Biomaterials – the merger of devices with biologicals

Paul Ducheyne

Center for Biomaterials and Tissue Engineering
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Human identity related to identity of language

Eric Zenmour

In: Mélancolie Française, March 2010

Science being an activity of man, let us use this unique identifier of the mind and ask the question how do we interpret the word “biomaterials”
“Eventually, large device companies will look more like Amgen, as we will need to have biologicals in our devices”

Stephen Oesterle, MD
Senior Vice President of Medicine and Technology
Medtronic
In: Genetic Engineering and News, August 1, 2008
Previously, orthopaedic implants were designed simply as mechanical devices; the biological aspects of the implant were a byproduct of stable internal/external fixation of the device to the surrounding bone or soft tissue. More recently, biologic coatings have been incorporated into orthopaedic implants in order to modulate the surrounding biological environment. While many of these coatings are still in the preclinical testing stage, bioengineers, material scientists and surgeons continue to explore surface coatings as a means of improving clinical outcome of patients undergoing orthopaedic surgery.

Stuart Goodman, et al.
The future of biologic coatings for orthopaedic implants
Biomaterials, p. 3174 (2013)
Biomaterials are hybrid materials

The illustration of bioceramics

- **Bioactive ceramics**
  Biologically reactive ceramics and glasses that **enhance** bone formation and **stimulate** tissue regeneration
- Inorganic controlled release materials, **including sol gel glasses**
- (Inert ceramics for wear resistance)
Problem: Bone Formation

- Biological Grafts
  - Autografts
    - Donor site morbidity
    - Limited donor bone supply
    - Anatomical and structural mismatch
  - Allografts
    - Immunological response
    - Disease transmission
- Synthetic grafts (Biocompatible ceramics and glasses)
  - Hydroxyapatite (HA)
  - Tricalcium Phosphate (TCP)
  - Bioactive glass (BG 45S5, ...)
- Tissue Engineering
  - Scaffolds
  - Molecules (growth factors BMP-2, OP-1, ...)
  - Cells
Non-Structural Bone Graft U.S. Market Overview

2001 $360 MM Market

- Autograft: 46%
- Non-structural Allograft / DBM: 10%
- Synthetic Scaffolds & Cells: 44%

2007 $1,000 MM Market

- Autograft: 32%
- Non-structural Allograft / DBM: 47%
- Synthetic Scaffolds & Cells: 21%

Source: Data Monitor, Market Dynamics: Bone Substitutes and Growth Factors, December 2002
Spinal fusion
Bony defect resulting from tumor surgery
Bioactive ceramics and glasses
Key references

Hench et al., 1972
Aoki, 1972
Jarcho et al., 1976
De Groot, 1980
Ducheyne et al., 1980
Kokubo, Yamamuro, et al., 1985
The effect of hydroxyapatite impregnation on skeletal bonding of porous coated implants

Statistically significant differences
at 2 weeks: $p < 0.01$

at 4 weeks: $p < 0.001$

No meaningful difference
at 12 weeks: $p < 0.5$

Effect of PS-HA on Ti

Canine Push-Out Testing

(Cook et al., 1988)
HA Coated Fibermetal Mesh

Pull Out Shear Strength

(Rivero et al., 1988)
The effect of calcium phosphate coating characteristics on early postoperative bone tissue ingrowth

\( \tau \) (MPA)

- Control
- CAP 1
- CAP 2
- CAP 3

Time after Implantation (wks)

2 wk  4 wk  6 wk

Ducheyne et al., Biomaterials 11, 531 (1990)
The effect of calcium phosphate coating characteristics on early postoperative bone tissue ingrowth

Statistically significant differences
(CAP 3 vs. control)

at 2 weeks: \( p < 0.005 \)
at 4 weeks: \( p < 0.01 \)
at 6 weeks: \( p < 0.001 \)
Mechanisms of bioactivity

- surface reactivity
  - Ca – P layer formation
  - preferential Fn adsorption
  - Fn binding effect
  - growth factor adsorption
- solution effect
  - ionic dissolution product
  - cell produced osteogenic factors
- porosity effect
Bioactive Glass (BG) Surface Modification

Protein

Ca-P

Glass (45% SiO₂)
Cell Detachment Apparatus (CDA)

Spinning disk in an infinite fluid:

- linear range of shear stresses to attached cells
  \[ \tau = 0.8r\sqrt{\rho \mu \omega^3} \]
- uniform chemical environment at the surface

Fibronectin Adsorption

\[ \Gamma \text{ (ng/cm}^2\text{)} \]

\[ [Fn] \text{ (\mu g/ml)} \]
Cell detachment experiments
Profiles for substrates coated with 0.1 μg/ml Fn

- CG
- BGO
- BG1d
- BG7d
- sHA

adherent fraction

shear stress (dyne/cm²)

- No CaP present
- CaP formed on BG
- Slowly reacting CaP
Intracellular component of the activated integrin receptor participates in the cell signaling cascade.

- Fibronectin
- Integrin receptor
- Plasmic membrane
- Chromosomes
- Nucleus
Proliferation

ng DNA

Time (Days)

Cells on SM-BG *5

Cells on HA *5

Differentiation

AP activity

nMole/min/μg DNA

Time (Days)

Mechanisms of bioactivity

- surface reactivity
  - Ca – P layer formation
  - preferential Fn adsorption
  - Fn binding effect
  - growth factor adsorption
- solution effect
  - ionic dissolution product
  - cell produced osteogenic factors
- porosity effect
Bioassay with Stromal Marrow Cells

Alkaline Phosphatase Activity

Normalized AP Activity
(nmol pnpp/min/μg DNA/cm²)

- C
- C-BT
- SG
- SG-BT
- SG-BP
- SG-BI

6 days

10 days

Mechanisms of bioactivity

- surface reactivity
  - Ca – P layer formation
  - preferential Fn adsorption
  - Fn binding effect
  - growth factor adsorption
- solution effect
  - ionic dissolution product
  - cell produced osteogenic factors
- porosity effect
Differentiation of osteoprogenitor cells
3 months implantation in the dog mandible

- **bioactive glass granules** of narrow size range **cause differentiation** of osteoprogenitor cells to bone forming cells (osteoblasts)
- **unique finding** for a synthetic bone graft material
- each granule acts as a **nucleus** for new bone formation

Schepers et al., 1991
“....New bone was deposited in pockets or excavation chambers filled with proliferating osteoprogenitor cells. The process began within a few weeks and was complete in a few months. How much new bone could be attributed to the osteoconduction of cells growing in from the walls of the host bone, and how much could be accounted for by osteogenic induction, was not apparent from experiment on normal, healthy bones....”
Non-bioactive and bioactive materials with constant pore structure
Microsphere-based discs

PLGA

PLGA-30%BG

Fine BG powder in the polymer

Formation of Ca-P layer - SEM
PLGA-30%BG composite: 14 days of immersion in TE
Osteogenesis of rat marrow stromal cells

*In vitro* evaluation - AP activity

Osteogenesis of rat marrow stromal cells cultured on porous PLGA scaffold or PLGA-30%BG scaffold for 7 and 9 days. Dex = 10 nM
Solution mediated effect

Cell culture system

Cells were physically separated from scaffolds, which were either not seeded or seeded with cells (exclude the effect of direct contact with scaffold)
Osteogenesis of rat marrow stromal cells

*In vitro* evaluation - AP activity

Osteogenesis of rat marrow stromal cells on plastic wells for 9 days. Cells were separated from scaffolds by membranes. D: distant cells
Biomaterials are hybrid materials

The illustration of bioceramics

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Bioassay with Stromal Marrow Cells

Alkaline Phosphatase Activity

Normalized AP Activity (nmol pNp/pmin/µg DNA/cm²)

6 days

10 days

Tissue Repair and Regeneration
with delivery from
Silica xerogels (Silica sol-gels)

Room-temperature process
- Highly porous materials – porosity at the nanoscale
- Release properties controlled by the nanostructure

Sol-gel processing steps
Hydrolysis
\[ \equiv \text{Si-OR} + \text{H}_2\text{O} \leftrightarrow \equiv \text{Si-OH} + \text{ROH} \]
Organosilane
Condensation
\[ \equiv \text{Si-OH} + \text{HO-Si} \leftrightarrow \equiv \text{Si-O-Si} \equiv + \text{H}_2\text{O} \]
\[ \equiv \text{Si-OR} + \text{HO-Si} \leftrightarrow \equiv \text{Si-O-Si} \equiv + \text{ROH} \]
Silicon Oxide

Mechanisms of release
- Biologically active molecules diffuse out
- Fluid penetrates 2 nm size pores

Desorption Resorption Diffusion
First references

- Nicoll et al., *Biomaterials*, 1997
- Bottcher et al., *J. Sol Gel Science & Technology*, 1997
- Sieminska et al., *J. Sol Gel Science & Technology*, 1997
- Falaize et al., *J. Am. Ceramic Soc.*, 1999
### Infection treatment and control (I)
- Thin films on fracture fixation material for the controlled release of antibiotics
- Bactericidal thin films on percutaneous external fixator pins
- Thin films on joint prostheses for the treatment of joint arthroplasty revisions
- MRSA treatments

### Pain treatment and control (P)
- Post-surgical pain
- Abuse resistant controlled delivery of opioids
- Wound dressings for the delivery of analgesics

### Controlled release of biological molecules (BM)
- Controlled release of BMP-2
- Controlled release of TGF-β
- Controlled release of monoclonal antibodies
- Treatment of compartment syndrome

### Delivery of anti-proliferative drugs
- Controlled release of antiproliferative agents (Rapamycin)
- Thin films for drug eluting stents

### Combination treatments
- Combined delivery of antibiotics and growth factors for the treatment of oral pathologies (I, BM)
- Wound dressings for pain and infection control (I, P)

### Scaffolds for tissue engineering
- Large volume bone tissue engineering
Benefits of silica xerogels (sol-gels)

- excellent film to substrate adherence (for fundamental reasons)
- upon release, therapeutic efficacy maintained
- control of release of molecules varying in size from small (<1 kDa) to large (>70 kDa)
- burst release avoided by virtue of processing flexibility
- release can be controlled for a duration ranging from hours up to one year
- Poorly soluble molecules can be delivered

Nicoll et al. (1998), Falaize et al. (1998), Santos et al. (1999), Radin et al. (2002)
Benefits of silica xerogels (sol-gels), cont...

- molecules are protected while in the sol gel (compare to the \textit{in vivo} half life – e.g PDGF: 2 minutes)
- various molecules can be delivered simultaneously
- substantially full release of therapeutic agents
- resorbable
- biocompatible
- room temperature processing

Nicoll et al. (1998), Falaize et al. (1998), Santos et al. (1999), Radin et al. (2002)
Infection treatment and control (P)
- Thin films on fractures
- Bactericidal thin films
- Thin films on joint prostheses
- MRSA treatments

Pain treatment and control (P)
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Controlled release of biological molecules (BM)
- Controlled release of BMP-2
- Controlled release of TGF-8
- Controlled release of monoclonal antibodies
- Treatment of compartment syndrome

Delivery of anti-proliferative drugs
- Controlled release of antiproliferative agents (Rapamycin)
- Thin films for drug eluting stents

Combination treatments
- Combined delivery of antibiotics and growth factors for oral pathologies (I, BM)
- Wound dressings for pain and infection

Scaffolds for tissue engineering
- Large volume bone tissue engineered
Dose response curve
Vancomycin release

Zone of inhibition size (mm)

Vancomycin concentration (µg/ml)

- standard solutions
  $r^2 = 0.999$
- released Vancomycin
  $r^2 = 0.995$

Materials science questions

- Growth factor delivery (20 kDa and up)
- Zero order release kinetics (or about)
  - Nanoparticles (120 nm)
  - Composite wound dressings
- Thin films (1–2 μm)
- Thin films – long duration of release (3 mths)
- Thin film to metal substrate adhesion

Large volume bone tissue engineering
Cumulative Trypsin Inhibitor (20 kDa) release
TMOS-derived xerogel

- Time- and load-dependent release
- 20% released by 9 weeks

Santos et al. (1999)
Macromolecules – Release kinetics
Nanostructural control
Modeling experiments

• dextran, a hydrophilic polysaccharide, is used as a model molecule having different sizes
• molecular weight can vary from 3 to 500 kDa
• this eliminates the effect of molecule chemistry on
  » sol gel processing
  » release properties
• molecular weight used: 10, 40 and 70 kDa
Effect of nanopore size - Variation of catalysis pH

One dextran weight used: 70 kDa

larger nanopores (3.5 nm)

smaller nanopores (1.8 nm)
Effect of molecular weight
Larger nanopores used (acid-base catalysis at pH 5)
Cumulative Trypsin Inhibitor (TI) Release from PEG-coated Mesoporous Silica Nanoparticles (MSN)

Properties
120 nm average diameter
3.5 nm average pore size

Results
- the absence of an initial burst
- sustained near zero order release over 4 weeks

Bhattacharyya et al., Acta Biomaterialia (2012)
Composite Xerogel-Copolymer Wound Dressings
Composites enable controlled, zero-order release of Bupivacaine for 1 week

Release rates controlled by:
- Tyrosine-based monomer hydrophilicity and PEG content
- Xerogel porosity, particle size and drug loading

Costache et al., Biomaterials (2010)
**In vitro release**

Coatings on fixation pins

Release from coatings composed of 5 layers with 10% triclosan (<300 Da)

- long-term release, up to 60 days
- Initially: first order release (up to 7 days)
- followed by sustained, near zero-order release
- Extrapolation:
  - release continues beyond 90 days (3 months)

Qu et al. (2015)
# Mechanical properties of thin sol-gel films

<table>
<thead>
<tr>
<th></th>
<th>Crack onset strain (%)</th>
<th>Crack saturation strain (%)</th>
<th>Critical cracking stress (GPa)</th>
<th>Interfacial shear strength (MPa)</th>
<th>Film fracture energy (J/m²)</th>
<th>Interfacial fracture energy (J/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polished Metallic surface</td>
<td>10</td>
<td>10</td>
<td>6.10</td>
<td>519.72</td>
<td>688.59</td>
<td>61.02</td>
</tr>
<tr>
<td>Sandblasted (0.28 MPa) Metallic surface</td>
<td>5</td>
<td>16</td>
<td>3.05</td>
<td>153.37</td>
<td>112.43</td>
<td>156.21</td>
</tr>
</tbody>
</table>
Preclinical and clinical questions

- **Infection treatment and control**
  - Infection treatment and control (I)
    - Thin films on fracture fixation material for the controlled release of antibiotics
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    - Thin films on joint prostheses for the treatment of joint arthroplasty revisions
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  - Thin films for drug eluting stents
- **Combination treatments**
  - Combined delivery of antibiotics
  - Wound dressings for pain and infection
- **Scaffolds for tissue engineering**
  - Large volume bone tissue engineering

- **Infection treatment and control**
  - **Internal fracture fixation**
  - **Percutaneous pins**
Bone infections
The clinical issue

- Risk of infection is considerable in open fractures
- Prevention is challenging when fracture fixation material is used
- *Bacteria adhere to internal fixation nails and form a biofilm*
- High resistance of the biofilm to systemic antibiotic treatment may require further surgical procedures
In vitro bactericidal activity

- Samples
  - Controls
    » IM nails (metallic surface)
    » Sol-gel coated IM nails without vancomycin
  - Treatment
    » Sol-gel coated IM nails with vancomycin
- Staphylococcus aureus cultures
  » $1 \times 10^3$ cfu, $37^\circ C$, 24h
- Evaluation
  » bacterial counts
  » live/dead staining - confocal laser microscopy
**In vitro** bactericidal effect

In comparison to controls, number of S. aureus colonies were reduced by three orders of magnitude on sol-gel/vancomycin films.
In vitro bacterial inhibition
Confocal microscopy

- Uncoated samples (left)
  » viable bacteria adhere and proliferate

- Sol-gel/vancomycin coated (right)
  » bacterial adhesion is prevented
In vivo bactericidal activity

- Wistar rats
- Implants: sol gel coated with and without vancomycin
- Surgery
  - intramedullary femoral canal
  - insertion via the intercondylar groove of the knee
  - one side: sol gel / vancomycin coated (12 implants)
  - other side: sol gel coated controls (12 implants)
In vivo bactericidal activity

- At time of surgery
  inoculation with a suspension of S. aureus
  (150 μl of $10^4$ cfu/ml)
- Sacrifice
  » 1, 2, 3 and 4 weeks
- Evaluation
  » cultures on retrieved IM nails
  » radiographic
In vivo bacterial inhibition – ex vivo cultures

- Number of S. aureus colonies cultured from bacteria, if any, present on IM nails in vivo harvested after 1 week and 2 weeks

- In comparison to control: sol gel / vancomycin film is bactericidal and inhibits bacterial adhesion
**In vivo** infection inhibition  
4 weeks of implantation

- Sol-gel/vancomycin film (top)
  - inhibits infection
- Infection on control side (bottom)
  - evidenced by
    - change in size
    - periosteal reaction (arrow)
    - lytic lesions & bone abscesses (*)
    - extensive bone remodeling

*S. Radin et al., Key Engr. Mater. 2006; C. Adams et al., J. Orthop. Res. 2009*
For animals (sheep) inoculated with $10^6$ or $10^8$ CFU *S. aureus*, the X-ray (top) and micro-CT (bottom) results show that SGV coating (left) but not SG coating (right) inhibits osteomyelitis (arrows) one month post-implantation.
Local tissue vancomycin concentration

- The local vancomycin concentrations exceed MIC 24 hours after implantation;
- The local vancomycin concentrations exceed MIC for 16 days, the longest time point measured;
- The plasma vancomycin concentrations are below the detection limit (50ng/ml).
External fracture fixation
A major health care issue

- 2,000,000 fracture fixation devices implanted annually in the USA
  » Overall postoperative infection rate 5%
- External fixation
  » Overall incidence of deep infection 16.2%
  » Overall chronic osteomyelitis rate 4.2%
  » Infection of femoral fractures, up to 32.2%
External fracture fixation
Constant release (zero order) over 3 months
*In vitro* - triclosan

Cumulative Irgasan release from coatings on grit blasted 4-mm 316L fixator pins (%)

- Triclosan (size: 289.55 Da)
  - Powerful antibacterial agent extensively used in hospitals
  - Disables the activity of the enzyme ENR vital in building cell membranes of many bacteria
  - Insoluble in water but soluble in ethanol

```
C_{12}H_{7}Cl_{3}O_{2}
```

*H. Qu, C. Knabe, et al., (2015)*
**In vitro** bactericidal efficacy

- Pins inoculated with *Staphylococcus aureus*
  - $1 \times 10^5$ cfu (first), $1 \times 10^8$ cfu (next)
  - cultured at 37°C, 24h
  - bacteria count

- Samples:
  - Controls: uncoated pins
  - Sol gel/triclosan coated 4-mm fixator pins
**In vitro** bactericidal efficacy

Number of S. aureus colonies compared to uncoated controls
(initial time point)

- sol-gel/ 10% triclosan coated pins prevented bacterial colonization - reduction by the maximum - 5 orders of magnitude
*In vitro* bactericidal efficacy

Number of *S. aureus* colonies compared to uncoated controls

(initial time point)

Sol-gel/20% triclosan coated pins reduced bacterial colonization - reduction by 7 orders of magnitude
In vitro bactericidal efficacy
Number of S. aureus colonies compared to uncoated controls (up to 4 weeks of elution)

Sol-gel/triclosan coated pins retained bactericidal effect over 4 weeks -
reduction by 5 orders of magnitude after 1 week
3 orders of magnitude after 4 weeks

Percutaneous pins – *in vivo*
peroperative photograph

rabbits
distal tibia
inoculation with *S. Aureus*
## Infection rates percutaneous pins – *in vivo*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Animals for evaluation</th>
<th>Animals with clinical signs of infection*</th>
<th>Animals with radiographic signs of infection†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1wk)</td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Sol-gel (1wk)</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control (2wk)</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Sol-gel (2wk)</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control (4wk)</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Sol-gel (4wk)</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* animal showing either of these signs at the time of sacrifice was checked as infected: serious discharge, superficial cellulitis and deep infection.
† a radiograph revealing osteomyelitis at the time of sacrifice is graded as infected.
Percutaneous pins – *in vivo*

Pocket depth

- **Sol-gel**
- **Control**

<table>
<thead>
<tr>
<th>Week</th>
<th>Pocket depth (mm)</th>
</tr>
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<tbody>
<tr>
<td>1wk</td>
<td>4.5 (4.0, 5.0)</td>
</tr>
<tr>
<td>2wk</td>
<td>4.5 (4.0, 5.0)</td>
</tr>
<tr>
<td>4wk</td>
<td>4.5 (4.0, 5.0)</td>
</tr>
</tbody>
</table>

*H. Qu, C. Knabe, et al., (2015)*
Control (A and C) and sol-gel coated (B and D) implants. Control implant (A) shows extensive epithelial downgrowth (green arrows) and bacterial colonization and infiltration of S. aureus in the subcutaneous tissue (C, white arrows). Coated implant (B & D) shows excellent bone integration of the pin (orange arrows) and positive osteocalcin expression (white arrow), extensive new woven bone formation (red arrows), excellent attachment of the subcutaneous tissue to the implant surface (yellow arrows) as well as epithelial attachment (green arrows).
Benefits of silica xerogels (sol-gels), cont..

- molecules are protected while in the sol gel (compare to the in vivo half life – e.g PDGF: 2 minutes)
- various molecules can be delivered simultaneously
- substantially full release of therapeutic agents
- resorbable

- biocompatible
  - room temperature processing

Nicoll et al. (1998), Falaize et al. (1998), Santos et al. (1999), Radin et al. (2002)
In vivo biocompatibility study

• New Zealand white rabbits
• Experimental groups:
  » sol gel discs (8 mm in diameter, 2 mm thick) subcutaneously in the back
  » sol gel granules (710 - 1000 μm) defects in the iliac crest
    (5 mm in diameter, 2 mm deep)
  » controls: sham surgery (defects without material)
• Implantation time: 2 & 4 weeks
Resorption –
Mean granule size vs implantation time

Mean granule size vs. implantation time

Gradual reduction in granule size reflects time-dependent granule resorption

Minimal inflammatory response

Radin et al. (2002)
Methods – animal model (*)

- 1500 mg of resorbable glass granules (300-355 μm) were implanted into the paraspinal muscle of 4 kg NZW rabbits for 24 weeks
- Clinically relevant dose of 30 cc for a 70 kg human (e.g. large compression fracture in the proximal tibia)
- Muscle implantation site heals faster than bone and possibly leads to an enhanced resorption rate in comparison to bone

(*) One of two models
Other model: bone implantation site
Silicon Excretion in Urine

![Graph showing silicon excretion rate over time for sham and implant groups.](image)

- **Y-axis:** Urinary Si Excretion Rate (mg/day)
- **X-axis:** Time (weeks)
- **Legend:**
  - Sham
  - Implant

- **Label:** n = 7

The graph illustrates the decrease in urinary silicon excretion rate over time for both sham and implant groups. The excretion rate decreases as time progresses.
Results - creatinine

- Creatinine: natural metabolite found in urine. Its production is proportional to total muscle mass. Excretion rate reflects kidney function.
- Excretion rates remained normal and stable for both implanted and sham groups. Kidney function was normal throughout experiment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Creatinine (mg/day/kg)</th>
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<tbody>
<tr>
<td>sham</td>
<td>38.3±5.1</td>
</tr>
<tr>
<td>implant</td>
<td>36.8±6.5</td>
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</table>
Discussion

- Increased urinary Si supports the hypothesis that the silica gel from the granules is dissolved into the bloodstream and removed by the kidney.
- The Si release product in the blood must be small enough to pass through kidney filtration (18 Å). *(Berne and Levy, Physiology, 1988)*
- The concentrations of silicon in the urine were well below saturation.
- Elevated Si was not found in any of the major organs. This corroborates findings of previous histological analyses in other glass granule implant studies revealing no pathological changes in kidney, liver or spleen.
- Creatinine excretion rates were similar between implanted and sham rabbits. Therefore, kidney function was not adversely affected by the surgical procedure and the dose of granules.
<table>
<thead>
<tr>
<th>Category</th>
<th>Concept stage</th>
<th>In vitro feasibility testing</th>
<th>In vivo proof of concept demonstrated</th>
<th>In vivo efficacy model 1 demonstrated</th>
<th>In vivo efficacy model 2 demonstrated</th>
<th>Clinical</th>
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<td><strong>Controlled release of biological molecules (BM)</strong></td>
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<td>Controlled release of BMP-2</td>
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<td>Controlled release of TGF-β</td>
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<td>Controlled release of monoclonal antibodies</td>
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<td>Treatment of compartment syndrome</td>
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<td><strong>Delivery of anti-proliferative drugs</strong></td>
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<td>Controlled release of antiproliferative agents (Rapamycin)</td>
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<td>Thin films for drug eluting stents</td>
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<td><strong>Combination treatments</strong></td>
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<td>Combined delivery of antibiotics and growth factors for the treatment of oral pathologies (I, BM)</td>
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<td>Wound dressings for pain and infection control (I, P)</td>
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<td><strong>Scaffolds for tissue engineering</strong></td>
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<td>Large volume bone tissue engineering</td>
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Background

• Methicillin resistant staphylococcus aureus infection has significantly increased 
  (Matar et al. (2010), Rivera et al. (2011))
  » From 1999 through 2005, infections outside the lungs or blood tripled
  » MRSA strains account for >50% of all S. aureus strains causing clinical disease

• Surgical site infection (SSI)
  » Treating SSI is complicated by how bacteria colonize implants
  » Bacteria adhere to implant surface and form a **biofilm**
  » High resistance of the biofilm to systemic antibiotic treatment
Farnesol as an adjuvant to vancomycin

- Natural quorum-sensing alcohol molecule
- Antimicrobial activity against *S. aureus* biofilms and synergistic with vancomycin (Gomes et al. (2011))
- It probably damages the cell membrane and impairs ergosterol synthesis
- Insoluble in water

Water insolubility compromises the **bio-availability** of farnesol along with vancomycin at the **site of infection** *in vivo*

**Objective:**
To design an efficient therapeutic strategy which can deliver both antibiotic and an adjuvant **simultaneously** at the **infection site** in controlled fashion
**In vitro** bactericidal effect (MRSA)

- Film with 10 wt% and 20 wt% vancomycin can reduce the MRSA growth by 10 and $10^2$ fold.

- When 10 wt% vancomycin combined with 30 wt% farnesol MRSA growth reduced by $10^3$ fold.

- When 20 wt% vancomycin is combined with 30 wt% farnesol, MRSA growth is completely suppressed (~$10^6$-fold reduction compared to control).
Working mechanism

- Farnesol penetrates the biofilm and gains access to the bacterial cell
- Farnesol accumulates in the cell membrane, increases the permeability and porosity of the biofilm and of the bacterial cell membrane
- Allows more vancomycin to enter the bacterial cell with greater ease. Vancomycin binds to the cell wall where its normal mode of action results in cell death
Health care cost > $1,000,000,000
Prevention of infection ~ $1,000,000,000
Annual medical cost [NIH, CDC, IoM] ~ $5,000,000,000
Hospital stay cost: ~ 20% less
Global surgical pain control market ~ $6,000,000,000
An amputation every 30 seconds
Mergers - a business term
Business models

- VW
- Mercedes
Evert Schepers, KU Leuven, Belgium
David Kohn, U Michigan
Kevin Healy, UC Berkeley
Tim Topoleski, U Maryland
Elsie Effah, U Ghana, Accra, Ghana
Charles Cohen, Gentis, Inc.
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Helen Lu, Columbia U
Jun Yao, Morphotek Inc.
Rong Chen, Sichuan U, Chengdu

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Qing-Qing Qiu, RTI Biologics
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Shula Radin
Haibo Qu
Sanjib Bhattacharyya

Christine Knabe
*Philipps University, Marburg, Germany*
thoroughbred Ferrari

Thank you