“Innovation for Encapsulation 2017”, 8 December, Burlington House, London

Mesoporous $CaCO_3$ crystals - a powerful tool for bio-friendly encapsulation

Dmitry Volodkin

Group „Active-Bio-Coatings“

Prof Assoc

School of Science and Technology, Nottingham Trent University
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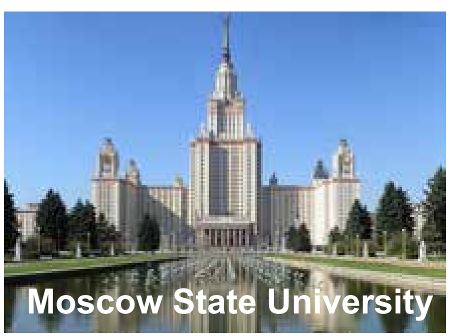

NOTTINGHAM TRENT UNIVERSITY

The content

1. Motivation

Seeking sacrificial templates for bio-friendly protein encapsulation

2. Mesoporous vaterite $CaCO_3$ crystals

Synthesis, control over size and porosity

3. Protein particles

One-component compact/porous protein beads

4. Multilayer capsules

Biomolecule encapsulation into finely-tuned polymer shells

5. Polymer scaffolds

Porous 3D scaffold for cell culture and tissue engineering

Proteins as effective therapeutic agents

Antibodies, Hormones, Growth factors, Enzymes, Cytokines, Vaccines, Peptides, etc

Increased number of effective therapeutic proteins available on market

Forecast: $315.90 billion by 2025*

Protein encapsulation:

- protection against biodegradation, local pH changes
- complexation with biomolecules that block the protein activity
- proper administration route
- targeted delivery and controlled release

Traditional encapsulation technologies

Crystallization, emulsification, spray- and freeze-drying, incorporation into polymeric and lipid matrices

- mechanical stresses (shear forces), creating additional interfaces
- high or low temperature, organic solvents, salt, pH
- surfactants or polymers

The challenge - develop effective encapsulation approaches to <u>keep protein bioactivity</u> and finely <u>tune a size and physical-chemical properties of a carrier</u>

* - https://globenewswire.com/news-release/2017/01/26/911299/0/en/Protein-Therapeutics-Market-Analysis-and-Trends-Report-2016-Therapeutic-Proteins-Application-Function-Forecast-to-2025-for-the-315-9-Billion-Market.html

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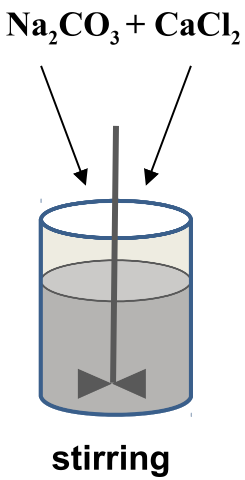
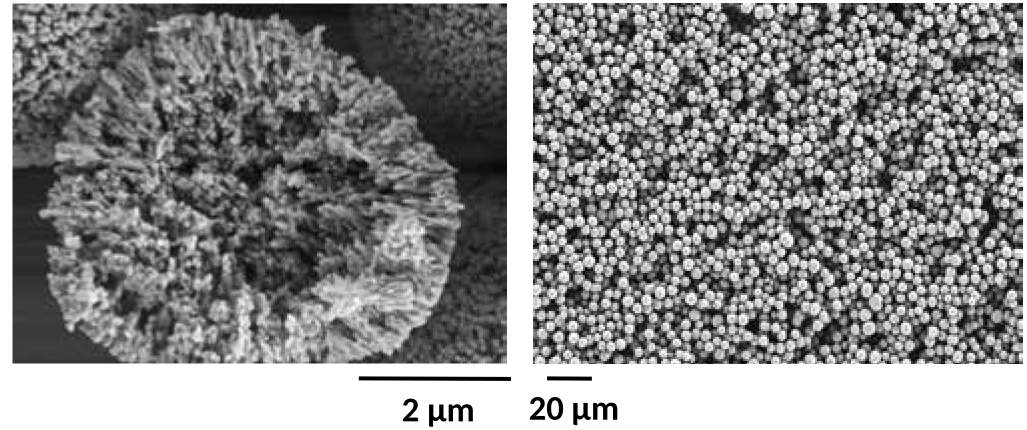
Porous $CaCO_3$ microcrystals





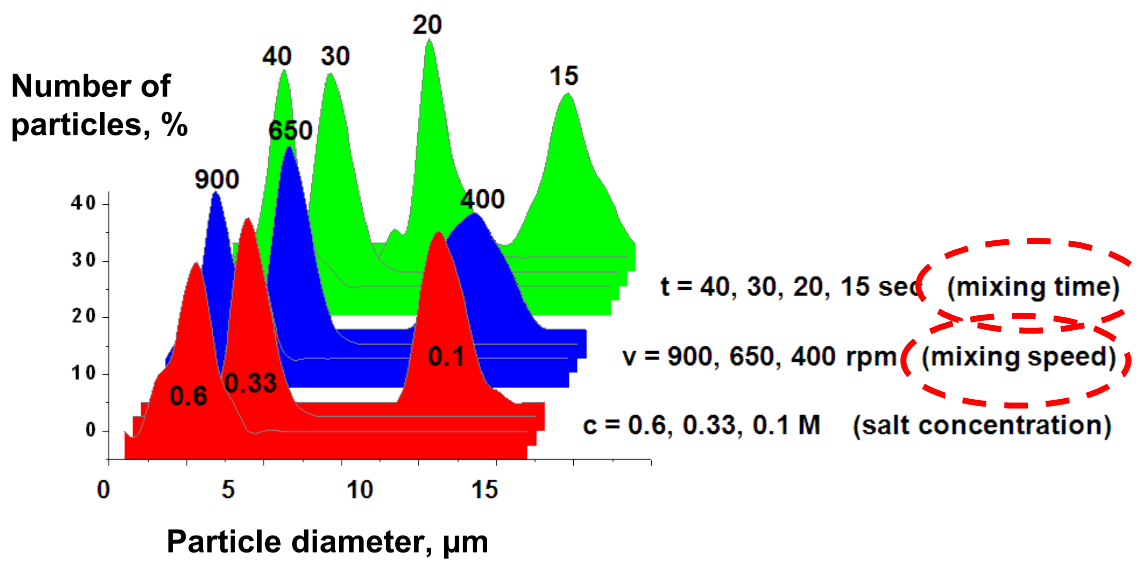
High porocity, ~ 10 m²/g

Pore diameter range: 10-60 nm

Homogeneous (vaterite form)

Biocompatible, inexpensive

Mild decomposition (EDTA, pH<7)

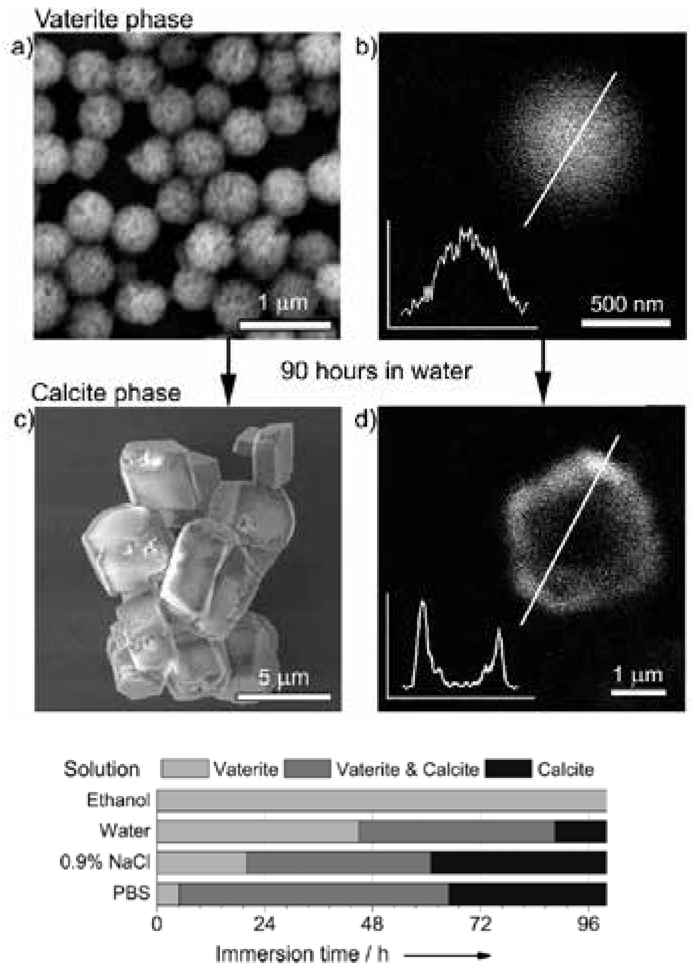


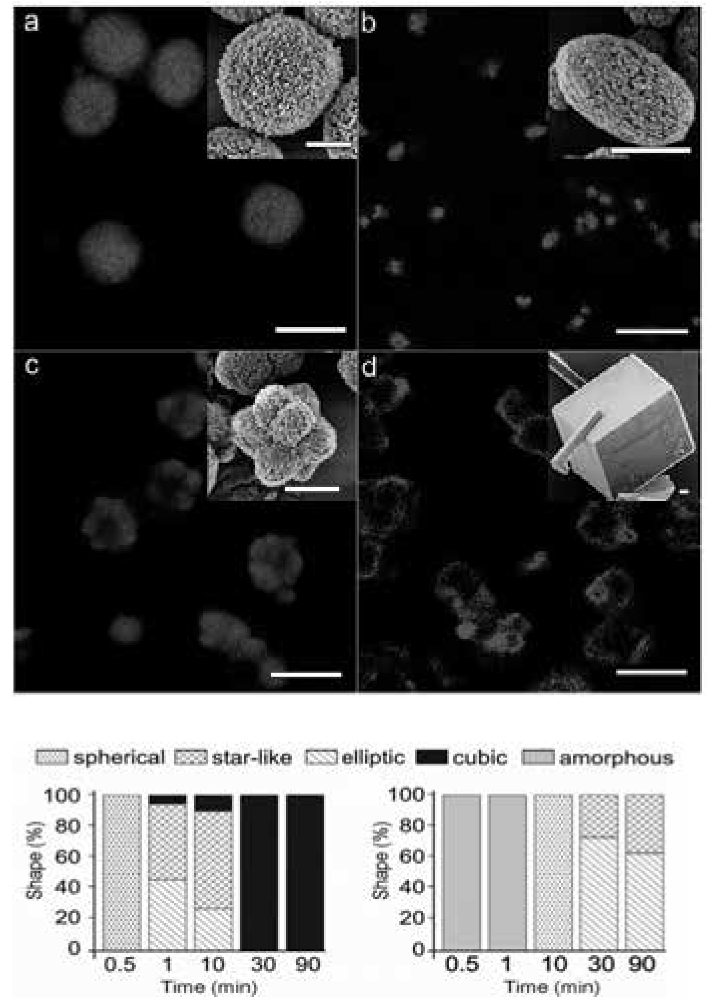
Volodkin DV. et al ***Langmuir*** 2004
Volodkin DV. et al ***Biomacromolecules*** 2004

Schmidt S. et al ***J. Mater. Chem***. 2013
Schmidt S. et al ***Adv Func Mater*** 2013

Nanometer sized $CaCO_3$ crystals. Variation of shape

430 nm crystals are stabilized by ethylene glycol
(supresses ion transport and nucleation rate)



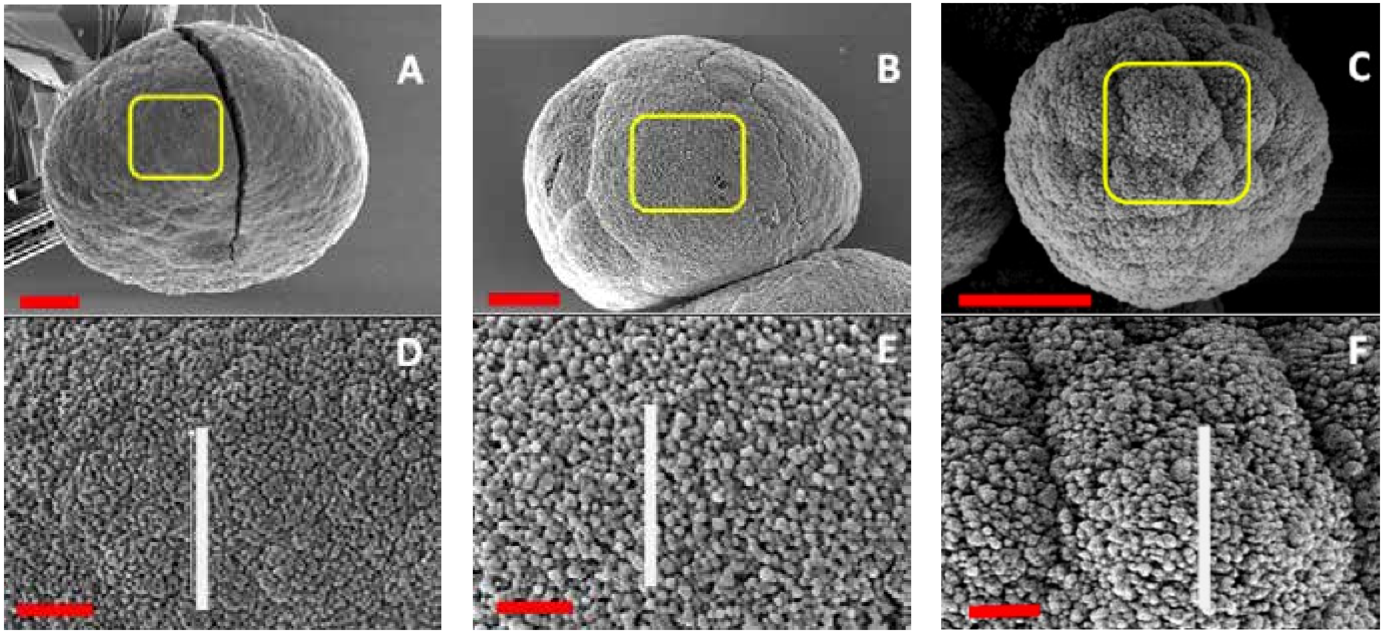


water

83% ethylene glycol

Parakhonskiy B et al ***Angew Chem Int Ed*** 2012; ***Chem Phys Chem*** 2014

Porosity control

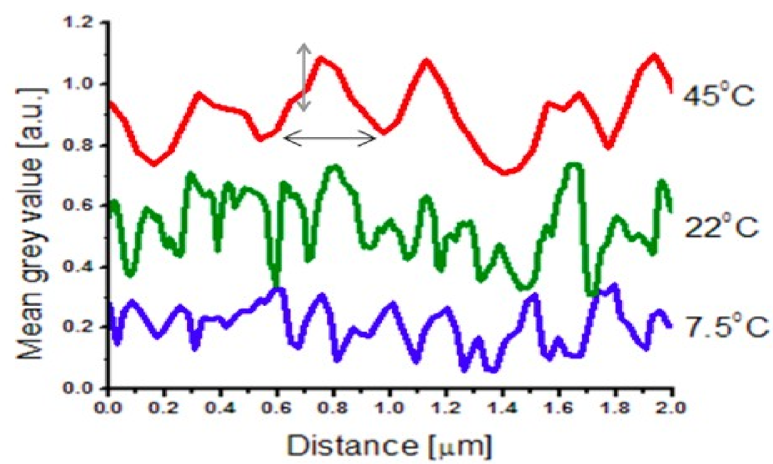


7.5 °C

22 °C

45 °C

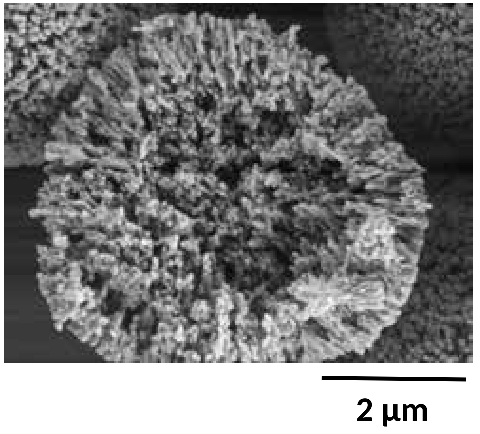
Preparation T

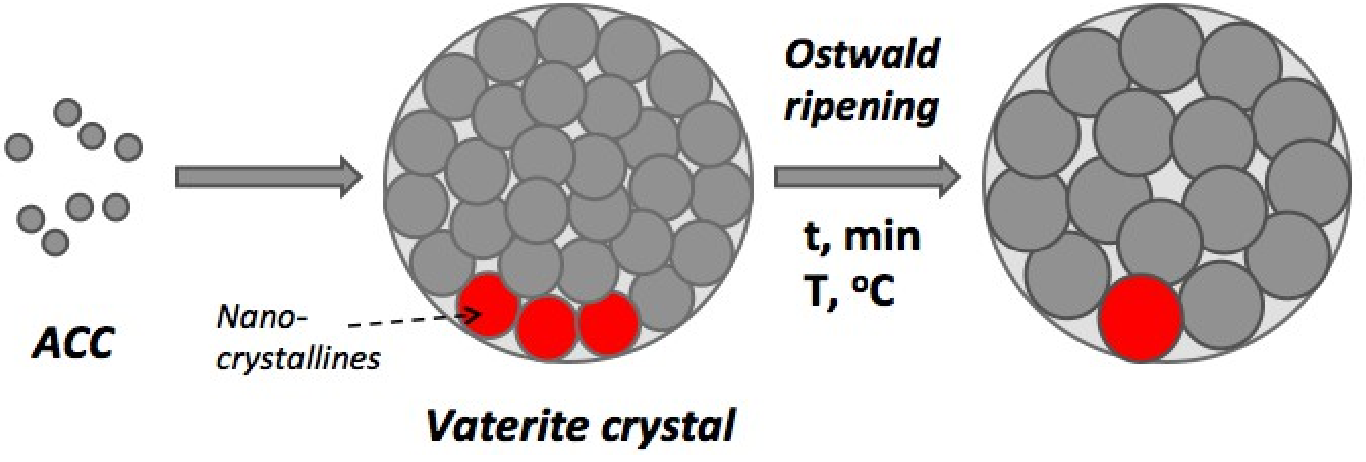


Feoktistova N. et al ***Langmuir*** 2016

Porosity control

Spherulitic growth





Higher T results in faster Ostwald ripening of nano-crystallines

Feoktistova N. et al ***Langmuir*** 2016

Pieter Bots et al ***Cryst. Growth Des***. 2012

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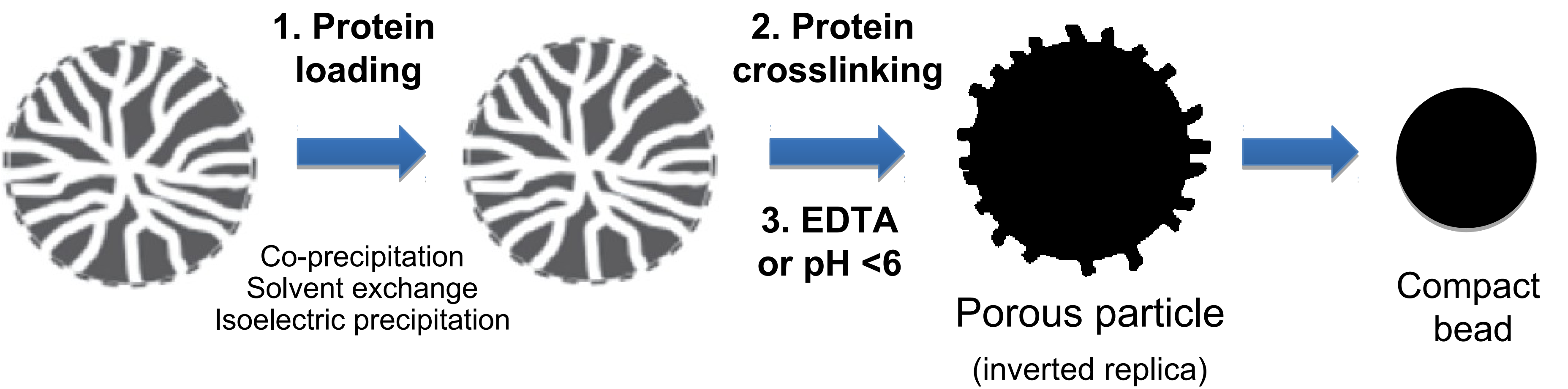
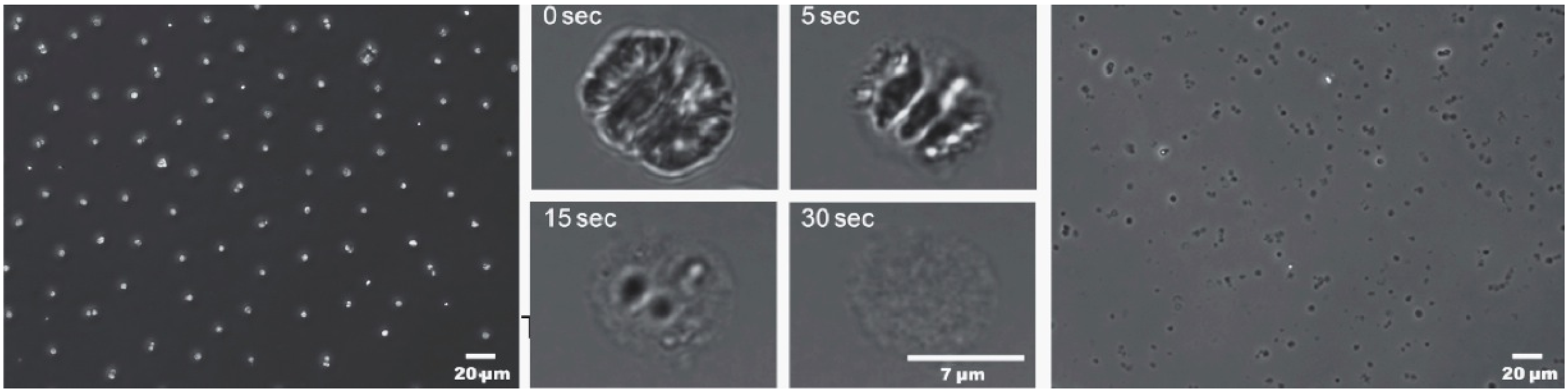
Protein beads



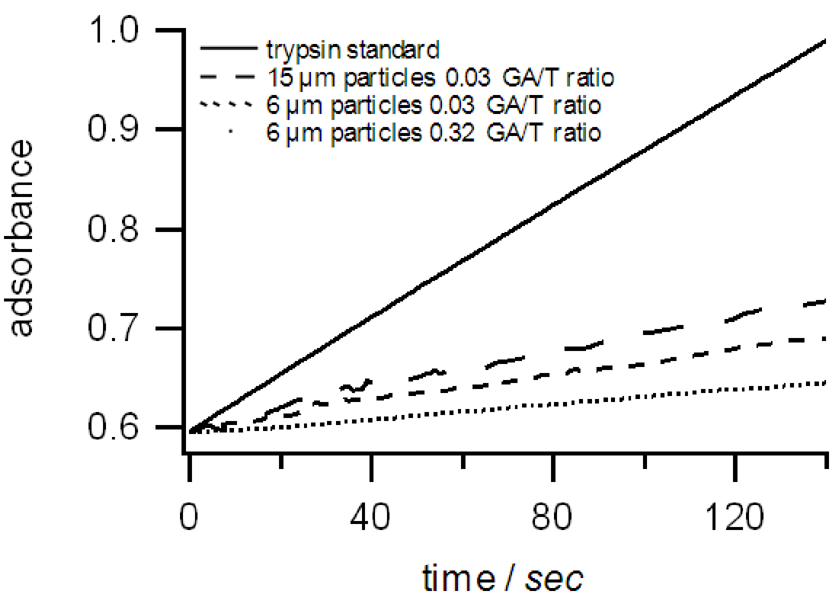


D. V. Volodkin *et al* ***Angew Chem Int Ed*** *2010*
D. V. Volodkin *et al* ***Adv Func Mater*** *2012*

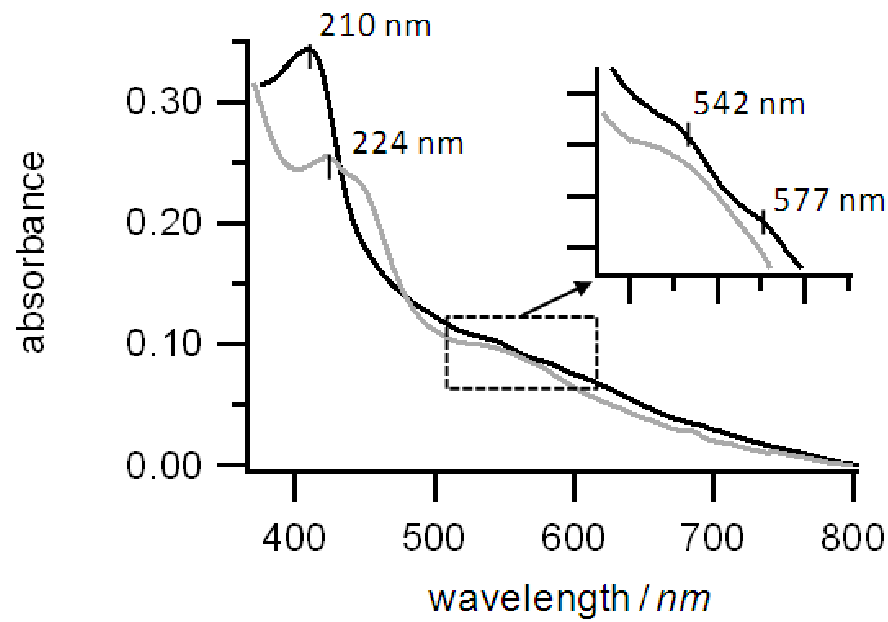
S. Schmidt, et al ***Adv Func Mater*** *2013*
S. Schmidt et al ***J Mater Chem*** *2013*

Catalysis and blood substitute

Trypsin



Hemoglobin

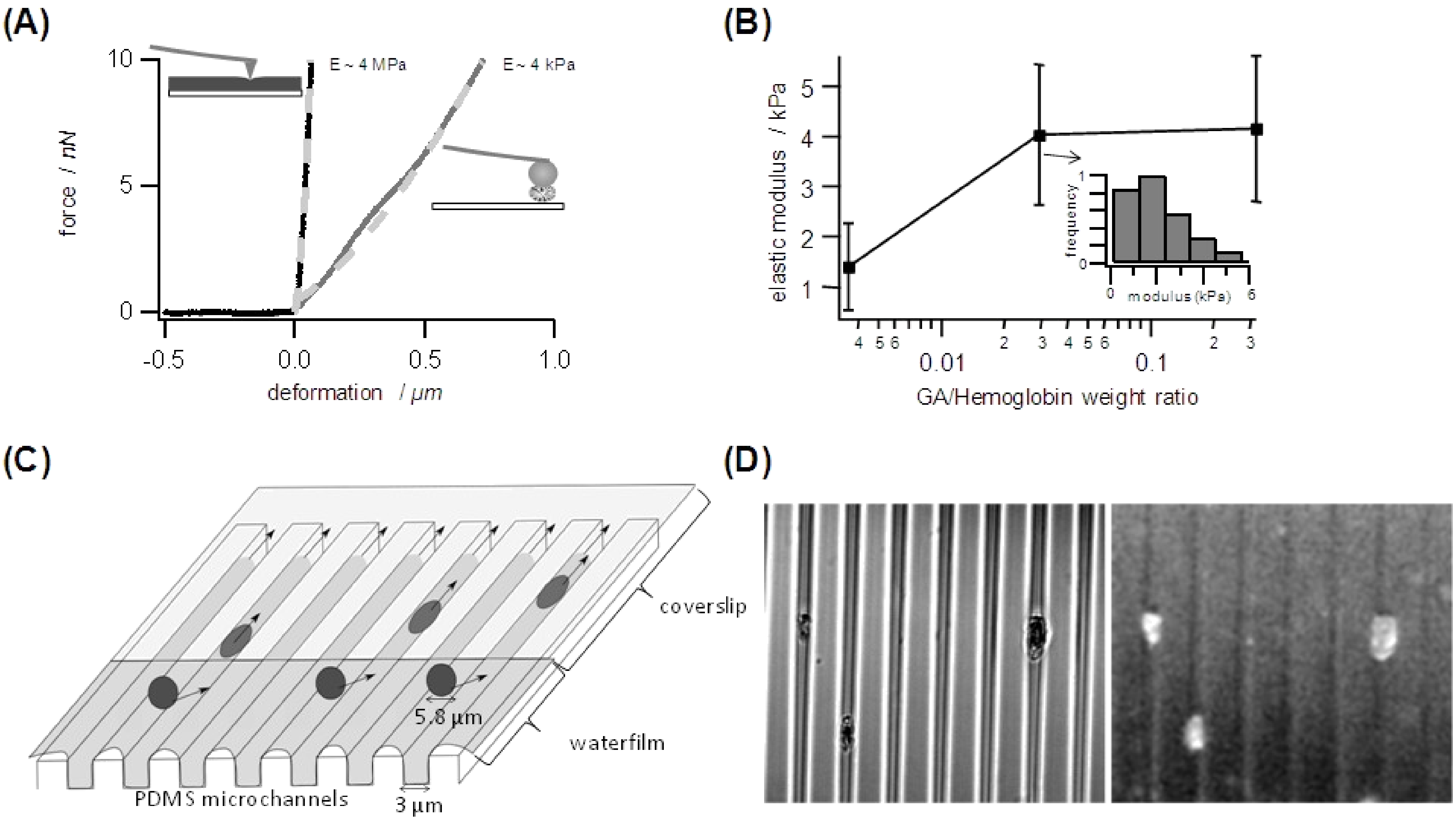


Deoxyhemoglobin (grey)
Oxyhemoglobin particles (black)

S. Schmidt, et al ***Adv Func Mater*** *2013*

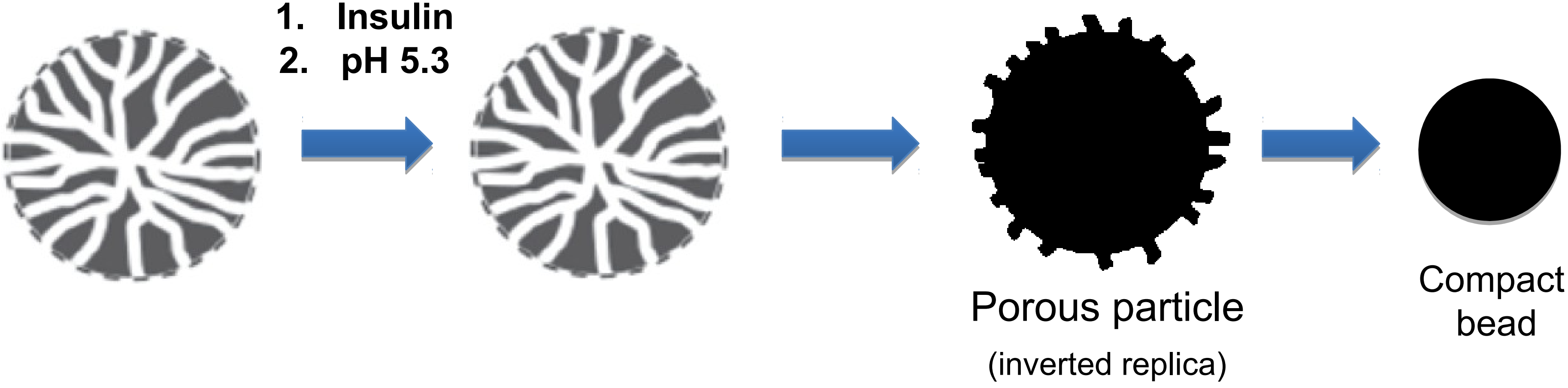
Hemoglobin porous beads as blood substitute

Mechanical properties – deformation in microchannels



S. Schmidt, et al ***Adv Func Mater*** *2013*

Compact insulin microbeads



One step fabrication

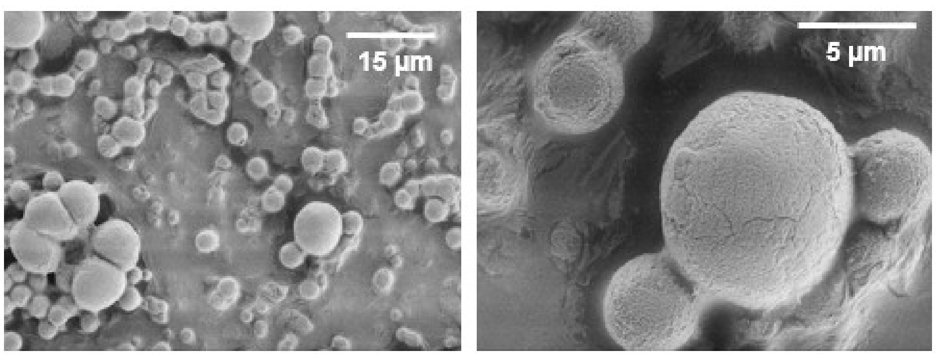
D. V. Volodkin *et al* ***Angew Chem Int Ed*** *2010*

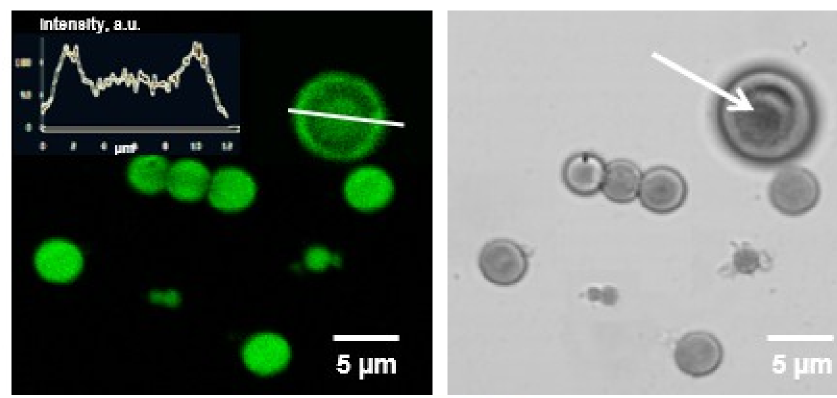
Compact insulin microbeads

$CaCO_3$ microcore diameter, µm (average pore diameter, nm)		**3.0** ± 0.9 (28 ± 4 nm)	**5.5** ± 0.6 (25 ± 3 nm)	**15.2** ± 3.8 (26 ± 4 nm)
Insulin microbeads	**Diameter**, µm by CLSM (SEM)	**2.2** ± 0.4 (2.0 ± 0.8)	**3.5** ± 0.4 (3.9 ± 0.9)	**10.5** ± 3.8 (9.7 ± 2.9)
	Diameter shrinkage coefficient	**1.36**	**1.34**	**1.45**
	Protein density, g/cm³	**0.34** ± 0.09	**0.33** ± 0.07	**0.36** ± 0.10
	Aerodynamic diameter (d_a)* , µm	**1.3** ± 0.2	**2.0** ± 0.2	**6.3** ± 2.3

* $d_a = d\,(\rho/\rho_{water})^{1/2} = d\,(\rho)^{1/2}$ ρ – protein density d_a for pulmonary delivery 1-6 µm

SEM, beads mixture



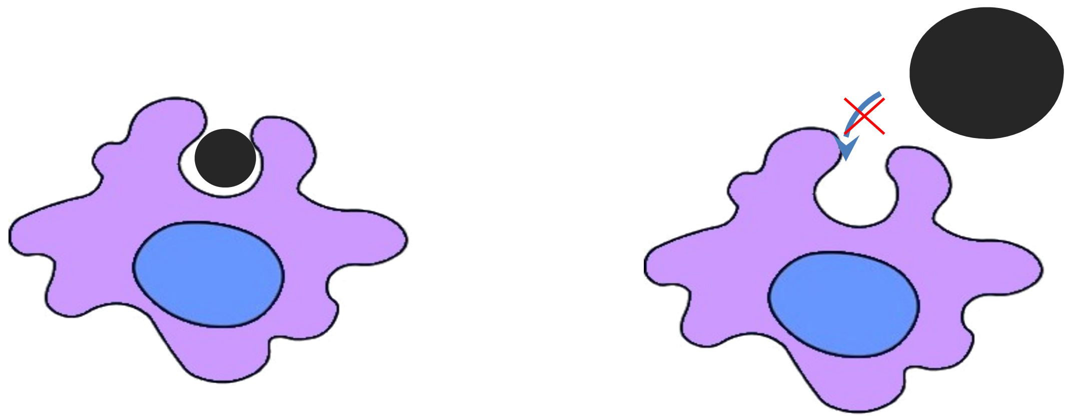


CLSM

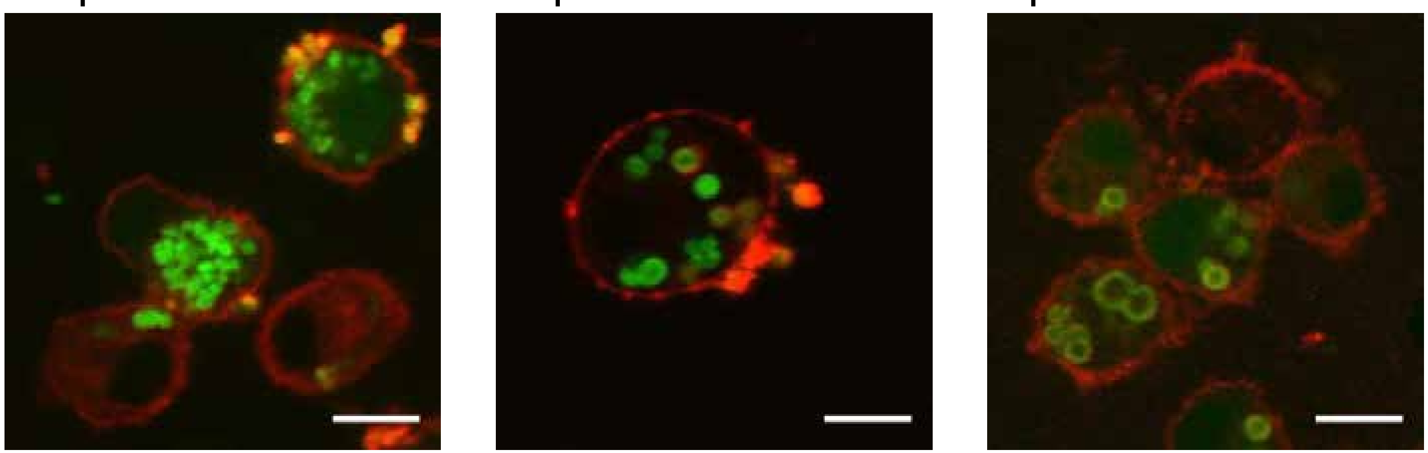
D. V. Volodkin *et al* ***Angew Chem Int Ed*** *2010*

Phagocytosis with alveolar macrophages

Idea: reduce phagocytic clearance by increasing the insulin particles size



1.5 µm 3 µm 5 µm



Uptake of insulin particles of different sizes by alveolar Macrophages NR8383.

Schmidt, S., et al ***Acta Biomat****erialia* 2014

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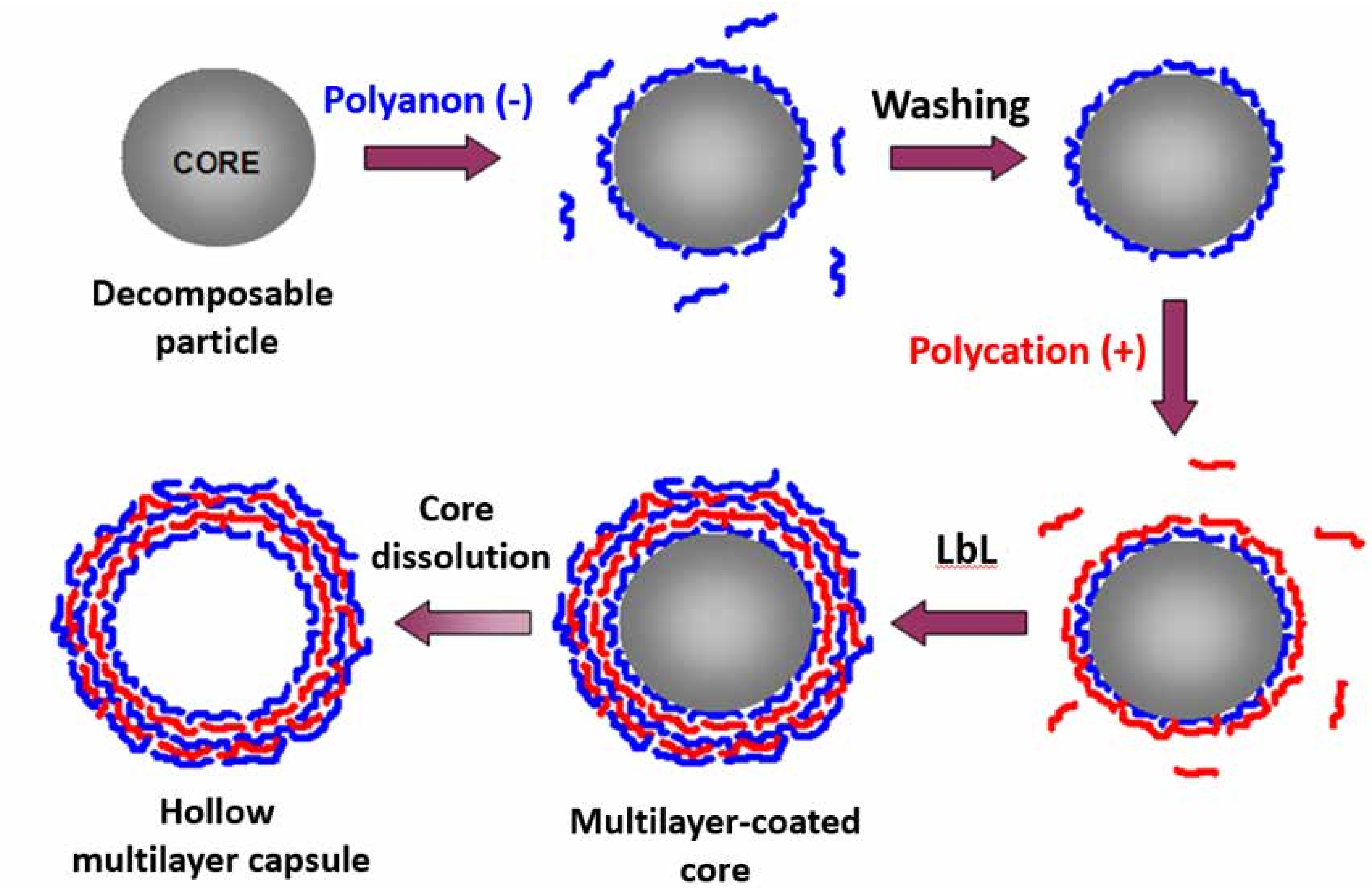
Layer-by-Layer assembled multilayer capsules

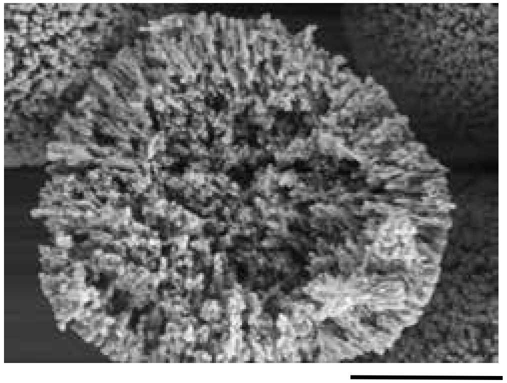


G. B. Sukhorukov, et al ***Colloid Surf. A-Physicochem. Eng. Asp.***,1998.

Bio-friendly cores for capsule formation

Table 1: Templates used for the preparation of hollow polyelectrolyte capsules.

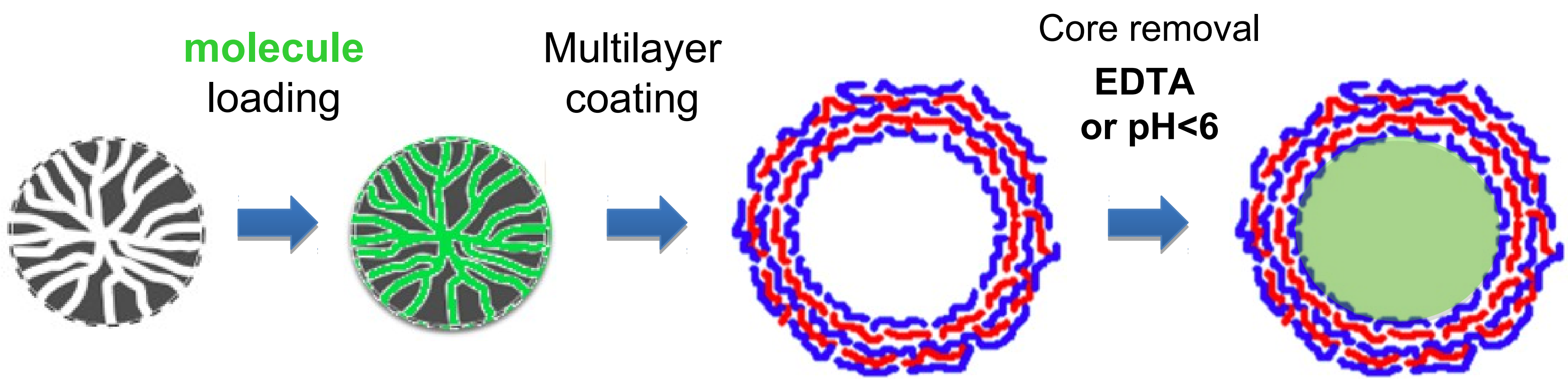
Parameter	Melamine formaldehyde[18]	Polystyrene latex[58]	Silica[163]	Erythrocytes[162]	$CdCO_3$, $MnCO_3$, $CaCO_3$	PLA/PLGA[160]
Size (µm)	0.3–10	0.1–5	0.03–100	5.5–7.5	3–8	0.2–20
shape	spherical	spherical	spherical	discocytes	crystalline, porous	spherical
monodispersity	excellent	excellent	good–excellent	good	medium	low
commercial availability	+	+	+	+	–	+/–
price	very high	medium	low	low	–	low
problems upon dissolution	mechanical stress; residues	mechanical stress; residues	aggregation	chemical stress; wall destruction	no stress	residues
Decomposition medium	0.1M HCL	organic solvent	HF	HCLO	**pH<7 or EDTA**	organic solvent



2 µm

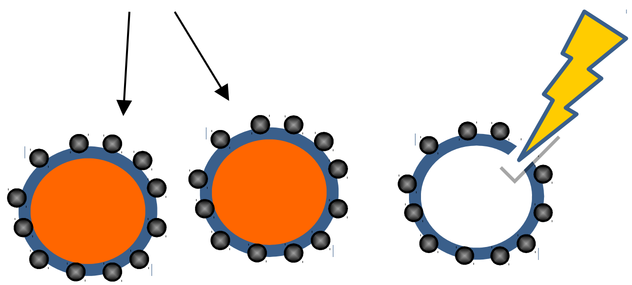
Peyratout, C. S. et al ***Angew Chem Int Ed*** *2004*

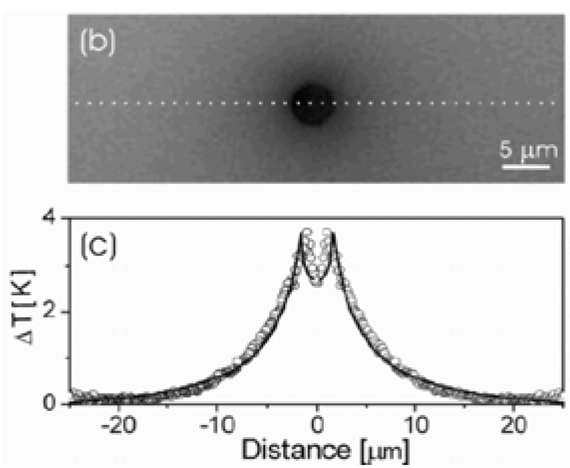
Encapsulation through porous $CaCO_3$



Light-triggered release (capsule shell overheating)

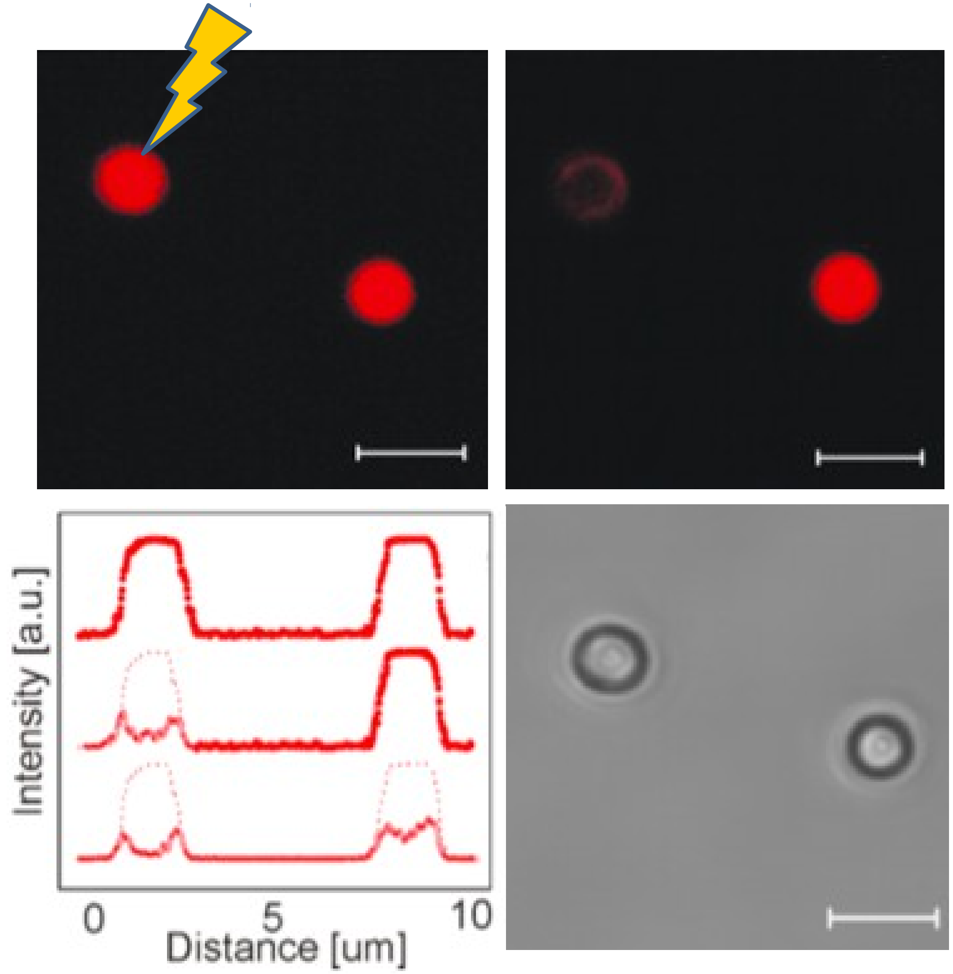
Dextrane filled (PDADMAC/PSS)$_4$ capsules (2 µm) coated with Au NPs





Skirtach AG et al ***Nano Lett*** 2005

830 nm



Volodkin et al ***Langmuir*** 2009
Volodkin et al ***ACS Appl. Mater. Int.*** 2009

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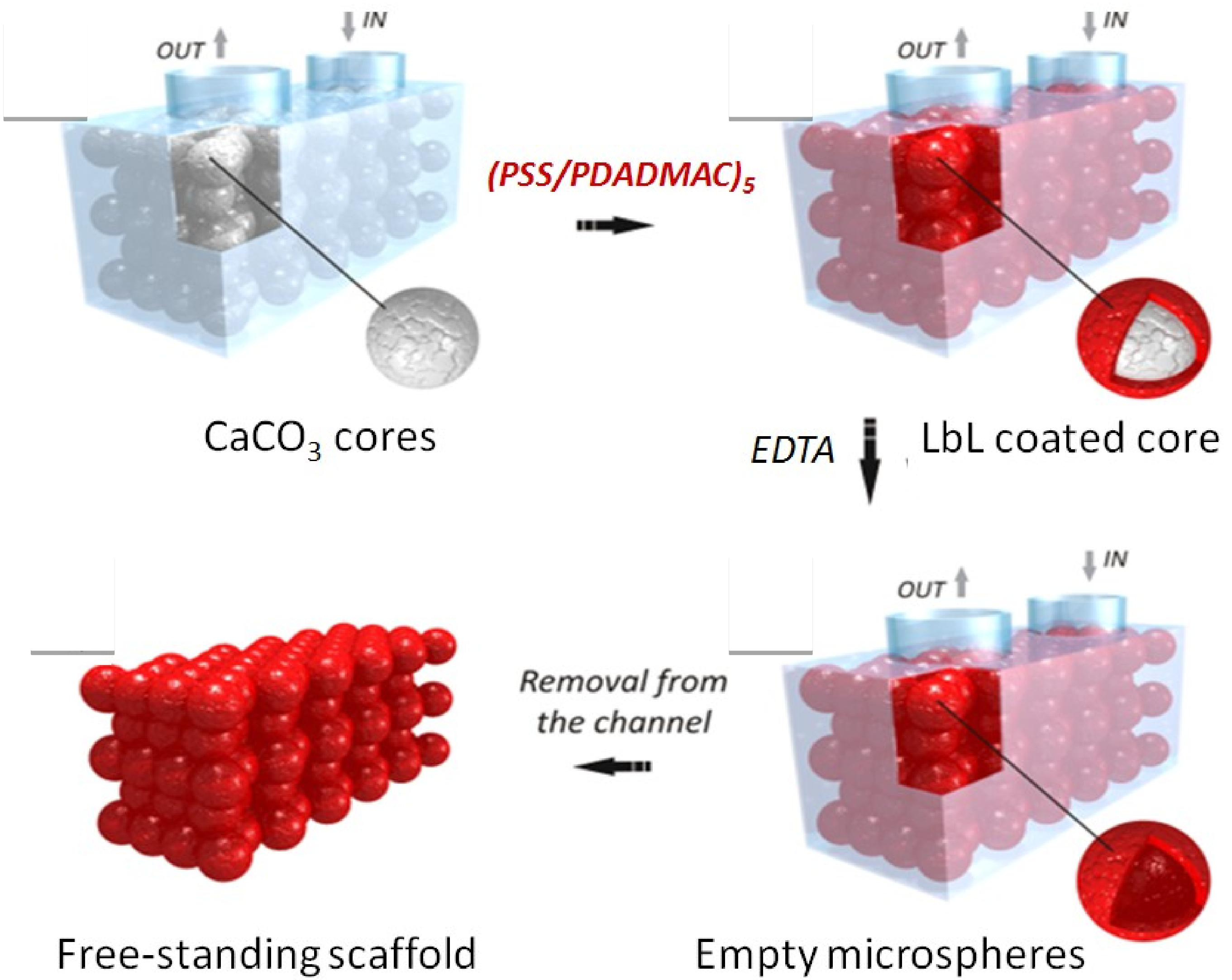
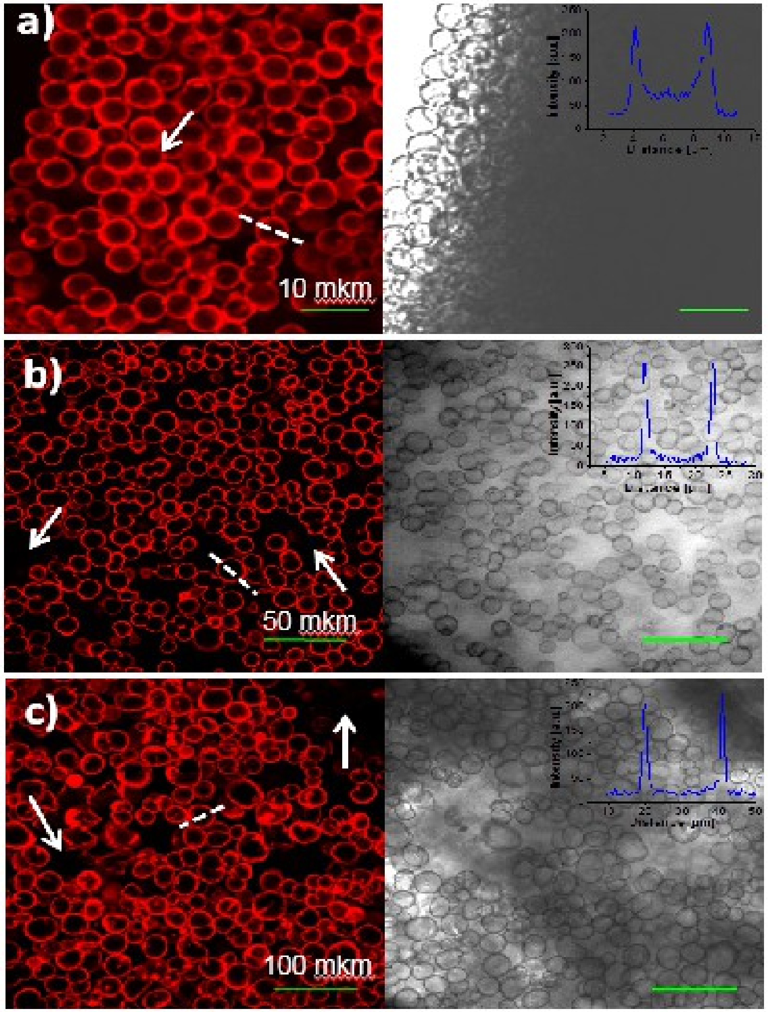
Porous polymer scaffolds templated on $CaCO_3$ cores

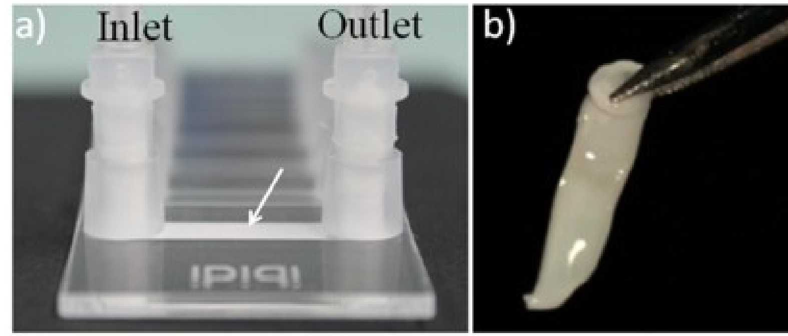


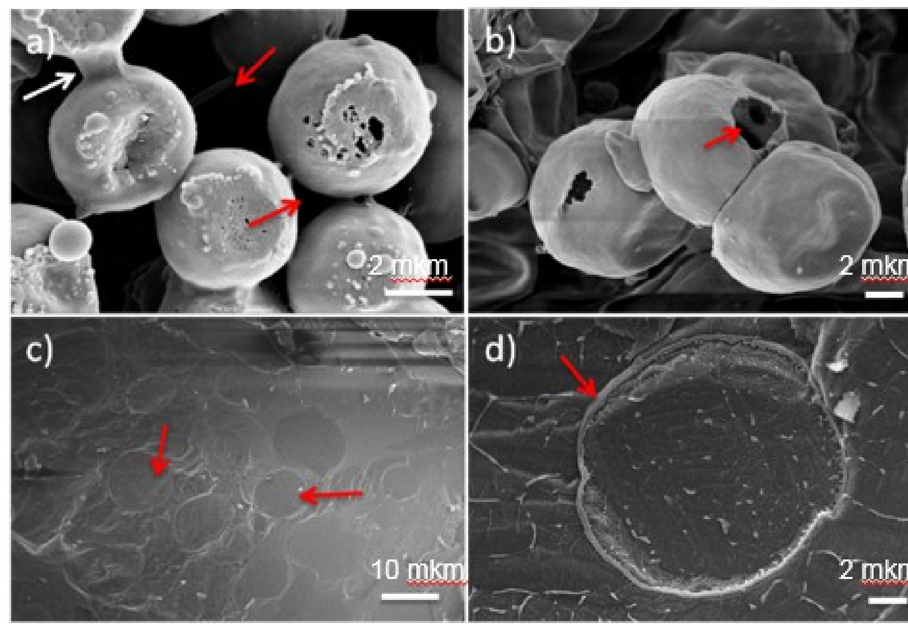
Paulraj T. et al ***Macromol Rapid Commun*** 2014

Porous polymer scaffolds templated on $CaCO_3$ cores



CLSM

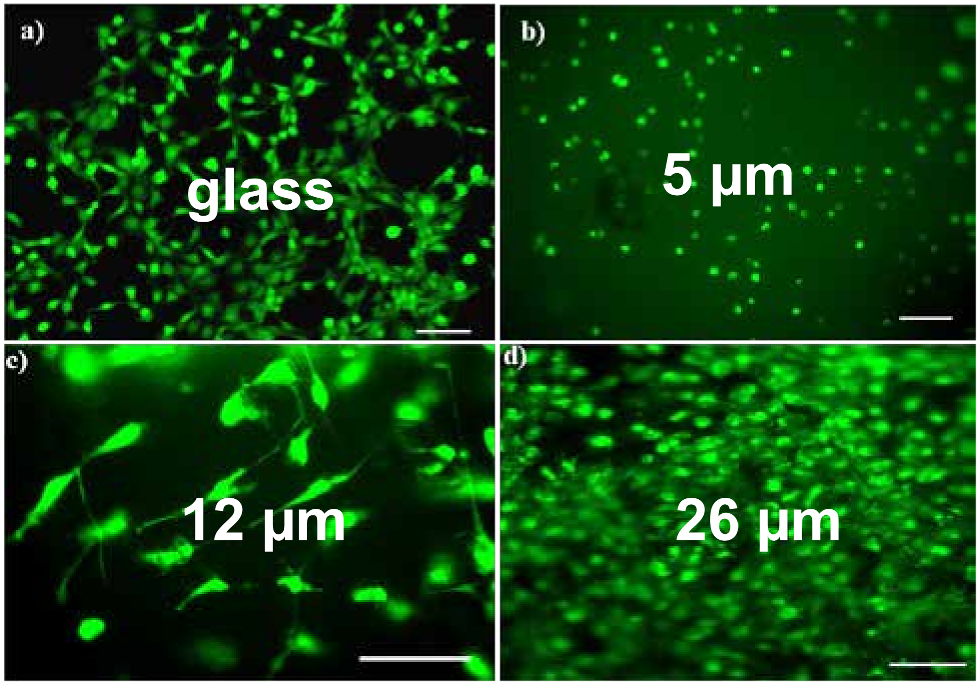




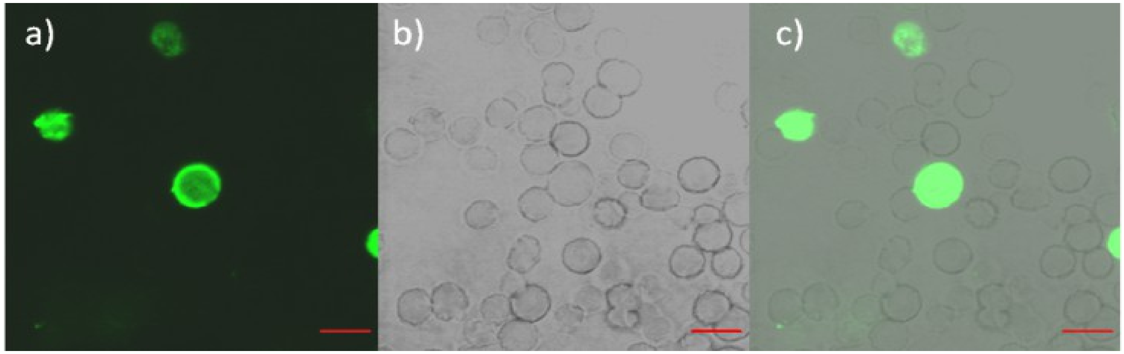
SEM, cryo-SEM

Paulraj T. et al ***Macromol Rapid Commun*** 2014

Cellular adhesion and protein encapsulation



3T3 fibroblasts, 3 days, calcein stained

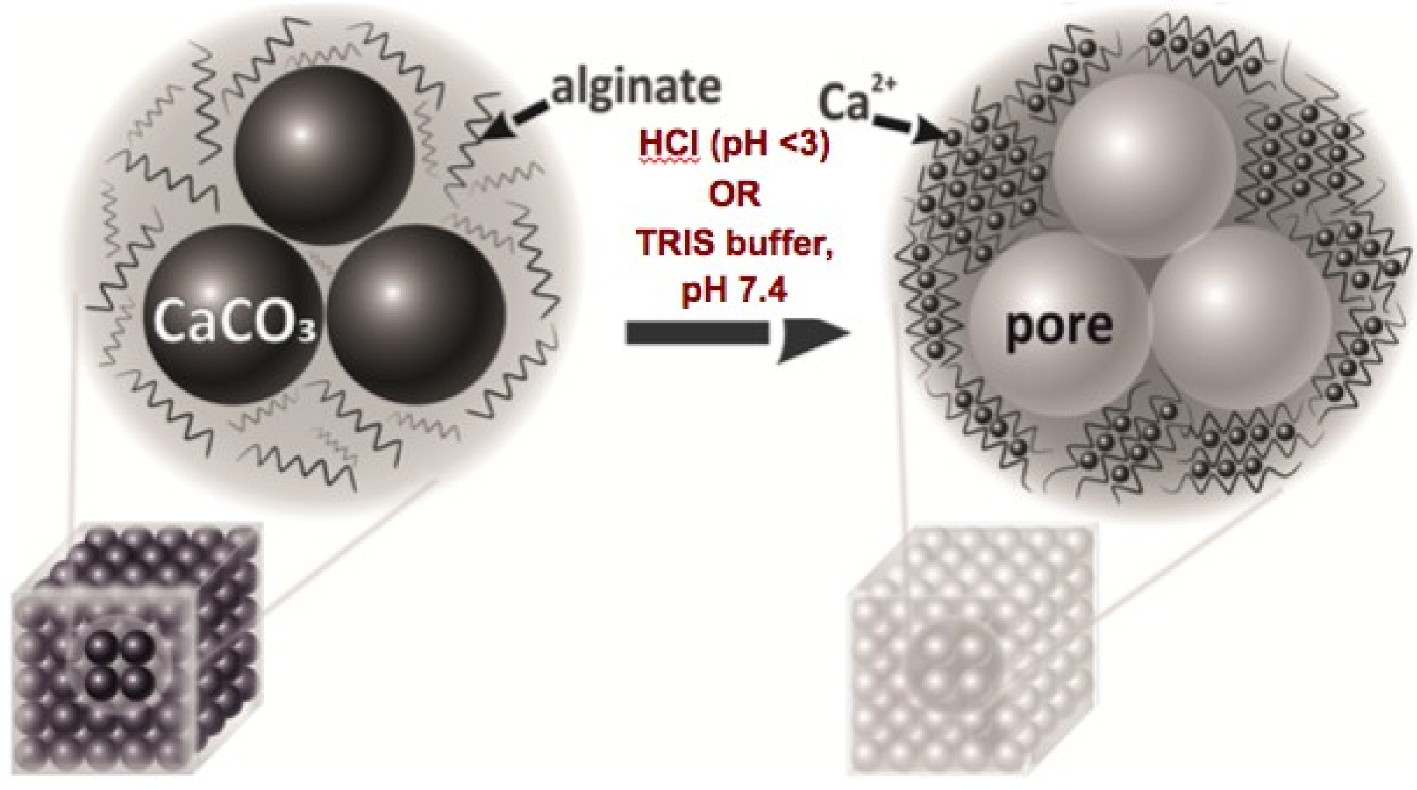


Microspheres loaded with BSA-FITC by co-precipitation into initial $CaCO_3$ cores

$CaCO_3$:$CaCO_3$-BSA = 5:1

Paulraj T. et al ***Macromol Rapid Commun*** 2014

Porous Ca-Alginate Scaffolds (PAS)



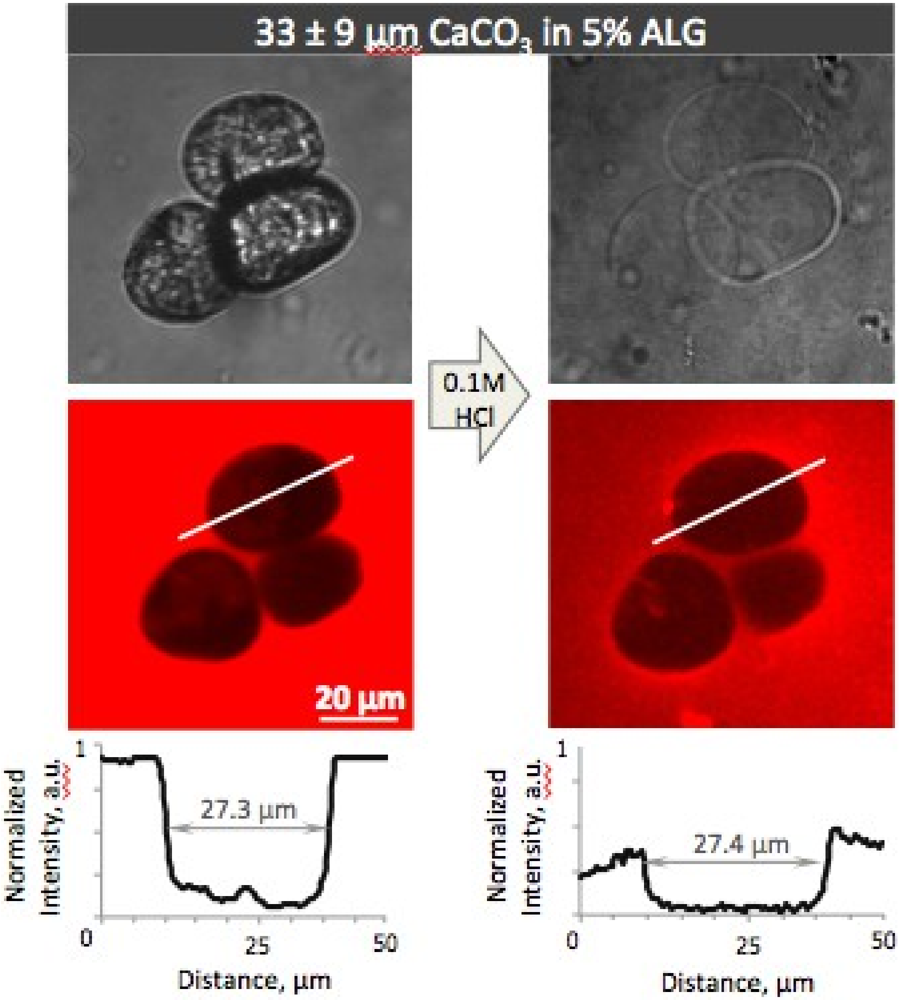
CaCO$_3$ crystals:

1. **Ca^{2+} source**
2. **Porogens**
3. **Carriers to load the pores with biomolecules trapped in the crystals**

Sergeeva, A. et al *Advanced Materials Interfaces* **2015,** *Acs Applied Materials & Interfaces* **2015**, *Langmuir* **2015**

Pore size = $CaCO_3$ size

Pores are identical to removed particles:
gel cross-linking and release of Ca^{2+} ions do not result in pore shrinkage and swelling, respectively

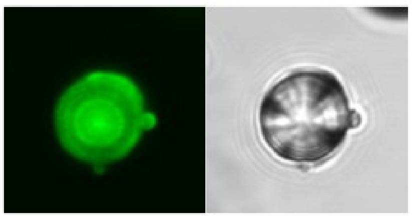


Sergeeva, A. et al *Advanced Materials Interfaces* **2015,** *Acs Applied Materials & Interfaces* **2015**, *Langmuir* **2015**

Pore size = $CaCO_3$ size

Pores do not swell <u>even if achieved cumulative osmotic pressure</u> induced by both Ca^{2+} ions (estimated as ~10^2 MPa) and by pore-encapsulated dextrans (5×10^{-3}–5×10^{-1}MPa)

Dextrans encapsulated into $CaCO_3$ templates



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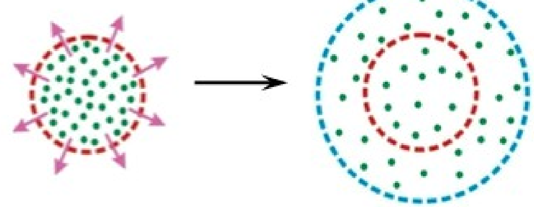
Induce **additional** osmotic pressure during particle dissolution (~ 1 sec)



→

pore swelling

?

Vant Hoff's equation for osmotic pressure

$$\pi = iCRT \quad ^{*}$$

Einstein's equation for biaxial diffusion

$$x = \sqrt{2Dt} \quad ^{**}$$

Osmotic pressure π induced if dissolving 8-µm-$CaCO_3$ particles

	π_{max}(t=0 sec), MPa	π_{diffus}(t=1 sec), MPa
$CaCO_3$ (Ca^{2+} ions)	**$1.3\cdot10^{2}$**	**$1.3\cdot10^{-1}$**
Dextran^{FITC} MW 70kDa	$5.0\cdot10^{-1}$	$1.8\cdot10^{-2}$
Dextran^{FITC} MW 500kDa	$7.2\cdot10^{-2}$	$4.9\cdot10^{-3}$

* *i* – Vant Hoffs' factor,
C – molar concentration (M),
R – absolute gas constant (J mol^{-1} K^{-1}),
T – temperature (K).

** *x* – distance which ions/molecules can travel within a time *t* (cm),
t – time (s),
D – diffusion coefficient for a given ion/molecule (cm^2 s^{-1}).

Sergeeva, A. et al *Advanced Materials Interfaces* **2015**, *Acs Applied Materials & Interfaces* **2015**, *Langmuir* **2015**

Acknowledgement

The group

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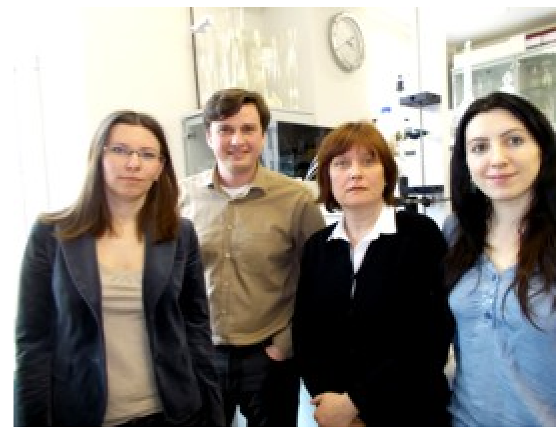

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Bell Michael (NTU)
Ashwell Ryan (NTU

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Thanks for attention!