Closed and open scaffolds for bone regeneration

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\textsuperscript{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

Replacement strategies (biomaterials/ artif. organs)

Organ transplantation

Pharmaceuticals

Tissue transplantation

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

biopsy; cell isolation / expansion

seeding in scaffolds

in vitro culture; Implantation

biochemical and mechanical stimuli

peripheral nervous tissue

cartilage

bone

blood vessels

skin

tendon ligament
Featured Letter:
Designing Biomaterials for Tissue Engineering Based on the Deconstruction of the Native Cellular Environment
Natural based polymers

polysaccharides

neutral
  starch
  dextran

polycationic
  chitin
  chitosan

polyanionic
  alginate
  carboxylated
  hyaluronic acid
  carragenan (κ, ι, λ)
  sulfated chondroitin sulfate
  ulvan
  gellan gum

proteins
  silk fibroin
  collagen
  fibronectin

polyesters
  poly(hydroxybutyrate)

J.F. Mano +, J.R.Soc.Interface ’07

ICVS/3B's
3D supports for cells in tissue engineering

OPEN

- porous scaffolds
- mass, cells

CLOSED

- compartmented cells
- soluble cues
- liquified/elastic environment
- mass:
  - immune cells, antibodies
  - nutrients, O₂
  - waste products, metabolites
- cells+biomaterials+soluble factors may be retained in the same volume.
- self-regulated strategies.
- Use of non-autologous cells.
- Injectable systems.

3B’s
Rapid Prototyping Technique

- Human Body
- X-Ray
- CAD software
- RP machine
- Scaffold with tissue
- Cell culture
- 3D Scaffold
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

Layer-by-layer methodology using polyelectrolytes solutions

- Substrate
- Polycation
- Wash
- Polyanion
- Wash

$n \times$ multilayered film

Decher (1992)
**Platelets**

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
- TGFβ
- BMP-2, BMP-4, ...
- IGF-1

**Antigen receptors**
- PECAM-1
- P-selectin

**Adhesion proteins**
- Fibronectin
- Vitronectin
- Trombospondin
- Vit-D binding protein

**Chemokines**
- IL-1β
- IL-8
- PF-4
- SDF-1α

**Hemostatic factors**
- Fibrinogen
- Protein S

**Others**

**EDMOND ALEXANDER, Shannon Associates LLC**

**3B’s**

**ICVS/3B’s**
multilayers containing PL

**Platelet Lysate**

*Platelets*

<table>
<thead>
<tr>
<th>PPP</th>
<th>PRP</th>
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<tbody>
<tr>
<td>Red Blood Cells</td>
<td></td>
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</tbody>
</table>

**Layer-by-Layer with PL**

**Polyelectrolytes**
- Chitosan (-NH$_2$; -OH)
- Alginate (-COOH; -OH)
- k-carrageenan (-OSO$_3$H$_x$; OH)
- l-carrageenan (-OSO$_3$H$_x$2)
- λ-carrageenan (-OSO$_3$H$_x$3)
- Heparin (-OSO$_3$H$_x$2; -NSO$_3$H$_x$1; -OH; -COOH)

**Ellipsometry data (6 bilayers)**

<table>
<thead>
<tr>
<th>(Alg/PL)$_6$</th>
<th>(k Car/PL)$_6$</th>
<th>(l Car/PL)$_6$</th>
<th>(λ Car/PL)$_6$</th>
<th>(Hep/PL)$_6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control: no PL</td>
<td>Positive sulfuration</td>
<td></td>
<td></td>
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</tbody>
</table>

S.M. Oliveira+, Biomaterials, '15
Incorporating human platelet lysates in the multilayers

Layer-by-Layer with PL and freeze-drying

CHITOSAN (Chi)
\(\text{\textregistered}\) L-CARRAGEENAN (Car)

PCL scaffolds
Bioplotter™

SAMPLES

Before

After

PCL = unmodified

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

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

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

hASCs P1: $0.12 \times 10^6$/scaffold
α mem 4D

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

S.M. Oliveira+, ACS Biomater. Sci. & Eng., ‘15
Hierarchical scaffolds containing PLs: osteogenic potential

**IMMUNODETECTION OF OSTEOCALCIN**

<table>
<thead>
<tr>
<th>+Dex</th>
<th>TCPS</th>
<th>PCL</th>
<th>PCL LbL</th>
<th>PCL LbL PL</th>
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</thead>
</table>

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

S.M. Oliveira+, ACS Biomater. Sci.&Eng., ‘15
Relevant properties influencing the behaviour of encapsulated cells

L. Gasperini, J.F. Mano, R.L. Reis, J.R.Soc.Interface \textsuperscript{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.

- Chitosan microparticle
- Chemical modification with biotin-NHS
- Conjugation with streptavidin
- Conjugation with biotinylated antibodies

- In situ scaffold formation
- Cell expansion
- Selection and attachment of target cells
- Combination with a mixed cell population
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
- Cells
- Permselective membrane
  - Poly-(L-lysine), alginate and chitosan
  - 12-layers, 0.5mg/mL
- Microparticles
- Layer-by-layer assembly
- Solvent evaporation
  - Poly-(L-lactic) acid
  - Collagen I coating
  - Diameter: 20-100µm

- Excellent diffusion
- Shape-freedom
- Controlled environment
  - (e.g. adhesive sites for cells)
Preparation of liquified capsules

- microparticle
- cells
- alginate solution

CaCl₂ bath

ionotropic gelation

alginate bead embedding the cargo

Layer-by-layer

Poly(L-lysine) solution

Alginate solution

Chitosan solution

Alginate solution

EDTA

core liquefaction

nutrients and oxygen

waste products and metabolites

time
hierarchical (liquified) capsules

(a) PLLA particles - diameter 45.6 ± 13.5 µm

(b) capsules - diameter 1.8 ± 0.1 mm.

C.R. Correia+, *Biomacromolecules* '13
Liquified capsules: Cell adhesion and proliferation studies

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).

DAPI-phalloidin fluorescence assay

DNA quantification assay

Cell density: 1x10^6 cells/mL alginate

DNA content (µg/mL)

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C.R. Correia+, *Biomacromolecules* '13
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 -

Isolated cells phenotype & co-encapsulation analysis

**in vitro**

- MONO capsules
- CO capsules

**Alizarin red**

**DAPI / Osteopontin**

**Osteopontin**

50µm
Chondrogenic Capsules

Collagen II-TGF-β3 coated PLLA microparticles

magnetite-nanoparticles incorporated into the multilayered membrane
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).


# 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.
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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