Biomimetic Biomaterials for Tissue Regeneration: Physico-chemical, Biochemical, and Construct Strategies

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Aim of the lecture

- To highlight the societal and clinical needs for new biomaterials
- To understand the main phases characterising tissue regeneration
- To explore biomimetic strategies by models (examples)
- To examine their pros and cons
- To evaluate their industrial feasibility
The impact of science in our life

- Increasing longevity has been one of the major successes achieved by science and technology in the second half of the 20th century
- At the beginning of the 20th century life expectancy was of 58 years
- Today life expectancy has risen to 78 years and this figure is expected to increase sharply in the near future
The impact of technology in our life

- In 1900 the top three causes of death in the US were pneumonia/influenza, tuberculosis etc.
- Since 1940’s, most deaths in the US have results from heart disease, cancer, and other lifestyle diseases.
- By the end of the 1990’s, lifestyle diseases accounted for more than 60% of all deaths
Lifestyle diseases

- Cardiovascular diseases
  - Atherosclerosis
  - Heart attack
  - Stroke
- Cancer
- Diabetes
- Osteoporosis/Osteoarthritis
- Nephritis
Biomedical implants & clinical needs

- Longer implantation times
- Longer life expectancy
- Insurgence of pathologies in younger subjects
- Implantation in pathological tissues
- Impaired tissue regeneration
Paradigm shift

- **Tissue (re)placement**
  - Conventional biomaterials substitute the damaged area

- **Tissue (re)generation**
  - Bioactive/bioreponsive biomaterials and tissue engineering products able to intervene in the wound healing process and stimulate the physiological formation of new tissue
Paradigm shift

- **Complete regeneration**
  - Tissue engineering approach

- **Interfacial regeneration in re-placement approaches** (e.g. load-bearing applications)
  - Regenerative surfaces
  - Regenerative coatings
Tissue regeneration process

Fibrin

1. Clot formation
   - Platelets

2. Inflammatory response
   - Macrophages

Hyaluronan

3. New matrix formation
   - Osteoblasts

4. Tissue regeneration
   - Mineralised tissue

Growth Factors

Collagen
The inflammatory cells

- Neutrophils (acute inflammation)
- Monocytes/Macrophages (acute and chronic inflammation)
- Giant cells (chronic inflammation)
Macrophages dominate the implant surface

Antigen presentation to lymphocytes

IL-1
PDGF
TGF-β

macrophage

Fibroblast migration, proliferation, activation

ECM remodelling, wound contraction

endothelial and smooth muscle proliferation angiogenesis
Events following implantation

- acute
- chronic
- granulation tissue

- neutrophils
- macrophages
- neovascularisation
- Foreign body giant cells
- fibroblasts
- fibrosis

- hours
- days
- weeks
- months
The foreign body reaction

“...the body react similarly to nearly all materials that we call biocompatible and walls them off in an avascular, tough, collagenous bag, roughly 50-200 mm thick.”

The fibrotic capsule formation

a-c: PHEMA
d-f: PHEMA/Gelatin IPN

Santin M., Huang S.J., Iannace S., Nicolais L.,
Ambrosio L. & Peluso G.
Synthesis and Characterization of Poly(2-Hydroxyethylmethacrylate)-Gelatin Interpenetrating Polymer Network.
Biomaterials, **17**, 1459-1467, 1996.
Blood: the early medium

Photos by Dr. A. Merolli
Universita’ Cattolica
Rome, Italy
Tissue integration: a race to the surface

Implant

Proteins (mSec)

Lipids (mSec)

Cells (h)

Ions (mSec)
Tissue regeneration approaches

➢ To implant a scaffold encouraging cell adhesion, proliferation and differentiation (*in situ* regeneration)
  ➢ Non-active scaffolds
  ➢ Bioactive scaffolds (releasing growth factors or genes)

➢ To deliver stem cells and differentiated cells (biohybrid)
  ➢ Individual cells
  ➢ Small cell aggregates
  ➢ Injected
  ➢ Combined with a scaffold

➢ To implant a tissue previously engineered *in vitro* (bioreactor)
Ideal features of bio-active/responsive biomaterials

- To regulate the regeneration of the damaged tissue by
  - minimising the inflammatory response
  - Balancing the proliferation/differentiation of tissue cells
Introduction: Ideal characteristics of a biodegradable biomaterial

- To promote the in-growth of the damaged tissue
- To resorb at a rate comparable to the growth of the surrounding tissue
- To minimise the inflammatory response
- To release by-products which do not elicit inflammatory response or toxicity
Biomaterials composition and engineering

- Biomaterials
  - Polymers
  - Metals
  - Ceramics

- Engineering
  - Composites/blends/interpenetrated polymer networks
  - Coatings
  - Moulding
  - Surface grafting of biofunctional molecules
  - Surface morphology modifications
Types of biomimetic strategies

- Biomimicking of animal structures and functions
- Biomimicking of common natural strategies
Biomimetic strategies

- Biomimicking of cell components
  - Glycocalyx
  - Lipids
- Biomimicking of extracellular matrix components
  - Fibrin
  - Hyaluronic acid
  - Collagen
  - Apatite of mineralized tissues
- Biomimicking of biochemical signalling pathways
  - Growth factors

1. Clot formation
   - Platelets

2. Inflammatory response
   - Macrophages

3. New matrix formation
   - Osteoblasts

4. Tissue regeneration

Cell component biomimicking

The polycarboxybetaine model
The carboxybetaine model

- Betaines are a class of plant amino acids able to retain water in the cell
- Used in biotechnology as cryoprotectants
Polycarboxybetalaine: advantages/disadvantages

- **Advantages**
  - Inducing water structuring thus preserving the native conformation of the proteins
  - Absorbing wound exudates

- **Disadvantages**
  - Poor mechanical properties
  - Inertness
  - Biocompatibility?
  - Biodegradation rate?
Cell component biomimicking

The phosphorylcholine model
The phosphorylcholine model

- Mimicking of the red cell membrane
- Relatively inert
- Suitable as monolith or as coating for several applications
  - Ophthalmology devices
  - Cardiovascular devices
  - Drug delivery devices
The phosphorylcholine polymers: limitations

- **Advantages**
  - Reduced inflammatory response
  - Reduced bacterial adhesion
  - Matrix/coating for drug delivery (i.e. drug eluting cardiovascular stents)

- **Disadvantages**
  - Inert
  - Non-bioactive
  - Non-biodegradable
Cell component biomimicking

The phosphatidylserine model

or

The calcium-binding phospholipid model
The matrix vesicle model: the mineralization potential of calcium-binding phospholipids

Growth factors → Collagen → Matrix Vesicles

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Figure 5
Role of matrix vesicles in bone formation

Matrix vesicle → Ca\(^{2+}\) influx → Collagen → ALP → Phosphate → Apatite

Typical Phosphatidylserine Structure

1,2-Dimyristoyl-sn-Glycerol-3-[Phospho-L-Serine] (Sodium Salt)
Biomineralization strategies

Forming crystal *nucleus*

Calcium binding molecule layer with high capacity and low affinity
Calcium-binding liposomes

Phosphatidylcholine : X : Cholesterol
7 : 2 : 1
X= Phosphatidylserine, phosphatidylinositol

T.E.M. of PC:X:C liposomes
in Simulated Body Fluid (SBF)
(Santin M et al, data not published)

Heywood BR, Eanes ED
Calcif. Tissue Int. 50, 149-156, 1992
Calcium-binding phospholipid model

Calcium-driven cross-linking of phospholipid bilayers

Wilschut J, Hoekstra D, 1986
Calcium-binding phospholipid model

Cryo-SEM of PC:PS:C titanium coatings:
- a = 7-days incubation in calcium-depleted SBF
- b = 7-days incubation in SBF
- c = 7-days incubation in SBF after 1-h pre-conditioning in serum

Santin M et al.
Calcium-binding phospholipids model

Incubation of PC:PS:C partially-coated titanium disk in SBF
a = visual inspection after 7- days incubation
b = Cryo-SEM after 1-h incubation
c = Cryo-SEM after 1-h incubation in serum-enriched SBF

Santin M et al.
Journal of the Royal Society Interface
3, 277-281, 2006
Calcium-binding phospholipids model

Cryo-SEM of PS coating after 7-days incubation in SBF
a = exposed surface
b = fracture surface
c = relative EDX analysis

Calcium-binding phospholipids model: \textit{in vitro} biocompatibility

Monocytes/macrophages adhesion and activation

Osteoblast adhesion & mineralization

Bosetti M, Lloyd AW, Santin M, Denyer SP, Cannas M Effects of phosphatidylserine coatings on titanium on inflammatory cells and cell-induced mineralization \textit{in vitro} Biomaterials, \textbf{26}, 7572-7578, 2005
Dr. A. Merolli
Catholic University
Rome, Italy
Calcium-binding phospholipids model: 
*in vivo* biocompatibility

Merolli A, Giannotta L, Tranquilli-Leali P, Lloyd AW, Denyer SP, 
Rhys-Williams R, Love WG, Gabbi C, Cacchioli A, Bosetti M, 

a=Implant at 4 wks 
b-d=Implant interfaces 
c= 8 wks 
d= 26 wks
Calcium-binding phospholipid model: advantages

- Liposomes containing calcium binding phospholipids are able to form aggregates with collagen and gelatin gels and induce their fast mineralization.
- Calcium-binding phospholipids are able to self-aggregate in presence of calcium to form highly mineralized hydrogels when incubated in SBF.
- Calcium-binding phospholipids can be used as bone fillers or coating materials:
  - Controlling the inflammatory response
  - Promoting the deposition of bone mineral phase
  - Favouring the osteoblast adhesion and mineralization potential
  - Inducing implant integration \textit{in vivo}
Calcium-binding phospholipid model: disadvantages

- Poor mechanical properties
- Eliciting of the host response tightly linked to the gel composition
- Slow degradation/resorption
Biomimicking of extracellular matrix components

Functionalised hydrogels
Poly(ethylene glycol) injectable biomimetic gel for bone tissue engineering

- PEG characteristics:
  - RGD-modified (Arg-Gly-Asp)
  - Proteolytically-sensitive (metalloproteinase-sensitive)
  - Carriers for bone marrow stem cells

Sakiyama-Elbert SE, Hubbell JA et al.
Relevant biomimetic sequences

- RGD (cell adhesion, integrin, non-specific)
  - $10^5$ bioligand/cell
  - 140 nm spacing
- YIGSR (cell adhesion, laminin receptor)
- REVD (endothelial cells adhesion)
- FHRRIKA (osteoblast migration)
- GVGVP (monocyte recruitment)
Relevant biomimetic sequences

- GAGAS/GVGVP (silk/elastin mimicking undergoing liquid/solid phase transition in aqueous medium, Cappello et al.)
- Self assembling peptide systems able to form gels in vivo (Stupp et al., Leon et al.)
Biomimetic hydrogels

- **Advantages**
  - Highly controlled 3D biomimicking environment
  - Application of the technology to different clinical needs

- **Disadvantages**
  - Poor mechanical properties
  - Storage conditions
  - Scale up/ costs
Biomimicking of extracellular matrix components

Functionalised metals
Metal biomimetic functionalisation

**Kokubo’s method**

\[
\text{TiO}_2 \xrightarrow{\text{NaOH}} \text{HTiO}_3\text{Na} \xrightarrow{\text{SBF}} \text{Ti-OH}
\]


**Nanci’s method**

\[
\text{TiO}_2 \xrightarrow{\text{P}} \text{RP} \xrightarrow{\text{P}} \text{R= Si, S, etc}
\]

Nanci A. et al. (1998) US Patent 5,824,651
Surface functionalisation with calcium-binding moieties

Metal biomimetic functionalisation

Biomimicking of the biochemical signalling pathways

The growth factor models
Growth Factors

- Transforming Growth factor superfamily
  - Transforming Growth Factor-β1
  - Bone Morphogenetic Proteins/Osteopontins
- Growth Differentiation Factors
- Vascular Endothelial Growth Factor
- Platelet Derived Growth Factor
- Fibroblast Growth Factor/bFGF
- Epithelial Growth Factor
- Nerve Growth Factor
Growth factors advantages

- To modulate the different phases of the tissue regeneration process
- To favour the selective regeneration of tissues
Growth factor disadvantages

- Effective at relatively high concentrations
- Delivery to be controlled
- Risk of tumours (?)
- Unstable at room conditions
- Expensive
Combined biomimicking of extracellular matrix and biochemical signalling pathways

The soybean biomaterial model
Soybean composition

- Carbohydrates: 38%
- Minerals: 4%
- Oil: 18%
- Proteins: 40%

Isoflavones:
- Genistein
- Genistin
- Daidzein
- Daidzin
Isoflavone effect on eukaryotic cells

- Inhibits immunocompetent cells
- Plasmalemma receptors
  (Tyrosine kinase inhibition)
Isoflavone effect on eukaryotic cells

- Inhibits proliferation and induces differentiation of tissue cells
- Nuclear membrane receptors
  (Oestrogen receptor $\beta$)
  - Inhibition of topoisomerase II and block of the G2/M phase
  - Osteoblast differentiation inducer (ALP, osteoprogeterin, BMP2)
Isoflavone effect on eukaryotic cells

- osteoclast inhibition
  - Indirect: Through osteoblast synthesis of osteoprotegerin and increased osteoprotegerin/RANKL ratio
  - Direct: through tyrosine kinase inhibition?
Scheme of Biomaterial Preparation by Thermosetting

De-fatted Soybean milk → Coagulation by calcium → Soybean curd → setting → Films Membranes Blocks

Plastic biodegradable material → granules

patent PCT/GB01/03464
Conclusions

- The understanding and modulation of the “Interface Biology” (J. Kirkpatrick) is key to improve both biomaterial and tissue engineering products.
- The development of a new generation of biomimetic/bio-responsive biomaterials is key to improve the performances of biomedical devices.
- They have to respond to the needs for a better quality of life of the population worldwide…