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A Review on Biocompatibility of Dental Restorative and Reconstruction Materials

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Abstract

Purpose of Review Confusion exists on the correct terminology and definitions associated with biocompatibility, including terms such as toxicity, health effects, and allergies. Therefore, this review aims to provide clarity by structuring and summarizing the current terminology, outlining the existing testing methods for each concept, and offering examples within dental material groups.

Recent Findings New materials, such as nanomaterials and engineered living materials (ELM), have entered the dental field, requiring a deeper understanding of their biocompatibility. Additionally, recent regulatory changes, such as the European Medical Device Regulation (EU MDR), underscore the importance of standardized terminology and testing methods in this evolving landscape.

Summary Measurements in biocompatibility are essential in biomedical applications, involving the interaction between materials and living tissues (host). Testing methods include in vitro, in vivo, clinical, and ex vivo approaches. While thresholds and guidelines, such as NOEL and LOAEL, ensure safe biomaterial use, dental materials, such as alloys, polymers, ceramics, and nanomaterials, exhibit varying biocompatibility and toxicity levels influenced by factors such as release rates, degradation, and chemical interactions. Nanoparticles hold promise but raise concerns about oxidative stress and long-term health effects. Regulatory bodies (i.e., FDA and EU MDR) play crucial roles in ensuring product safety. In conclusion, the dynamic field of dental materials requires ongoing adaptation, rigorous testing, and adherence to regulations for the safe and effective use of emerging technologies in dentistry.

Keywords Dental materials · Biocompatibility · Toxicity · Allergy · Biomaterials · Biological performance

Introduction

Dental materials in restorative and reconstructive dentistry have undergone tremendous progress during the last decades [1–4]. While dental industry and pharmacy seek and produce what clinicians demand for and beyond, there are national and international legal regulations to control dental materials and devices. Their most important task is hazard identification, the implementation of control systems, and definition of a risk plan, in short: the regulatory organs ensure safety for the producer and for the end user [5]. In order to avoid malpractice in dental practice, dental products should be approved and certified by the regulatory organs, and also be applied as indicated by the patient's needs [6]. Even though dental materials, their safety, biocompatibility, and effectiveness are subject to regulatory systems, manufacturers need the fundamental knowledge for adequate production and handling of the materials and dentists benefit on knowledge about the different risks for each material and the basic adverse effect reporting.

Nowadays, new materials, such as nanomaterials, are introduced to the market and in the dental field, which require even more knowledge and some basic understanding [7]. Nanoscale materials show unique physical, chemical, and biological properties, and differ significantly from larger bulk materials [8]. Though their definition is clearly stated with dimensions up to 100 nm, many products are

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entitled "nano," even though they are not truly in nanoscale and therefore do not hold nanoscale properties. The same confusion counts for the brand-new engineered living (tissue) materials (ELM), which are even more difficult to unite in the current terminology. Attempts to produce new inert biomaterials are almost deprecated and displaced by novel bioactive, instructive, and immunomodulatory trends [9]. With the new European Medical Device Regulation (EU MDR) in 2017 and all progresses in the abovementioned new medical product groups, a clear terminology and systematic test methods seems to be more important than ever.

Therefore, this review aims to summarize the current terminology related to biocompatibility, toxicity, and health effects. Furthermore, current test methods for each term and examples of dental material groups will help the clinician to differentiate and understand the subject of dental materials biocompatibility and their legal regulations.

Biocompatibility of Dental Materials

Biocompatibility, in principle, relates to the interplay between living tissues and non-living substances. The professor J. Black, known for his work in orthopedic surgery and bioengineering, penned a seminal book on the biological performance of materials and the underlying principles of biocompatibility [10••]. The FDA's Biocompatibility Guidance (Food and Drug Administration), informed by this book and grounded in the standards of ISO 10993–1 (International Standardization Organisation), consequently defined biocompatibility as "The capacity of a device material to function harmoniously with an appropriate host response in a specific context." [11].

In relation to this, a couple of key terms were further clarified:

- Biomaterial: A (nonviable) substance utilized in a medical device, purposed to interact with biological systems.
- Host Response: The response from a living organism to the introduction of a material.
- Biocompatibility (or Biological Performance): The capability of a substance to function cohesively with an appropriate host response in a specific situation.
- Toxicity: The ability to damage a biological system by chemical means [12••].
- Cytotoxicity: The ability of a substance to harm or kill cells by disrupting their function or causing cell death.
- Health Effects: Consequences of substance exposure, including local reactions (due to substances, bacteria, or physical stimuli), systemic toxicity (adverse reactions away from the application site), and other effects, like allergies, which can occur at lower substance concentrations than systemic toxicity.

Ultimately, the core focus lies on the triadic interaction among the biomaterials, the host, and the surrounding environment, where the biomaterial can be either inert or tolerable. However, it is crucial to acknowledge that "biocompatibility" is not an absolute term, and thus, it is advisable to use its synonym "biological performance" or "tissue compatibility." [5, 10••, 12••]. The central concern of biocompatibility is not strictly about the potential adverse biological reactions to a material, but more importantly, whether that material performs adequately (as intended) in its proposed biomedical application. Therefore, only then can it be deemed a successful biomaterial (Fig. 1).

Testing for Biocompatibility

Control regulations are delineated into three regulatory classes: in vitro testing, in vivo testing, and clinical testing (Fig. 2).

While in vitro tests are quick and easy to perform, the results cannot be directly transferred to patients. The in vivo animal tests are usually closer to the clinics and offer a good control; however, animal tests are cost-intensive and need experimental animals [13]. The clinical tests are the gold standard and closest to the clinical situation, but are expensive, time-consuming, and may not always represent the average clinical practice [14••]. Interesting alternatives are nowadays evolving by a new categorization called ex vivo testing [15]. These tests use chick chorioallantois membrane (CAM) assays to evaluate its vascularization after contact to the test material. These tests can be ranked between the in vitro and the in vivo tests. Other interesting alternatives include in silico testing methods, which are computer-based simulations and modeling to predict and assess behavior, properties, or outcomes in a virtual environment, reducing the need for physical experiments.

For a better overview of all these potential experimental groups, and their underlying questions, Schmalz and

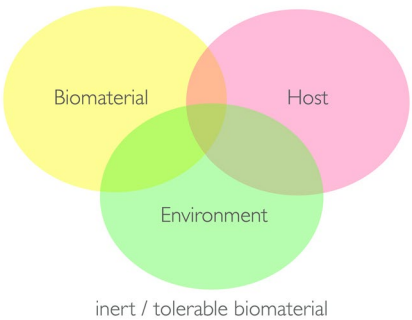

Fig. 1 Triadic interaction among the biomaterials, the host, and the surrounding environment

Fig. 2 Overview and short description of the three testing procedures for the biocompatibility

Arenholt-Bindslev presented a systematic classification (Fig. 3 [12••]).

In Vitro *Testing for Biocompatibility*

In vitro tests employ a variety of cell culture methodologies to determine cytotoxic reactions, inflammatory responses, or mutagenicity. Mouse fibroblasts or other target tissue cells serve as primary culture and are incubated with the test material. The outcome is often the surviving of the cells, protein synthesis, enzyme activity, or inflammatory mediators [16]. Other common tests in dentistry are dentin barrier tests or the so-called two-chamber tests, in which dentin disks or roots are put between the test material and target cells, which perfuse with the growth medium [17]. The probably most widely used bacterial DNA mutation test is the "Salmonella/mammalian microsome test". This test is also called the AMES test, after his founder Bruce Ames, who leads laboratories in Berkeley California. Nowadays, it is suspected, that over 90% of known carcinogens are directly

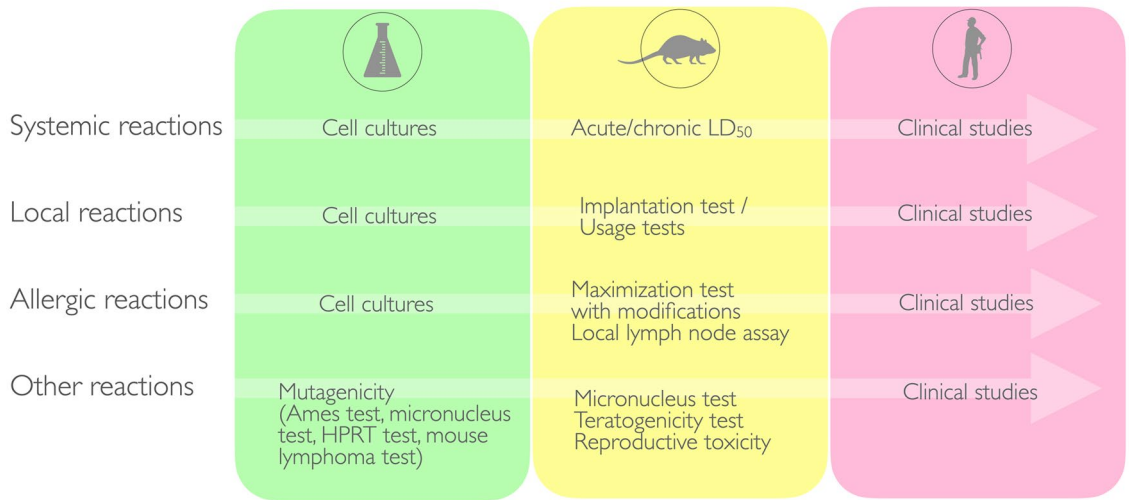

Fig. 3 Modified overview from Schmalz and Ahrenholt-Bindlev [12••] of all three testing procedures from in vitro (green), over in vivo (yellow), and the clinical testing procedures (orange). Common respective testing procedures are listed for different reactions, such as systemic, local, allergic or all others from top to bottom

acting in DNA (positive Ames test). This simple testing procedure serves therefore as a surrogate endpoint for carcinogenicity [18, 19]. All these in vitro tests therefore often serve as the initial foundation for identifying potential risks and the general performance of the test materials.

In VivoTesting for Biocompatibility

Subsequent to in vitro experiments, in vivo tests are generally employed, which involve animal-based usage tests where the test materials are applied to dental pulp or gingival/mucosal tissues [13]. In dentistry, "Class V tests" are also conducted, in which the test material is applied to cervical lesions in non-human primates. These specimens are later examined histologically. The primary focus is usually on the cellular-level response of the pulp, bacterial leakage, or tertiary dentin formation [20, 21]. Other animal models are used as endodontic usage test, where the test materials are applied into the root canal. Histologic (and metagenomic) evaluations are thereafter performed to analyze the periodontal tissues [22]. Another form of the in vivo test method is the implantation test in which subcutaneous, intramuscular implantation are performed in test animals [23]. The investigated outcome is hereby the inflammation and foreign body reaction. Further assessments relate to the body reaction and degradation of the applied test material [24].

Clinical Testing for Biocompatibility

If the in vitro and in vivo test methods revealed promising results, the next level of testing procedures are clinical tests, in which the test materials are applied on humans in defined study settings. The gold standard is the controlled randomized clinical trial. However, it should be mentioned that biocompatibility is usually not the main focus of clinical studies. More common, efficiency tests or investigations on wear and longevity are the primary objectives, and only secondary objectives are usually pulp sensitivity, postoperative pain, tissue, or mucosal reactions.

Threshold Values in Biocompatibility

In order to allow interpretations of the abovementioned testing procedures in biocompatibility, some terms were defined as reference:
NOEL: no observed effect level.
NOEAL: no observed adverse effect level.
LOEL: lowest observed effect level.
LOAEL: lowest observed adverse effect level.

These threshold values help to assess the safety profile of a biomaterial. They help establish the highest level at which a biomaterial can be used without causing any adverse biological response, and the lowest level at which a biomaterial starts causing any adverse biological reaction. These values are essential for determining the safety and appropriate usage levels of new biomaterials.

Examples in Biocompatibility of Dental Materials

Most of the dental alloys, which are used in dentistry, were already excessively tested for biocompatibility [25–29]. Newer tests investigate mainly the effects of the fabrication techniques within the same materials [27]. While explicit threshold values were defined, many biocompatibility tests still do not refer to the terminology. Common outcomes in the tests either compare different materials within their biocompatibility [28]. One example to mention is the in vitro biocompatibility of nickel–chromium (Ni–Cr) alloys, with three different manufacturing techniques (casting, selective laser melting (SLM), and soft milling (SM)) [27]. The authors describe the excessive impact of the applied fabrication technique in relation to wear resistances (nanohardness, elastic modulus, Vickers hardness) and biocompatibilities (cell proliferation, cytotoxicity, cell apoptosis tested on L-929 mouse fibroblasts in medium with CAST or SLM extracts) of the Co-Cr dental alloys. Superior outcomes in both, wear resistance and biocompatibilities, were measured for the SLM technique compared to the CAST technique for fabricating Co-Cr dental alloys.

Regarding the material group "Ceramics," literature and most product data sheets attest acceptable biocompatibility, while adverse effects were described in relation to the cementation or bonding products used [30]. Solubilized lithium content of dental ceramics are discussed in the "Examples in Toxicity of Dental Materials" section.

Biocompatibility test methods of polymers in dentistry showed very different results between the different monomers [31–35]. It was shown that some polymers beyond the dental field, such as bisphenol A (BPA) and di (2-ethylhexyl) phthalate (DEHP), act as hormonally active agents. These results have generated public interest due to their presence in several consumer products [31]. The public pressure led to an abundance of BPA in most newborn products. Similar to how dental alloy biocompatibility test methods are described, various polymer tests adopt a comparison strategy: Becher et al. for instance compare aqueous extracts of cured compomers to evaluate their potential to induce necrosis and apoptosis in primary rat alveolar macrophages and the J744A1 macrophage cell line. Their findings show that GDMA (glycerol dimethacrylate) exhibits the most cytotoxic effects compared to other monomers, followed by TEGDMA (triethylene glycol dimethacrylate), while HEMA demonstrates higher apoptotic cell accumulation [32]. Many studies analyzing the effects of composite resins' monomers measure cytotoxicity. Consequently, the dental industry aims to develop products that yield lower percentages

of unbound free monomers after curing to enhance their in vitro biocompatibility.

Toxicity of Dental Materials

The toxicity of a material describes the ability to damage a biological system by chemical means [12••]. It can be distinguished between the systemic toxicity, and a local toxicity. In general, substances are released from all dental materials into the oral cavity. If adverse reactions appear at the application site, local toxicity occurs. Substances in their plain form or degraded as by-product can, however, also find their way to different organs via blood circulation. This means that the application site (oral cavity) may differ from the effect site, where the substance unfolds its toxic effects which is called systemic toxicity. If certain threshold levels are reached, interactions may occur with the function of a specific organ. These toxic reactions can be acute, within 24 h of the substance release, subacute, within 3 months, or chronic. Moreover, immunotoxicity can be defined as impairment of the host defense or as a tissue damage, such as by chronic inflammation [36]. The immune function of the host is altered and an increased sensitivity to infections and cancers may happen, as a matter of polarization of the immune response by immunotoxic agents. The term cytotoxicity describes the toxic effects of substances, to cause cell death.

Health Effects and Toxicity

Exposure to a substance can result in local reactions. However, local reaction may also be triggered by bacterial accumulation or physical stimuli, like pressure or irritation. These health effects can be categorized as follows [12••].:

- Local reactions: These are caused by the release of substances, bacterial presence, or mechanical/physical stimuli.
- Systemic toxicity: This refers to adverse reactions occurring distant from the application site.
- Allergies and others: This encompasses various health effects. Interestingly, a difference between systemic toxicity and allergies and others is based on the concentration of the substance to induce adverse effects. Allergic effects require less doses to induce health effects, than the systemic toxicity.

A famous quote attributed to Paracelsius states that "the dose makes the poison" [37], implying that the harmfulness of a substance is determined by its concentration. However, this notion is challenged by research in endocrinology and clinical medicine. Hormonally active compounds, for instance, can have dose–response curves where low doses induce effects opposite to those at high doses. This indicates that the relationship between substance exposure and health effect is more complex than a simple dose-dependent pattern [38].

Testing Toxicity

Traditional methods for acute toxicity testing measure the median lethal dose (LD_{50}) or the median lethal concentration (LC_{50}). Pure chemicals are applied to test animals, such as to mice, rats or rabbits, either orally, dermally, or are inhaled or injected [39••, 40]. The median lethal dose is described, using the 95% confidence interval and estimates the death of 50% of the test animals. Different conventional testing methods were introduced over the last 100 years, such as the Kärber or Reed and Muench method in 1931 and 1938 [41, 42]. The tests differ in the number of required animals, their simplicity to perform, and their expenditure. These conventional methods alike are in need of a high number of animals to sacrifice. Due to ethical reasons, the principle of 3Rs, namely reduction, replacement, and refinement, was introduced in science and industrial practice [43]. The aim is to reduce animals in laboratory testing procedures (reduction), to find alternatives, whenever possible, and to minimize potential pain and suffering during the experiments (refinement). This standard caused several new measurement methods, such as the fixed dose procedure (FDP) in 1992, the acute toxic class method in 1996, an the up- and down procedures (UDP) in 1998. These newer methods require less test primates and are approved by regulatory organs as adequate testing method. The test outcome are still signs of toxicity and death [44].

Testing Toxicity Today

A newer method to test the toxicity is called limit test, where a fixed dose of a test substance (for instance 2000 or 5000 mg/kg body weight) is applied to the test animals [45]. If this concentration is not high enough to reach the LD_{50}, no further tests are applied [39••]. It is important to acknowledge that toxicological tests, including LD tests, are predominantly conducted on animals, typically rodents, rabbits, and guinea pigs. However, it is essential to remain cautious about extrapolating the results directly to humans and consider their relevance carefully [46]. In some rare cases, extensive sever toxicity is expected from chemicals, and tests should be avoided to reduce the risk of contact. For these special cases, in silico toxicology test methods are described as suitable alternative [47]. As ethical concerns continue to grow, computational modeling methods are emerging as promising alternatives.

Threshold Values in Toxicity

The classical acute LD50 refers to the median lethal dose, which is the calculated dose of a substance expected to cause death in 50% of the test population. LD_{50} values are expressed as the amount of substance administered per unit of body weight (mg/kg). Lower LD_{50} values indicate higher acute toxicity, meaning that a smaller amount of the substance can cause harm. Each substance has its own toxicity thresholds, and these thresholds may vary depending on the regulations of different countries or organizations.

Examples in Toxicity of Dental Materials

Most acute LD_{50} values are documented in the safety data sheet of the respective substances under toxicological information. The LD_{50} (lethal dose) or LC_{50} (lethal concentration) with additional information on the test location (oral, dermal) and information in the test population (rat, rabbit) are given, in most of the cases.

Toxicity of Dental Alloys

In the 1970s, research on dental alloys and their acute LD50 values was conducted, but these studies have gained renewed interest today, primarily due to advancements in nanotechnology and its diverse impacts. For mercuric chloride (Hg), published acute LD50 values range from 25 to 78 mg/kg (rat), indicating its relatively higher toxicity. On the other hand, palladium appears to be less toxic, with LD50 values around 200 mg/kg [48]. Metal and metallic oxide nanoparticles are a growing trend in dental applications, known for their unique shape-dependent properties, bio-physio-chemical functionalization, antimicrobial activity, and biocompatibility. Copper nanoparticles, being cost-effective and stable, are widely used in dentistry and can improve various dental materials' physical and chemical properties [49]. They intermix well with other materials and have been employed in dental amalgams, cements, adhesives, resins, endodontic solutions, dental implants, and orthodontic components. With an LD_{50} value of 413 mg/kg, 23.5 nm copper nanoparticles are considered moderately toxic [50]. Among dental alloy materials, silver (Ag) is considered the most problematic component in Au alloy restorations due to its toxicity and composition. It was estimated, that on average, adults could have up to four tooth surfaces restored with Au alloy before exceeding the reference exposure level (REL) for Ag [51•].

Toxicity of Dental Polymers

Toxicological measurements of polymers are another extensively researched topic. Methacrylate monomers, for instance, are commonly used in dentistry as polymer-based composite restorative materials and can cause irritation and allergic reactions due to incomplete polymerization. Their toxicity is related to their reactivity with nucleophiles like glutathione (GSH) [52]. Acrylates, with higher electrophilic reactivity, are known to be more toxic. Monomers and co-monomers are released into the oral cavity and pulp, which may then enter the bloodstream, affecting various organs. This potential release of substances raises concerns about adverse biological effects. In contrast to classical acute toxicity tests, polymer research relates to short-term release of free monomers during conversion and to long-term release of leachable substances by erosion and degradation. Efforts have reduced the percentage of unbound monomers, but complete conversion during polymerization is yet to be achieved. Residual monomers, even in small quantities (1.5–5%), can cause significant cytotoxic effects [53]. The quantity of monomers released and their cytotoxic effects vary depending on the polymerization parameters [54]. Dentin permeability and thickness influence the reaction, with residual dentin absorbing some unbound monomers [55].

Toxicity of Dental Composite Resins

Composite resins exposed to oxygen during curing produce nonpolymerized surface layers containing formaldehyde, adding to cell toxicity [56]. Various monomers and additives have been identified in dental composites, showing moderate to severe cytotoxic effects, especially at early intervals [57•]. The cytotoxicity results vary depending on the material and cells used for testing. Human periodontal ligament and pulp fibroblasts were observed to be more sensitive than 3T3 and gingival fibroblasts. While resin-containing restorative materials are generally considered cytotoxic, less cytotoxic alternatives could be identified for several highly cytotoxic composite components [56, 58].

The long-term effects due to leachable substances and their chemical characteristics determine their diffusion through the polymer network, resulting in a release due to degradation or erosion over time. Chemical degradation can be caused by hydrolysis or enzymatic catalysis, such as by human saliva-derived esterases [59•]. Resin composites can release bis-HPPP (2,2-bis [4(2,3-hydroxypropoxy)-phenyl]propane) and TEGMA (triethyleneglycol-dimethacrylate) when incubated with cholesterol esterase. Water or solvents entering the polymer result in erosion and weight loss, facilitating the long-term diffusion of unbound monomers by softening the Bis-GMA (bisphenol-A glycidyldimethacrylates) matrix [60].

Toxicity of Dental Ceramics

Regarding the material group of ceramics, limited research has been published on the chemical exposures and risks

associated with dental ceramic materials, especially when compared to dental alloys or composite resins [51•]. Observations indicate that lithium, the most problematic content of ceramic restorations, has the potential to solubilize from ceramic materials [61, 62]. Adults could have up to 15 tooth surfaces restored with ceramics before surpassing the reference exposure level for lithium. In terms of relative risks of chemical exposures from dental materials, the order is as follows: Amalgam > Au alloys > ceramics > composite resins [51•]. Hydrofluoric acid is used in dentistry to condition glass ceramics for cementation or repair which poses potential hazards, with acute symptoms like skin or nail burns, and chronic effects involving systemic toxicity, eye injuries, and respiratory or ingestion-related symptoms, and can be fatal. The aggressive nature of HF makes it harmful to soft tissues, and symptoms may not immediately manifest after exposure [63].

Nanoscience and Toxicity of Dental Materials

Nanoscience has developed from laboratory research to applied technology, with nanomaterials now widely used in various products. Limited studies on the long-term health effects of nanoparticles exist, prompting concerns about their potential toxicity [64]. Understanding the body's ability to eliminate nanomaterials (< 100 nm) and prevent particle build-up in tissues is still a subject of research.

Nanoparticles (NPs) can cause toxicity in the body by generating an excess of reactive oxygen species (ROS). This occurs during the dissolution of iron-based NPs, resulting in oxidative stress [50]. Moreover, certain inert nanomaterials can induce ROS production by specifically targeting mitochondria under specific biological conditions. ROS play essential roles in cellular events but can also be harmful, damaging cells and potentially causing various diseases like cancer, renal disease, and neurodegeneration [65]. According to Rallo et al. [66], nanoparticle-induced oxidative stress affects cell signaling in three stages: low stress enhances defense gene transcription, higher stress activates inflammation signaling, and very high stress leads to apoptotic pathways and necrosis. Reactive oxygen species (ROS) from nanoparticles can cause DNA damage, including double-strand breaks and mitochondrial DNA damage.

Regulations of Medical Devices

Regulatory organs address mainly the safety and the general biocompatibility of the materials and devices as their key targets. These regulatory systems are well defined and specified in almost all countries, and most often rely on superordinate organizations, such as the International Organization for Standardization (ISO). All medical materials and devices are subjected to certain principles to guarantee their safety and biocompatibility for the end user prior to marketing. Certifications are necessary to allow their use and start the post-market stage after testing. In the USA, the Food and Drug Administration (FDA) is responsible for the protection of public health and controls the medical product development and manufacturing. FDA-approved medical devices were allowed to be used worldwide, while the European's Conformité Européenne (CE) mark was less powerful. Scandals in the medical product industry (such as the PIP breast implant scandal), however, triggered the European control organs to publish the new European Medical Device Regulation (EU MDR) and the In Vitro Diagnostic Medical Devices

Table 1 Overview of EU MDR Risk classifications of various dental medical products, according Regulation on Medical Devices 2017/745 (MDR) Annex VIII, date: 29.12.2022

Product description	Class		Comments
Adhesives	IIa	Rule 8	Placed "in teeth"
All-ceramics	IIa	Rule 8	
Alloys (for crowns, bridges, inlays, prosthetics)	IIa	Rule 8	
Bone replacement materials	IIa	Rule 8	Not resorbable
	IIb	Rule 8	Resorbable
Dental implants and abutments	IIb	Rule 8	Long-term use, not placed "in teeth" therefore not class IIa
Dental implants, biological coated	III	Rule 8, 3rd indent	If biological action is claimed
Filling materials (composite, glass-ionomer-cements, ceramic inlays, galvano inlays)	IIa	Rule 8	If nanomaterial, internal exposure negligible
	IIa	Rule 19	
Materials for guided tissue regeneration	IIb	Rule 8	Not resorbable
	IIb	Rule 9	Resorbable
Suture materials	IIa	Rule 7	Not resorbable
	III	Rule 7, 14 or 18	Resorbable

Regulation (IVDR) in 2017. The Unique Device Identification (UDI) is one of the changes related to both EU regulations. The traceability of all medical devices can be facilitated with this unique code for each medical device. This identifier counteracts fake origin medical devices and improves patient safety. The new regulations in the EU and the FDA regulations seem to be more comparable since the EU MDR, but differ in some key points.

The medical device classifications, which are the first step towards medical device marketing, differ between the FDA and MDR: The FDA classifies the device risk with class I (low risk), and class II (moderate risk), which fall mostly under the 510 (k) pathway to market. Product manufacturers can provide the FDA with documented evidence, that the new medical device is equivalent to a previous device, which is already on the market. If the 510 (k) pathway is not suitable, the pre-market authorization (PMA) takes place. This pathway applies to all Class III medical devices and needs non-clinical and clinical studies prior to marketing (Homepage: https://www.fda.gov/medical-devices/overview-device-regulation/classify-your-medical-device Date: 29.12.2022). In contrast, the EU MDR risk classification counts 4 device categories (Homepage: https://eumdr.com/classification/ Date: 29.12.2022): non-invasive, invasive, active, and special medical devices which include contraceptive, disinfectant, or radiological diagnostic medical devices. Another classification is based on the risk assessment, which is categorized as class I: non-sterile or no measuring function (low risk); class I: sterile and a measuring function (low/medium risk); class IIa (medium risk); class IIb (medium/high risk); class III (high risk). All medical products have to fulfill special testing requirements, according to their risk classification. Representative examples of the risk classification of different dental products are shown in Table 1.

Limitations/Concluding Remarks

Biocompatibility and toxicity considerations in the field of dental materials are of utmost importance. An essential balance exists between the applied materials and living tissues. To analyze potential harmful interactions and risks, research and regulations follow predefined testing methods due to standardization. The methodologies range from in vitro and in vivo to clinical and ex vivo approaches, each offering distinct advantages and accompanied by inherent limitations. However, these predefined and standardized methodologies face several challenges. The rapid advancement in materials, especially in nanotechnology, leads to new material groups and undefined product classes. Also, medical device regulations can hinder innovation and the introduction of new products. Moreover, the absence of standardized testing procedures poses difficulties in classifying hazardous levels of materials. For instance, the term "biomaterial" traditionally referred to nonviable materials used in medical devices, but with advancements such as engineered living materials (ELM), the material can be considered viable in a medical device. In material science and technologies, 3D printing of living tissues holds a promising potential. These developments however call for continuous adaptation and improvement in biocompatibility research along with the regulations to ensure safe and effective medical applications of evolving technologies.

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Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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