Biomaterials in bone tissue regeneration and biofabrication: advances and challenges

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OUTLINE

- Tissue engineering/regeneration: scaffold requirements
- Bioactive scaffolds based on bioactive glass/polymer composites
- “Multifunctional” bioactive scaffolds
- Biofabrication for superior scaffolds
- Conclusions and the future
Key factors involved in scaffold design

Structural properties
(porosity, pore size, pore interconnection, mechanical properties)

Materials
(synthetic and natural polymers, ceramics and glasses)

Bioactivation
(chemical modification, surface activation, controlled release)

Signalling molecules
(soluble and insoluble signals)

Cells
(stem cells, marrow stromal cells, osteoblasts, chondrocytes and fibroblasts)

Biological requirements
(cell adhesion, proliferation and differentiation)

(Guarino et al., Expert Rev. Med. Devices (2007))
“... at present the challenge in tissue engineering bone and cartilage is the design and fabrication of reproducible bioreabsorbable 3-D scaffolds, which are able to function for a certain period of time under load-bearing conditions.”

Is this statement still valid in 2014?
Bioactive composite scaffolds for tissue engineering

**Biodegradable polymer scaffold**
(PDLLA, PLGA, PCL, natural polymers ...)

- Fibres
- Meshes
- Foams

**Bioactive material**
(Hydroxyapatite, Bioglass®, TiO₂ ... particles or fibres)

- Filler
- Coating

Composites for tissue engineering scaffolds


“3rd Generation Biomaterials”

Bioactive composite scaffolds for tissue engineering

Biodegradable polymer scaffold (PDLLA, PLGA, PCL, natural polymers...)
- Fibres
- Meshes
- Foams

Bioactive material (Hydroxyapatite, Bioglass®, TiO₂... particles)
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Composites for tissue engineering scaffolds

“3rd Generation Biomaterials”
A (nanoscale) bioactive phase ...

will improve osteoconductivity of a scaffold enabling the formation of hydroxyapatite (HA) on the surface and a site for bone re-growth

might be used to control the degradation rate of the polymer and to counteract its acidic degradation

enhances mechanical properties (composites approach)

possesses the ability to simulate the surface and/or chemical properties of bone

Nanoscale materials in tissue engineering

-Much work on understanding how nanostructures and scales affect the material-cell/tissue interactions

- Protein adsorption on nanostructured, patterned and/or functionalised surfaces

Relatively little is known about the link between 2D in-vitro behaviour of cells on nanoscale topographies and their behaviour on nanostructured 3D matrices and scaffolds (Challenge I)
Making 3D bioactive glass scaffolds: Foam replica technique

Chen & Boccaccini, patent WO2007017756 (2007)
Biodegradable polymer coatings (PDLLA, PHB, gelatine, ...) on Bioglass® scaffolds

→ Improved mechanical properties
  (mimicking bone “composite” structure) *(Challenge II)*

→ Added functionalities
  (e.g. addition of biomolecules/growth factors to the polymer, drug delivery function, sensing elements, nanoparticles/carbon nanotubes for controlled surface roughness / electrical –magnetic properties, nanotopography, ...)

Bioglass®/polymer scaffolds with interpenetrating network microstructure: improvement of mechanical behaviour

Why bioactive glasses?

"Better" than other bioactive ceramic materials?

45S5 Bioglass® [composition in wt%: 45% SiO₂, 24.5% Na₂O, 24.5% CaO, 6% P₂O₅] has been confirmed to greatly enhance bone regrowth compared to HA (Wheeler, 2001, etc.)
Bioglass® enhances bone formation through a direct control over genes that regulate cell cycle induction and progression.


Intracellular effects: Enhanced differentiation and proliferation of bone stem cells via gene activation.

Extracellular effects: Adsorption and desorption of growth factors without loss of conformation and biological activity.

--> the result is rapid regeneration of bone.
Bioactive glasses as carrier / delivery platform for therapeutic ions

Use of bioactive glasses as vehicle for controlled delivery of ions that act on cellular behaviour (Zn, Fe, Sr, Cu, Co, Ag, Mg, Ga, B, etc.)

Silicate glasses offer a more versatile alternative than crystalline materials (e.g. hydroxyapatite)

Ions can be incorporated in the glass melting stage, after scaffold fabrication by ion exchange processes or by sol-gel methods.
Most common specific targets of relevant metallic ions (*bioinorganics*) in their role as therapeutic agents.

Mourino et al., *J. Royal Soc. Interface*, 2012
Angiogenesis and bone regeneration (Challenge III)

Bioactive glass as angiogenic agent?
PLGA/Bioglass® composite mesh – In-vivo assessment of neovascularization

The presence of Bioglass® stimulates neovascularization from the surrounding tissue

Increased amount of VEGF secretion in presence of Bioglass®


H. Keshaw, et al., Biomaterials 26 (2005) 4171-4179


PLGA/Bioglass® mesh fully cellularized at 14 days. Collagen (pink staining) is deposited between the woven mesh fibres (arrows) and blood vessels (large arrow)
Angiogenesis and bone regeneration

• Materials-based approaches can lead to effective angiogenic / osteogenic responses
  – Reproducible
  – Less risk than direct cellular therapy?

• Link between angiogenesis and bone formation
  – Composite materials combining Bioglass®, biodegradable polymers (and growth factors, e.g. VEGF?)

Day et al., *Tissue Eng.* (2009)
Gerhardt et al., *Biomaterials* (2011)
Arkudas et al., *Tissue Eng.* (2013)
VEGF secretion of human fibroblasts after 72 hrs (Gerhardt et al., Biomaterials 2011)

Enzyme-linked immuno-sorbent assay (ELISA)

VEGF = signalling protein, which regulates angiogenesis

Bioglass® stimulated angiogenic signalling in fibroblasts, 2-5 times higher VEGF secretion compared to PDLLA films
**In-vivo study in AV-loop**

AV-loop filled with Bioglass® “granules” in a fibrin matrix

µCT analysis after explantation after 3 weeks

Arkudas, Boccaccini, et al.: 
*Evaluation of Angiogenesis of Bioactive Glass in the AV Loop Model. Tissue Engineering C, 2013*

(Kneser, Arkudas, Horch, et al., Erlangen)
Bioactive glasses as carriers of therapeutic ions

Table 1
Effect of selected metallic ions on human bone metabolism and angiogenesis: summary of literature studies.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Biological response in vivo/in vitro</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si</td>
<td>essential for metabolic processes, formation and calcification of bone tissue</td>
<td>[58,59]</td>
</tr>
<tr>
<td></td>
<td>dietary intake of Si increases bone mineral density (BMD)</td>
<td>[84]</td>
</tr>
<tr>
<td></td>
<td>aqueous Si induces HAp precipitation</td>
<td>[83]</td>
</tr>
<tr>
<td></td>
<td>Si(OH)₄ stimulates collagen I formation and osteoblastic differentiation</td>
<td>[60]</td>
</tr>
<tr>
<td>Ca</td>
<td>favours osteoblast proliferation, differentiation and extracellular matrix (ECM) mineralization</td>
<td>[54]</td>
</tr>
<tr>
<td></td>
<td>activates Ca-sensing receptors in osteoblast cells, increases expression of growth factors, e.g. IGF-I or IGF-II</td>
<td>[55,56]</td>
</tr>
<tr>
<td>P</td>
<td>stimulates expression of matrix la protein (MGP), a key regulator in bone formation</td>
<td>[57]</td>
</tr>
<tr>
<td>Zn</td>
<td>shows anti-inflammatory effect and stimulates bone formation in vitro by activation protein synthesis in osteoblasts</td>
<td>[51]</td>
</tr>
<tr>
<td>Mg</td>
<td>increases ATPase activity, regulates transcription of osteoblastic differentiation genes, e.g. collagen I, ALP, osteopontin and osteocalcin</td>
<td>[92]</td>
</tr>
<tr>
<td>Sr</td>
<td>stimulates new bone formation</td>
<td>[80]</td>
</tr>
<tr>
<td></td>
<td>increases bone cell adhesion and stability (probably due to interactions with integrins)</td>
<td>[80,101]</td>
</tr>
<tr>
<td>Cu</td>
<td>shows beneficial effects on bone cells and bone formation in vivo</td>
<td>[50,65]</td>
</tr>
<tr>
<td></td>
<td>significant amounts of cellular Cu are found in human endothelial cells when undergoing angiogenesis</td>
<td>[88]</td>
</tr>
<tr>
<td></td>
<td>promotes synergetic stimulating effects on angiogenesis when associated with angiogenic growth factor FGF-2</td>
<td>[94]</td>
</tr>
<tr>
<td>B</td>
<td>stimulates proliferation of human endothelial cells</td>
<td>[95]</td>
</tr>
<tr>
<td></td>
<td>induces differentiation of mesenchymal cells towards the osteogenic lineage</td>
<td>[96]</td>
</tr>
<tr>
<td></td>
<td>stimulates RNA synthesis in fibroblast cells</td>
<td>[102,103]</td>
</tr>
</tbody>
</table>
Bioactive Metal Ions: Cu$^{+/2+}$

**Cu$^{2+}$ ions affect**

- **Angiogenesis**
  - Cu is found in human *endothelial cells* when undergoing angiogenesis
  - promotes synergetic stimulating effects on angiogenesis when associated with angiogenic growth factor FGF-2
  - stimulates proliferation of *human endothelial cells*
  - an increase in blood vessels formation in bioactive glass scaffolds seeded with MSCs and/or 0.4 wt.% doping Cu

- **Osteogenesis**
  - induces differentiation of mesenchymal cells towards the osteogenic lineage

A. Hoppe, PhD, 2014 Erlangen
Multifunctional scaffolds
Multifunctional bioactive composite scaffolds

- *Improved* (time dependant) *mechanical properties*

- *Drug delivery + ion delivery / therapeutic function*

- *Electrical conductive*

- *Nanostructured in 3D*
Tissue engineering scaffolds with drug / growth factor delivery function

Bioactive glasses as carriers for bioactive molecules and therapeutic drugs: a review

Jasmin Hum · Aldo R. Boeveceni

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Abstract Bioactive glasses (BG) show great promise for bone tissue engineering based on their key properties, e.g., biocompatibility, biodegradability, osteoconductivity as well as osteogenic and angiogenic potential, which make them excellent candidates for bone tissue scaffolds and bone substitute materials. Recent work has shown that dissolution products of bioactive glasses have the potential to induce angiogenesis in addition to their known effect of influencing gene expression and promoting osteoblastic differentiation. One of the most interesting features of BG is their ability to bond both to soft and hard tissues, depending on their composition. To intensify the positive impact of BG for medical applications, there are considerable research efforts on using bioactive glass based platforms as carriers for the encapsulation, delivery and controlled release of bioactive molecules and therapeutic drugs. Different types of bioactive glasses have been considered in combination with different therapeutic drugs, hormones, growth factors and peptides. Using bioactive glasses as drug delivery system combines thus the effectiveness of therapeutic drugs (or bioactive/signaling molecules) with the intrinsic advantages of this inorganic bionmaterial. Considering research carried out in the last 15 years, this review presents the different chemical compositions and morphologies of bioactive glasses used as carrier for bioactive molecules and therapeutic drugs and discusses the expanding potential of BG with drug delivery capability focusing in the field of bone tissue engineering.

1 Introduction

1.1 Bioactive glasses and bone tissue engineering

The treatment of bone defects caused by tumors or trauma is still a great challenge for orthopedics and surgeons and there is continuous and increasing request for bone substitute materials (BSM) which cannot be covered only by autogenous or allogenic bone grafts. These grafts are associated with some disadvantages like immunological reactions, possible transmission of infections and limited availability. For some time now synthetic BSM have broadened the spectrum of possibilities to regenerate bone defects and have important applications in dental and orthopedic surgery [1–4]. One relatively new therapeutic approach for the treatment of bone defects and disorders is tissue engineering [5]. Bone tissue engineering (BTE) aims at assembling a bone substitute based on combination of cell biology and engineering principles [3, 4]. BTE usually relies on three main components, namely a macroporous 3D scaffold, relevant cells and signaling biomolecules (e.g., growth factors, proteins). In a suggested BTE approach, cells are seeded on a scaffold, they attach to the surface, differentiate and build new healthy bone in a biochemical suitable environment (e.g., provided by growth factors and signaling molecules), while the scaffold is degrading in vivo. A schematic diagram of the BTE approach is shown in Fig. 1.

Great attention has been paid to inorganic materials (bioactive ceramics) like hydroxyapatite or bioactive glasses as BSM and bone scaffolds because they have the ability to build a strong interface between the material surface and bone tissue [3, 6–9]. This bone bonding results from the bioactive glass dissolution products that react with the
(Nano-)structuring 3D scaffold surfaces

1. Interpenetrating biodegradable polymer composite scaffolds; e.g. PDLLA and P(3HB)

2. Nanostructured surface Using PDLLA/Nd-HA or PDLLA/IONP (slurry dipping or EPD?).

SEM image of the structure of a typical Bioglass® scaffold

PDLLA fibres deposited by electrospinning
Simple fabrication technique for multilayered stratified composite scaffolds suitable for interface tissue engineering

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Fig. 2. Sample morphological analysis by SEM: chitosan-based sample (F.D.90-C), and zoom on the interface area (A), chitosan-based sample (F.G.) (B), alginate-based sample (F.D.90-A) (C), chitosan-based sample (F.D.24) (D) and representative sample top view (E).
“Tissue Engineering: the biofabrication approach”

- Cells (including different cell types)
- Bioactive molecules, other elements, e.g. functional nanoparticles
- Soft Matrix

Biofabrication

- Cell/biomolecule containing 3D scaffold

Biomaterials Erlangen
Different strategies

2 Approaches:

**Cells seeded on a scaffold**
- Cell must attach on the biomaterial surface
  - Complex seeding techniques
  - Cell cultivation necessary
- Co-cultures difficult to realise
- Scaffold processing may be time consuming (shaping, sintering, etc.)
- All biocompatible materials can be used and combined
  - High mechanical stability
  - Controlled degradation

**Cells encapsulated**
- Cell seeded in a soft-matrix:
  - Cell distribution
  - Higher efficiency
  - Close simulation of the natural environment of cells
  - Nanotopography and stiffness of gels can support stem cell differentiation
- Co-cultures can readily be created (relevant for vascularisation)
- Drugs easy to incorporate
  - Injectable approaches
- Rapid prototyping/additive manufacturing:
  - Bioprinting of desired geometry
  - Complex structures
  - Different tissues can be combined
- Use of printed tissues/organs for replacement of in vivo test
Hydrogels as tissue engineering scaffolds

Ease of handling, a highly hydrated tissue-like environment for cell and tissue growth

Important properties of hydrogels
- Biocompatibility
- Swelling
- Mechanical properties
- Degradation
- Diffusion

Alginate + Gelatin?
Evaluation cell behaviour in a 3D Hydrogel-Matrix

Encapsulation process of cells

- Pressure range: 2.3 - 3.5 bar
- Cell-loaded alginate-gelatin mixture
- Nozzle diameter: 200μm
- 0.1 M CaCl$_2$
- Hydrogel capsules
Human adipose tissue derived stem cells (hADSC)

Actin cytoskeleton : Red  
Nuclei : Green  
Optimal composition for cell spreading, 40:60  
After 4 weeks
Conclusions

- Bioactive glass based scaffolds (also in composites) are materials of choice for bone tissue engineering
  - More relevant in vivo studies needed

- Bioactive glass compositions with addition of metallic ions ("bioinorganics") for enhanced cellular response /angiogenesis:
  - Expanding and relevant research field ("more biology")

- Advanced 3D scaffolds concepts based on biofabrication approaches and "soft matrices" may help to bridge the gap between regenerative medicine and cancer research
One never notices what has been done; one can only see what remains to be done...

Marie Curie

Thank you!