Smart biological films for orthopaedic implants

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Implants: Current medical practices for bone regeneration

Ceramics
- BONE GRAFTS
- Hydroxyapatite/
  Tricalcium phosphates
- Ca/P
- Osteo-conduction

Metals
- SPINE Plates
- Dental implant

Polymers
- SPINE Fusion cage
- Custom-made design
**BMP : Body Morphogenetic Proteins**

- **BMPs** – 25 family members
  - Induce bone formation
    - Ulrist, *Science* 1965
  - Clinics
    - BMP-2 (Medtronic – since 2003)
    - BMP-7 (Olympus Biotech – 2004/2014)

**Brain**
- BMP2, BMP4, Noggin
- Glioma, Alzheimer’s disease

**Pulmonary artery**
- BMP2, BMP4, BMP7
- PAH

**Heart**
- BMP2, BMP4, BMP10, GDF15
- Hypertrophy, PAH

**Kidney**
- BMP7, USAG1
- Chronic renal diseases, renal fibrosis

**Pancreas**
- BMP6
- Diabetes

**Gastrointestinal tract**
- BMP2, Noggin
- JPS, colorectal cancer

**Muscle**
- BMP2, myostatin
- Muscular dystrophy, FOP

**Bone and joint**
- BMP2, BMP7, GDF5
- Osteoporosis, arthritis

P. Knaus, *Science Signalling* 2010
Bone regeneration is a time-regulated process

BMP-2 present in all steps of the repair

Challenges
- Boost bone regeneration (kinetics)
- Struture of new bone (maturation)
- Avoid bacterial infection/side effets (safety)

Improved patient’s quality of life
Decrease societal cost (hospital stay)

From Reis RL et al. Tissue Eng. B 2013
**In situ** bone tissue engineering

- IMPLANT + BMPs

- **Nanoreservoir**
- **Spatially localized delivery**

- **FILM**

- **Stem cells**
Polyelectrolyte multilayer films as surface coating

- Very simple self-assembly process

  Substrate (titanium, glass, gold…)
  Decher, Science, 1997
  Caruso, Science 2014

  Advantages:
  - Versatility (self-assembly, building blocks)
  - Applicable to any type of substrate
  - Aqueous process (green)
  - Cost effective

Different forms:

- Cell sheets
- Cell coating
- Hollow capsules

ETH Zurich
USA, Japan
Germany, Australia, China, Portugal

Surface coating of implants

Engineering of BMP nanoreservoir Poly(L-lysine)/Hyaluronan multilayer films

1. Biomimetic film
   - Dip coating
   - Number layers tunable

Poly(L-lysine) (PLL)
- Polyamino acid

Hyaluronan (HA)
- Cartilage, skin, eye and synovial fluids...
- Used in bone & cartilage tissue eng, plastic surgery

Robot
Confocal microscopy vertical section
PLL $^{	ext{FITC}}$

~ 1.5 µm to 5 µm
EDC cross-linking of (PLL/HAl films

1. Biomimetic film  2. EDC Cross-linking

Chemical : FTIR spectroscopy

% reduction in COO- vs EDC concentration (mg/mL)

Mechanical: AFM nano-indentations

Elastic modulus E0 (kPa) vs EDC concentration (mg/mL)

From ~ 5 kPa to 500 kPa

Covalent amide bond

COO- + NH3+
Tunable doses of BMP-2 in loaded in nanoreservoir

1. Biomimetic film  
2. EDC Cross-linking  
3. Post-loading of BMPs

BMP-2 loading

Depends on [BMP-2]i

BMP-2 release

BMP release depends on EDC

Crouzier et al. Small, 2009; Bouyer et al, Biomaterials, 2016
BMP-2 delivery to cells: soluble versus matrix-bound

**Cell Biology**
Soluble BMP-2
sBMP-2

**Materials Science**
Matrix-bound BMP-2
bBMP-2

<table>
<thead>
<tr>
<th>BMP-2</th>
<th>Stiffness</th>
<th>Presentation</th>
<th>Lifetime</th>
<th>Diffusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fixed</td>
<td>Soluble</td>
<td>~13h</td>
<td>Free</td>
</tr>
</tbody>
</table>

Biophysical studies
Engineered biomaterials and biomimetic plateforms to understand cell signalling by bBMP-2

In collaboration with IAB Grenoble
Team DYSAD
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Laure Fourel
Amaris Guevera
Olivier Destaing
Cyril Boyault

C. ALBIGES-RIZO
L. FOUREL
bBMP-2 delivered by the films is bioactive

Biomimetic film

SMAD

- sBMP-2  bBMP-2

pSMAD

actin


Shore EM, Nat Rev Rheumat. 2010

BMP-2

ALP

Crouzier et al. Small. 2009 + Patent
bBMP-2 is internalized by cells
Understanding the molecular mechanisms leading cell differentiation to bone

Cooperation between BMPR and adhesion receptors

Micropatterning for single cell studies


Bone tissue engineering

Implant + Matrix-bound BMP-2

BIOPHYSICS
MOLECULAR
MECHANISM

REGENERATIVE
MEDICINE
BONE
REGENERATION

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Can we repair bone with the bioactive film coating containing bBMP-2?

1. OSTEOINDUCTION
   Rat muscle
   ectopic bone induction
   6 weeks

2. BONE REPAIR
   Rat femur
   Bone regeneration
Osteoinduction in ectopic site (intramuscular in rat)

TCP/HAP granules
From Medtronic

Granule + film bBMP-2

 Titanium alloy

Ti + film

Ti + film bBMP-2

Bone


**Repair of a critical size femoral defect (rat) using a hollow tube**

**Lactosorb® PLGA**
Maxillo-facial surgery (1996)

**Diagram A**
- PLGA sheet
- Cutting
- Heating
- Molding
- Film buildup
- Crosslinking

**Diagram B**
- BMP-2 loading
- 6 mm defect
- Implantation for 2 months

**Diagram C**
- Analysis: X-rays, microcomputed tomography, histology
Pre-screening of BMP-2 dose

<table>
<thead>
<tr>
<th>EDC (mg/mL)</th>
<th>BMP (µg/mL)</th>
<th>5</th>
<th>25</th>
<th>50</th>
<th>100</th>
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<tr>
<td>10</td>
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<td>70</td>
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**Bony shell**

n=2 per group

**Negative controls**
- No implant (n=2)
- Bare PLGA (n=8)
- PLGA + ads BMP-2 (n=8)
Dose-dependent regeneration of femoral bone

PLGA

BMP2

Bone volume ratio

BMP25  BMP50  BMP100

Bouyer, M. Biomaterials, 2016
Histology: bone maturation and vascularization

Vascularization

PLGA / film - interface

Endothelial cells

vessels / red blood cells

Polarized microscopy

Cortical bone

PLGA tube

rbc
New bone imaged by high resolution µCT

ESRF

BMP25  BMP50

Cortical bone

Around the tube

Trabecular bone

Inside the tube

Cortical bone

BMP25  BMP50

B

Ct - bone volume (mm³)

0 5 10 15 20 25 30

**

200 250 300 350

C

Ct - thickness (µm)

150

BMP25  BMP50

*
In summary

- An osteoinductive surface (2D) combined with a hollow PLGA tube can regenerate a VOLUMIC critical size bone defect (3D).

- The new bone $v$ depends on the BMP-2 dose delivered via the film.

- Both cortical & trabecular bones are formed + vascularization.

Perspectives in personalized medicine

Modularity: customized implants / optimized BMP-2 dose
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