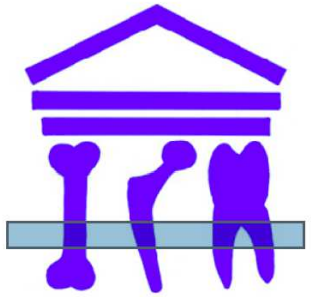
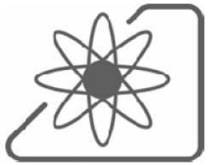


TRAINING SCHOOL

NON LIVING MATERIALS MEET LIVING BIOLOGY

Patras, Greece – 9-12 May 2017



COST MP1301

NEWGEN

The Concept of Biocompatibility

Reto Luginbuehl

reto.luginbuehl@ipromedai.net

Agenda

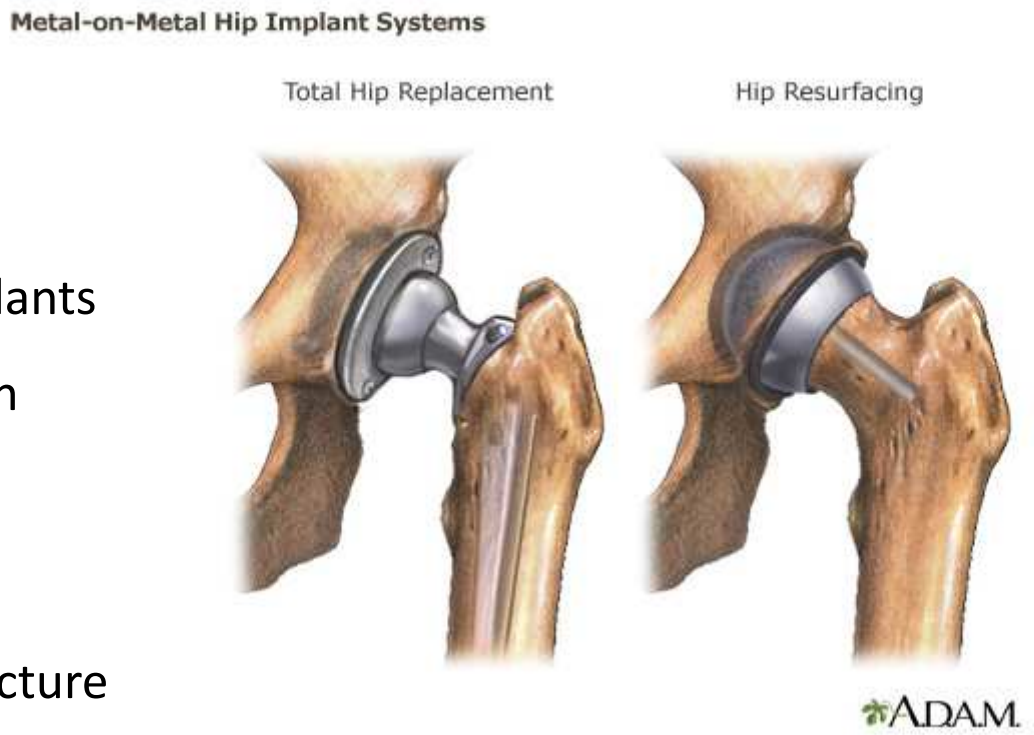
1. The failure of biocompatibility testing exemplified with metal-on-metal articulation
2. Definition of Biomaterials and Biocompatibility
3. Establishing Biocompatibility according to the risk management process as described in ISO 10993
4. Why does academia fails to translate their results to products?

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

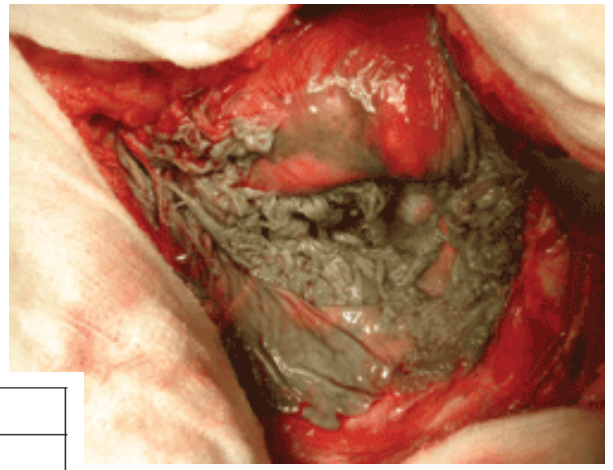
Survival Rate of Hip implants:

- 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.

https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/ImplantsandProsthetics/MetalonMetalHipImplants/default.htm

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.



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

- Some of the metal ions (e.g. cobalt and chromium) from the metal implant or from the metal particles will enter the bloodstream.

Cobalt and Chromium ions are considered (> 1-5 ng/mL):

- cytotoxic, acute toxic, genotoxic, carcinogenic

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

› DEFECTIVE MEDICAL DEVICES › DEPUY HIP IMPLANTS

DePuy ASR Hip Replacement Recall

DePuy/Johnson and Johnson

In the past few years, Johnson and Johnson – one of the largest pharmaceutical and medical device manufacturers in the world – has been forced to recall 11 drugs and medical devices. The most recent recall involved 29 models of a prosthetic hip implant, the DePuy ASR XL Acetabular System, which were recalled by the FDA in August, 2010 because of evidence of high failure rate. Failed implants cause pain to patients and often necessitate additional surgeries. If you are experiencing pain or have had to undergo surgery for a hip implant, contact one of our skilled Florida DePuy hip replacement lawyers.

Johnson and Johnson's ASR XL Acetabular System is a metal-on-metal (MoM)

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

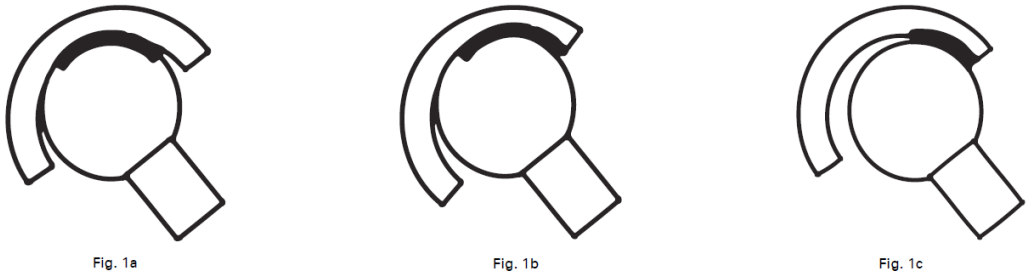


Fig. 1a Fig. 1b Fig. 1c

Diagrams showing the effect of malposition of the acetabular component or femoral head on the position of the tribological contact patch (thick dark line). In the normal position (a) the contact patch lies within the bearing area of the acetabular component; with rotational malposition of the acetabular component (b; steep inclination) its rim intersects the contact patch; and with translational malposition of the head or acetabular component (c) its rim intersects the contact patch.

- 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

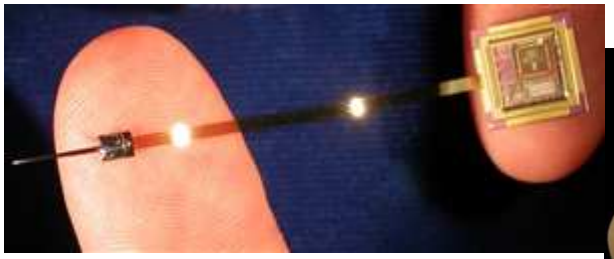
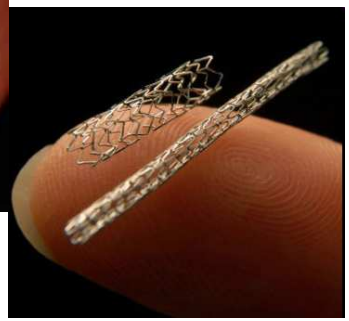
J. Fisher J Bone Joint Surg [Br] 2011;93-B:10 01-4 Instructional Review.

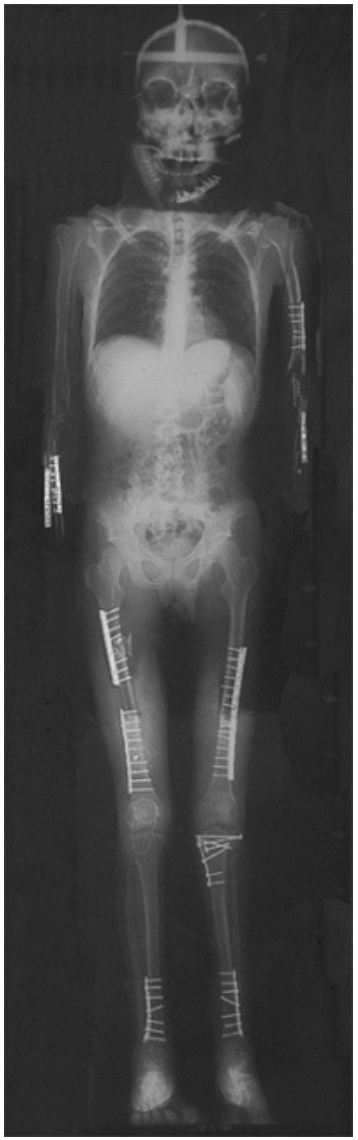
What is a Biomaterial?

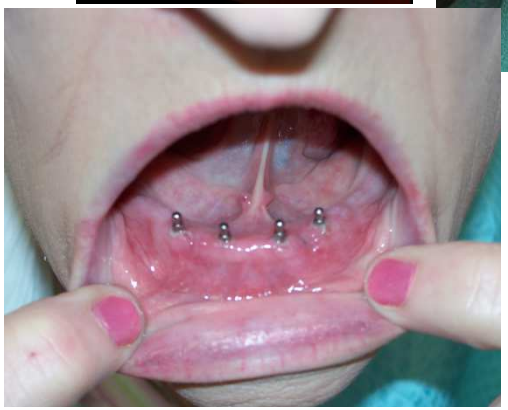
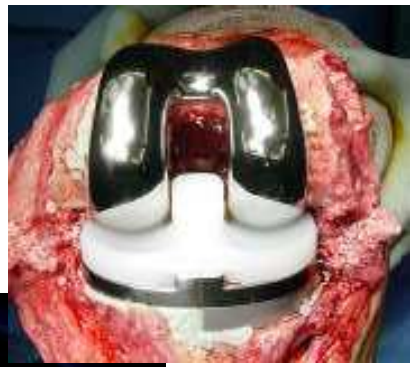
Definition of Biomaterial:

What are Medical Devices?

A material implanted for **restoring functions:**

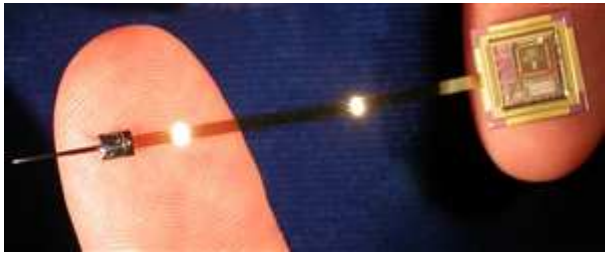



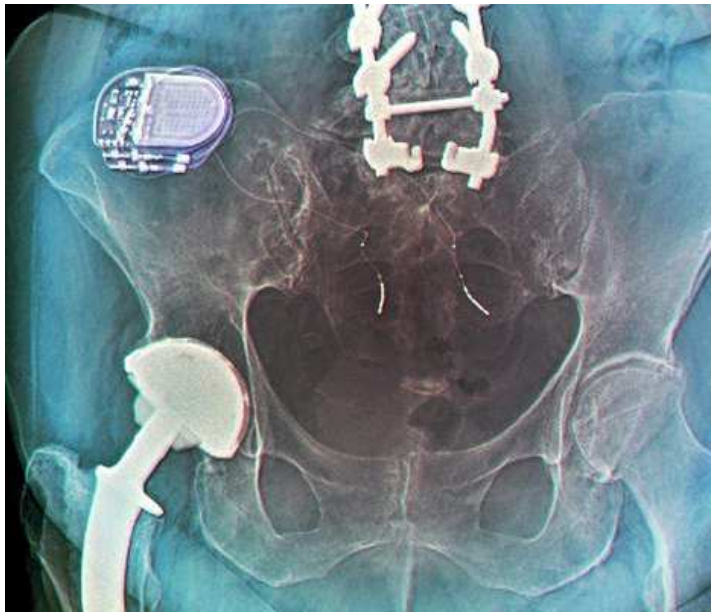


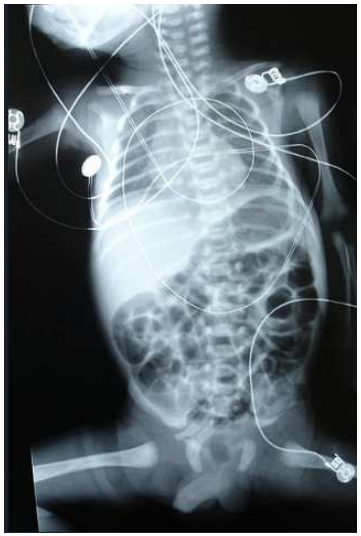



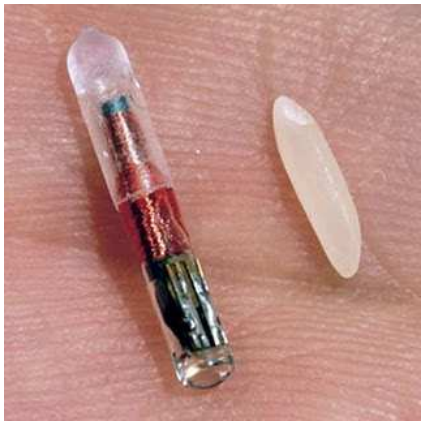

What are Medical Devices?

A material with **active functions:**



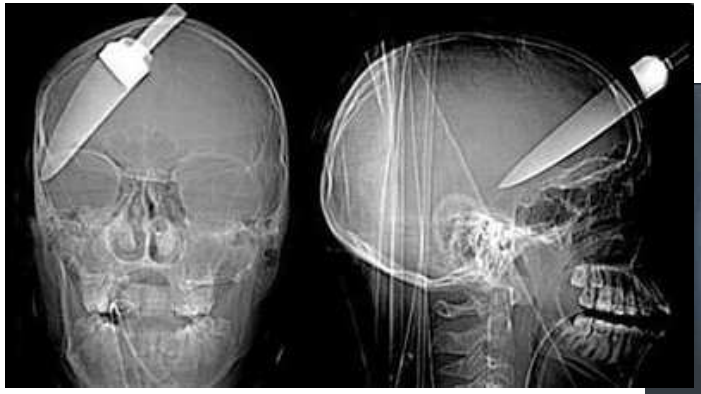


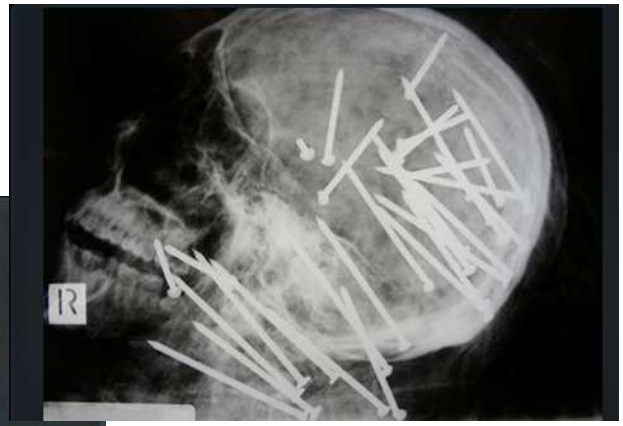


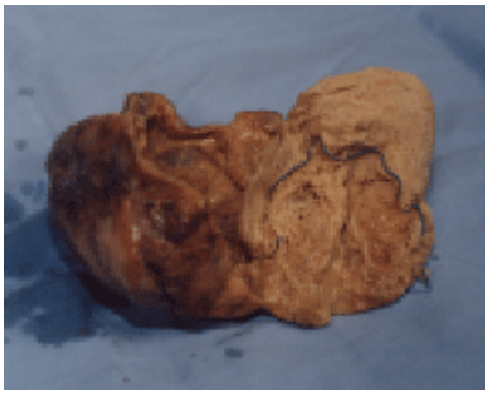


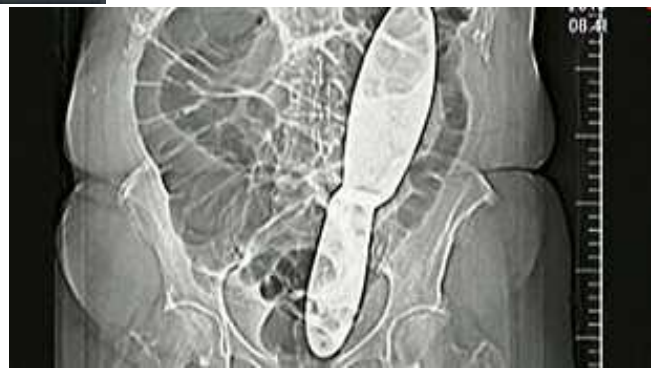
What are Medical Devices?

A material implanted **“unintentionally”: No**



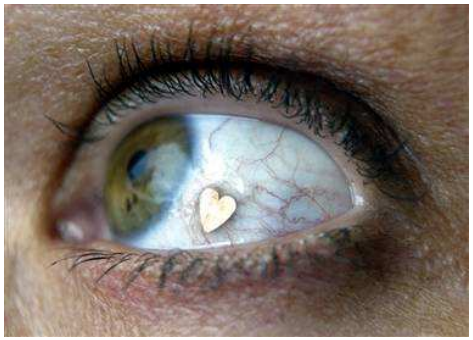


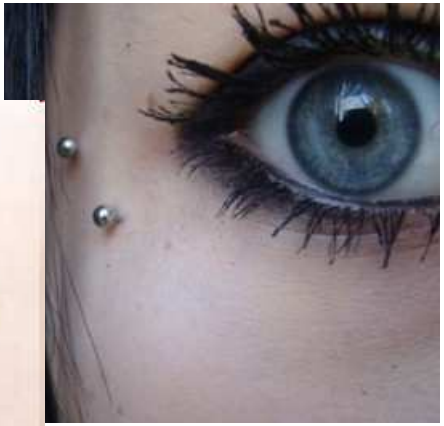


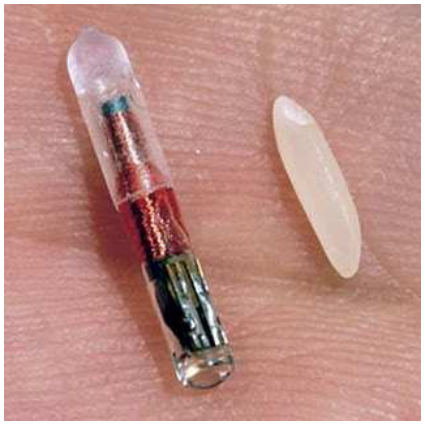


What are Medical Devices?

A material implanted for **cosmetically or commercial functions: No***

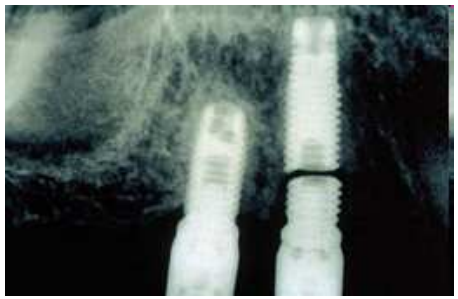


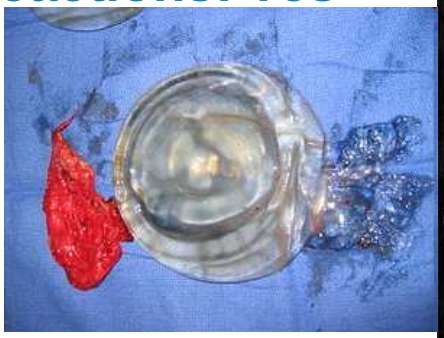


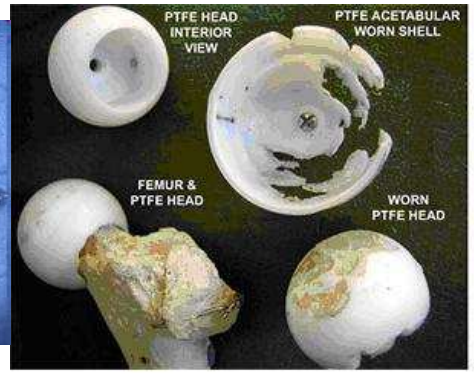


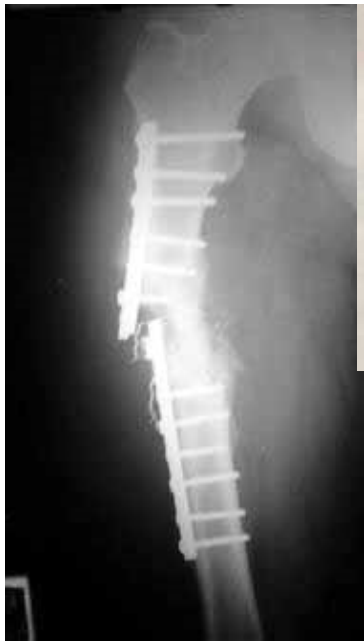
What are Medical Devices?

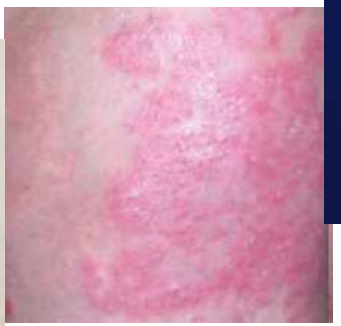
Materials that **may fail or elicit host reactions: Yes**

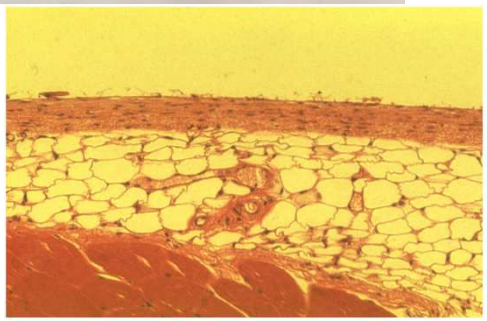


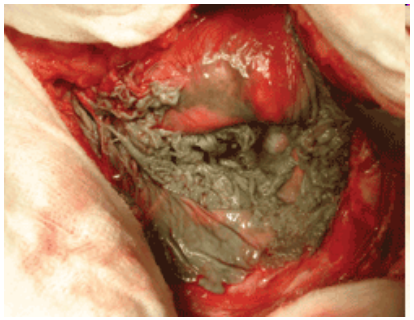












The MDR and IVDR of 2017

The ***Medical Device Directive (MDD)*****,** in force sicne 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.

D. Williams, Biomaterials 35, 2014
http://dx.doi.org/10.1016/j.biomaterials.2014.08.035

What is a Biocompatibility?

Definition of Biocompatibility:

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)

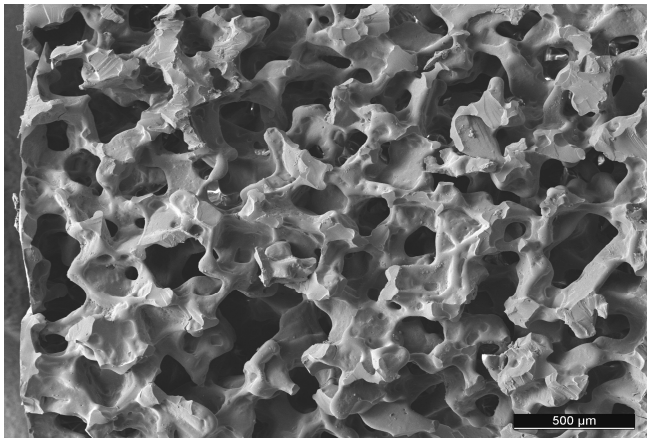
Questions from an Engineering Point of View

- **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,
criystallinity



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

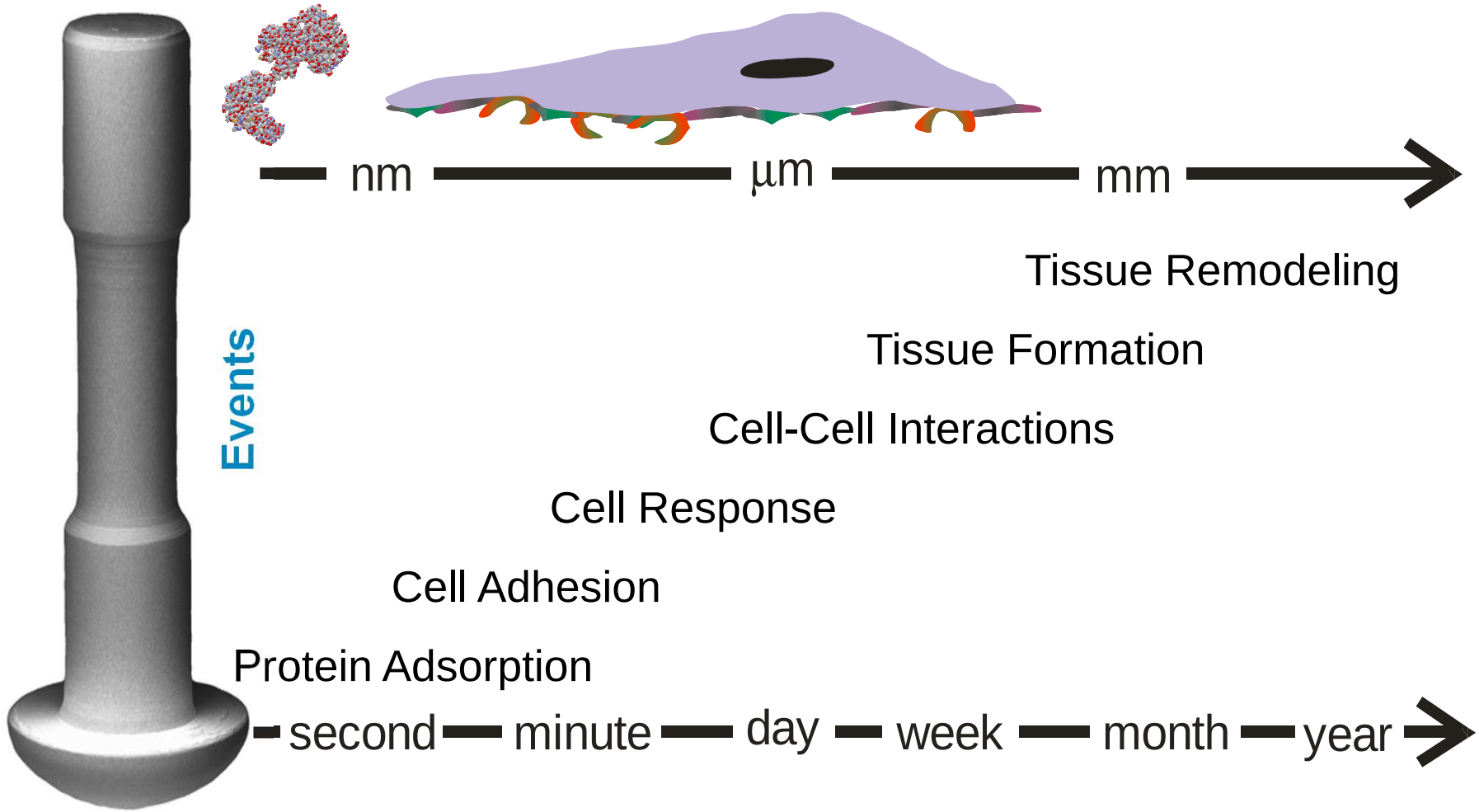
d) Leachable compounds from the bulk materials

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

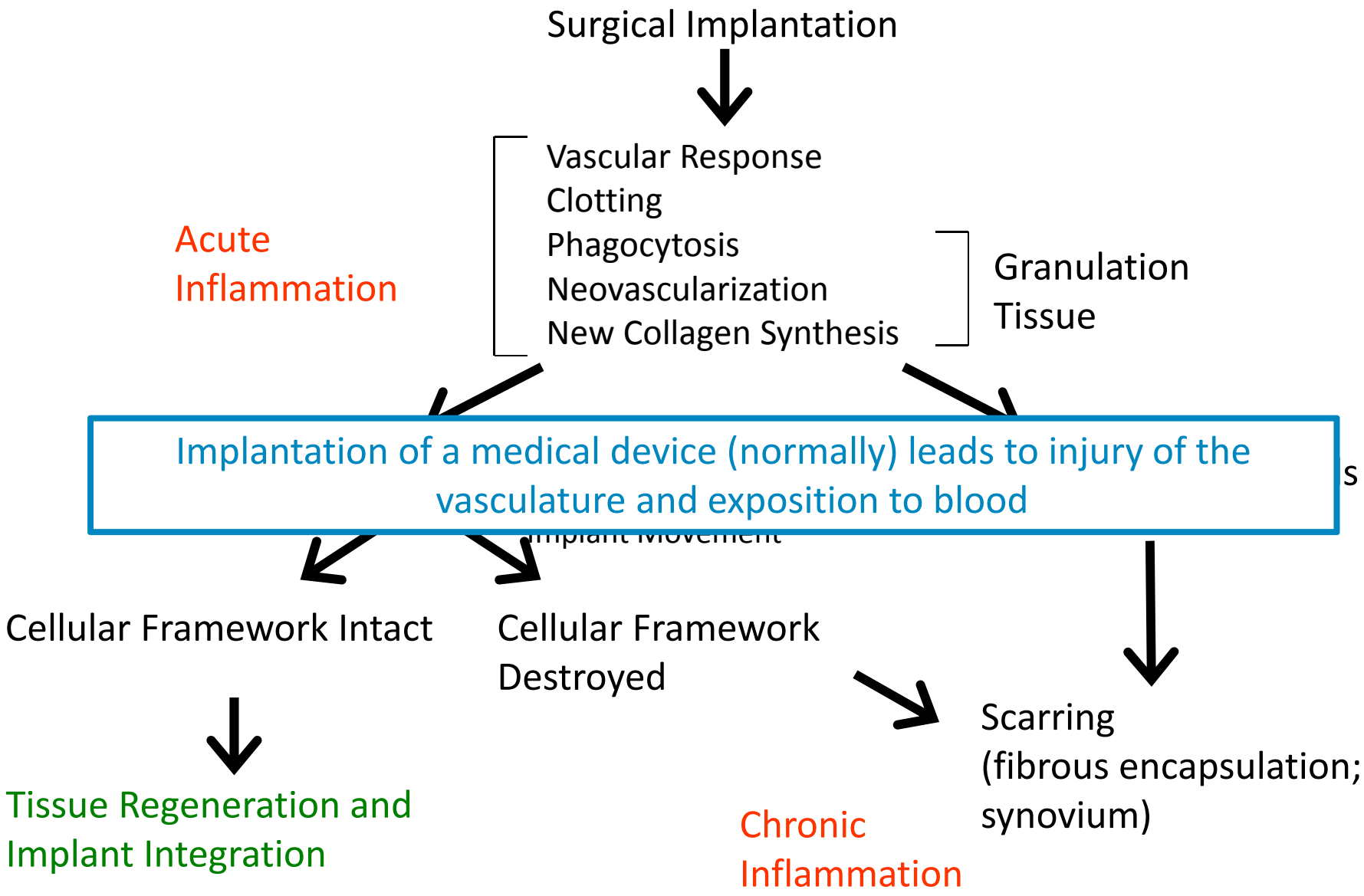
Material meets Tissue



Sequence of Local Events upon Device Implantation

Phase	Normal Wound Healing	Wound Healing as Response to Implants
Injury	• mechanical injury/damage to vasculature /Implantation	
Acute Inflammation	• Blood coagulation-clot formation • Platelet activation and degranulation • Inflammation-oedema	
Chronic Inflammation	• Removal of damaged matrix and necrotic cell components • Cell proliferation and recruitment including endothelial, epithelial, stromal and inflammatory cells • Continued removal of matrix	
Regeneration and remodeling	• Angiogenesis • Matrix synthesis and deposition	
	• Epithelialization and wound contraction • Decrease in cellularity-apoptotic pathway • Tissue remodeling-elastin synthesis	• **Foreign body reaction** • **Macrophages and FBGCs at the material-tissue interface** • **Fibrosis and Fibrous capsule formation**

Implantation of a Biomaterial

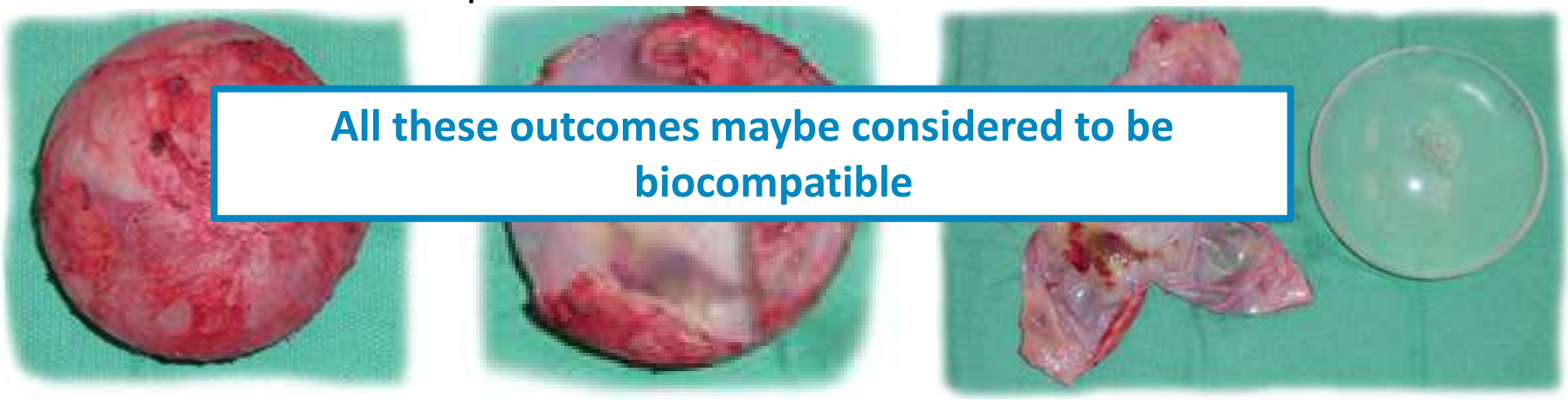


Possible outcomes for the implant:

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

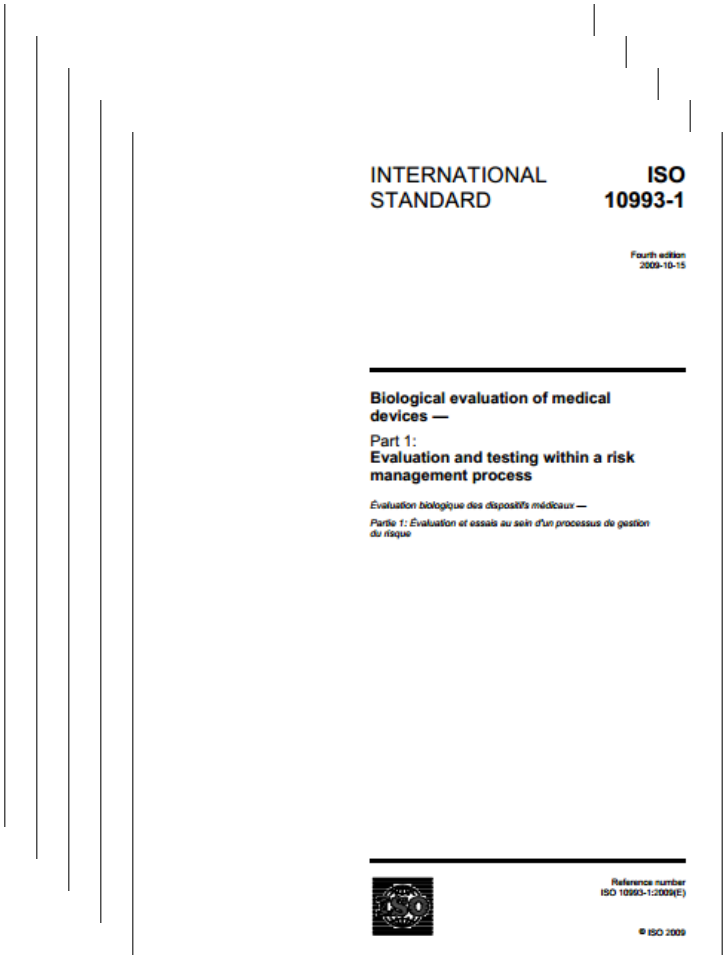
Basic Approach with Standards and Regulations

- 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

Sterilization Residuals

Part 7:	Ethylene oxide sterilization residuals

Animal Welfare

Part 2:	Animal welfare requirements

Reference Materials

(Part 8:	Selection of reference materials)
Part 12:	Sample preparation and reference materials

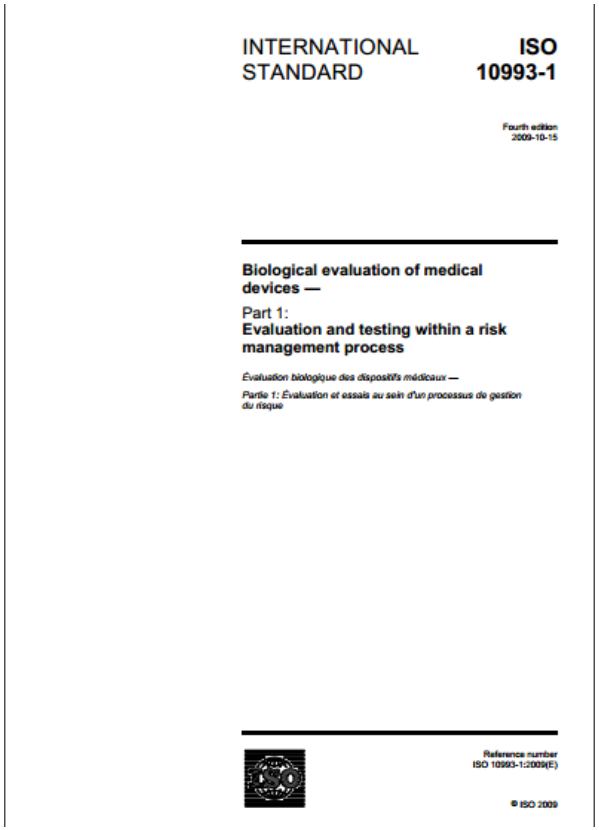
Degradation Products

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

Materials Characterization

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

Testing According to ISO 10993-1



INTERNATIONAL STANDARD **ISO 10993-1**

Fourth edition
2009-10-15

Biological evaluation of medical devices —

Part 1:
Evaluation and testing within a risk management process

Évaluation biologique des dispositifs médicaux —

Partie 1: Évaluation et essais au sein d'un processus de gestion du risque

Reference number
ISO 10993-1:2009(E)

© ISO 2009

Fundamental classification according to:

a) Intended use

b) Contact duration

Those two factors define the extend of required
in vitro and *in vivo* testing.

Testing According to ISO 10993-1

Contact Duration:

differentiation	duration
Short term contact (A)	< 24 hours
Intermediate contact (B)	24h to 30 days
Long term/permanent contact (C)	> 30 days

Testing According to ISO 10993-1

Intended use:

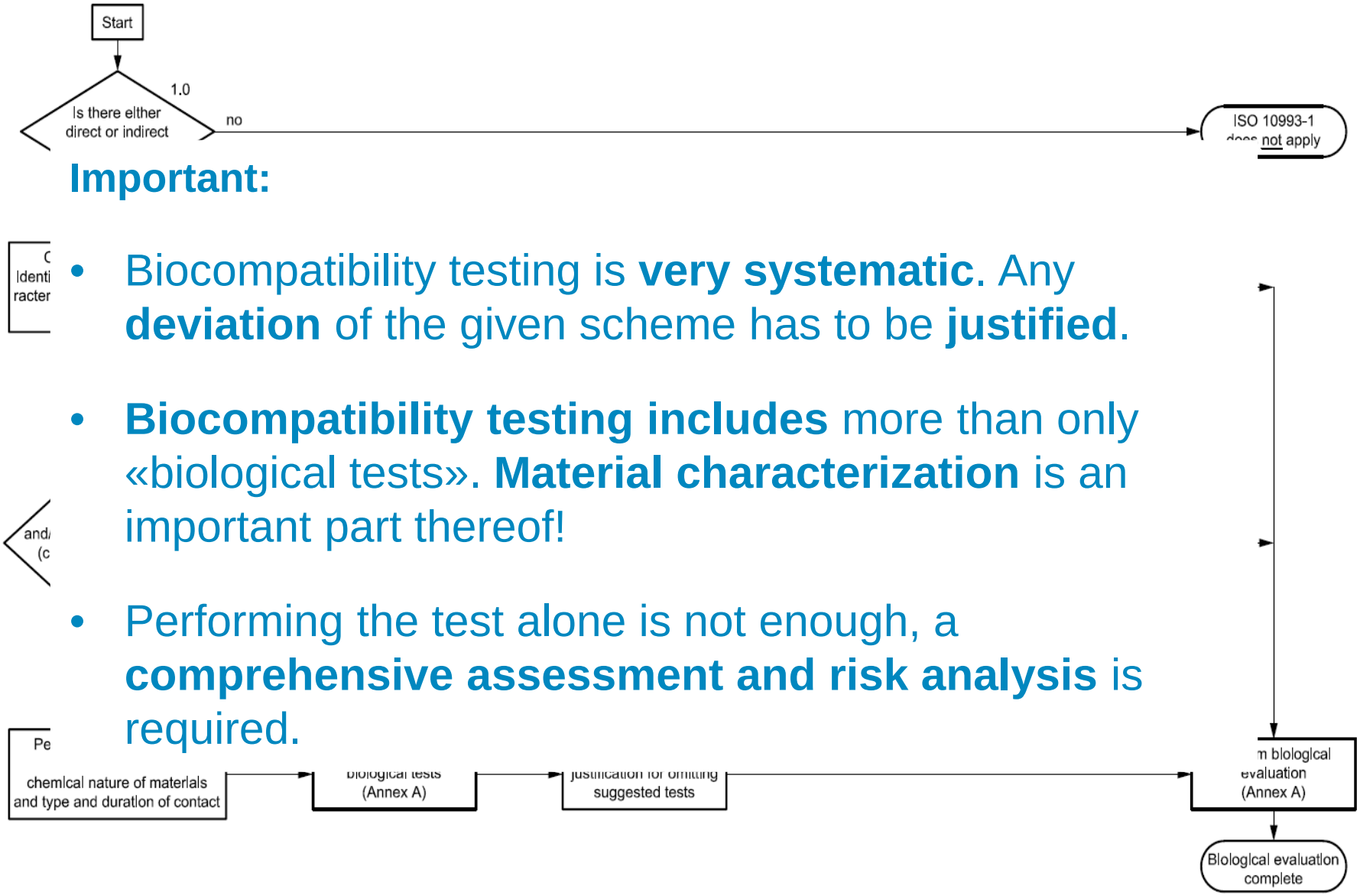
Type of contact	Affected tissue	example
Surface Contact (external)	Skin (healthy/intact)	Skin electrodes, US probe, leg prosthesis
	Mucosa (intact)	stomach probe, contact lenses, dental fixtures, urinary catheter
	Breached or compromised surfaces	Wound bandage
External communicating devices	Blood path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

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**!

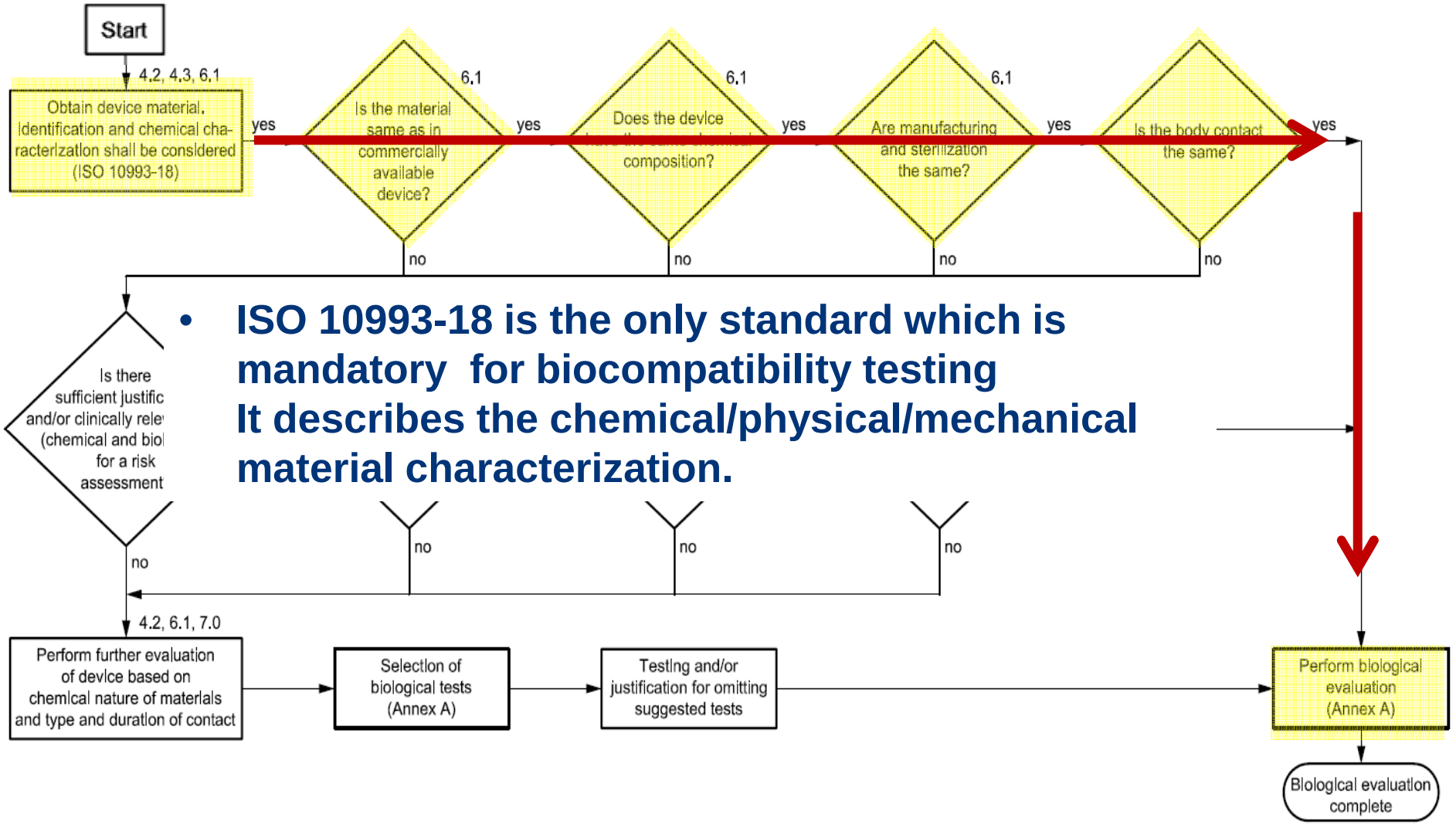
Testing According to ISO 10993-1



Important:

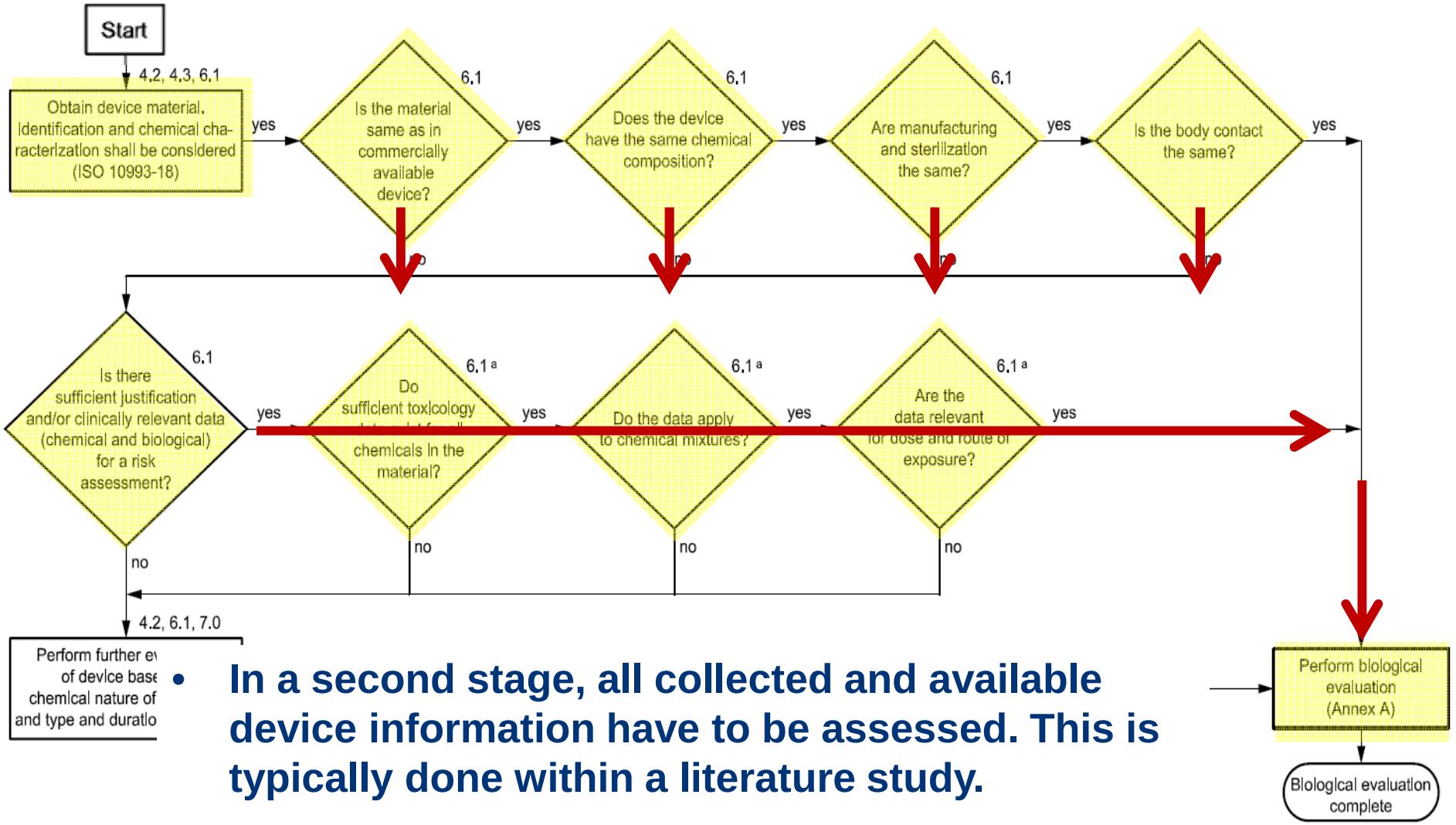
- Biocompatibility testing is **very systematic**. Any **deviation** of the given scheme has to be **justified**.
- **Biocompatibility testing includes** more than only «biological tests». **Material characterization** is an important part thereof!
- Performing the test alone is not enough, a **comprehensive assessment and risk analysis** is required.

Testing According to ISO 10993-1



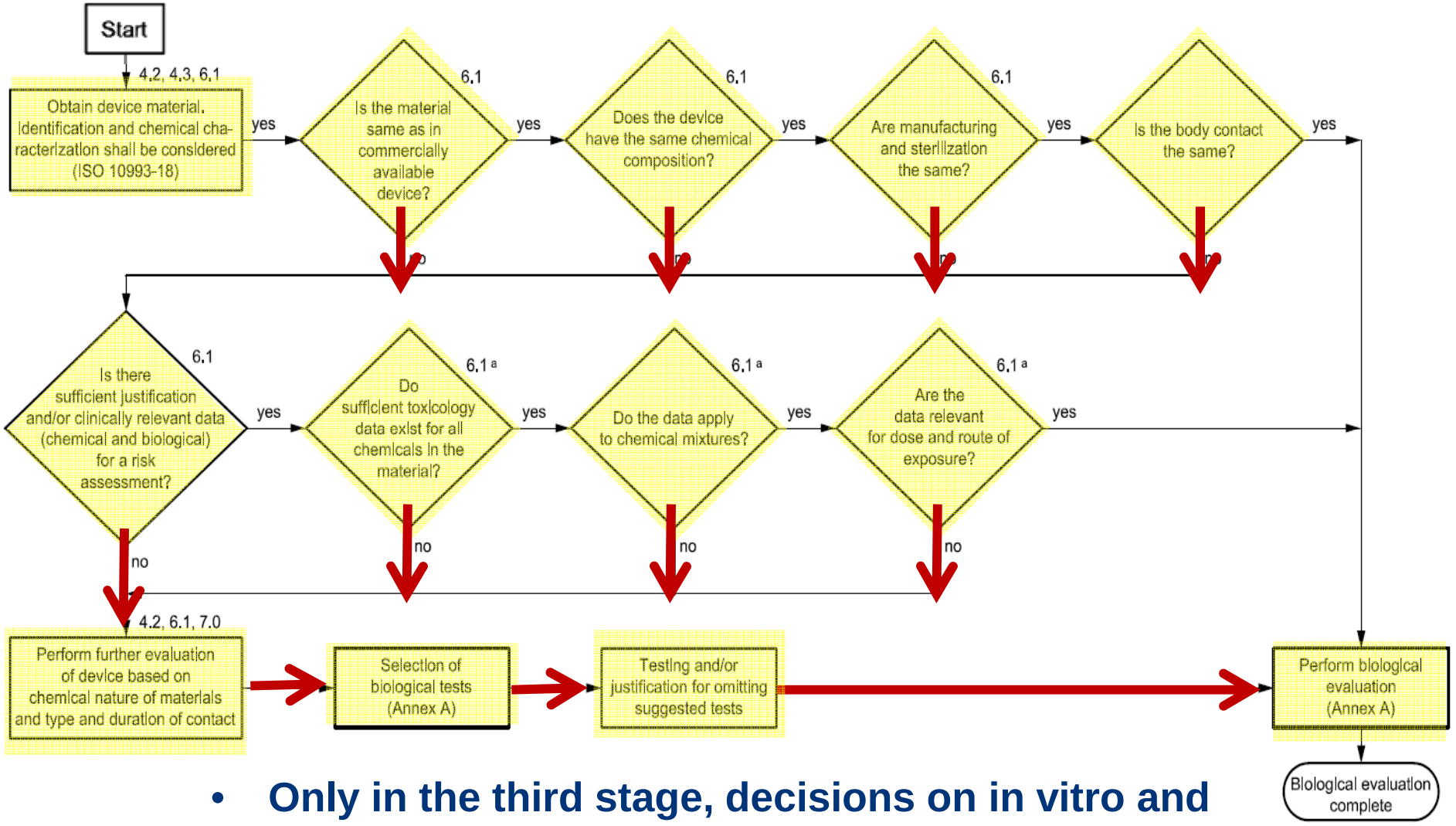
- **ISO 10993-18 is the only standard which is mandatory for biocompatibility testing**
  It describes the chemical/physical/mechanical material characterization.

Testing According to ISO 10993-1



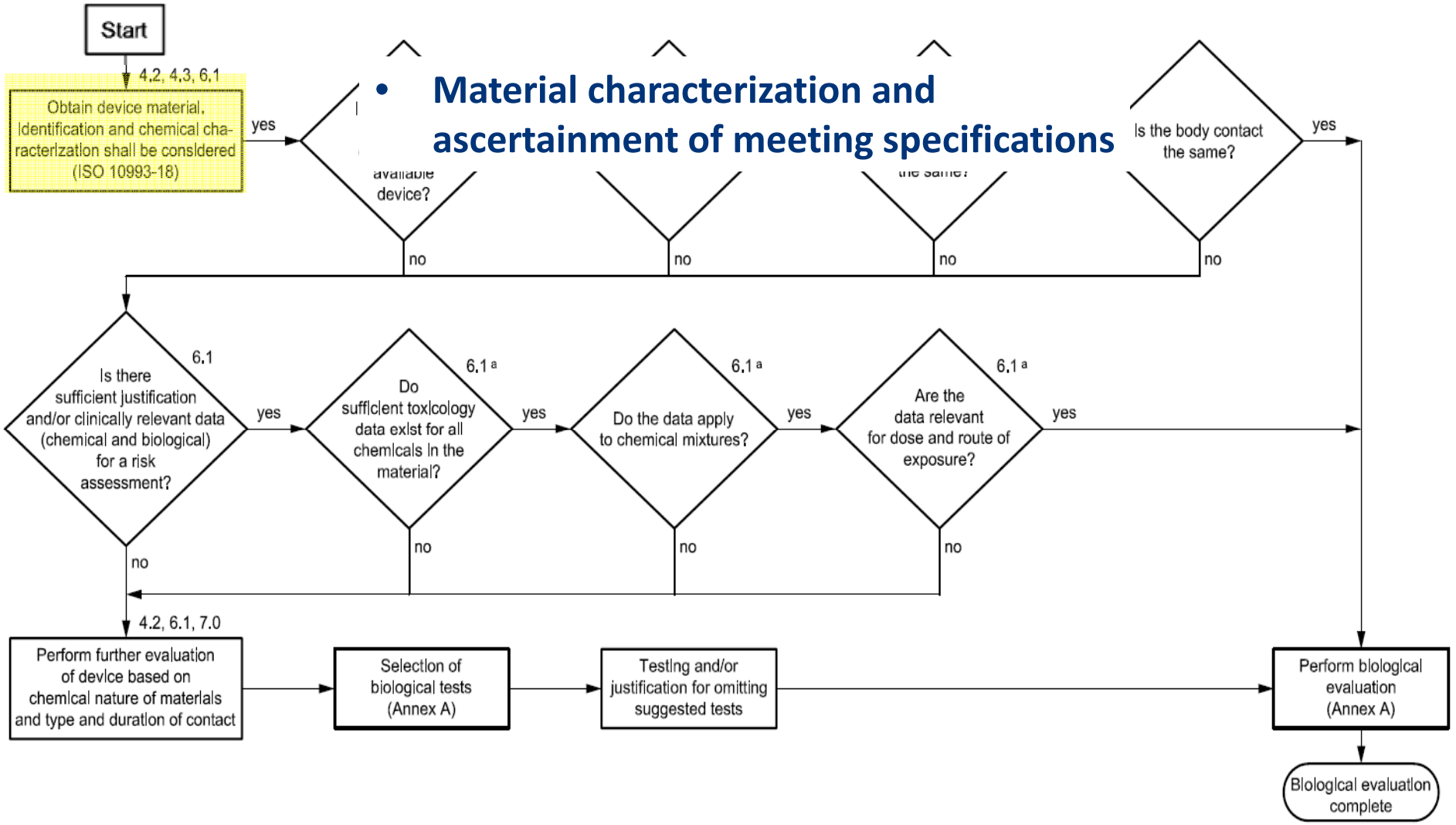
- **In a second stage, all collected and available device information have to be assessed. This is typically done within a literature study.**

Testing According to ISO 10993-1



- **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**

Chemical and physical Analyses according to ISO 10993-18

Tabelle 2 — Parameter und Untersuchungsverfahren für die Analyse von Polymeren

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

Beispiele zu untersuchender Parameter	Beispiele von Verfahren (nicht umfassend oder ausschließend)	Qualitativ	Quantitativ
Identifikationsfeststellung	Kolorimetrie	X	—
	2D PAGE	X	X
	GPC	X	—
chemische Struktur	Analyse und Sequenzierung von Aminosäuren	X	X
	FTIR	X	—
	^{13}C ^{1}H- und ^{13}C-*NMR*	X	—
Konfiguration der chemischen Ketten	Titration	—	X
— analytische Bestimmung der Seitenketten	Spektroskopie	X	X
physikalische Konfiguration der Ketten			
1 Taktizität	Spektroskopie (^{13}C-*NMR*)	X	X
	DSC	X	—
2 Vernetzungsgrad	Sol-Gel-Extraktion	X	—
	Analyse der Disulfidbrücken	X	X
3 Verzweigung	*DMTA*	—	X

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

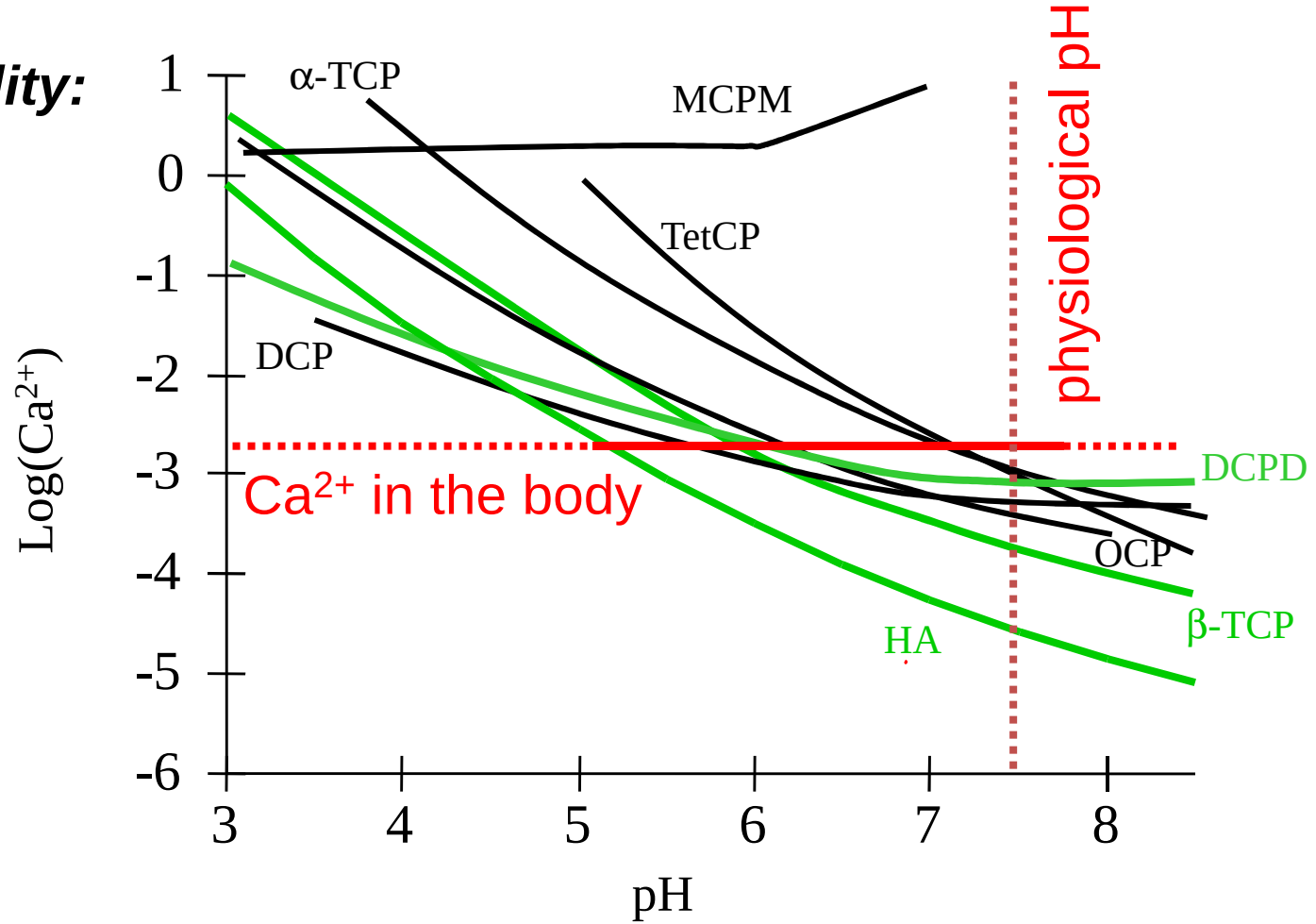
Poly(1,3-Trimethylene Carbonate)

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

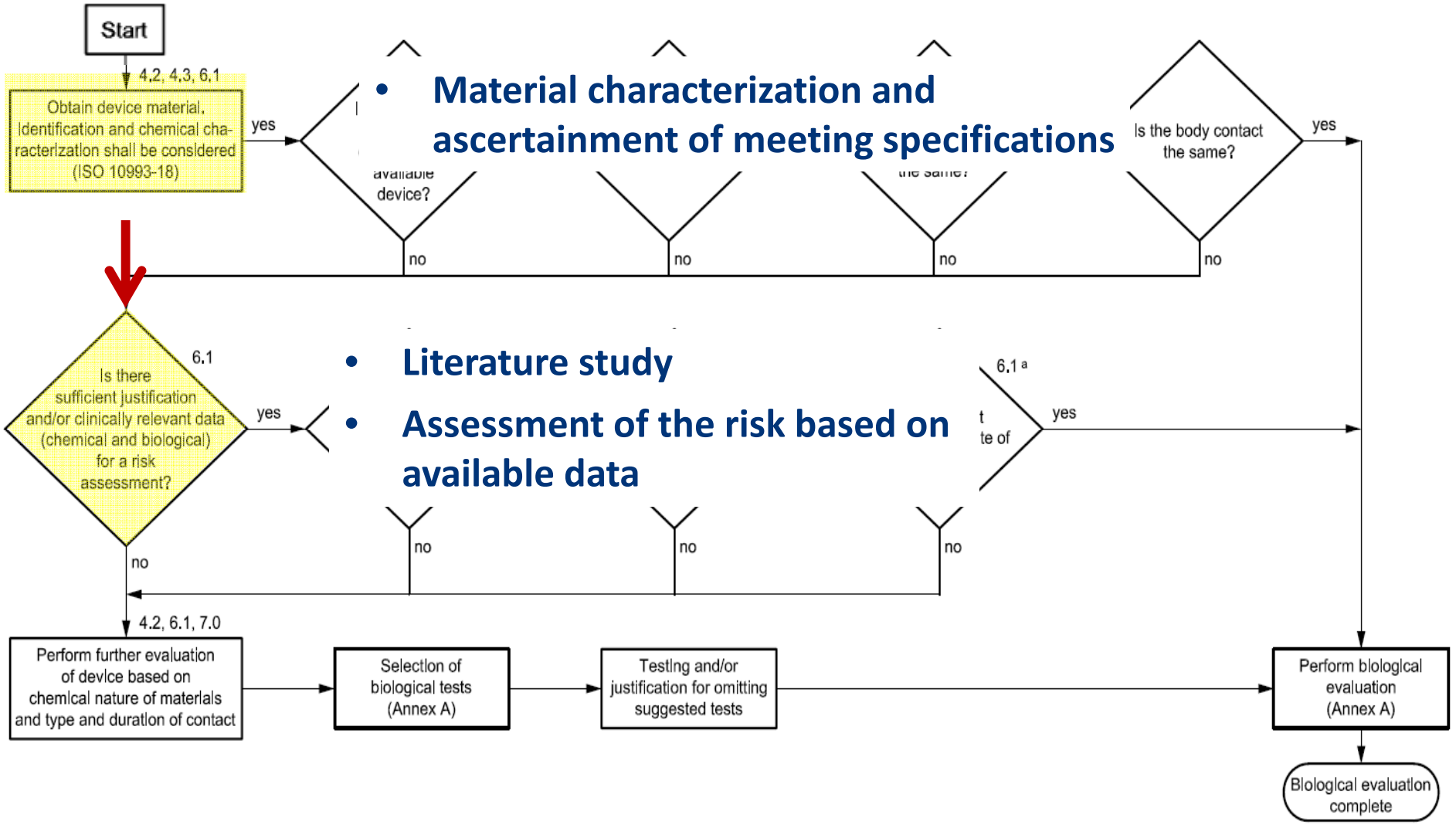
e.g. what is the morphology and exact composition of the CaP?
It will define the absorption behavior in the host

Solubility:



e.g. Fernandez et al , *Journal of Materials Science: Materials in Medicine* 10, 169-176 (1999).

Testing According to ISO 10993-1



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

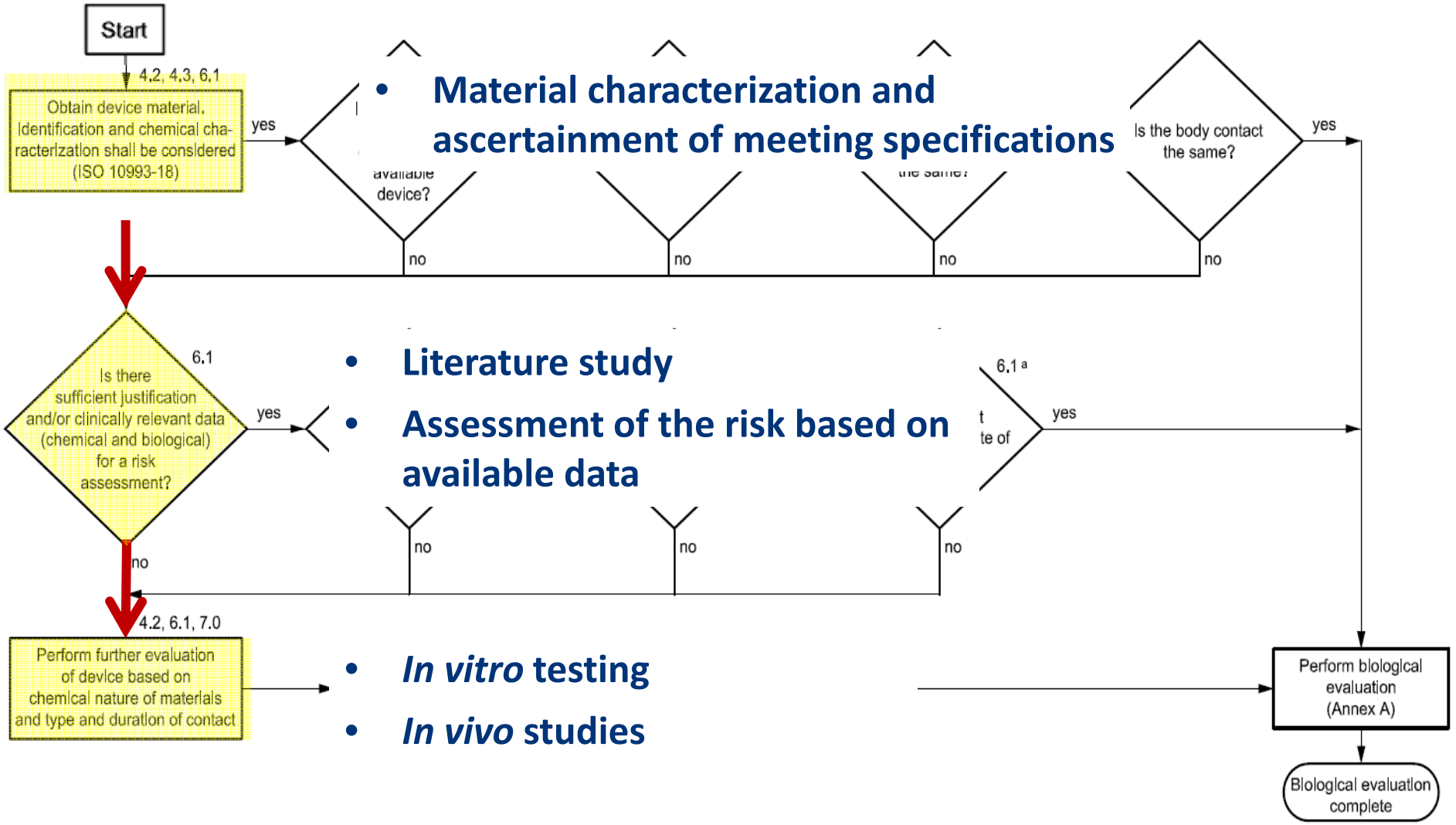
Literature Study According to ISO 10993-1

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

Device Categorization			Biological Effect							
Category	**Contact**	**Duration** **A – limited** (<24h) **B – prolonged** (>24h, <30d) **C- permanent** (>30d)	**Cytotoxicity**	**Sensitization**	**Irritation**	**Systemic Toxicity (acute)**	**Subchronic Toxicity**	**Genotoxicity**	**Implantation**	**Haemocompatibility**
Surface device		A	X	X	X					
		B	X	X	X					
		C	X	X	X					
	Mucosal Membrane	A	X	X	X					
		B	X	X	X					
		C	X	X	X		X	X		
	Breached or compromised surface	A	X	X	X					
		B	X	X	X					
		C	X	X	X		X	X		
External communicating device	Blood Path, indirect	A	X	X	X	X				X
		B	X	X	X	X				X
		C	X	X		X	X	X		X
	Tissue/bone/ dentin	A	X	X	X					
		B	X	X	X	X	X	X	X	
		C	X	X	X	X	X	X	X	
	Circulating blood	A	X	X	X	X				X
		B	X	X	X	X	X	X	X	X
		C	X	X	X	X	X	X	X	X
Implant device	Tissue/bone	A	X	X	X					
		B	X	X	X	X	X	X	X	
		C	X	X	X	X	X	X	X	
	Blood	A	X	X	X	X		X	X	X
		B	X	X	X	X	X	X	X	X
		C	X	X	X	X	X	X	X	X

ISO 10993-1 table A.1

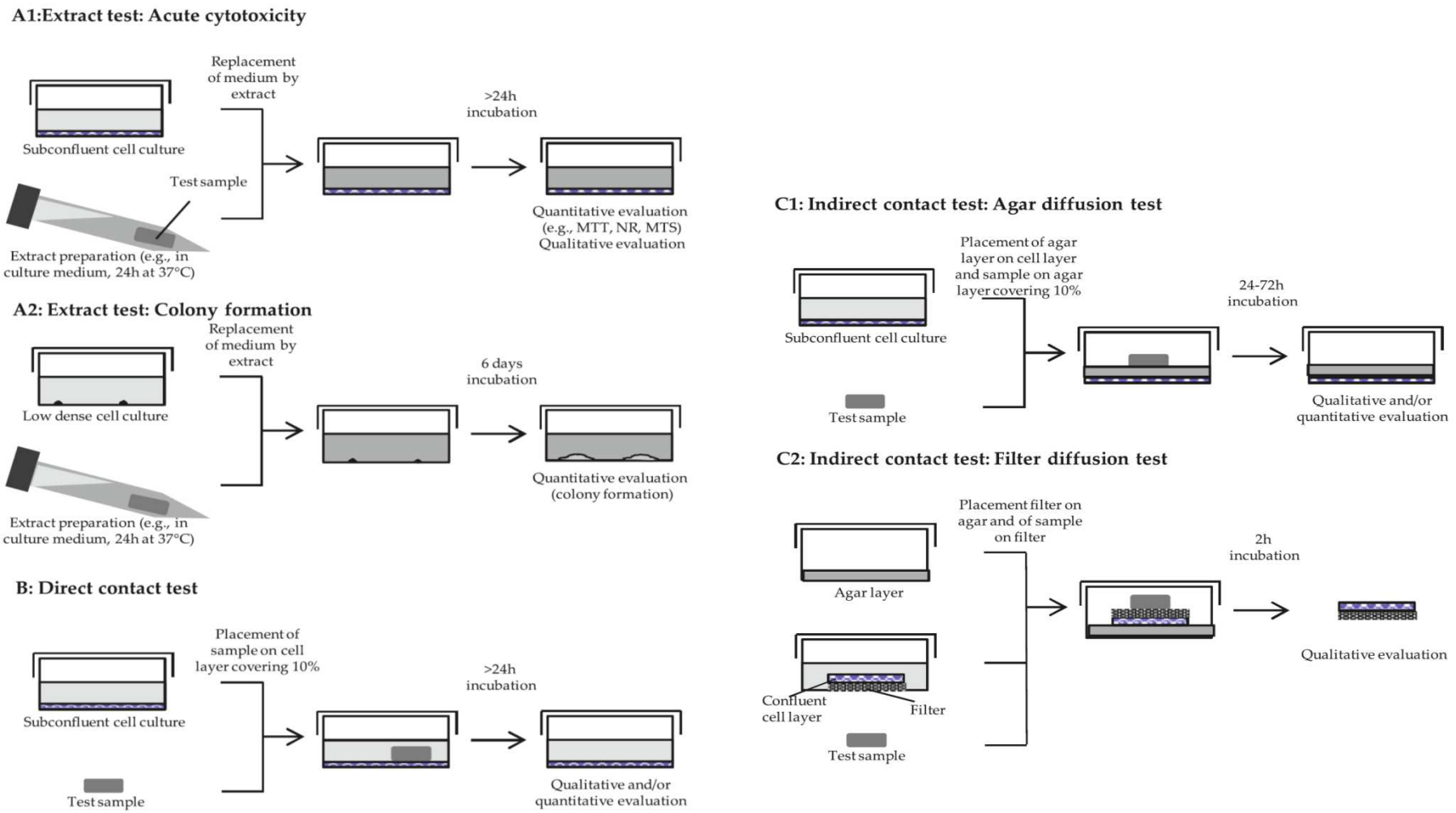
Testing According to ISO 10993-1

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

Standard Cytotoxicity Testing: ISO 10993-5

- *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

Standard Cytotoxicity Testing: ISO 10993-5



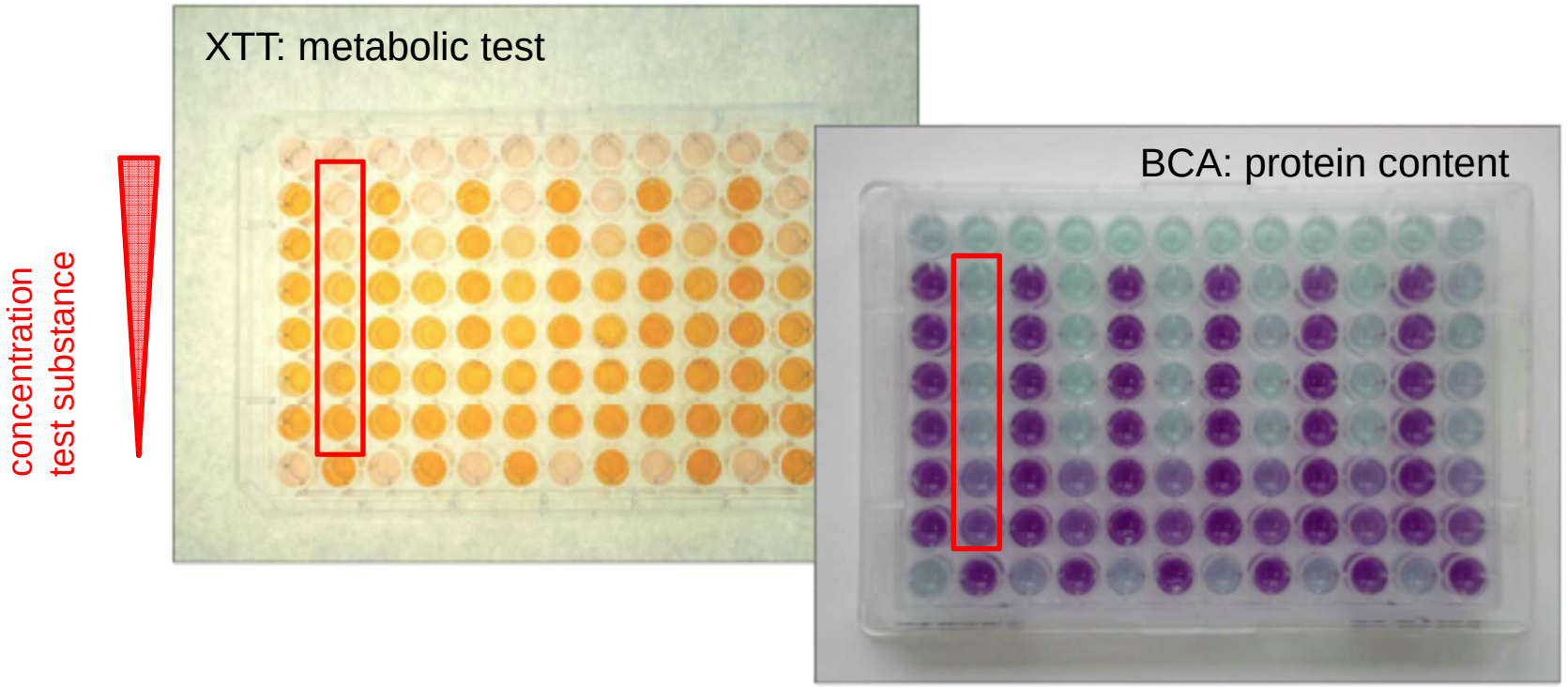
(Bruinink und Luginbuehl, Adv. Biochem. Engin/Biotechnol 2011)

Standard Cytotoxicity Testing: ISO 10993-5

- 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		2 ➔ mild effect	20-50%	4 ➔ severe effect	70-100%
1 ➔ slight effect	1- 20%	3 ➔ moderate effect	50-70%	each standard uses different classification	

Definition of Cytotoxic Effects

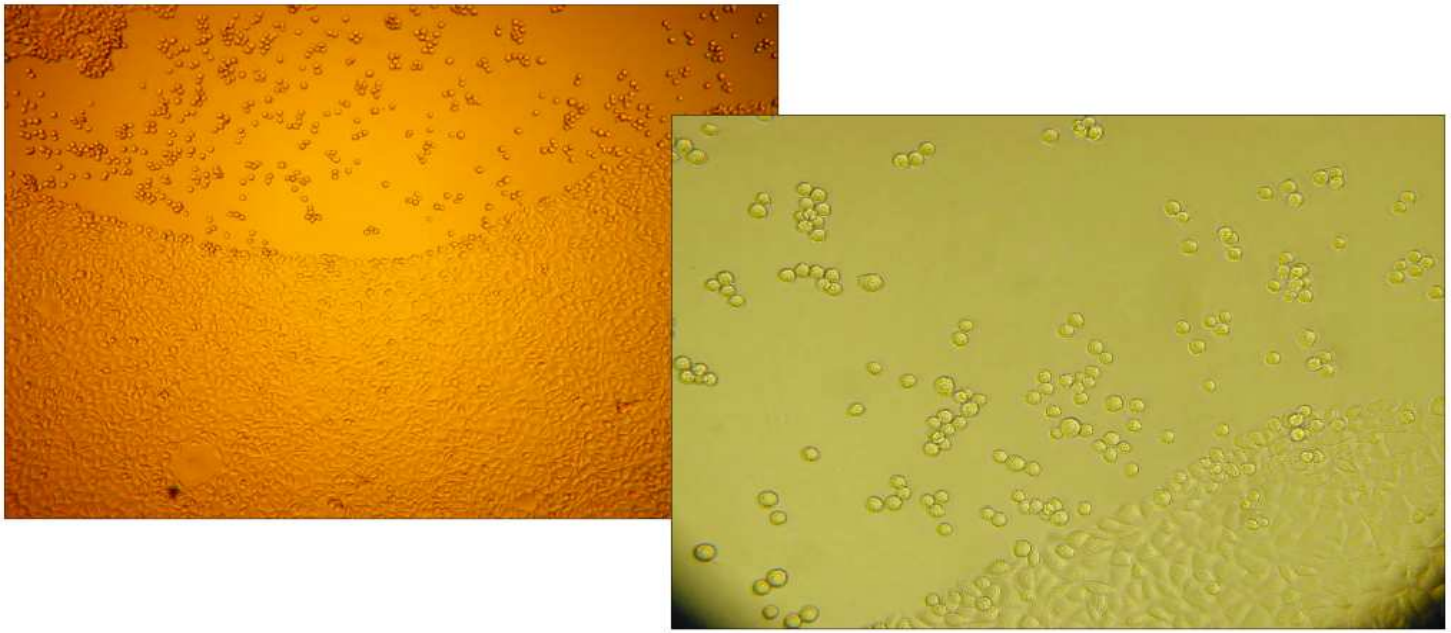
Quantitative assessment:
Reduction of cell viability/function by more than 30% considered cytotoxic.

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):

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

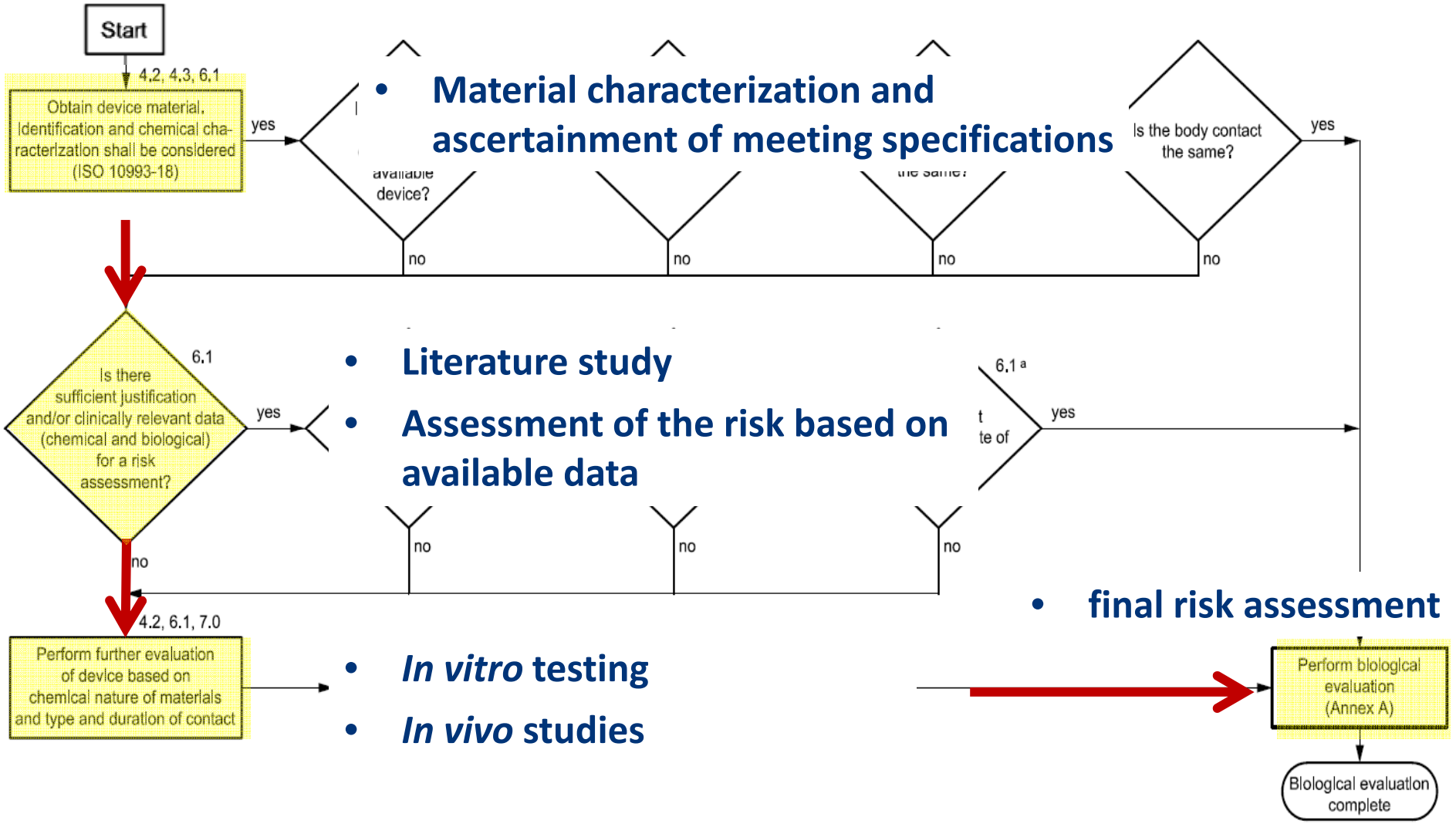
- Irritation (local body reaction; skin, intracutaneous, ocular…)
  (ISO 10993-10)
- Sensitization (systemic body reaction; allergic reactions)
- Acute, sub-acute, sub-chronic and chronic toxicity (i.v., i.p, dermal, oral…)
  (ISO 10993-11)
- Implantation / local tolerance (orthopedic implants, drug 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
- *In vitro* testing
- *In vivo* studies
- final risk assessment

Is in vivo Testing Predictive?

Subcutaneous implantation

9 Different Materials:

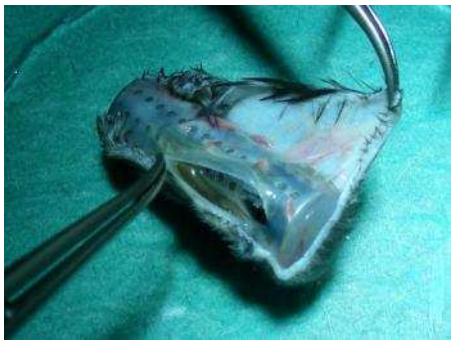
- Polyethylene
- Hydroxyapatite
- Polyurethane
- Silicone
- pHEMA
- PTFE (Gore-tex)
- Pyrolytic carbon
- Gold
- Titanium

Short term reaction:

- Differential protein adsorption
- Varied activation of host response

Long term reaction:

- Fibrous encapsulation



Hydrophilic
Hydrophobic
Metal
Polymer
Hard/Soft

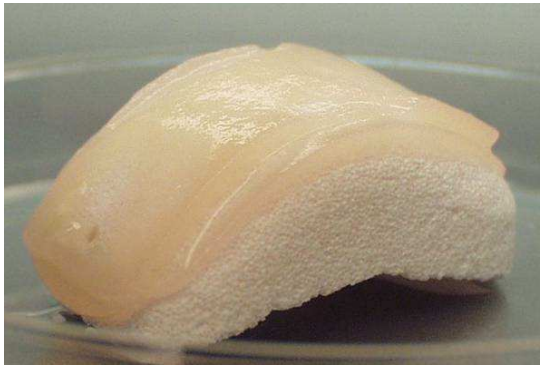
All have the same endpoint, but all materials can be considered biocompatible if no other host reaction occurs and device performance is not at risk

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

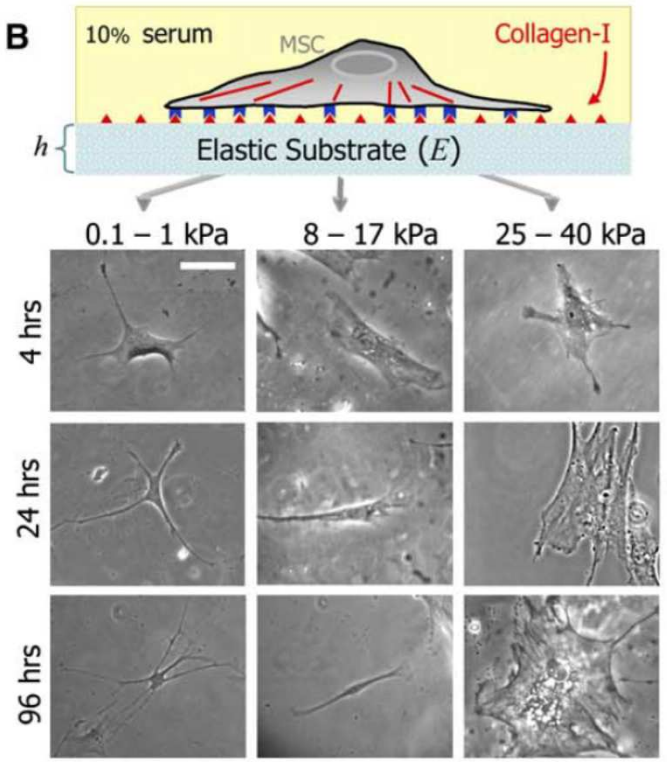
Cells: autologous or allogenous, progenitor cells or differentiated, pre-cultivated versus peri-harvested

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!

Cell Response to Modulation of Scaffold Elastic Properties

"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."



Engler et al, Cell, 126 (2006)

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*)."

Cell Response to Modulation of Scaffold Elastic Properties

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

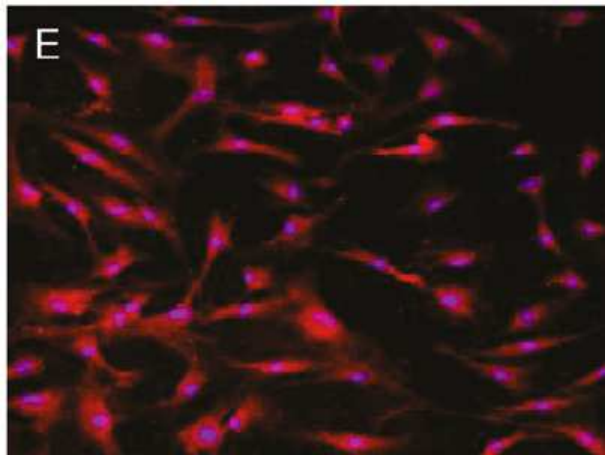
(based on original paper and cited papers)

The unknown side of the scaffold include:

- polymerization condition, e.g. time, temperature, monomer quality and stabilizator concentration, final composition incl. monomer content
- swelling behavior, porosity, cristallinity, molar mass
- real viscoelastic properties incl. bulk modulus and creep
- behavior of the MSC on control/reference material

Functionalization of Scaffold Materials

"**Surface functionalization of hydroxyapatite (HA) and β-tricalcium phosphate (TCP)** bioceramics with chemical ligands containing a pyrrogallol 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

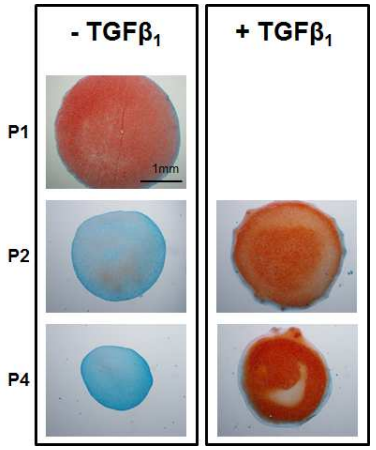
- nothing is known on the HA and TCP scaffold except to „densely sintered discs"
- no reference materials were used
- no cytotoxicity test according to international standards

(Borcard et al ,CHIMIA, 67/4, 2013)

Vast Selection of Scaffold Materials in Tissue Engineering

Table 2.2 Classification of polymeric scaffolds in cartilage regeneration

Class	Subclass	Chemical name	Acronym	Reference
Synthetic polymers		Polyester (general)		Mahmood et al. (2005)
		Polyamide		Deschamps et al. (2003), Springer et al. (2001)
		Polyurethane	PU	Eyrich et al. (2007), Gorna and Gogolewski (2006), Grad et al. (2005), Lee et al. (2005)
		Polymethylmetacrylate	PMMA	Barry et al. (2004)
		Polyethylene glycol (and copolymers)	PEG	Bryant and Anseth (2002), Deschamps et al. (2004), Holland et al. (2005), Lotz et al. (2009), Malda et al. (2005), Park et al. (2004)
		Polyethylene polypropylene	PE/PP	Schoen et al. (2009), Zhu et al. (2010)
		Polyethylene glycol terephthalate	PEGT	Malda et al. (2005), Woodfield et al. (2005)
		Polyhydroxybutyrate	PHA	Chen and Wu (2005), Deng et al. (2002), Valappil et al. (2006)
	Poly alpha hydroxyl acids	Polyvinylalcohol	PVA	Charlton et al. (2008), Cho et al. (2005), Grant et al. (2006), Jeong et al. (2008), Kondo et al., 2009, Lee et al. (2005, 2009), Oh et al. (2003), Serino et al. (2007)
		Polylactic acid	PLA	Agrawal and Ray (2001), Barnes et al. (2007), Coutts et al. (2001), Liu and Ma (2004), Lu et al. (2001), Ma et al. (2003), Piskin et al. (2007), Schek et al. (2004), Sherwood et al.



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”.

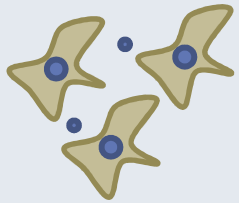
In most cases, the scaffolds were poorly characterized!

(Egli et al, International Review of Cell and Molecular Biology, Vol. 289, 2011)

The Future of *in vitro* Testing?

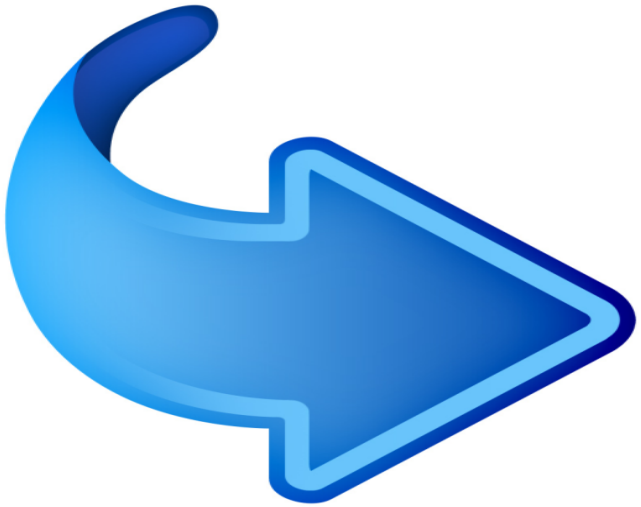
Today:

2D



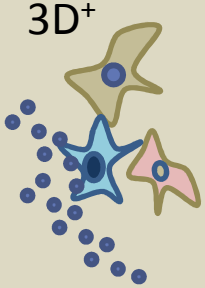
Too simple to be of prognostic value

In vitro tests



The Future:

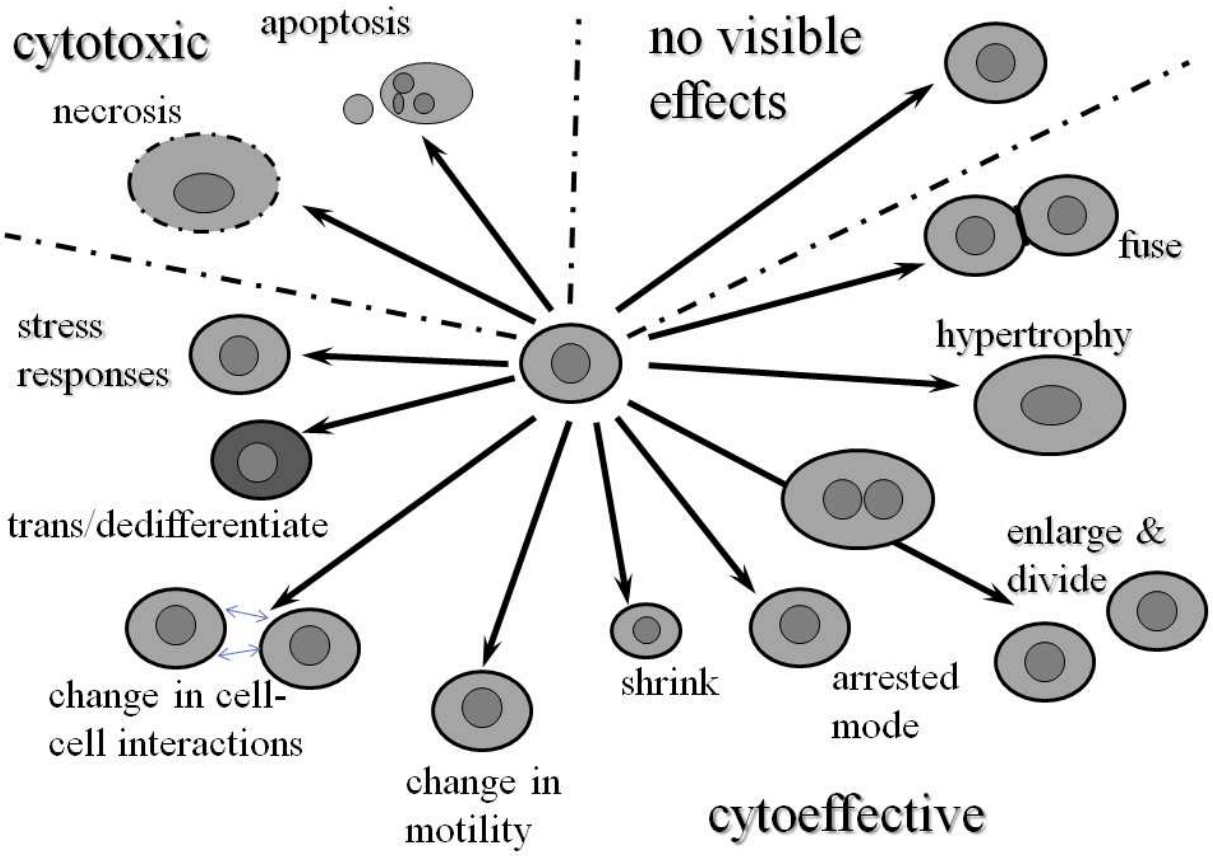
3D+



just as complex as required to be near physiological

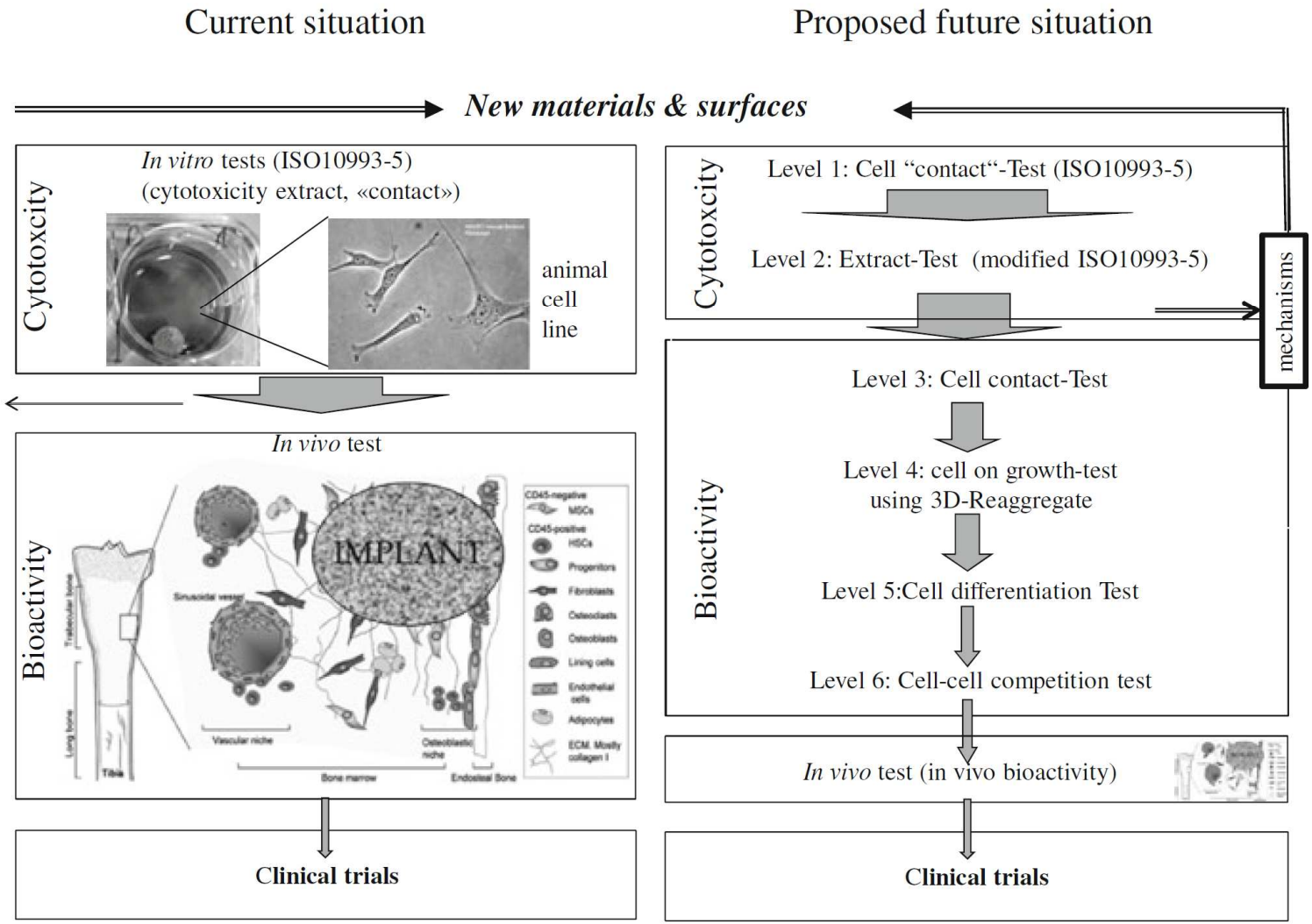
Bruinink and Luginbuehl, *Adv Biochem Engin/Biotechnol*, 2011

Cell Response to Materials and Pharmaceuticals



Bruinink and Luginbuehl, *Adv Biochem Engin/Biotechnol*, 2011

The Future of Biocompatibility Testing?



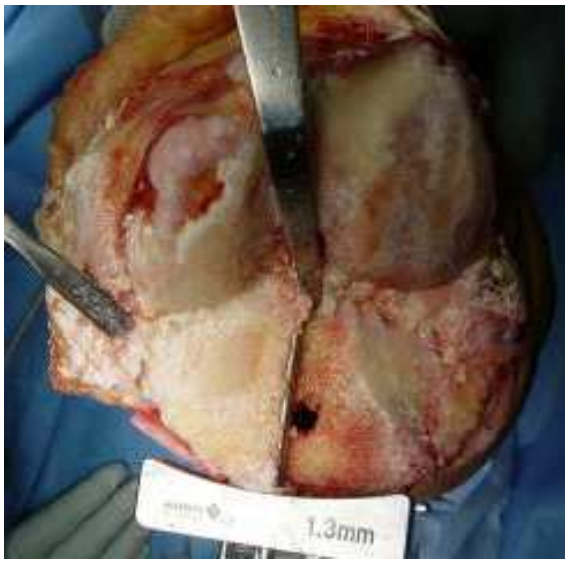
Bruinink and Luginbuehl Adv Biochem Engin/Biotechnol, Spring Verlag 2011

Is there such a Thing as a Biocompatible Material?

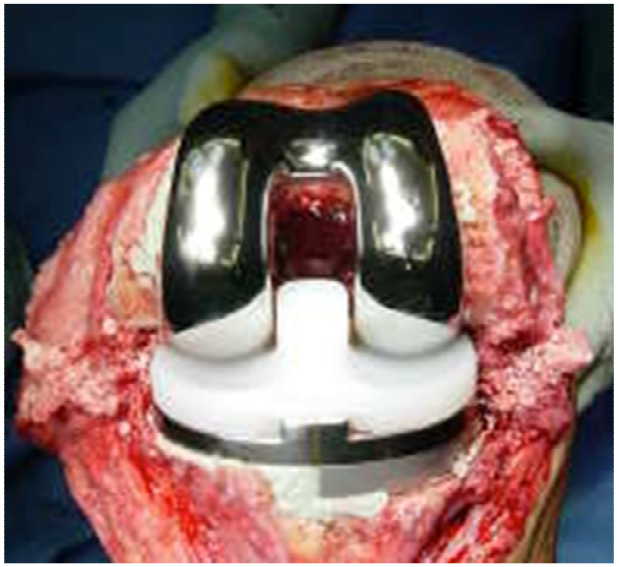
Biocompatibility subsumes a collection of individual phenomena and is impossible to quantify. There can be no scale of biocompatibility; therefore it is scientific nonsense to consider certain materials as 'biocompatible', occupying the ground at one end of a non-existent scale, and other materials as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

Today's Orthopedic Implants



Total Arthroplasty is an orthopedic success story, enabling hundreds of thousands of people to live fuller, more active lives.

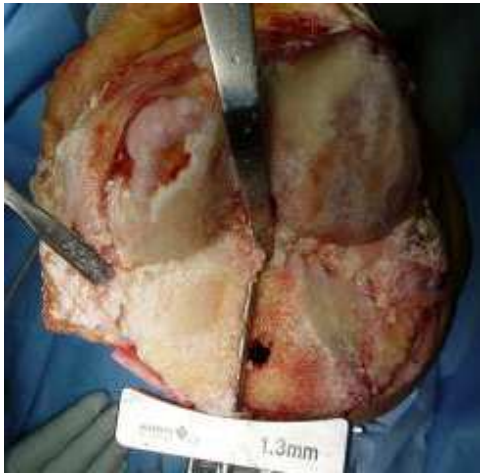


(photo courtesy MDs Schär, Zumstein Inselspital 2010)

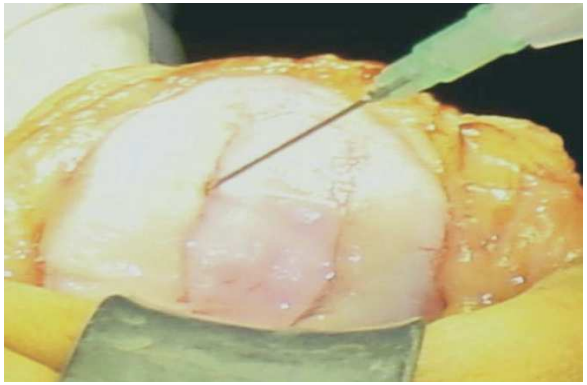
Total Arthroplasty is **a pure engineering solution** centered on material selection. Key issues preventing the perfect solution include:

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.



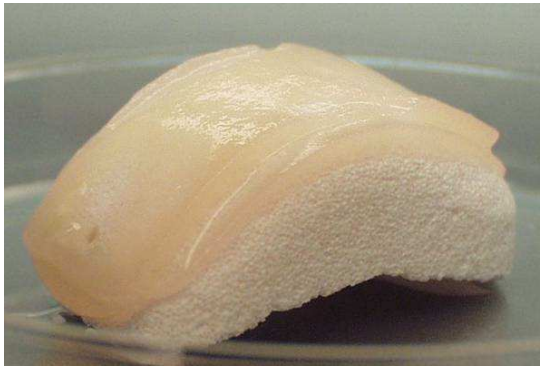
(photo Gibson et al, Operative Techniques 2006)

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 i**mpossible** 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