The Concept of Biocompatibility

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Agenda

1. The failure of biocompatibility testing exemplified with metal-on-metal articulation
2. Definition of Biomaterials and Biocompatibility
3. Establishing Biocompatibility according to the risk management process as described in ISO 10993
4. Why does academia fails to translate their results to products?

Aim:

Don’t misuse anymore the term biocompatibility in the future
MoM Implants: The Promises

Metal-on-Metal (MoM) hip implants consist of a ball, stem and shell, all made of metal materials. MoM hip implants were designed to offer the following benefits:

- Less device material wear is generated when the ball and socket rub against each other in comparison to other hip implants
- Decreased chance of dislocation when the ball of the thighbone (femur) slips out of its socket in the hip bone (pelvis)
- Decreased chance of device fracture

There are two types of MoM hip implants:

- Traditional total hip replacement systems
- Resurfacing hip systems

https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/ImplantsandProsthetics/MetalonMetalHipImplants/default.htm
MoM Implants: The Statistics

Survival Rate of Hip implants:

• Data from the Australian and United Kingdom Orthopedic device registries (the largest of its kind), indicate that approximately **95 percent** of patients with any kind of total hip replacement have not undergone revision surgery for **seven years** after the initial implantation.

• More than **85 percent** of patients with MoM total hip replacements from the U.K registry and more than **92 percent** of patients with MoM total hip replacements from the Australian registry did not have a revision for **seven years** after the initial implantation.
MoM Implants: The Facts

- Metal release will cause some tiny metal particles to wear off of the device into the space around the implant.
- Wear and corrosion at the connection between the metal ball and taper of the stem may also occur.

<table>
<thead>
<tr>
<th>Orthopedic Status</th>
<th>[Serum]</th>
<th>[Blood]</th>
<th>[Synovium]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unexposed:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co (ng/mL):</td>
<td>&lt;0.9</td>
<td>&lt;1.8</td>
<td>(&lt;0.9 est)</td>
</tr>
<tr>
<td>Cr (ng/mL):</td>
<td>&lt;0.9</td>
<td>&lt;2.0</td>
<td>(&lt;0.9 est)</td>
</tr>
<tr>
<td>Unaffected Implants:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co (ng/mL):</td>
<td>4-10</td>
<td>&lt;40</td>
<td>13-770</td>
</tr>
<tr>
<td>Cr (ng/mL):</td>
<td>1-20</td>
<td>&lt;2</td>
<td>180-550</td>
</tr>
<tr>
<td>Affected Implants:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co (ng/mL):</td>
<td>5-70</td>
<td>&gt;100</td>
<td>110-5,120</td>
</tr>
<tr>
<td>Cr (ng/mL):</td>
<td>10-90</td>
<td>&gt;100</td>
<td>155-29,000</td>
</tr>
</tbody>
</table>

Cobalt and Chromium ions are considered (> 1-5 ng/mL):
- cytotoxic, acute toxic, genotoxic, carcinogenic

• Some of the metal ions (e.g. cobalt and chromium) from the metal implant or from the metal particles will enter the bloodstream.
MoM Implants: The Consequences

Concerns:

• MoM are recalled from the market by all major manufactures
• There are lawsuits to be settled with claims in the billions
• There huge cost in the future for the public health care
**MoM: Why didn’t we realize it before?**

- Low wear in MoM bearings had been defined as a wear rate of < 1 mm$^3$ per million cycles associated with a combined serum metal ion level of < 10 ppb / metal ion level of < 5 ppb in vivo.

- Due to the design of the devices, they are very difficult to place correctly and translational malposition is very frequently and higher wear occurs.

- Due to the wear, the CrOx layer is destroyed and corrosion of the metals, i.e. of Co occurs.

- New designs may solve the problems, but the risk of intoxication upon higher wear remains and not company will take that risk for the years to come.

- Testing schemes are required to account for worst-case situations.

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What is a Biomaterial?

Definition of Biomaterial:
What are Medical Devices?

A material implanted for **restoring functions**:
What are Medical Devices?

A material with **active functions:**
What are Medical Devices?

A material implanted “unintentionally”: No
What are Medical Devices?

A material implanted for cosmetically or commercial functions: No*
What are Medical Devices?

Materials that may fail or elicit host reactions: Yes
The MDR and IVDR of 2017

The Medical Device Directive (MDD), in force since 1993, is now replaced by the Medical Device Regulation (MDR) and the In Vitro Diagnostics Regulation (IVDR) in 2017.

They are intended to harmonise the laws relating to medical devices and in vitro diagnostics within the European Union.

- They define the requirements to be met for a manufacturer how to proceed to legally place a medical device or a in vitro diagnostic product on the European market.
- Manufacturers' products that meet those requirements are considered conform to the regulation.
- The Medical Device Directive (MDR) differentiates between 4 classes of material according to their invasiveness and risk potential in application Class (I) ; Class (IIa) and (IIb) ; Class (III).
- It defines how industry has to prepare their documentation.
- Products conforming with the MDR are CE marked.
There is no such thing as a biocompatible material

*Biocompatibility subsumes a collection of individual phenomena and is impossible to quantify. There can be no scale of biocompatibility; therefore it is scientific nonsense to consider certain materials as ‘biocompatible’, occupying the ground at one end of a non-existent scale, and other materials as ‘non-biocompatible’ or ‘bioincompatible’ existing at the other end.*
What is a Biocompatibility?

Definition of Biocompatibility:

“The ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response in that specific situation, and optimizing the clinically relevant performance of that therapy” (Definition ASTM 2011)
What is a Bioactive?

Definition:

*Bioactive agents, n*
any molecular component in, on, or with the interstices of a device that is intended to elicit a desired tissue or cell response.

DISCUSSION
Growth factors, antibiotics, and antimicrobials are typical examples of bioactive agents. Device structural components or degradation byproducts that evoke limited localized bioactivity are not included.

(Definition ASTM 2011)
Questions from an Engineering Point of View

- Is it possible to engineer materials that don’t provoke unwanted host responses?
- Can we predict the host performance?
- Can we predict the harmlessness of a biomaterial?
Materials Meets Life @ the Scaffold Interface

Morphology/Design
macro/micro/nano roughness, 
2D/3D structural features, porosity

Physical
electric properties, 
crystallinity

Chemical
composition, functionalities, active molecules, water uptake

Mechanics
elastic moduli, creep, stress shielding, anisotropies

Stability
absorption/degradation, particle release, release of ions/monomers
Host Response versus a Biomaterial

Cells, Tissue, Organs respond to:

a) Inert bulk materials
b) Debris of inert bulk materials
c) Degradation products being absorbed
d) Leachable compounds from the bulk materials
e) Contamination on the surface of the materials

➔ The response depends on the cause, it may provoke a local or a systemic host reaction
➔ Any implantation is a injury of tissue and initiates a healing response in the host
The Concept of Biocompatibility

Material meets Tissue

Material  Interface  Cell/Tissue

Events

- Protein Adsorption
- Cell Adhesion
- Cell Response
- Cell-Cell Interactions
- Tissue Formation
- Tissue Remodeling

- second  minute  day  week  month  year

nm  μm  mm
## Sequence of Local Events upon Device Implantation

<table>
<thead>
<tr>
<th>Phase</th>
<th>Normal Wound Healing</th>
<th>Wound Healing as Response to Implants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injury</td>
<td>• mechanical injury/damage to vasculature /Implantation</td>
<td></td>
</tr>
<tr>
<td>Acute Inflammation</td>
<td>• Blood coagulation-clot formation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Platelet activation and degranulation</td>
<td>• Inflammation-oedema</td>
</tr>
<tr>
<td>Chronic Inflammation</td>
<td>• Removal of damaged matrix and necrotic cell components</td>
<td>• Cell proliferation and recruitment including endothelial, epithelial, stromal and inflammatory cells</td>
</tr>
<tr>
<td></td>
<td>• Cell proliferation and recruitment including endothelial, epithelial, stromal and inflammatory cells</td>
<td>• Continued removal of matrix</td>
</tr>
<tr>
<td>Regeneration and remodeling</td>
<td></td>
<td>• Angiogenesis</td>
</tr>
<tr>
<td></td>
<td>• Matris synthesis and deposition</td>
<td>• Decrease in cellularity-apoptotic pathway</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Tissue remodeling-elastin synthesis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Implantation of a medical device (normally) leads to injury of the vasculature and exposition to blood.

- Acute Inflammation
- Vascular Response
  - Clotting
  - Phagocytosis
  - Neovascularization
  - New Collagen Synthesis
- Granulation Tissue
- Tissue of Labile and Stable Cells
- Tissue of Permanent Cells
- Scarring (fibrous encapsulation; synovium)
- Chronic Inflammation
- Implant Movement
- Cellular Framework Intact
- Cellular Framework Destroyed
- Tissue Regeneration and Implant Integration
- Surgical Implantation
Possible outcomes for the implant:

a) **integration:**
   very limited occurrence in practice; close approximation of normal host tissue to the implant without an intervening capsule (e.g. implantation of pure titanium in bone)

b) **absorption:**
   if the implant is absorbed then the implant site eventually resolves to a collapsed scar or, in the case of bone, may completely disappear

c) **encapsulation:**
   the most usual response

All these outcomes maybe considered to be biocompatible
Basic Approach with Standards and Regulations

- Is it possible to engineer materials that don’t provoke unwanted host responses?
- Can we predict the host performance?
- Can we predict that the biomaterial is harmlessness in the therapy?

Standards are

- documents **developed by experts** in the field (academia/industry/authorities/notified bodies)
- internationally **recognized** by **authorities** and used by industry to fulfil regulation requirements
- **revised** regularly and **adapted** to **new insights**
- **guidance** , **test methods**, or **specifications** documents
Basic Approach with Standards and Regulations

- Is it possible to engineer materials that don’t provoke unwanted host responses?
- **Can we predict the host performance?**
- **Can we predict that the biomaterial is harmlessness in the therapy?**

Standards

- **can not cover all aspects** of all devices
- **aim** to **reduce the resulting risk** by applying standardized schemes and risk assessments
- guarantee for a **minimal quality** of devices
- may help to **compare the performance of different devices** regarding composition, design, functionality and potential risks
The ISO 10993 Series

- It a Series of more than 20 standards
- High level guidance on how to conduct a biological evaluation
- Detailed test methods for investigation of different aspects of biological safety
- Supporting guidance on materials characterisation, use of reference materials, animal welfare, and more.
- Reference to other test methods and guidances in Pharmacopoeia and national standards.

Those guidance documents have taken almost 25 years to develop.
The different Types of ISO 10993 Documents

**Test Methods (in vitro and in vivo)**
- Part 5: Cytotoxicity
- Part 10: Irritation & hypersensitivity
- Part 11: Systemic toxicity
- Part 3: Genotoxicity, carcinogenicity and reproductive toxicity
- Part 6: Implantation and local effects
- Part 4: Blood compatibility
- Part 16: Toxicokinetic study design for leachables and degradation products
- Part 20: Principles and methods for immunotoxicology testing

**Reference Materials**
- Part 8: Selection of reference materials
- Part 12: Sample preparation and reference materials

**Degradation Products**
- Part 9: Framework for Identification and quantification of degradation products
- Part 13: Identification and quantification of polymeric degradation products
- Part 14: Identification and quantification of ceramic degradation products
- Part 15: Identification and quantification of metallic degradation products
- Part 17: Establishment of allowable limits for leachables

**Sterilization Residuals**
- Part 7: Ethylene oxide sterilization residuals

**Materials Characterization**
- Part 18: Chemical characterization of materials
- Part 19: Physico-chemical, morphological and topographical characterization

**Animal Welfare**
- Part 2: Animal welfare requirements
Fundamental classification according to:

a) Intended use
b) Contact duration

Those two factors define the extend of required \textit{in vitro} and \textit{in vivo} testing.
Contact Duration:

<table>
<thead>
<tr>
<th>Differentiation</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short term contact (A)</td>
<td>&lt; 24 hours</td>
</tr>
<tr>
<td>Intermediate contact (B)</td>
<td>24h to 30 days</td>
</tr>
<tr>
<td>Long term/permanent contact (C)</td>
<td>&gt; 30 days</td>
</tr>
</tbody>
</table>
### Intended use:

<table>
<thead>
<tr>
<th>Type of contact</th>
<th>Affected tissue</th>
<th>example</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surface Contact (external)</strong></td>
<td>Skin (healthy/intact)</td>
<td>Skin electrodes, US probe, leg prosthesis</td>
</tr>
<tr>
<td></td>
<td>Mucosa (intact)</td>
<td>stomach probe, contact lenses, dental fixtures, urinary catheter</td>
</tr>
<tr>
<td></td>
<td>Breached or compromised surfaces</td>
<td>Wound bandage</td>
</tr>
<tr>
<td><strong>External communicating devices</strong></td>
<td>Blood path indirect</td>
<td>Infusion and transfusion devices</td>
</tr>
<tr>
<td></td>
<td>Tissue/bone/dentin</td>
<td>arthroscope, staples, dental fillings, wound drainage</td>
</tr>
<tr>
<td></td>
<td>Circulating blood</td>
<td>Central venous catheter, dialysis devices</td>
</tr>
<tr>
<td><strong>Implantable devices</strong></td>
<td>tissue/bone</td>
<td>Orthopedic implants, pacer makers, breast implants</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>Heart valve devices, stents</td>
</tr>
</tbody>
</table>
Testing According to ISO 10993-1

Some basic rules:

• The contact duration is summed up upon repeated contact.

• The highest / most stringed requirements apply if a device falls in different categories.

• All states have to be assessed if a medical device is transformed during its life time (e.g. upon in situ polymerization, absorption of a device).

• The properties of the medical device has to be ensured during the whole live time.

• The biocompatibility has to be tested on the final product!
Testing According to ISO 10993-1

**Important:**

- Biocompatibility testing is **very systematic**. Any **deviation** of the given scheme has to be **justified**.

- **Biocompatibility testing includes** more than only «biological tests». **Material characterization** is an important part thereof!

- Performing the test alone is not enough, a **comprehensive assessment and risk analysis** is required.

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33 The Concept of Biocompatibility
ISO 10993-18 is the only standard which is mandatory for biocompatibility testing. It describes the chemical/physical/mechanical material characterization.
In a second stage, all collected and available device information have to be assessed. This is typically done within a literature study.

- Obtain device material, identification and chemical characterization shall be considered (ISO 10993-18).
- Is the material same as in commercially available device?
- Is the body contact the same?
- Are manufacturing and sterilization the same?
- Are the data relevant to evaluate the risk of exposure?
- Do the data apply to chemical mixtures?
- Do sufficient toxicology chemicals in the material?
- Is there sufficient justification and/or clinically relevant data (chemical and biological) for a risk assessment?
- Perform further evaluation of device based on chemical nature of and type and duration.
- Perform biological evaluation (Annex A).

Biological evaluation complete
Only in the third stage, decisions on in vitro and in vivo testing have to be taken.
Testing According to ISO 10993-1

- Material characterization and ascertainment of meeting specifications

The Concept of Biocompatibility
### Chemical and physical Analyses according to ISO 10993-18

#### The Concept of Biocompatibility

#### Tabelle 2 — Parameter und Untersuchungsverfahren für die Analyse von Polymeren

<table>
<thead>
<tr>
<th>Parameter zu untersuchender Parameter</th>
<th>Beispiele von Verfahren (nicht umfassend oder ausschließlich)</th>
<th>Qualitativ</th>
<th>Quantitativ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identifikationsfeststellung</td>
<td>Kolorimetrie, 2D PAGE, GPC</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>chemische Struktur</td>
<td>Analyse und Sequenzierung von Aminosäuren, FTIR, 13C-1H- und 13C-NMR</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Konfiguration der chemischen Ketten</td>
<td>Titration, Spektroskopie</td>
<td>—</td>
<td>X</td>
</tr>
<tr>
<td>physikalische Konfiguration der Ketten</td>
<td>Spektroskopie (13C-NMR), DSC</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>1 Taktizität</td>
<td>Sol-Gel-Extraktion, Analyse der Disulfidbrücken, DMTA</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>2 Vernetzungsgrad</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Verzweigung</td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

#### Tabelle 3 — Parameter und Untersuchungsverfahren für die Analyse von Metallen und Legierungen

#### Tabelle 4 — Parameter und Untersuchungsverfahren für die Analyse von Keramiken

#### Tabelle 5 — Parameter und Untersuchungsverfahren für die Analyse natürlicher Makromoleküle

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The Concept of Biocompatibility
Chemical and physical Analyses according to ISO 10993-18

• Comprehensive analysis is important to ascertain the function of the device
• For absorbable products, the mechanisms have to be understood

e.g. absorption versus degradation in polymers
Poly(1,3-Trimethylene Carbonate)
High molar mass = hydrophobic ➔ enzymatic degradation by lipases and absorption
Low molar mass = hydrophilic ➔ acidic hydrolysis and clearing by lymphatic system or blood
Chemical and physical Analyses according to ISO 10993-18

e.g. what is the morphology and exact composition of the CaP? It will define the absorption behavior in the host

**Solubility:**

![Graph showing solubility of different CaP phases](image)

- Log\((\text{Ca}^{2+})\)
- pH

**Ca^{2+} in the body**

Testing According to ISO 10993-1

- Material characterization and ascertainment of meeting specifications
- Literature study
- Assessment of the risk based on available data

The Concept of Biocompatibility
General requirements:

• All available information has to be included
• All data sets have to be compared
• The information has to be assessed regarding the relevance versus the medical device, in particular versus performance and safety

• Biological assessments must include information of earlier preclinical and clinical studies and all published literature
• The whole process has to be documented in details according to appendix C of ISO 10993-1.
Testing According to ISO 10993-1

Material characterization and ascertainment of meeting specifications

- Literature study
- Assessment of the risk based on available data

- In vitro testing
- In vivo studies
## Testing According to ISO 10993-1

<table>
<thead>
<tr>
<th>Device Categorization</th>
<th>Biological Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Category</strong></td>
<td><strong>Duration</strong></td>
</tr>
<tr>
<td></td>
<td>A – limited (&lt;24h)</td>
</tr>
<tr>
<td></td>
<td>B – prolonged (&gt;24h, &lt;30d)</td>
</tr>
<tr>
<td></td>
<td>C – permanent (&gt;30d)</td>
</tr>
<tr>
<td>Surface device</td>
<td>Surface device</td>
</tr>
<tr>
<td>Mucosal Membrane</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Breached or compromised surface</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>External communicating device</td>
<td>Blood Path, indirect</td>
</tr>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Tissue/bone/dentin</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Circulating blood</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Implant device</td>
<td>Tissue/bone</td>
</tr>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Blood</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>C</td>
</tr>
</tbody>
</table>

ISO 10993-1 table A.1

The Concept of Biocompatibility
Testing According to ISO 10993-1

• Typical *in vitro* testing methods are:
  • Cytotoxicity
  • Genotoxicity
  • Haemocompatibility
  • Reproductive / development toxicity (Teratogenicity)
  • (acute systemic toxicity)

• *In vitro* determination of the cytotoxic potential of medical device (finished product) or of the material used for manufacturing the medical device.

• Comparison of the cytotoxic potential against negative and positive controls.

• Testing options:  
  - Extracts  
    - Direct contact  
    - Indirect contact, diffusion

The Concept of Biocompatibility

A1: Extract test: Acute cytotoxicity

Replace medium by extract

>24h incubation

Quantitative evaluation (e.g., MTT, NR, MTS)

Qualitative evaluation

Extract preparation (e.g., in culture medium, 24h at 37°C)

Subconfluent cell culture

Test sample

A2: Extract test: Colony formation

Replace medium by extract

6 days incubation

Quantitative evaluation (colony formation)

Extract preparation (e.g., in culture medium, 24h at 37°C)

Low dense cell culture

Subconfluent cell culture

Test sample

B: Direct contact test

Placement of sample on cell layer covering 10%

>24h incubation

Subconfluent cell culture

Test sample

C1: Indirect contact test: Agar diffusion test

Placement of agar layer on cell layer and sample on agar layer covering 10%

24-72h incubation

Subconfluent cell culture

Test sample

C2: Indirect contact test: Filter diffusion test

Placement filter on agar and of sample on filter

2h incubation

Agar layer

Filter

Subconfluent cell layer

Test sample

Qualitative and/or quantitative evaluation

Qualitative and/or quantitative evaluation

Qualitative evaluation

The Concept of Biocompatibility

• Extract obtained by incubation of the medical device in cell culture medium containing serum
  => hydrophilic as well as some hydrophobic compounds can be extracted (see as well ISO 10993-12)

• Defined extraction conditions:
  - **Surface/ volume ratio**
  - Mass/ volume ratio
  - Time (24h-72h)
  - **Temperature (37°C)**
  - **Extraction solvents**

• Extract in dilution series (no more necessary) to assess growth inhibition, colony forming capacity, and viability of cells

• Exposure to mouse fibroblasts L929 during 72 h

• Quantification of cell growth with either XTT, MTT, BCA, etc.
Standard Cytotoxicity Testing

Quantitative testing

Quantitative assessment:
Data are normalized to negative controls (no cytotoxic effect).
Up to 30% cytotoxic effect is acceptable
Standard Cytotoxicity Testing

Qualitative testing

- no effect – intact cell layer
- severe effect – only few cells

Microscopical assessment (according to US Pharmacopeia):

- 0 ➔ no effect
- 1 ➔ slight effect 1-20%
- 2 ➔ mild effect 20-50%
- 3 ➔ moderate effect 50-70%
- 4 ➔ severe effect 70-100%

Each standard uses different classification.
Definition of Cytotoxic Effects

Quantitative assessment:
Reduction of cell viability/function by more than 30% considered cytotoxic.

Qualitative assessment:
Effect of more than grade 2 (> 50%) considered cytotoxic.

Preference of quantitative assessment
BUT
qualitative assessments are allowed by the ISO standards
Standard Cytotoxicity Testing: Direct Contact

- A planar piece of sample material is placed on top of an established cell layer.
- Cytotoxic substances will affect cell growth and/or induce cell lysis underneath or within diffusion distance to the sample.
- Lipophylic substances are in direct contact with the cells.

Microscopical assessment (according to US Pharmacopeia):

<table>
<thead>
<tr>
<th></th>
<th>Effect Description</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>no effect</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>slight effect</td>
<td>1-20%</td>
</tr>
<tr>
<td>2</td>
<td>mild effect</td>
<td>20-50%</td>
</tr>
<tr>
<td>3</td>
<td>moderate effect</td>
<td>50-70%</td>
</tr>
<tr>
<td>4</td>
<td>severe effect</td>
<td>70-100%</td>
</tr>
</tbody>
</table>

Each standard uses different classification.
A Note on *in vitro* Cytotoxicity Testing

- Cell Culture Cytotoxicity Assays—This test evaluates *in vitro* toxicity of substrate materials to cultured cells.

- The **direct relation between results of cytotoxicity testing and biocompatibility** of materials **has not been documented** and there is some **controversy** as to the value of the testing since some good materials may be excluded and some others that are not biocompatible may pass this test.

- Cytotoxicity testing is recommended as an early screening test and also to provide information that will aid in the development of cytotoxicity tests predictive of *in vivo* performance.

(ASTM F748-06(2010))
**In vivo Assessments**

- All known possible biological hazards shall be taken into account for every material and final product, but this does not imply that testing for all possible hazards will be necessary or practical (ISO 10993-1/2009)
- Prior to application to animals, all relevant alternative methods have been considered and used wherever possible (ISO 10993-2/2006)
- It has be ascertained that no similar *in vivo* assessments had been performed before (ISO 10993-2)
- The need to perform animal tests is justified and any pain, suffering, distress or lasting harm that is caused during essential animal tests is minimized
- 3 R’s: - Replace
  - Reduce
  - Refine

The best science and the best animal welfare are inseparable
Biocompatibility Testing: *In vivo*

- Irritation (local body reaction; skin, intracutaneous, ocular...)
  (ISO 10993-10)
- Sensitization (systemic body reaction; allergic reactions)
- Acute, sub-acute, sub-chronic and chronic toxicity (i.v., i.p, dermal, oral...)
  (ISO 10993-11)
- Implantation / local tolerance (orthopedic implants, drug application systems, tissue engineering products, cardiovascular implants; subcutaneous, muscle, bone)
  (ISO 10993-6)

*Note: Please use protocols that are well established and standardized!*
*e.g. critical size defect models according to ASTM F2721 or for infection “Handbook of Animal Infection Models”*
**In vivo Assessments**

- Proper selection of the animal model is essential
- The physiologic of some organs or pathways is closer in certain animals than in others.
- The genetic variability is a hurdle in large animal models
- All models have to functional and reflect human use.
Testing According to ISO 10993-1

- Material characterization and ascertainment of meeting specifications
- Literature study
- Assessment of the risk based on available data
- Final risk assessment
- In vitro testing
- In vivo studies
9 Different Materials:
- Polyethylene
- Hydroxyapatite
- Polyurethane
- Silicone
- pHEMA
- PTFE (Gore-tex)
- Pyrolytic carbon
- Gold
- Titanium

Short term reaction:
- Differential protein adsorption
- Varied activation of host response

Long term reaction:
- Fibrous encapsulation

Subcutaneous implantation

All have the same endpoint, but all materials can be considered biocompatible if no other host reaction occurs and device performance is not at risk.

Is in vivo Testing Predictive?
The Interface between Engineering and Biological Sciences

When **engineering meets biology**, research results in:
- >75,000 citation on tissue engineering
- >30,000 citations on tissue engineering and scaffolds

Research in **Tissue Engineering and Regeneration** includes **ALWAYS**:

**Scaffolding:** ceramic or polymer, natural or synthetic, solid or porous, stable or absorbable

**Cells:** autologous or allogenous, progenitor cells or differentiated, pre-cultivated versus peri-harvested

**Cultivation System:** 2D versus 3D, mechanical stimulation, supplements, environmental conditions, etc.

In any case: the **SCAFFOLD is a KEY ELEMENT** as cells and culture conditions!
“Microenvironments appear important in stem cell lineage specification but can be difficult to adequately characterize or control with soft tissues. .... 

....Soft matrices that mimic brain are neurogenic, stiffer matrices that mimic muscle are myogenic, and comparatively rigid matrices that mimic collagenous bone prove osteogenic.”

Substrate Preparation

“Cells were plated on variably compliant polyacrylamide gels, according to a previously established protocol by Pelham and Wang (Pelham and Wang, 1997), creating gels that were 70–100 mm thick as measured by microscopy. To produce thin gels, a protocol from Engler and coworkers was used (Engler et al., 2004b). Type 1 collagen was used at 0.25–1 mg/cm2 (BD Biosciences), as quantified using fluorescent collagen for calibration (per Engler et al., 2004a).”
What do we know of the scaffold’s properties?

- ratio of initiator, monomer to form the polyacrylamide
- immobilization of collagen
- elastic properties as measured by AFM technique

(based on original paper and cited papers)

The unknown side of the scaffold include:

- polymerization condition, e.g. time, temperature, monomer quality and stabilizer concentration, final composition incl. monomer content
- swelling behavior, porosity, crystallinity, molar mass
- real viscoelastic properties incl. bulk modulus and creep
- behavior of the MSC on control/reference material
“Surface functionalization of hydroxyapatite (HA) and β-tricalcium phosphate (TCP) bioceramics with chemical ligands containing a pyrogallol moiety was developed to improve the adhesion of bone cell precursors to the biomaterials. Fast and biocompatible copper-free click reaction with azido-modified human fetal osteoblasts resulted in improved cell binding to both HA and TCP bioceramics, opening the way for using this methodology in the preparation of cell-engineered bone implants.”

• Excellent description of ligand synthesis
• Good description of cell culture assays

but

• nothing is known on the HA and TCP scaffold except to „densely sintered discs“
• no reference materials were used
• no cytotoxicity test according to international standards

(Borcard et al ,CHIMIA, 67/4, 2013)
Findings:
Despite the vast variety of materials that have been described to date for cartilage tissue engineering, the outcome is always positive and the researches materials is “superior”. In most cases, the scaffolds were poorly characterized!

The Future of *in vitro* Testing?

Today:

2D

Too simple to be of prognostic value

The Future:

3D+ just as complex as required to be near physiological

Cell Response to Materials and Pharmaceuticals

The Future of Biocompatibility Testing?

**Current situation**

**Cytotoxicity**
- *In vitro* tests (ISO10993-5)
  - cytotoxicity extract, "contact"
- Animal cell line

**Bioactivity**
- *In vivo* test

**Proposed future situation**

**New materials & surfaces**

**Cytotoxicity**
- Level 1: Cell "contact"-Test (ISO10993-5)
- Level 2: Extract-Test (modified ISO10993-5)

**Bioactivity**
- Level 3: Cell contact-Test
- Level 4: Cell on growth-test using 3D-Reaggregate
- Level 5: Cell differentiation Test
- Level 6: Cell-cell competition test

*In vivo* test (in vivo bioactivity)

Bruinink and Luginbuehl Adv Biochem Engin/Biotechnol, Spring Verlag 2011
Is there such a Thing as a Biocompatible Material?

Biocompatibility subsumes a collection of individual phenomena and is impossible to quantify. There can be no scale of biocompatibility; therefore it is scientific nonsense to consider certain materials as ‘biocompatible’, occupying the ground at one end of a non-existent scale, and other materials as ‘non-biocompatible’ or ‘bioincompatible’ existing at the other end. (D. Williams)

• Worldwide, a standard series of tests for ‘biological safety’ are used by companies to establish the safety of their products.

• Many of these tests are long established, and even though the information they yield is very limited.

• It is a simplified and relative assessment which may not reflect the final in human performance.

• We have to move forward from trying to ensure that a medical device does no harm to prove that the medical device performs at its best – but also considering industrial constrains!
Total Arthroplasty is an orthopedic success story, enabling hundreds of thousands of people to live fuller, more active lives.

Total Arthroplasty is a pure engineering solution centered on material selection. Key issues preventing the perfect solution include:

**Technical issues:** wear, corrosion, implant fracture, dislocation

**Biological reasons:** material sensitivity, loosening, tissue degradation, tissue fracture (near implant), infection

**Surgical issues:** misalignment, instability
The Future of Orthopedic Implants: Regeneration

Future solutions of orthopedic surgery entail therapies supporting regeneration of skeletal tissues.

Future therapies are based on an orchestrated interplay between engineered biomaterials and biological sciences:

**Tissue Engineering:** only limited importance in orthopedic settings

**One Stage Procedures:** peri-operative preparation, loading of scaffolds with cells

**Early Intervention:** articulation of tissue versus synthetic materials

But it is a long way as the current implant concepts are successful.
Summary

• The industrial **approach** to establish **biocompatibility** is given by **standard guidelines** and **standard tests**

• The **interplay** between **material** chemistry and engineering design and the **biological structures** on a molecular, cellular, and tissue level is **well-recognized**

• Today, it is very **costly** and **time consuming** to **introduce new materials** and new **processing methods** for medical device applications

• A **failure** of a **new concept** that results in a **recall** of products leads to avoidance of that material/concept/design by industry for a long time

• In academia, materials used for biomedical purposes are – unfortunately – often not well or not at all characterized

• **Comparision** of results is most often **impossible** due to **missing reference materials** and **standard protocols**

• Our research costs billions of tax Dollars – therefore we should try to our best for the profit of all and that includes that we know exactly what material we use
Question