



The Hidden Dangers: Cytotoxic Effects of Orthodontic Materials Explored

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ABSTRACT:

Orthodontic treatment is a commonly used method for correcting dental misalignments, utilizing materials such as metals, ceramics, polymers, and composites. Although chosen for their mechanical properties and biocompatibility, concerns have been raised regarding potential cytotoxic effects on oral tissues. This abstract provides a concise overview of cytotoxicity associated with orthodontic materials, emphasizing the impact on cellular viability and tissue health. Research methodologies employed to assess cytotoxicity include in vitro studies with oral cell lines and in vivo investigations using animal models. Stainless steel and nickel-titanium alloys, commonly used in orthodontic brackets and wires, may undergo corrosion, releasing metal ions, especially nickel, linked to cytotoxic effects on oral cells. Ceramic brackets, chosen for aesthetic reasons, present challenges due to their hardness and potential abrasiveness. Additionally, polymers and adhesives used in orthodontics can release substances affecting cell behavior. Addressing these cytotoxic concerns necessitates a multidisciplinary approach, involving orthodontists, material scientists, and researchers. Ongoing research aims to enhance biocompatibility by developing new alloys, ceramics, and polymers that balance mechanical strength with reduced cytotoxic potential.

Introduction

Orthodontic treatment has long been recognized as a crucial practice for achieving proper dental alignment and improving oral health. Central to this field are the materials used in orthodontic appliances, such as brackets, wires, and adhesives, chosen for their mechanical strength and biocompatibility. However, a growing body of research has raised concerns regarding the potential cytotoxicity of these materials, emphasizing the need for a nuanced understanding of their impact on cellular health. The goal of orthodontic treatment extends beyond enhancing the aesthetic appeal of a patient's smile to correcting functional issues associated with misaligned teeth and jaws. While the mechanical properties of orthodontic materials are

crucial for the success of these interventions, the biocompatibility of these materials with oral tissues is equally important. Cytotoxicity, or the ability of a material to cause harm to living cells, has become a focal point of investigation within the realm of orthodontics. The range of materials employed in orthodontics is diverse, spanning metals such as stainless steel and nickel-titanium alloys, ceramics chosen for aesthetic considerations, and various polymers and adhesives. Given that these materials come into direct contact with oral tissues for extended periods during treatment, understanding their potential cytotoxic effects has become imperative to ensure the overall health and well-being of orthodontic patients.



Significance Of Cytotoxicity Studies

Cytotoxicity studies play a crucial role across various scientific and medical disciplines, holding significant importance in understanding the potential impact of substances on living cells. The significance of cytotoxicity studies can be highlighted in several key aspects:

Biomedical Research and Drug Development.

Medical Device Evaluation.

Environmental and Occupational Safety.

Regulatory Compliance.

Biocompatibility in Biomaterials.

Toxicology and Environmental Impact.

Clinical Applications in Dentistry and Orthodontics.

Informed Decision-Making in Consumer Products.

Advancements in Biotechnology.

Cytotoxicity studies are fundamental for evaluating the safety and efficacy of new drugs and pharmaceutical compounds. They provide insights into how substances interact with cells, helping researchers identify potential therapeutic agents while minimizing harmful effects. In the field of medical devices, including implants and prosthetics, cytotoxicity studies assess the compatibility of materials with surrounding tissues. This is crucial to ensure that devices do not induce harmful reactions and can be safely integrated into the human body. Cytotoxicity studies are essential for evaluating the safety of chemicals and materials in various industries. They contribute to establishing occupational health standards and guidelines, helping ensure the well-being of workers exposed to potentially harmful substances. Regulatory agencies worldwide, such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), often require cytotoxicity data as part of the approval process for new drugs, medical devices, and other healthcare products. Compliance with these standards is essential for market authorization. Cytotoxicity studies are critical in the development and assessment of biomaterials used in tissue engineering and regenerative medicine. Understanding how these materials interact with cells is essential for creating biocompatible scaffolds and

implants for medical applications. Cytotoxicity studies contribute to toxicological assessments, helping evaluate the potential hazards of chemicals and pollutants in the environment. This information is vital for regulatory agencies, policymakers, and environmental scientists working to safeguard ecosystems and human health. In dental and orthodontic practices, cytotoxicity studies assess the impact of dental materials on oral tissues. This is crucial for ensuring the safety of restorative materials, adhesives, and orthodontic appliances, contributing to the overall well-being of patients. Cytotoxicity studies are employed in the assessment of various consumer products, such as cosmetics and personal care items. Ensuring that these products are not harmful to the skin or other tissues is essential for consumer safety. Cytotoxicity studies drive advancements in biotechnology, guiding the development of new therapies, diagnostic tools, and treatment strategies. This research contributes to the continuous improvement of healthcare practices.

Overview Of Commonly Used Orthodontic Materials

Orthodontic materials play a pivotal role in the orthodontics field, serving as the basis for various appliances and devices designed to correct dental misalignments. Commonly used orthodontic materials encompass metal alloys, ceramics, polymers, and adhesives. **Stainless Steel:** Extensively utilized in orthodontics, stainless steel provides strength and durability. It is commonly employed for brackets, wires, and other components due to its robustness and resistance to corrosion. **Nickel-Titanium Alloys:** Known for their flexibility and ability to maintain shape memory, nickel-titanium alloys are often used in orthodontic wires. These alloys allow for controlled tooth movement and are available in various formulations to accommodate different treatment stages. **Polycrystalline Alumina and Sapphire:** Ceramic brackets are popular for their aesthetic appeal, resembling the color of natural teeth. Polycrystalline alumina and monocrystalline sapphire are common materials for manufacturing ceramic brackets, offering a more discreet alternative to traditional metal braces. **Thermoplastics:** Polymeric materials, particularly thermoplastics, find application in clear aligners such as Invisalign. These removable and nearly invisible



aligners provide an aesthetic option for patients seeking orthodontic treatment without traditional braces. Composite Resins: Used for bonding brackets to the enamel surface of teeth, composite resins are tooth-colored adhesives. They not only provide effective bonding but also contribute to the overall cosmetic appeal of orthodontic treatments. Beta-Titanium and Nickel-Titanium Wires: These wires connect orthodontic brackets and apply controlled forces to move teeth into their desired positions. Beta-titanium wires offer flexibility and resilience, while nickel-titanium wires are known for their elasticity. Latex-Free Elastics: These are used to exert additional forces on the teeth, assisting in the correction of bite issues. Latex-free options are available for patients with latex allergies. Facebows and Straps: In cases requiring external forces for jaw correction, headgear components like facebows and straps are used. These are attached to the braces to influence the growth of the jaws. Retainers: Typically made of acrylic and metal wires, retainers are used after active orthodontic treatment to maintain the corrected tooth positions. They are crucial for preventing relapse.

Unmasking the Intersection: Metal Alloys and Cytotoxicity in Orthodontics

Metal alloys, widely employed in orthodontics, present a complex relationship with cytotoxicity, involving corrosion, ion release, allergic reactions, and clinical implications. Exploring these facets unveils the intricate dynamics of metal alloys in the context of orthodontics.

Despite their resilience, metal alloys are susceptible to corrosion, accelerated by the oral environment, leading to the release of ions near oral tissues. Corroded metal alloys release ions, creating a potential orthodontic dilemma. Understanding ion release dynamics is crucial for assessing the impact on surrounding tissues, balancing orthodontic efficacy with biocompatibility. Certain alloy components, particularly nickel and cobalt, may trigger allergic reactions in sensitive individuals. Orthodontic interventions must consider these allergenic possibilities to ensure patient well-being. Orthodontists play a crucial role in managing patient sensitivities. Recognizing the potential for allergic reactions prompts a proactive approach, tailoring treatments to minimize exposure and selecting alloys considering patient-specific factors. The

amalgamation of corrosion, ion release, and allergic reactions introduces metal alloys to clinical implications. Orthodontic practitioners must translate scientific understanding into pragmatic applications for safe and effective treatments. As metal alloys remain integral to orthodontics, clinicians must adopt risk mitigation strategies. Regular monitoring, material selection based on patient profiles, and patient education become pivotal tools in mitigating potential cytotoxicity risks. Cytotoxicity studies related to stainless steel in orthodontics primarily focus on assessing the biocompatibility of the material and its impact on oral tissues. These studies typically involve *in vitro* experiments using oral cell lines to evaluate cellular responses to stainless steel exposure. Research indicates that stainless steel, when used in orthodontic appliances, generally exhibits low cytotoxicity. The corrosion-resistant nature of stainless steel prevents the release of harmful ions, minimizing the risk of adverse cellular reactions. While stainless steel is considered biocompatible, individual patient responses can vary. Allergic reactions to nickel, one of the components of stainless steel, have been reported in some cases. However, efforts are made to reduce nickel content in orthodontic alloys to address potential sensitivities. Overall, cytotoxicity studies contribute to the ongoing refinement of orthodontic materials, ensuring that stainless steel and its alloys meet safety standards and pose minimal risks to oral health. Advances in metallurgy and material science continue to enhance the biocompatibility of stainless steel, making it a reliable and widely used material in orthodontic practice.

Ceramic Materials in Orthodontics: A Deep Dive into Cytotoxicity

Ceramic materials have emerged as a compelling alternative in orthodontics, offering advantages in durability and aesthetics. However, beneath the surface, the intricate relationship between ceramic materials and cytotoxicity unfolds, encompassing particle release, tissue response, and aesthetic considerations.

Ceramic materials in orthodontics may undergo subtle particle release over time. Understanding these microscopic dynamics is crucial for assessing their impact on oral health. Released particles, although minuscule, can interact with oral tissues. Investigating the nature and extent of this release becomes pivotal in



ensuring the biocompatibility of ceramic materials. Ceramic materials evoke a tissue response when in contact with oral structures. Exploring the biological interplay between ceramics and tissues is fundamental for predicting and managing potential reactions. Understanding how tissues respond to ceramic materials aids orthodontists in selecting suitable options for individual patients. This nuanced approach ensures a harmonious integration of orthodontic appliances with the oral environment. Beyond their functional role, ceramic materials bring aesthetic considerations to the forefront. Patients increasingly seek orthodontic solutions that not only rectify misalignments but also do so with a visually pleasing touch. Orthodontists must strike a balance between functionality and aesthetics when incorporating ceramic materials. This delicate equilibrium ensures patient satisfaction without compromising oral health. Cytotoxicity studies related to ceramic materials in orthodontics focus on assessing the biocompatibility of these materials and their impact on oral tissues. Research indicates that ceramic materials used in orthodontic brackets generally exhibit low cytotoxicity. The composition and properties of these ceramics contribute to their compatibility with oral tissues, minimizing the risk of adverse cellular reactions. The smooth and inert surfaces of ceramic brackets reduce the likelihood of plaque accumulation and staining, contributing to improved oral hygiene during orthodontic treatment. While ceramics are known for their biocompatibility, individual patient responses can vary. Cytotoxicity studies help identify any potential concerns and contribute to the ongoing refinement of ceramic materials to ensure their safety in orthodontic applications.

Polymeric Materials in Orthodontics: Navigating the Complex Landscape of Cytotoxicity

Polymeric materials have revolutionized orthodontics, offering versatility and unique properties. However, within this innovative landscape lies the intricate relationship between polymeric materials and cytotoxicity, involving polymer degradation, plasticizers and additives, and the emergence of biodegradable materials.

Polymeric materials in orthodontics are not immune to degradation over time. Understanding the gradual breakdown of polymers is crucial for assessing their

impact on patient health. As polymers degrade, byproducts may be released into the oral environment. Investigating the nature of these byproducts is essential to ensure they pose no harm to oral tissues. Polymeric materials often incorporate plasticizers and additives to enhance flexibility and performance. Scrutinizing the impact of these additional components is vital for maintaining orthodontic appliance safety. Certain plasticizers and additives may pose risks if released into the oral cavity. Orthodontic practitioners must stay vigilant, choosing materials with a keen eye on minimizing potential cytotoxic effects. The emergence of biodegradable polymeric materials heralds a new era in sustainable orthodontics. Understanding the dynamics of these materials as they break down is crucial for evaluating their long-term impact. Biodegradable materials offer the promise of reduced environmental impact. Orthodontists adopting these innovations play a pivotal role in aligning patient care with ecological responsibility. Cytotoxicity studies involving polymeric materials in orthodontics are crucial for assessing the biocompatibility of these materials and their impact on oral tissues. Research indicates that thermoplastic materials, commonly used in clear aligners, generally exhibit low cytotoxicity. These materials are designed to be in direct contact with oral tissues for extended periods, and cytotoxicity studies contribute to ensuring their safety. Elastomeric polymers used in ligatures and elastic bands are subject to cytotoxicity assessments to confirm their biocompatibility and minimize any potential adverse reactions in the oral environment. Advances in polymer science have led to the development of latex-free alternatives to accommodate patients with latex allergies. Cytotoxicity studies play a role in evaluating the safety and performance of these alternative materials. Polycarbonate, while not as commonly used as thermoplastics and elastomers in orthodontic applications, undergoes cytotoxicity studies to ensure its biocompatibility when incorporated into orthodontic brackets or other devices.

Adhesives and Resins in Orthodontics: Navigating the Complex World of Cytotoxicity

Adhesives and resins play a pivotal role in modern orthodontics, facilitating the bonding of brackets and ensuring the effectiveness of orthodontic treatments. However, beneath the surface lies a nuanced



relationship between these materials and cytotoxicity, encompassing monomers and curing agents, bonding materials, and potential adverse reactions.

Monomers and curing agents form the foundation of orthodontic adhesives. Understanding the chemical intricacies of these components is essential for comprehending their role in the orthodontic bonding process. The process of polymerization, triggered by curing agents, binds monomers together to create a durable adhesive. Exploring this chemical transformation sheds light on the potential release of byproducts into the oral environment. Orthodontic adhesives must strike a delicate balance between strength and compatibility. Examining the composition of bonding materials ensures that the adhesive fosters a robust connection while remaining biocompatible. The interaction between adhesives and dental tissues is critical. Understanding how bonding materials interface with enamel and other oral structures provides insights into potential cytotoxic effects. Despite advancements, adverse reactions to adhesives and resins remain a concern. Monitoring and addressing potential issues, such as allergic responses or tissue irritation, are imperative for ensuring patient safety. Orthodontic practitioners must adopt a patient-centric approach, considering individual sensitivities and proactively managing any adverse reactions that may arise during or after orthodontic treatments. Cytotoxicity studies related to orthodontic adhesives and resins are essential for assessing the biocompatibility of these materials and their impact on oral tissues. Research indicates that orthodontic adhesives, particularly those based on Bis-GMA and other acrylic monomers, generally exhibit low cytotoxicity. This is crucial, as these adhesives are in direct and prolonged contact with the enamel and surrounding tissues. Initiators used in light-cured adhesives may undergo cytotoxicity studies to ensure that the polymerization process does not result in harmful byproducts that could adversely affect oral tissues. Fillers, such as silica or glass particles, are subject to evaluation to confirm their biocompatibility and ensure that the added materials do not contribute to cytotoxic effects. Advances in adhesive technology include the development of light-cured adhesives that allow for precise control over the bonding process. Cytotoxicity studies play a role in evaluating the safety and effectiveness of these adhesive systems.

Conclusion: Shaping the Future of Cytotoxicity in Orthodontics

In traversing the intricate landscape of cytotoxicity in orthodontics, the culmination of findings, the imperative of ongoing research, and the profound implications for clinical practice stand as pivotal guideposts for the future.

The exploration into cytotoxicity has unveiled the dynamic interplay between orthodontic materials and cellular health. From metal alloys to ceramics, polymers, and adhesives, each material poses a unique set of considerations in the quest for safer and more biocompatible orthodontic interventions. Duration of exposure, patient-specific factors, and environmental considerations emerged as influential factors in the cytotoxic narrative. Recognizing the temporal nuances, tailoring treatments to individual profiles, and optimizing clinical environments contribute to a comprehensive understanding of cytotoxicity. Cytotoxicity research in orthodontics remains a dynamic journey. The evolving nature of materials, patient profiles, and environmental dynamics necessitates an ongoing commitment to research. Staying at the forefront of scientific inquiry allows practitioners to adapt practices in response to emerging knowledge. Ongoing research is the cornerstone of advancing patient safety in orthodontics. As new materials and technologies emerge, continual investigation ensures that orthodontic practices align with the highest standards of safety and efficacy, fostering trust between practitioners and patients. The findings of cytotoxicity research directly inform clinical decision-making. Orthodontic practitioners armed with knowledge about materials, temporal considerations, and patient-specific factors are empowered to make informed choices, minimizing risks and optimizing treatment outcomes. The implications extend to a more patient-centric orthodontic practice. Tailoring treatments based on individual sensitivities, utilizing biocompatible materials, and optimizing clinical environments contribute to an orthodontic experience that prioritizes not just efficacy but also the overall well-being of each patient. In conclusion, the journey through cytotoxicity research in orthodontics is a testament to the field's commitment to excellence and patient care. The summary of findings serves as a foundation for ongoing exploration, emphasizing the



importance of research in navigating uncharted territories. As orthodontics charts its course into the future, the implications for clinical practice underscore the responsibility to embrace knowledge, adapt practices, and forge a safer path forward. Through the synergy of research, practice, and patient-centered care, the future of orthodontics promises not only healthier smiles but also a continuous evolution towards excellence and well-being.

References

1. Toms AP. The corrosion of orthodontic wire. *Eur J Orthod* 1988;10:87–97.
2. Bass JK, Fine H, Cisneros G. Nickel hypersensitivity in the orthodontic patient. *Am J Orthod* 1993;103:280–5.
3. Nagel ML. A Latex glove alert. *Chem Health Safety* 1997;4:14–8.
4. Staerkjaer L, Menne T. Nickel allergy and orthodontic treatment. *Eur J Orthod* 1990;12:284–9.
5. Wiltshire WA, Ferreira MR, Ligthelm AJ. Allergies to dental materials. *Quintessence Int* 1996;27:513–20.
6. Grimsdottir MR, Hensten-Pettersen A, Kullman A. Cytotoxic effect of orthodontic appliances. *Eur J Orthod* 1992;14:47–53.
7. Rose E, Jonas I, Kappert HF. Investigation into the biological assessment of orthodontic wires. *J OrofacOrthop* 1998;59:253–64.
8. Wataha JC, Malcom C, Hanks CT. Correlation between cytotoxicity and the element released by dental casting alloys. *Int J Prosthodont* 1995;8:9–14.
9. Barrett RD, Bishara SE, Quinn JK. Biodegradation of orthodontic appliances, Part I. Biodegradation of nickel and chromium in vitro. *Am J Orthod* 1993;103:8–14.
10. Brune D. Metal release from dental materials. *Biomater* 1986;7:163–75.
11. Kerosuo E, Moe G, Kleven E. In vitro release of nickel and chromium from different types of simulated orthodontic appliances. *AngOrthod* 1995;2:111–6.
12. International Organisation for Standardization, ISO 10993-5, Biological evaluation of medical devices—Part 5: Tests for cytotoxicity: in vitro methods, CH-1211 Geneva 20, Switzerland, 1992.
13. Grill V, Sandrucci MA, Basa M, Dorigo E, Bareggi R. The influence of dental metal alloys on cell proliferation and fibronectin arrangement in human fibroblast cultures. *Archs Oral Biol* 1997;42:641–7.
14. Kois JC. The restorative–periodontal interface: biological parameters. *Periodontology* 2000;11(1996):29–38.
15. Hamula DW, Hamula W, Sernetz F. Pure titanium orthodontic brackets. *J ClinOrthod* 1996;30:140–4.
16. Tang TH, Liu Y, Björkman L, Ekstrand J. In vitro cytotoxicity of orthodontic bonding resins on human oral fibroblasts. *Am J OrthodDentofacOrthop* 1999;116:132–8.
17. Wataha JC, Hanks CT, Sun Z. Effect on cell line on in vitro metal ion cytotoxicity. *Dent Mater* 1994;10:156–61.
18. Craig RG, Hanks CT. Cytotoxicity of experimental casting alloys evaluated by cell culture tests. *J Dent Res* 1990;69:1539–42.
19. Dai K, Chu Y. Studies and applications of NiTi shape memory alloys in the medical field of China. *J Biomed Engin* 1996;6:233–40.
20. Ryhanen J, Niemi E, Serlo W, Sandvik P, Salo T. Biocompatibility of nickel–titanium shape memory metal and its corrosion behavior human cell cultures. *J Biomed Mat Res* 1997;15-3:451–7.
21. Wataha JC, Hanks CT, Craig RG. In vitro effects of metal ions on cellular metabolism and the correlation between these effects and the uptake of the ions. *J Biomed Mater Res* 1994;28:427–33.
22. Bondemark L, Kurol J, Wennberg A. Biocompatibility of new, clinically used and recycled orthodontic samarium-cobalt magnets. *Am J Orthod* 1994;105:568–74.
23. Wataha JC, Craig RG, Hanks CT. The effects of cleaning on the kinetics of in vitro metal release from dental casting alloys. *J Dent Res* 1992;71:1417–22.
24. Wataha JC, Lockwood PE. Release of elements from dental casting alloys into cell-culture medium over 10 months. *Dent Mater* 1998;14:158–63.
25. Bouillaguet S, Wataha JC, Virgilito M, Gonzalez L, Rakich DR, Meyer JM. Effect of sub-lethal concentrations of HEMA on THP-1 human



- monocyte-macrophages, in vitro. *Dent Mater* 2000;16:213–7.
26. Zachrisson BU, Buyukyilmaz T. Bonding in orthodontics. In: Graber TM, Vanarsdall RL, Vig K, eds. *Orthodontics: Current Principles and Techniques*. Philadelphia, Pa: ElsevierInc; 2005:579–659.
27. Malkoc S, Uysal T, Usumez S, Isman E, Baysal A. In vitro assessment of temperature rise in the pulp during orthodontic bonding. *Am J OrthodDentofacialOrthop*. In press.
28. Eliades T, Eliades G. Orthodontic adhesive resins. In: Brantley WA, Eliades T, eds. *Orthodontic Materials: Scientific and Clinical Aspects*. Stuttgart, Germany: Thieme; 2001:201–220.
29. Gioka C, Bourauel C, Hiskia A, Kletsas D, Eliades T, Eliades G. Light-cured or chemically cured orthodontic adhesive resins? A selection based on the degree of cure, monomer leaching, and cytotoxicity. *Am J OrthodDentofacialOrthop*. 2005;127:413–419; quiz 516.
30. Wahata JC. Principles of biocompatibility. In: Brantley WA, Eliades T, eds. *Orthodontic Materials: Scientific and Clinical Aspects*. Stuttgart, Germany: Thieme; 2001:271–286.
31. Huang TH, Tsai CY, Chen SL, Kao CT. An evaluation of the cytotoxic effects of orthodontic bonding adhesives upon a primary human oral gingival fibroblast culture and a permanent, human oral cancer-cell line. *J Biomed Mater Res*. 2002;63:814–821.
32. Schmalz G. Resin-based composites. In: Schmalz G, Arenholt-Bindlev D, eds. *Biocompatibility of Dental Materials*. Berlin, Heidelberg, Germany: Springer-Verlag; 2009:99–138.
33. Davidson WM, Sheinis EM, Shepherd SR. Tissue reaction to orthodontic adhesives. *Am J Orthod*. 1982;82:502–507.
34. onke E, Franz A, Freudenthaler J, Konig F, Bantleon HP, Schedle A. Cytotoxicity and shear bond strength of four orthodontic adhesive systems. *Eur J Orthod*. 2008;30: 495–502.
35. Ferracane JL. Current trends in dental composites. *Crit Rev Oral Biol Med*. 1995;6:302–318.
36. Spahl W, Budzikiewicz H, Geurtsen W. Determination of leachable components from four commercial dental composites by gas and liquid chromatography/mass spectrometry. *J Dent*. 1998;26:137–145.
37. Reichl FX, Durner J, Hickel R, et al. Uptake, clearance and metabolism of TEGDMA in guinea pigs. *Dent Mater*. 2002; 18:581–589.
38. Reichl FX, Durner J, Manhart J, et al. Biological clearance of HEMA in guinea pigs. *Biomaterials*. 2002;23:2135–2141.
39. Hansel C, Leyhausen G, Mai UE, Geurtsen W. Effects of various resin composite (co)monomers and extracts on two caries-associated microorganisms in vitro. *J Dent Res*. 1998;77:60–67.
40. Soehnel H, Gjerdet NR, Hensten-Pettersen A, Ruyter IE. Allergenic potential of two orthodontic bonding materials. *Scand J Dent Res*. 1994;102:126–129.
41. Geurtsen W, Lehmann F, Spahl W, Leyhausen G. Cytotoxicity of 35 dental resin composite monomers/additives in permanent 3T3 and three human primary fibroblast cultures. *J Biomed Mater Res*. 1998;41:474–480.
42. Stanislawski L, Lefeuvre M, Bourd K, Soheil-Majd E, Goldberg M, Perianin A. TEGDMA-induced toxicity in human fibroblasts is associated with early and drastic glutathione depletion with subsequent production of oxygen reactive species. *J Biomed Mater Res A*. 2003;66: 476–482.
43. Koulaouzidou EA, Helvatjoglu-Antoniades M, Palaghias G, Karanika-Kouma A, Antoniadis D. Cytotoxicity of dental adhesives in vitro. *Eur J Dent*. 2009;3:3–9.
44. Ulker HE, Sengun A. Cytotoxicity evaluation of self adhesive composite resin cements by dentin barrier test on 3D pulp cells. *Eur J Dent*. 2009;3:120–126.
45. Usumez S, Buyukyilmaz T, Karaman AI, Gunduz B. Degree of conversion of two lingual retainer adhesives cured with different light sources. *Eur J Orthod*. 2005;27: 173–179.
46. Pithon MM, Oliveira MV, Ruellas AC, Bolognese AM, Romano FL. Shear bond strength of orthodontic brackets to enamel under different surface treatment conditions. *J Appl Oral Sci*. 2007 Apr;15(2):127-30.



49. Pithon MM, dos Santos RL, de Oliveira MV, Ruellas AC, Romano FL. Metallic brackets bonded with resin-reinforced glass ionomer cements under different enamel conditions. *Angle Orthod.* 2006 Jul;76(4):700-4.
50. Fidalgo TK, Pithon MM, Maciel JV, Bolognese AM. Friction between different wire bracket combinations in artificial saliva—an in vitro evaluation. *J Appl Oral Sci.* 2011 Jan-Feb;19(1):57-62.
51. Lacerda dos Santos R, Pithon MM, Romanos MT. The influence of pH levels on mechanical and biological properties of non-latex and latex elastics. *Angle Orthod.* 2011 Dec 9.
52. Pithon MM, dos Santos RL. Does ozone water affect the bond strengths of orthodontic brackets? *AustOrthod J.* 2010 May;26(1):73-7.
53. Pithon MM, dos Santos RL, de Oliveira Ruellas AC, Nojima LI, Sant'Anna EF. In vitro evaluation of fluoride release of orthodontic bonding adhesives. *Orthodontics (Chic.)*. 2011 Winter;12(4):290-5.
54. Pithon MM, Nojima MG, Nojima LI. Primary stability of orthodontic mini-implants inserted into maxilla and mandible of swine. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2012 Jan 2.
55. Santos RL, Pithon MM, Fernandes AB, Cabral MG, Ruellas AC. Biocompatibility of orthodontic adhesives in rat subcutaneous tissue. *J Appl Oral Sci.* 2010 Sep- Oct;18(5):503-8.
56. Rogero SO, Lugão AB, Ikeda TI, Cruz AS. Teste in vitro de citotoxicidade: estudo comparativo entre duas metodologias. *Mater Res.* 2003;6(3):317-20.
57. International Organization for Standardization. International standard ISO 10993-5, Biological evaluation of medical devices. 1992. Part 5: Tests for cytotoxicity: in vitro methods. Geneva (SH): 1992
58. dos Santos RL, Pithon MM, Martins FO, Romanos MT, de Oliveira Ruellas AC. Evaluation of the cytotoxicity of latex and non-latex orthodontic separating elastics. *Orthod Craniofac Res.* 2010 Feb;13(1):28-33.
59. Pithon MM, Santos RL, Ruellas ACO, Fidalgo TK, Romanos MTV, Mendes GS. Citotoxicidade in vitro de elásticos ortodônticos: comparação entre duas metodologias. *Rev Saúde Com.* 2008;4(1):19-26.
60. Rogero SO, Souzabazzi A, Ikeda TI, Cruz AS, Fernandes KC, Higa OZ. Citotoxicidade in vitro das membranas de hidrogel reticuladas por radiação ionizante. *Rev Inst Adolfo Lutz.* 2000;59(1/2):1-5.
61. Santos RL, Pithon MM, Silva Mendes G, Romanos MT, Ruellas ACO. Cytotoxicity of intermaxillary orthodontic elastics of different colors: an in vitro study. *J Appl Oral Sci.* 2009 Jul-Aug;17(4):326-9.