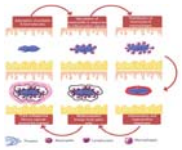
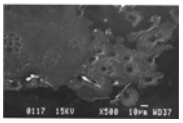
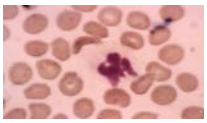
Introduction to BioMEMS & Medical Microdevices

Biocompatibility, FDA and the ISO 10993

Prof. Steven S. Saliterman, http://saliterman.umn.edu/







Topics

- Biocompatibility
- Foreign Body Giant Cells (FBGS)
- Biofouling
- FDA & ISO 10993 biocompatibility assessment
- Biological effects.

Biocompatibility

- Biocompatibility testing answers two fundamental questions:
  - Is the material safe?
  - Does it have the necessary physical and mechanical properties for its proposed function?
- The extent to which a material needs to be characterized depends on:
  - Type of material,
  - End use of the device (is it a medical device?),
  - Function of the material within the device.
  - Availability of existing data on the material.

Foreign Body Reaction

1. Phagocytic attack and encapsulation of a device.
2. When an implanted material contacts blood, a layer of host proteins adsorb to the material surface.
   - Proteins fibrinogen, fibronectin, and vitronectin; immunoglobulin G (IgG); and the complement-activated fragment C3b.
3. Neutrophil infiltration then leads to inflammation
   - Neutrophils normally phagocytose microorganisms and foreign bodies.
   - Monocytes, macrophages and lymphocytes lead to chronic inflammation.
   - Granulation tissue develops as endothelial cells and fibroblasts proliferate.

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Bryers, J., CM Giachelli BD Ratner. Engineering biomaterials to integrate and heal: the biocompatibity paradigm shifts. Biotechnology and Bioengineering. 109(8) 2012.

4. Macrophages fuse forming foreign body giant cells.
5. Fibrous encapsulation of the device occurs.

- All materials inside and outside the device, including materials encountered during the manufacturing and preservation process have a potential to evoke a *foreign body response*.

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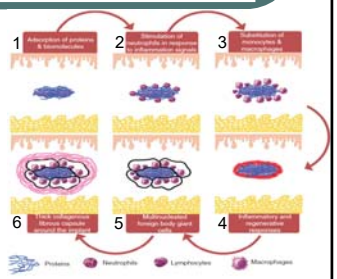
Host Foreign Body Response…

1. Adsorption of proteins & biomolecule,
2. Stimulation of neutrophils in response to inflammation signals.
3. Substitution of monocytes and macrophages.
4. Inflammatory & regenerative responses.
5. Multinucleated FNGC
6. Fibrous encapsulation.



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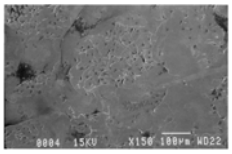
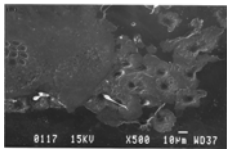
Barkam, S, et al. Fabricated micro-nano devices for in vivo and in vitro biomedical applications. WIREs Nanomed Nanobiotechnol 2013, 5:544–568

Foreign Body Giant Cells…

- SEM photomicrographs showing *fusion of macrophages into foreign body giant cells*:
  - Individual macrophage aggregation on silicon dioxide (day 7) (left).
  - Enlarging giant cell with fusion of cytoplasm and consolidation of nuclei (day 14) (right).





Voskerician, G., et al., Biocompatibility and biofouling of MEMS drug delivery devices. Biomaterials 24(11), 2003

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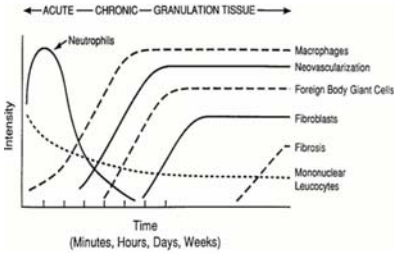
Cellular response to implanted materials…



Bryers, J., CM Giachelli BD Ratner. Engineering biomaterials to integrate and heal: the biocompatibiity paradigm shifts. Biotechnology and Bioengineering, 109(8) 2012.

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Biofouling

1. Biofouling is the process whereby functioning of a medical device is interfered with by the biological response of the host.
2. This commonly occurs when *macrophages* and *foreign body giant cells (FBGCs)* attach to the implanted device, accumulate, grow and interfere with normal operation.
3. Surface coating of biomaterials seems one good approach to lessen the inflammatory response, lessen macrophage adhesion and FBGCs growth, and improve wound healing.
4. The foreign body response by the host is largely independent of the material's being polymeric, ceramic or metallic; being hydrophobic or hydrophilic; or being hard or soft.

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5. The status of the proteins on a material surface is believed to determine the ultimate biocompatibility of a given polymer.
6. Producing a more biocompatible surface requires achieving specific responses between the polymer surface and the adjacent cells and to reduce non-specific interactions.
   - Methods include passivating the polymer surfaces to minimize non-specific protein interaction.
   - Functionalizing the polymer surface with biomolecules to induce specific protein adsorption and cell responses.

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Chen, H. et al. Biocompatible polymer materials: Role of protein–surface interactions. Progress in Polymer Science 33, pp 1059-1087 (2008).

Non-Fouling Surfaces…

- Non-fouling (i.e. protein adsorption-resistant) polymer coatings for biomaterials provides a more rigorous approach to reduce inflammatory responses. Such polymer surface coatings must satisfy the following constraints:
  - Use of nontoxic (biocompatible) materials:
  - Effectively inhibit in vivo biofouling,
  - Appropriate thickness and permeability to allow analyte transport,
  - Techniques to deposit coating onto a variety of materials and architectures.
  - Must be mechanically, chemically, and electrically robust to withstand surface deposition, sterilization methods, implantation procedures, and in vivo environment.
  - Polyethylene glycol (PEG), $HO(-CH_2CH_2O-)_nH$, is an example.

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Historical Observations…

- Hydrophobic surfaces tend to absorb proteins.
  - From unfolding of proteins on the surface and release of bound water molecules.
- Cationic proteins bind to anionic surfaces and anionic proteins bind to cationic surfaces.
- Proteins tend to adsorb in monolayers.
- A minimum PEG molecular weight is required to provide good protein repulsion (500-2000).
  - Mechanism may be resistance of the polymer coil to compression.

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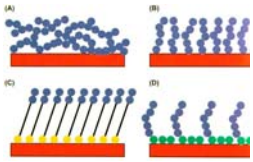
Producing Non-Fouling Surfaces…



Purple dots are non-fouling chemical moiety such as $(CH_2CH_2O)_n$ (Polyethylene glycol)

(A) Cross-linked network of long polymeric chains.
(B) Polymers grown off the surface.
(C) Oligo-non-fouling headgroups on a self-assembled monolayer (such as thiol)
(D) Surfactant absorbed to the surface (green dots are hydrophobic tails).

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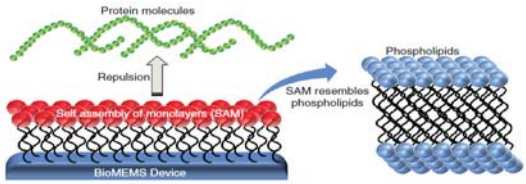
Ratner, B.D., A.S. Hoffman, F.J. Schoen, J.E. Lemons, *Biomaterials Science*, Academic Press, New York (2013).

Surface Engineering…



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FDA (US Food & Drug Administration)…

Use of International Standard ISO 10993-1, "Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process"

Guidance for Industry and Food and Drug Administration Staff

U.S. FOOD & DRUG ADMINISTRATION

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ISO 10993 Biocompatibility Assessment

- The ISO 10993 International Standard pertains to:
  - Surface devices on the skin, mucosal membranes, breached or compromised surfaces.
  - External communicating devices with blood, tissue, bone, dentin.
  - Implantable devices.
- Its purpose is to protect humans and to serve as a framework for selecting tests to evaluate biological responses.
- In so doing consideration has been given to minimize the number and exposure of test animals.

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Table A.1: Biocompatibility Evaluation Endpoints

Medical device categorization by			Biological effect														
Nature of Body Contact		Contact Duration	Cytotoxicity	Sensitization	Irritation or Intracutaneous Reactivity	Acute Systemic Toxicity	Material-Mediated Pyrogenicity	Subacute/Subchronic Toxicity	Genotoxicity	Implantation	Hemocompatibility	Chronic Toxicity	Carcinogenicity	Reproductive/Developmental Toxicity#	Degradation#
Category	Contact	A – limited (≤24 h) B – prolonged (>24 h to 30 d) C – permanent (> 30 d)															
Surface device	Intact skin	A	X	X	X												
		B	X	X	X												
		C	X	X	X												
	Mucosal membrane	A	X	X	X												
		B	X	X	X	O	O	O		O							
		C	X	X	X	O	O	X	X	O			O				
	Breached or compromised surface	A	X	X	X	O	O										
		B	X	X	X	O	O	O		O							
		C	X	X	X	O	O	X	X	O			O	O			
External communicating device	Blood path, indirect	A	X	X	X	X	O				X						
		B	X	X	X	X	O	O			X						

Biological Effects
Cytotoxicity
Sensitization
Irritation – Intracutaneous Reactivity
Acute Systemic Toxicity
material-Mediated Pyrogenicity
Subacute/Subchronic Toxicity
Genotoxicity
Implantation
Hemocompatibility
Chronic Toxicity
Carcinogenicity
Reproductive/Development
Degradation

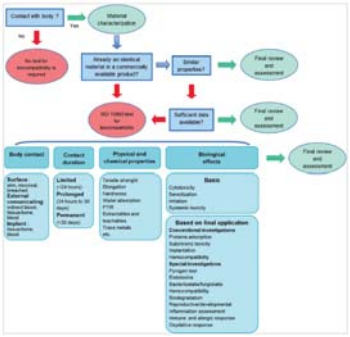
Use of International Standard ISO 10993-1, "Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process", Guidance for Industry and Food and Drug Administration Staff Document issued on: September 4, 2020

Medical device categorization by			Biological effect														
Nature of Body Contact		Contact Duration	Cytotoxicity	Sensitization	Irritation or Intracutaneous Reactivity	Acute Systemic Toxicity	Material-Mediated Pyrogenicity	Subacute/Subchronic Toxicity	Genotoxicity	Implantation	Hemocompatibility	Chronic Toxicity	Carcinogenicity	Reproductive/Developmental Toxicity#	Degradation#
Category	Contact	A – limited (≤24 h) B – prolonged (>24 h to 30 d) C – permanent (> 30 d)															
External communicating device (continued)		C	X	X	O	X	O	X	X	O	X	O	O				
	Tissue*/bone/ dentin	A	X	X	X	O	O										
		B	X	X	X	X	O	X	X	X							
		C	X	X	X	X	O	X	X	X		O	O				
	Circulating blood	A	X	X	X	X	O		O		X						
		B	X	X	X	X	O	X	X	X	X						
		C	X	X	X	X	O	X	X	X	X	O	O				
Implant device	Tissue*/bone	A	X	X	X	O	O										
		B	X	X	X	X	O	X	X	X							
		C	X	X	X	X	O	X	X	X		O	O				
	Blood	A	X	X	X	X	O		O	X	X						
		B	X	X	X	X	O	X	X	X	X						
		C	X	X	X	X	O	X	X	X	X	O	O				

Biological Effects
Cytotoxicity
Sensitization
Irritation – Intracutaneous Reactivity
Acute Systemic Toxicity
material-Mediated Pyrogenicity
Subacute/Subchronic Toxicity
Genotoxicity
Implantation
Hemocompatibility
Chronic Toxicity
Carcinogenicity
Reproductive/Development
Degradation

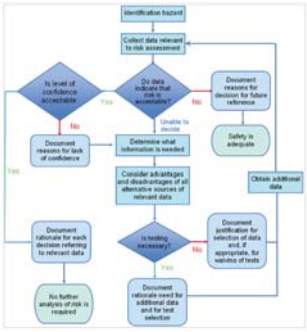
X = ISO 10993-1:2009 recommended endpoints for consideration*
O = Additional FDA recommended endpoints for consideration*

Decision Tree According to Use (All Materials)



Bernard, M, E. Jubeli, D Pungente and N Yogoubi. Biocompatibility of polymer-based biomaterials and medical devices – regulations, in vitro screening and risk-management. Biomater. Sci., 2018, 6, 2025–2053

Approach to Documentation (All Materials)



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Characterization Methods

- Identification of a materials constituents and:
  - Changes of the material over time,
  - Changes with exposure to different environments,
  - Lot-to-lot consistency for manufacturing purposes.
- Methodologies:
  - Infrared spectral analysis (IR),
  - Thermal analysis,
  - Density analysis,
  - Molecular weight distribution,
  - Mechanical properties,
  - Surface properties,
  - Extract Characterization – Gas & Liquid Chromatography.

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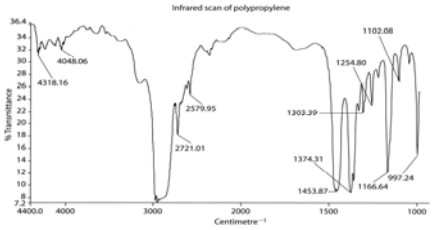
Infrared Spectral Analysis (IR)…

Wave number vs % Transmission



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Albert, DE. "The important role of material and chemical characterization in device evaluation. *Medical Device Technology*, Octo Media Ltd, June 2004

- Useful in material identification and for following polymer degradation.
- Molecules absorb specific frequencies that are characteristic of their structure. These absorptions are resonant frequencies, i.e. the frequency of the absorbed radiation matches the transition energy of the bond or group that vibrates.
- A basic IR spectrum is essentially a graph of infrared light absorbance (or transmittance) on the vertical axis vs. frequency or wavelength on the horizontal axis.
- Typical units of frequency used in IR spectra are reciprocal centimeters (sometimes called wave numbers), with the symbol cm^{-1}

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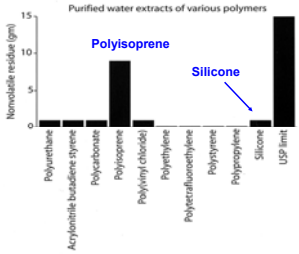
Purified Water Extracts…



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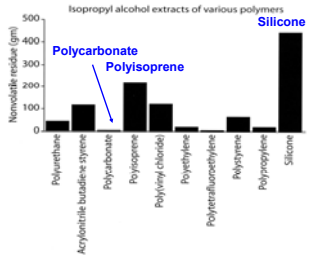
Isopropyl Alcohol Extracts…



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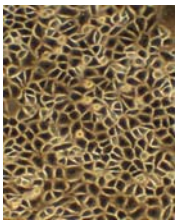
Cytotoxicity

- Cytotoxicity refers to cell damage caused by materials, either by direct contact or by leachable substances (extracts).
- Cell damage may occur by a variety of means including activation of the complement system.
  - The complement system involves serum factors that are activated in the presence of antigen-antibody binding, bacteria and viruses, or foreign materials.

Mouse Fibroblast Cells…



Normal

Cytotoxicity
Exposure to Toxic Extract

Image Courtesy of NAMSA - North American Science Associates

Sensitization

- Sensitization refers to a materials ability to *induce* specific *delayed-type hypersensitivity* in the body upon initial exposure:
  - Haptens,
  - Langerhans cells and T-cell lymphocytes,
  - Lymphokines.
- Testing:
  - Guinea pig maximization test (GPMT),
  - Closed-patch test (Buehler test),
  - Murine Local Lymph Node Assay.

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Guinea Pig Maximization Test …

- For the induction phase, a sample volume of 0.1 mL is injected at the prepared site. Seven days later the topical induction is performed.
  - A saturated filter paper or gauze is applied over the skin injection site by an occlusive dressing and torso wrap for a period of two days.
- The challenge phase is performed two weeks later by administering the sample topically to sites not previously used for the induction phase.
  - The dressings are applied for one day and removed.
  - The appearance of the skin is then reviewed and graded at one and two days.

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Irritation

- Irritation refers to a *non-specific inflammatory* response to a single, repeated or continuous application of a material.
- Areas tested:
  - Skin,
  - Eyes,
  - Oral mucosa,
  - Genitalia,
  - Rectum.
- Rabbits and human subjects are often used.

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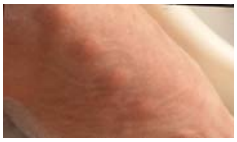
Dermal Irritation…



- Shown is erythema and edema (swelling) about the intracutaneous injection sites on a rabbit test animal.
- For rabbits, the samples may be solid, powders or liquids and are applied directly to the skin for 4 hours.
- The appearance at each application site is assessed at 1, 24, 48 and 72 hours. Repeated exposure may be performed.

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Systemic Toxicity – Whole Body

- Systemic toxicity (body at large):
  - *Acute toxicity* - within 24 hours,
  - *Subacute toxicity* - single dose or multiple doses of a test sample during a period from 14 to 28 days,
  - *Subchronic toxicity* - at 90 days, but not exceeding 10% of the life cycle of the device,
  - *Chronic toxicity* - single or multiple exposures to medical devices, materials and extracts during at least 10% of their lifespan of the test animal.

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Genotoxicity

- Gene or point mutations, small deletions, mitotic recombination or microscopically visible chromosome changes.
- Studies available:
  - Ames bacterial reverse mutation assay,
  - Mouse lymphoma assay,
  - Chinese hamster ovary cells,
  - Mouse bone marrow micronucleus test.

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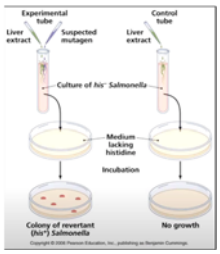
Ames Test…



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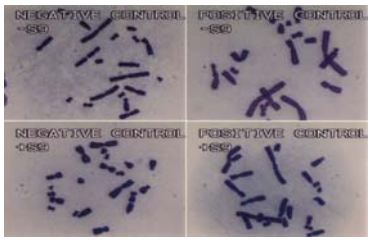
https://youtu.be/9sCHHD5dqy0

Chromosomal Aberrations…



On the left are negative controls, and on the positive controls, the latter showing a karyotype with chromosomal aberrations.

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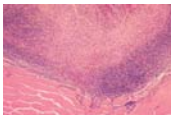
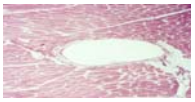
Image Courtesy of NAMSA

Implantation Histological Changes

Slight irritation - mild infiltration of lymphocytes



Severe lymphohistiocytic response.



Fibrous encapsulation around a previously implanted test material.

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Image courtesy of NAMSA

Types of Histological Findings…

- The extent of fibrous capsular involvement around the device and adjoining tissue.
- Tissue inflammatory changes, including polynuclear leucocytes, lymphocytes, plasma cells, eosinophils, macrophages and multinucleated cells.
- Presence of tissue necrosis, capillary wall breakdown or other deterioration.
- Material debris, fatty infiltration and granuloma formation.
- Quality and quantity of tissue ingrowth into porous materials.

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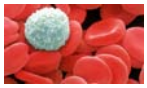
Hemocompatibility

- Hemocompatiblity tests evaluate the effects of medical devices or materials that are in contact (or indirect contact) with blood, on blood components.
  - Hemolysis is the abnormal breakdown of blood cells.
  - Thrombosis is the clotting of blood with obstruction of a blood vessel and potential for embolization.



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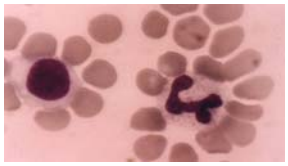
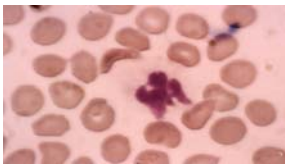
msn. Encarta

Toxin Exposure…



Normal Red and White Blood Cells



White Blood Cell Karyorrhexis (breakdown of the nucleus)

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Image courtesy of NAMSA

Degradation

- Degradation is the unwanted breakdown of implanted medical device materials.
- Ideally in an implanted device all materials of degradation are ultimately removed by the body without toxicity.
- Include:
  - Polymer degradation.
  - Ceramic degradation.
  - Metal and alloy electrochemical effects,

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Summary

- Biocompatibility
- Foreign Body Giant Cells (FBGS)
- Biofouling
- FDA & ISO 10993 biocompatibility assessment
- Biological effects.
- Addendum:
  - Supplemental material on biological effects.
  - Tested materials.

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ISO Definition of a Medical Device

- Any instrument, apparatus, appliance, material or other article, including software, whether used alone or in combination, *intended by the manufacturer to be used for human beings (in-vivo)* solely or principally for the following purposes:
  - Diagnosis, prevention, monitoring, treatment or alleviation of disease;
  - Diagnosis, monitoring, treatment, alleviation of or compensation for an injury or handicap;
  - Investigation, replacement or modification of the anatomy or of a physiological process;
  - Control of conception.

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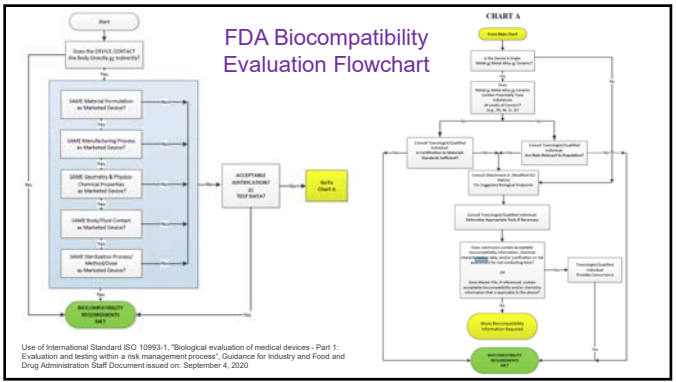
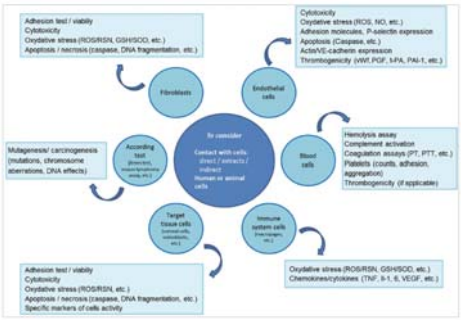
FDA Biocompatibility Evaluation Flowchart



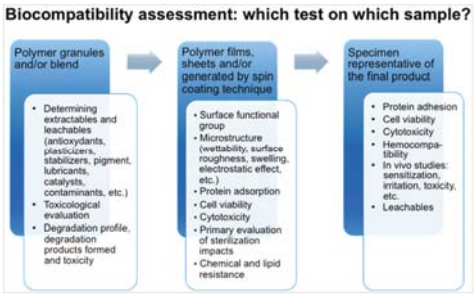
Use of International Standard ISO 10993-1, "Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process", Guidance for Industry and Food and Drug Administration Staff Document issued on: September 4, 2020

In-Vitro Tests for Biocompatibility Assessment and Conventional Cell Model Choice (All Materials)



Bernard, M, E. Jubeli, D Pungente and N Yogoubi. Biocompatibility of polymer-based biomaterials and medical devices – regulations, in vitro screening and risk-management. Biomater. Sci., 2018, 6, 2025–2053

Polymers as an Example



Bernard, M, E. Jubeli, D Pungente and N Yogoubi. Biocompatibility of polymer-based biomaterials and medical devices – regulations, in vitro screening and risk-management. Biomater. Sci., 2018, 6, 2025–2053

Cytotoxicity Assessment…

- Determination of cytotoxicity includes:
  - Microscopic (qualitative) evaluation
    - Morphology
    - Vacuolization
    - Detachment
    - Cell lysis
    - Membrane integrity
  - Quantitative evaluation
    - Cell death
    - Inhibition of cell growth
    - Cell proliferation
    - Cellular secretions

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Implantation

- Tests for assessment of the local effects of implant material on living tissue.
- Comparison is made with reactions observed to medical devices whose clinical acceptability has already been established.
- Short term studies of less than 12 weeks implantation, and long-term studies of greater than twelve weeks may be performed.
- Solid implant materials for testing must be prepared in the same manner as they are intended for implantation, including form, density, hardness, surface finish, sterilization, and handling.
- Non-solid materials such as liquids, pastes and particulates may also be used, and be contained in polyethylene, polypropylene or polytetrafluoroethylene tubes. Controls of similar size, shape of surface finish should be used.

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Polymer Degradation

- Chemical bond scission due to hydrolytic and oxidative processes.
- Enzymes, proteins and other cellular activity can alter the rate and nature of degradation.
- Ultraviolet cleavage of chemical bonds.
- Gamma and electron radiation that cause embrittlement, discoloration and thermal instability.
- Metal induced degradation from impurities, additives or hybrid construction.

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Tested Materials

- Biosensors
- Stents
- Micro-Nano-Needles
- Micro-Nano-Reservoirs
- Micro-Nano-Pumps
- Micro-Nano-Actuators
- Tissue Engineering
- Coatings
- Self-Assembled Monolayers
- ISO 10993 Parts
- Biocompatibility Testing of Polymers

NFS Compositions…

TABLE I.2.10.2 "Non-Fouling" Surface Compositions

Synthetic Hydrophilic Surfaces
- PEG polymers and surfactants
- Neutral polymers
  poly(2-hydroxyethyl methacrylate)
  polyacrylamide and poly(N-methyl acrylamide)
  poly(N-vinyl 2-pyrrolidone)
  poly(N-isopropyl acrylamide) (below 31°C)
- Anionic polymers
- Phosphoryl choline polymers
- Sulfobetaines, carboxybetaines, taurine-functionalized materials
- Poly(2-methyl-2-oxazoline)
- Gas discharge-deposited coatings (especially from PEG-like monomers)
- Self-assembled n-alkyl molecules with oligo-PEG headgroups

Natural Hydrophilic Surfaces
- Passivating proteins (e.g., albumin and casein)
- Polysaccharides (e.g., hyaluronic acid)
- Liposaccharides
- Phospholipid bilayers
- Glycoproteins (e.g., mucin)

Other Molecules (see Ostuni et al., 2001).

Ratner, B.D., A.S. Hoffman, F.J. Schoen, J.E. Lemons, *Biomaterials Science*, Academic Press, New York (2013).

Thermodynamics of Protein Absorption…

TABLE I.2.10.1 Thermodynamics of Protein Adsorption

Favoring Adsorption

ΔH_{ads}
- van der Waals interactions (short range)
- ion–ion interactions (long range)

ΔS_{ads}
+ desorption of many H_2O's
+ unfolding of protein

Opposing Adsorption

ΔH_{ads}
+ dehydration (interface between surface and protein)
+ unfolding of protein
+ chain compression (PEO)

ΔS_{ads}
- adsorption of protein
- protein hydrophobic exposure
- chain compression (PEO)
- osmotic repulsion (PEO)

Ratner, B.D., A.S. Hoffman, F.J. Schoen, J.E. Lemons, *Biomaterials Science*, Academic Press, New York (2013).

Biosensors

Devices	Material	Functionality	*In Vivo* and *In Vitro* Testing Model	Results
Biosensor	Zinc oxide nanowire	Intracellular K^+ detection	*In vitro* on human adipocytes and frog oocytes cells	Successfully measured K^+ ions concentration with small interference from other species
	Epoxy-polyurethane membrane	Protective membrane for Ag/AgCl reference electrode	*In vivo* subcutaneously in rats	Stable for 4–8 months
	Polydimethyl siloxane (PDMS)	Noninvasive contact lens	*Ex vivo* on rabbit eyes	Successfully measured glucose concentration
	Sulfonic acid functionalized hydroxyl-terminated hyperbranched polyester	Antibiofouling coating	*In vitro* test on blood	Successfully integrated biocompatible, antibiofouling, and anticoagulation coating on biosensor
	Parylene probes with Platinum electrode	Cyborg eyes	*In vivo* on Zophobas morio beetles	Exhibited stable chronic insect sensory recordings
	Stent-316 stainless steel, Ti-Ni-Ta alloy. Au-Cr film	Stent-type thermal flow for measuring nasal respiration	—	Response time 260 milliseconds. and output frequency of 2 Hz

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Barkam, S, et al. Fabricated micro-nano devices for in vivo and in vitro biomedical applications. *WIREs Nanomed Nanobiotechnol* 2013, 5:544–568

Stents

Devices	Material	Functionality	*In Vivo* and *In Vitro* Testing Model	Results
Stents	Poly (D,L-lactic-co-glycolic acid) (PLGA) with sirolimus and triflusal	Bare metal stent	*In vivo* on porcine carotid artery model	Exhibited significant reduction in restenosis
	Formula 418 Cook medical	FDA approved stent	*In vivo* on American Yorkshire pig	Successful reception of wireless data up to a range of 50 cm
	Liquid crystal polymer (LCP)	Hermetic packaging for wireless housing	*In vivo* on domestic pigs	Enabled precise and accurate measurements of wireless transmitted data
	Polyethylene stent with magnetoelastic sensor array	Wireless transmission	*In situ* on female domestic swine	Successful reception of wireless signals up to a range of 7.5 cm

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Micro-Nano Needles

Devices	Material	Functionality	*In Vivo* and *In Vitro* Testing Model	Results
Micro-Nano needles	Carbon nanosyringe arrays	Intracellular delivery	*In vitro* on cancer cells and human mesenchymal stem cells	Successfully delivered different cargos in cytoplasm of cell
	AFM silicon tip to nanoneedle	Intracellular delivery	*In vitro* on HeLa cells	Delivered proteins directly in cell
	MWCNT nanoneedle attached to tungsten tip	Intracellular biosensing	—	Exhibited detection of Glucose, ascorbic acid, cytochrome in volumes <1 pL
	Silicon based microneedle array	Intradermal delivery	*In vivo* on mice skin	Successfully delivered protein to dermal layer
	Electrodeposited metal microneedle	Intradermal delivery	*In vivo* on guinea pig skin	Delivered 90% of cargo within the skin
	Ultra-sharp silicon microneedle	Transdermal delivery	*In vivo* on human skin	Force of insertion of single microneedle less than 10nN.
	Hollow silicon microneedles	Transdermal delivery	*In vivo* on diabetic rat	Unsuccessful delivery of insulin

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Micro-Nano-Pumps

Devices	Material	Functionality	*In Vivo* and *In Vitro* Testing Model	Results
Micro-Nano pumps	Polyetheretherketone (PEEK)	Material for tubing and fittings	Single cell manipulation	Achieved 98.5% cell manipulation success rate
	Poly(methyl methacrylate) (PMMA)	Diaphragm material	Single cell manipulation	1.3 mm thick diaphragm and dispensed volume varied from 500 pL to 250 nL at flow rate of 250 nL/s.
	Parylene C	Material for bellow fabrication, housing for actuator assembly in electrochemical pumps	*In vivo* subcutaneous drug delivery in Mice	Measured flow rates varied from 1 μL to 34 μL/min and power required is less than 3 mW.
	Silicone	Material for catheter	*In vivo* subcutaneous drug delivery in Mice	Minimized flow resistance and demonstrated optimal delivery to site with one sided valve in catheter to avoid back flow
	SAM-N-(triethosilylpropyl)-O-polyethylene oxide Urethane (PEOU)	Antithrombogenic coating	*Ex vivo* on rats	Imparted hemocompatibility to silicon based device

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Micro-Nano-Actuators

Devices	Material	Functionality	*In Vivo* and *In Vitro* Testing Model	Results
Micro-Nano actuators	Electrostatic comb-drive	Mechanical testing of single cells	*In vitro* on epithelial cells	Measured stiffness, hysteresis, and visco-elasticity of adherent cells
	Polyvinylidene fluoride with silver electrode encapsulated in PMMA	Induces bone formation	*In vivo* in right hind limb of Merino ewe	Piezoelectric actuator stimulated bone growth and exhibited bone area increment
	C2C12-collagen film integrated with Silicon MEMS device	Molecular actuator utilizing glucose as power source	—	Depicted successful locomotive motion
	Parylene C encapsulated nickel based magnetic actuator	Clearing biological accumulation on catheter pore	*In vitro* on murine vascular smooth muscle cell line	Magnetic actuator successfully removed cellular accumulation
	Polypyrole based electrostatic actuation	Actuation controlled catheter tip movement	—	Required faster actuation speed to image using catheter.

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Micro-Nano-Reservoirs

Devices	Material	Functionality	*In Vivo* and *In Vitro* Testing Model	Results
Micro-Nanoreservoirs	Gold	Sacrificial anode in controlled-release microchip	*In vivo* subcutaneously in Female rats	Gold corrosion found to be biocompatible. Voltage application reduced leukocyte concentration to control level
	Poly(l-lactic acid) (PLLA)	Biodegradable microreservoir body	*In vivo* subcutaneously in female Sprague-Dawley rats	PLA showed slow degradation as compared to the PLGA membrane.
	Poly(lactic-co-glycolic acid) (PLGA)	Biodegradable membrane for microreservoir	*In vivo* subcutaneously in female Sprague-Dawley rats	Different molecular weights of membrane degrades over a span of time and showed pulse drug delivery behavior
	Carbon nanotubes coated by Poly (lactide)-poly(ethylene glycol) CNT-PLA-PEG	Nanoreservoir	*In vivo* in C57BL/6 mice	Biocompatible polymer coated CNTs showed less toxicity in *in vivo* and *in vitro*. Increased the drug efficiency from 12 to 50%.

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Barkam, S, et al. Fabricated micro-nano devices for in vivo and in vitro biomedical applications. *WIREs Nanomed Nanobiotechnol* 2013, 5:544–568

Tissue Engineering

Devices	Material	Functionality	*In Vivo* and *In Vitro* Testing Model	Results
Tissue engineering	Core-polyethylene glycol/polyacrylic acid	Artificial cornea	*In vitro* for core-corneal epithelium cells for periphery-corneal fibroblasts cells	Successfully demonstrated cell growth with contingency on covalent tethering of collagen
	Periphery poly(hydroxyethyl acrylate)			
	Resorbable chitosan	Increases implants stiffness, adds hemostatic and antiseptic properties and improves adhesion	*In vivo* in Wistar rats	Successful recording of physiological signals for over 12 months
	Stroma tissue	Artificial human cornea	*In vitro* fibroblast culture to reconstruct stromal tissue	Successfully bioengineered cornea and seeded endothelial and epithelial cells.
	Biodegradable polyurethane scaffolds	Enhancement of mechanical properties	*In vivo* on skin of Wistar rats	Completely resorbed scaffold after 3 months with slight inflammatory response

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Barkam, S, et al. Fabricated micro-nano devices for in vivo and in vitro biomedical applications. *WIREs Nanomed Nanobiotechnol* 2013, 5:544–568

Coatings

Devices	Material	Functionality	*In Vivo* and *In Vitro* Testing Model	Results
Coatings	Dexamethasone loaded nitrocellulose coatings	Anti-inflammatory coating for neural implants	*In vivo* on adult male Sprague-Dawley rats	Sustained drug release for over 16 days
	Micropatterned titanium dioxide	Anti-inflammatory	*In vitro* on human neutrophils from blood	Pattern size dependent anti-inflammatory coating
	Poly(N-isopropylacrylamide) hydrogel microparticles	Anti-inflammatory	*In vivo* on murine peritoneal cavity	Reduced leukocyte adhesion and attenuated the expression of pro-inflammatory cytokines
	Nanosilver	Anti-inflammatory	*In vivo* on Sprague-Dawley female rat	Reduced cytokine concentration and lowered lymphocyte and mast cell infiltration
	Perfluorocarboxylate ionomer (PFCI)	Anticracking	*In vivo* subcutaneously in Guinea pigs	Reduced mineralization on coating and exhibited better cracking resistant property

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Barkam, S, et al. Fabricated micro-nano devices for in vivo and in vitro biomedical applications. *WIREs Nanomed Nanobiotechnol* 2013, 5:544–568

Coatings Continued

Devices	Material	Functionality	*In Vivo* and *In Vitro* Testing Model	Results
	Carboxymethyl-PEG-carboxymethyl	Antibiofouling	*In vitro* on blood samples	Exhibited hemocompatibility
	NDGA porous collagen scaffolds	Angiogenesis stimulating antibiofouling and	*In vivo* subcutaneously in rats	Coating showed stability for 4 weeks
	Boophilin coated ethylene glycol	Hemocompatible	*In vitro* activity assay	Better anti fouling performance as compared to PEO brush

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Barkam, S, et al. Fabricated micro-nano devices for in vivo and in vitro biomedical applications. *WIREs Nanomed Nanobiotechnol* 2013, 5:544–568

Self-Assembled Monolayers

Devices	Material	Functionality	*In Vivo* and *In Vitro* Testing Model	Results
Self-assembled monolayer	Flufenamic acid coated 11-mercapto-1-undecanol	Late stent thrombosis	*In vitro* on human aortic endothelial cell (HAECs) cultures	Favored endothelialization
	Carboxyl terminal	Deploying probes	*In vivo* on adult rats	Successfully deployed PDMS probes

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ISO 10993 Parts

Part 1: Evaluation and testing within a risk-management process

Part 2: Animal welfare requirements
Part 3: Tests for genotoxicity, carcinogenicity, and reproductive toxicity
Part 4: Selection of tests for interactions with blood

Part 5: Tests for *in vitro* cytotoxicity

Part 6: Tests for local effects after implantation

Part 7: Ethylene oxide sterilization residuals

Part 9: Framework for identification and quantification of potential degradation products

Part 10: Tests for irritation and sensitization
Part 11: Tests for systemic toxicity

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Reeve L, Baldrick P. Biocompatibility assessments for medical devices - evolving regulatory considerations. *Expert Review of Medical Devices*. Feb 2017;14(2):161-167. doi:10.1080/17434440.2017.1280392

ISO 10993 Parts…

Part 12: Sample preparation and reference materials
Part 13: Identification and quantification of degradation products from polymeric medical devices
Part 14: Identification and quantification of degradation products from ceramics
Part 15: Identification and quantification of degradation products from metals and alloys

Part 16: Toxicokinetic study design for degradation products and leachables

Part 17: Establishment of allowable limits for leachable substances
Part 18: Chemical characterization of materials

Part 19: Physicochemical, morphological, and topographical characterization of materials

Part 20: Principles and methods for immunotoxicity testing of medical devices

Part 22: Guidance on nanomaterials
Part 33: Guidance on tests to evaluate genotoxicity – supplement to ISO 10993-3

Steven S. Saliterman

Reeve L, Baldrick P. Biocompatibility assessments for medical devices - evolving regulatory considerations. *Expert Review of Medical Devices*. Feb 2017;14(2):161-167. doi:10.1080/17434440.2017.1280392

Other Relevant Standards…

ISO/NP TR 15499: Biological evaluation of medical devices – guidance on the conduct of biological evaluation within a risk-management process
ISO/DTR 21582: Pyrogenicity – principle and method for pyrogen testing of medical devices
ISO/NP TS 21726: Biological evaluation of medical devices – application of the TTC for assessing biocompatibility of extractable substances from medical devices
ISO/AWI TS 29741: Biological evaluation of medical devices – development of tolerable intake values for DEHP
ISO/TR 37137: Cardiovascular biological evaluation of medical devices – guidance for absorbable implants
ISO/TR 37,137-2: Cardiovascular biological evaluation of medical devices – guidance for absorbable implants – Part 2: standard guide for absorbable metals

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Reeve L, Baldrick P. Biocompatibility assessments for medical devices - evolving regulatory considerations. *Expert Review of Medical Devices*. Feb 2017;14(2):161-167. doi:10.1080/17434440.2017.1280392

How to Proceed (e.g. Polymers)

Bernard, M, E. Jubeli, D Pungente and N Yagoubi. Biocompatibility of polymer-based biomaterials and medical devices – regulations, in vitro screening and risk-management. Biomater. Sci., 2018, 6, 2025–2053