What is Biocompatibility?

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Introduction

Pittsburgh Plastics Mfg. (PPM) is a contract manufacturer of products for medical, foot care, safety and other industrial markets with focus on polymeric cushioning solutions. Medical positioning pads, gel insoles, helmet pads, and vibration damping parts are product examples for each of the major markets. These examples show that many of the products manufactured at PPM are in direct contact with human (or animal) skin. For this reason, PPM's engineering team is commonly questioned about material biocompatibility. The purpose of this technical paper is to help our customers better understand biocompatibility and how PPM establishes that a material or product is safe for skin contact applications.

Discussion

The word biocompatibility refers to the interaction of a living system or tissue with a finished medical device or component materials. In the simplest sense, a biocompatible material or device does not harm the patient. A common dictionary definition is "the quality of being compatible with living tissue or a living system by not being toxic or injurious and not causing immunological rejection". In a regulatory sense, biocompatibility is testing to determine the potential toxicity resulting from bodily contact with a material or medical device. Biocompatibility is vital for medical devices. Both local and systemic reactions are evaluated. A systemic reaction affects parts of the body beyond the local part that contacted the material or device.

A medical device may be comprised of materials that are biocompatible; however, the device itself requires biocompatibility testing. For example, a band aid is made of at least three materials: the adhesive, plastic and gauze. Even if these materials are biocompatible, testing of the band aid itself is required because it shows the effects of material interaction.

All testing is performed on the final version of the product. Microbes or contaminants may influence test results, so test articles are cleaned and/or sterilized with the same method as planned for production. However, sterility is different from biocompatibility. Sterility is the absence of `living organisms (such as bacteria) from a material's surface.

FDA Approval

Biocompatibility testing is an important part of obtaining FDA's approval to market a medical device. The first step of the approval process is to confirm that a product is a medical device as defined by section 201(h) of FD&C Act.

The FDA groups devices into three classes, so the second step is to classify the device. Class I devices have the lowest risk and class III the highest. Examples are exam gloves (class I), biopsy forceps (class II) and artificial heart valve (class III). Classification is obtained from the FDA website. Go to "Medical Device Databases" under Tools & Resources. Search under "Product Classification" (general product name/keyword) or "Registration & Listing" (company name that makes a similar product or proprietary product name).

The third step is to collect appropriate data. Most class I devices are 510(k) exempt. Most class II devices require a 510(k) application. Most class III devices require a premarket approval application. For most PMA applications, clinical trials are required.

Novel medical devices or devices made with new, unfamiliar materials were automatically made class III. The FDA has created a new class, De Novo, for low and moderate risk devices that previously were made class III because of their novelty. The De Novo application is less demanding than the PMA application.

For premarket notification route, chemical characterization and biocompatibility test data is collected for the 510(k). The data supports the claim that the device is safe and substantially equivalent to a legally marketed device. For premarket approval route, test data is collected to obtain an investigational device exemption (IDE). An IDE allows the device to be used in a clinical study in order to obtain safety and effectiveness data to support a PMA application.

Reference [1] is a helpful technical bulletin from the Alliance for the Polyurethane Industry on compliance of new medical devices to FDA regulations. The bulletin notes that the majority of medical devices entering today's market were cleared by the FDA without clinical trials/data. In fact, greater than 95% of medical devices used in the United States were never tested in clinical trials. They were brought to market either under the 510K program or they were on the market prior to 1976 when the safe medical device act started. Nevertheless, the FDA is interested in the biocompatibility of raw materials and finalized medical devices.

Standards

USP Class VI
United States Pharmacopeia (USP) is an independent, non-governmental, science-based organization that promotes the public health by establishing testing standards that ensure the quality of medicines and other health care technologies. USP standards prescribe animal use to test for impurities or contaminants in drugs, biologics, or biological products and to assess the toxic potential of plastics or leachable components of implanted medical devices.

The U.S. Pharmacopeia website states that "The United States Pharmacopeia–National Formulary (USP–NF) is a book of public pharmacopeial standards. It contains standards for medicines, dosage forms, drug substances, excipients, medical devices, and dietary supplements." USP–NF combines two official compendia, the USP and NF. The standards in USP-NF are updated in official monographs, and these standards and procedures are enforceable by the U.S. FDA.

The relevant monograph for polymeric materials is USP General Chapter <88> Biological Reactivity Tests, In Vivo. The monograph states that "The following tests are designed to determine the biological response of animals to elastomerics, plastics and other polymeric material… Six plastic classes are defined. This classification is based on responses to a series of *in vivo* tests for which extracts, materials, and routes of administration are specified. These tests are directly related to the intended end-use of the plastic articles." Of the six classes, Class VI must pass the most stringent testing. The tests measure and determine the biological response of animals to plastic material by either direct or indirect contact, or by injection of specific extracts prepared from the material under test. The tests are described as:

<u>Systemic Toxicity Test</u>
Extracts of the plastic material are injected intravenously or intraperitoneally (in the body cavity). Systemic tests evaluate the toxicity of leachables to biological systems such as nervous or immune systems.

<u>Intracutaneous Test</u>
Extracts of the plastic material are injected under the skin. Intracutaneous tests are used to assess local inflammatory or irritation reactions to leachable substances.

<u>Implantation Test</u>
The device or plastic material is implanted at an appropriate site. Implantation tests are used to evaluate the local response of living tissue to implanted material. Scoring is based on both microscopic and macroscopic parameters.

The above tests are “in vivo” (Latin: within the living), meaning that testing is performed using whole, living organisms. In contrast, “in vitro” (Latin: within glass) testing is performed on isolated components of an organism.

For Class VI systemic and intracutaneous testing, four different extract solutions (NaCl, 5% EtOH, cotton seed oil and polyethylene glycol) are used to ensure capture of any substances that leach from the material.

Although USP Class VI testing is widely used and accepted in the medical products industry, some view it as the minimum requirement a material must meet to be considered for use in health care applications. USP Class VI testing does not fully meet any category of ISO 10993-1 testing guidelines.

ISO 10993

The International Organization for Standardization was established to determine uniform worldwide standards. It is a non-governmental network of national standards institutes of 162 countries, and forms a bridge between private and public sectors. International Organization for Standardization is abbreviated ISO from the Greek word “isos” meaning equal.

In 1995, the organization published ISO 10993, a series of standards for biological evaluation of medical devices and dental materials. ISO 10993 currently has 20 parts, and its structure is shown in Table 1.

Table 1. Structure of ISO 10993

Part	Title
1	Evaluation and testing within a risk management process
2	Animal welfare requirements
3	Tests for genotoxicity, carcinogenicity and reproductive toxicity
4	Selection of tests for medical devices that interact with blood
5	Tests for in vitro cytotoxicity
6	Tests for local effects after implantation
7	Ethylene oxide sterilization residuals
8	Selection and qualification of reference materials for biological tests
9	Framework for identification and quantification of potential degradation products
10	Tests for irritation and skin sensitization
11	Tests for systemic toxicity
12	Sample preparation and reference materials
13	Identification and quantification of degradation products from polymeric medical devices
14	Identification and quantification of degradation products from ceramics
15	Identification and quantification of degradation products from metals and alloys
16	Toxicokinetic study design for degradation products and leachables
17	Establishment of allowable limits for leachable substances
18	Chemical characterization of materials
19	Physico-chemical, morphological and topographical characterization of materials
20	Principles and methods for immunotoxicology testing of medical devices

The introduction to ISO 10993-1 states: “The primary aim of this part of ISO 10993 is the protection of humans from potential biological risks arising from the use of medical devices… The role of this part of ISO 10993 is to serve as a framework in which to plan a biological evaluation which… minimizes the number of exposures to test animals by giving preference to chemical constituent testing and *in vitro* models…” Therefore, this part of the standard provides a methodology for choosing the proper biological evaluation test program. ISO 10993-1:2009 describes:

- the general principles governing the biological evaluation of medical devices within a risk management process (framework or methodology for planning a biological evaluation program);

- the general categorization of devices based on the nature and duration of their contact with the body;
- the evaluation of existing relevant data from all sources;
- the identification of gaps in the available data set on the basis of a risk analysis;
- the identification of additional data sets necessary to analyze the biological safety of the medical device;
- the assessment of the biological safety of the medical device.

Section 6 of ISO 10993-1 is titled "Biological evaluation process" and states the following: "Material characterization is a crucial first step in the biological evaluation process. The extent of chemical characterization required depends on what… data exist, and on the nature and duration of body contact with the medical device; but, as a minimum, the characterization shall address the constituent chemicals of the device and possible residual process aids or additives used in the manufacture. Material characterization is described in ISO 10993-18 and ISO/TS 10993-19… If the combination of all materials, chemicals, and processes has an established history of safe use in the intended application, then further characterization and biological evaluation might not be necessary." Materials influence biocompatibility through surface topography and chemistry and by the presence of extractable chemical compounds or foreign particulates. Material characterization is useful for screening new materials and evaluating manufacturing processes.

The biological evaluation test program depends on the ISO 10993 device category of which there are three: surface, external communicating and implant. For each device category there are options for contact mode. For the surface device category, for example, options are skin, mucous membranes and breached or compromised surfaces. In each category, the exposure period of the material is also used to determine the test program. ISO 10993 arrays contact duration into three categories: limited (<24 hours), prolonged (24 hours to 30 days) and permanent (>30 days). Table 2 in the appendix describes device categories with associated biocompatibility testing [3].

Once a device category, contact mode, and contact duration are determined, ISO 10993 suggests the biological testing for biocompatibility validation. ISO 10993 is not a formal checklist, but a guide to the typical information required to establish biocompatibility. For example, one device is made of materials that are well characterized chemically and physically in published literature and have a long history of safe use. Another device is for a specialized application or is made of less characterized materials. Tests that are appropriate for the former device are not the same as for the latter.

Biocompatibility Testing at PPM

A common device category for PPM's products is skin contact, possibly compromised skin, for limited duration. Table 2 shows that cytotoxicity, sensitization, irritation/intracutaneous reactivity and acute systemic toxicity are required by the FDA; ISO requires only the first three.

Cytotoxicity – ISO 10993-5
Cytotoxicity is the most sensitive of the biocompatible tests, and is the only *in vitro* test of those listed for ISO 10993-5, 10 and 11 standards. Cytotoxicity assays are used to assess the toxicity (quality of being poisonous) of the test material when in contact with a specific cell culture. ISO 10993 states: "Cytotoxicity tests employing cell culture techniques shall be used to determine the lysis of cells (cell death), the inhibition of cell growth, colony formation, and other effects on cells by medical devices, materials and/or their extracts…" Results of cytotoxicity tests correlate fairly well with short-term implant studies. Antibiotics may be added to the cell culture medium to remove potential interference from microbial contamination.

Historically, if a sample fails only one of the biocompatibility tests, 90% of the time cytotoxicity fails. For this reason, PPM uses cytotoxicity to screen materials or devices. The agar diffusion cytotoxicity test is ideal as a screening tool because it costs less than other cytotoxicity tests and requires a short amount of time to perform. The following descriptions of agar diffusion and other tests were reproduced from Toxikon with permission [3].

Agar Diffusion
This qualitative assay determines the biological reactivity of a monolayer of the L929 mammalian cell in response to a test material. The test is designed for a variety of solid and liquid test materials. The cells are allowed to grow to approximately 80% confluency in cell culture dishes and then overlaid with an agarose layer. The test material is placed over the agar layer, which protects the cells from mechanical damage while allowing the diffusion of leachables from the test material onto the cell layer. The plates are incubated for forty-eight hours at 37°C in a 5% CO2 incubator and scored for reactivity at twenty-four and forty-eight hours on a scale from Grade 0 (no reactivity) to Grade 4 (severe reactivity). The test item is considered non-cytotoxic if none of the cultures exposed to the test item shows greater than mild reactivity (Grade 2).

If a material or device passes agar diffusion, then the following tests are performed according to the FDA's standard for skin contact, possibly compromised skin, for limited duration. PPM uses MTT cytotoxicity to quantitatively evaluate cytotoxicity.

MTT Cytotoxicity
The *in vitro* biological reactivity of the L929 mouse fibroblast cell culture is quantitatively determined in response to an extract of the test material in 6 replicates. The cells are allowed to grow to semi-confluency in tissue culture plates. An extract of the test material is prepared in Minimum Essential Media (MEM), which is transferred onto the cell layer. The plates are incubated for forty-eight hours at 37°C in a 5% CO2 incubator, and quantitatively analyzed for viability via the reduction of the water-soluble, yellow MTT by the mitochondrial reductases enzymes into an insoluble, blue-violet formazan. The number of viable cells correlates to the color intensity determined by photometric measurements after dissolving the formazan. The test item is considered non-cytotoxic if the percentage of viable cell is equal to or greater than 70% of the untreated control.

Sensitization – ISO 10993-10
The sensitization tests are used to determine the allergic or sensitizing capacity to the repeated or prolonged exposure of a test material. Sensitization is characterized by the fact that reactions are delayed, not localized, and independent of dose. ISO 10993 states: "These tests are important because exposure or contact to even minute amounts of potential leachables can result in allergic or sensitization reactions." As an illustration of delayed type hypersensitivity, no reaction occurs the first time someone is exposed to poison ivy; however, a second exposure may result in contact dermatitis. PPM uses Kligman maximization to test for sensitization.

Kligman Maximization Test
The Kligman Maximization Test evaluates the allergenic potential or sensitizing capacity of the test article in guinea pigs. The test article will be exposed to the test system directly or through test article extracts. Extracts of the test material are prepared in a polar (saline) and/or non-polar (cottonseed oil) solution. The test begins (day 0) with intradermal (within the layers of the skin) injections of Freund's Complete Adjuvant (FCA) and the test article. Seven days later (day 7), the injection sites are exposed to a topical application (gauze soaked with test article extract). Then on day 23, a new site is challenged with a second topical application of the test article. The test guinea pigs are scored during the following 48 hours for irritation or any reaction compared to controls. A sensitization reaction to the test article is scored based on the defined evaluation criteria in ISO 10993-10.

Irritation – ISO 10993-10
The irritation tests are *in vivo* screening tests to evaluate the potential of test materials to cause irritation on an exposed part of the body. Irritation testing is similar to sensitization testing, but tests for an immediate irritation reaction. The test reveals whether a device or material creates an immediate reaction when in contact with a patient. Standard studies are single exposure evaluations. ISO states: "Irritation tests shall be used to estimate the irritation potential of medical devices, materials and/or their extracts, using an appropriate site for application such as skin, eye and mucous membrane in a suitable model." PPM uses intracutaneous injection to test for irritation.

Intracutaneous Injection
The intracutaneous injection test is designed to evaluate local responses to solutions or extracts following intracutaneous (directly under the skin) injections into rabbits. The test article will be exposed to the test system directly or through test article extracts. Extracts of the test material are prepared in a polar (saline) and/or non-polar (cottonseed oil) solutions. Three rabbits are injected intracutaneously with the test article and control materials. The injected sites are examined over a seventy-two hour period for evidence of tissue reaction such as erythema, edema, or necrosis. Observations are scored according to ISO 10993 scoring system. At the end of the observation period, the scores are used to determine an overall mean reaction score for the test article versus the corresponding control article. The requirements of the test are met if the difference of the mean reaction score for the test article and the control article is 1.0 or less.

Systemic Toxicity – ISO 10993-11
Systemic toxicity is a toxic effect as a result of absorption and distribution of a toxicant to a site in the body that is distant from the exposure site. *In vivo* systemic tests evaluate the impairment of biological systems, rather than the impairment of individual cells or organs. Categories are based on duration of exposure and dose route. Acute systemic toxicity testing is the most commonly performed, and includes a single exposure with a 72-hour observation period. PPM uses acute systemic injection and material-mediated rabbit pyrogen to test for acute systemic toxicity.

Acute Systemic Injection
This test is designed to evaluate solutions and test article extracts for potential toxic effects as a result of a single-dose systemic injection in mice. Extracts of the test material are prepared in polar (saline) and/or non-polar (cottonseed oil) solutions. The test article is injected intravenously and/or intraperitoneally (into the body cavity) in groups of five mice. The animals are observed for seventy-two hours after administration for signs of biological reactivity. This test is considered negative if none of the animals injected with the test article show a significantly greater biological reaction than the animals treated with the control article. If two or more mice die, or show signs of toxicity, or if three or more mice lose more than 2g of body weight, the test article does not meet the requirements of the test.

Material-Mediated Rabbit Pyrogen
This test is designed to detect the risk of a fever reaction as a result of the administration of the test article. The test article is administered by intravenous injection into three New Zealand White rabbits. The rectal temperatures of the injected rabbits are compared with the temperature of a control rabbit similarly injected. The baseline temperatures of the rabbits, as determined no more than thirty minutes prior to injection of the test article, are used to exclude rabbits whose body temperatures vary by more than 1 ^{o}C from each other and whose temperatures are greater than 39.8 ^{o}C. Body temperatures are recorded in thirty-minute intervals between one and three hours subsequent to injection. If no rabbit exhibits a rise in temperature of 0.5 ^{o}C or more above its baseline temperature, the product meets the requirements for the absence of pyrogens (endotoxins, hormones or other substances that cause a febrile reaction).

References

1. The Use of Polyurethanes in Medical Device Applications, Alliance for the Polyurethanes Industry, Technical Bulletin, August 2001, AX146

2. ISO 10993-1, Biological evaluation of medical devices – part 1: Evaluation and testing within a risk management process, fourth edition 2009-10-5

3. Medical Device Testing Guide, Rev. June 2011, Toxikon website

Appendix

Table 2. Device categories with associated biocompatibility testing [3].

Device Categories		Biological Effects- Initial Evaluation									Biological Tests Supplemental Evaluation			
Body contact mode	Contact Duration	Cytotoxicity	Sensitization	Irritation/ Intracutaneous Reactivity	Acute Systemic Toxicity	Pyrogenicity	Subacute/ Subchronic Toxicity	Genotoxicity	Implantation	Hemocompatibility	Chronic Toxicity	Carcinogenicity	Reproductive/ Developmental Toxicology	Biodegradation
Surface Devices														
Skin	A	◊	◊	◊										
	B	◊	◊	◊										
	C	◊	◊	◊										
Mucosal membranes	A	◊	◊	◊										
	B	◊	◊	◊	●	●	●		●					
	C	◊	◊	◊	●	●	◊	◊	●		●			
Breached/ Compromised Surfaces	A	◊	◊	◊	●	●								
	B	◊	◊	◊	●	●	●		●					
	C	◊	◊	◊	●	●	◊	◊	●		●			
External Communicating Devices														
Blood Path, Indirect	A	◊	◊	◊	◊	◊				◊				
	B	◊	◊	◊	◊	◊	●			◊				
	C	◊	◊	●	◊	◊	◊	◊	●	◊	◊	◊		
Tissues/ Bones/ Dentin	A	◊	◊	◊	●	●								
	B	◊	◊	□	□	□	□	◊	◊					
	C	◊	◊	□	□	□	□	◊	◊		□	□		
Circulating Blood	A	◊	◊	◊	◊	◊		●		◊				
	B	◊	◊	◊	◊	◊	□	◊	□	◊				
	C	◊	◊	◊	◊	◊	◊	◊	□	◊	◊	◊		
Implant Devices														
Tissues/ Bones	A	◊	◊	◊	●	●								
	B	◊	◊	□	□	□	□	◊	◊					
	C	◊	◊	□	□	□	□	◊	◊		◊	◊		
Blood	A	◊	◊	◊	◊	◊	▪		◊	◊				
	B	◊	◊	◊	◊	◊	□	◊	◊	◊				
	C	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊		

Table 2 key

A	Limited Exposure (less than 24 hours)
B	Prolonged Exposure (24 hours to 30 days)
C	Permanent Exposure (More than 30 days)
▪	Evaluation Required by FDA, ISO, and MHLW
□	Evaluation Required by FDA and ISO
●	Evaluation Required by FDA
◊	Evaluation Required by ISO