Closed and open scaffolds for bone regeneration

João F. Mano[1,2]

[1] 3B´s Research Group - Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine. AvePark, Zona Industrial da Gandra, S. Cláudio do Barco, 4806-909 Caldas das Taipas, Guimarães, Portugal.

[2] ICVS / 3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal.

Repair of damaged/diseased tissues and organs

failure/loss/damaging of tissues and organs

- Chronic diseases / aged-linked degeneration
- Trauma / accidents
- congenital deformity

organ transplantation

tissue transplantation

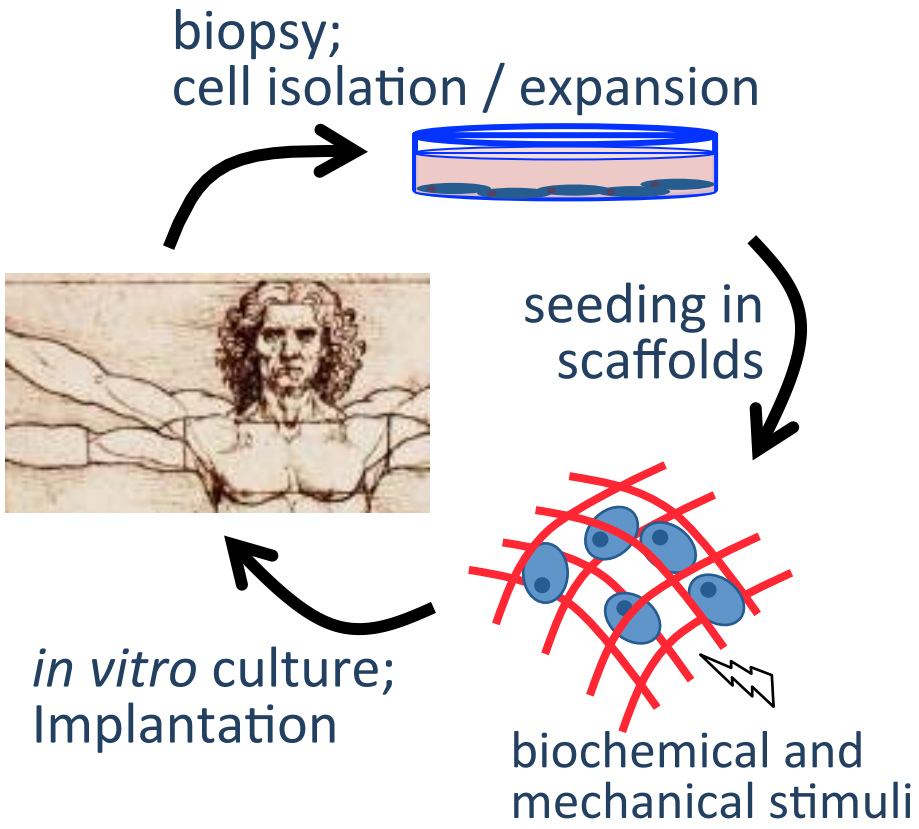
replacement strategies (biomaterials/ artif. organs)

pharmaceuticals

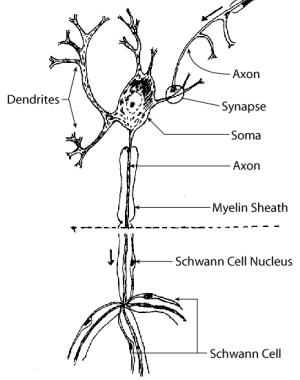
need for developing:

- novel strategies for restoring the structure and function of tissues and organs.
- methods of curing previously untreatable injuries and diseases.

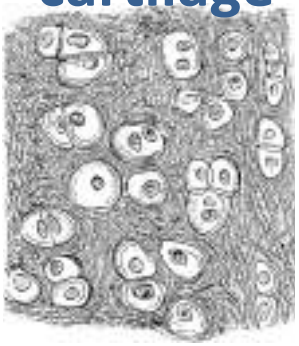
Tissue Engineering

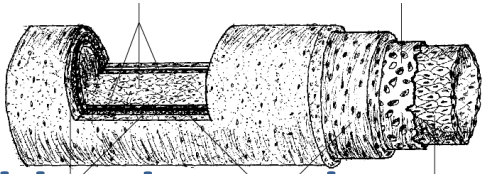


peripheral nervous tissue



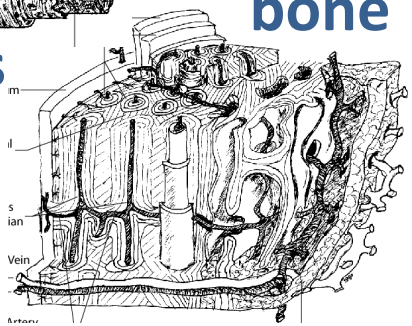
cartilage



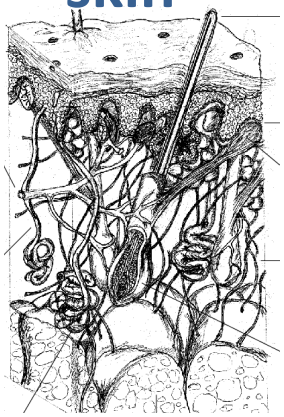


blood vessels

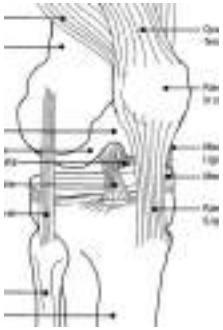
bone



skin



tendon
ligament



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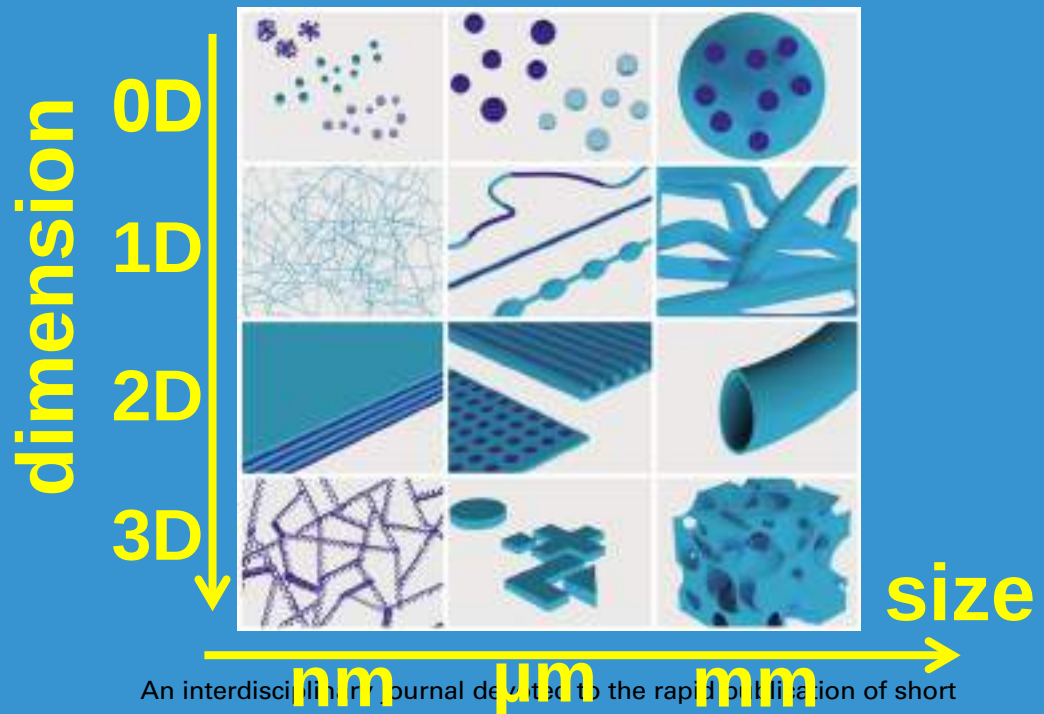
materials letters

Featured Letter:
Designing Biomaterials for Tissue Engineering Based on the Deconstruction of the Native Cellular Environment



An interdisciplinary journal devoted to the rapid publication of short communications on the science, applications and processing of materials

Editor-in-Chief: Aldo R. Boccaccini

J.F. Mano, *Mater. Lett.* '15
(featured letter)

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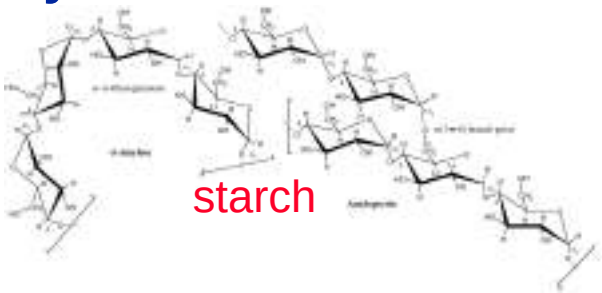
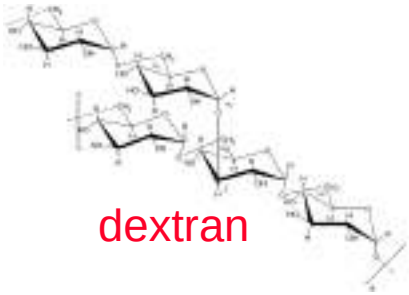
Natural based polymers

polysaccharides

neutral



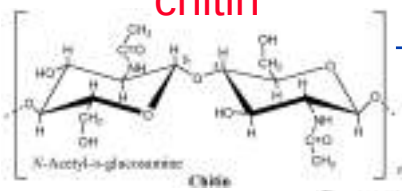
starch

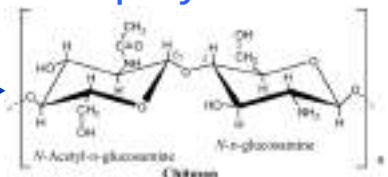


dextran

polycationic

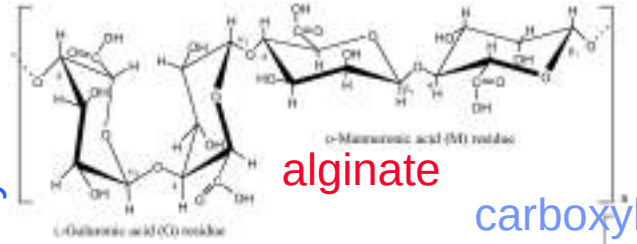
chitin



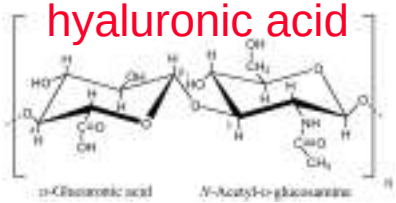


chitosan

polyanionic

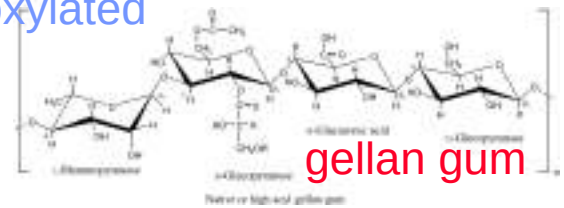


alginate

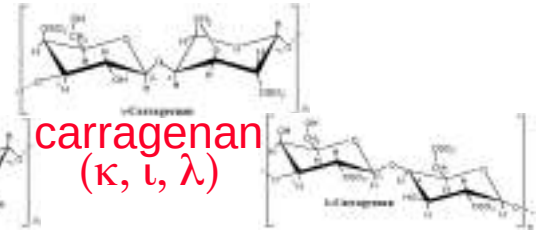


hyaluronic acid

carboxylated



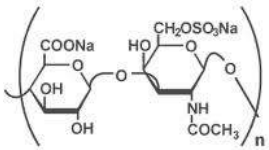
gellan gum



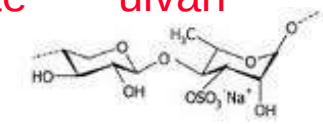
carragenan
(κ, ι, λ)

sulfated

chondroitin sulfate

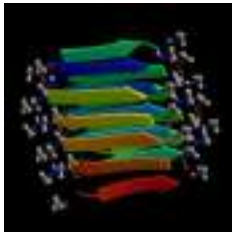


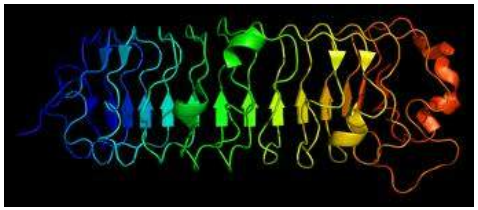
ulvan



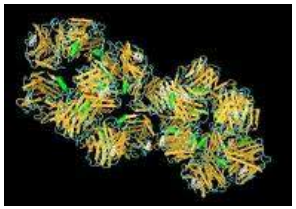
proteins

silk fibroin



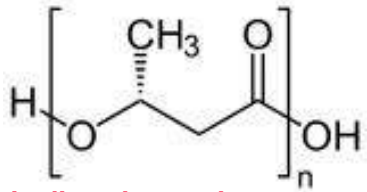


collagen



fibronectin

polyesters

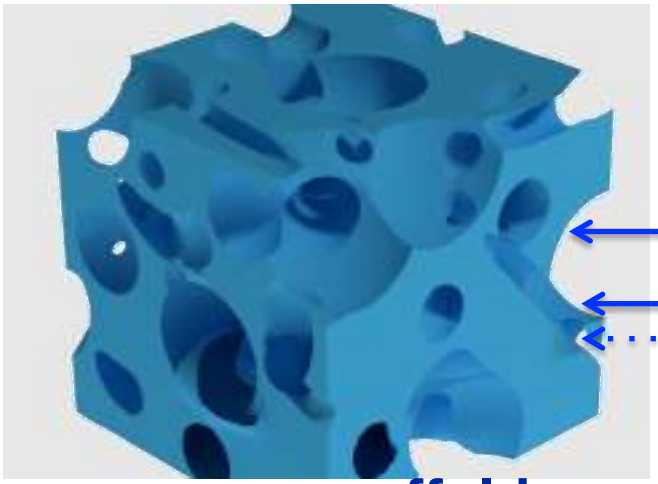


poly(hydroxybutyrate)

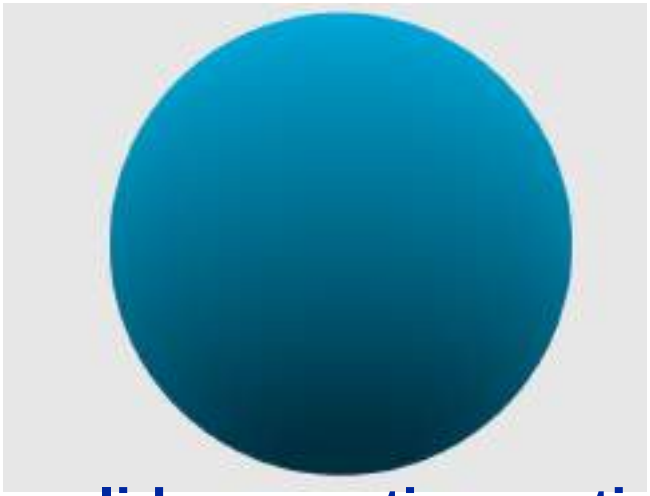
J.F. Mano +, *J.R.Soc.Interface* '07

3D supports for cells in tissue engineering

OPEN

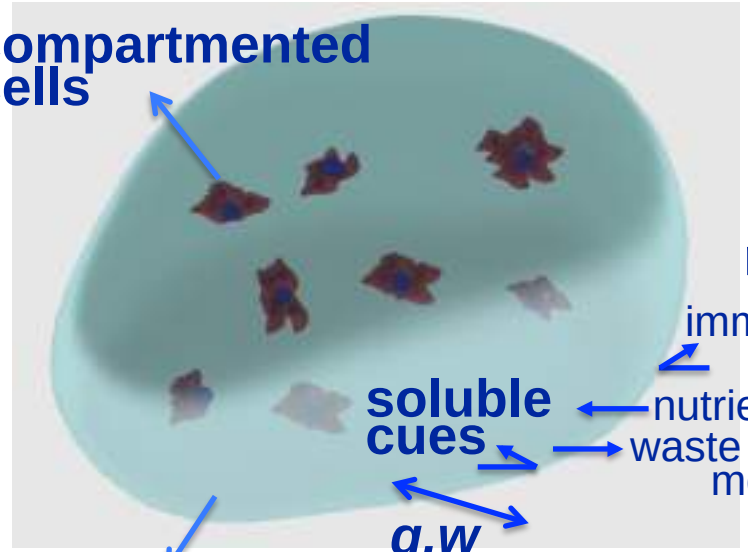


porous scaffolds



solid supportive particles

CLOSED

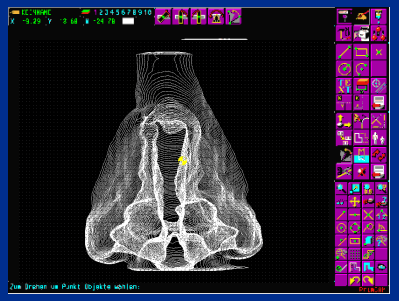


- cells+biomaterials+soluble factors may be retained in the same volume.
- self-regulated strategies.
- Use of non-autologous cells.
- Injectable systems.

Rapid Prototyping Technique

Human Body

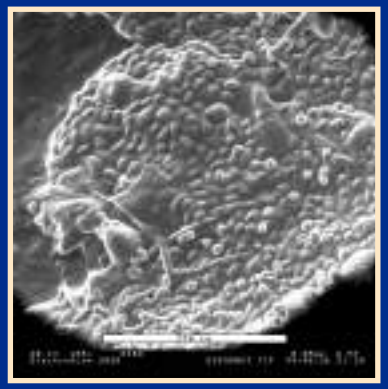
X-Ray



CAD software

RP machine

3D Scaffold



Cell culture

Scaffold with tissue

3B's

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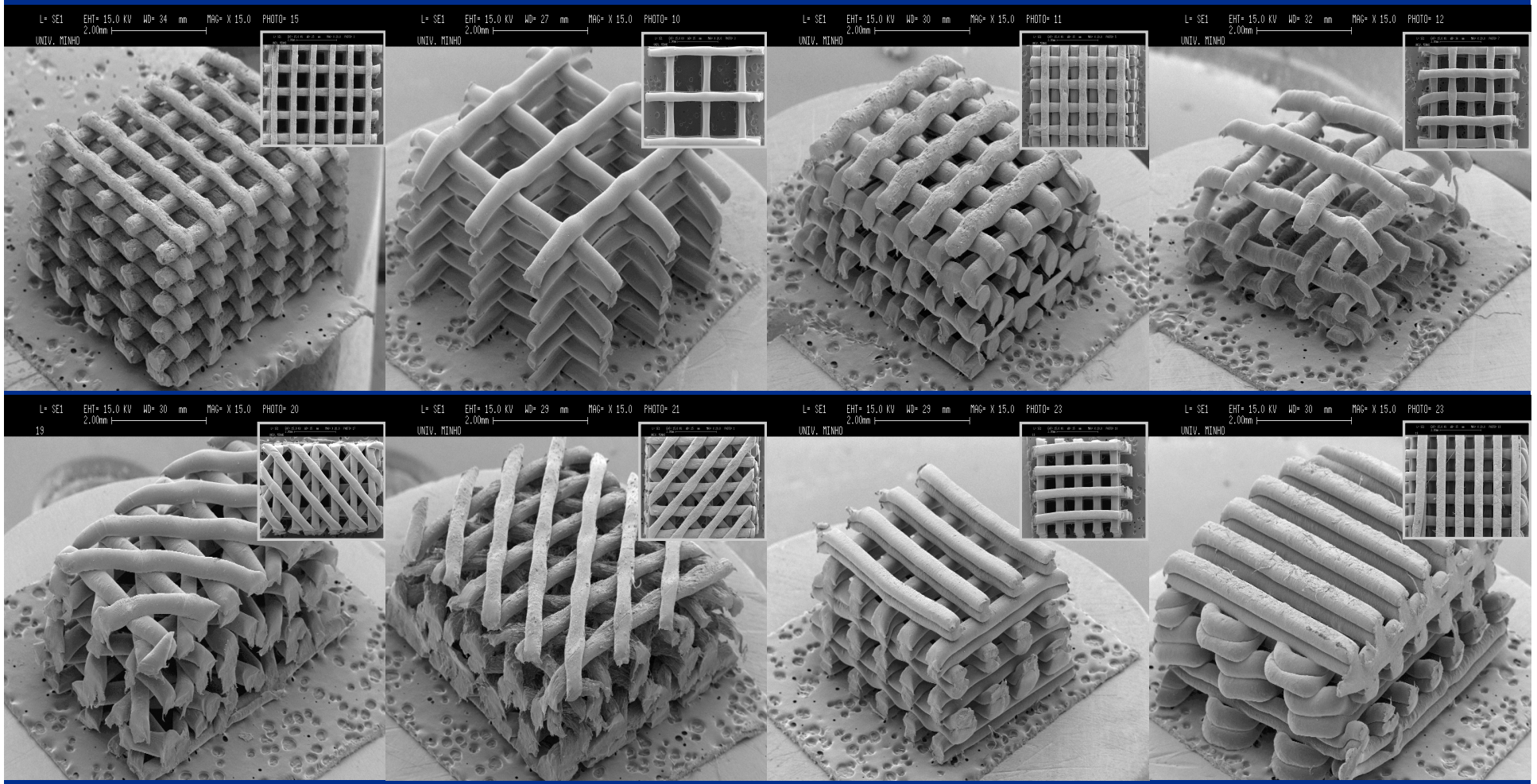
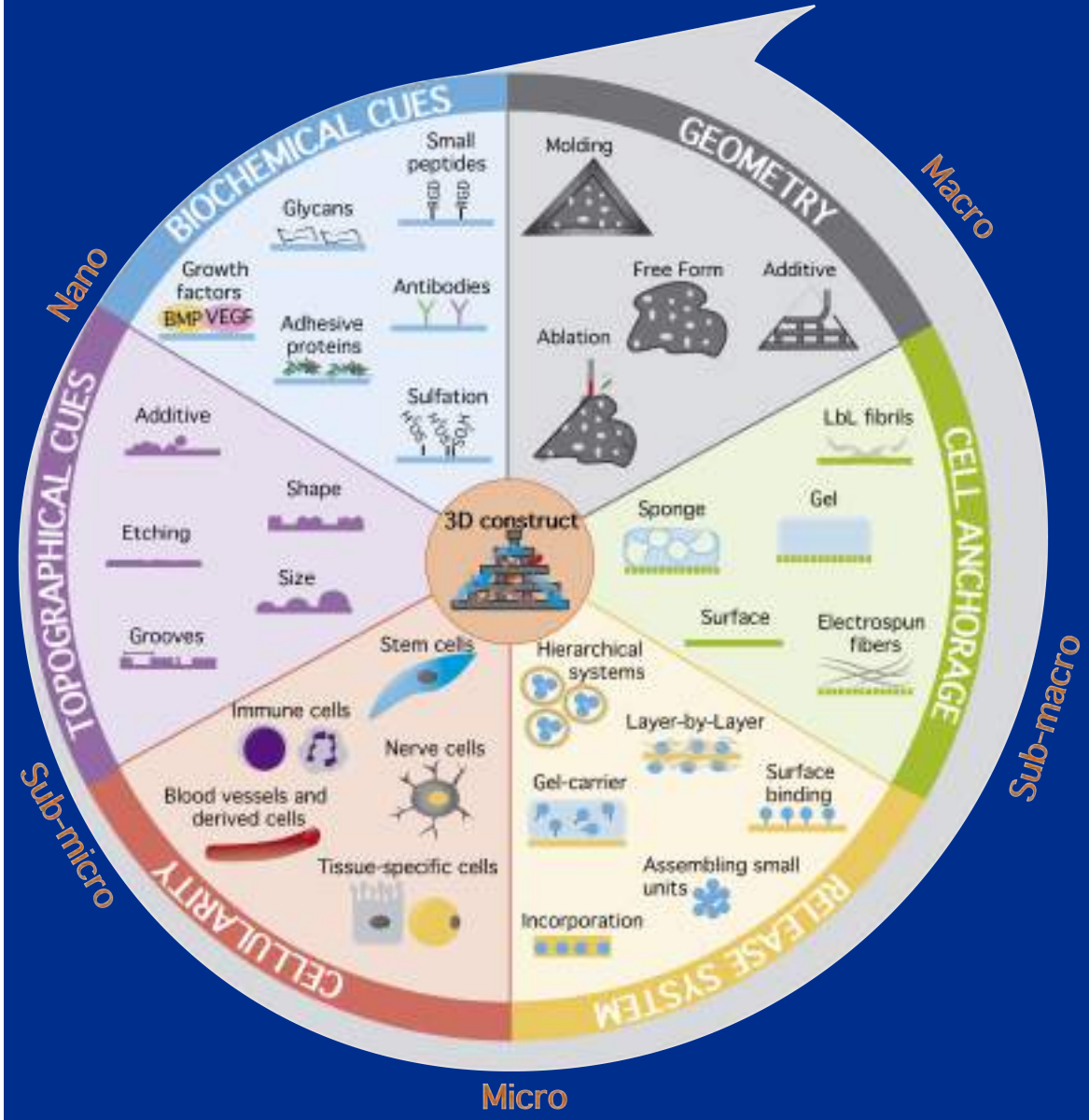
Rapid Prototyping Technique: control of morphology

Standard scaffold morphologies with porosities ranging between 55 and 85 %.



Multiscale Features/Properties for 3D Constructs Design

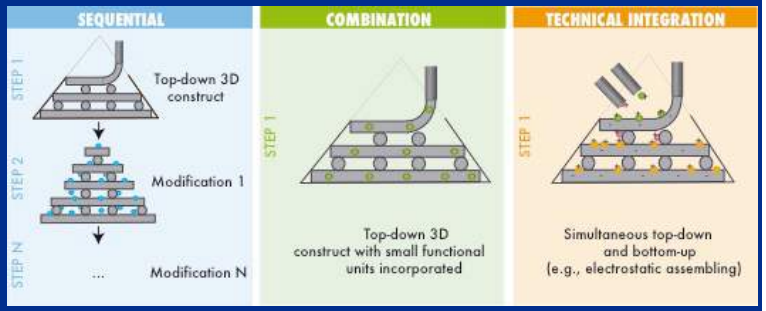


Top-down approaches + Bottom-up approaches

e.g., Freeze-drying, Electrospinning

e.g., Layer-by-Layer, Self-assembling

Integrative approaches



S.M. Oliveira+, *Biotechnol. Adv.* '15

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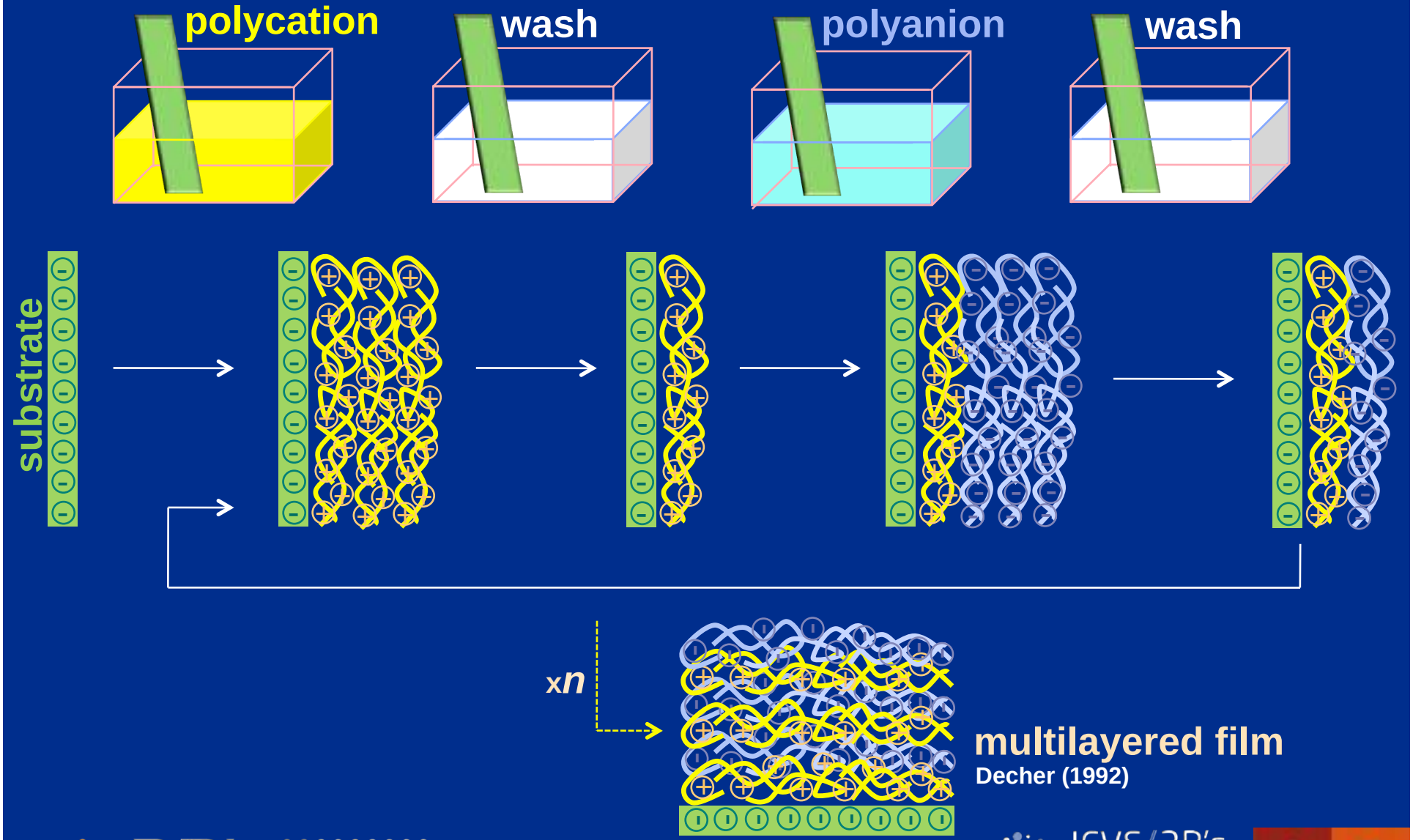
Layer-by-layer methodology using polyelectrolytes solutions

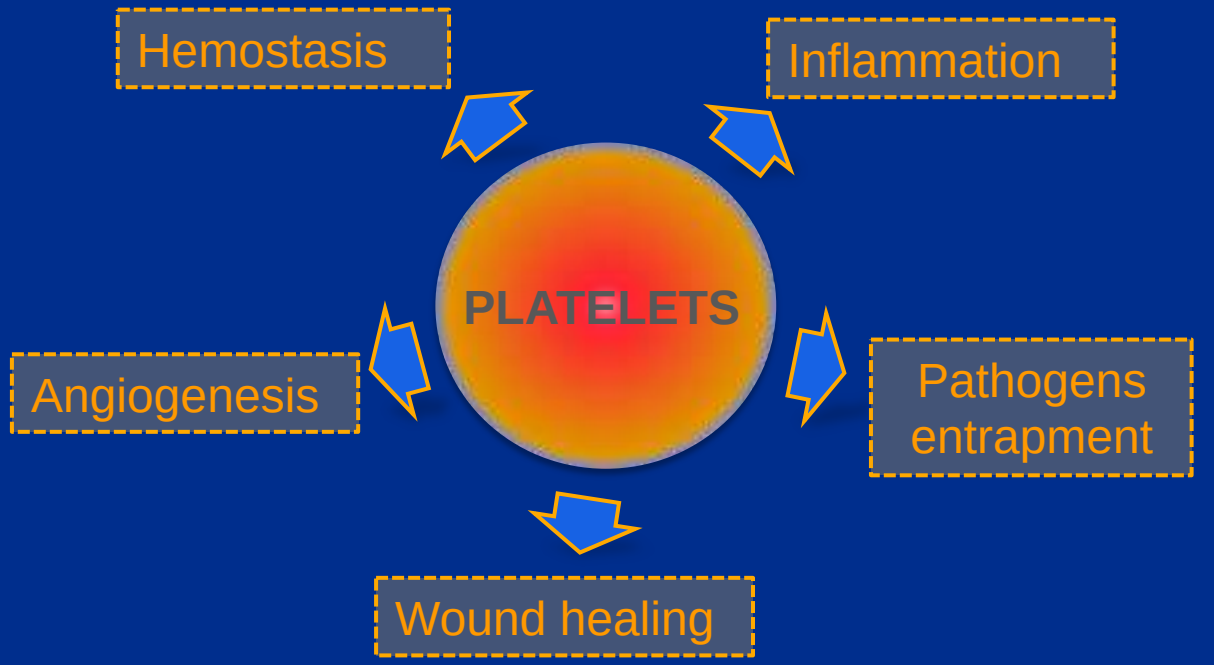


Platelets

EDMOND ALEXANDER, Shannon Associates LLC

Events trigger platelets activation and the release of contents:
Vascular disruption and/or tissue injury

- cost-effective autologous source of multiple growth factors.
- Involved in vivo in very important physiological functions.



Growth factors
FGF
VEGF
PDGF
TGF β
BMP-2, BMP-4,..
IGF-1
…

Antigen receptors
PECAM-1
P-selectin

Hemostatic factors
Fibrinogen
Protein S

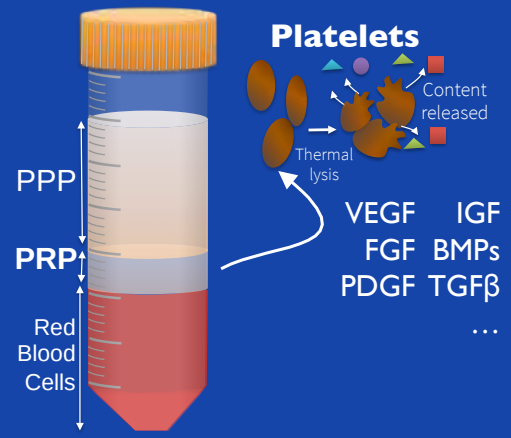
Adhesion proteins
Fibronectin
Vitronectin
Trombospondin
Vit-D binding protein

Chemokines
IL-1β
IL-8
PF-4
SDF-1α

Others

multilayers containing PL

Platelet Lysate



Layer-by-Layer with PL

Polyelectrolytes

- Chitosan (-**NH_2; -OH**)
- Alginate (-**COOH; -OH**)
- κ-carrageenan (-**OSO_3Hx1; OH**)
- ι-carrageenan (-**OSO_3Hx2**)
- λ-carrageenan (-**OSO_3Hx3**)
- Heparin (-**OSO_3Hx2; -NSO_3Hx1; -OH; -COOH**)

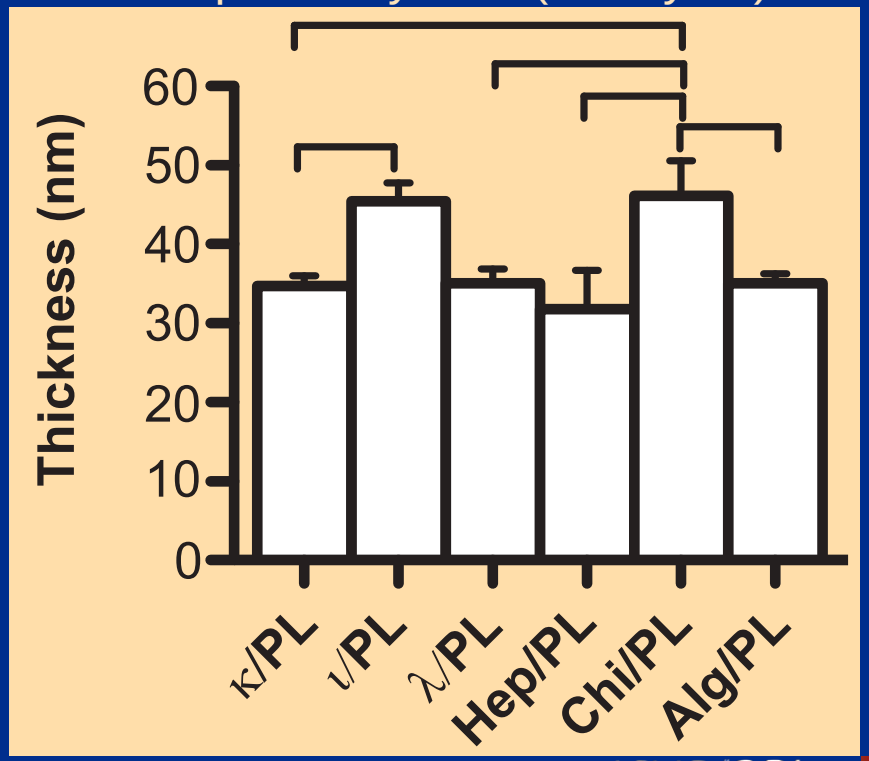
negative → sulfation

$(Alg/PL)_6$
$(\kappa Car/PL)_6$
$(\iota Car/PL)_6$
$(\lambda Car/PL)_6$
$(Hep/PL)_6$

$(Chi/PL)_6$
positive

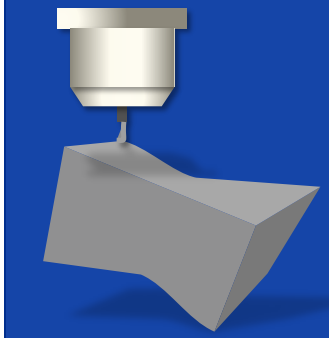
control: no PL

Ellipsometry data (6 bilayers)

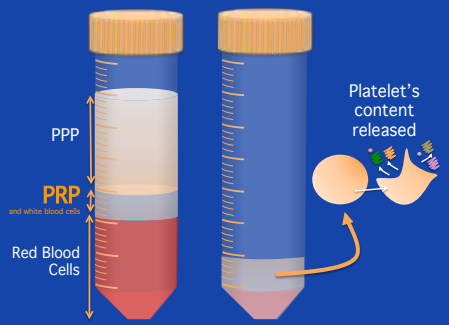


S.M. Oliveira+, *Biomaterials*, '15

Incorporating human platelet lysates in the multilayers



PCL scaffolds
Bioplotter™

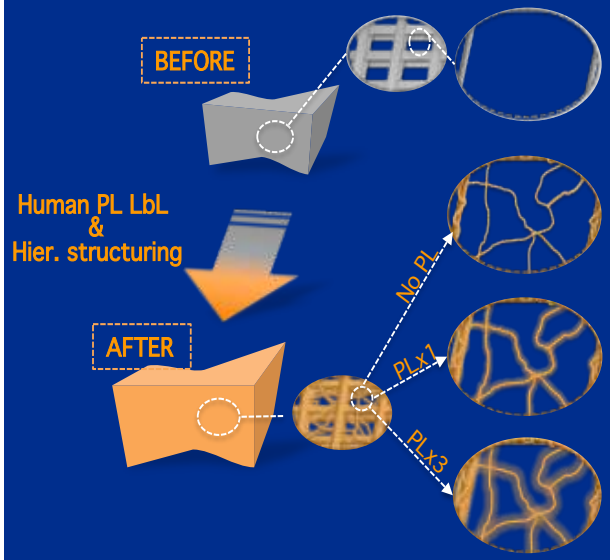


PL
Thermal Cycles

Layer-by-Layer with PL
and freeze-drying

Chitosan(Chi)
ι-carrageenan (Car)

SAMPLES



PCL =unmodified

PCL LbL $=(Car\text{-}Chi\text{-}Car\text{-}Chi)_{10}$

PCL LbL PL $=(Car\text{-}PL\text{-}Car\text{-}Chi)_{10}$

PCL LbL PL$_{x3}$ $=(Car\text{-}PL\text{-}Car\text{-}Chi)_{30}$

Last layers: PL or Car

Platelets:

- Events trigger platelets activation and the release of contents: Vascular disruption and/or tissue injury.
- Cost-effective autologous source of multiple growth factors

Hypothesis:

- PLs could be included in multilayers as a structural component and its presence could have beneficial biological implications.

S.M. Oliveira+, *ACS Biomater.Sci.&Eng.*, '15

3B's

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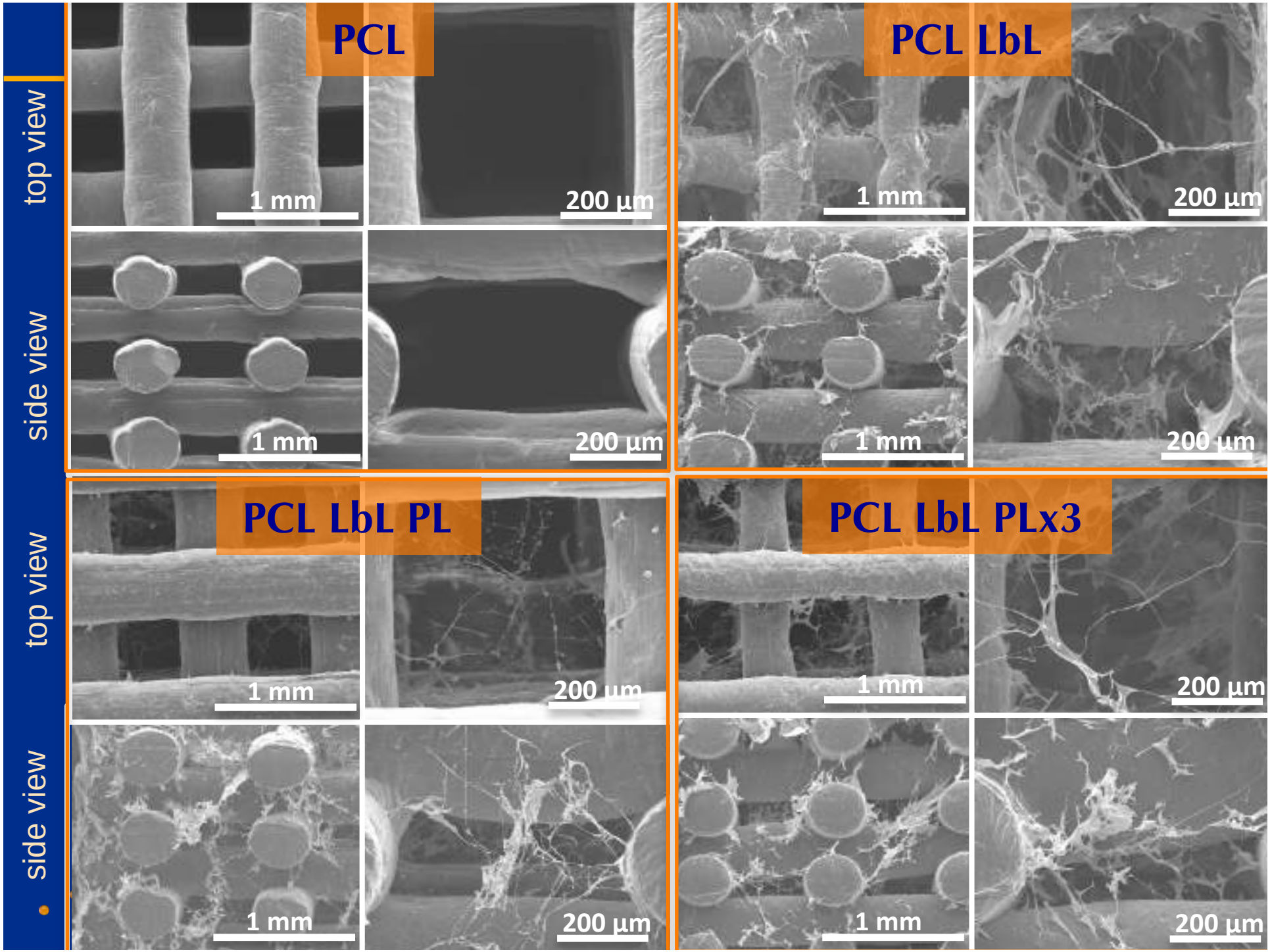
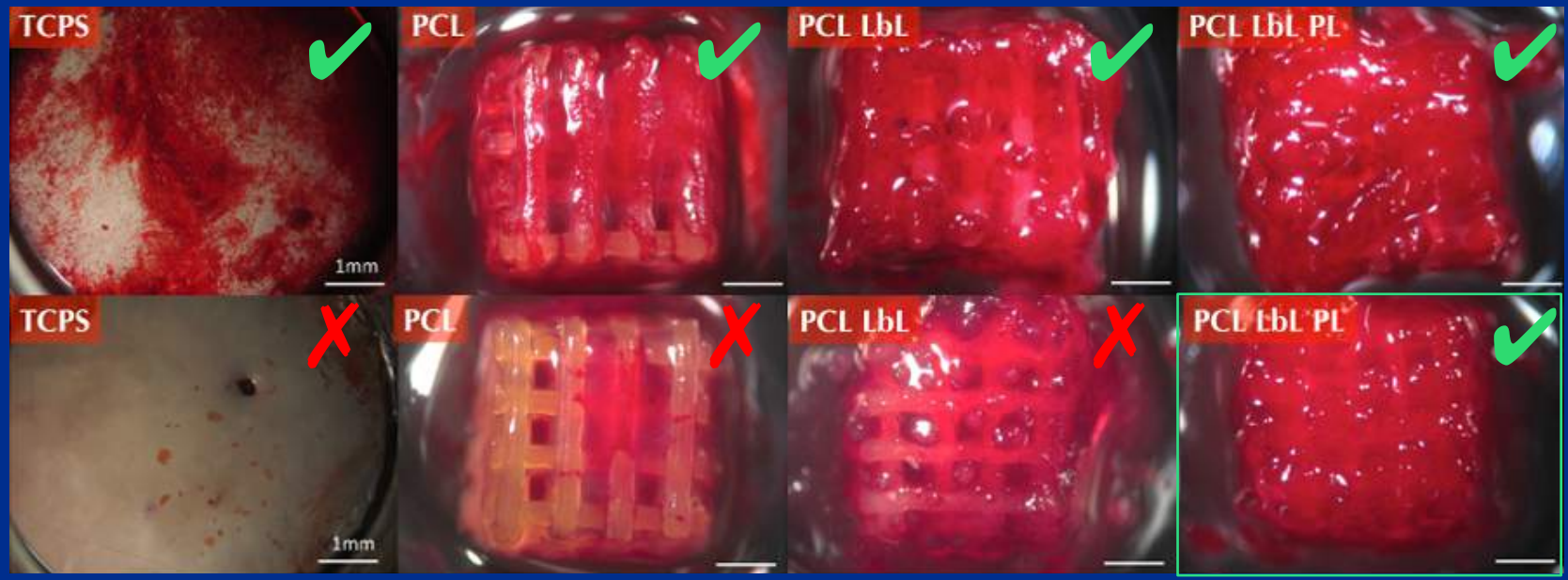

top view
side view
PCL
1 mm
200 µm
1 mm
200 µm
PCL LbL
1 mm
200 µm
1 mm
200 µm
top view
side view
PCL LbL PL
1 mm
200 µm
1 mm
200 µm
PCL LbL PLx3
1 mm
200 µm
1 mm
200 µm

Hierarchical scaffolds containing PLs: osteogenic potential

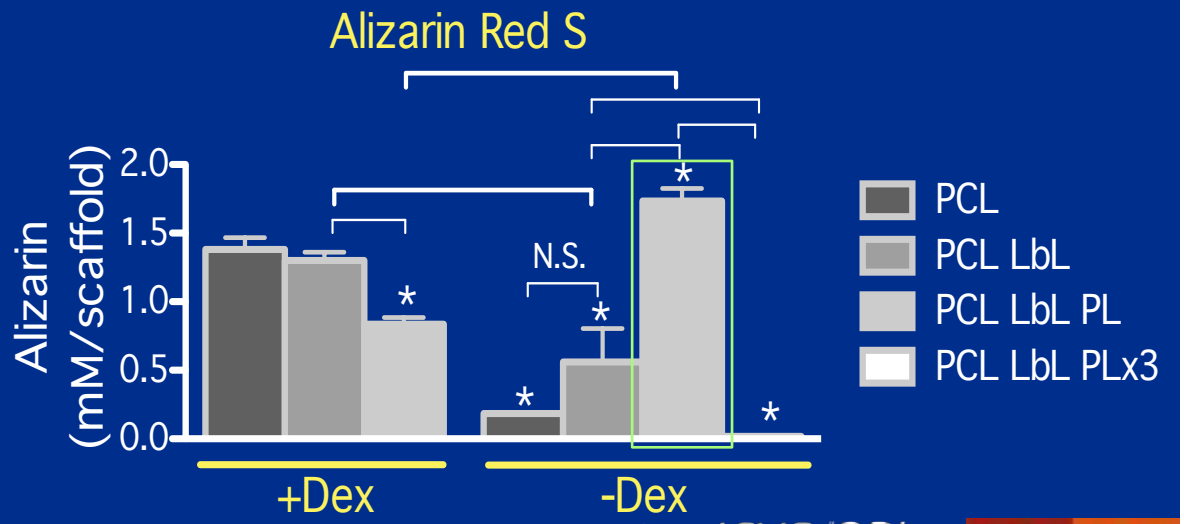
BIOMINERALIZATION



Cell studies

hASCs P1: 0.12x10^6/scaffold
α mem 4D

+ 28 days: L-AA + βGly, **with and without Dexamethasone (Dex)**



S.M. Oliveira+, *ACS Biomater.Sci.&Eng.*, '15

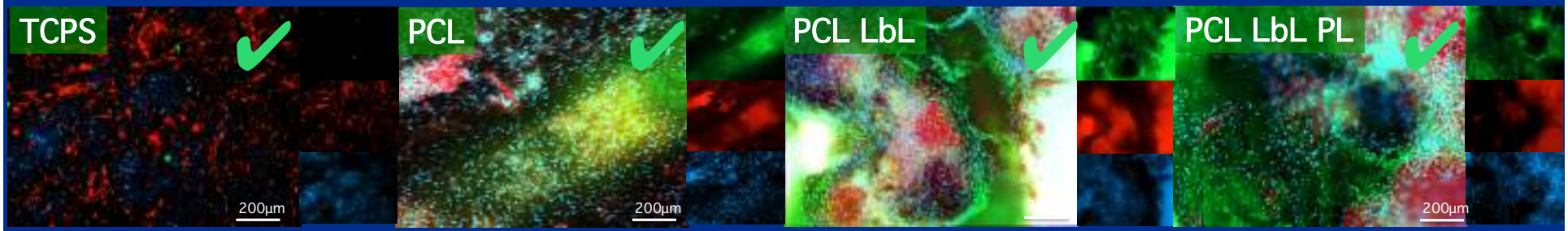
3B's

ICVS/3B's Associate Laboratory

Hierarchical scaffolds containing PLs: osteogenic potential

+Dex

IMMUNODETECTION OF OSTEOCALCIN



-Dex

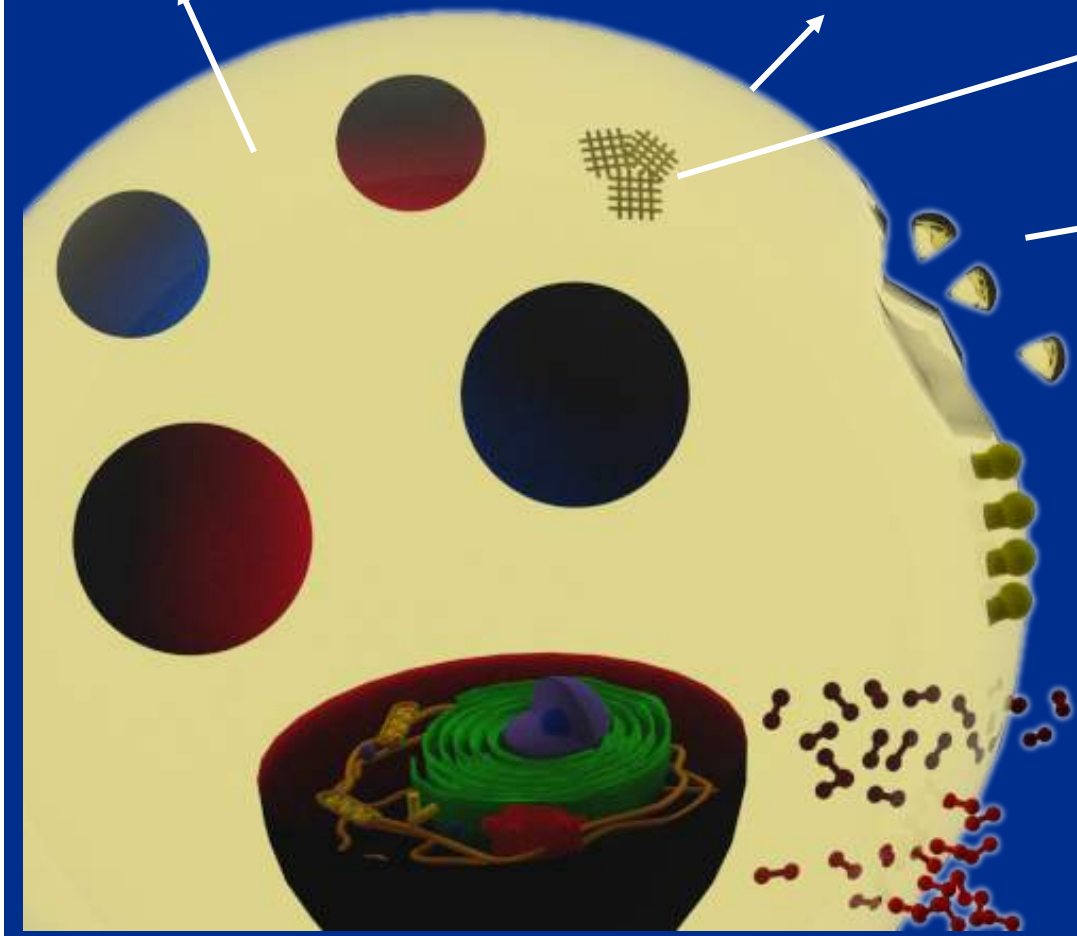
Green – Osteocalcin; Red – Alizarin Red S; Blue - Nuclei

Relevant properties influencing the behaviour of encapsulated cells

L. Gasperini, J.F. Mano, R.L. Reis, *J.R.Soc.Interface* '14

Bulk nature: water content, chemical composition, crosslinking density, stimuli responsiveness, mechanical properties

Size and shape



Core properties (internal microstructure, hierarchical organization, adhesion motifs)

Degradability

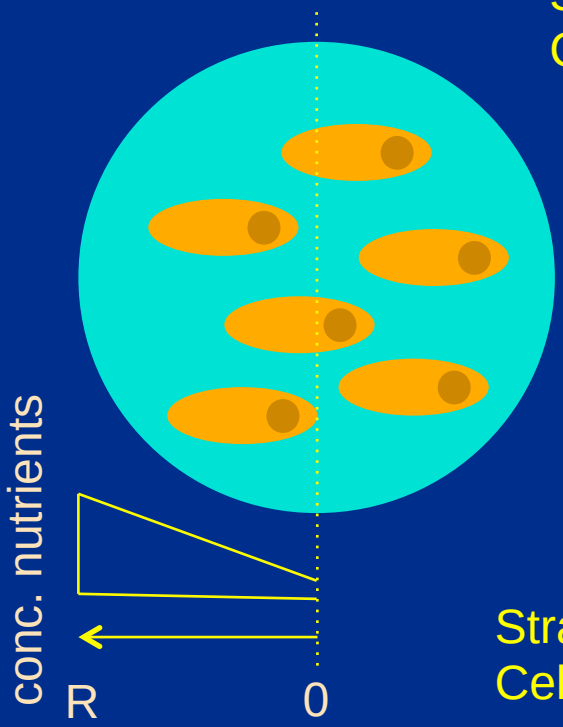
Surface properties (protein adsorption/adhesion to tissue, protective shell, bioinstructives motifs, topography)

Removal of waste,release of biomolecules

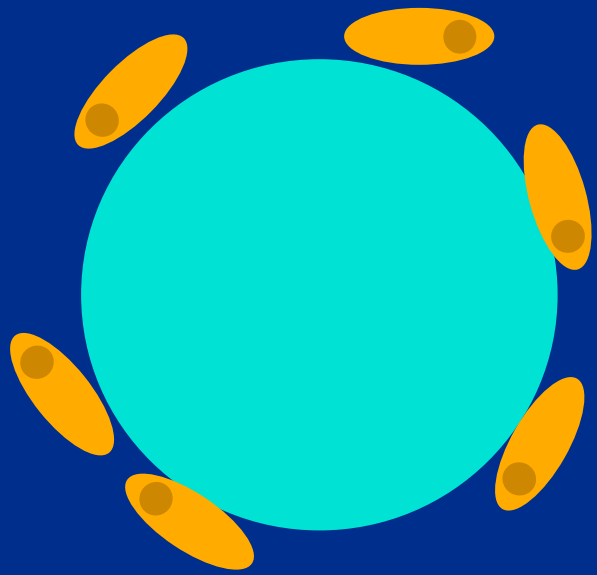
Diffusion of nutrients, oxygen, biomolecules

Permeability

Feeding the cells



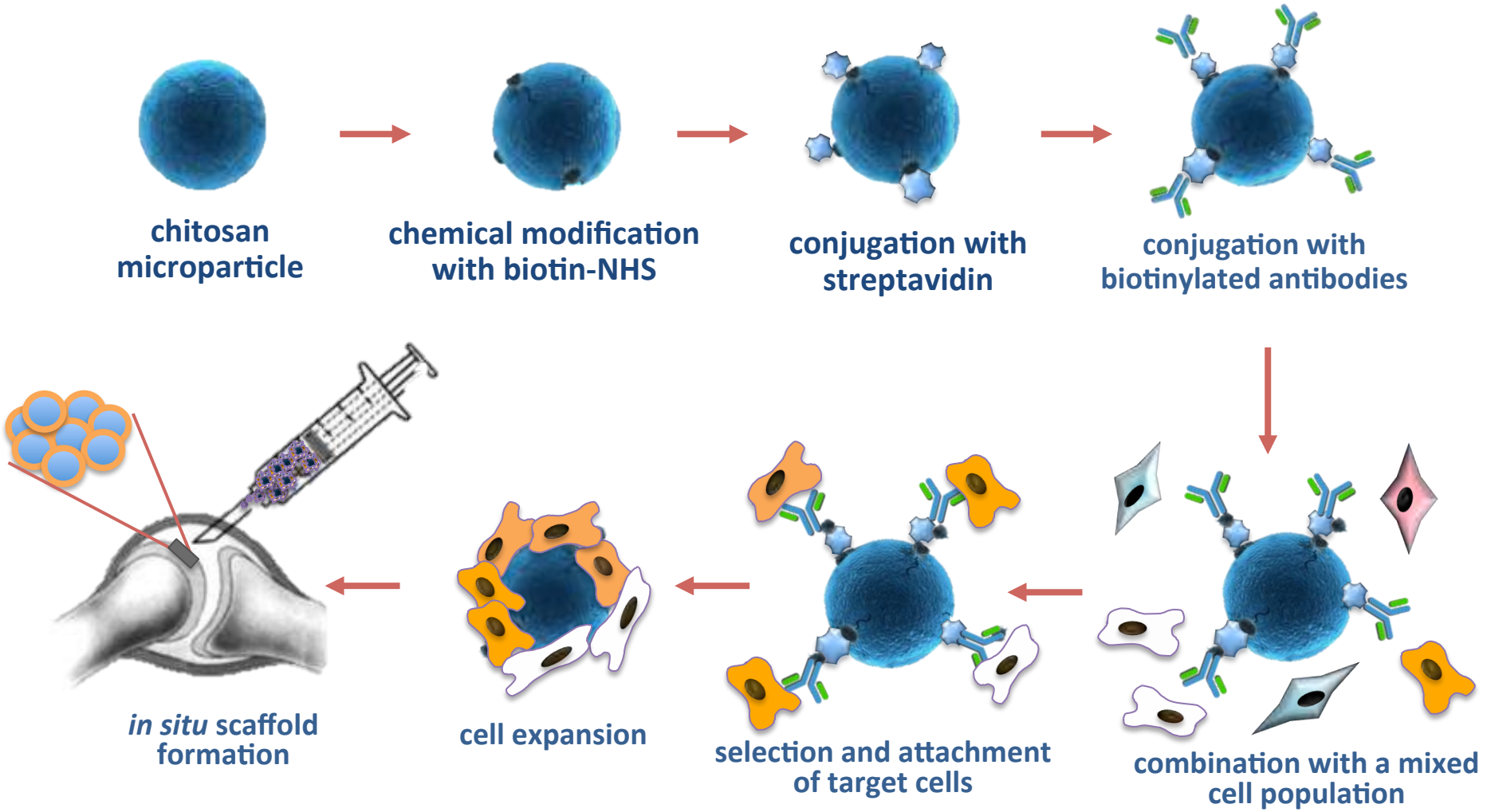
Strategy 1:
Cells over particles



Strategy 2:
Cells in liquefied capsules

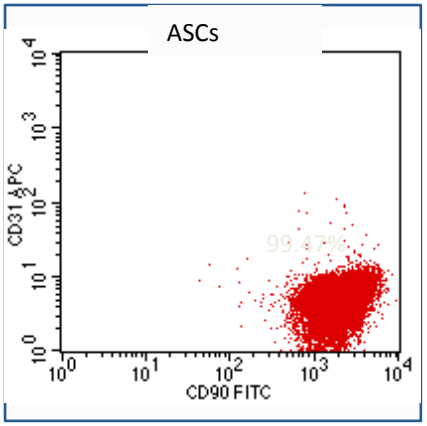
Bioinstructive particles

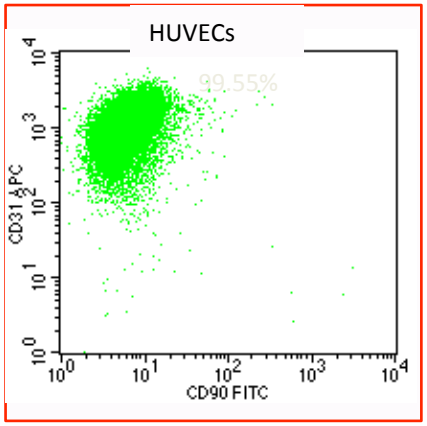
Concept: polymeric microparticles that are able to target specific cells through antibody–antigen interactions, while simultaneously allowing cell expansion of target cells.

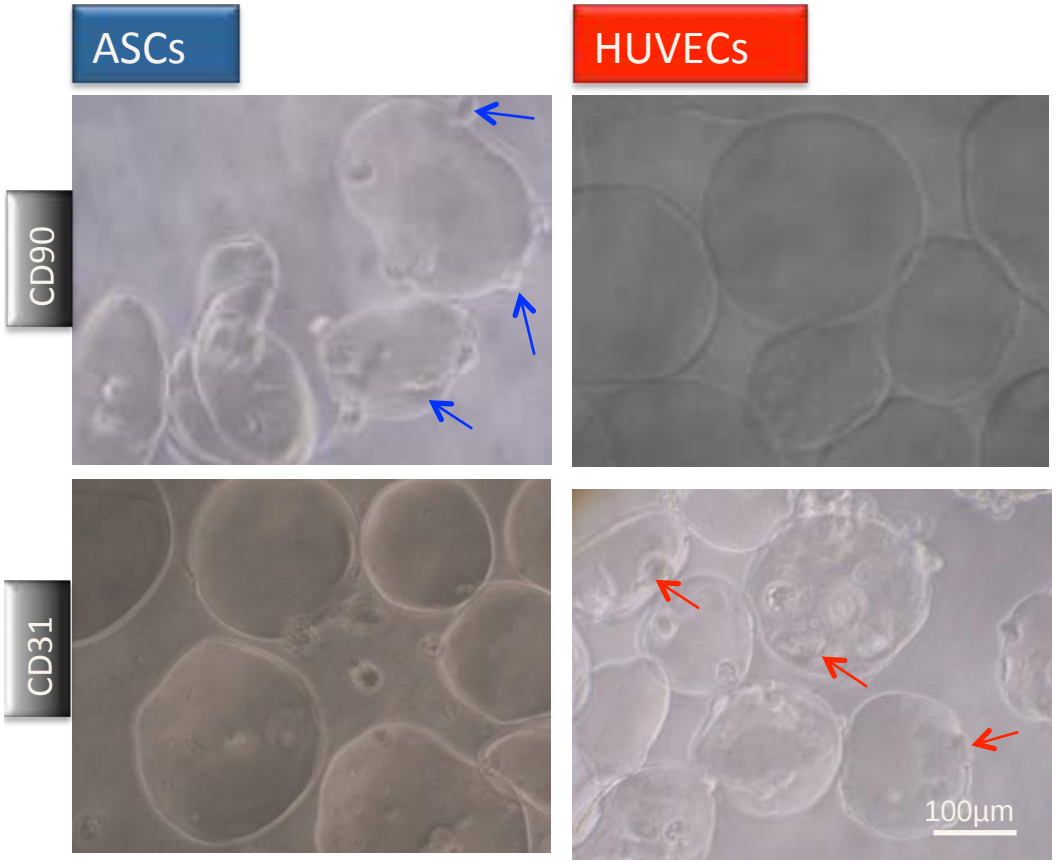


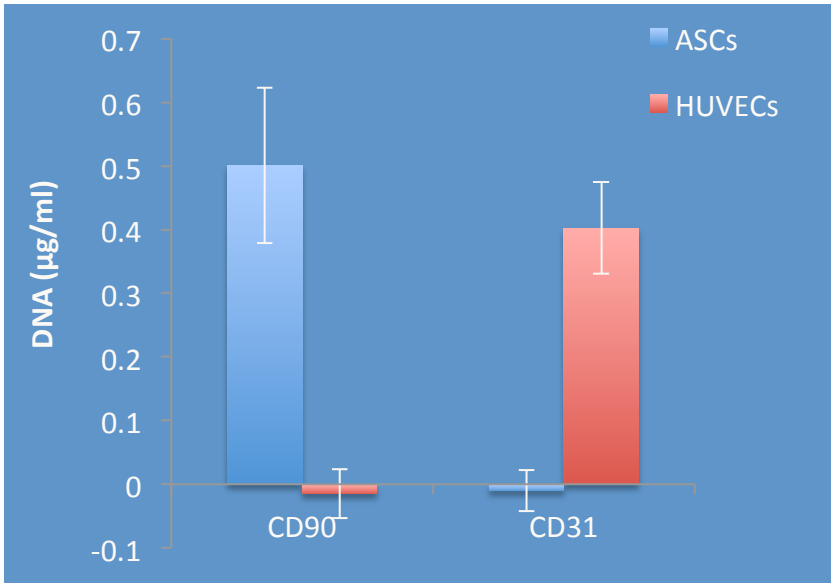
Particles with specific interactions with stem cells and endothelial cells

- CD90 is a cell surface glycoprotein that has been identified in stem cells.
- CD31 is found on the surface of endothelial cells.









ASCs adhere on CD90 particles.
HUVECs adhere on CD31 particles.

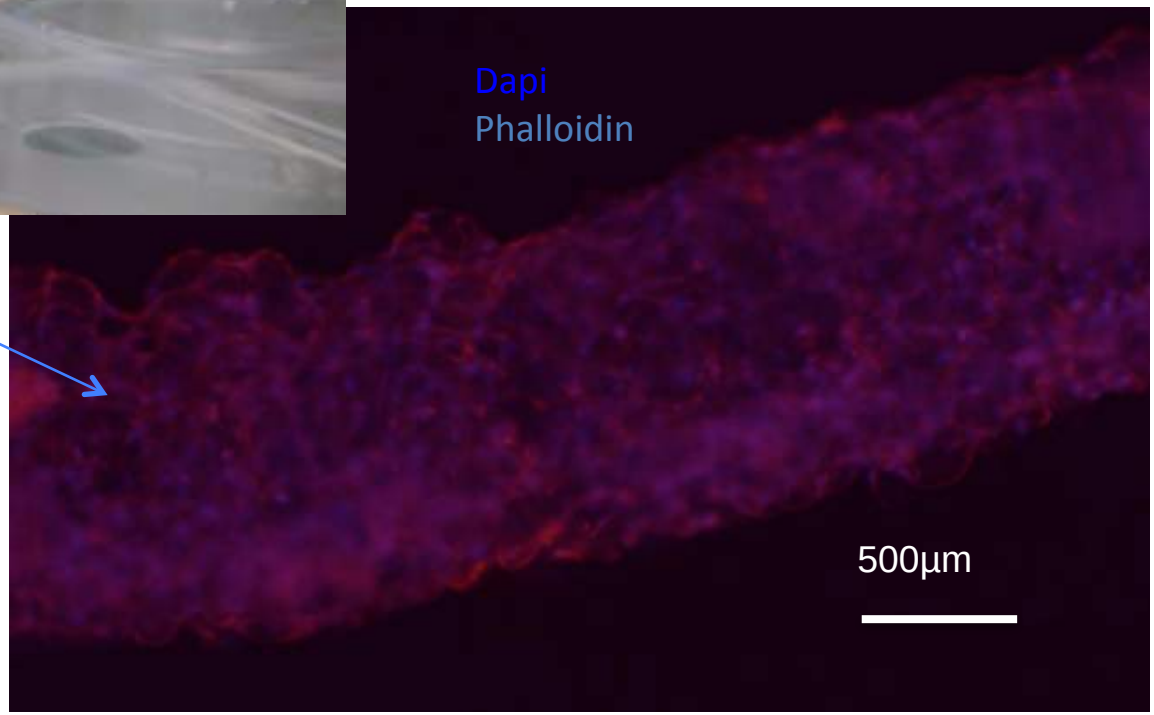
C.A. Custódio+, *Biomaterials* '15

Injectability and *in situ* scaffold formation

Hydrogel with a channel-shaped cavity

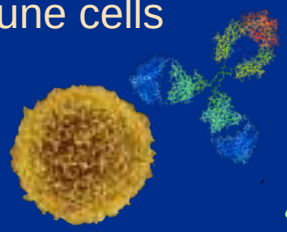
CD90 microparticles seeded with ASCs

Cell culture for 3 days



C.A. Custódio+, *Biomaterials* '15

Encapsulation of cells in microparticles-in-capsules



Antibodies,
immune cells

Waste products
Metabolites

Nutrients
Oxygen

Liquified
environment

Permselective
membrane

Layer-by-layer assembly
Poly-(L-lysine), alginate and chitosan
12-layers, 0.5mg/mL

Microparticles

Solvent evaporation
Poly-(L-lactic) acid
Collagen I coating
Diameter: 20-100µm

Cells

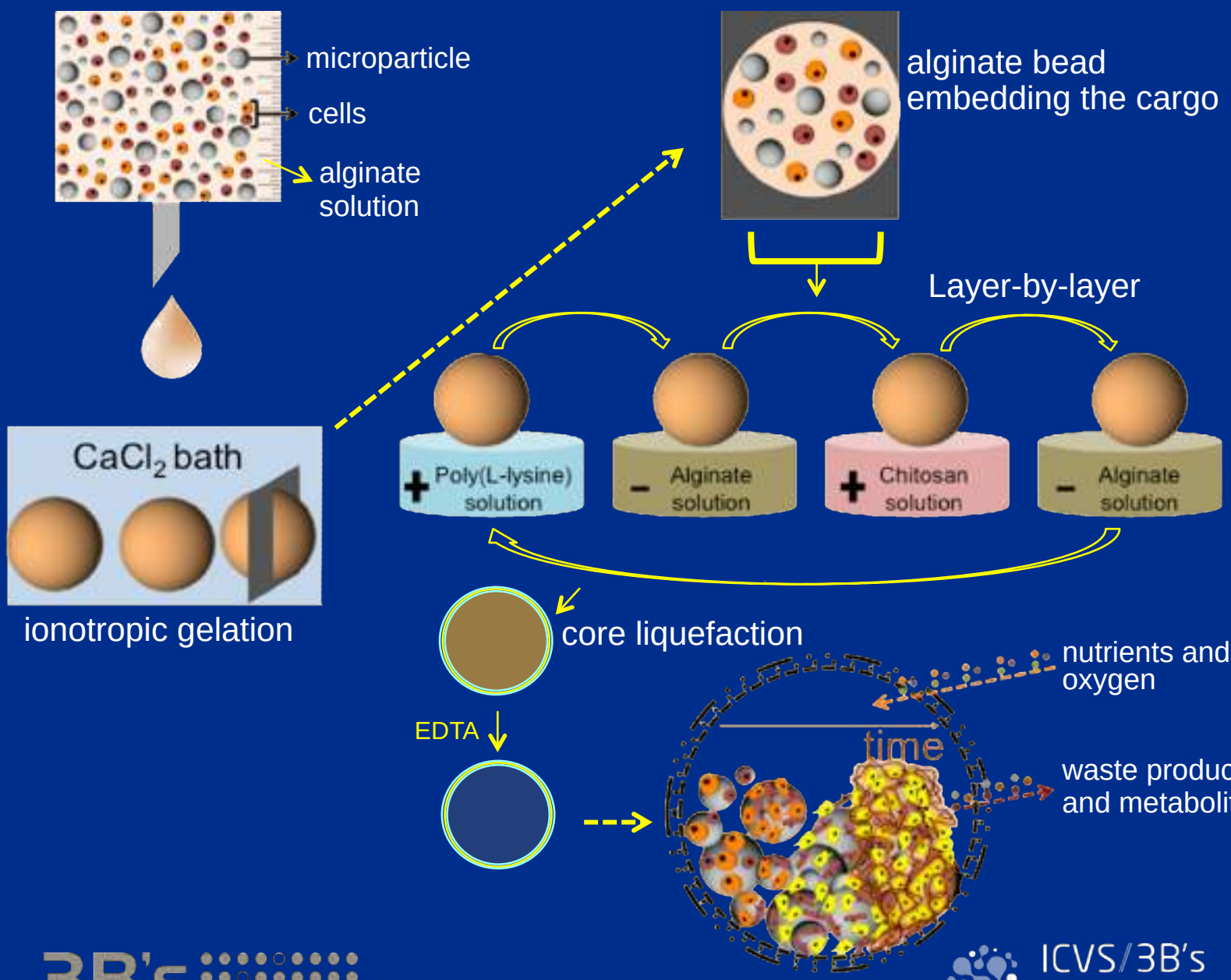
- Excellent diffusion
- Shape-freedom
- Controlled environment

(e.g. adhesive sites for cells)

3B's

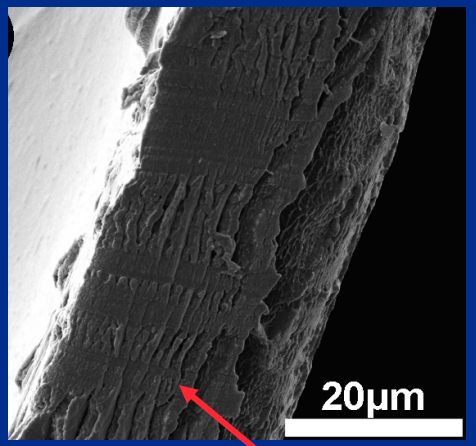
ICVS/3B's
Associate
Laboratory

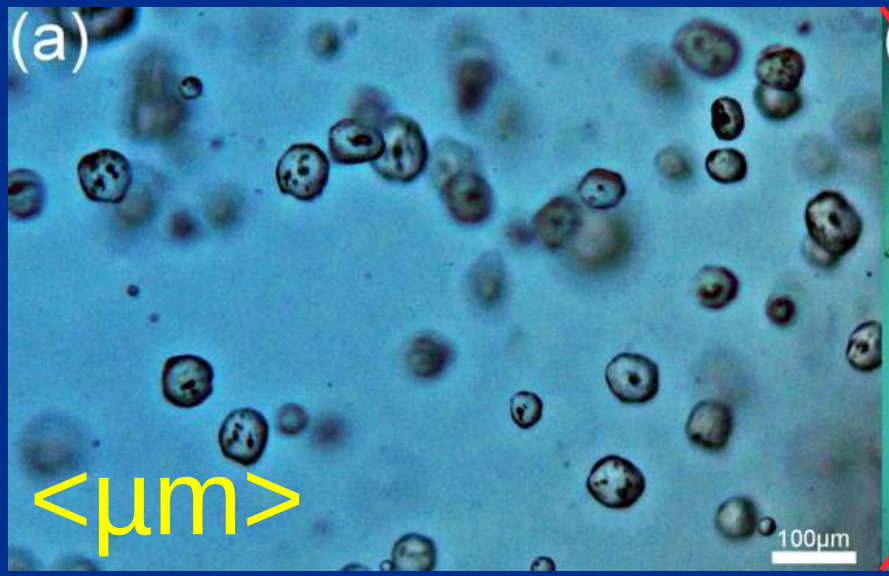
Preparation of liquified capsules



hierarchical (liquified) capsules

<nm>



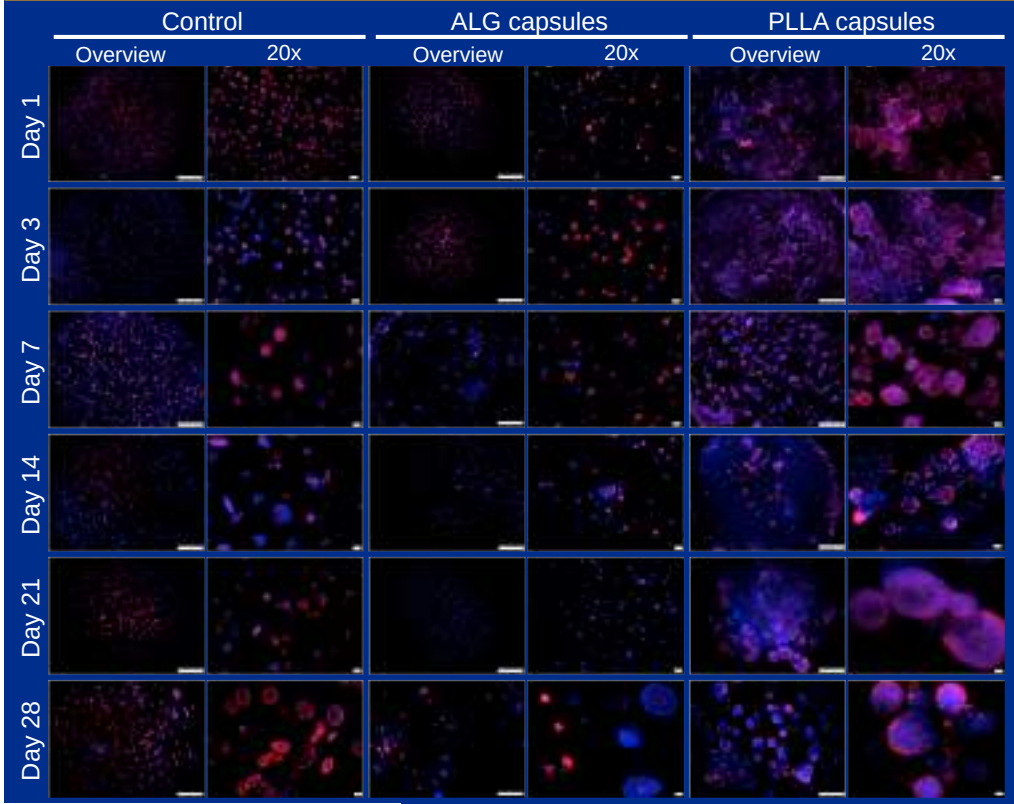


(a) PLLA particles - diameter 45.6 ± 13.5µm

(b) capsules - diameter 1.8 ± 0.1mm.

C.R. Correia+, *Soft Matter* '13
C.R. Correia+, *Biomacromolecules* '13

Liquified capsules: Cell adhesion and proliferation studies

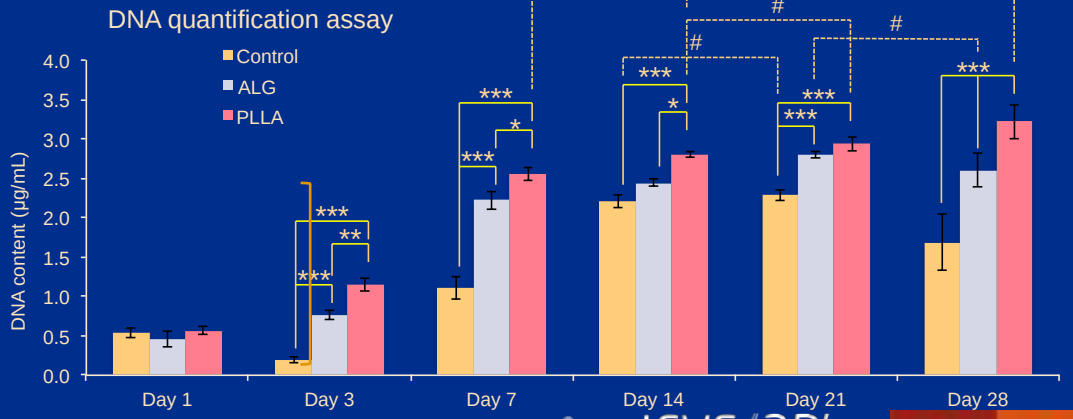


DAPI-phalloidin fluorescence assay

CONTROL: alginate particles without LbL nor EDTA treatment;
ALG: alginate particles after 6 bilayers and EDTA treatment (ALG capsules);
PLLA: alginate particles containing collagen I coated PLLA microparticles after 6 bilayers and EDTA treatment (PLLA capsules).

DNA quantification assay

Cell density: 1x10^6 cells/mL alginate



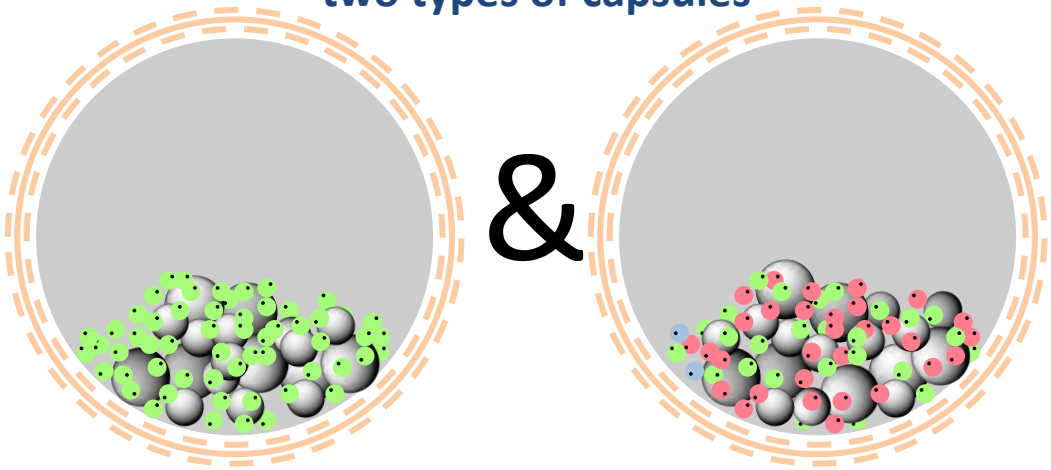
C.R. Correia+, *Soft Matter* '13
C.R. Correia+, *Biomacromolecules* '13

ICVS/3B's Associate Laboratory

Osteogenic (bone forming) Capsules

Co-cultures to explore the crosstalk existing between vascular cells and stem cells.

two types of capsules



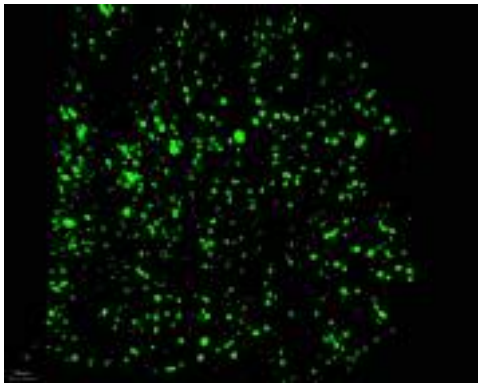
hASCs

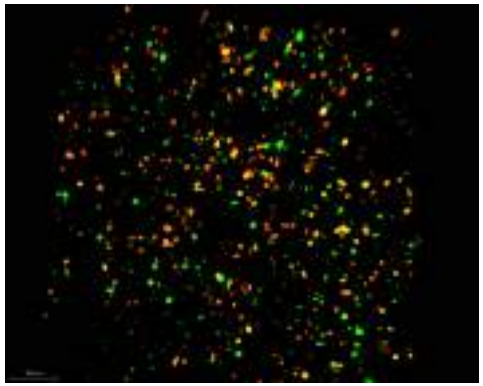
- MONO capsule -

hAMECs + hASCs (1:1)

Co-cultured capsule

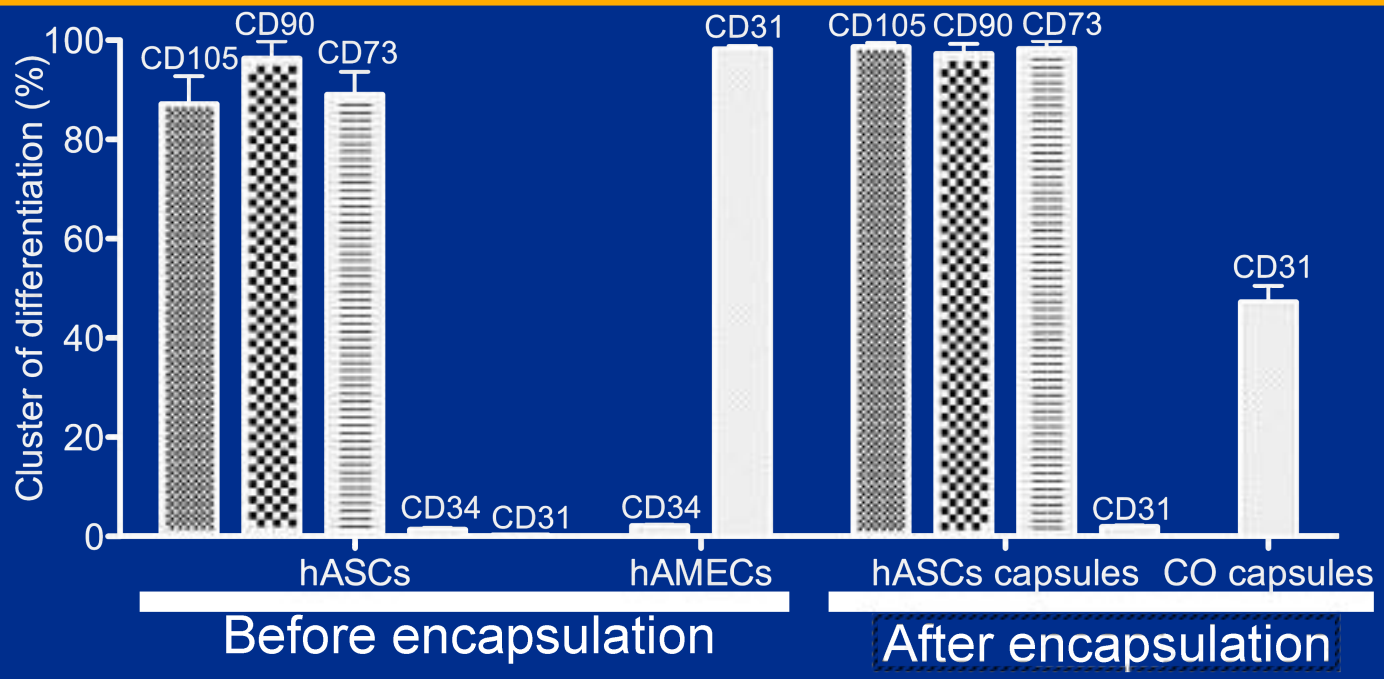
- CO capsule -

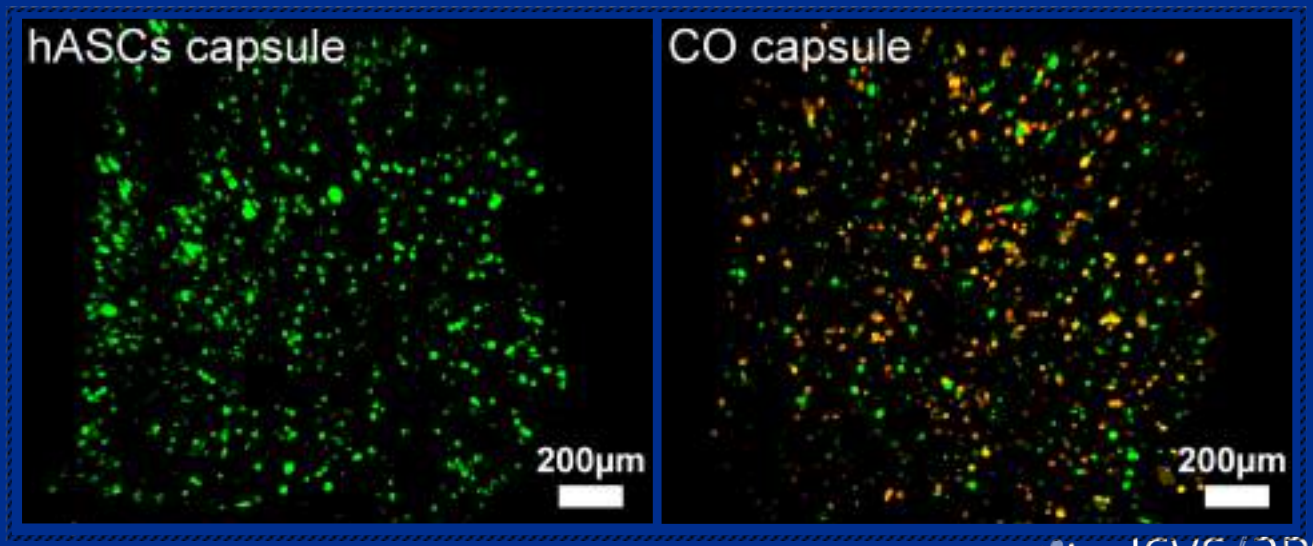




C.R. Correia+, *Sci.Rep.* '16

Isolated cells phenotype & co-encapsulation analysis



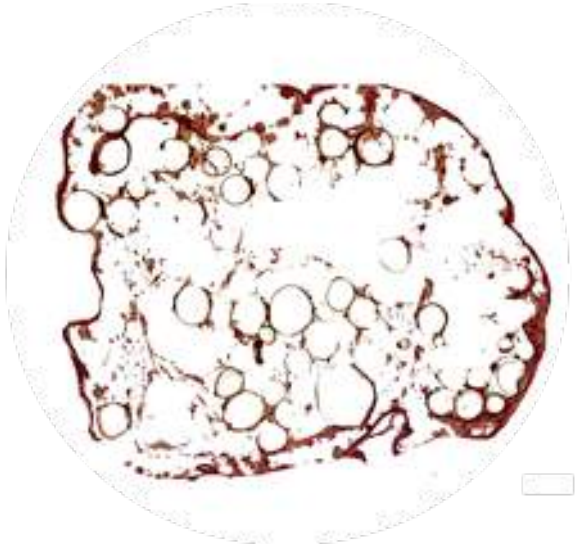


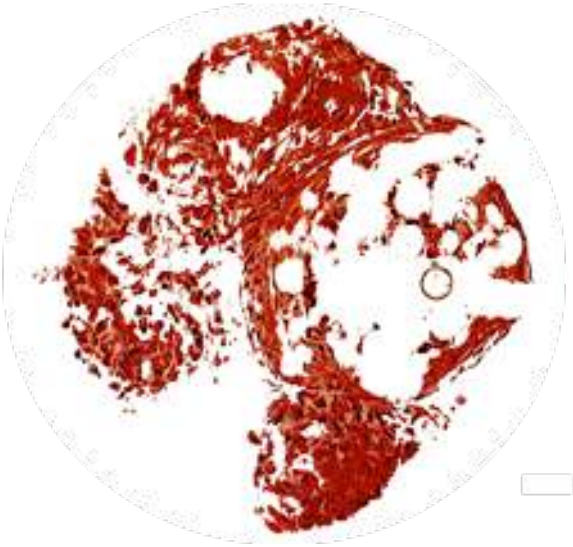
in vitro

MONO capsules

CO capsules

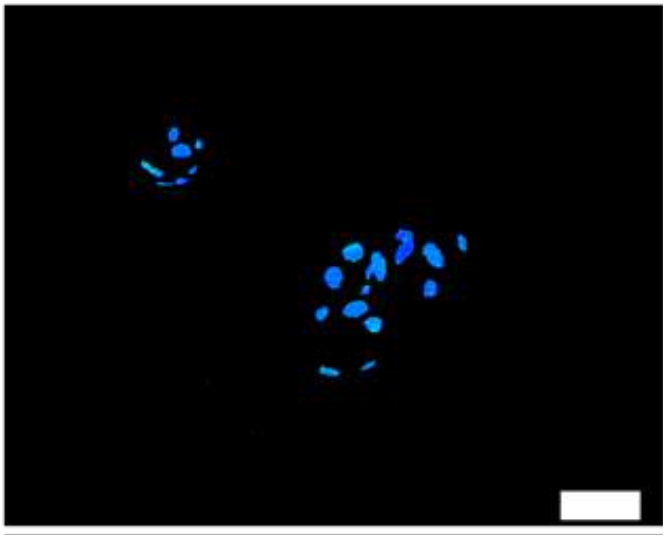
Alizarin red

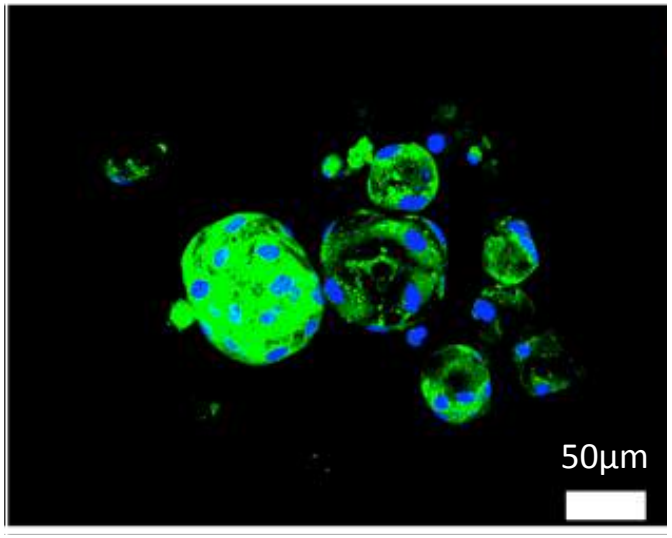




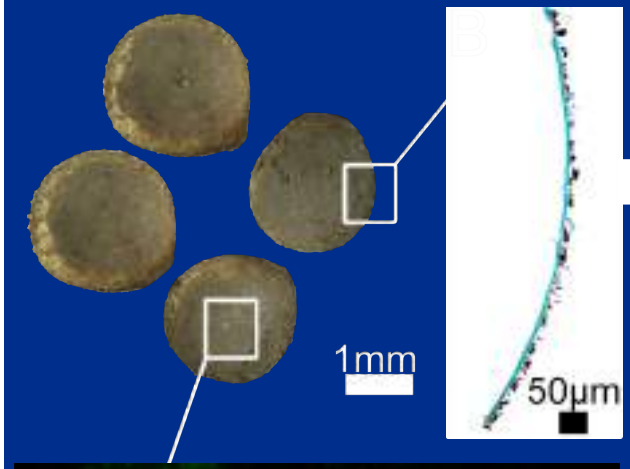
Osteopontin

DAPI Osteopontin

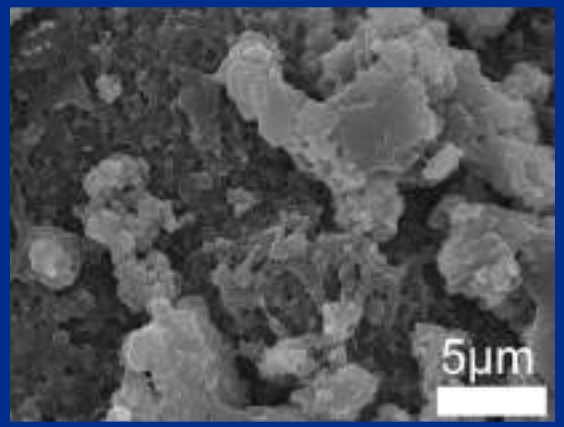


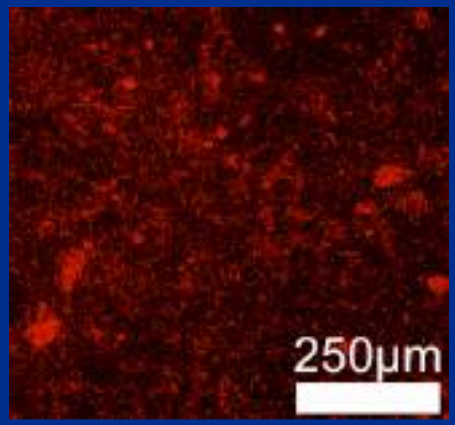


Chondrogenic Capsules

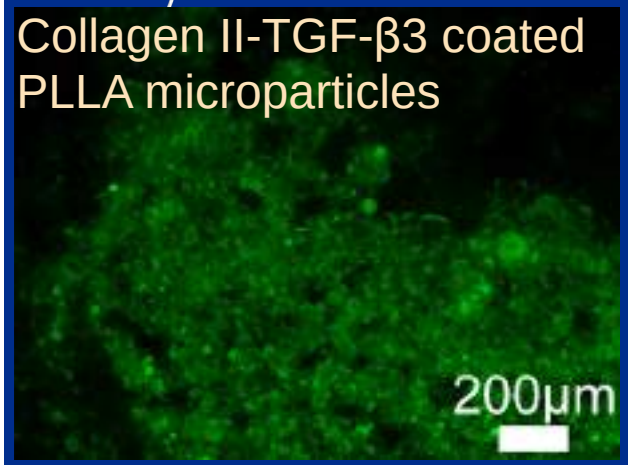


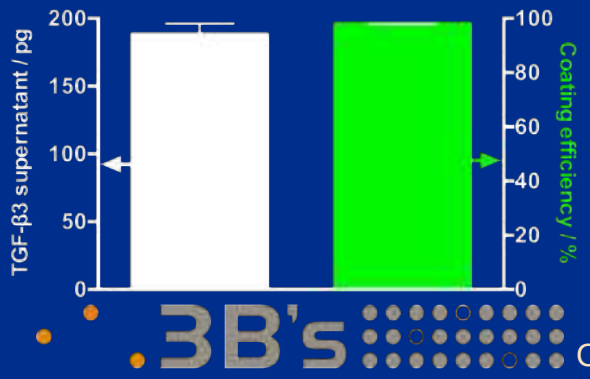
magnetite-nanoparticles incorporated into the multilayered membrane





Collagen II-TGF-β3 coated PLLA microparticles





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Encapsulated hASCs, using PLLA with two coatings:

- Col-II/TGF-β3 (cultured in TGF-β3 deprived medium);
- Col-II coating (cultured in medium containing TGF-β3).

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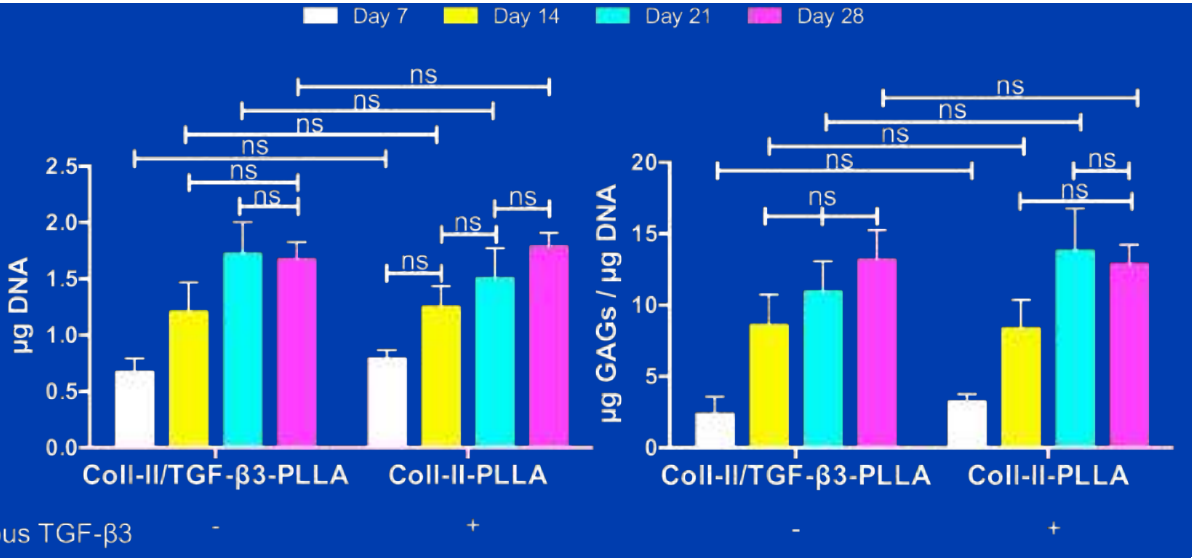
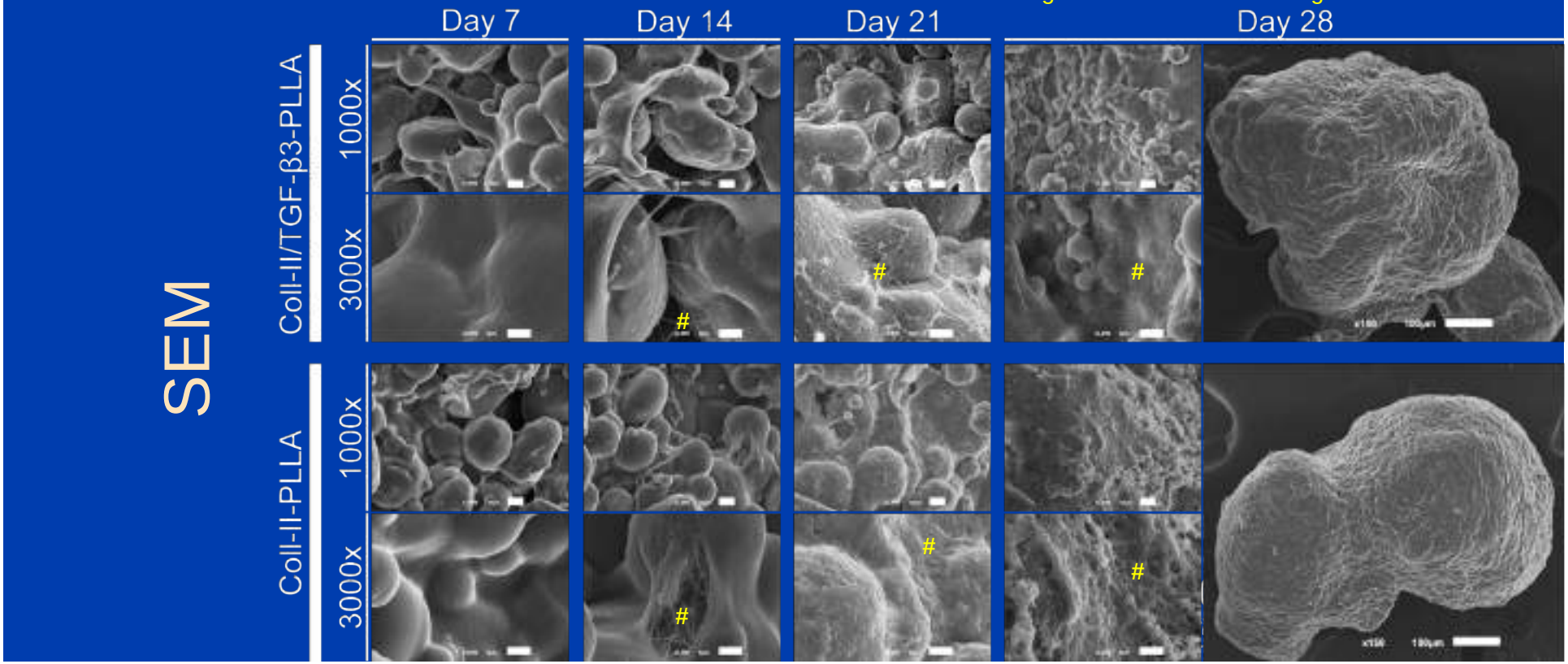
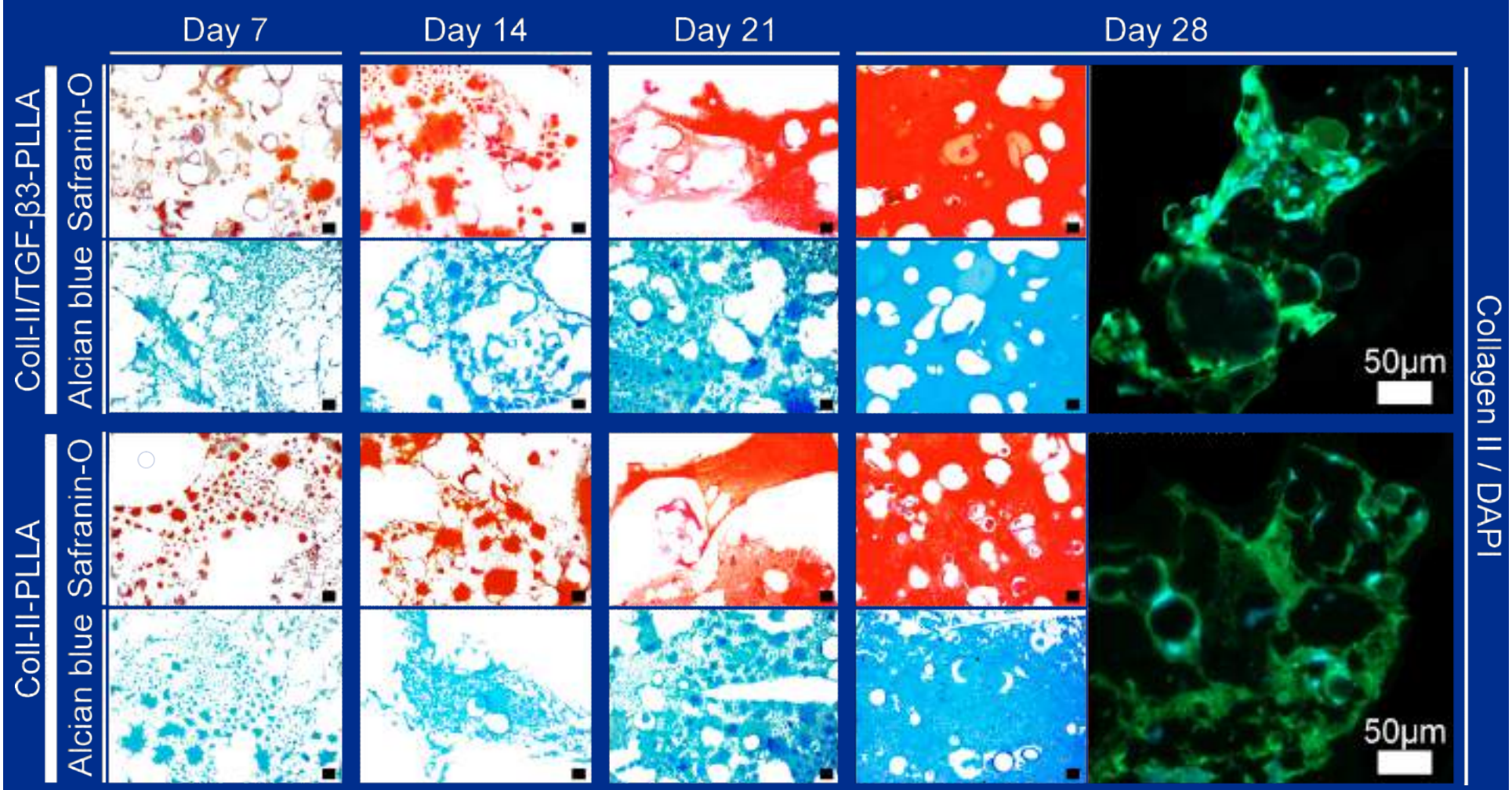


nanofibers in the newly deposited ECM resembles the collagen fibrils of native cartilage



Chondrogenic Capsules: histology



The presence of the major constituent of cartilage, collagen II, was detected by immunocytochemistry and afranin-O and alcian blue stainings revealed a basophilic ECM deposition (rich in glyco and proteoglycans), which is characteristic of neocartilage

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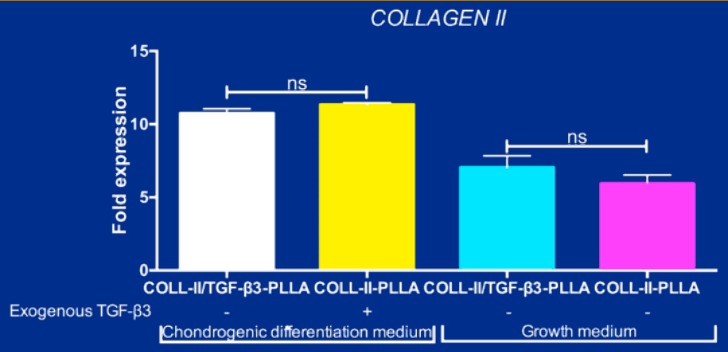
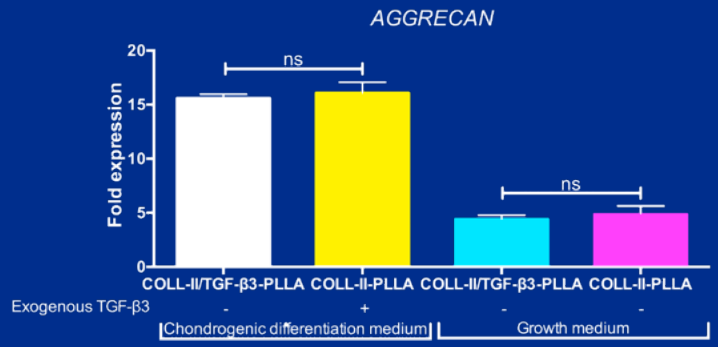
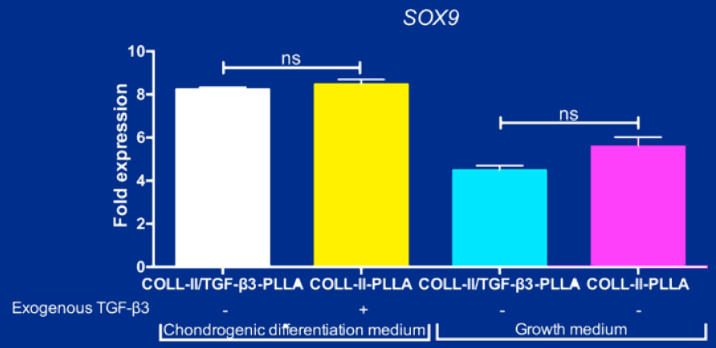
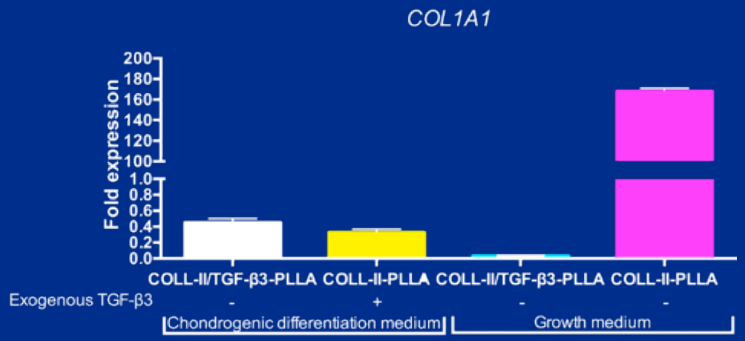
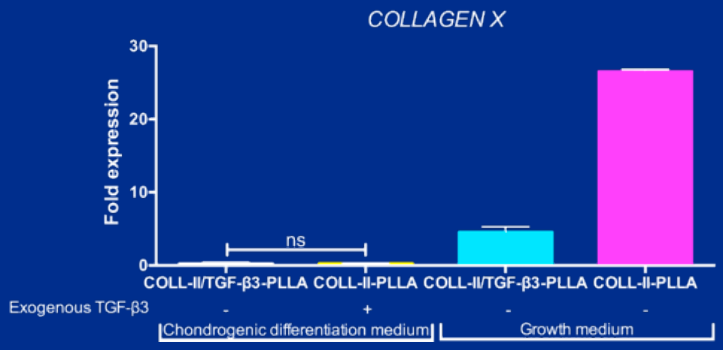
Chondrogenic Capsules: RT-PCR











The production of glycosaminoglycans and the expression of cartilage-relevant markers (collagen II, Sox9, aggrecan, and COMP) increased up to 28 days, while hypertrophic (collagen X) and fibrotic (collagen I) markers were downregulated.

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Students involved in the projects

International collaborators

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- CHEM2NATURE (TWINNING)
- ATLAS (ERC-AdG)