Biological responses of cells and tissues to biomaterials
AO Foundation

- Founded in 1958
- Medically guided, global network of surgeons
- World’s leading educational and research organisation for trauma and musculoskeletal treatment
- With more than 10,000 surgeons, in more than 100 countries, it is one of the most important and extensive networks in medicine
- Global knowledge network—interdisciplinary teamwork
- International faculty of over 3,000 experts
Outline

- Lexicon
- Classification of biomaterials
- Cell-material interactions
- Tissue-material interactions
- Examples of cell and tissue interactions with:
  - Ceramics
  - Metals
  - Polymers
- Summary
- Future areas of research
Lexicon

- Biomaterial
- Implant
- Primary and secondary stability
- Osseointegration
- Bone to implant contact
- Fibrous capsule
- Inflammation (acute and chronic)
- Osteoblast
- Stem cell
Biomaterial: evolution of the definition

- **Williams 1987**: “A biomaterial in a nonviable material used in a medical device, intended to interact with biological systems”

- **Williams 1999**: “Biocompatibility is the ability of a material to perform with an appropriate host response in a specific situation”

- **NIH**: “Biomaterial is any substance or combination of substances, other than drugs, synthetic or natural in origin, which can be used for any period of time, which augments or replaces partially or totally any tissue, organ or function of the body, in order to maintain or improve the quality of life of the individual”
Application of biomaterials

dental implant

ocular prosthesis

ear replacement

maxillofacial implant

cardiac valve

hip prosthesis

cartilage replacement

knee replacement

synthetic skin

mesh for hernia repair

tendon prosthesis

catheter drain

internal fixator

mammary prosthesis

vascular prosthesis

tube for neural regeneration

dental implant
Classification of biomaterials

**Composition**
- Metals & alloys → **Ss**, Co-Cr, **Ti**, **Ti-6Al-4V**, Ni-Ti, Mg
- Polymers → PMMA, PLA, PGA, PE, PEEK
- Ceramics & glasses → **Al₂O₃**, **ZrO₂**, CaP, BAG
- Composites → bone (unprocessed), BCP-PCL, BAG-PLA, PU-HA

**Structure**
- Bulk → **implants (stems, plates, screws)**
- Porous → scaffolds
- Surface → **topography (macro, micro, nano)**, bioactive coating on «bioinert» material

**Source**
- Natural → bone grafts, hyaluronic acid, fibrin, collagen, chitosan, cellulose
- Synthetic → PCL, PMMA, PEEK

**Response**
- Toxic
  - «Bioinert» → **Al₂O₃**, **ZrO₂**
- Bioactive → osteoconductive, osteoinductive → HA, TCP, BCP, BAG
- Bioresorbable → BCP, PCL, PU, PLA, PGA, Mg

**Function**
- Temporary → non biodegradable → **temporary implants (polished metals)**
- Temporary → biodegradable → maxillofacial screws
- Permanent → hip prostheses, spine cages
Which cells?
which species?
which location?
healthy or diseased?
primary or cell line?
how many donors?

- fibroblasts
- progenitor cells (stem cells)
- bone cells (osteoblasts, osteocytes, osteoclasts)
- macrophages
Which tissues?
which species?
which location?
healthy or diseased?

soft tissue
- tendon
- muscle
- skin

hard tissue
- bone

vascularised tissue
- bone

avascular tissue
- intervertebral disc

mechanically-loaded tissue

unloaded tissue
Which response?

Focus: bone

- cytocompatibility (in vitro)
- biocompatibility (in vivo)
- cell proliferation (metabolic assays, DNA)
- cell morphology (microscopy)
- gene expression (runx2, alkaline phosphatase, osteocalcin)
- protein expression (collagen type I, alkaline phosphatase, osteocalcin)
- tissue mechanics (micro-indentation)
- tissue structure (histology)
- mineral deposition (staining for Ca and P)
Biomaterial characteristics?

- Stability
- Sterilisation
- Reproducibility
  - Minimise material variability
- Cytocompatibility
- Shape & dimensions
- Porosity
  - Macro and micro
- Surface
  - Chemistry
  - Topography
  - Wettability
- Goal
- Number of samples
Surfaces ... what do you see?
Surfaces... what do cells see?
• Rough & smooth topography (micro/nano range)
Cell-material interactions

Interactions levels

Nano- Micro- Macro-

<100nm

1-300µm

>300µm

Protein

Cell

Tissue

Organ

Water – surface interaction

Proteins adsorption on surface

Cell attachment

Proliferation/migration

Differentiation/spreading

Maturation

Implantation/integration to tissue

$ t = 0$

ns

s

hrs

days
Molecular level events at implant surface

- **Chemistry** – determines the types of intermolecular forces, governing interaction with proteins.
- **Hydrophobicity** – hydrophobic surfaces often bind protein more strongly (can limit cell adhesion).
- **Heterogeneity** – surface non-uniformity, domains interact differently with proteins.
- **Potential** – influences ion distribution & interaction with proteins (dependant upon topography / chemistry).
- **Topography** – greater texture exposes discontinuities for interaction with proteins.
Cell-material interactions

Surface Chemistry
- Charge
- Hydrophobicity / Hydrophilicity
- Ligand binding

Topography
- Nano / Micro

Mechanical
- Stimulation
- e.g. Bulk Properties

3-D Structure

Adhesion

Morphology

ECM Synthesis

Proliferation

Migration

Phenotype

Apoptosis / Maturation

Tissue-material interactions

Gittens RA. *Acta Biomaterialia* 2014
Implant osseointegration and the role of microroughness and nanostructures:
Lessons for spine implants
Outcome of acute inflammation

ACUTE INFLAMMATION
- Vascular changes
- Neutrophil recruitment
- Mediators

RESOLUTION
- Clearance of injurious stimuli
- Clearance of mediators and acute inflammatory cells
- Replacement of injured cells
- Normal function

INJURY
- Infarction
- Bacterial infections
- Toxins
- Trauma

Progression

CHRONIC INFLAMMATION
- Angiogenesis
- Mononuclear cell infiltrate
- Fibrosis (scar)

FIBROSIS
- Loss of function

Adapted from Kumar V, Abbas AK, Aster JC eds. Robbins Basic Pathology 9th Ed. 2013
Surface roughness
Surface roughness

Gittens RA. *Acta Biomaterialia* 2014
Implant osseointegration and the role of microroughness and nanostructures: Lessons for spine implants
Ways to obtain surface roughness

Gittens RA. *Acta Biomaterialia* 2014
Implant osseointegration and the role of microroughness and nanostructures: Lessons for spine implants
Examples: “bioinert” ceramics
Synergic effect of micro & nanoroughness

MC3T3-E1 (murine cell line)

Wettability

Cell proliferation

ALP activity

Ito H. Dent Mater J 2013
Response of osteblast-like cells to zirconia with different surface topography

MS: mirror-polished, SB50: sand-blasted 50 µm
SB150: sand-blasted 150 µm, E: etched
Synergic effect of micro & nanoroughness

Hempel U. *Clin Oral Implant Res* 2009
Response of osteblast-like SAOS-2 cells to zirconia ceramics with different surface topographies
Synergic effect of micro & nanoroughness

As sintered

Ra = 0.13µm

Ra = 0.77µm

ALP/DNA (nmols/min/ug)

Time

DNA content (ug)

Day 1 Day 10 Day 20 Day 30

Time

Day 1 Day 10 Day 20 Day 30

ALP/DNA (nmols/min/ug)

Time

Day 1 Day 10 Day 20 Day 30

ALP/DNA (nmols/min/ug)

Time

Day 1 Day 10 Day 20 Day 30

ALP/DNA (nmols/min/ug)

Time
Zirconia vs. Ti in vivo

Depprich R. *Head & Face Medicine* 2008
Osseointegration of zirconia implants compared with titanium: an in vivo study.
Roualdes O. *Biomaterials* **2010**

*In vitro* and *in vivo* evaluation of an alumina-zirconia composite for arthroplasty applications.
Examples: “bioactive” ceramics
Biphasic calcium phosphates

In vitro

In vivo

Yuan H. *PNAS* 2010

Osteoinductive ceramics as a synthetic alternative to autologous bone grafting.
Biphasic calcium phosphates

Yuan H. *PNAS* 2010
Osteoinductive ceramics as a synthetic alternative to autologous bone grafting.
Biphasic calcium phosphates

212-300 µm

106-212 µm

45-106 µm

<45 µm

Effect of particle size on osteoinductive potential of microstructured biphasic calcium phosphate ceramic.
Biphasic calcium phosphates

“Cut-off” ~ 50 µm particle size/porosity

Vascularisation → nutrients and mesenchymal stem cell infiltration

Micropores are a pre-requisite for inductive bone formation → accumulation of growth factors

Particle-size mediated inflammation (initial stimulation and further protease/anti-protease balance)

Compared to previous studies (blocks instead of particles): earlier mineralisation (~half time)

Resorption: TCP prepared from calcium-deficient apatite did not resorb after 2.5 years of implantation


Effect of particle size on osteoinductive potential of microstructured biphasic calcium phosphate ceramic.
Examples: metals
Soft tissue reaction to metal surfaces: polished versus rough
• 1 in 6 fractures are distal radius fractures

• Tendons in contact with the implant may incur a cellular reaction, tendon adhesions, limited palmar flexion & rupture.

• Tendon damage & rupture more common with Ti & Ti alloy implants, compared to steel of similar design. (Sinicropi, M.S et al., 2001)

• Why?
**In vitro fibroblast cell behaviour**

Surface microtopography can control cell growth, spreading & behaviour.
Soft tissue reaction - cpTi surfaces

In vivo evaluation of defined polished titanium surfaces to prevent soft tissue adhesion.
Bone tissue reaction to metal surfaces: polished versus rough
Surface microtopography & osteoblast shape

TAN

**Cuboid shape**

**Fibroblast-like shape**

NES,TAN electropolished
NP-TAN polished
NS-TAN standard
Ss-Stainless steel

**Relative Fold Change in Osteocalcin Gene Expression at 21d on TAN**

Hayes JS *Eur Cell Mater* 2010.
The role of surface microtopography in the modulation of osteoblast differentiation.
Another example: cpTi

Labelling for cytoskeletal components. Red: actin, green: tubulin

Hayes JS Exp Reviews 2010
Surfaces to control tissue adhesion for osteosynthesis with metal implants.
Effect of surface on screw removal

3 biomaterials:

- Stainless steel (ISO 5832-1),
- Commercially pure titanium (cpTi; ISO 5832-2)
- Titanium alloy: Titanium-6%Aluminium-7%Niobium (TAN; ISO 5832-11)

5 surface treatments:

- SS - polished stainless steel
- TS - microrough Ti
- NS - microrough TAN,
- TE - electropolished Ti
- NE - electropolished TAN
Effect of surface on screw removal

Polishing significantly reduces the torque required for screw removal in both cancellous & cortical bone

<table>
<thead>
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<th>6 weeks</th>
<th>12 weeks</th>
<th>18 weeks</th>
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<tbody>
<tr>
<td><strong>Mean Peak Removal Torque - Rib</strong></td>
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<tr>
<td>SS-polished stainless steel</td>
<td></td>
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<td>TS-microrough Ti</td>
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<tbody>
<tr>
<td><strong>Mean Peak Removal Torque - Tibia</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SS-polished stainless steel</td>
<td></td>
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Biological reaction to bone – cpTi / TAN

Direct osseointegration

Courtesy of Geoff Richards
Biological reaction to bone - EPSS

Fibro-osseointegration
No issues with stability!
Smooth versus rough surface

18 months in sheep tibia

Hayes JS *Exp Reviews* 2010
Surfaces to control tissue adhesion for osteosynthesis with metal implants.
Smooth versus rough surface

Adapted from Hayes JS *Exp Reviews* 2010
Surfaces to control tissue adhesion for osteosynthesis with metal implants.
Biological reaction to bone - TAN

Difficult to remove IM nails, especially in young patients

IM Nail - 12 mo implantation

0.758 µm
Biological reaction to bone – TAN (polished)

IM Nail - 12 mo implantation

Courtesy of Geoff Richards
An in vivo evaluation of surface polishing of TAN IM nails for ease of removal.

Hayes JS *Eur Cell Mater* 2009
Effect of polishing

12 months, sheep tibia

IM: intramedullary; TAN: titanium-6% aluminium-7% niobium (wt%)

Hayes JS Exp Reviews 2010
Surfaces to control tissue adhesion for osteosynthesis with metal implants.
The effective roughness spectrum

Adapted from Hayes JS *Exp Reviews* 2010
Surfaces to control tissue adhesion for osteosynthesis with metal implants.

0.2 - 2 µm
Infection rates - surfaces

<table>
<thead>
<tr>
<th>LCP Type</th>
<th>n</th>
<th>Rate of Infection (%)</th>
<th>ID$_{50}$ (CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polished TAN</td>
<td>22</td>
<td>45</td>
<td>7.1 x 10$^6$</td>
</tr>
<tr>
<td>Standard TAN</td>
<td>21</td>
<td>38</td>
<td>6.3 x 10$^6$</td>
</tr>
<tr>
<td>Standard Ti</td>
<td>19</td>
<td>42</td>
<td>3.9 x 10$^6$</td>
</tr>
<tr>
<td>EPSS</td>
<td>22</td>
<td>54</td>
<td>3.2 x 10$^6$</td>
</tr>
<tr>
<td>Polished Ti</td>
<td>20</td>
<td>50</td>
<td>2.7 x 10$^6$</td>
</tr>
</tbody>
</table>

In a stable locking IF plate system **no large differences found bet materials** (cpTi, TAN, EPSS) **or surface roughness** for infection susceptibility **in vivo** **(without fracture or major tissue trauma)**

Moriarty TF. *Int J Artif Organs* 2009

Influence of material and microtopography on the development of local infection **in vivo**
Examples: polymers
Plasma-modified PEEK: in vitro
Survey of cell adhesion to materials (hydrophobicity on very smooth surface)

- Cells attach best to surfaces that are neither too hydrophobic or too hydrophilic.
- Manufacturing & contamination moves these boundaries.

Adapted graph courtesy (Harbers, G. M., & Grainger, D. W.)
Surface wettability of plasma treated PEEK

Untreated
~83º

Plasma Treated for 600s
~60º
XPS surface analysis of oxygen incorporation as a function of plasma treatment time

![Graph showing oxygen incorporation over plasma treatment time with untreated samples](image)

Repeating monomer

Long-term stability of surface treatment

![Graph showing surface oxygen concentration over time for 600s plasma treated and untreated samples.](image)

**600s plasma treated**

**Untreated**

Time after Surface Treatment [Months]

Surface Oxygen [atom %]

AFM of evaluation of surface topography

Plasma surface modification

- Unmodified
  - ~83°
- Modified
  - ~60°

Inj. Moulded PEEK

cpTi
Cell proliferation on PEEK

Data from 5 independent femoral heads, ± st. dev. GLM ANOVA with Tukey post-hoc, significance P<0.05

Surfaces to control tissue adhesion for osteosynthesis with metal implants.
Alkaline phosphatase activity on PEEK.

Surfaces to control tissue adhesion for osteosynthesis with metal implants.
Gene expression profile of HOB on PEEK

Osteonectin

Relative fold change

Time [Days]

Surfaces to control tissue adhesion for osteosynthesis with metal implants.
Nodule formation on PEEK

Surfaces to control tissue adhesion for osteosynthesis with metal implants.
Conclusions: *in vitro* study

- Oxygen plasma treatment has increased the **surface energy** of PEEK substrates.
- Surface treatment is **stable** for 26 months in air (also > 18 months in PBS at 37ºC).
- **Optimal levels** of surface treatment have been identified for HOB cells.
- **ALP expression** is more characteristic for hOB cells on the treated surfaces.
- **Nodule formation** was higher from day 7 on all treated surfaces compared to untreated PEEK.
- The influence of these surfaces on hOB cell **gene expression** indicates that the differentiation is up-regulated at earlier time points.

These *in vitro* findings indicate that this surface modification is likely to improve bone integration to PEEK implants.
Plasma-modified PEEK: 
in vivo
### Materials & methods – in vivo

#### Groups
- Machined PEEK Implant: PA
- Injection Moulded PEEK Implant: PO
- Plasma modified Machined PEEK Implant: PAm
- Plasma modified Injection Moulded PEEK Implant: POm

#### Ovine Model
- 24 Swiss Alpine Sheep
- Female, 60-65kg, 3-4yrs
- Cancellous bone of the proximal tibia and distal femur
- Cortical bone of the tibiae
- Time-points: 4, 12 and 26 weeks, 8 per time-point

#### Characterisations
- Surface analyses: XPS, WLP, AFM and WCA.
- In vivo analysis: Radiographs, Fluorochrome labelling
- Explant analyses: Radiographs, Mechanical push-out testing, histology and histomorphometry
Preclinical study

Schematic of the bilateral model implantation areas in the tibiae and femurs, where the implant sites are annotated and division between histology and mechanical testing is shown.

Custom made jig with k-wires

All 4 implants in place in the tibial diaphysis with 2 marker screws on either side
Push-out force

![Graphs showing force vs. displacement and force vs. weeks for cancellous and cortical bone]

AO Foundation
PEEK in vivo: new bone formation

Proximal tibia (cancellous bone). 4 weeks after implantation

Pink: bone, blue: soft tissue, white: bone marrow.

Giemsa-eosin

Intravital calcein green and xylenol orange

PA - machined PEEK, PAm- modified machined PEEK, PO- moulded PEEK, POm- modified moulded PEEK

Poulssohn AHC Biomaterials 2014

Osseointegration of machined, injection moulded and oxygen plasma modified PEEK implants in a sheep model.
**PEEK in vivo: new bone formation**

Tibial diaphysis (cortical bone). 4 weeks after implantation

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Poulsson AHC *Biomaterials* 2014
Osseointegration of machined, injection moulded and oxygen plasma modified PEEK implants in a sheep model.
Conclusions: *in vivo* study

- Limited inflammatory response for all materials
- Good osseointegration of all materials
- Micro-roughness (machining) has a significant influence on bone-to-implant contact and push-out force
- Oxygen plasma induced an improved osseointegration and implant stability at early time point in cancellous bone

(from *in vitro*  → *to in vivo* → *to the patient?* → *which patient?*)
Summary
Summary

Which cell?
- fibroblasts
- progenitor cells
- bone cells
- macrophages

Which tissue?
- soft tissue
- hard tissue
- vascularised tissue
- avascular tissue
- mechanically-loaded tissue
- unloaded tissue

Which response?
- cytocompatibility
- biocompatibility
- cell proliferation
- cell morphology
- gene expression
- protein expression
- tissue mechanics
- tissue structure
- mineral deposition

Biomaterial
- stability
- sterilisation
- reproducibility
- cytocompatibility
- porosity
- surface
- shape & dimensions
- number of samples

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Biomaterial
- stability
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- shape & dimensions
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Summary

- Definition of goal/research question is fundamental
- Experimental design is the next key step

The Importance of Experimental Design

Let's see if the subject responds to magnetic stimuli... ADMINISTER THE MAGNET!

Interesting...there seems to be a significant decrease in heart rate. The fish must sense the magnetic field.

http://www.hawaii.edu/fishlab/NearsideFrame.htm
What else?

- Gene level and protein level
- Short term vs. long term cultures
- In vivo veritas?
- How comparable are different studies?
- How important are the controls, the blanks (e.g. materials cultured in the same conditions but without cells) and the artifacts!
- Be critical:
  
  statistically significant difference 
  may be ≠
  biologically significant difference
- Controversies:
  - do not look only at one paper
  - high impact journal in the field is important
Future areas of research
Surfaces: What to mimic?
Swiss Mountain Mimetics

Courtesy of Geoff Richards
Future lines of research

• Advanced materials:
  o surface patterning
  o gradient materials
  o 3D printing

• More predictable in vitro tests

• Application of 3R principle to *in vivo* tests: https://www.nc3rs.org.uk/the-3rs

  ![Image of 3R principles]

  - Replacement: Methods which avoid or replace the use of animals
  - Reduction: Methods which minimise the number of animals used per experiment
  - Refinement: Methods which minimise suffering and improve animal welfare

• As complete documentation as possible, especially for *in vivo*

• Bridge the gap between *in vitro* and *in vivo* with *ex-vivo* models
Surface patterning

Yao X. *Advanced Materials* **2013**
Cell-material interactions revealed via techniques of surface patterning.

Peng R. *Biomaterials* **2012**
Gradient materials

Kunzler TP. *Biomaterials* 2007

Cell response of osteoblasts and fibroblasts to surface roughness was studied by means of gradient substrata with a continuously varying roughness value and similar topographical features.

Osteoblasts prefer the rougher part whereas fibroblasts favored the smoother part of the roughness gradient.

**Cell-material interactions are cell-type specific**

Michelmore A. *J Nanomater* 2012

Cells sense chemical gradients
Ex-vivo bone culture in a bioreactor

• Osteoarthritic human femoral heads (total hip replacement)

Viability of bone cores after 2 week culture

[C.M. Davies et al. (2006)]

[Lactate dehydrogenase][Calcein AM]

[M.J. Stoddart et al. (2006)]
Acknowledgements

Prof. Geoff Richards (Director AO Research Institute Davos)
Prof. Mauro Alini (vice-director AO Research Institute Davos)
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Dr. Alexandra Poulsson (post-doc AO Research Institute Davos)
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Acknowledgements
eCM conferences

www.ecmjournal.org

**eCM Next Events**

**2015 eCM XVI: Implant Infection (Orthopaedic & Musculoskeletal Trauma related)**  
24th - 26th June 2015, Congress Center, Davos, Switzerland

**2016 eCM XVII: Stem cells, Bone Fixation, Repair & Regeneration**  
20th – 23rd June 2016, Congress Center, Davos, Switzerland

**2017 TERMIS-EU Conference** (no eCM in 2017) **TERMIS-EU**  
26th-30th June 2017 Congress Center, Davos, Switzerland.  
Conference Chair: Prof. R. Geoff Richards, PhD Conference  
Program Chair: Prof. Mauro Alini, PhD

**2018 eCM XVIII: Cartilage & Disc: Repair and Regeneration**  
25th - 28th June 2018, Congress Center, Davos, Switzerland
Open Access, online only, preclinical research in musculoskeletal field.

Official Research Journal of:
AOCMF, AOTRAUMA,
European Orthopedic Research Society (EORS),
Swiss Society for Biomaterials (SSB),
Tissue & Cell Engineering Society (TCES)

5-year Impact Factor 2013 - 5.991
Yearly Impact Factor: 2013 - 4.887
Thank you for your attention!

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