1

Advances in Biocompatibility: A Prerequisite for Biomedical Application of Biopolymers

Matthew R. Jorgensen, Helin Räägel, and Thor S. Rollins

Nelson Laboratories, LLC, 6280 S Redwood Rd, Salt Lake City, UT 84123, USA

1.1 Introduction

Biocompatibility is a concept that, in one form or another, has existed since the dawn of medicine. At the base of Vesuvius in ancient Rome was the house of a surgeon, home to an impressive collection of medical instruments that were preserved by ash when the mountain exploded. Without a doubt, patrons of the ancient surgeon subjected themselves to these devices with the expectation and trust that they would be getting better – not worse – due to the treatment they received. While biocompatibility has not always been explicitly defined through history, the safety of a tool in a doctor's hand is central to the mission of the doctor. Following the industrial revolution, instruments have become mass-produced and marketed as effective tools for the practice of medicine, making doctors rely on the diligence of the manufacturer to ensure patient safety. Concurrently, our knowledge of toxicology has expanded through experience, and medical journals have become widely available to share clinical experiences. These platforms have been and are currently successfully used to notify doctors and also the public about medical instruments thought to be safe, but which actually did more harm than good, and discuss options for mitigating the risks associated with the use of these devices.

To protect patients from being harmed by medical devices, which for one reason or another might be unsafe due to negligence on the part of the device manufacturer, medical device safety has become regulated. These regulations require medical device manufacturers making a device or product to demonstrate that what they are producing performs appropriately when used as intended. Past experience and modern toxicology have identified what sorts of health risks are associated with the use of a given medical device. The most modern and comprehensive overview of biocompatibility is the suite of documents that make up the international standard ISO 10993; the first document in the series, *ISO 10993-1,* provides the high-level framework for evaluation of biocompatibility as a whole, while the other documents in the series explore specific topics in more detail.

The modern concept and definition of biocompatibility is the ability of a medical device (or material) to "perform with an appropriate host response" when

Biopolymers for Biomedical and Biotechnological Applications, First Edition.
Edited by Bernd H. A. Rehm and M. Fata Moradali.

used as intended. This means that the device or material should not cause an unacceptable biological risk when used, taking into account the nature of use in terms of contact site and duration, as well as the potential benefit of using the device. ISO 10993-1, Annex A, lists several key biological risks associated with specific types and durations of patient contact. As the contact duration goes up, and the devices or materials become more invasive, the types of potential risks multiply. For example, a device that is used on an intact skin is not very invasive, and therefore the associated risks are minimal; the skin is an organ effective at protecting the body from our natural environment that is often replete with biological risks. In contrast, consider a neurological stent; this invasive device is in permanent contact with brain tissues. For such a device, risks range from immediate toxicity to thrombosis to more chronic systemic toxicities like cancer. Therefore, even the more modern concept of biocompatibility encompasses the broader idea well captured by the oft-repeated phrase in medicine "First, do no harm," which certainly applies to the materials used with the intention of healing.

1.2 Biocompatibility Evaluation of Biopolymeric Materials and Devices

Biopolymers represent a special subset of materials useful in medicine, being derived or produced by living organisms or synthesized from basic biological building blocks. Compared with synthetic polymers, the advantages from the perspective of biocompatibility are clear: because these materials are made by living systems, from building blocks ubiquitous to life, it would seem like the potential for adverse biological reactions would be reduced. For implants, like biocomposite bone anchors used by Arthrex® in hip arthroscopy procedures (Figure 1.1), if the goal is to mimic the tissue being replaced, using a material made from

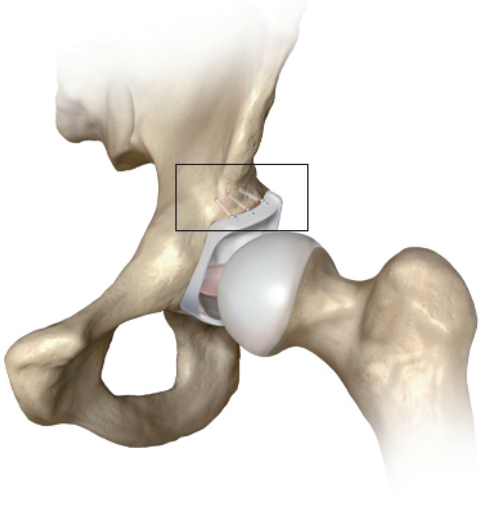

Figure 1.1 BioComposite Knotless SutureTak® anchor used in hip arthroscopy procedures. *Source:* Courtesy of Arthrex®.

natural building blocks is logical. The scope and range of biopolymers has been discussed in detail within this text and elsewhere in literature [1–3]. Briefly, they include polysaccharides (such as chitin, hyaluronic acid, and cellulose), polyesters (such as polylactic acid [PLA]), proteins (such as silk, collagen, and casein), and others like latex rubber and shellac. As varied as the possible biopolymers are their individual chemical properties; therefore, broad grouping of biopolymers for biocompatibility is not possible. Rather, these materials should be considered without special allowance, in terms of their intended use and durability in the body.

The biocompatibility evaluation process, in general, begins by determining what potential biological risks the use of the material would present. Once risks are determined, a plan to evaluate those risks should be developed. Often, the risk identification process begins by answering the following questions:

1. **What is the intended use of the device (or material)?**
   a. What tissues or fluids will it contact in the body (either directly or indirectly)?
   b. How long is the cumulative amount of time it may contact the body?
   c. Who will be exposed to the device (infants, pediatrics, adults)?
2. **What is known about the device materials and their fate in the body?**
   a. What processing, packaging, and sterilization are the materials exposed to?
   b. Are the materials known to degrade over time?
   c. What previous clinical experience is there with the device (or materials)?

Annex A in ISO 10993-1 contains a chart of biological risks for consideration, stratified by contact duration (limited ≤24 hours, prolonged >24 hours to 30 days, long term >30 days) and contact type. These risks can provide a starting point for understanding the risks presented by a device for both the device manufacturer and those who would in the end approve the device for use. To illustrate how Annex A is used, two commonly used biopolymeric devices are put through the thought process as examples:

- Device 1: A chitin-based hemostatic agent for acute treatment during massive hemorrhage in an open wound
- Device 2: A polycaprolactone (PCL) implant for infants, designed to degrade and resorb over a period of two to three years

How the description of Device 1 and Device 2 translates into a classification and set of biological risks is shown in Table 1.1.

The risks identified by ISO 10993-1, Annex A (outlined for the two devices in Table 1.1), are not necessarily all-inclusive or exhaustive. The spirit of the document is to provide a starting point and basis for a biological evaluation; if other potential biological or toxicological risks are known through clinical experience, those would also need to be addressed. For instance, if a medical instrument is known or has been shown to chip during a surgical procedure, leaving fragments of the device possibly permanently in the patient, this should be addressed in the biocompatibility assessment.

Table 1.1 Example classification and associated risks for two representative devices.

	Hemostatic	Implant
Contact tissues	Bleeding wound	Muscle and bone
Contact duration	Expected to be less than 24 h, but could extend beyond	Device resorbs over 2–3 yr
Target patient population	Adults	Infants
Classification per Annex A	Category: surface medical device Contact: breached or compromised skin Contact duration: prolonged	Category: implant medical device Contact: tissue/bone Contact duration: permanent
Biological risks to be addressed (per ISO 10993-1, Annex A)	• Cytotoxicity • Sensitization • Irritation • Material-mediated pyrogenicity • Acute systemic toxicity • Subacute toxicity • Implantation effects	• Cytotoxicity • Sensitization • Irritation • Material-mediated pyrogenicity • Acute systemic toxicity • Subacute toxicity • Subchronic toxicity • Chronic toxicity • Implantation effects • Genotoxicity • Carcinogenicity • Degradation

It should also be recognized that the risks identified by Annex A are not highlighted in the standard as an explicit "checklist for testing." Fortunately, the latest ISO 10993-1 released in 2018 more clearly defines this statement within the document. Based on the updated verbiage in the standard, each of the biological risks (or endpoints) can be evaluated using a risk-based approach, taking into consideration chemical and material information, existing endpoint-specific data, or a written rationale why testing or further data is not needed to address a particular risk. In any case, the biocompatibility of a device or material must be spelled out, addressing directly each of the specific risk identified, mitigating concern through testing results or written evaluation in a biological risk assessment.

1.3 Using a Risk-Based Approach to Biocompatibility

After the specific biological risks for a particular implementation of a device are identified, the strategy for how the biological safety will be proven must be decided. In the past, the expectation was that because devices are typically made by competitors in unique environments, and with proprietary processing, categorically calling a material "biocompatible" was not possible, and testing should be executed anew for each device coming to market. The list of biological risks

was pretty much a shopping list, more or less blindly ordered and executed. Since that time, there has been a dramatic shift toward a more thoughtful scientific approach to the evaluation of biocompatibility.

The shift from check-listing tests to a risk-based approach has been motivated by several factors:

- Consideration of animal welfare, with a charge to reduce animal testing as much as possible
- A broader and better consolidated body of data on materials and toxicology
- Better analytical chemistry tools to evaluate manufacturing residuals, material leachables, and degradation products

Knowing that the key is to protect patient safety by proving biocompatibility of a device to the skeptical reviewer while at the same time avoiding as much unnecessary testing as possible is the heart of evaluating biocompatibility using a risk-based approach. There is an art to a biocompatibility evaluation, balancing commonsense measures to ensure safety with currently available data on one hand and the expectations of regulatory bodies across the spectrum on the other. Understanding the role the material information has and how this broadly impacts the testing strategy (along with the cost and time burden of testing) is central to the strategy.

In the best case, material information and written assessment alone can be sufficient to mitigate and address all of the biological risks associated with a device. To be convincing, however, a great deal of detail is needed. Often, the question of biocompatibility is not about the bulk material itself at all, but rather about the processing of that material that takes place both upstream and downstream. Consider a polycaprolactone (PCL) implant, manufactured using 3D printing from a powder starting material. To the manufacturer, the name PCL along with its assigned chemical abstracts service (CAS) number defines the material. But there are many ways to synthesize PCL [4] that may influence its safety profile in terms of impurities that (while not obvious from bulk properties) will affect toxicology. Consider the PCL pipeline upstream from the device manufacturer:

1. Preparation of the monomer (either ε-caprolactone or 6-hydroxycaproic acid) at raw chemical supplier:
   a. ε-Caprolactone and 6-hydroxycaproic acid may be produced naturally by oxidation of cyclohexanol by microorganisms and then harvested and purified (all steps removing or introducing impurities to varying degrees).
   b. ε-Caprolactone can also be produced industrially through a reaction of cyclohexanone with peracetic acid.
2. The monomer is purified, packaged, sold, and shipped to the maker of the polymer without knowledge that the monomer will end up in a medical device:
   a. Purity and performance metrics are based on bulk properties (not toxicological endpoints).
3. The monomer is polymerized by another manufacturer:
   a. Polymerization occurs using a variety of different possible techniques, using different activators and/or catalysts, several of which are complex

organometallic complexes of questionable safety (see, for example, those contained in Ref. [4]).

4. The polymer is powdered and purified by the manufacturer using a proprietary cryogenic process.

Most (or all) of the details of the upstream process are unknown to the medical device manufacturer, yet they can impact device safety. It could matter, from a toxicological perspective, if the PCL in a device is manufactured using lithium diisopropylamide or *tert*-butoxy potassium as a catalyst. If the device manufacturer were to ask their polymer supplier what catalyst or what monomer is used and the method of manufacture, the information is likely considered intellectual property, and medical device manufacturers are typically not big enough customers of polymer manufacturers to be able to make demands. Therefore, in these cases, it is up to the device manufacturer to prove the biocompatibility of their materials acknowledging that very little is known about the impurity profile of their device.

Knowing what you do not know and how that gap in knowledge might be interpreted by a regulator or a patient receiving the device is key in developing a testing strategy for biocompatibility (Figure 1.2). Regulators have been witness to all sorts of mischief on the part of manufacturers, and patients have been injured by devices made from misunderstood materials, elevating further the concern for each device that is in the process of clearance for market. For biopolymer devices, it is typically not known what trace chemicals may be in the material. Another gap in knowledge is often how different processing steps influence the degradation rate of resorbable biopolymers. To answer those questions, we turn to chemistry.

1.3.1 Chemistry of Biopolymers and Risk

Based on their physicochemical properties, various biopolymers so far used in the medical industry can loosely be placed into three categories: polysaccharides, proteins, and polyesters. Some examples of common biopolymers are shown in Table 1.2.

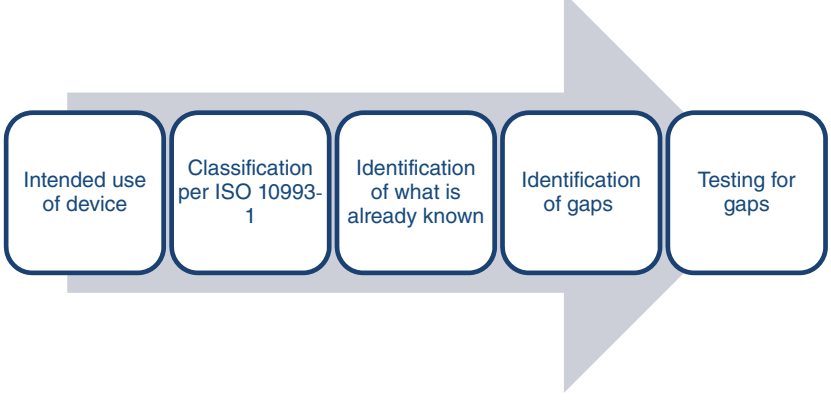

Figure 1.2 Thought process for using ISO 10993-1 for biological evaluation of medical devices.

Table 1.2 Examples of common biopolymers.

Classification	Example biopolymer	Notes on production	Risks
Polysaccharides	Hyaluronic acid, HA (polymer of D-glucuronic acid and *N*-acetylglucosamine)	Primarily produced using bacteria including *Streptococcus* [5–7]	Production by pathogenic bacteria coproduces myriad other potentially toxic biological products that must be removed during subsequent purification steps
	Cellulose (polymer of D-glucose)	From plant products, cellulose is dissolved from other plant materials in an alkali process, followed by purification. Produced bacterially using *Acetobacter xylinum* [8, 9]	Industrial purification steps can introduce impurities. Bacterial production coproduces myriad other potentially toxic biological products that must be removed during subsequent purification steps
Proteins	Silk Primarily fibroin, a repeating amino acid sequence of (Gly-Ser-Gly-Ala-Gly-Ala)	Primarily from the mulberry silkworm *Bombyx mori* [10]	Industrial post-processing and purification steps can introduce impurities
Polyesters	Polylactic acid	Primarily ring-opening polymerization of lactide (cyclic lactic acid dimer) [11]	Crude lactic acid contains many impurities (acids, alcohols, metals)

While the chemistry of biopolymers and the source of these materials' building blocks are very diverse, there is a commonality among them when it comes to potential patient risk: there is always concern over side products and manufacturing residuals. While it is accepted that biopolymers have an inherent advantage from being similar chemically to substances naturally found in the body, they also have the same disadvantage facing all medical device materials from being processed. For that reason, the chemical evaluation strategy used for medical devices made from biopolymers is very similar to what is used for devices made from fully synthetic materials. The heart of the strategy is acknowledging that the manufacturer of the device does not know what they do not know, and the only way to safeguard against unpleasant surprises is to screen for everything that might reasonably be in or on the device.

1.3.2 Chemistry Screening of Biopolymers

It is important to start the design of a chemistry testing strategy with the end goal in mind. In the case of chemistry for biocompatibility, the end goal is to be able to screen for unexpected contaminants with enough sensitivity and with enough

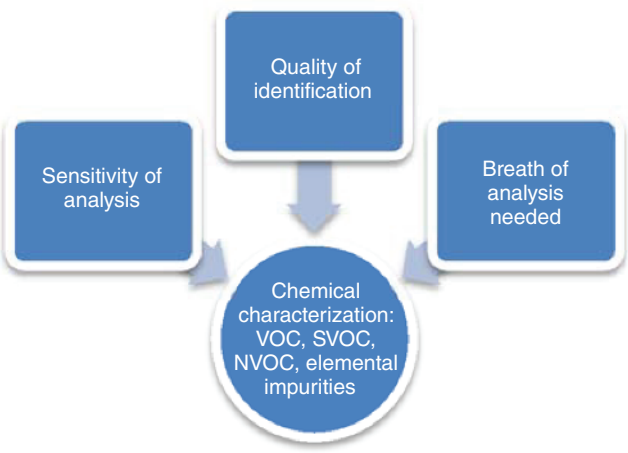

Figure 1.3 Important aspects for setting up a chemical characterization study.

accuracy that toxicological conclusions can be made based on the data produced (Figure 1.3). Determining the proper sensitivity can be a matter of debate but should be low enough so that any chemicals that are present – but not reported because they are below the sensitivity – are known to not be toxicologically concerning. In other words, a threshold of toxicological concern (TTC) is needed.

The TTC concept was developed to define an acceptable intake for any unstudied/understudied chemical that, if below the TTC, would pose a negligible risk of carcinogenicity, systemic toxicity, and reproductive toxicity. The concept was developed for chemicals present in the human diet and is accepted by the US Food and Drug Administration (FDA), International Conference on Harmonization (ICH), and the European Medicines Agency (EMA) for the evaluation of impurities in pharmaceuticals. It has also been used for assessing contaminants in consumer products and environmental contaminants. The methods upon which the TTC is based are generally considered very conservative since they involve data for the most sensitive species and most sensitive site induction (several "worst-case" assumptions). The TTC concept provides an estimate of safe exposures values for any compound not on the TTC exclusion list (i.e. metals, nitrosamines, and polycyclic aromatic hydrocarbons). The most conservative TTC value has been set at 1.5 µg/d and is assigned for greater than 10 years to a lifetime of exposure. A TTC of 120 µg/d has been proposed for genotoxic exposures limited to one month or less [12]. Exceeding the TTC is not necessarily associated with an increased risk given the conservative assumptions employed in the derivation of the TTC value [13–17]. When adequate evidence exists that a constituent is non-carcinogenic, a non-carcinogenic TTC value may be used to address the constituent (e.g. Cramer classification) [18, 19].

The TTC concept for medical devices was formalized in ISO 21726 published in February 2019. This brief international standard outlines the appropriate strategy for using the Cramer class and TTC. When adequate toxicological data is not available in the literature, the Cramer classification should be used for non-cancer

Table 1.3 Recommended TTC values from ISO 21726.

Medical device contact category	Limited (<24 h)	Prolonged (24 h to 30 d)	Long term[a] (>30 d)		
Duration of body contact	≤1 mo		>1–12 mo	>1–10 yr	>10 yr to lifetime
TTC for any one compound (μg/d)	120		20	10	1.5[b]

a) Considered permanent according to ISO 10993-1.
b) This value incorporates a 10^{-5} cancer risk for a 60 kg adult.

effects; for cancer-based effects, the ICH M7 TTC values should be used based on the contact duration of the device. Cramer classification stratifies compounds into three groups (I, II, and III, with III being the highest risk); the acceptable daily exposures are 1800 μg/d for class I, 540 μg/d for class II, and 90 μg/d for class III compounds. The TTC values from ISO 21726 for carcinogenic endpoints depend on contact duration and are shown in Table 1.3.

In addition to the sensitivity, the breadth of the analysis is critical. ISO 10993-12, ISO 10993-17, and ISO 10993-18 provide guidance on the sample preparation and scope of analysis to give the required breadth. The device should be extracted in multiple solvents covering a range of polarities to be representative of the range of matrices that are found in the body. Extraction conditions should be selected to appropriately exaggerate the amount of chemicals found. For example, extraction of the device at 50 °C for 72 hours is prescribed by ISO 10993-12 and is the most commonly used extraction condition. Typical extraction solvents are purified water, isopropyl alcohol, and hexane. Following extraction, the extracts must be analyzed for volatile organic compounds (VOCs), semi-volatile organic compounds (SVOCs), non-volatile organic compounds (NVOCs), and metals using a suite of techniques that are both qualitative and quantitative; these are almost always chromatography with mass spectroscopy (MS) for organic compounds and inductively coupled plasma for metals.

VOCs are typically analyzed for only in aqueous extracts, as semipolar and non-polar solvents are often VOCs themselves. Two main techniques are available for VOCs: headspace gas chromatography with mass spectroscopy (HS-GC/MS) and purge and trap GC/MS. HS-GC/MS measures the volatiles present in the gas above a water sample in a closed vial; the vial might be slightly heated to encourage volatiles to enter the gas phase above the liquid. The gas is directed through a gas chromatograph, which separates molecules in the gaseous mixture by polarity. Different molecular polarities are retained in the instrument for different amounts of time; how long a molecule remains in the instrument is referred to as the retention time. After separation, the molecules are identified using mass spectroscopy. Briefly, mass spectroscopy works by fragmenting molecules into electrically charged pieces and then measuring the weight of those pieces very precisely. With knowledge of both the retention time and mass fragmentation patterns, VOCs can almost always be positively identified by comparison with

large public or commercial databases. Purge and trap measurements differ from headspace only in the way compounds are sampled; first volatile organics are purged from the water by bubbling inert gas through the liquid and trapped in an adsorbent tube. VOCs are released from the tube into the GC/MS for analysis as with HS-GC/MS.

SVOC measurement methods provide the single broadest source of information regarding the content of extracts and are amenable to both aqueous and nonaqueous extraction matrices. The term SVOC is ill defined in the medical device community but generally is considered to be those compounds most well suited for analysis by direct injection GC/MS. The distinction of this definition is important, as there are many molecules amenable to direct injection GC/MS that are considered to be NVOCs by every other definition. The methods used for SVOCs by GC/MS are mostly characterized by the details of their sample preparation and rigor of data analysis; instrumental details of the GC/MS remain largely harmonized. Water extracts are prepared for analysis by first doing a solvent exchange to a solvent compatible with GC/MS. Typically this is accomplished by repeatedly shaking the extract with methylene chloride under acidic, neutral, and basic conditions. The methylene chloride can then be concentrated and directly injected into the instrument. Organic solvents do not need a solvent exchange and are typically concentrated and then directly injected.

NVOCs not amenable for analysis by GC/MS are most clearly those compounds that have such a high molecular weight or polarity that they are not capable of vaporization without decomposition. For these compounds, liquid chromatography with mass spectroscopy (LC/MS) must be used. Unlike GC/MS analyses, which have more or less standardized instrument parameters, LC methods are highly variable. Because of this variability, large public databases are of limited utility, and effective interpretation of data relies much more on the level of expertise of the analyst and internal experience of the analyzing lab. LC techniques coupled with advanced mass spectroscopy tools providing high-resolution accurate mass (HRAM) such as quantitative time of flight (qTOF) or Orbitrap can be a significant advantage, as these more sensitive methods can greatly narrow down the number of possible compounds in the identification process.

One of the key variables in chemical analysis for toxicological risk assessment and biocompatibility is the degree of certainty in the identification and quantification of compounds. Quality of identification can range from a fully automated comparison to a public database, without peer review of the results to fully confident identification. Fully automated identification can lead to scenarios where compounds with very low match scores are reported as compounds for which they are almost certainly not. On the other end of the identification spectrum is a fully validated identification where the compound in question has been injected using a standard on the same instrument and under the same conditions and under expert review. Of course, in practice, results can be a mix. It is not possible to inject standards for every compound that might occur from a biomaterial. With respect to quantification, results can vary based on the amount of evidence that is present to support the accuracy and precision of the presented results. On one end of the spectrum, results can be fully validated with calibration curves

and precision and accuracy measurements. On the other end, results may be estimates based only on the concentration of an internal standard. Because patient safety may hinge on the result, often toxicologists want something more than a blind estimate of concentration of the compound is on the edge of being considered safe.

Chemistry results must be evaluated and assessed through the lens of toxicology to understand the possible systemic risks associated with the findings and the route of exposure of the device per ISO 10993-17. This assessment should complement the results of traditional biocompatibility tests performed on biopolymeric device materials.

1.4 Specific Biological Endpoint Evaluations

For most biological endpoints per ISO 10993-1, a biopolymer would be tested very similarly to any other polymer. The main concern with a biopolymer is the degradation profile and the impact of the degradation on the test system. The testing system that needs the most consideration for the individual degradation profile of a material is in cytotoxicity, systemic toxicity, implantation, and material/chemical characterization.

1.4.1 Cytotoxicity

In general, cytotoxicity tests are a broad range of assays that look for the impact of a substance on individual cells grown under *in vitro* conditions. The test can be performed on different cell lines and can look at (qualitatively) or assess (quantitatively) different cellular endpoints. The various internationally accepted cytotoxicity assays are summarized in part 5 of the ISO 10993 series (i.e. ISO 10993-5). All the tests usually run using the L929 mouse fibroblast cell line. Although it is possible to use other cell lines for testing, the L929 cell line is the one that has historically been used and is therefore recommended for comparison. Additionally, despite the availability of many different versions of cytotoxicity tests, the standard testing for biocompatibility of medical devices consist of either MEM elution, MTT/XTT assays, or neutral red uptake assay. Each assay has different cytotoxicity evaluation endpoints and sensitivity, so comparing results from one assay to the other has proven to be difficult.

The cytotoxicity test is a very sensitive test and is the most likely test to cause trouble with any medical device, but specifically with biopolymers. This trouble comes from the fact that some biopolymers lack the mechanical properties and stability in the extraction fluid that is used to prepare a sample for the cytotoxicity test. This lack of stability may be caused a high concentration of ions in the extraction fluid that could result in a cytotoxic response in the assay. Crosslinking can be used in the attempt to improve the results, but this can also cause potential cytotoxicity as these crosslinking agents themselves can be cytotoxic (e.g. glutaraldehyde).

Therefore, the best approach for assessing cytotoxicity of biopolymers is a risk-based approach. As mentioned before, the cytotoxicity test is historically

the most sensitive test available and is thus often used as a screening test for materials, process residuals, and the final device configuration. In the ANSI/AAMI/ISO 10993-5 Guidance section 10, it states "Any cytotoxic effect can be of concern. However, it is primarily an indication of potential for *in vivo* toxicity and the device cannot necessarily be determined to be unsuitable for a given clinical application based solely on cytotoxicity data." When elevated cytotoxicity results are seen, a risk assessment should be performed to identify the source of observed cytotoxicity. Then on, the risk assessment should evaluate the toxic potential of the material or compound to determine the clinical impact. The investigation should include a review of the procedures to determine the effectiveness of the test system, additional testing to evaluate clinical risk of the results, and then a clinical risk assessment of the toxicity using additional animal testing along with chemical analysis and toxicological assessment of the detected compounds.

Based upon examination of the biopolymer, its history of use in medical industry, inherent surface properties of the device material, surface area in contact with the user, use and contact type, duration of contact, and the route of exposure, this cytotoxicity failure may not be clinically relevant, and subsequently it can be concluded that adverse effects in patients are unlikely to develop.

1.4.2 Systemic Toxicity (Acute, Subacute, Subchronic, and Chronic)

Systemic toxicity is a potential adverse generalized response including organ or organ system effects that can result from the absorption, distribution, and metabolism of leachates from the device or its materials to parts of the body that are not in direct contact with the device or material. The type of test recommended per ISO 10993 is dependent on the duration of exposure to the patient:

- Acute toxicity is defined as an adverse systemic effect occurring at any time within 72 hours after single, multiple, or continuous exposures of a test sample for 24 hours.
- Subacute toxicity is defined as an adverse effect occurring after multiple or continuous exposure between 24 hours and 28 days. The term subacute might be somewhat misleading since generally "sub" is understood as less, and subacute would, based on this logic, be considered as less than acute. Since this term is confusing, it is best to consider subacute toxicity as any adverse effects occurring within a short-term repeated exposure during a systemic toxicity study. This is generally done with time intervals between 14 and 28 days for intraperitoneal injection studies; intravenous studies are generally defined as treatment durations or exposure of more than 24 hours but less than 14 days.
- Subchronic toxicity is any adverse effect occurring after the repeated or continuous administration of an extract of a material or device for (typically) 90 days in rodents or in other species for duration of exposure that does not exceed 10% of the life span of the test animal. Subchronic intravenous studies are generally defined as treatment durations of 14–28 days for rodents and non-rodents, respectively.

Table 1.4 Standard device extraction ratios used for biocompatibility (per ISO 10993-12).

Thickness (mm)	Extraction ratio
<0.5	6 cm^2/ml
0.5–1.0	3 cm^2/ml
>1.0	3 cm^2/ml
>1.0 (elastomeric devices)	1.25 cm^2/ml
Irregular solid devices	0.2 g/ml
Irregular porous devices	0.1 g/ml

- Chronic toxicity is any adverse effect occurring after the repeated or continuous administration of a test sample for a major part of the test animal's life span; these are usually studies with duration of 6–12 months.

The main consideration point for systemic toxicity and biopolymers is regarding the dose. The standard biocompatibility test is performed on the basis of surface area or mass to volume; these ratios are spelled out in Table 1.4.

As Table 1.4 points out, the more surface area or mass a device has, the more extraction volume is added to the device during sample preparation. This approach works well for solid, stable materials such as metals and hard plastics but can be challenging with materials such as biopolymers, especially if they are produced with a porous microarchitecture or are biodegradable.

Another giant gap in the approach that uses surface area or mass for calculating the extraction volume is that it does not take into consideration the actual dose that a single patient will be exposed to. Typically, each biological test requires a certain minimal volume of fluid to run, and because of this limitation the sample amount needed for the testing is directly portioned to the logistics demanded by the test itself and not on the actual clinical use of the device. For example, let us say during a surgical procedure, a patient will only receive one PLA screw that is 0.5 g in weight. For the biocompatibility assessment of the screw, a standard subacute study was run. For testing, up to 112 screws were included in order to conform to the required sample volumes that were repeatedly dosed to the test animals, resulting in an exposure that is in actuality multiple times the clinical mass to body weight dose. This leads to a vast overestimation of the exposure risks of the biopolymer.

A better way to design the different systemic toxicity studies of biopolymers is based on dose per body weight of the patient. The standard weights per patient population are described in Table 1.5. In this case, one would determine the appropriate worst-case target population for the medical device or material and determine a dose per kg of body weight based on that criterion. Subsequently, the testing would be done with a sample size that would expose the specific animal to a safety-factor-corrected dose that represents the appropriate clinical dose.

An example of a test design according to the clinical dose approach would be as follows: a surgical procedure where up to two screw PLA screws (each weighing

Table 1.5 Standard body weight parameters.

Population	Standard body weight used (kg)
Adult man	70
Adult woman	58
Children	10
Neonates (<1 yr)	3.5

Table 1.6 Example of specific population doses for 1 g PLA screw.

Population	Gram of screw per kg body weight	With 10X safety factor
Adult man	0.01	0.14
Adult woman	0.02	0.17
Children	0.10	1.00
Neonates (<1 yr)	0.29	2.86

0.5 g) will be implanted into a patient, the worst-case exposure per patient will be 1 g of PLA, and the specific clinical prescribed doses are outlined in Table 1.6.

In a rat subchronic study, if the worst-case target population is adult women and the test rat weighs 500 g, the dose would be calculated as follows:

Desired ratio with safety factor = 0.17 g of screw per body weight

$$\frac{0.17\text{ g of screw}}{\text{kg body weight}} \times \frac{1\text{ kg}}{1000\text{ g}} \times \frac{500\text{ g}}{1\text{ Rat}} = \frac{0.085\text{ g of Screw}}{\text{Rat}}$$

This approach would ensure an accurate exposure dose to the animal and would present a more clinically relevant evaluation for the risks of systemic toxicity for the device.

1.4.3 Implantation

The most difficult and complex test design for many biopolymers revolves around implantation risks. It is important not to walk into an implant study with haste and without careful planning. Indeed, in this case, failing to plan could lead to a failing test. It is important that the study is planned in sufficient detail such that all relevant information can be extracted from the study, as the implant test is usually the longest test in the biocompatibility suite, and therefore, it is imperative to have the design right up front.

The main issue with testing a biopolymer in an implant test is the absorption profile. Physical characteristics (such as form, absorption rate, metabolism characteristics, density, and surface hardness) can all influence the tissue response to

the test material. Also, the choice of control articles should be matched as closely as reasonably possible to the test sample physical characteristics. This is recommended in order to allow comparison of the specific tissue reaction(s) with that of a similar material whose clinical acceptability and biocompatibility characteristics have been established to determine acceptance criteria for the test.

Another key consideration for the implant test for a biopolymer is with the implantation time points. ISO 10993-6 states: "For absorbable materials, the test period shall be related to the estimated degradation time of the test product at a clinically relevant implantation site. When determining the time points for sample evaluation, an estimation of the degradation time shall be made." Usually, in practice we try to estimate the absorption profile based on the specific metabolism rate and method of the material and the implant system. After this, we set three time periods: one where we first see degradation (usually between two and four weeks), second when half the sample is degraded, and third when we see a "steady state" in the sample material. A steady state is defined as a point in time where the body is no longer interacting with the material and no additional changes are happening. For example, *in vivo* implantation tests with a PLLA density scaffold demonstrated fast degradation in the first three weeks, after which the degradation rate progressively decreased [20]. This milestone is reached when the body has either encapsulated or otherwise dealt with the foreign material or when full degradation of the material has occurred.

As mentioned above, an appropriate control is the basis for the acceptance criteria of the test itself, making it an essential component for a relevant and applicable test system. The implantation test is set up so that the evaluation is conducted by comparing the result of the test site histopathology with the control site. Thus, if the chosen control article is a hard piece of metal or plastic that would not induce interaction with the surrounding tissues, then the comparison with the implant site of the biopolymer would probably not be favorable, leading to a higher tissue reactivity and making it look like the test material is non-biocompatible. However, if an appropriate control is used, then the histopathological comparison of the test and control article sites can be made with confidence, and a correct understanding of the implantation risk of the material can be drawn.

1.5 Conclusion

Biopolymers occupy a unique and advantageous space as a medical device material. Devices made from these naturally occurring or biomimetic substances have the distinct advantage that the material itself is akin to those tissues the device contacts. From a bulk perspective, there is no concern regarding the material as a foreign body. Biopolymers also have environmental and manufacturing advantages as they are often produced not from petroleum derivatives but by living systems.

In contrast to the major advantages presented by biopolymers within the context of biocompatibility, there are a couple of key concerns that must be

addressed. The natural origin of these materials does not mean that they are free from manufacturing residuals. Contact with solvents through manufacturing and purification steps can introduce contamination, as can contact with storage and primary packaging materials. Chemical analysis screening for these compounds can be complicated by the complex organic nature of the device material. Additionally, many biopolymers are degradable or resorbable by the body. While this is, in principle, a positive therapeutic effect, it can be difficult to prove that the safety of the device does not change over the degradation lifetime.

The pallet of materials afforded by biopolymers allows an even broader spectrum of medical devices with huge potential to help mankind. The biocompatibility principles discussed in this chapter can be applied to biopolymers to address concerns with regard to their safety. Use of thoughtful risk-based testing strategies can conservatively mitigate risk, allowing more of these devices to reach full maturity in development and arrive on the market.

References

1 Rebelo, R., Fernandes, M., and Fangueiro, R. (2017). Biopolymers in medical implants: a brief review. *Procedia Engineering* 200: 236–243.

2 Yadav, P., Yadav, H., Shah, V.G. et al. (2015). Biomedical biopolymers, their origin and evolution in biomedical sciences: a systematic review. *Journal of Clinical and Diagnostic Research: JCDR* 9 (9): ZE21.

3 Sahana, T. and Rekha, P. (2018). Biopolymers: applications in wound healing and skin tissue engineering. *Molecular Biology Reports* 45: 2857–2867.

4 Labet, M. and Thielemans, W. (2009). Synthesis of polycaprolactone: a review. *Chemical Society Reviews* 38 (12): 3484–3504. https://doi.org/10.1039/B820162P.

5 Chien, L.J. and Lee, C.K. (2007). Enhanced hyaluronic acid production in *Bacillus subtilis* by coexpressing bacterial hemoglobin. *Biotechnology Progress* 23 (5): 1017–1022.

6 Chien, L.J. and Lee, C.K. (2007). Hyaluronic acid production by recombinant *Lactococcus lactis*. *Applied Microbiology and Biotechnology* 77 (2): 339–346.

7 Liu, L., Wang, M., Du, G., and Chen, J. (2008). Enhanced hyaluronic acid production of *Streptococcus zooepidemicus* by an intermittent alkaline-stress strategy. *Letters in Applied Microbiology* 46 (3): 383–388.

8 Helenius, G., Bäckdahl, H., Bodin, A. et al. (2006). In vivo biocompatibility of bacterial cellulose. *Journal of Biomedical Materials Research Part A* 76A (2): 431–438.

9 Hoenich, N.A. (2007). Cellulose for medical applications: past, present, and future. *BioResources* 1 (2): 270–280.

10 Rockwood, D.N., Preda, R.C., Yücel, T. et al. (2011). Materials fabrication from *Bombyx mori* silk fibroin. *Nature Protocols* 6 (10): 1612–1631.

11 Auras, R.A., Lim, L.-T., Selke, S.E., and Tsuji, H. (2011). *Poly(lactic Acid): Synthesis, Structures, Properties, Processing, and Applications*. Wiley.

12 ISO/TS 21726:2019 (2016). Biological evaluation of medical devices – Application of the threshold of toxicological concern (TTC) for assessing biocompatibility of medical device constituents. Standardization IOf.
13 Kroes, R., Kleiner, J., and Renwick, A. (2005). The threshold of toxicological concern concept in risk assessment. *Toxicological Sciences* 86 (2): 226–230.
14 Munro, I., Renwick, A., and Danielewska-Nikiel, B. (2008). The threshold of toxicological concern (TTC) in risk assessment. *Toxicology Letters* 180 (2): 151–156.
15 Hennes, E. (2012). An overview of values for the threshold of toxicological concern. *Toxicology Letters* 211 (3): 296–303.
16 Guideline IHT (2014). Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk. *International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)*, Geneva. Citeseer (31 May 2017). http://academy.gmp-compliance.org/guidemgr/files/M7_R1_ADDENDUM_STEP_4_2017_0331.PDF
17 European Food Safety Authority and World Health Organization (2016). Review of the Threshold of Toxicological Concern (TTC) Approach and Development of New TTC Decision Tree. EFSA Supporting publication 2016:EN-1006. https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/sp.efsa.2016.EN-1006
18 Cramer, G., Ford, R., and Hall, R. (1976). Estimation of toxic hazard – a decision tree approach. *Food and Cosmetics Toxicology* 16 (3): 255–276.
19 Munro, I.C., Ford, R.A., Kennepohl, E., and Sprenger, J. (1996). Correlation of structural class with no-observed-effect levels: a proposal for establishing a threshold of concern. *Food and Chemical Toxicology* 34 (9): 829–867.
20 Weng, W., Song, S., Cao, L. et al. (2014). A comparative study of bioartificial bone tissue poly-L-lactic acid/polycaprolactone and PLLA scaffolds applied in bone regeneration. *Journal of Nanomaterials* 2014: 236.