FACULDADE DE
MEDICINA DENTÁRIA
UNIVERSIDADE DO PORTO

Integrated Master in Dental Medicine, Faculty of Dental Medicine, University of Porto

RESEARCH MONOGRAPHY

EVALUATION OF THE POTENTIAL FOR IRRITATION OF DIFFERENT DENTAL ADHESIVES

João Francisco Oliveira Figueiredo

Supervisor: Prof. Dr. Pedro de Sousa Gomes

Porto, 2022

Dissertation submitted to the Faculty of Dental Medicine, University of Porto, for the degree of Master of Dental Medicine

Author

João Francisco Oliveira Figueiredo

Fifth year student of the Integrated Master in Dental Medicine at the Faculty of Dental Medicine, University of Porto

up201706184@edu.fmd.up.pt

Supervisor

Prof. Dr. Pedro de Sousa Gomes

Associate Professor with Aggregation at the Faculty of Dental Medicine, University of Porto

Porto, 2022

U.PORTO
FACULDADE DE MEDICINA DENTÁRIA
UNIVERSIDADE DO PORTO

“Na hora de pôr a mesa
Seremos sempre quatro
Enquanto um de nós estiver vivo
Seremos sempre quatro”

Readaptação de um poema de José Luís Peixoto

Acknowledgments

Aos meus pais. “Máxima liberdade, máxima responsabilidade”. Muito obrigado por toda a dedicação, presença e amor que demonstraram para comigo durante estes 22 anos. São um exemplo para tudo o que faço e o meu maior motivo para continuar no caminho certo dada a enorme vontade de vos orgulhar.

Ao meu irmão e melhor amigo. Uma amizade fraterna de muitas horas e aventuras juntos. Aprendi contigo muito o que é ser uma pessoa de princípios e valores corretos. Além disso, sem ti ainda não sabia atar as sapatilhas ou abrir a porta de casa.

Aos meus amigos, aos novos e aos de sempre. Agradeço a compreensão, o companheirismo e a amizade que partilham comigo. Uma família que escolhi e que estou certo ter tomado uma decisão correta.

À faculdade, por ser uma segunda casa. Durante cinco anos, todos os elementos da minha faculdade, souberam receber-me e ser parte integrante da minha vida. Obrigado a todos os docentes e não docentes, pelo carinho e pelas lutas conjuntas que travamos.

À AEFMDUP e a todos os dirigentes associativos com os quais cresci em conjunto. Foram três anos de momentos desafiantes, mas recompensantes. Das jornadas mais bonitas da minha vida.

Por último, mas não menos especial ao meu orientador. Além de todo o esforço e trabalho que despendeu durante a realização deste projeto de investigação, agradeço por todo o trabalho conjunto e vivências que partilhamos durante estes cinco anos.

Abstract

Introduction: The development of adhesive systems, capable of making the composite resin substrate/dental structure achievable, defined a turning point in dentistry. Over time, clinicians demanded more versatile and user-friendly products that would allow the selection of the most appropriate adhesion strategy. In fact, dental adhesives, due to their application in intimate and prolonged contact with vital dentin, have a critical influence on pulp tissue. This influence depends widely on their chemical composition and the concentration in which they are applied. Biological characterization of universal adhesives has been extensively performed in vitro with different cell types, focusing on the assessment of cytotoxicity, suggesting a concentration-dependent toxicity. Nevertheless, the potential for irritation is influenced by a variety of factors such as pulpal health, thickness of remaining dentin and the composition of the dental adhesives and remains widely neglected.

Objective: This study aims to analyze the irritation potential of eight distinct commercially available Universal Adhesives with dissimilar chemical and physical formulations, through an *in vivo* assay the Hen's Egg Test – Chorioallantoic Membrane Test Method assay.

Methodology: Fertilized chicken eggs were placed in an automatic rotating incubator – *Briensa Octagon Advance* - at 37°C in a humidified atmosphere until testing, on day 9. Any defective or nonviable eggs were discarded before testing. At this time, the chorioallantoic membrane was accessed by removing the shell above the air sac and with the inner egg membrane carefully detached. The test was performed according to ICCVAM-Recommended Test Method Protocol: Hen's Egg Test – Chorioallantoic Membrane Test Method, with the adhesives being placed directly over the chorioallantoic membrane , in an area defined by an o-ring. Adhesives were characterized prior and after complete polymerization, to mimic the complete spectra of the clinical intervention. The biological response to the adhesives was assayed for 300 seconds, according to the test standards, being scored for hemorrhage, vascular lysis and coagulation, upon microscopic

evaluation. A positive control - with NaOH – and a negative control – with NaCl - were also performed for validation of the assay.

Results: The analysis of the vascularization of the membrane showed that none of the eight adhesives studied induced significant vascular adverse changes – in a similar way to the negative control - and, no differences were recorded between them, either regarding the non-polymerized or the polymerized materials. In contrast, NaOH (positive control) induced significant changes in CAM vascularization, with vascular lysis, coagulation and hemorrhage occurred early, within the 300 seconds period of the assay.

Conclusion: The dental adhesives analyzed in this investigation showed no irritation potential and consequently an adequate biological response, within the limited frame of this assay. Nevertheless, further studies are required due to the complexity of the biological evaluation process of materials aiming contact with the human organism, further including cytotoxicity, genotoxicity, and carcinogenicity, among other end points, to address the full spectra of the biological assessment process.

Keywords: Adhesion; Dental adhesives; Universal adhesives; Potential for irritation; Adverse effects; Cytotoxicity; Genotoxicity.

Resumo

Introdução: O desenvolvimento de sistemas adesivos, capazes de tornar possível a interação eficaz entre o substrato de resina composta e a estrutura dentária, definiu um ponto de viragem na Medicina Dentária. Ao longo do tempo, os clínicos exigiram produtos mais versáteis e fáceis de utilizar que lhes permitissem escolher a estratégia de adesão mais adequada. De facto, os adesivos dentários, devido à sua aplicação em contacto íntimo e prolongado com a dentina vital, têm uma influência crítica no tecido pulpar. Esta influência depende amplamente da sua composição química e da concentração em que são aplicados. A caracterização biológica dos adesivos universais tem sido amplamente realizada *in vitro*, com diferentes tipos de células, concentrando-se na avaliação da citotoxicidade, sugerindo uma toxicidade dependente da concentração. Apesar de o potencial de irritação ser influenciado por uma variedade de fatores como a saúde pulpar, espessura de dentina remanescente e a composição dos adesivos dentários, este parâmetro continua a ser amplamente negligenciado.

Objetivos: Este estudo visa analisar o potencial de irritação de oito adesivos universais distintos disponíveis comercialmente com formulações químicas e físicas diferentes, através do ensaio in vivo, de Hen's Egg Test – Chorioallantoic Membrane Test Method.

Metodologia: Os ovos fertilizados de galinha foram colocados numa incubadora de rotação automática - Briensa Octagon Advance - a 37°C numa atmosfera com humidade controlada até ao teste, no dia 9. Todos os ovos defeituosos ou não viáveis foram descartados antes da realização dos testes. Neste período, a membrana corioalantóide foi acedida através da remoção da casca situada por cima da câmara de ar e a membrana interna do ovo foi cuidadosamente descolada. O teste foi realizado de acordo com o ICCVAM-Recommended Test Method Protocol: Hen's Egg Test – Chorioallantoic Membrane Test Method, com os adesivos a serem colocados diretamente sobre a membrana corioalantóide numa área definida por um O-ring. Os adesivos foram testados antes e após a sua polimerização, procurando mimetizar o espetro completo da sua utilização

clínica. A resposta biológica aos adesivos foi avaliada durante 300 segundos, de acordo com os padrões do teste, sendo analisada a presença de hemorragia, lise vascular e coagulação, após avaliação microscópica. Foi também realizado um controlo positivo - com NaOH - e um controlo negativo - com NaCl - para validação do ensaio.

Resultados: A análise da resposta vascular da membrana demonstrou que nenhum dos oito adesivos estudados induziu alterações vasculares significativas – de forma similar à resposta observada no controlo negativo - e não foram registadas diferenças entre a resposta aos adesivos universais, na forma não polimerizada ou polimerizada. Em contraste, o controlo positivo - NaOH - promoveu alterações significativas na vascularização da membrana corioalantóide, observando-se lise vascular, coagulação e hemorragia, que ocorreram precocemente, dentro do período de 300 segundos do ensaio.

Conclusão: Os adesivos dentários analisados nesta investigação não mostraram potencial de irritação e, consequentemente, uma resposta biológica adequada, dentro do quadro limitado deste ensaio. No entanto, são necessários mais estudos devido à complexidade do processo de avaliação biológica dos materiais destinados ao contacto com o organismo humano, incluindo ainda a citotoxicidade, genotoxicidade e carcinogenicidade, entre outros, por forma a englobar todo o espetro de ensaios de resposta biológica.

Palavras-chave: Adesão; Adesivos dentários; Adesivos universais; Potencial de irritação; Efeitos adversos; Citotoxicidade; Genotoxicidade.

Table of Contents

List of Figures

List of Tables

List of Terms and Acronyms

HAp	Hydroxyapatite
HET-CAM	Hen's Egg Test – Chorioallantoic Membrane Test Method
CAM	Chorioallantoic membrane
Bis-GMA	Bisphenol A diglycidylmethacrylate
HEMA	Hydroxyethyl methacrylate
MDP	Methacryloyloxydecyl dihydrogen phosphate
UDMA	Urethane Dimethacrylate
TEGDMA	Triethylene glycol dimethacrylate
ROS	Reactive Oxygen Species
ICCVAM	Interagency Coordinating Committee on the Validation Alternative Methods
IS	Irritation Score
GSH	Glutathione
IL-1β	Interleukin 1 beta
IL-8	Interleukin 8
MMP3	Matrix metalloproteinase-3

INTRODUCTION

1. Introduction

1.1 Adhesion

Adhesion can be defined as the joint between two different structures when placed in intimate contact (maximum distance: 0.0007 μm) allowing intermolecular forces activity, as well as mechanical retention, or due to a third lodged material (1–3).

The feature of adhesion, capable of making dental composite resin/dental substrate tangible, has defined a turning point in dentistry and characterizes the beginning of a new era for clinical practice. Adhesion to teeth structures, is the result of processes of diverse nature such as chemical, physical and mechanical, and as it seems dependent on the presence of irregularities in the substrate, the ability of monomers to diffuse through these roughnesses, the possibility of air escaping from the pores as the material percolates, and the polymerization capacity of that region.

Additionally, three factors are considered fundamental in order to achieve intimate contact between the adhesive system and the substrate (tooth structure) (1–3):

A. Wettability - which may be characterized by the contact angle formed when a drop of adhesive is dispersed over the substrate surfaces – enamel or dentin. The more the liquid spreads over the surface, the closer the contact angle will be to zero and the greater the wettability of the adhesive. In order to favour the lowest angle possible, it is critical to avoid the contamination of the substrate by fluids as saliva that induce the surface energy and compromise wettability;
B. Viscosity – the adhesive is effective when it spreads easily and quickly over the substrate;
C. Surface roughness – increases the potential for adhesion by increasing the contact area.

1.1.1 Differences between enamel adhesion and dentin adhesion

Adhesion to enamel is still considered the “gold standard” of adhesion in dentistry, while adhesion on dentin is much more challenging for several reasons (1,4).

Mature enamel presents a hard crystalline structure and is composed of approximately 96% hydroxyapatite (HAp) that forms enamel prisms, with high surface energy (1,4). The remainder consists of water and organic material. Additionally, it is a dry substrate without vital structures, which makes enamel with almost the ideal characteristics to establish a strong bond (4,5). Clinically, when the phosphoric acid is applied over enamel surface – during routine preparation, the HAp is selective dissolved, with approximately 10 μm of the surface being removed, and with the formation of pores 5 to 10 μm deep (2). Upon application, the monomers of the bonding agent flow into the irregularities of the micro-retentions by capillary attraction and copolymerize with each other, forming micro or macro “prism-like” resin tags. Thus, creating a micromechanical interlocking that can effectively seal the restorations margins in the long-term, providing immediate resistance to adhesive debonding (1,4).

On the other hand, dentin is a complex biocomposite structure and less predictable due to dynamic compositional disparities. The mineral composition of dentin corresponds on average to 70%, whereas the organic matrix is around 20%, of which 85 % is type 1 collagen, the remainder being composed of water (1,6). Dentin adhesion is affected by many factors including clinical and biological factors that include on one side the experience and procedure of the clinician, and on the other side the patient’s age, location of the tooth in the mouth, presence of sclerotic and/or carious dentin, dentin depth, amongst others factors. (4)

Moreover, physiological/pathological changes, resulting from dentin ageing or in response to different “aggressive” stimuli, for example a caries process, increase the degree of mineralization of dentin and its thickness, reducing its permeability, which interferes directly on dentin bond capabilities (4,6,7).

Furthermore, the preparation of the cavity alters the composition of the most superficial layer of tooth tissue, covering its surface with a layer of debris, called smear

layer. This smear layer is broadly composed of HAp, denatured collagen, saliva, blood, bacteria and by their products. Its thickness can vary from 1 to 2 μm (3,8,9). Clinically, it behaves as a true physical barrier that reduces the dentin permeability by 86%, since it occludes the orifices of the dentin identified as smear plugs, which have the capacity to extend up to 10 μm inside the dentin tubules. In addition, the oxygen and water in the dentin fluid cannot be completely removed which interferes with the polymerization of the adhesive (1,3,10).

The adhesive monomers penetrate between collagens fibrils and create a resin-impregnated dentin zone after polymerization, defined as the hybrid layer. As a matter of fact, the hybrid layer is a conjugation of residual monomers, collagen, HPa, residual solvents and dentin. The more heterogenous and compact it is, the more stable the adhesion will be (1,5,8,11,12). Nonetheless, occlusal forces, expansion and contraction stresses caused by temperature changes within the oral cavity, acidic chemical agents, as well as the incomplete polymerization, seem to affect resin stability and can compromise the adhesion and longevity of the restoration (11).

1.2 Dental adhesives

Adhesive systems have undergone several changes in terms of composition, application's methodology, and biocompatibility profile, over the years since their initial appearance, with the seminal works of Buonocore in 1952 (3,5).

Dental adhesives are solutions of resin monomers that enable adhesion to dental tissue. They are composed of hydrophobic groups that allow interaction and co-polymerization, as well as hydrophilic groups that enhance wettability. The differences between these groups lie imminently in the chemical composition of their monomers and solvents. In addition, their chemical composition also includes polymerization initiators, inhibitors or stabilizers, solvents and in some cases inorganic fillers (4,8,13)

Dental adhesives also feature the need for a less extensive tooth preparation, since it is not necessary to overextend the cavity to provide mechanical retention in order to retain the restorative material. In this way, it allows the preservation of a high quantity of

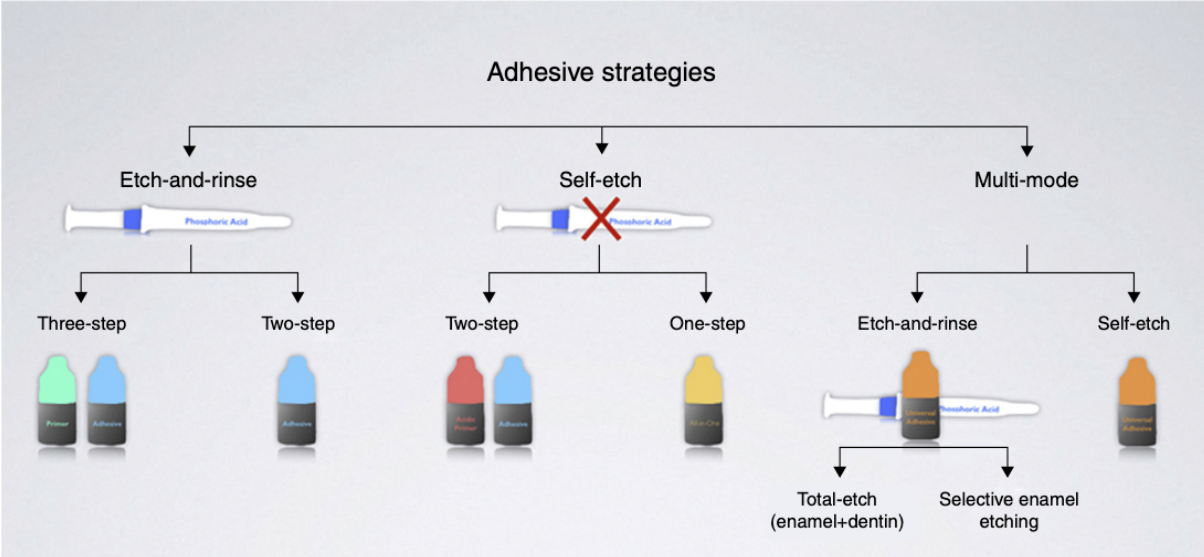
healthy tooth structure. Further reduces the microleakage, reducing the amount of secondary caries (8,14).

Dental adhesives can be classified according to their generation, which is the most widely system used by the industry, despite lacking objectivity, since it provides insufficient information on the adhesive system composition, not being classified according to scientific criteria. On the other hand, the classification proposed by Van Meerbeek *et.al* categorizes adhesives systems according to their interaction with the tooth structure, and more precisely how they interact with the smear layer. It is also informative about the number of steps required during the clinical procedure (3–5,14,15).

Table 1 – Generational classification. Adapted from (8)

Generation	Number of steps	Surface pre-treatment	Components	Shear bond strength (MPa)
1st	2	Enamel etch	2	2
2nd	2	Enamel etch	2	5
3nd	3	Dentine conditioning	2-3	12-15
4th	3	Total etch	3	25
5th	2	Total etch	2	25
6th	1	Self-etch adhesive	2	20
7th	1	Self-etch adhesive	1	25
8th	1	Self-etch adhesive	1	Over 30

Table 2 - Classification by Van Meerbeeck *et.al.* Adapted from (1)



1.2.1 Universal adhesives

Over time, clinicians have demanded more versatile, user-friendly products that allow them to choose the most suitable bonding strategy. Manufacturers, therefore, developed adhesive systems that enable clinicians to select the most appropriate protocol for each situation. Therefore, universal adhesives are also known as multi-mode, since they can be applied as self-etch (do not require a separate etching step) or etch and rinse: total etch (requires a separate phosphoric acid step to etch enamel and dentin), selective enamel etching technique (separate phosphoric acid to etch only enamel), among other techniques (5,10,16).

This new bonding philosophy, ideally, preaches a single bottle, which is much more challenging due to the inherent differences between dental substrates, the polyvalence of using different bonding strategies depending on the clinicians' preference and the clinical situation, simplifying its clinical application and improving its performance, prospectively increasing the longevity of the restorations (4,5,8).

Another property of universal adhesives is that they have a broad range of indications such as direct restorations, indirect restorations, zirconia primmer, silane coupling agent for ceramic glass matrices, core buildups and dentin desensitizer (4,15).

1.3 Biological response of dental adhesives

The dental pulp can be exposed to various irritants that are potentially detrimental to the health and function of this tissue. Some common irritants include dental caries, cavity preparation procedures, traumatic injuries, and chemicals such adhesive systems (17). In the frame of this dissertation, we will following focus on the potential biological alterations induced by adhesive systems.

The components of the adhesive systems on their own have the capacity to induce deleterious damage to the pulp tissue. Several studies suggest the disturbance of the redox state of pulp cells with the production of reactive oxygen species (ROS), decreased cell viability, depleted vital cellular functions, increased number of apoptotic cells, decreased metabolic rate, modifications in the cell cycle with a shrinkage of cells with

proliferative capacity, morphological changes - with more rounded and anucleate forms, and even induction of immunosuppression leading to a decrease in resistance to infectious agents (18–23).

Indeed, dental adhesives, due to their application in sustained contact with vital dentin, have a decisive influence on pulp tissue. This influence is broadly dependent on their chemical composition and on the concentration in which they are administered. Several components are accountable for inducing the cytotoxicity of dentin adhesives, such as the presence of free residual monomers like Bisphenol A diglycidylmethacrylate (bis-GMA) and Hydroxyethyl methacrylate (HEMA); photo-initiators as camphorquinone; pH; and light-curing parameters (24–29).

Nevertheless, one of the most preponderant factors to differentiate the biological response is the remaining dentin thickness, since the thinner it is, the closer to the pulp tissue and consequently the stronger capacity to induce an adverse response the adhesive system has. Hence, the deeper the cavity, the thinner the dentin and the larger the diameter and number of dentinal tubules, allowing adhesives penetration. Therefore, dentin permeability is increased and adhesive toxic compounds can diffuse more effortlessly through these tubules and consequently access the pulp tissue (17,18,22).

1.4 Hen's Egg-chorioallantoic membrane test method

The Hen's egg-chorioallantoic membrane test method (HET-CAM) is an alternative toxicological method that permits to evaluate, in a simple and reproducible way, the irritation potential of different substances. In this test, incubated hen's eggs are opened carefully and the chorioallantoic membrane (CAM) is exposed. Test substances are following placed directly over the exposed CAM and the membrane is inspected visually for vascular alterations through time, and quantitatively scored (30–32). The test has been validated as an alternative to the Draize test (Eye irritation test previously performed on an animal model) (33).

1.5 Study Objectives

This study aims to analyze the irritation potential of eight distinct commercially available Universal Adhesives with dissimilar chemical and physical formulations, through the HET-CAM assay, in order to disclose the ones with the least irritation potential and associated with an enhanced biocompatible profile.

By conducting this research, it is intended to answer the following null (research) hypotheses:

H0: The assayed Universal adhesive systems have no irritation potential, according to the HET-CAM assay.

H1: The assayed Universal adhesive systems have an irritation potential, according to the HET-Cam assay.

MATERIALS AND METHODS

2. Materials and Methods

In the present study eight universal dental adhesives were assayed, with different chemical formulations. Two different assays were performed for each universal adhesive. In the first, irritation was determined after light-curing of the adhesives. The second test evaluated the irritation potential, in direct contact of the adhesives, during polymerization, through exposure with ambient light.

2.1 Materials

The manufacturer, polymerization procedure, pH and composition of the eight universal adhesives assayed, are listed in Table 3.

Table 3 - Manufactures, pH and components of eight Universal Adhesives

Adhesive	Manufacturer	Polymerization	pH	Composition
All- Bond Universal	Bisco; Schaumburg, IL, USA	Light 10 s	3.2	Bis-GMA, ethanol, Methacryloyloxydecyl dihydrogen phosphate (MDP), HEMA
NormoBond xse	SanaPro Dental Gmbh	Light 20 s	*	Metacrylathe, photoiniators, ethanol, MDP
Universal Adhesive	Proclinic Expert	Light 20 s	*	Modified methacrylates, MDP, catalysts, stabilizers in ethanol.
Prime & Bond Active	Dentsply Sirona	Light 20 s	*	Phosphoric acid modified acrylate resin; Multifunctional acylate; Bifunctional acrylate; Acidic acrylate; Isopropanol; Water; Initiator; Stabilizer
Clinibond	Clinix Dentistry	Light 20 s	*	Triethylene glycol dimethacrylate (TEDGMA), Aliphatic polyester urethane acrylate, Adhesive accelerator, Hydroxyethyl, Photoinitiators, Acetone
Unibond	R&S	Light 10-20 s	*	Acetone, Fumed silica, 4-META, Water, Urethane Dimethacrylate (UDMA) Additives;
Futurabond M+	VOCO	Light 10 s	*	Bis-GMA, ethanol, acidic adhesive monomer, catalyst
Futurabond U	VOCO	Light 10	2.3	Bis-GMA, HEMA, acidic adhesive monomer, UDMA catalyst

*Not provided by the manufacturer

Figure 1 – Universal Adhesives used in the study

2.2 Preparation of the adhesives for the irritation assay

For the purpose of this investigation, O-rings, 1 mm thick and 5 mm in diameter were obtained. The O-rings were initially degreased and decontaminated with the aid of a gauze moistened with Ethanol 70%, left to dry on a sterile gauze.

For the first test (assessment of irritation after polymerization of the adhesives), 8 glass slides (1 for each dental adhesive studied) were degreased and decontaminated. A small aluminum sheet was following degreased, again using a gauze moistened with 70% ethanol, and placed on top of the glass, to avoid that during polymerization, the disk formed would remain stuck to the glass slide. Each glass slide was properly identified with the name of the universal adhesive to be applied. Subsequently, 3 O-rings were placed on top of each glass slide and 0.3 ml of each dental adhesive was consecutively applied inside the O-rings and light-cured for the period recommended by the manufacturer using a photopolymerizer (Coltene Spec 3 LED). Samples of each universal adhesive were performed separately to avoid premature polymerization and to avoid errors that could bias the final outcome.

After polymerization, the disks and O-rings were separated from the glass slides, visually inspected to ensure that they had no voids and their surface was as smooth as possible to guarantee adequate visualization of the CAM in the next procedure. Thereafter, they were stored in Petri dishes and properly identified.

Figure 2: a) O-Ring; b) Photopolymerizer Spec 3 Coltene; c) Petri Dish of an Universal Adhesive - Futurabond M+

2.3 In vivo HET-CAM assay for irritation assessment

This assay was performed as described in previous studies, with minor modifications (20,31–33). Fertilized chick eggs were sourced from a local farmer's and placed in an automatic rotating incubator – *Briensa Octagon Advance* - at 37°C in a humidified atmosphere until testing, on day 9. Any defective or nonviable eggs were discarded before testing. Eggs that have not been fertilized or have not undergone embryogenesis were discarded. CAM was accessed by removing the shell above the air cell (revealed using a light and marked with a pencil) with surgical scissors, and the inner egg membrane carefully removed with forceps moistened with physiological saline, ensuring that the CAM was not injured. None of the adhesives studied were diluted prior to the two tests. Firstly, light-cured universal adhesives were applied using the O-rings.

Alternatively, the universal adhesives were applied directly on the CAM and inside the O-rings to avoid diffusion of the substance through the egg. The chemicals (universal adhesives) were applied directly onto de CAM. In the two instances, 0.3 ml of each solution were applied using a micropipette. The O-rings were removed at the end of the exposure time.

A positive control test (1% NaOH) and a negative control (sterile saline - 0.9% NaCl) were included to serve subsequently as a comparison with the outcomes achieved with the test substances and for validation of the assay.

The biological response to the adhesives was assayed for 300 seconds, according to the test standards, being scored for hemorrhage, vascular lysis and coagulation, upon microscopic evaluation (ZEISS Stemi 508 Stereo Microscope).

In accordance with Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), irritation potential was evaluated by the occurrence of specific effects on the vascular network of the CAM (lysis – blood vessel disintegration, coagulation - intra and extra-vascular protein denaturation, hemorrhage – bleeding from the vessels) in the referenced periods (31).

a) b)

Figure 3: a) Shell aboved the air cell removed. Inner membrane is visible; b) Briensa Octagom Advance (automatic rotating incubator)

2.4 Evaluation of the test results

The data output recommended by the ICCVAM for prospective studies on the HET-CAM assay is the Irritation Score IS (A) analysis method, which is based on developing each of the three HET-CAM endpoints at fixed time intervals of 0.5, 2, and 5 minutes.

The time-dependent numerical scores for lysis, hemorrhage/bleeding, and coagulation (Table 4) are summed to give a single numerical value indicating the irritation potential of the test substance on a scale with a maximum value of 21.

When using the IS analysis method, a severe irritancy rating is attributed for a test chemical when the value is higher than nine.

Table 4 - Scoring Scheme for Irritation Testing with the HET-CAM Test Method – ICCVAM. Adapted from (31)

Effect	Score		
	0.5 min	2 min	5 min
Lysis	5	3	1
Hemorrhage	7	5	3
Coagulation	9	7	5

RESULTS

3. Results

In the present study, two different assays were performed. The first trial used already polymerized universal adhesives. And the second trial sought to study the potential for irritation during light-curing.

3.1 First assay – polymerized adhesives

In the first assay, it was decided to evaluate the biological response of the eight universal adhesives under conditions closer to clinical practice, that is, after light-curing. The light-curing time applied was the one recommended by the manufacturer to reduce potential errors related with the polymerization status. In addition, all were light-cured by the same photopolymerizer (Coltene Spec 3 LED). Thus, changes were recorded over 300 seconds, and for each universal adhesive, the 3 endpoints (0,5 min, 2 min, and 5 min) were registered.

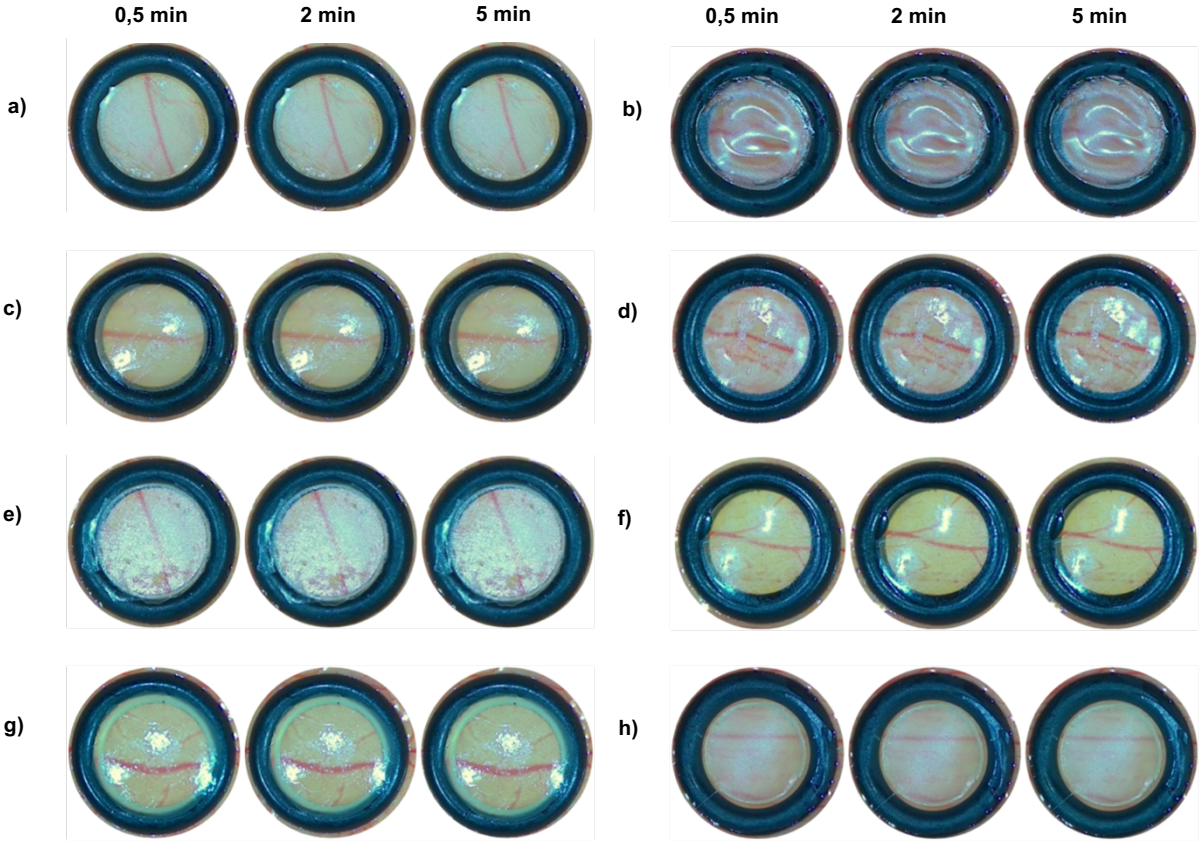


Figure 4:Observations recorded at 0,5, 2 and 5 minutes of each universal adhesive, upon microscopic evaluation (ZEISS Stemi 508 Stereo Microscope)

a) All Bond Universal; b) Clinibond; c) Futurabond M+; d) Futurabond U; e) NormoBond xse; f) PrimeBond; g) Proclinic Expert Universal; h) Unibond

After an appropriate visual analysis of all the endpoint images (Fig.4), it is possible to verify the absence of any relevant vascular modifications on the membrane (CAM). The application of polymerized universal adhesives directly on CAM did not induce any vascular reaction such as coagulation, haemorrhage or lysis despite the exposure time. The IS of the eight universal adhesives after polymerization are described in table 5.

Table 5 - Irritation Score of eight universal adhesives after polymerization

Adhesive	Effect	IS Score
All Bond Universal	None	**0**
Clinibond	None	**0**
Futurabond M+	None	**0**
Futurabond U	None	**0**
NormoBond xse	None	**0**
PrimeBond	None	**0**
Proclinic Expert	None	**0**
Unibond	None	**0**

3.2 Second assay – during polymerization

In the second trial, the potential for irritation of the eight adhesives was tested during light-curing under ambient light, to assess the direct contact of the adhesives with the CAM. Therefore, after application of the O-ring to a suitable area within the egg, samples of the different adhesives were applied inside, and the reactions were evaluated for 300 seconds according to test standards. Vascular changes were recorded at the endpoints, 0,5 min, 2 min and 5 minutes.

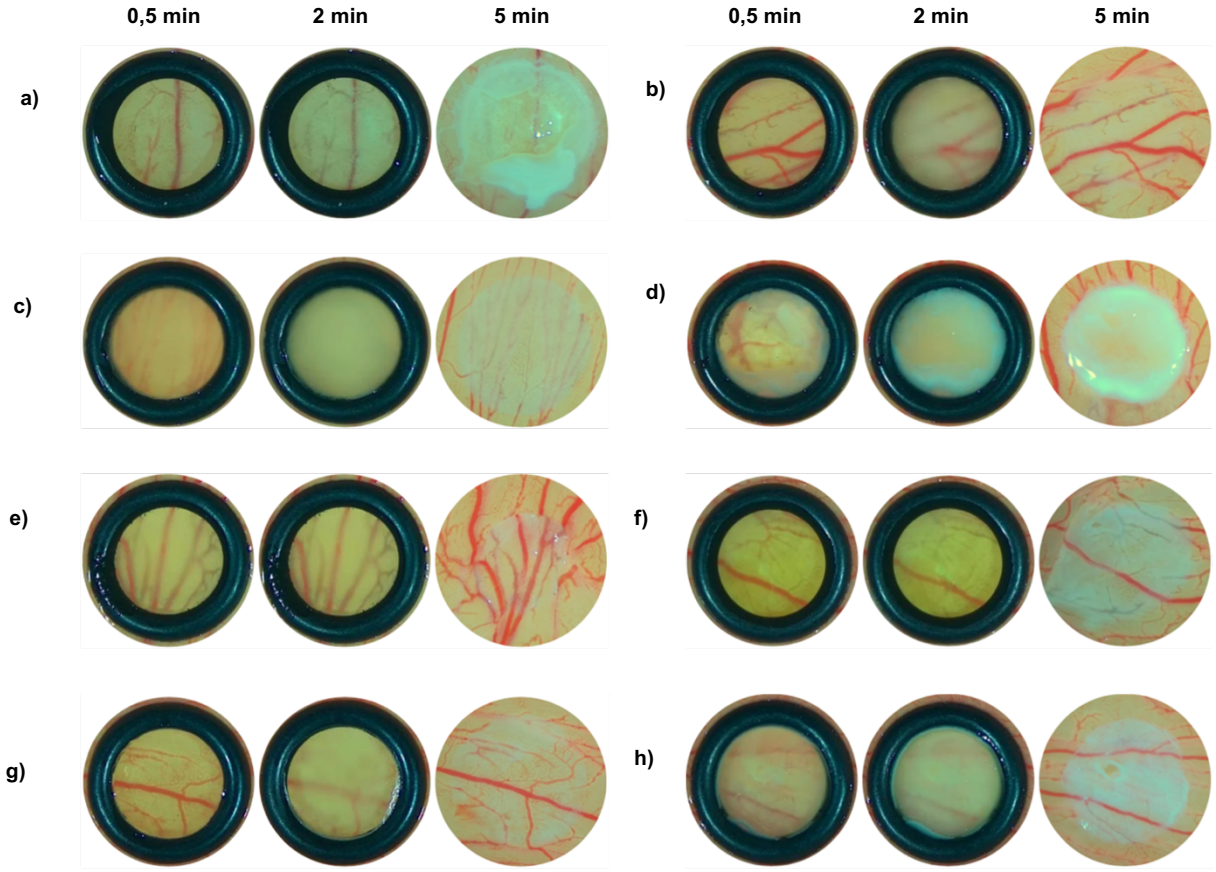


Figure 5: Observations recorded at 0,5, 2 and 5 minutes of each universal adhesive, upon microscopic evaluation (ZEISS Stemi 508 Stereo Microscope)

a) All Bond Universal; b) Clinibond; c) Futurabond M+; d) Futurabond U; e) NormoBond xse; f) PrimeBond; g) Proclinic Expert Universal; h) Unibond

After careful and rigorous analysis of the endpoint images there were no significant modifications on the CAM vascular response. Although polymerization was occurring and there was a higher quantity of residual free monomers, these did not interfere significantly with the CAM vascular functionality, to the extent of inducing changes in the assayed endpoints.

The 5 minutes images were taken after the removal of the O-rings to facilitate visualization and allow a better analysis of the vascularized membrane of the egg, since during polymerization the film created became too thick, interfering with observation.

The IS of the eight universal adhesives during polymerization are described in table 6.

Table 6 - Irritation Score of eight universal adhesives during polymerization

Adhesive	Effect	IS Score
All Bond Universal	None	**0**
Clinibond	None	**0**
Futurabond M+	None	**0**
Futurabond U	None	**0**
NormoBond xse	None	**0**
PrimeBond	None	**0**
Proclinic Expert	None	**0**
Unibond	None	**0**

3.3 Controls

3.3.1 Negative control

A negative control was incorporated into both assays to ensure the reliability of the results, as well as to serve as a comparative method. According to ICCVAM the negative control recommended is sterile saline (0.9% NaCl) (31).

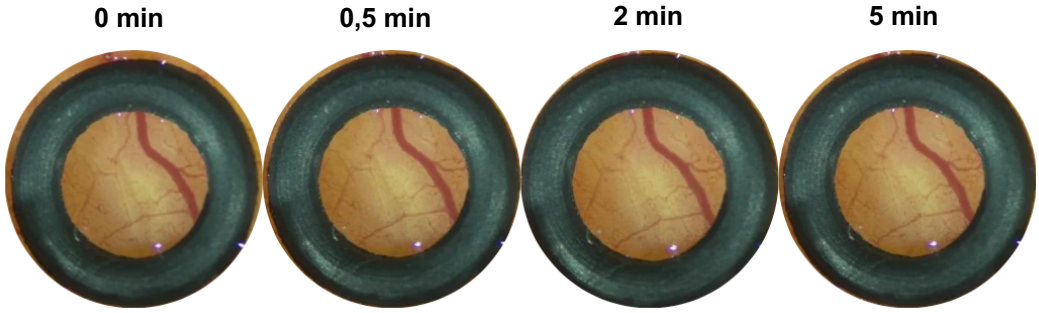


Figure 6: Negative control – 0,9% NaCl. Alterations recorded at 0, 0,5, 2 and 5 minutes, upon microscopic evaluation (ZEISS Stemi 508 Stereo Microscope)

The IS of the negative control is described in table 7.

Table 7 - Scoring Scheme of NaCl Irritation Testing with the HET-CAM Test Method

	Effect	IS Score
0,9 % NaCL	None	**0**

3.3.2 Positive Control

Not only to validate the tests previously performed, but also to serve as a comparison with the results a positive control was carried out. The positive control should be a substance that induces a severe response and therefore be highly irritating to the CAM vascularization. The ICCVAM recommended the use of 1% NAOH (31).

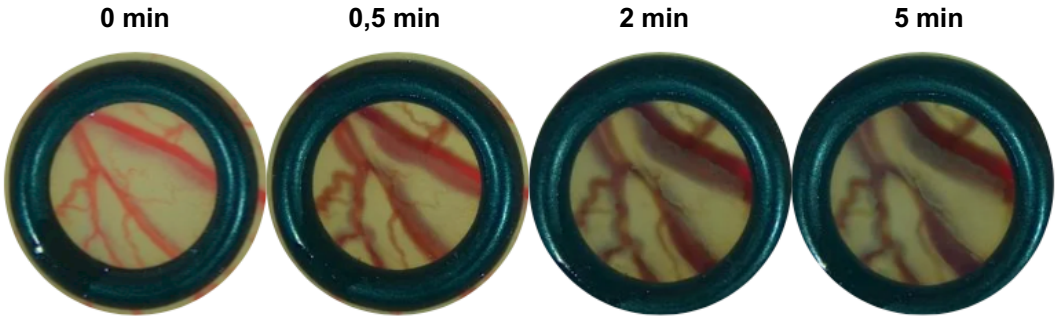


Figure 7: Positive control - 1%NaOH. Alterations recorded at 0, 0,5, 2 and 5 minutes, upon microscopic evaluation (ZEISS Stemi 508 Stereo Microscope)

The IS of the positive control is described in tables 8 and 9.

Table 8 - Scoring Scheme of NaOH Irritation Testing with the HET-CAM Test Method

Effect	Score		
	0.5 min	2 min	5 min
Lysis	5	3	1
Hemorrhage	7	5	3
Coagulation	9	7	5

Table 9 - Irritation Score of 1%NaOH (Positive control)

	Effect	IS Score
1% NaOH	Hemorrhage - 0,5 min Coagulation – 2 min	**14**

IS (1% NaOH) = 14

Thus, it can be assumed that given the irritating score greater than 9, NaOH is considered a severe irritant and hence can be used as a positive control. The vascular changes recorded over these 5 minutes are extremely different from those observed for the eight universal adhesives.

DISCUSSION

4. Discussion

The emergence of universal adhesives revolutionized and marked a rupture point with what was practiced in restorative dentistry previously (8). Universal adhesives, besides having all the advantages of the adhesive systems previously developed - as the reduction on the teeth preparation allowing the maintenance of healthy dental tissue; the absence of need of mechanical retention for the restorations – allows clinicians to decide on the most appropriate strategy for each clinical situation (14). As previously mentioned, the new generation of dental adhesives - universal adhesives - has the potential to significantly simplify and expedite adhesive protocols, reducing application time and improving standardization with a special focus on its user-friendliness, versatility and less technique-sensitive application (3,10,11,15).

To ensure adhesion to different dental substrates, as well as to a panoply of restorative materials, universal adhesives have a complex composition that is significantly different from each other regarding the presence and concentration of monomers, pH, ethanol or acetone, water, and other elements (4,10). Table 3 perfectly illustrates this premise, since the eight universal adhesives manifest clear differences in their composition.

Actually, all these different nuances and compounds may contribute to a different biological response that depends not only on factors external to the adhesives, such as the thickness of the remaining dentin - as studies have shown an inverse relationship between thickness and inflammatory response of the pulp, with 0.5 - 0.7 mm being sufficient thickness to ensure pulp protection against the diffusion of toxic substances - but also on factors intrinsic to adhesives, such as their composition and concentration (17,34–36).

Globally, several studies indicate that when universal adhesives are applied to cell cultures, including fibroblasts, human leukocyte cell lines or odontoblasts, similar general responses were recorded, safeguarding differences related to the composition and concentration, at which the different adhesives were applied. Briefly, a decreased cell viability, increased number of apoptotic cells, intense morphological alterations, cell

membrane damage, alterations in the cell cycle with decreased cell function and decreased proliferation capacity, damage at the DNA level, disturbance of the redox state of the cells with oxidative damage at the lipid, protein and nucleic acid level, which can result in alterations in the transduction pathways and in gene expression up to mutagenesis and cell death, were reported (18,19,21,22,37). An analysis of the composition of the eight universal adhesives studied reveals the presence of methacrylate monomers such as bis-GMA, UDMA, HEMA and TEGDMA, which are the main potentially cytotoxic elements, since their presence promotes direct cell damage, and, at the tissue level, chronic inflammatory response and a capacity for immunosuppression, that can even decrease host resistance to infectious agents (36).

Bis-GMA has a limited ability to diffuse through dentin due to its high molecular weight, however given the susceptibility to hydrolysis, its products may induce loss of cell membrane permeability. In addition, it can alter cell cycle progression and enhance oxidative stress and induce apoptosis in a concentration-dependent manner. It can also provoke prostanoid production that induces pulpal cell cytotoxicity (19,27,29). UDMA is also a methacrylate monomer present in universal adhesives (Unibond), with the capacity to induce cellular alterations that promote pathological phenotypes and cell death. Its toxicity is related to ROS induction, necrosis and/or apoptosis induction, glutathione (GSH) depletion and cell cycle disruption (19,22,37). On the other hand, HEMA has high capacity to diffuse through the dentinal tubules due to its low molecular weight and composition as it is easily released in an aqueous solution. This monomer has the ability to suppress the growth and morphological changes of various cell types, increase ROS levels and activate apoptosis since it induces DNA strand breaks (36–39). Finally, TEDGMA due to its lipophilic character has the ability to penetrate the cytosol and some cell membrane compartments. It also has the potential to induce an intracellular decrease of GSH and to cause DNA damage. In effect, the synergistic interaction between these elements can lead to amplification of the response when compared to the monomers individually (19,22).

Another important factor to the analyses is the pH of the universal adhesives. It ranges from 2.2 to 3.2 (10). Unfortunately, only two of the eight adhesives studied provided the pH value on their data sheets (All-Bond Universal - 3.2 and Futurabond U - 2.3). In fact, in tests performed with sufficient dentin thickness the pH was not able to

induce cytotoxicity, but when the thickness is reduced, lower than 0,5 mm, then it becomes critical, also because the deeper the cavity the greater the number of dentinal tubules and their diameters (18).

10-MDP, a component present in most of the universal adhesives studied in this trial, has also been demonstrated to have the potential to suppress odontoblastic differentiation as well as develop an inflammatory response since it enhances the expression of pro-inflammatory cytokines such as Interleukin 1 beta (IL-1β) and Interleukin 8 (IL-8) (19,40). Moreover, photoinitiators, present in most of the adhesives studied, such as camphorquinone may also induce cytotoxicity in pulpal cells, since they have the capacity to increment the expression of IL-6 and IL-8 and of matrix metalloproteinase-3 (MMP3), all potent pro-inflammatory cytokines. On the other hand, they have also demonstrated the ability to inhibit pulpal cell differentiation and mineralization in *in vivo* studies (19,41).

A final factor to take into consideration is polymerization. Indeed, some factors can be considered within the polymerization, such as light intensity and type, as well as distance. All these can influence the polymerization and therefore the amount of residual monomers and their toxicity (19,35). In order to try to avoid this variant as much as possible within our assay, we applied the polymerization time recommended by the manufacturer and the photopolymerizer used was the same for all adhesives. Nevertheless, there are studies indicating that the polymerization time is not enough to induce cytotoxicity, instead the composition of the adhesives (38).

Although the above-mentioned changes and adverse effects have been demonstrated in both animal models and cell cultures, limited attention has been devoted to the potential for irritation of the material, despite being one of the requirements of ISO standards. Chemical irritants display the ability to induce reversible inflammation or irritation of a body surface such as the eyes or mucous membranes like oral mucosa. It is also worth noting that some irritants are sensitizers and therefore may on repeated low-level exposure induce allergic reactions and on the other hand may show delayed symptoms. The degree of irritation depends on the chemical concentration, the duration of contact and personal factors like medical condition. All these factors apply imminently

to dental adhesives as they are of long contact duration and often the patients treated in dentistry are polymedicated and with several systemic pathologies (42,43).

Notwithstanding the different chemical formulations, polymerization time and pH, all the universal adhesives studied did not demonstrate differences among them in promoting irritation to the egg CAM, neither in the test after polymerization nor in the test during polymerization. During the 300 seconds of each assay it is verifiable that no vascular alterations of the CAM occurred, and therefore the adhesives studied present an adequate biocompatibility profile, within the limits of the framework of our test. It is important to mention that in cases in which the visualization of the CAM may have been hindered by the adhesive film created, the analysis was performed on the vessels surrounding the O-ring, and there, too, no vascular alterations of any kind were observed. The removal of the O-rings after 5 minutes also allowed concluding that the vascularization of the CAM remained intact, without changes caused by the adhesives, not even when there were residual monomers in direct contact with the CAM - 2nd test.

Another important evaluation is in fact the comparison with the positive control - 1% NaOH. By analyzing the positive control endpoints, it is easy to notice significant vascular changes immediately after 30 seconds, a totally different pattern from the universal adhesives. After 30 seconds significant bleeding of vessels is observed and after 2 minutes coagulation of some vessels.

An *in vivo* study of 2007, assessing the potential of dental adhesives to induce mucosal irritation using the HET-CAM assay found that out of a sample of 36 different adhesive solutions, only 2 had no irritation potential. 17 were considered moderate irritants and 16 strong irritants. In fact, a completely different result, that may be related to the fact that were studied adhesives from an earlier generation, as universal adhesives only appeared in 2011 (30). The same author had already conducted a study with the same purpose in 1999, with the following results: only three as non-irritant, 6 slight irritants, 4 moderate irritants, 6 strong irritants. In both studies the adhesive were classified as non-irritant (score 0-0,9), slight irritant (score 1-4,9), moderate irritant (score 5-8,9) and strong irritant (score 9-21) (33). These details suggest that the manufacturers intended to improve the irritation potential of the adhesives, and consequently, to reduce the irritability of their compounds.

CONCLUSION

5. Conclusion

The evidence gathered in this experimental work suggests that universal adhesives have no associated irritation potential, notwithstanding the evident compositional differences, within the limited frame of this assay. Furthermore, it shows that historically dental adhesives of previous generations presented superior genotoxicity, cytotoxicity, and irritation potential, which reveals carefulness from manufacturers in promoting materials with a more adequate biocompatibility profile and with acceptable biological response.

Nevertheless, further studies are required due to the complexity of the biological evaluation process of materials that aim at contact with the human organism, also including cytotoxicity, genotoxicity, and carcinogenicity, among other endpoints.

BIBLIOGRAPHY

6. Bibliography

1. Sezinando A. Looking for the ideal adhesive - A review. Rev Port Estomatol Med Dent e Cir Maxilofac [Internet]. 2014;55(4):194–206.
2. Reis A, Loguercio AD. Materiais dentários diretos : dos fundamentos à aplicação clínica. 2007. p. 423.
3. Vinagre Alexandra, Ramos J. Adhesion in Restorative Dentistry. 2016.
4. Perdigão J. Current perspectives on dental adhesion: Dentin adhesion – not there yet. Jpn Dent Sci Rev. 2020;56(1):190–207.
5. Sebold M, André CB, Sahadi BO, Breschi L, Giannini M. Chronological history and current advancements of dental adhesive systems development: a narrative review. J Adhes Sci Technol. 2021;35(18):1941–67.
6. Avery JK, Chiego DJ. Essentials of Oral Histology and Embryology, 3rd Edition. 2006. 178–195 p.
7. Carvalho RM, Tjäderhane L, Manso AP, Carrilho MR, Carvalho CAR. Dentin as a bonding substrate. Endod Top. 2009;21(1):62–88.
8. Eshrak Sofan, PhD Afrah Sofan, PhD Gaspare Palaia, PhD Gianluca Tenore, MD, DDS Umberto Romeo, MD, DDS Guido Migliau, MD D. Classification review of dental adhesive systems: from the IV generation to the universal type. Ann Stomatol (Roma). 2017;8(1):1–17.
9. Saikaew P, Senawongse P, Chowdhury AA, Sano H, Harnirattisai C. Effect of smear layer and surface roughness on resin-dentin bond strength of self-etching adhesives. Dent Mater J. 2018;37(6):973–80.
10. Alex G. Universal adhesives: the next evolution in adhesive dentistry? Compend Contin Educ Dent. 2015;36(1).
11. Scotti N, Cavalli G, Gagliani M, Breschi L. New adhesives and bonding techniques. Why and when? Int J Esthet Dent. 2017;12(4):524–35.
12. Bayne SC, Ferracane JL, Marshall GW, Marshall SJ, van Noort R. The Evolution of Dental Materials over the Past Century: Silver and Gold to Tooth Color and Beyond. J Dent Res. 2019;98(3):257–65.
13. Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, et al. Systematic review of the chemical composition of contemporary dental adhesives. Biomaterials. 2007;28(26):3757–85.

14. Dionysopoulos D, Gerasimidou O, Papadopoulos C. Current modifications of dental adhesive systems for composite resin restorations: a review in literature. J Adhes Sci Technol 2022;36(5):453–68.
15. Perdigão J, Araujo E, Ramos RQ, Gomes G, Pizzolotto L. Adhesive dentistry: Current concepts and clinical considerations. J Esthet Restor Dent. 2021;33(1):51–68.
16. Banjar AA, Nassar HM. Universal Dental Adhesives: Cost-Effectiveness and Duration of Use. Appl Sci. 2022;12(1).
17. de Oliveira MAVC, Quagliatto PS, Magalhães D, Biffi JCG. Effects of bleaching agents and adhesive systems in dental pulp: A literature review. Brazilian J Oral Sci. 2012;11(4):428–32.
18. Cardoso M, Coelho A, Marto CM, Gonçalves AC, Paula A, Bela A, et al. Scotchbond ™ Universal in Odontoblasts. 2021;1–15.
19. Wawrzynkiewicz A, Rozpedek-Kaminska W, Galita G, Lukomska-Szymanska M, Lapinska B, Sokolowski J, et al. The toxicity of universal dental adhesives: An in vitro study. Polymers (Basel). 2021;13(16):1–11.
20. Al RH, Dahl JE, Morisbak E, Polyzois GL. Irritation and cytotoxic potential of denture adhesives. Gerodontology. 2005;22(3):177–83.
21. Demirci M, Hiller KA, Bosl C, Galler K, Schmalz G, Schweikl H. The induction of oxidative stress, cytotoxicity, and genotoxicity by dental adhesives. Dent Mater. 2008;24(3):362–71.
22. Demirel G, Kaya Demirsoy FF, Irmak Ö. Cytotoxicity evaluation of eluates from universal adhesives by real-time cell analysis. Dent Mater J. 2020;39(5):815–24.
23. Scarano A, Manzon L, Di Giorgio R, Orsini G, Tripodi D, Piattelli A. Direct capping with four different materials in humans: Histological analysis of odontoblast activity. J Endod. 2003;29(11):729–34.
24. Pagano S, Lombardo G, Costanzi E, Balloni S, Bruscoli S, Flamini S, et al. Morpho-functional effects of different universal dental adhesives on human gingival fibroblasts: an in vitro study. Odontology. 2021;109(2):524–39.
25. Adam Wawrzynkiewicz 1, Wioletta Rozpedek-Kaminska 1, Grzegorz Galita 1, Monika Lukomska-Szymanska 2 , Barbara Lapinska 2 JS 2 and IM 1. The Cytotoxicity and Genotoxicity of Three Dental Universal Adhesives—An In Vitro St. International Journal of Molecular Sciences. 2020.

26. Leite ML de A e. S, Costa CA de S, Duarte RM, de Andrade AKM, Soares DG. Bond strength and cytotoxicity of a universal adhesive according to the hybridization strategies to dentin. Braz Dent J. 2018;29(1):68–75.
27. Arun M, Silja PK, Sheeja LE, Geetha CS, Mohanan P V. Molecular level toxicological evaluation of a dental composite implanted in albino rats. Toxicol Environ Chem. 2012;94(4):760–8.
28. Krifka S, Seidenader C, Hiller KA, Schmalz G, Schweikl H. Oxidative stress and cytotoxicity generated by dental composites in human pulp cells. Clin Oral Investig. 2012;16(1):215–24.
29. Nagem-filho H. Effect of dental adhesives on the exudative phase of the inflammatory process in subcutaneous tissue of rats Efeito dos adesivos dentais na fase exsudativa do processo inflamatório em tecido subcutâneo de ratos. 2003;17(2):109–12.
30. Dahl JE. Potential of dental adhesives to induce mucosal irritation evaluated by the HET-CAM method. Acta Odontol Scand. 2007;65(5):275–83.
31. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). Recommended Test Method Protocol : Hen ' s Egg Test – Chorioallantoic Membrane (HET-CAM) Test Method. ICCVAM Test Method Eval Rep. 2010;13(10):B30–8.
32. Irritation E, Description E. Hen ' s Egg Test on the Chorioallantoic Membrane (HET-CAM) INVITTOX n ° 96 PROCEDURE DETAILS , July 1994. 2007;(June 2007):1–18.
33. Dahl JE. Irritation of dental adhesive agents evaluated by the HET-CAM test. Toxicol Vitr. 1999;13(2):259–64.
34. Pashley DH, Tay FR, Breschi L, Tjäderhane L, Carvalho RM, Carrilho M, et al. State of the art etch-and-rinse adhesives. Dent Mater. 2011;27(1):1–16.
35. About I, Camps J, Burger AS, Mitsiadis TA, Butler WT, Franquin JC. Polymerized bonding agents and the differentiation in vitro of human pulp cells into odontoblast-like cells. Dent Mater. 2005;21(2):156–63.
36. Hebling J, Giro EMA, Costa CAS. Human pulp response after an adhesive system application in deep cavities. J Dent. 1999;27(8):557–64.
37. Pupo YM, de Freitas Bernardo CF, de Souza FF de FA, Michél MD, de

Morais Ribeiro CN, Germano S, et al. Cytotoxicity of etch-and-rinse, self-etch, and Universal Dental adhesive systems in fibroblast cell line 3T3. Scanning. 2017;2017.

38. Elias ST, dos Santos AF, Garcia FCP, Pereira PNR, Hilgert LA, Fonseca-Bazzo YM, et al. Cytotoxicity of universal, self-etching and etch-and-rinse adhesive systems according to the polymerization time. Braz Dent J. 2015;26(2):160–8.
39. Nayyar S, Tewari S, Arora B. Comparison of human pulp response to total-etch and self-etch bonding agents. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology. 2007;104(2):45–52.
40. Kim EC, Park H, Lee SI, Kim SY. Effect of the Acidic Dental Resin Monomer 10-methacryloyloxydecyl Dihydrogen Phosphate on Odontoblastic Differentiation of Human Dental Pulp Cells. Basic Clin Pharmacol Toxicol. 2015;117(5):340–9.
41. Kim RH, Williams DW, Bae S, Lee RS, Oh JE, Mehrazarin S, et al. Camphorquinone inhibits odontogenic differentiation of dental pulp cells and triggers release of inflammatory cytokines. J Endod. 2013;39(1):57–61.
42. APA. Guia de Classificação de Resíduos. Agência Port do Ambient [Internet]. 2020;123.
43. (EHRS) EH& RS. PennEHRS. 2021. SOP: Irritants; 17 nov 2021 [cited at 10 may 2022. Available from: https://ehrs.upenn.edu/health-safety/lab-safety/chemical-hygiene-plan/standard-operating-procedures/sop-irritants

ATTACHMENTS

7. Attachments

7.1 Repository statement

U.PORTO
FACULDADE DE MEDICINA DENTÁRIA
UNIVERSIDADE DO PORTO

DECLARAÇÃO
Mestrado Integrado em Medicina Dentária

Monografia/Relatório de Estágio

Identificação do autor

Nome completo João Francisco Oliveira Figueiredo

N.º de identificação civil 14974710 N.º de estudante 201706184

Email institucional up201706184@up.pt

Email alternativo figueiredo.joaomd@gmail.com Tlf/Tlm 915010655

Faculdade/Instituto Faculdade de Medicina Dentária da Universidade do Porto

Identificação da publicação

Dissertação de Mestrado Integrado (Monografia) [X] Relatório de Estágio [ ]

Título completo
Evaluation of the potential for imitation of different dental adhesives

Orientador Prof. Drº Pedro Gomes

Coorientador

Palavras-chave ; ; ; ;

Autorizo a disponibilização imediata do texto integral no Repositório da U.Porto: (x)

Não Autorizo a disponibilização imediata do texto integral no Repositório da U.Porto : X (x)

Autorizo a disponibilização do texto integral no Repositório da U.Porto, com período de embargo, no prazo de:

6 Meses: ; 12 Meses: ; 18 Meses: ; 24 Meses: X ; 36 Meses: ; 120 Meses: .

Justificação para a não autorização imediata Elaboração de um artigo científico

Data 23 / 05 / 2022

Assinatura João Figueiredo

7.2 Author's Statement

U.PORTO
FACULDADE DE MEDICINA DENTÁRIA
UNIVERSIDADE DO PORTO

DECLARAÇÃO

Monografia/Relatório de Estágio

Declaro que o presente trabalho, no âmbito da Monografia/Relatório de Estágio, integrado no MIMD, da FMDUP, é da minha autoria e todas as fontes foram devidamente referenciadas.

23/05/2022

João Figueiredo
O/A Estudante

7.3 Supervisor's Statement

U.PORTO
FACULDADE DE MEDICINA DENTÁRIA
UNIVERSIDADE DO PORTO

Informo que o Trabalho de Monografia/Relatório de Estágio desenvolvido pelo(a)

Estudante João Francisco Oliveira Figueiredo

com o título: Evaluation of the potential for imitation of different dental adhesives

está de acordo com as regras estipuladas na FMDUP, foi por mim conferido e encontra-se em condições de ser apresentado em provas públicas.

23/05/2022

O(A) Orientador(a)/Coorientador(a)