, it seems that biodegradation is achieved by hydrolysis of synthetic polymers or by enzymatic degradation of natural polysaccharides or polypeptides. It is clear now that the first contact with the cell is by electrostatic interaction with the extracellular matrix, i.e, the proteoglycans glucosaminoglycans. Their impact occurs through macropinocytosis, endocytosis or membrane perturbations me-

chanisms.

The next step in cellular internalization involves rather a specific receptor (receptor-mediated endocytosis) or a non specific electrostatic attraction which can mediate the internalization by adsorptive endocytosis (e.g., polylysine) [8]. Polyelectrolytes like the polypeptides poly-L-asparginine and poly-L-arginine are also susceptible to enzymatic hydrolysis by pronase [3]. However, actual techniques to measure cell penetrating peptidess inside the cell are not really effective since there are many clearance mechanisms (lysosomal degradation, cellular target interactions, cytoplasmatic degradation, etc.) that affect the reading of this parameter [8].

Finally, there is a long way to walk in the field of nanotechnology. However, the doubt of how much deeper can we go inside the human body still remains. Instead of questioning what could be the long term effects of penetrating into the cells components and what mechanism is involved, it is now important to ask though: what can we do to prevent it?

REFERENCES

1. Yang, Y.-W. and Hsu, P.Y.-J., The effect of poly(d,l-lactide-co-glycolide) microparticles with polyelectrolyte self-assembled multilayer surfaces on the cross-presentation of exogenous antigens. Biomaterials, 2008. 29(16): p. 2516-2526.
2. De Koker, S., DE Geest, B.G., Singh, S.K., De Rycke, R., Naessens, T., Kooyk, Y.V., Demeester J., De Smedt, S.C., Grooten J., Polyelectrolyte Microcapsules as Antigen Delivery Vehicles To Dendritic Cells: Uptake, Processing, and Cross-Presentation of Encapsulated Antigens. Angewandte Chemie International Edition, 2009. 48(45): p. 8485-8489.
3. De Geest, B.G., De Koker, S., Sukhorukov, G.B., Kreft, O., Parak, W.J., Skirtach, A.G., Demeester, J., De Smedt, S.C., Hennink, W.E., Polyelectrolyte microcapsules for biomedical applications. Soft Matter, 2009. 5(2): p. 282-291.
4. Baldassarre, F., Vergaro, V., Scarlino, F., De Santis, F., Lucarelli, G., Torre, d. A., Ciccarella G., Rinaldi, R., Giannelli, G., Leporatti, S., Polyelectrolyte Capsules as Carriers for Growth Factor Inhibitor Delivery to Hepatocellular Carcinoma. Macromolecular Bioscience, 2012. 12(5): p. 656-665.
5. González-Aramundiz, J., Lozano, M.V., Sousa-Herves, A., Fernandez-Meiga, E., Csaba, N., Polypeptides and polyaminoacids in drug delivery. Expert Opinion on Drug Delivery, 2012. 9(2): p. 183-201.
6. De Geest, B.G., Willart, M.A., Lambercht,B.N., Pollard, C., Vervaet, C., Remon J.P., Grooten J., De Koker, S., Surface-Engineered Polyelectrolyte Multilayer Capsules: Synthetic Vaccines Mimicking Microbial Structure and Function. Angewandte Chemie International Edition, 2012. 51(16): p. 3862-3866.
7. An, Z., Kavanoor, K., Choy, M.L., Kaufman, L.J., Polyelectrolyte microcapsule interactions with cells in two- and three-dimensional culture. Colloids and Surfaces B: Biointerfaces, 2009. 70(1): p. 114-123.
8. Patel, L., J. Zaro, W.-C. Shen, Cell Penetrating Peptides: Intracellular Pathways and Pharmaceutical Perspectives. Pharmaceutical Research, 2007. 24(11): p. 1977-1992.
9. Hillaireau, H., P. Couvreur, Nanocarriers' entry into the cell: relevance to drug delivery. Cellular and Molecular Life Sciences, 2009. 66(17): p. 2873-2896.
10. De Temmerman, M.-L., Rejman, J., Vandenbroucke, R.E., De Koker, S., Libert, C., Grooten, J., Demeester, J., Gander, B., De Smedt, S., Polyelectrolyte layer-by-layer microcapsules versus PLGA microparticles for immunization with a protein antigen. Journal of Controlled Release, 2012. 158(2): p. 233-239.
11. 1De Koker, S., Naessens, T., De Geest, B.G., Bogaert, P., Demeester, J., De Smedt, S., Grooten, J., Biodegradable Polyelectrolyte Microcapsules: Antigen Delivery Tools with Th17 Skewing Activity after Pulmonary Delivery. The Journal of Immunology, 2010. 184(1): p. 203-211.
12. De Koker, S., De Geest, B.G., Cuvelier, C., Ferdinande, L., Deckers, W., Hennik, W.E., De Smedt, S., Mertens, N., In vivo Cellular Uptake, Degradation, and Biocompatibility of Polyelectrolyte Microcapsules. Advanced Functional Materials, 2007. 17(18): p. 3754-3763.

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ARTICLE

MICROFLUIDIC PRODUCTION OF BIOPOLYMER-BASED JANUS MICROBEADS

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WHY TO PRODUCE BIOPOLYMER BASED JANUS MICROBEADS USING MICROFLUIDICS?

During the past decade, multicompartment and anisotropic particles, have received significant attention due to their novel morphologies and diverse potential applications [1]. Janus particles have two distinguishable surface areas of equal size, which makes them suitable for applications in switchable display devices, interface stabilizers, self-motile microparticles, and smart nanomaterials, such as biological sensors, nanomotors, antireflection coatings, and anisotropic building blocks for complex structures [2-4].

Biphasic particles were first reported by Xerox society in 1970s with black and white plastic hemispheres for use in twisting-ball display.

Janus particles are currently produced by templating methods, colloidal assembly, particle lithography techniques, glacing-angle deposition, nanosphere lithography, and capillary fluid flow. Capillary flow based approach such as microfluidic devices offer a number of advantages over conventional flow control technology because they ensure highly versatile geometry and can be used to produce monodisperse spherical polymeric microparticles with diameters ranging from several tens to several hundreds of micrometers [5].

In most of the previous works, Janus particles produced by microfluidics were obtained from the polymerization of organic monomers by fast UV illumination. This strategy is, however, limited to light sensitive compounds.

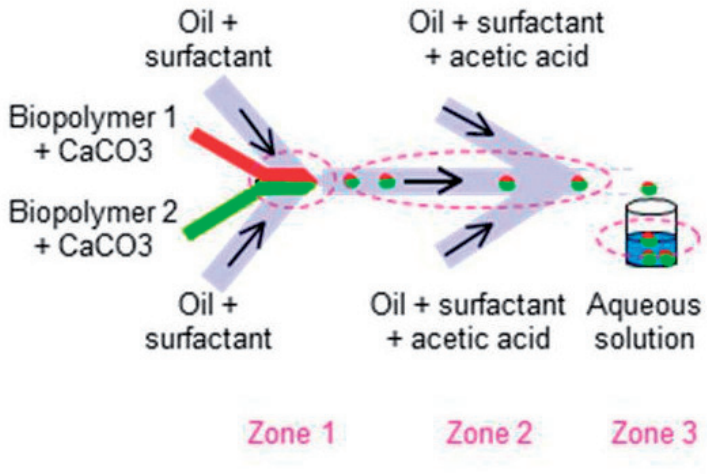

Figure 1. Schematic representation of the microfluidic device for Janus droplet generation using a micro flow focusing device with inlets for the two biopolymers mixed with an inactive form of the cross-linking agent (CaCO3) emulsified in oil. Droplet gelation was induced by the diffusion of acetic acid from the oil phase to the droplets, where the resulting pH decrease inside the droplets led to calcium bridging and, thus, to biopolymer gelation. Zone 1: junction; zone 2: time residence of droplets in the continuous phase; zone 3: beads collect.

Hydrogel-based microparticles, in contrast, are hydrophilic polymer networks with a high affinity for water. Pectin and alginate are environmentally friendly because they are highly water-soluble, biocompatible, and biodegradable. One feature of these biopolymers is their high content of carboxylic groups that can be ionically cross-linked to achieve the formation of gels.

We describe here a microfluidic device for the generation of monodisperse homo and hetero Janus microbeads using pectin–pectin and pectin–alginate hydrogels. As the polysaccharides used are completely miscible in a wide range of concentrations, the challenge in microfluidic design was to obtain a well-defined interface between the two biopolymer hemispheres in the homo and hetero Janus microbeads. Further experiments were carried out to achieve the gel degradations in two independent steps by directing enzymatic hydrolysis according to the biopolymer compositions of the Janus microbeads.

MICROFLUIDICS TECHNIQUE FOR BIOPOLYMER BASED JANUS MICROBEADS PRODUCTION.

We first showed that in bulk phases a gelation time of 30 s was too short to produce an alginate gel, while a complete gelation of pectin solution occurred. In both cases, the polysaccharide solutions had completely gelled after a contact time of 2 min. This time of gelation was in agreement with our microfluidic design where both time residence of droplets in the continuous phase and droplets gelation times allowed the onset of microbeads formation.

A schematic representation of the microfluidic device for Janus droplet generation and microbeads generation

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is depicted on Figure 1. Although the microfluidics process used to obtain Janus particles takes advantage of laminar flow [2], it is important to note that diffusive intermixing may occur with miscible fluids in a two-phase stream before the droplets break up. In fact, the low Reynolds number in the channel before the junction (R_e < 1) indicates that nonconvective transport can occur across the two parallel streams. In this context, the channel containing the two miscible phases of the biopolymers (FA-pectin and Bodipy-pectin or FA-alginate and Bodipy-pectin), the geometry of the flow focusing junction and the central channel were therefore adjusted so as to minimize diffusive intermixing. Other details concerning the device optimizations for the generation of Janus microbeads can be found in [6]. The microbeads were then collected at the end of the microcircuit in an aqueous solution of CaCl2 to ensure maximal cross-linking and to limit the coalescence of Janus microbeads. The average size of microbeads was of 90 µm using flow rates for biopolymer solutions and oil of 1 and 18 µL/min, respectively.

STRUCTURAL CHARACTERIZATION OF BIOPOLYMER-BASED HOMO AND HETERO-JANUS MICROBEADS.

Fluorescently labeled (Fluoresceinamine, FA and Bodipy Tr cadaverine, Bodipy) pectins were prepared to visualize the coflowing aqueous stream and the production of fluorescent homo Janus particles. The fluorescence micrographs after gelation (Figure 2) showed fluorescently labeled hemispheres within the droplets, composed of FA-labeled and Bodipy-labeled pectin.

Initial observations by fluorescence microscopy showed that FA-pectin and Bodipy-pectin were concentrated on opposite sides of the hemispheres. However, the interface was not clearly defined even though convection phenomena were controlled by having a large central channel and rapid gelation, which suggested that some diffusive intermixing occurred. Analysis of the fluorescence intensity profile across the microbead clearly revealed a superimposition of fluorescence labeling near the interface.

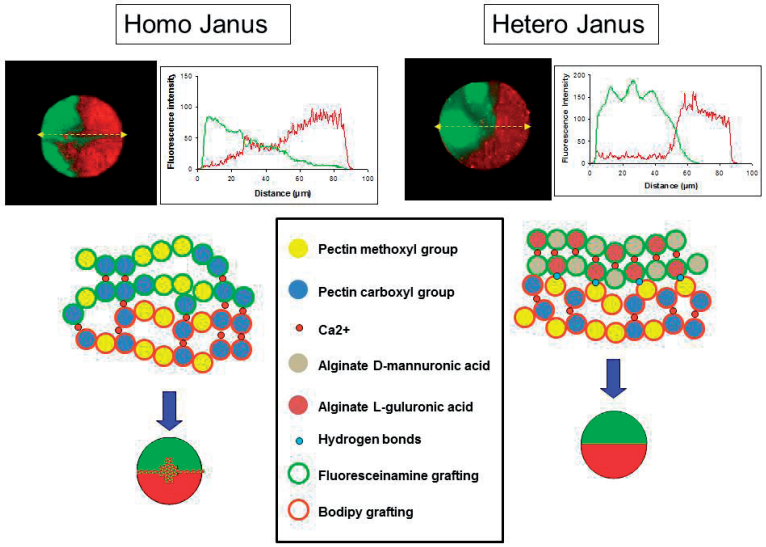

Figure 2. Fluorescence confocal microscopy images and profiles of the fluorescence intensities for FA-pectin/Bodipy-pectin homo Janus and FA-alginate/Bodipy-pectin hetero Janus microbeads. FA and Bodipy excitations were set at 488 and 561 nm, respectively, while emission fluorescence was recorded between 500 and 530 nm (green) and between 570 and 620 nm (red). Scale bars: 50 µm. The schematic explanations relative to the interface for both Janus microbeads are also given (see text for details).

Fluorescent FA-labeled alginate and Bodipy-labeled pectin microbeads were prepared under the same conditions as for homo Janus microbeads. The alginate/pectin microbeads also displayed two distinct hemispheres but the interface was well defined, clearly indicating that only limited diffusive intermixing occurred. The sharper definition of the interface in the hetero Janus particles was confirmed by the analysis of fluorescence intensity.

This difference in interface definition between hetero and homo Janus microbeads led us to reflect on the mechanisms occurring during droplet and gel formation. The most likely reason probably stems from the chemical structure of each polysaccharide and the interactions taking place during the gelation process. In the case of pectin gelation, only the carboxyl groups from the galacturonic acid repeat units are involved in binding with calcium ions, while the methanol-esterified carboxyl groups create steric repulsions between adjacent pectin chains. This intricate balance between long-range attractions and short-range repulsions would produce a less well-defined, irregularly shaped interface in homo Janus microbeads due to repulsive forces between the methoxyl groups, not involved in the ionic gelation process, on both sides of the hemispheres [7]. These conformational constraints, coupled with the convective and interdiffusive mixing phenomena, would generate invaginations at the homo Janus interface, as revealed by the fluorescent confocal image (Fig. 2). In the case of alginate gelation, L-guluronic and D-mannuronic acid repeat units are involved in the ionic gelation process with divalent ions. In addition, pectin and alginate have been previously shown to be able to interact with each other as a result of the formation of hydrogen bonds between the methoxyl groups in pectin and the hydroxyl groups in the guluronic acids of alginate [8]. This specific interaction would lead to a decrease in long-range molecular motions at the interface and, therefore, reduce the linear distance of diffusive intermixing, thus, creating a better separation at the interface of hetero Janus microbeads (Fig. 2).

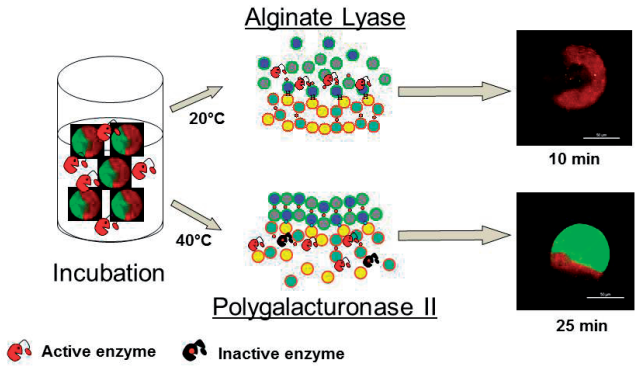

Figure 3. Schematic representation of the selective degradation of hetero Janus microbeads using alginate lyase and polygalacturonase II and related confocal images of Janus microbeads after alginate and pectin degradations. Scale bars: 50 µm.

ENZYMATIC HYDROLYSIS OF HETERO-JANUS MICROBEADS.

The feasibility of selective degradation was demonstrated and the Janus architecture confirmed by investigating the effect of specific enzymes against each of the polysaccharides present in the hetero Janus microbeads.

A polygalacturonase (PGII) from Aspergillus niger was used to hydrolyze the pectin backbone while alginate degradation was achieved by using an alginate lyase (AL) from Sphingobacterium multivorum. The enzymatic hydrolyses clearly revealed the degradation of the desired hemisphere in the microbeads (Fig. 3). Indeed, FA-alginate/Bodipy-pectin microbeads mixed with PGII showed a single fluorescent hemisphere, implying total degradation of the pectin, after 25 min of incubation.

The persistence of red fluorescence at the end of the experiment could be due to a progressive decrease of PGII activity due to the release of calcium ions from the pectin network [8]. On the other hand, microbeads mixed with alginate lyase displayed a single fluorescent hemisphere, which suggested the total degradation of alginate, after 10 min of incubation. Moreover, the presence of either PGII or AL did not affect the stability of the nondegraded hemispheres in the hetero Janus microbeads over time.

CONCLUSION.

This study demonstrated the use of microfluidics to generate Janus microbeads from polysaccharides. The design of the microdevice, coupled with optimization of the chemical route of biopolymer gelation, allowed the production of homo and hetero Janus microbeads from pectin–pectin and pectin– alginate with a well-defined interface, particularly in the case of hetero Janus microbeads, thanks to specific interactions between the two polysaccharides. The increased flexibility of microbeads derived from polysaccharides open up new opportunities for the release of two active substances in a controlled environment and could find applications in food, medicine, and cosmetics.

REFERENCES

1. Du, J. and O'Reilly, R. K. Anisotropic particles with patchy, multicompartment and Janus architectures: preparation and application. Chem. Soc. Rev., 40 (2011) 2402–2416
2. Nisisako, T., Torii, T., Takahashi, T. and Takizawa, Y. Synthesis of monodisperse bicolored Janus particles with electrical anisotropy using a microfluidic co-Flow System. Adv. Mater., 18 (2006) 1152–1156.
3. Walther, A., Hoffmann, M. and Mueller, A. H. E. Emulsion polymerization using Janus particles as stabilizers. Angew. Chem. Int. Ed., 47 (2008) 711–714.
4. Glotzer, S. C. and Solomon, M. J. Anisotropy of building blocks and their assembly into complex structures. Nat. Mater., 6 (2007) 557–562.
5. Liu, K., Ding, H. J., Liu, J., Chen, Y. and Zhao, X. Z. Shape-Controlled Production of Biodegradable Calcium Alginate Gel Microparticles Using a Novel Microfluidic Device. Langmuir, 22 (2006) 9453–9457.
6. Marquis, M., Renard, D. and Cathala, B. Microfluidic generation and selective degradation of biopolymer-based Janus microbeads. Biomacromolecules, 13 (2012) 1197-1203.
7. Braccini, I., Rodriguez-Carvajal, M.A. and Perez, S. Chain-Chain Interactions for Methyl Polygalacturonate: Models for High Methyl-Esterified Pectin Junction Zones. Biomacromolecules, 2 (2005) 1322-1328.
8. Gohil, R.M. Synergistic Blends of Natural Polymers, Pectin and Sodium Alginate. J. Appl. Polym. Sci., 120 (2010) 2324-2336.

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