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Polymer Biocompatibility

Isabel Cristina Celerino de Moraes Porto

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1. Introduction

Polymers are very versatile materials and are used in many applications including pharmaceutical applications. Natural polymers, modified natural polymers, and synthetic polymers are used as excipients in the manufacture of cosmetics and systems for conventional and modified delivery of drugs, by altering the composition and physical properties such as molecular weight, polydispersity, crystallinity, and thermal transitions. They can be prepared to provide a wide range of degradation rates and mechanical properties (Amaral, 2005; Villanova & Oréfice, 2010).

More recently, polymers have been developed that can modulate and deliver drugs to target areas. Biodegradable polymers, bioadhesives, biomimetic materials, and responsive hydrogels have been included in pharmaceutical formulations (Villanova & Oréfice, 2010). Naturally derived polymers offer several advantages compared with synthetic polymers, namely biocompatibility, biodegradability, and biological activity, as most of them are present in the structural tissues of living organisms. However, the low mechanical strength and high rates of degradation of natural polymers often result in their use in combination with ceramics, or in subsequent cross-linking reactions to reduce the degradation rates. Synthetic polymers can be tailored to meet an absorption time requirement, potentially facilitating reproducibility and scale-up, with no concerns about disease transmission, which often constitutes a problem with naturally occurring polymers (Amaral, 2005).

Synthetic polymers present an attractive avenue for biocompatible biomaterials because of their well-studied syntheses and modifiable properties (Ouchi & Ohya, 2004; Puskas & Chen, 2004). Biocompatible polymers can be made into devices directly or incorporated into devices by coating to reduce the chance of rejection when incorporated into the body. There have been significant developments in recent years in shape memory materials, tissue engineering, and coronary stents for use as biocompatible materials (Quansah, 2004).

Biodegradable polymers, especially those belonging to the family of polylactic acid and polyglycolic acid, play an increasingly important role in orthopedics. These polymers degrade by hydrolysis and enzymatic activity and have a range of mechanical and physical properties that can be engineered to suit a particular application. Their degradation characteristics depend on several parameters including their molecular structure, crystallinity, and copolymer ratio. These biomaterials are also rapidly gaining recognition in the fledgling field of tissue engineering because they can be fashioned into porous scaffolds or carriers of cells, extracellular matrix components, and bioactive agents. Although their future appears to be bright, several questions regarding the biocompatibility of these materials linger and should be addressed before their wide-scale use (Athanasίου et al., 1996). The most important requirement for a biodegradable polymer to be used in medical applications is its compatibility not only in terms of physical and chemical properties but also the properties that define their behavior at the time they come into contact with the body (Silva et al., 2004).

2. Biocompatibility

In general, a biomaterial is defined as any substance, except food and medications, that can be used for a length of time as part of a system that aims to treat or to replace any tissue, organ, or body function. Few materials, if any, are totally inert from a physiological standpoint; most materials present a variety of components with potential toxic or irritating properties. In addition, chemical reactions that occur during setting of the material may also produce noxious effects (Anusavice, 2003).

Biomaterials need to satisfy a number of prerequisites before that can be used in applications, including biocompatibility. To verify this feature, its components should be subjected to different tests (Schmalz, 2002), performed as recommended by various organizations and federations. These tests consist of a sequence of research protocols, described and regulated in many countries, for correct use of experimental materials under evaluation, thereby determining their safety for clinical application in humans (Costa, 2001).

Biocompatibility may be defined as (Williams, 2008):

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

Biocompatibility can also be defined as the relationship between a material and the organism so that neither produces undesirable effects. Biocompatibility has been mentioned in many works with increasing interest in evaluating the characteristics of medical and dental materials and devices and responses caused by its components. An ideal pattern for determining these properties has not yet been determined, however, various methods have been suggested for this purpose.

Biocompatibility is a term that encompasses many aspects of the material, including its physical, mechanical, and chemical properties, as well as potential cytotoxic, mutagenic, and allergenic effects, so that no significant injuries or toxic effects on the biological function of cells and individuals arise (Costa, 2001; Lemmons & Natiella, 1986; Schmalz, 2002).

Until the biocompatibility of a material is proven, it must be subjected to various studies ranging from in vitro assays to clinical trials, involving distinct areas such as pharmaceuticals, biology, chemistry, and toxicology.

Biomaterials or their degradation byproducts should not trigger an adverse inflammatory reaction or immune response once implanted. Surface properties such as chemistry, roughness, and surface energy play a major role in cell–material interactions, particularly when considering an absorbable material, which is always presenting a new surface. These surface characteristics determine not only how biological molecules adsorb to the surface, but also their spatial orientation on adsorption. This is of particular importance in host–implant interactions, as the adsorption of proteins present in physiological fluids, such as albumin, immunoglobulin, fibrinogen, and fibronectin, dictate the subsequent inflammatory response and the fate of the implant (Amaral, 2005).

2.1. Methods of biocompatibility testing

Biocompatibility testing has sought to standardize biological tests for biomaterials, to find an effective and safe testing protocol that is more reliable for comparing results from different studies.

These tests are divided into 3 groups, corresponding to primary (level I), secondary (level II), and preclinical (level III) tests, which include analysis of the cytotoxicity and irritant potential of systemic toxicity in animals through intramuscular and subcutaneous implants, and usage tests by observation of tissue reactions after insertion of the material, for example, in human teeth.

Level I tests can be done both in vitro and in vivo. In vitro tests assess the properties of the material directly in cultured cells that react to the effects of the experimental material. Many constituents judged initially as cytotoxic may be modified or have their use controlled by manufacturers to prevent cytotoxicity. In vivo tests are mainly based on the implantation of materials into subcutaneous or intramuscular areas in rats and rabbits to evaluate the tissue response to the implanted material after a period of observation (Anusavice, 2003).

To conduct these tests, it is necessary to involve health researchers for research methodology development and evaluation of the tissue and researchers for development of materials and their properties, such as engineers and chemists.

2.2. In vitro and in vivo tests

The ideal biological research methodology consists of in vivo experiments, despite the ethical aspects involved. Nevertheless, although in vitro studies provide responses limited

by the absence of biological and physiological components that are impossible to reproduce entirely, they continue to be used and are suitable for determining whether a material contains significant quantities of extractable biologically harmful components (Porto et al., 2011).

Clinically, the results are divergent. Studies in vitro could report no damage (Büyükgüröl & Cereli, 2008), moderate damage (Nayyar et al., 2007) or intense damage (Costa et al., 2002). This reinforces the effect of the physiology present in in vivo systems, but does not diminish the relevance of in vitro research. In vitro research remains important for pointing out pathways for studies of the adverse reactions recorded clinically.

In in vitro cell cultures, the complex physiology of an organism performing various functions simultaneously is not present. Thus, the buffer capacity of complex humoral and cellular systems in the intact organism is absent; a biomaterial may not work well in the in vitro test, but may be biocompatible in vivo (Kirkpatrick et al., 2005; Libonati et al., 2011). This highlights the necessity of integrated in vitro and in vivo tests for valuable predictive estimation of the toxicity of complex materials (Libonati et al., 2011).

2.2.1. *In vitro biocompatibility testing*

In the field of biomaterials, it is necessary to consider aspects of biosecurity, such as elimination of cytotoxicity and other harmful effects of the material to be used (Kirkpatrick et al., 2005). By definition, the cytotoxicity of a material or device refers to the toxicological risks caused by a material or its extract in a cell culture (Cao et al., 2005). To perform these tests, mammalian cells, usually of mouse or human origin, obtained from a commercial supplier, are cultured in the laboratory in flasks using nutrient culture media. These cultured mammalian cells reproduce by cellular division and can be subcultured to produce multiple flasks of cells for use in evaluating the cytotoxicity of materials. For cytotoxicity tests in vitro, permanent cell lines or primary cultures are recommended. Although there are difficulties with isolation and maintenance, the primary cells are very important for biological assays because of their similarity to the original tissue (Wallin, 1998).

Cytotoxicity tests are considered a rapid, sensitive and standardized method to determine the toxicity of a material or if it contains significant amounts of biologically harmful leachable compounds. The presence in cultures of isolated cells and the absence of important physiological effect present in vivo systems, which help to protect cells within the body, produces a test with high sensitivity. Culture medium of mammalian cells is the preferred method for the extraction of substances that can be released from a material, because it is a physiological solution capable of extracting a wide range of chemical structures, not only those soluble in water.

Several types of cells can be used for cytotoxicity tests such as cell lines - human and mouse fibroblasts, lymphocytes, queratinocytes, mouse odontoblast-like cells, mouse macrophages, rat submandibular salivary gland acinar cells - (Bakoupoulou et al., 2007; Franz et al., 2009; Kostoryz et al., 2003; Lin et al., 2007; Roll et al., 2004; Samuelsen et al., 2008), and primary cell types - human lymphocytes, polymorphonuclear leukocytes, and mixed leukocytes,

mouse blastocysts, mouse macrophages, and mouse embryo cells - (Becher et al., 2006; Huang & Chang, 2002; Jonhson et al., 1985; Libonati et al., 2011; Porto et al., 2009, 2011; Prica et al., 2006).

Macrophages present throughout the body, including the oral tissues, are involved in inflammation and presentation of antigen during the reaction to infectious agents and foreign bodies. They also amplify the inflammatory response by signaling other cells, and play a central role in the pathogenesis of inflammatory response, therefore are relevant for testing the biocompatibility of materials *in vitro* (Becher et al., 2006). When stimulated, the macrophages are subjected to a process known as macrophagic activation in which they increase their metabolic, motility, and phagocytic activity. Many new proteins are synthesized under activation, including inducible nitric oxide synthase. Nitric oxide (NO), a product of nitric oxide synthase, plays an important role in the defensive function of macrophages (Parslow et al., 2001). Therefore, possible factors such as toxic levels of leachable compounds from materials or devices can be indicated by nitric oxide secretion, cell death, reduction in or loss of cellular function.

Interactions of materials and their components with cells at a molecular level are responsible for tissue reactions, such as inflammation, necrosis (Accorinte et al., 2005), immunological alterations, genotoxicity (Kleinsasser et al., 2004), and apoptosis (Paranjpe et al., 2005).

Among the 3 categories of tests for assessing cytotoxicity that are listed in ISO 10993-5 (2009) (extract test, direct contact test, indirect contact test), it is possible apply a wide variety of experimental protocols. The choice of one or more of these categories depends on the nature of the sample to be evaluated, the potential site of use, and the nature of the use. This choice then determines the details for the preparation of the samples to be tested, the preparation of the cultured cells, and the way in which the cells are exposed to the samples or their extracts. At the end of the exposure time, evaluation of the presence and extent of the cytotoxic effect is undertaken.

The various methods and parameters used in determining cytotoxicity can be grouped into the following categories of evaluation: assessment of cell damage by morphological means, measurement of cell damage; measurement of cell growth; measurement of specific aspects of cellular metabolism.

There are several ways to produce results in each of these 4 categories. The investigator should be aware of the test categories and into which category a particular technique fits, so that comparisons can be made with other results on similar devices or materials at both the intra- and interlaboratory levels. Quantitative evaluation of cytotoxicity can be done using cell death, inhibition of cell growth, cellular proliferation and colony formation, cell number, amount of protein, enzyme released, vital dye release, vital dye reduction, or any other measurable parameters that can be quantified by objective means (ISO 10.993-5, 2009). The biochemical methods (DNA synthesis, protein synthesis, and ATP activity) demonstrated good agreement in toxicity ranking of the materials, regardless of which cell culture was used, and the cell cultures responded similarly for each method. Methods that measured the

functional characteristics of cells (adhesion and phagocytosis) were highly sensitive but had low toxicity ranking agreement and reproducibility. Assays (defined as method and cell culture combinations) using cell lines were more reproducible than assays using primary cell types. Significant differences in sensitivity were noted among the assay systems for particular material types (Johnson et al., 1985).

Relative sensitivity of in vitro biocompatibility test systems was explored by Johnson et al. (1983) and showed cellular responses of 12 standardized cell lines to 20 materials representing a range of toxicity. Results of the tissue culture assays were compared with those obtained for the same materials in vivo using a 5-day rabbit intramuscular implant assay. Methods involving measurement of cellular growth (colony counts or presence of confluence) in serum-fortified media extracts of test samples were generally more sensitive and discriminating than those in which test materials were placed directly in cell cultures (measurement of zone of growth inhibition). Based on sensitivity, reproducibility, ability to discriminate materials, and grader agreement, 4 of the 12 cell lines and 2 of the 4 test methods appeared most suitable for screening and evaluation of materials. Agreement of results using these 4 cell lines with intramuscular implantation tests for the 30 materials ranged from 60 to 90% (Johnson et al., 1983).

The pattern of apoptotic response, cellular glucose, oxygen consumption, and gene expression after exposure to the various compounds, single substances or biomaterials has been used to evaluate cytotoxicity in previous studies (Becher et al., 2006; Lin et al., 2007; Nooca et al., 2007; Sangsanon et al., 2007) and can be used to assess cytopathic effects of multicomponent extracts from cured polymers. The use of different methods to evaluate cytotoxicity may result provide data for a knowledge base to clarify how experimental materials affect cell behavior (Porto et al., 2009).

Using the MTT assay, cell viability can be assessed by the cytochemical demonstration of succinic dehydrogenase enzyme activity, which is a measure of the mitochondrial respiration of the cells. Polymers and its components may alter the enzyme activity of primary cells (Becher et al., 2006; Chen et al., 2003; Porto et al., 2009, 2011) or immortal cell lines (Demirci et al., 2008; Lin et al., 2007; Poskus et al., 2009).

One of the most studied alternative in vitro testing methods for identification of developmental toxicity is the embryonic stem cell test. A study conducted by Van Dartel and Piersma (2011) presents the progress that has been made with regard to the prediction of developmental toxicity using the embryonic stem cell test combined with transcriptomics. Although the embryonic stem cell test has been formally validated, the applicability domain as well as the predictability of the model needs further study to allow its successful implementation as an alternative testing method in regulatory toxicity testing.

Development of additional aspects required for further optimization of the embryonic stem cell test, including kinetics, the use of human embryonic stem cells and computational toxicology, and the current and future use of the embryonic stem cell test model for prediction of developmental toxicity in testing strategies and in regulatory toxicity

evaluations should be also discussed. Genomics technologies have already provided proof of principle of their value in identification of toxicants such as carcinogenic compounds. Within the embryonic stem cell test, gene expression profiling has shown its value in the identification of developmental toxicity and in the evaluation of factors critical for risk assessment, such as dose and time responses. It is expected that the implementation of genomics in the embryonic stem cell test will provide a more detailed end point evaluation compared with the classic morphological scoring of differentiation cultures. Therefore, genomics may contribute to improvement of the embryonic stem cell test, both in terms of the definition of its applicability domain and its predictive capacity (Van Dartel & Piersma, 2011).

The embryonic stem cell test is a high-throughput in vitro screening assay for developmental toxicity free of animal use. The embryonic stem cell test uses the ability of murine embryonic stem cells to differentiate into the mesodermal cardiac lineage in combination with 2 cytotoxicity test systems. Validation of the embryonic stem cell test showed that the test system is very promising as an alternative to animal testing, however to optimize predictability and increase knowledge on the applicability domain of the embryonic stem cell test, improvements to the method were proposed and studied. The authors discuss the first definition of the embryonic stem cell test and the innovative approaches that have been proposed to increase the predictivity of the embryonic stem cell test, including implementation of molecular end points in the embryonic stem cell test, such as OMICS technologies and the addition of alternative differentiation models to the testing paradigm, such as neural and osteoblast differentiation and the use of human stem cells. These efforts to improve the embryonic stem cell test increase the value of embryonic stem cells used as in vitro systems to predict developmental toxicity (Theunissen & Piersma, 2012).

2.2.2. *In vivo biocompatibility testing*

Level II tests are based on tissue assessment of animals that received implants subcutaneously and intramuscular injection of a material with potential to cause systemic toxicity by inhalation, skin irritation, among other responses. Dermal toxicity tests are important because of the large number of chemicals with which we have daily contact. When a material, product, or toxic component is identified, it can be replaced, diluted, or neutralized to reduce the level of toxicity.

Despite their high cost, controversy, and ponderous bureaucratic challenges, animal tests are critical for assessing the biological responses to a new material before it is used in humans. Many aspects of clinical biological responses cannot be modeled by in vitro tests (Anderson, 2001). Animal tests offer evidence about these types of effects without putting humans at risk. Animal tests may be structured to mimic human clinical use to some degree, are commonly less expensive than human clinical trials, can be finished more quickly in many cases, and can be controlled to a greater degree. Animals may be exposed to materials or their degradation products with routes of administration or doses that

would be unethical to consider in humans. Animal tests may be used to determine responses that are difficult or impossible to ethically test in humans and may be tested at many phases of life (for example, embryos or 'children') in a way that is not possible in humans (Wataha, 2012).

Functional properties and biocompatibility are basic issues in the development of biomaterials. There are several methodologies to analyze these properties, however, in all cases the human host has been the ultimate test (Natiella & Lemmons, 1986).

Biocompatible materials cannot be mutagenic or influence inflammatory mediators causing systemic responses, including toxicity, tissue injury, teratogenic or carcinogenic effects. Such materials must be free of agents that may cause allergic responses to individuals sensitive to these substances. After a material has successfully passed the tests for levels I and II, it should be tested in humans (level III test) to evaluate its performance and the favorable or unfavorable reactions that may present under normal clinical conditions (Anusavice, 2003).

3. Biocompatibility for specific polymeric systems

3.1. Chitosan

Chitosan is a biopolymer type polysaccharide and has a chemical structure similar to vegetal fiber cellulose. It is derived from chitin, a polysaccharide that is extremely abundant in nature and is the main component of the exoskeletons of insects and crustaceans, and is found in the cell walls of some species of fungi. Chitosan has several important uses due to its antifungal and antimicrobial activity, its ability to inhibit tumor cells and act as a controlled releaser of drugs in the body.

VandeVord et al. (2002) examined the biocompatibility of chitosan scaffolds using a mammalian implantation model. Early migration of neutrophils into the implantation area, which resolved with increasing implantation time, was reported. Besides this early accumulation of neutrophils, cells that are usually associated with acute inflammation, no evidence of other signs associated with an inflammatory response, such as erythema and edema, were found.

Endotoxins were not detected and a chronic inflammatory response did not develop. In addition, a very low incidence of specific immune reactions was observed, as determined by lymphocyte proliferation assays and antibody binding responses measured using ELISA techniques. Formation of normal granulation tissue associated with accelerated angiogenesis, the typical healing response, was observed. The results from this study indicated that chitosan has a high degree of biocompatibility. These results are in accordance with previous studies (Rao & Sharma, 1997; Tomihata & Ikada, 1997), in which the biocompatibility of chitosan films with different degrees of acetylation is reported, even for highly deacetylated chitin derivatives.

A wide variety of cells have been successfully cultured in/on chitosan matrices, among them keratinocytes (Chatelet et al., 2001), chondrocytes (Denuziere et al., 1998), osteoblasts (Lahiji et al., 2000), endothelial cells, hepatocytes (Elçin et al., 1998), Schwann cells (Yuan et al., 2004), and NH3T3 cells (Li et al., 2012), indicating the cytocompatibility of chitosan toward these cells. In addition, no cytotoxic products are released from chitosan matrices, as shown by the maintenance of cell metabolic activity (Amaral, 2005).

3.2. Dental polymers

In vitro and in vivo studies have demonstrated that the cytotoxic effects of polymeric dental materials, such as light-cured methacrylate polymers, depend on the quantity of unconverted resinous monomers that remain after polymerization (Annunziata et al., 2006; Falconi et al., 2007).

Among the components capable of being lixiviated from dental polymers are bisphenol A-glycidyl methacrylate (bis-GMA), triethylene glycol dimethacrylate (TEGDMA), and 2-hydroxyethyl methacrylate (HEMA) (Lin et al., 2007). These monomers can alter cellular metabolism at concentrations well below the toxic level. The changes induced can be considered a potential mechanism for clinical and subclinical effects (Nooca et al., 2007).

Bis-GMA is the most toxic among them. A mechanism suggested for the cytotoxicity of this monomer is alteration of the lipid layer of the cell membrane, which affects membrane permeability (Lefebvre et al., 1996). In addition, bis-GMA undergoes hydrolysis, producing methacrylic acid as a metabolite. Methacrylic acid is soluble in water and capable of inducing cytotoxicity via stimulation of tumor necrosis factor alpha release (Kostoryz et al., 2003).

HEMA is an amphoteric monomer that displaces water in dentin and is capable of diffusing rapidly through the dentin (Bouillaguet et al., 1996). It is also miscible with most of the monomers used in composites (Bouillaguet, 2004). Therefore, the possibility of other components of the adhesive system acting synergistically with HEMA to increase cytotoxicity needs to be investigated, especially because other relatively hydrophobic resinous composites are soluble in HEMA and may be carried through the dentin (Bouillaguet et al., 1996).

HEMA has been shown to be a potent mediator of cell death by apoptosis at concentrations ranging from millimolar to micromolar (Cetinguç et al., 2007). If released at low concentrations for a prolonged period of time, HEMA could reduce the rate of cellular proliferation and result in apoptosis, possibly as a response to DNA damage (Samuelsen et al., 2008).

Dental adhesive and composites components may alter the succinate dehydrogenase enzyme activity of primary cells (Becher et al., 2006; Chen et al., 2003; Porto et al., 2009, 2011) or immortal cell lines (Demirci et al., 2008; Lin et al., 2007; Poskus et al., 2009).

Despite have adverse effects measured in vitro tests, dental polymers may or not may trigger noxious reactions in vivo, and it depends on the amount released compounds, concentration and other factors. Thus after many in vitro tests and animal tests be completed is possible that the hazards and risks are acceptable for human usage.

3.3. Polylactide acid and polyglycolide acid

Synthetic polymers, such as polylactic acid and polyglycolic acid and their copolymer are becoming more and more attractive for tissue engineering applications and have already been approved by the US Food and Drug Administration. Since the 1960s, polylactic acid and polyglycolic acid have been applied in medicine as controlled drug delivery systems, mostly injectable as microspheres, orthopedic fixation devices such as pins and screws, and as scaffold to mimic the extracellular matrix for cells (Ashammaki & Rokkanen, 1997; Gunatillake & Adhikari, 2003; Jain, 2000; Richardson et al., 2001; Thomson et al., 1995).

Depending on the lactic and glycolic portion of the polylactide-co-glycolide copolymer, various physical and mechanical properties can be produced and the degradation rate can be specifically regulated (Wintermantel & Ha, 2002). Polylactic acid shows a high crystallinity and is attractive as a biodegradable polymer because degradation products naturally occur in the body and are resorbed through the metabolic pathway. Both polymers demonstrate a similar degradation rate caused by random hydrolysis of their ester linkage producing either lactic acid from polylactic acid or glycolic acid from polyglycolic acid, which can be excreted in urine. The degradation rate of the material depends on various criteria besides the copolymer ratio, such as configurational structure, crystallinity, morphology, stress, the amount of residual monomer, porosity, and the site of the implantation (Jain, 2000). The desired mechanical properties are also based on the polymer ratio and on the molecular weight and crystallinity of the scaffold (Mauth et al., 2007).

Various in vitro and in vivo studies have approved the biocompatibility and the biodegradability of these polymers. Mild inflammatory reactions have been observed after a massive release of acidic degradation in vivo depending on the amount and degradation rate of the material (Bostman et al., 1990; Grayson et al., 2004; Holy et al., 1999; Hutmacher, 2000; Thomson et al., 1999; Young et al., 2002). However, the use of synthetic biocompatible material has the enormous advantage of reproducible synthesis and the mechanical and chemical properties including the structure, size, viscosity, and porosity, as well as degradation rate of the desired scaffold can be controlled. Furthermore, bioactive molecules can be incorporated and locally applied by controlled release of the biodegradable polylactic acid, polyglycolic acid, or polylactide-co-glycolide copolymer system and so influence the cell phenotype expression (Mauth et al., 2007).

In their study Athanasiou et al. (1996) undertook an extensive literature review on the toxicity and biocompatibility of polylactic acid-polyglycolic acid biomaterials. In general, polylactic acid/polyglycolic acid biomaterials showed satisfactory biocompatibility,

although some reduction in cell proliferation has been noted. Many studies have successfully demonstrated biocompatibility of polylactic acid/polyglycolic acid biomaterials in vivo (Athanasίου et al., 1996).

4. Conclusion

For the biocompatibility of a material to be proved, it must be subjected to various studies ranging from in vitro assays to clinical trials and involving distinct areas such as pharmaceuticals, biology, chemistry, and toxicology. The use of standardized tests allows better comparison between the results of different studies to clarify the behavior of the materials and their safety in relation to cells and tissues.

For biodegradable polymers, long-term tests should be prioritized to enable the evaluation of the effects of continuous release of metabolites resulting from their degradation and to observe the type and extent of local and systemic changes.

Only a combination of various in vitro and in vivo tests can provide an overview of the interaction of biomaterials with the host.

Author details

Isabel Cristina Celerino de Moraes Porto

Federal University of Alagoas, CESMAC University Center, Brazil

5. References

- Accorinte, M.L.R., Loguercio, A.D., Reis, A., Muench, A., & Araújo, V.C. (2005). Adverse effects of human pulps after direct pulp capping with the different components from a total-etch three-step adhesive system. *Dental Materials*, Vol. 21, No. 7, (July 2005), pp. 599–607, ISSN 0109-5641.
- Amaral, I.M.S.R.F. (2005). Chitosan matrices for cell-based bone regenerative therapies. PhD thesis, Faculdade de Engenharia, Universidade do Porto, Portugal.
- Anderson, J.M. (2001). Biological responses to materials. *Annual Review of Materials Research*, Vol. 31, pp. 81-110.
- Annunziata, M., Aversa, R., Apicella, A., Annunziata, A., Apicella, D., Buonaiuto, C., & Guida, L. (2006). In vitro biological response to a light-cured composite when used for cementation of composite inlays. *Dental Materials*, Vol. 22, No. 12, (December 2006), pp. 1081–1085, ISSN 0109-5641.
- Anusavice, K.J. (2003). *Phillip's Science of Dental Materials* (11th edition), Saunders, ISBN 9780721693873, Philadelphia, PA.
- Ashammakhi, N. & Rokkanen, P. (1997). Absorbable polyglycolide devices in trauma and bone surgery. *Biomaterials*. Vol. 18, No. 1, pp. 3–9.

- Athanasίου, K.A., Agrawal, C.M., Barber, F.A., & Burkhart, S.S. (1998). Orthopaedic applications for PLA-PGA biodegradable polymers. *Arthroscopy*, Vol. 14, No. 7, (October 1998), pp. 726–737, ISSN 0749-8063.
- Athanasίου, K.A., Niederauer, G.G., Agrawal C.M. (1996). Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/ polyglycolic acid copolymers. *Biomaterials*, Vol. 17, No. 2, (January 1996), pp. 93–102, ISSN 0142-9612.
- Bakopoulou, A., Tsiftoglou, A., Galaktidou, G., Markala, D., Triviai, I., Garefis, P. (2007). Patterns of cell death and cell cycle profiles of cultured WEHI 13 var fibroblasts exposed to eluates of composite resins used for direct and indirect restorations. *European Journal of Oral Sciences*, Vol. 115, No. 5, (October 2007), pp. 397–407.
- Becher, R., Kopperud, H.M., Al, R.H., Samuelsen, J.T., Morisbak, E., Dahlman, H.J., et al. (2006). Pattern of cell death after in vitro exposure to GDMA, TEGDMA, HEMA and two compomer extracts. *Dental Materials*, Vol. 22, No. 7, (July 2006), pp. 630–640, ISSN 0109-5641.
- Bostman, O., Hirvensalo, E., Mäkinen, J., & Rokkanen, P. (1990). Foreign-body reactions to fracture fixation implants of biodegradable synthetic polymers. *Journal of Bone and Joint Surgery British Volume*, Vol. 72, No. 4, pp. 592–596.
- Bouillaguet, S. (2004). Biological risks of resin-based materials to the dentin-pulp complex. *Critical Reviews in Oral Biology and Medicine*, Vol. 15, No. 1, (January 2004), pp. 47–60, ISSN 1045-4411.
- Bouillaguet, S., Wataha, J., Hanks, C.T., Ciucchi, B., & Holz, J. (1996). In vitro cytotoxicity and dentin permeability of HEMA. *Journal of Endodontics*, Vol. 22, No. 5, (May 1996), pp. 244–248, ISSN 0099-2399.
- Büyükgür, B. & Cehreli, Z. (2008). Effect of different adhesive protocols vs calcium hydroxide on primary tooth pulp with different remaining dentin thickness: 24-month results. *Clinical Oral Investigations*, Vol. 12, No. 1, pp. 91–96, ISSN 1432-6981.
- Cao, T., Saw, T.Y., Heng, B.C., Liu, H., Yap, A.U., & Ng, M.L. (2005). Comparison of different test models for assessment of cytotoxicity of composites resins. *Journal of Applied Toxicology*, Vol. 25, No. 2, pp. 101–108.
- Çetinguç, A., Olmez, S., & Vural, N. (2007). HEMA diffusion from dentin bonding agents in young and old primary molars in vitro. *Dental Materials*, Vol. 23, No. 3, pp. 302–307.
- Chatelet, C., Damour, O., & Domard, A. (2001). Influence of the degree of acetylation on some biological properties of chitosan films. *Biomaterials*, Vol. 22, No. 3, pp. 261–268.
- Chen, R.-S., Liu, C.-C., Tseng, W.-Y., Jeng, J.-H., & Lin, C.-P. (2003). Cytotoxicity of three dentin bonding agents on human dental pulp cells. *Journal of Dentistry*, Vol. 31, No. 3, pp. 223–229.
- Costa, C.A.S. (2001). Testes de citotoxicidade em culturas de células. In: *Metodologia Científica. Ensino e Pesquisa em Odontologia*, Estrela, C. (Ed.), pp. 145–160. São Paulo: Artes Médicas.
- Costa, C.A.S., Nascimento, A.B.L., & Teixeira, H.M. (2002). Response of human pulps following acid conditioning and application of a bonding agent in deep cavities. *Dental Materials*, Vol. 18, pp. 543–551.

- Demirci, M., Hiller, K.-A., Bosl, C., Galler, K., Schmalz, G., & Schweikl, H. (2008). The induction of oxidative stress, cytotoxicity, and genotoxicity by dental adhesives. *Dental Materials*, Vol. 24, No. 3, pp. 362–371.
- Denuziere, A., Ferrier, D., Damour, O., & Domard, A. (1998). Chitosan-chondroitin sulfate and chitosan-hyaluronate polyelectrolyte complexes: biological properties. *Biomaterials*, Vol. 19, No. 14, pp. 1275–1285.
- Elçin, Y.M., Dixit, V., & Gitnick, G. (1998). Hepatocyte attachment on biodegradable modified chitosan membranes: In vitro evaluation for the development of liver organoids. *Artificial Organs*, Vol. 22, No. 10, pp. 837–846.
- Falconi, M., Teti, G., Zago, M., Pelotti, S., Breschi, L., & Mazzotti, G. (2007). Effects of HEMA on type I collagen protein in human gingival fibroblasts. *Cell Biology and Toxicology*, Vol. 23, No. 5, (September–October 2007), pp. 313–322, ISSN 0742-2091.
- Franz, A., König, F., Lucas, T., Watts, D.C., Schedle, A. (2009). Cytotoxic effects of dental bonding substances as a function of degree of conversion. *Dental Materials*, Vol. 25, No. 2, pp. 232–239.
- Grayson, A.C., Voskerician, G., Lynn, A., Anderson, J.M., Cima, M.J., & Langer R. (2004). Differential degradation rates in vivo and in vitro of biocompatible poly(lactic acid) and poly(glycolic acid) homo- and co-polymers for a polymeric drug-delivery microchip. *Journal of Biomaterials Science Polymer Edition* Vol. 15, No. 10, pp. 1281-1304.
- Gunatillake, P.A. & Adhikari, R. (2003). Biodegradable synthetic polymers for tissue engineering. *European Cells & Materials*, Vol. 5, pp. 1–16.
- Holy, C.E., Dang, S.M., Davies, J.E., & Shoichet, MS. (1999). In vitro degradation of a novel poly(lactide-co-glycolide) 75/25 foam. *Biomaterials*, Vol. 20, No. 13, pp. 1177–1185.
- Huang, F.M., Chang, Y.C. (2002). Cytotoxicity of dentine-bonding agents on human pulp cells in vitro. *International Endodontic Journal*, Vol. 35, No. 11, pp. 905-909.
- Hutmacher, D.W. (2000). Scaffolds in tissue engineering bone and cartilage. *Biomaterials*, Vol. 21, No. 24, pp. 2529–2543.
- ISO: No. 10993-1 (2009). Biological evaluation of medical devices
- Jain, R.A. (2000). The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. *Biomaterials*, Vol. 21, No. 23, pp. 2475–2490.
- Johnson, H.J., Northup, S.J., Seagraves, P.A., Garvin P.J., & Wallin, R.F. (1983). Biocompatibility test procedures for materials evaluation in vitro. I. Comparative test system sensitivity. *Journal of Biomedical Materials Research*, Vol. 17, No. 4 (July 1983), pp. 571–586.
- Johnson, H.J., Northup, S.J., Seagraves, P.A., Atallah, M., Garvin, P.J., Lin, L. et al. (1985). Biocompatibility test procedures for materials evaluation in vitro. II. Objective methods of toxicity assessment. *Journal of Biomedical Materials Research*, Vol. 19, No. 5, pp. 489–508, doi: 10.1002/jbm.820190503.
- Kirkpatrick, C.J., Peters, K., Hermanns, M.I., Bittinger, F., Krump-Konvalinkova, V., Fuchs, S. et al.. (2005). In vitro methodologies to evaluate biocompatibility: status quo and perspective. *ITBM*, Vol. 26, No. 3, pp. 192–199
- Kleinsasser, N.H., Wallner, B.C., Harréus, U.A., Kleinjung, T., Folwaczny, M., Hickel, R. et al. (2004). Genotoxicity and cytotoxicity of dental materials in human lymphocytes as

- assessed by the single cell microgel electrophoresis (comet) assay. *Journal of Dentistry*, Vol. 32, No. 3, pp. 229–234.
- Kostoryz, E.L., Eick, J., Chappelow, C.C., Glaros, A.G., Wetmore, L., & Yourtee, D.M. (2003). In vitro effect of light-cure dental adhesive on Il-6 release from LPS-stimulated and unstimulated macrophages. *Journal of Biomedical Materials Research Part A*, Vol. 65, No. 1, pp. 89–94.
- Lahiji, A., Sohrabi, A., Hungerford, D.S., & Frondoza, C.G. (2000). Chitosan supports the expression of extracellular matrix proteins in human osteoblasts and chondrocytes. *Journal of Biomedical Materials Research*, Vol. 51, No. 4, pp. 586–595.
- Lefebvre, C.A., Schuster, G., Rueggeberg, F.A., Tamareselvy, K., & Knoernschild, K.L. (1996). Responses of oral epithelial cells to dental resin components. *Journal of Biomaterials Science Polymer Edition*, Vol. 7, No. 11, pp. 965–976.
- Lemmons, J. & Natiella, J. (1986) Biomaterials, biocompatibility and peri-implant considerations. *Dental Clinics of North America*, Vol. 30, No. 1 (January 1986), pp. 3–23.
- Li, X., Kong, X., Zhang, Z., Nan, K., Li, L., Wang, X. et al. Cytotoxicity and biocompatibility evaluation of N,O-carboxymethyl chitosan/oxidized alginate hydrogel for drug delivery application. *International Journal of Biological Macromolecules*, 2012, doi:10.1016/j.ijbiomac.2012.03.008.
- Libonati, A., Marzo, G., Klinger, F.G., Farini, D., Gallusi, G., Tecco, S. et al. (2011). Embryotoxicity assays for leached components from dental restorative materials. *Reproductive Biology and Endocrinology*, 2011, pp. 9:136. doi:10.1186/1477-7827-9-136.
- Lin, N.J., Bailey, L.O., Becker, M.L., Washburn, N.R., & Henderson, L.A. (2007). Macrophage response to methacrylate conversion using a gradient approach. *Acta Biomaterialia*, Vol. 104, No. 2, pp. e45–e52.
- Mauth, C., Huwig, A., Graf-Hausner, U., & Roulet, J.-F. (2007). Restorative applications for dental pulp therapy. In: *Topics in Tissue Engineering*, Ashammakhi, N., Reis, R., & Chiellini, E. (Eds.), Vol. 3, Chapter 3.
- Nayyar, S., Teari, S., & Arora, B. (2007). Comparison of human pulp response to total-etch and self-etch bonding agents. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, Vol. 104, No. 2, pp. e45–e52.
- Nocca, G., De Palma, F., Minucci, A., De Sole, P., Martorana, G.E., Callà, C. et al. (2007). Alterations of energy metabolism and glutathione levels of HL-60 cells induced by methacrylates present in composite resins. *Journal of Dentistry*, Vol. 35, No. 3, pp. 187–194.
- Ouchi, T. & Ohya, Y. (2004). Design of lactide copolymers as biomaterials. *Journal of Polymer Science. Part A Polymer Chemistry*, Vol. 42, No. 3, (February 2004). pp. 453–462, ISSN 1099-0518.
- Paranjpe, A., Bordador, L.C., Wang, M.Y., Hume, W.R., Jewett, A. (2005). Resin monomer 2-hydroxyethyl methacrylate (HEMA) is a potent inducer of apoptotic cell death in human and mouse cells. *Journal of Dental Research*, Vol. 84, No. 2, pp. 172–177.
- Parslow, T.G., Stites, D., Terr, A.I., & Imboden, I.B. (2001). *Medical Immunology*. New York: Lange Medical Books/McGraw-Hill Medical Publishing Division.

- Porto, I.C., Andrade, A.K., Guênes, G.M., Ribeiro, A.I., Braz, R., Castro, C.M. (2009) In vitro potencial cytotoxicity of an adhesive system to alveolar macrophages. *Brazilian Dental Journal*, Vol. 20, No. 3, pp. 195–200.
- Porto, I.C., Oliveira D.C., Raele, R.A., Ribas, K.H., Montes, M.A., & De Castro, C.M. (2011). Cytotoxicity of current adhesive systems: In vitro testing on cell cultures of primary murine macrophages. *Dental Materials*, Vol. 27, No. 3, (March 2011), pp. 221–228.
- Poskus, L.T., Lima, R.S.M.S., Lima, I.R., Guimarães, J.G.A., Silva, E.M., & Granjeiro, J.M.G. (2009). Cytotoxicity of current adhesive systems: *in vitro* testing on cell culture of L929 and balb/c 3T3 fibroblasts. *Revista Odonto Ciências*, Vol. 24, No. 2, pp. 129–134.
- Prica, D., Galić N, Želježić D, Prica A. (2006). Genotoxicity evaluation of five different dentin bonding agents by chromosomal aberration analysis. *Journal of Oral Rehabilitation*, Vol. 33, No. 6, pp. 462–471.
- Puskas, J.E. & Chen, Y. (2004). Biomedical application of commercial polymers and novel polyisobutylene-based thermoplastic elastomers for soft tissue replacement. *Biomacromolecules*, Vol. 5, No. 4, (July–August 2004), pp. 1141–1154, ISSN 1525-7797.
- Quansah, J.K. Synthetic polymers for biocompatible biomaterials. *Materials literature seminar*. Sep. 23 2004. Available in: www.chemistry.illinois.edu/research/materials/seminar_abstract_/2004_2005/Quansah.Abstract.LitSeminar.pdf. April 13 2012.
- Rao, S.B. & Sharma, C.P. (1997). Use of chitosan as a biomaterial: studies on its safety and hemostatic potential. *Journal Biomedical Materials Research*, Vol. 34, No. 1, pp. 21–28.
- Richardson, T.P., Murphy, W.L., & Mooney, D.J. (2001). Polymeric delivery of proteins and plasmid DNA for tissue engineering and gene therapy. *Critical Reviews in Eukaryotic Gene Expression*, Vol. 11, Nos. 1–3, pp. 47–58.
- Roll EB, Dall J, Runningen G, Morisbak E. (2004). In vitro cell death induced by irradiation and chemicals relevant for dental applications; dose–response and potentiation effects. *European Journal of Oral Sciences*. Vol. 112, No. 3, pp. 273–279.
- Sangsanog, P., Waleetorncheepsawat, S., Suwantong, O., Wutticharoenmongkol, P., Weeranantanapan, O., Chuenjitbuntaworn, B., et al. (2007). In Vitro Biocompatibility of Schwann Cells on Surfaces of Biocompatible Polymeric Electrospun Fibrous and Solution-Cast Film Scaffolds. *Biomacromolecules*, Vol. 8, pp. No. 5, 1587–1594.
- Samuelsen, J.T., Holme, J.A., Becher, R., Karlsson, S., Morisbak, E., & Dahl, J.E. (2008). HEMA reduces cell proliferation and induces apoptosis in vitro. *Dental Materials*, Vol. 24, No. 1, pp. 134–140.
- Schmalz, G. (2002). Material science: biological aspects. *Journal of Dental Research*, Vol. 81, No. 10, pp. 660–663.
- Silva, G.A., Marques, A.P., Gomes, M.E., Coutinho, O.P., & Reis, R.L. (2004). Cytotoxicity screening of biodegradable polymeric systems. In *Biodegradable Systems in Tissue Engineering and Regenerative Medicine*, Reis, R.L. & San Róman, J. (Eds.), Chapter 19. Boca Raton, FL: CRC Press.
- Theunissen, P.T. & Piersma, A.H. (2012). Innovative approaches in the embryonic stem cell test (EST). *Frontiers in Bioscience*, Vol. 17, (January 2012), pp. 1965–1975. DOI:<http://dx.doi.org/10.2741/4032>.

- Thomson, R.C., Wake, M.C., Yaszemki, M.J., & Mikos, A.G. (1995), Biodegradable polymer scaffolds to engineer trabecular bone. *Journal of Biomaterials Science Polymer Edition*, Vol. 7, No. 1, pp. 23–38.
- Thomson, R.C., Mikos, A.G., Beahm, E., Lemon, J.C., Satterfield, W.C., Aufdemorte, T.B. et al. (1999). Guided tissue fabrication from periosteum using preformed biodegradable polymer scaffolds. *Biomaterials*, Vol. 20, No. 21, pp. 2007–2018.
- Tomihata, K. & Ikada, Y. (1997). In vitro and in vivo degradation of films of chitin and its deacetylated derivatives. *Biomaterials*, Vol. 18, No. 7, pp. 567–575.
- Van Dartel, D.A. & Piersma, A.H. (2011). The embryonic stem cell test combined with toxicogenomics as an alternative testing model for the assessment of developmental toxicity. *Reproductive Toxicology*, Vol. 32, No. 2, (September 2011), pp. 235–244.
- VandeVord, P.J., Matthew, H.W.T., DeSilva, S.P., Mayton, L., Wu, B., & Wooley, P.H. (2002). Evaluation of the biocompatibility of a chitosan scaffold in mice. *Journal of Biomedical Materials Research*, Vol. 59, No. 3, pp. 585–590.
- Villanova, J.C.O. & Oréfice, R.L. (2010). Aplicações farmacêuticas de polímeros. *Polímeros: Ciência e Tecnologia*, Vol. 20, No. 1, pp. 51–64.
- Wallin, R.F. A practical guide to ISO 10993-5: cytotoxicity. *Medical device & diagnostic industry magazine*. (1998). Available in: www.mddionline.com/article/practical-guide-iso-10993-5-cytotoxicity. March 10 2012.
- Wataha, J.C. (2012). Predicting clinical biological responses to dental materials. *Dental Materials*. Vol. 28, No. 1, pp. 23–40.
- Williams, D.F. (2008). On the mechanisms of biocompatibility. *Biomaterials*. Vol. 29, No. 20, pp. 2941–2953.
- Wintermantel, E. & Ha, S.W. (2002). *Medizintechnik mit biokompatiblen Werkstoffen und Verfahren*, Vol. 3, pp. 192–232. Berlin: Springer.
- Young, C.S., Terada, S., Vacanti, J.P., Honda, M., Bartlett, J.D., Yelick, P.C. (2002). Tissue engineering of complex tooth structures on biodegradable polymer scaffolds. *Journal of Dental Research*, Vol. 81, No. 10, pp. 695–700.
- Yuan, Y., Zhang, P.Y., Yang, Y.M., Wang, X., Gu, X.S. (2004). The interaction of Schwann cells with chitosan membranes and fibers in vitro. *Biomaterials*, Vol. 25, No. 18, pp. 4273–4278.