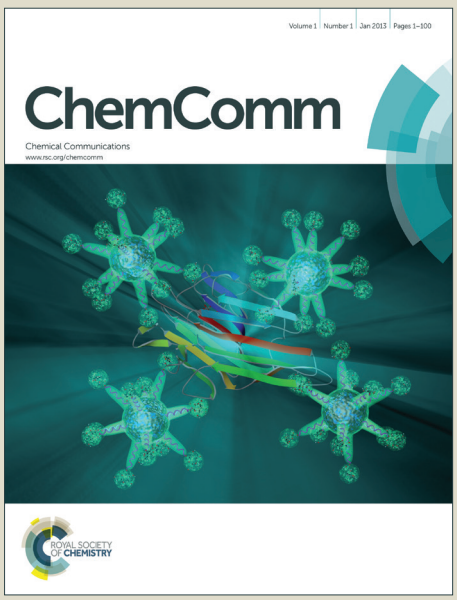
ChemComm

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Journal Name

RSCPublishing

COMMUNICATION

Compartmentalization of Bacteria in Microcapsules

Cite this: DOI: 10.1039/x0xx00000x

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Judith van Wijk[1], Tiaan Heunis[2], Elrika Harmzen[3], Leon M.T. Dicks[2], Jan Meuldijk[1], Bert Klumperman[3,*]

Lactobacillus plantarum **strain 423 was encapsulated in hollow *poly*(organosiloxane) microcapsules by templating water-in-oil Pickering emulsion droplets via the interfacial reaction of alkylchlorosilanes. The bacteria were suspended in growth medium or buffer to protect the cells against pH changes during the interfacial reactions with alkylchlorosilanes. The results of this work open up novel avenues for the encapsulation of microbial cells.**

Encapsulation of viable microbial cells has several novel applications in pharmaceutical, food and agricultural industries.[1–3] Critical for these potential applications is that the microcapsules are permeable for small molecules and even macromolecules, but impermeable for the encapsulated microbial cells. It is also a prerequisite that the encapsulated cells remain viable during and after encapsulation. This is challenging, as most microorganisms are sensitive to changes in environmental conditions such as temperature, pH and the presence of cytotoxic chemicals. Viable cells have previously been encapsulated in gels, often in the form of microbeads. These include hydrogels[4,5], calcium alginate[6] and sol-gel products[7–9]. Hollow calcium carbonate capsules produced by a layer-by-layer technique have been used to encapsulate individual *E. coli* cells[10]. These methods trap the bacteria in a matrix or just encapsulate one individual bacterium. Contrarily, in the present work, multiple bacteria suspended in Tris-HCl buffer (pH 8.0) are encapsulated in microcapsules. Here we report on an encapsulation method, which results in minimum contamination of the dispersed phase, causing bacteria to remain viable inside the microdroplets. Contamination caused by, *e.g.* a pH change, would result in cell death.. Contamination that causes a change in pH should thus not exceed the buffer capacity. Beneficial characteristics of sol-gel silica as encapsulation material include the controllable porosity and mechanical properties of the shell. These characteristics can be controlled by the selection of precursors, modification agents and synthesis conditions.[11] Pickering emulsion droplets are known to be suitable templates for microcapsules because of their high colloidal stability and their controllable droplet size.[12–14] This paper reports on the encapsulation of *L. plantarum* in hollow poly(organosiloxane) microcapsules. To the authors' knowledge, this is the first time that viable bacteria have been encapsulated in microcapsules templated on a Pickering emulsion. These microcapsules are synthesized from water-in-oil emulsions stabilized by silica microparticles by an interfacial reaction of alkylchlorosilanes, see Figure 1A. The alkylchlorosilanes have hydrophobic properties, *i.e.* they are not miscible with water before and after hydrolysis. Alkylchlorosilanes are very reactive towards water and contribute to the stabilization of the water droplets by the microparticles. Hence, the monomer is oil-soluble and rapidly polymerizes upon contact with the stabilizing particles or with water at the interface of the droplets. The viability of the bacteria was determined in the Pickering emulsion droplets and in the microcapsules by using confocal fluorescence microscopy.

The method used to prepare microcapsules is schematically presented in Figure 1. The objective of this work was to encapsulate viable bacteria and as a consequence, a water-in-oil (or inverse) Pickering emulsion was required, since bacteria generally do not survive in organic media, with only very few exceptions.[15] The three-phase contact angle that the particles have at the oil-water interface, when used in a Pickering emulsion, is a strong indication of the stability of the emulsion.[16] The most stable inverse Pickering emulsions are produced when the stabilizing particles have a three-phase contact angle between 94° and 110°, meaning the particles are slightly hydrophobic.[17] Silica microparticles that are modified by alkylsilanes are known to have a three-phase contact angle suitable for inverse Pickering stabilization.[18] Water was added to the dispersion of modified particles in heptane to form the inverse

ChemComm Accepted Manuscript

Pickering emulsion (Figure 1). The water, oil and particle concentration in the emulsion were calculated according to Salari *et al*. [19] and the target droplet radius was always set at 25 µm, unless indicated otherwise. The Pickering emulsion was produced manually, by shaking the mixture of water, *n*-heptane and the hydrophobic SiO_2 particles for 30 seconds. The reason for choosing this methodology is that bacteria are not able to survive high shear or ultra-sonication, which is usually applied to effect emulsification. Alkylchlorosilanes were added to the inverse Pickering emulsion that reacted with water at the interface (Figure 1B). The reaction of these alkylchlorosilanes proceeds first by a rapid hydrolysis of the monomer with water, producing a surfactant-resembling monomer (Eq 1).[20] The hydrolysis is followed by the condensation reactions (Eq 2), producing the shell around the microemulsion droplets, as depicted in Figure 1B.

$$RSiCl_3\ (liq) + H_2O \rightarrow RSi(OH)_3\ (interface) + HCl\ (aq)$$
Hydrolysis reaction (1)

$$2\ RSi(OH)_3\ (interface) \rightarrow R(OH)_2SiOSi(OH)_2R\ (interface) + H_2O$$
Condensation reaction (2)

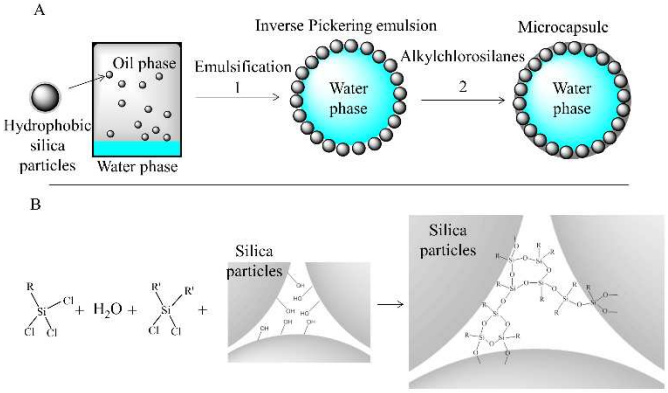


Figure 1. Methodology for the synthesis of microcapsules. (A) An inverse Pickering emulsion is produced by the emulsification of water in a dispersion of hydrophobic silica particles in *n*-heptane (1). The addition of alkylchlorosilanes to the inverse Pickering emulsion results in an interfacial reaction with water to produce polymer, thus forming microcapsules (2). (B) The reactive silanol groups on the primary stabilizing silica particles will also react with the poly(alkyl siloxane).

The particles that stabilize the inverse Pickering emulsion were produced by the Stöber method.[21] These silica particles were used in a seeded silica polymerization to produce monodisperse particles in the micron-size range.[22] After centrifugation, the particles were air-dried and re-dispersed in *n*-heptane. Subsequently, the SiO_2 particles obtained were modified with 3-(trimethoxysilyl)-propyl methacrylate (MPTS) in *n*-heptane to give them a hydrophobic character,[18] see Electronic Supporting Information (ESI) Equation S1.

The average concentration of reactive silanol groups on silica particles that are produced by the Stöber method is equal to 8 $\mu mol \cdot m^{-2}_{particle}$.[23] Besides reactive silanol groups, physically adsorbed water is also present at the surface of the silica microparticles. Amorphous silica is well known to physically adsorb water and the adsorbed water will also react with the modification agent.[24] Hence, it is assumed that after the modification step, the surface of the silica microparticles is covered with a layer of MPTS. This again resulted in residual reactive hydroxy groups from the MPTS. Figure 2A is a Scanning Electron Microscope (SEM) image of silica microparticles that were produced by the Stöber method, followed by a seeded polymerization technique. Before modification, the surface area of the microparticles was calculated by using the freeware package ImageJ.[25] Water was added to the particle dispersion after modification, followed by manual vigorous shaking of the mixture to produce a stable inverse Pickering emulsion (Figure 2B and C).

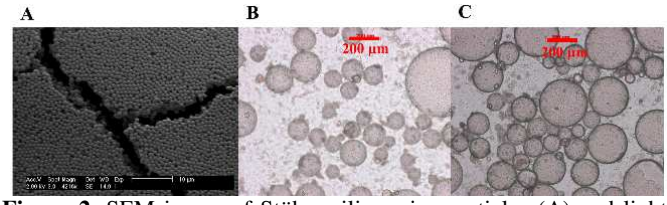


Figure 2. SEM image of Stöber silica microparticles (A) and light microscopy images of inverse Pickering emulsion droplets stabilized by hydrophobized silica microparticles (B and C).

When the microparticles used to stabilize the Pickering emulsion are monodisperse and have the proper three-phase contact angle, they arrange in a hexagonal close packing on the water-oil interface. For this reason Pickering emulsions with a narrow size distribution can be produced with a distribution of $D_z/D_v \approx 1.1$.[13] However, it takes a certain amount of energy to transfer a particle to the interface and a specific time to bring them all at the interface. When emulsification takes place manually, the amount of energy and the dispersion time are not sufficient to create a Pickering emulsion with narrow size distribution, which is clear from the estimated polydispersity index $D_z/D_v \approx 5.3$ based on Figure 2B and C. Despite the broad droplet size distribution, the emulsions were stable and macroscopic phase separation was not observed over the course of 7 days.

Alkylchlorosilanes are frequently used for the modification of silica surfaces to improve dispersion properties, or to effect crosslinking for the immobilization of catalysts or biomolecules.[26] When alkylchlorosilanes are used to modify surfaces in the presence of water, they are known to yield a variety of nanostructures, besides a smooth polymer layer on the relevant surface.[27–29] The underlying reason for the formation of the nanostructures is the hydrolysis and self-condensation of the alkylchlorosilanes in the presence of water that occurs in parallel to reactions with the surface-bound silanol groups.[27] The formation of the nanostructures is further dependent on the alkyl chain length of the alkylchlorosilane and the size, shape and concentration of the nanostructures is equally dependent on the chain length.[27]

Reaction of the alkylchlorosilanes with the silanol groups on the particles and the network formation between the silica particles should be sufficient to produce a stable microcapsule when a solid shell around an inverse Pickering emulsion is produced. Hydrolysis of a chlorosilane leads to a hydrophilic intermediate due to the produced hydroxy groups (see Equations 3 and 4). It is important that the intermediates do not migrate into the emulsion droplets, but stay at the interface. To restrict the reaction to the interface and to avoid migration into the emulsion droplet, octadecyltrichlorosilane (OTC) was used. To stimulate complete network formation, dimethyldichlorosilane (DMDCS) was used, which is more reactive, but also partially hydrophobic after hydrolysis. After a stable Pickering emulsion was produced, a solution of OTC and DMDCS in *n*-heptane was added (Figure 1). Silica-based condensation products that have not reacted with silica particles or with the newly formed silica shell are hydrophobic and will therefore stay in the continuous oil phase.

Light microscopy (LM) and SEM images of microcapsules produced after the addition of OTC and DMDCS to a stable Pickering emulsion is shown in Figure 3. A polyalkylsiloxane shell is formed around the microdroplets, as a result of the interfacial reaction. The

ChemComm Accepted Manuscript

This journal is © The Royal Society of Chemistry 2012

reaction of the monomers at the interface with each other, and with the silanol groups at the surface of the particles, also results in particle detachment from the interface because of their increased hydrophobicity (Figure 3A). Nevertheless, this particle detachment did not destabilize the emulsion to such an extent that macroscopic phase separation took place, before encapsulation. The newly produced polymer surrounding the initially stabilizing silica microparticles can be clearly distinguished when focusing on the shell (Figure S1F). In contrast to the inverse Pickering emulsion droplets, the microcapsules do not appear to be perfectly spherical (Figure 3). Deformation is due to the low glass transition temperature (T_g) of the polymer of -95 °C as determined by Differential Scanning Calorimetry (DSC) analysis, see Figures S2 and S3. The capsules need to be dried before SEM analysis, and the analysis itself takes place under high vacuum. Consequently, broken capsules and collapsed capsules were expected as a result of evaporation of water from the capsules, see Figures 3B-C and S1.

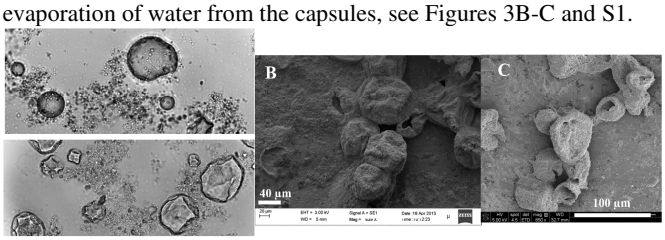


Figure 3. Light microscopy (A) and SEM (B and C) images of the produced microcapsules. Microcapsules were synthesized by the interfacial reaction of octadecyltrichlorosilane and dimethyldichlorosilane at the interface.

Microcapsules and the method used for encapsulation of bacteria need to have certain characteristics. For example, the capsules should provide a favorable environment for the bacteria to ensure survival and, in view of future applications, the capsules should also be permeable to allow transport of molecules into the external environment. The latter could be tested by following the release of fluorescent markers with different sizes from the capsules.[30] The focus of the current study is to develop a method to encapsulate viable *L. plantarum* 423 cells[31] and to ensure viability throughout and after encapsulation. An inverse Pickering emulsion was produced by using a *L. plantarum* 423 suspension in De Man, Rogosa, and Sharpe (MRS) broth as the dispersed phase. The growth medium for the bacteria, besides other ingredients, also contains salts and the surfactant Tween 80 that could have an influence on the different interfacial energies in the system. However, the presence of these ingredients did not influence the three-phase contact angle to such an extent that macroscopic phase separation took place. Classical microbiological techniques, such as plating out to determine colony-forming units (CFUs), could not be used to determine viability, as the bacteria will be exposed to *n*-heptane after destabilization of the droplets. We therefore utilized a staining technique based on fluorescent dyes, to evaluate viability of *L. plantarum* 423 upon dispersion in the droplets of a Pickering emulsion. 4',6-diamidino-2-phenylindole (DAPI, blue) and bis-benzimide trihydrochloride (SYTO 9, green) were added, which should stain the bacteria, although, interestingly, SYTO9 only stained the silica particles (Figure 4). In addition, propidium iodide (red) was used, which can only penetrate a cell when the cell membrane is compromised, which is indicative of non-viable bacteria (Figure 6).

To determine the viability of *L. plantarum* 423 in the emulsion droplets, positive and negative controls were also imaged (Figure 4A and E). In these control experiments, the bacterial cells were dispersed in a growth medium with SYTO9 (green) and propidium (red dye). Confocal fluorescence microscopy (CFM) was used to image the viable bacteria (Figure 4A). Non-viable cells were visualized after the addition of ethanol (Figure 4E). After the addition of *n*-hexylamine to an inverse Pickering emulsion, all bacteria inside the microdroplets died. This image served as a negative control for bacteria in emulsion droplets (Figure 4F).

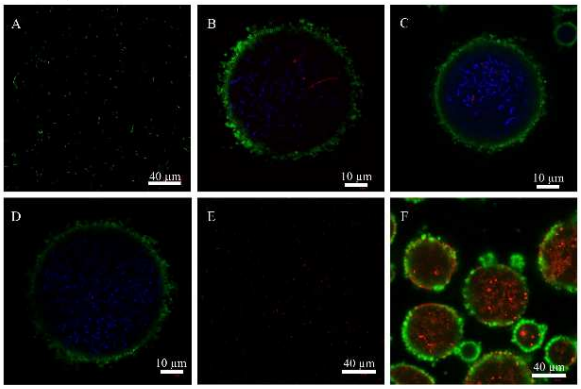


Figure 4. Inverse Pickering emulsion with *L. plantarum* 423 imaged by confocal fluorescence microscopy (CFM). A: positive control, B-D: encapsulated *L. plantarum* 423 in an inverse Pickering emulsion, E: negative control, F: negative control for an inverse Pickering emulsion. Blue: DAPI, stains the nucleus of all cells; Red: propidium iodide, stains cells with a permeable membrane (non-viable); Green: SYTO9, stains the stabilizing silica particles.

When comparing *L. plantarum* 423, dispersed in MRS broth and emulsified in the Pickering emulsion droplets with the different control samples, it became apparent that at least 90% of the bacteria remained viable in the emulsion droplets after the emulsification procedure (Figure 4B-D). The experiment was repeated three times.

Finally, after a stable inverse Pickering emulsion, containing viable *L. plantarum* 423 was produced, alkylchlorosilanes were added to create a shell around the droplets for final encapsulation. A pH buffer was added to the water phase to counteract the pH change that would otherwise be caused by the formation of hydrochloric acid during the polymerization of alkylchlorosilanes. Fluorescent dyes were added before emulsification and the interfacial reaction (Figures 5 and S4).

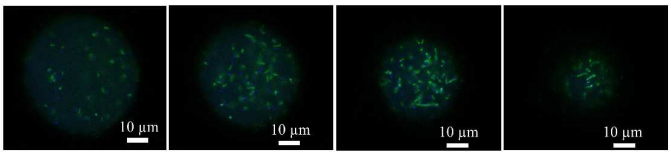


Figure 5. Confocal Fluorescence Microscopy images (so-called Z-stack) of isolated *L. plantarum* 423 bacteria in microcapsules. Microcapsules were synthesized by the interfacial reaction of octadecyltrichlorosilane and dimethyldichlorosilane at the interface of inverse Pickering emulsion droplets. Colors are the same as in Figure 4.

The encapsulation procedure resulted in viable *L. plantarum* 423 cells inside the microcapsules (Figure 5 and S4). The staining technique used is similar to the one used for the Pickering emulsion, using fluorescent dyes, to evaluate viability of *L. plantarum* 423. Again DAPI (blue) and SYTO 9 (green) were added, which should stain the bacteria. In addition, propidium iodide (red) was used, which can only penetrate a cell when the cell membrane is compromised, see Figure 4E-F. After the interfacial reaction of alkylchlorosilanes, the produced silica capsule wall was not stained by SYTO 9 as in Figure 4B-D and F. The reason for this different

ChemComm Accepted Manuscript

behavior is that the formed polysiloxanes possess very different characteristics from amorphous silica. Again the viable bacteria could be recognized by the blue and green color caused by DAPI and SYTO 9 and the non-viable bacteria by their red color via the addition of propidium iodide. From the CFM images it could be concluded that after the interfacial reaction a large fraction of the bacteria remained viable (Figure 5 and 4S). Figure 5 is a series of 2D focus stacking images of an individual capsule. This means that images were made by focusing up and down through the sample/microcapsule.[32] The images indicate that the bacteria mostly reside on the bottom of the capsules, most likely caused by gravity. In a separate experiment, the viability of the bacteria after the encapsulation process has been monitored. There is no loss of viability within the first three hours after encapsulation (Figure S5), which means that the encapsulation process and the environment inside the capsule after encapsulation are benign to the bacteria.

Conclusions

In this contribution we reported the microencapsulation of *L. plantarum* 423 and showed that the majority of the cells remained viable during and after encapsulation. Encapsulation of *L. plantarum* 423 was accomplished by formation of a Pickering emulsion of an aqueous suspension of bacteria in *n*-heptane stabilized by hydrophobized silica particles, followed by the interfacial reaction of alkylchlorosilanes at the interface of inverse the Pickering emulsion droplets that were stabilized by hydrophobized silica microparticles. The bacteria remained viable during and after microcapsule synthesis. This research opens up novel avenues for the encapsulation of bacteria, enzymes and viable cells.

Notes and references

[1] Eindhoven University of Technology, Department of Chemical Engineering and Chemistry, P.O. Box 513, 5600 MB Eindhoven University of Technology, the Netherlands
[2] Department of Microbiology, Stellenbosch University, Private Bag X1, 7602 Matieland, South Africa
[3] Department of Chemistry and Polymer Science, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa
e-mail: bklump@sun.ac.za

Electronic Supplementary Information (ESI) available: Experimental procedures and some supporting figures. See DOI: 10.1039/c000000x/

(1) Vidhyalakshmi, R.; Bhakyaraj, R.; Subhasree, R. S. Encapsulation " The Future of Probiotics " -A Review. *Adv. Biol. Res. (Rennes).* **2009**, *3*, 96–103.
(2) Keating, C. D. Inorganic Protocells: Gated Access to Microreactors. *Nat. Chem.* **2013**, *5*, 449–451.
(3) Bu, Z.; Callaway, D. J. E. *Proteins Move! Protein Dynamics and Long-Range Allostery in Cell Signaling.*; 2011; Vol. 83, pp. 163–221.
(4) Jen, a C.; Wake, M. C.; Mikos, a G. Review: Hydrogels for Cell Immobilization. *Biotechnol. Bioeng.* **1996**, *50*, 357–364.
(5) Rossow, T.; Heyman, J. A.; Ehrlicher, A. J.; Langhoff, A.; Weitz, D. A.; Haag, R.; Seiffert, S. Controlled Synthesis of Cell-Laden Microgels by Radical-Free Gelation in Droplet Microfluidics. *J. Am. Chem. Soc.* **2012**, *134*, 4983–4989.
(6) Sugiura, S.; Oda, T.; Izumida, Y.; Aoyagi, Y.; Satake, M.; Ochiai, A.; Ohkohchi, N.; Nakajima, M. Size Control of Calcium Alginate Beads Containing Living Cells Using Micro-Nozzle Array. *Biomaterials* **2005**, *26*, 3327–3331.
(7) Coiffier, A.; Coradin, T.; Roux, C.; Bouvet, O. M. M.; Livage, J. Sol–gel Encapsulation of Bacteria: A Comparison between Alkoxide and Aqueous Routes. *J. Mater. Chem.* **2001**, *11*, 2039–2044.
(8) Nassif, N.; Bouvet, O.; Noelle Rager, M.; Roux, C.; Coradin, T.; Livage, J. Living Bacteria in Silica Gels. *Nat. Mater.* **2002**, *1*, 42–44.
(9) Livage, J.; Coradin, T.; Roux, C. Encapsulation of Biomolecules in Silica Gels. *J. Phys. Condens. …* **2001**, *673*, R673–R691.
(10) Flemke, J.; Maywald, M.; Sieber, V. Encapsulation of Living E. Coli Cells in Hollow Polymer Microspheres of Highly Defined Size. *Biomacromolecules* **2013**, *14*, 207–214.
(11) Gill, I.; Ballesteros, a. Bioencapsulation within Synthetic Polymers (Part 1): Sol-Gel Encapsulated Biologicals. *Trends Biotechnol.* **2000**, *18*, 282–296.
(12) Pickering, S. U. Pickering: Emulsions. *J. Chem. Soc.* **1907**, *91*, 2001–2021.
(13) Van Wijk, J.; Salari, J. W. O.; Zaquen, N.; Meuldijk, J.; Klumperman, B. Poly(methyl Methacrylate)–silica Microcapsules Synthesized by Templating Pickering Emulsion Droplets. *J. Mater. Chem. B* **2013**, *1*, 2394–2406.
(14) Bon, S. A. F.; Chen, T. Pickering Stabilization as a Tool in the Fabrication of Complex Nanopatterned Silica Microcapsules. *Langmuir* **2007**, *23*, 9527–9530.
(15) Sardessai, Y.; Bhosle, S. Tolerance of Bacteria to Organic Solvents. *Res. Microbiol.* **2002**, *153*, 263–268.
(16) Pieranski, P. Two-Dimensional Interfacial Colloidal Crystals. *Phys. Rev. Lett.* **1980**, *45*, 569–572.
(17) Kaptay, G. On the Equation of the Maximum Capillary Pressure Induced by Solid Particles to Stabilize Emulsions and Foams and on the Emulsion Stability Diagrams. *Colloids Surfaces A Physicochem. Eng. Asp.* **2006**, *282-283*, 387–401.
(18) Kulkarni, S. a; Ogale, S. B.; Vijayamohanan, K. P. Tuning the Hydrophobic Properties of Silica Particles by Surface Silanization Using Mixed Self-Assembled Monolayers. *J. Colloid Interface Sci.* **2008**, *318*, 372–379.
(19) Salari, J. W. O.; Jemwa, G. T.; Wyss, H. M.; Klumperman, B. Reconstruction of the 3D Structure of Colloidosomes from a Single SEM Image. *Soft Matter* **2011**, *7*, 2033–2041.
(20) Parikh, A. N.; Schivley, M. A.; Koo, E.; Seshadri, K.; Aurentz, D.; Mueller, K.; Allara, D. L.; Pennsyl, V.; Uni, S.; Park, U. V. N - Alkylsiloxanes : From Single Monolayers to Layered Crystals . The Formation of Crystalline Polymers from the Hydrolysis of N - Octadecyltrichlorosilane. *Am. Chem. Soc.* **1997**, *7863*, 3135–3143.
(21) Stöber, W.; Fink, A.; Bohn, E. Controlled Growth of Monodisperse Silica Spheres in the Micron Size Range. *J. Colloid Interface Sci.* **1968**, *69*, 62–69.
(22) Bogush, G.; Tracy, M.; Iv, C. Z. Preparation of Monodisperse Silica Particles: Control of Size and Mass Fraction. *J. Non. Cryst. Solids* **1988**, *104*, 95–106.
(23) Zhuravlev, L. T. The Surface Chemistry of Amorphous Silica. Zhuravlev Model. *Colloids Surfaces A Physicochem. Eng. Asp.* **2000**, *173*, 1–38.
(24) Zhuravlev, L. T. Surface Characterization of Amorphous Silica—a Review of Work from the Former USSR. *Colloids Surfaces A Physicochem. Eng. Asp.* **1993**, *74*, 71–90.
(25) Abràmoff, MD., Magalhaes, PJ., Ram, S. Image Processing with ImageJ. *Biophotonics* **2004**, 1997–2014.
(26) Silane coupling agents, connecting across bounderies www.gelest.com (accessed Apr 7, 2014).
(27) Jin, M.; Li, S.; Wang, J.; Liao, M.; Zhao, Y. Controllable Fabrication of Organosilane Nano-Architectured Surfaces with Tunable Wettability. *Appl. Surf. Sci.* **2012**, *258*, 7552–7555.
(28) Fadeev, A. Y.; McCarthy, T. J. Self-Assembly Is Not the Only Reaction Possible between Alkyltrichlorosilanes and Surfaces: Monomolecular and Oligomeric Covalently Attached Layers of Dichloro- and Trichloroalkylsilanes on Silicon. *Langmuir* **2000**, *16*, 7268–7274.
(29) Khoo, H. S.; Tseng, F.-G. Engineering the 3D Architecture and Hydrophobicity of Methyltrichlorosilane Nanostructures. *Nanotechnology* **2008**, *19*, 345603.
(30) Neubauer, M. P.; Poehlmann, M.; Fery, A. Microcapsule Mechanics: From Stability to Function. *Adv. Colloid Interface Sci.* **2014**, *207*, 65–80.
(31) Van Reenen, C. A.; Dicks, L. M.; Chikindas, M. L. Isolation, Purification and Partial Characterization of Plantaricin 423, a Bacteriocin Produced by Lactobacillus Plantarum. *J. Appl. Microbiol.* **1998**, *84*, 1131–1137.
(32) *LSM 710, LSM 780 Operating Manual*; 2010; pp. 0–138.

ChemComm Accepted Manuscript

 This journal is © The Royal Society of Chemistry 2012