

**"ASSESSMENT MODALITIES FOR ORTHODONTIC MATERIALS: A
COMPREHENSIVE REVIEW OF TESTING METHODS"**

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Orthodontic materials play a pivotal role in modern dental practice, necessitating rigorous evaluation of their performance and biocompatibility. Tests of orthodontic materials encompass a comprehensive assessment of mechanical properties, such as tensile strength, hardness, and resilience. These evaluations aid in determining the materials' durability and ability to withstand the demanding oral environment. Biocompatibility tests are equally crucial, as orthodontic materials are in prolonged contact with oral tissues. Cytotoxicity assays evaluate the materials' potential to induce harmful cellular reactions, while sensitization tests assess their allergenicity. Furthermore, biocompatibility tests extend to evaluating the materials' impact on soft and hard tissues, ensuring they do not provoke adverse inflammatory responses or hinder the natural healing process. Comprehensive biocompatibility assessments ensure patient safety by minimizing the risk of allergic reactions, tissue irritation, or other detrimental effects. Therefore a meticulous understanding of these materials' mechanical properties, alongside their interactions with oral tissues, empowers dental practitioners to make informed decisions, ultimately leading to enhanced orthodontic care.

Keywords: orthodontic tests, biocompatibility , tensile test, bend test.

INTRODUCTION

Orthodontic treatment has evolved significantly over the years due to advancements in technology and materials. With the availability of newer materials and techniques, orthodontic treatment has become more efficient and comfortable for patients.¹ Most commonly used orthodontic archwires have high tensile strength and are available in various diameters and configurations. Ceramic brackets are made from high-strength ceramics and are aesthetically pleasing as they blend with the tooth colour.² Elastics and elastomerics are have a variety of uses as ligatures , e chains , ethreads etc due to their physical properties.³ Testing of these materials is essential to ensure their effectiveness, longevity and safety for use Mechanical properties, corrosion resistance, biocompatibility, and other factors are commonly evaluated through various test.

Organisations like ASTM (American Society for Testing and Materials) and ISO (International Organisation for Standardisation) have developed standardised protocols to guarantee uniformity and accuracy in testing methods. ⁴⁻⁵ This article will provide a brief review of the various tests to assess the physical properties as well as biocompatibility of orthodontic materials.

TESTS FOR PROPERTIES OF ORTHODONTIC MATERIALS

There are several tests to evaluate mechanical attributes as well as biocompatibility of orthodontic materials to determine if they are reliable and appropriate for clinical usage.

A.TENSILE TEST

A test specimen is put under a tensile tension that increases gradually until it breaks or fractures. performed by Instron on a Universal Testing Machine.⁶ following are the steps

Sample preparation → Mounting the specimen → Zeroing the machine → Applying a tensile load → Measuring load and deformation → Determining mechanical properties → Interpreting the findings

The appropriateness of the material for its intended use is then determined by comparing the findings with standard values or specifications.

B.BENDING TEST

Bend strength, is a material's ability to resist deformation under pressure. Flexural strength is a measure of the material's greatest internal stress at the moment of rupture. A material's elastic modulus of bending, flexural stress, and flexural strain may all be calculated using bending experiments. The mechanical properties of orthodontic wires are frequently evaluated under bending conditions since it is believed that this type of deformation is more therapeutically

applicable than the conventional tension test. This concept gave rise to American Dental Association specification no. 32, which evaluates orthodontic wires free of precious metals using a cantilever bending test. ⁷

Types of bending test:-

Cantilever bending test

As recommended ADA Specification No.32, done with the help Olsen stiffness tester. ⁸ Involves clamping a specimen by one end and applying a controlled force on the free end. The distance that exists between the clamp and that point at which the controlled force is applied is referred to as the test span. While the opposite end of the test span is being bent by a bending plate, the specimen's fixed end rotates. A motor drive gradually applies the strain, and an incredibly precise indication of the load and consequent bend angle are provided. For 25 mm test spans of large diameter (d) SS and Co-Cr-Ni archwires, the Olsen stiffness tester was used to calculate the modulus of elasticity (E), which produces agreement with value of E obtained under tension.: $E = \frac{l M}{2I \theta}$

In this scenario, l denotes the length of test span, I denotes moment of inertia, and M/ denotes the slope of bending moment-angular deflection plot. ⁹

Three-point bending tests

This test subject the samples to severe tensile as well as compressive stress in their plane, as well as shear loads that range from most at neutral axis to 0 at the outermost surface. Two pins that are separated from one another by a particular distance support the sample. For three-point bending tests, the maximum deflection (y) of the beam is : $y = \frac{FL^3}{48 EI}$

Where F = applied force

L = test span length.

Disadvantage :- The bending moment varies linearly from its peak at the loading point to 0 at the both supports. The site of a specimen's failure can be predicted by this loading pattern. ⁹

Four-point bending test

For the 4-point bending tests, there exists a constant bending moment between 2 inner loading points. While mechanical testing is done in order find structural flaws, the specimen in this case is

permitted to fail at weakest place, which is extremely helpful characteristic.

$$\text{maximum deflection } (y) = \frac{FaL^3}{24EI}(3L^2-4a^2)$$

a=distance between the two inner loading point & outer support

F= applied force at inner loading point

Five point bending tests

The 5-point bending test is a variant of the 3-point bending test that was suggested by Nikolai et al. two loading points are present at either end of the section to represent the pair. This promotes the arch wire in the bracket to engage. The centre of the wire section serves as the fifth loading point.¹⁰

C.FRACTURE TOUGHNESS TEST

The amount of stress that is present at the point when a crack or fault first begins to spread through a material in an unstable way is referred to as fracture toughness. This characteristic has been linked to a biomaterial's capacity to withstand wear and fracture propagation in oral environments. It gives an estimate of the force or energy needed to cause a fracture to spread.¹¹ Fracture toughness test methods included:-

1.Vickers indentation techniques

Also known as the Palmqvist toughness test.¹² This method, which was first put out in the late 1970s, was created to determine the fracture toughness of ceramic materials by gauging the lengths of fractures that emerge from Vickers indents.¹³ The bases of the brackets are polished using a variety of abrasives, with a sub-micrometer diamond paste serving as the last step. The brackets are implanted in metallographic resin. The polished specimen surface is indented using the Vickers hardness testing apparatus. The fracture toughness (K_{Ic}) from the relationship is calculated using the length of the fractures extending from the corners of the depression.

The ideal indenting load will result in cracks that are at least as long as the indentation's diagonal.¹³ A Vickers indentation's palmqvist toughness is determined by counting all the cracks that extend from its four corners. The harder the metal is, for a certain indentation load, the shorter the crack. The test has no established standards, and surface preparation techniques have a significant impact on the test's outcomes. Due to the possibility of considerable crack expansion over time, errors in the calculation of K_{Ic} may arise if the measurement of fracture length is postponed.

2. Single edge-notch techniques

This method uses a beam with a limited tip radius notch. The outcomes vary depending on the preparation technique and notch width. First, a beginning crack must be produced. Next, the specimen must undergo cyclic fatigue loading to produce a sharp extension of the original fracture. Finally, the entire crack length must be measured under a microscope. Throughout the test, the load-displacement curve is noted and utilised to determine the material's fracture toughness. But it was shown to be extremely technique-sensitive. Specimen information: There is a prepared rectangular bar specimen with a centrally positioned notch of length a , a width W , a breadth B , and a test span length S between the two supports. The dimensional requirements in the ASTM standard for the test specimen include :

W/B ratio is 2;

The a/W ratio must be between 0.45 and 0.55

Both a and B must exceed $2.5 \times K_{Ic} / YS^2$,

where YS is the yield strength

$$K_{Ic} = \frac{PS}{BW^{3/2}} f\left(\frac{a}{W}\right)$$

This test is recommended by ISO 6872:200815, which outlines the specifications and test procedures for dental ceramic materials.¹⁴ ASTM Standard E399(1983) provides an outline of the single-edge notched.¹⁵

3. Compact tension-specimen techniques

The compact tension specimen (CTS) is one of the often used test specimens for estimating the stress intensity factor, K_{Ic} . By applying equal and opposing pressures through the specimen's two holes, the compact tension fracture toughness test is carried out in order to spread the initial, acute break. Compact tension specimen technique, as opposed to single edge-notch technique, provides the benefit of reduced specimen dimensions that satisfy plane-strain criteria. By applying cyclic loads through pins put into the sample's holes using a laboratory fatigue test machine, a notched sample is used to induce a fatigue fracture. On the notch's point, the fatigue crack will start, and it will continue through the sample. Typically, the length of the crack is measured directly using an optical microscope.¹⁶

4. Short-rod technique along with a chevron notch.

A chevron-notched specimen was introduced by L.M. Barker (1977) to assess fracture toughness under plane strain circumstances. In this procedure, a chevron notch is machined into one end of a short rod.¹⁷ ASTM Standard E 1304 (1989) provides a description of the short rod test design. A cylinder along with a chevron-shaped notch is loaded under tension during the test using a short rod design. The short-rod test results in a stable cracking zone that acts as a pre-crack for the eventual development of an unstable fracture. In order to load the CNSRB, three points are bent. In a CNSRB test, the specimen should be secured in a three-point bending fixture, with the top loading roller aligned with the notched plane and the bottom support rollers symmetrically positioned on either side of the notch plane.¹⁸ The chevron-notched specimens are utilised for a fracture toughness test of small-size specimens. The short rod chevron-notch specimen has the advantages of (I.) The chevron tip developed a crack at the initial test loading. (II.) a simple method for calculating K_{Ic} using the maximum test load and a calibration factor that is only dependent on the specimen shape and loading method. (III.) doesn't call for pre-cracking¹⁸

5. Double-torsion technique

The slow crack development and fracture toughness are tested using the double-torsion testing method. In the test setting, symmetric four-point load is applied to a crack or notch on one end of a rectangular plate, leading to torsional deformation in both plate halves. This loading design stands out because the factor that determines stress intensity is, independent of fracture length for a range of crack lengths in the test specimen. This shows that double torsion testing is best suited for evaluating opaque & non-reflective materials as it may be difficult to determine the fracture length in these materials.¹⁹ In double-torsion testing, fracture toughness can be assessed by quickly loading a test specimen that has already cracked and measuring the maximum load at failure

D. BOND STRENGTH MEASUREMENTS

It is a way to gauge how well an adhesive bond holds a bracket on etched tooth enamel or another surface. The bond's ability to withstand tensile, shear/peel, and torsional loads has been measured. The bond strengths of adhesive systems are chosen based on laboratory testing results. These bond strengths can forecast how long a bound complex would last. There are two types of bond strength measuring testing: qualitative screening tests and quantitative tests. Quantitative tests forecast the bond's load capacity and longevity, whereas qualitative testing investigate bond failures. Bond strength can be evaluated using clinical outcomes and laboratory techniques. Depending on the size of the bond region, it can be statically tested using a macro- or micro-test setup. By dividing the highest applied force by the bonded cross-sectional area, the nominal bond strength is determined.²⁰⁻²¹ Laboratory tests can be static or dynamic tests.

1. Static tests

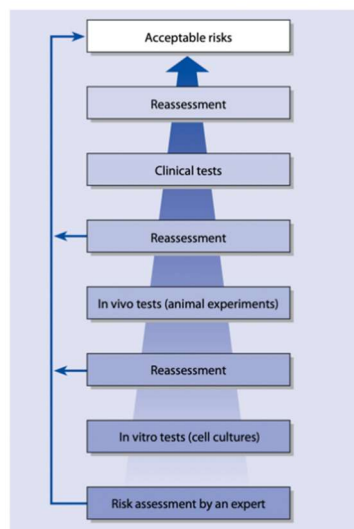
In this the load is delivered while the specimen is stationary. Static testing are divided into macro-tests with a bond area greater than 3 mm² and micro-tests with a bond area less than 3 mm².²² The macro-bond strength can be assessed using push-out, shear, or tensile testing.

2. Dynamic tests

For a more accurate simulation of clinical functioning, dynamic fatigue data must be included to static bond-strength data. Different fatigue techniques, such as the Macro/Micro push-out, Macro/Micro shear, Micro-tensile, and Micro-4-point-bend tests, are utilised.²³⁻²⁴ Variables influencing the results of macro-bond strength test- Substrate related factor, Source of the teeth, Type of the teeth used, Dentin depth & its permeability, Smear layer, Substrate location, Tooth donor-age, Storage conditions and time etc. Variables that are related to the properties of test specimen - Bonding area, Elastic modulus of resin composite. Variables that are related to the specimen preparation for testing the bond strength - Aging media and time, Thermal cycling, Mechanical cycling, Operator's skill & technique sensitivity. Variables that are related to the test mechanics- Type of loading and Speed of cross head.²²

E. BIOCOMPATIBILITY TESTS

The term "biocompatibility" (sometimes referred to as "tissue compatibility") describes a substance's ability to work with the adequate and accepted host response when employed as intended. A material's biocompatibility is mostly determined by the compounds that are released through corrosion or dissolution. The adverse effects produced by biomaterials may range from toxicity and allergic reaction to any systemic toxicity. Materials that contact surface can be biofunctionalized, eg coating of a titanium surface using signalling protein like the BMP, this improves its attachment to surrounding bone.



1. Cell culture

For these assays, isolated cells produced in culture plates from human or animal tissues are employed.²⁵⁻²⁶ The materials or their extracts are applied to ("incubated") these cell cultures. A variety of other characteristics will then be assessed, including the quantity of the surviving cells, the activity of enzymes, inflammatory mediator production and protein synthesis,. One of the techniques for measuring cell damage related to the material was based on "neutral red", the dye. Vital cells are stained by this dye, whereas cells with damaged membranes are not stained. MTT test, which uses a colour change reaction to measure the activity of mitochondrial enzymes photometrically can also be used.²⁷ Dentin-

barrier assays are more contemporary methods that mimic tooth circumstances by sandwiching test material and target cells with a dentin disc. The target cells can be immortalised pulp fibroblast cultures in three dimensions. Growth media is continuously infused into the cultures, preserving their viability for up to a few weeks. In that approach, certain circumstances won't require doing animal research.²⁸⁻²⁹ It is always important to analyse and evaluate a substance in comparison to comparable substances with documented clinical behaviour (relative toxicity study)²⁷

2. Implantation test

The material is implanted into an experimental animal (eg rabbits, rats, etc.) in the bone, , intramuscularly or subcutaneously for implantation experiments. The neighbouring tissue is examined macroscopically and microscopically following various implantation times (between 1 week to several months). The main evaluation will be of implant-circumscribed inflammation following a brief implantation period (1-2 weeks). Implantation investigations, as compared to cell culture test, also reveal data regarding evacuation of hazardous compounds from the tissue and about the overall organism's defence response, such as through an inflammatory reaction. Consequently, compared to cell culture trials, this form of research is more in-tune with patients.

3. Mucosal damage & Mucosa usage test

For assessing mucosal compatibility (oral mucosa test), many cell cultures or animal models can be used.³⁰⁻³¹ Use of In vitro generated skin analogues like skin fibroblasts and keratinocytes are cultivated in in vitro co-cultures are recently being used for these testings. Tests of oral mucosa aren't taken into account in the majority of national and international standards due to their technical constraints therefore additional tests are employed to assess possible mucosal injury

4. Intraosseous Implant test

Test animals had intraosseous implants placed in their jaws with materials used for dental implants. For this, experimental animals are used to replicate the treatment of patients by penetrating the epithelial barrier. Primates, dogs, guinea pigs, rats, dogs, and small pigs are examples of appropriate animals. The tissue in touch with implants is of interest when evaluating tissue reactivity histologically³²

5. Diagnostic tests

Diagnostic test are done on patients in order to thoroughly analyse alleged or actual undesired adverse effects in an individual. certain materials may induce isolated incidences of diseases that are allegedly or really connected to the materials. These observations serve as the foundation to assume an individual's compatibility with dental materials. To uncover a workable explanation regarding the various symptoms, to carry out a causative treatment, or even to avoid these symptom, it is necessary to undertake a study of an individual's compatibility of biomaterials being used.

6. Allergy test

The patch test is one of the most significant allergy tests in relation to dental biomaterials. It was first created and reported by Jadassohn This test can be used to determine whether type IV reactions of the delayed kind are what's causing an allergic contact dermatitis³² The prick test can be used to identify immediate responses (type I reactions, like asthma). You can employ the radioallergosorbent test (RAST) in addition to the finger prick test.

In patch test The skin on the patient's back is applied with tapes that contain the probable allergen at high concentrations capable of causing allergic reactions that are non irritating³⁴ Following the removal of the tape, skin is examined for any responses such as redness, presence of blisters, itching etc. The Skin's responses are evaluated at the 2nd and 3rd day but further examinations at 5th and 7th days are also required. The major approach for identifying a type IV response allergies to dental biomaterials is the patch test.

Type I responses, or "immediate-type" allergies, are identified with prick test. The skin is first "pricked" via the allergen drop after it has been administered to it. The skin reaction is evaluated (development of redness, weals, etc.) after 5 to 30 minutes. Although extremely unlikely, there is a very small chance that the test itself might cause an instant allergic reaction. Therefore, only qualified employees should conduct this exam.

The RAST is a member of the class of in vitro tests used to identify allergies. Presence of IgE specific to allergens in patient's blood is utilised to detect immediate-type allergies (IgE mediated). RAST is in-vitro test, therefore patients won't be at danger of being sensitised as a result of the test. However, the test may produce results that may be at odds with clinician's findings in atopic individuals or through other antibodies.

7. Immunotoxicological test Methods

These methods are utilised to determine how a chemical or material affects one or more of the parts of immune system. The activation of certain sensitizations, development of autoimmune responses, or suppression or encouragement of cellular immune defence are examples of its components. The LTT (lymphocyte transformation test) is one of these examinations. To conduct LTT, blood sample from patient who has a possible allergy is obtained, and the T-cell proliferation in the presence of allergens is assessed. A variation of the LTT is "memory lymphocyte immunostimulation assay" (MELISA). The MELISA uses monocytes that were obtained from the patient's blood. These techniques have not yet received scientific approval for use in standard testing due to inconsistencies in results ³⁵⁻³⁶

8. Analysis of presence of Metals in saliva and Biopsy

After chemical pulping, a predetermined amount of the "morning saliva" is collected (prior to any meal or any drink consumption or before engaging in any oral hygiene procedures) and then examined, for example by atomic absorption spectrometry (AAS). The metal content of biopsies, like those that were collected from gingiva that is present next to the metal restoration, was determined after chemical pulping, metal concentrations in the biopsy collected are analysed by AAS. ³⁷⁻³⁸

CONCLUSION

Factors such as sample size, loading rate, and specimen geometry can all have a significant impact on the outcome of mechanical tests. While no single test can provide a complete characterization of orthodontic materials, a combination of tests can be used to obtain a more comprehensive understanding of their mechanical properties. This includes evaluating not only the strength and stiffness of the material but also its fatigue resistance, fracture toughness, and wear behaviour as well as its inertness in the oral environment.

The importance of comprehensive testing in the development and evaluation of orthodontic materials is necessary for standardization of material properties. By utilizing a variety of tests and carefully considering test parameters, researchers and clinicians can obtain a better understanding

of the mechanical properties of these materials and make informed decisions about their use in clinical practice.

REFERENCES

1. Eliades T, Bourauel C. Intraoral aging of orthodontic materials: the picture we miss and its clinical relevance. *Am J Orthod Dentofacial Orthop.* 2005;127(4):403-412.
2. Nelson B, Hirschi R, Kennedy DB. Elastomeric modules: an investigation of tooth movement, anchorage, and friction. *Am J Orthod Dentofacial Orthop.* 1991;100(2):149-156.
3. Kusy RP, Whitley JQ. Resistance to sliding of orthodontic appliances in the dry and wet states: influence of archwire alloy, interbracket distance, and bracket engagement. *Journal of Biomedical Materials Research.* 1989 May;23(5):535-51.
4. ASTM F2516-14, Standard Test Method for Tension Testing of Nickel-Titanium Superelastic Materials.
5. Burstone CJ, Goldberg AJ. Beta titanium: A new orthodontic alloy. *Am J Orthod.* 1980 Feb;77(2):121–32.
6. Asgharnia MK, Brantley WA. Comparison of bending and tension tests for orthodontic wires.
7. Council on Dental Materials and Devices. New American Dental Association specification no. 32 for orthodontic wires not containing precious metals. *J Am Dent Assoc* 1977; 5: 1169±71.
8. Brantley WA, Augat WS, Myers CL, Winders RV. Bending deformation studies of orthodontic wires. *J Dent Res* 1978; 57: 609±15.
9. Yoshikawa DK, Burstone CJ, Goldberg AJ, Morton J. Flex-ure modulus of orthodontic stainless steel wires. *J Dent Res* 1981; 60: 139±45.
10. Nikolai RJ, Anderson WT, Messersmith ML. Structural re-sponses of orthodontic wires in flexure from a pro-posed alternative to the existing specification. *Am J Orthod Dentofac Orthop* 1988; 93: 496±504.
11. ANSTIS GR, CHANTIKUL P, LAWN BR, MARSHALL DB. A Critical Evaluation of Indentation Techniques for Measuring Fracture Toughness: I, Direct Crack Measurements. *Journal of the American Ceramic Society.* 1981 Sep;64(9):533–8.
12. Kruzic JJ, Kim DK, Koester KJ, Ritchie RO. Indentation techniques for evaluating the fracture toughness of biomaterials and hard tissues. Vol. 2, *Journal of the Mechanical Behavior of Biomedical Materials.* Elsevier BV; 2009. p. 384–95.

13. LAWN BR, EVANS AG, MARSHALL DB. Elastic/Plastic Indentation Damage in Ceramics: The Median/Radial Crack System. *Journal of the American Ceramic Society*. 1980 Sep;63(9–10):574–81.
14. International Organization for Standardization. ISO 6872: 2008: Dental ceramics, Switzerland; 2008 .
15. Fujishima A, Ferracane JL. Comparison of four modes of fracture toughness testing for dental composites. Vol. 12, *Dent Mater*. 1996.
16. Newman JC; YY; JMA. Newman, J. C.; Yamada, Y.; James, M. A. (2011). “Back-face strain compliance relation for compact specimens for wide range in crack lengths”. *Engineering Fracture Mechanics*. 78 (15): 2707–2711.
17. Barker LM. A simplified method for measuring plane strain fracture toughness. *Engineering Fracture Mechanics*. 1977;9:361-369. DOI: 10.1016/0013-7944(77)90028-5.
18. Bubsey RT, Munz D, Pierce WS, Shannon JL. Compliance calibration of the short rod chevron-notch specimen for fracture toughness testing of brittle materials. Vol. 18, *International Journal of Fracture*. 1982.
19. Shyam A, Lara-Curzio E. The double-torsion testing technique for determination of fracture toughness and slow crack growth behavior of materials: A review. In: *Journal of Materials Science*. 2006. p. 4093–104.
20. Versluis A, Tantbirojn D, Douglas WH. Why do shear bond tests pull out dentin? *J Dent Res*. 1997;76:1298–307.
21. Oilo G. Adhesion of dental materials to dentine: Debonding tests. In: Thylstrup A, Leach SA, Qvist V, editors. *Dentine and dentine reactions in the oral cavity*. Oxford: IRL Press Ltd.; 1987. pp. 219–24.
22. Van Meerbeek B, Peumans M, Poitevin A, Mine A, Van Ende A, Neves A, et al. Relationship between bond-strength tests and clinical outcomes. *Dent Mater*. 2010;26:e100–21.).
23. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, et al. A critical review of the durability of adhesion to tooth tissue: Methods and results. *J Dent Res*. 2005;84:118–32.
24. Ruse ND, Shew R, Feduik D. In vitro fatigue testing of a dental bonding system on enamel. *J Biomed Mater Res*. 1995;29:411–5 Braem M. Microshear fatigue testing of tooth/adhesive interfaces. *J Adhes Dent*. 2007;9:249–53.
25. Kawahara, H., Shiota, M., Yamakawa, Y.: Studies on the effects of dental metals upon the mesenchymal cells in tissue culture. *J Osaka Odontol Soc* 18, 343–348 (1955). .
26. Maizumi, H., Sauerwein, E.: Die Wirkung verschiedener Vital- erhaltungs- und Wurzelfüllmittel auf Gewebekulturen. [Effect of various endodontic materials on cell cultures] *Dtsch Zahnärztl Z* 17, 1628–1634 (1962).
27. Schmalz, G.: The use of cell cultures for toxicity testing of dental materials – advantages and limitations. *J Dent* 22 (suppl. 2), 6–11 (1994). .

28. Schmalz, G., Schuster, U., Nützel, K., Schweikl, H.: An in vitro pulp chamber with three-dimensional cell cultures. *J Endod* 25, 24–29 (1999) .
29. Schmalz, G., Schweikl, H.: Characterization of an in vitro dentin barrier test using a standard toxicant. *J Endod* 20, 592–594 (1994).
30. Klötzer, W.T., Langeland, K.: Tierexperimentelle Prüfung von Materialien und Methoden der Kronen- und Brückenprothetik. [Testing of materials and methods for crown and bridge prosthesis on animals] *Schweiz Monatsschr Zahnheilkd* 83, 163–244 (1973) .
31. Wirthlin, M.R., Armitage, G.C., Rao, S., Fritzinger, B., Phillips, S., Heller, J.: A mucosal irritancy test device for intraoral use in dogs. *J Periodontol* 68, 746–749 (1997).
32. Donath, K.: The diagnostic value of the new method for the study of undecalcified bones and teeth with attached soft tissue [Säge- Schliff (sawing and grinding) technique]. *Pathol Res Pract* 179, 631–633 (1985).
33. Cohen, D.E.: Contact dermatitis: a quarter-century perspective. *J Am Acad Dermatol* 51 (1), 60–61 (2004) .
34. Korting, H.C., Sterry, W.: Diagnostische Verfahren in der Dermatologie. [Diagnostic Methods in Dermatology] Blackwell Wissenschafts-Verlag, Berlin 1997 .
35. Bieger, W.P.: Immuntoxikologie der Metalle. Labordiagnostik der Quecksilber- und Dentalmetall-Sensibilisierung. [Immunotoxicology of metals. Laboratory diagnosis of mercury and dental alloy sensitization] *Clin Lab* 42, 243–255 (1996) .
36. Loftenius, A., Skoglund, A., Ekstrand, J., Hovmark, A., Möller, E.: No evidence for specific in vitro lymphocyte reactivity to HgCl₂ in patients with dental amalgam related contact lesions. *J Oral Pathol Med* 28, 364–370 (1999).
37. Schmalz, G., Garhammer, P.: Biological interactions of dental cast alloys with oral tissues. *Dent Mater* 18, 396–406 (2002) .
38. Wirz, J., Vock, M., Schmidli, F.: Splittertest – ein zuverlässiges Diagnosehilfsmittel bei Abklärungen von Metallunverträglichkeit. [The particle test – a reliable diagnostic tool for detection of a metal incompatibility] *Quintessenz* 47, 1373–1384 (1996).