Scholars Journal of Dental Sciences
Abbreviated Key Title: Sch J Dent Sci
ISSN 2394-4951 (Print) | ISSN 2394-496X (Online)
Journal homepage: https://saspublishers.com/journal/sjds/home

OPEN ACCESS

In Vitro Assessment of Cytotoxicity of Orthodontic and Dental Composite Resins using Human Gingival Fibroblasts

Raoul Bationo[1*], Ablassé Rouamba[2], Abdoulaziz Diarra[3], Monique Lydie Beugré-Kouassi[4], Fabienne Jordana[5], Jean Bertin Beugré[4]

[1]Bogodogo Teaching Hospital, Burkina Faso; University Felix Houphouet Boigny, Abidjan, Côte d'Ivoire
[2]Laboratory of Applied Biochemistry and Chemistry, University Joseph Ki-Zerbo, 03 BP 7021, Ouagadougou, Burkina Faso
[3]Municipal Center of Oral Health of Ouagadougou, Burkina Faso
[4]Department of Odontology, University Felix Houphouet Boigny, Abidjan, Côte d'Ivoire
[5]Department of Odontology, University of Nantes, 1 Quai de Tourville, 44035 Nantes, France

DOI: 10.36347/sjds.2019.v06i07.006 | **Received:** 24.06.2019 | **Accepted:** 02.07.2019 | **Published:** 22.07.2019

***Corresponding author**: Raoul Bationo

Abstract **Original Research Article**

Objective: The aim of this study was to evaluate and compare the biological effects of seven composite resins (Clearfil Majesty ES-2, Clearfil Majesty ES Flow, Grengloo, Blugloo, Transbond XT, Transbond LR and Filtek Supreme XTE) on human gingival fibroblasts. ***Methods:*** Human gingival fibroblasts were cultured up to confluence. Cultures were exposed to composite resins for 24 hours and cell viability measured by MTT assay. ***Results:*** All composite resins showed reduced values (85 to 94%) of cell viability compared to the control. ***Conclusions:*** Orthodontic and dental composite resins are toxic to human gingival fibroblasts. Dental materials that are used in dentistry should be harmless to oral tissues, so they should not contain any leachable toxic and diffusible substances that can cause some side effects.

Keywords: Cytotoxicity, MTT assay, Gingival fibroblast, Light-cured composite resin.

INTRODUCTION

Dental composites are complex mixed materials which generally consist of an organic polymerizable matrix, reinforcing fillers, which are mainly inorganic and a silane-coupling agent [1]. The polymerizable matrix contains one or more monomers: Bis-GMA and/or UDMA, co-monomers (EGDMA, DEGDMA, TEGDMA) and various additives like initiator, coinitiator, inhibitor of polymerization and photostabilizer [2].

Various components may be released from composite resins into the oral environment. It has been shown that these released components can cause several adverse effects such as mucosal irritation, epithelial proliferation, oral lichenoid reaction, hypersensitivity, and anaphylactoid reactions [3].

Composite resins may cause different reactions in the oral soft tissues such as gingiva. Composites are initially very cytotoxic in in vitro tests of direct contact with fibroblasts. The cytotoxicity seems to be, in the early phase, from the not-polymerized components in the air-inhibited layer that leach out from the materials [4]. Other in vitro studies, which have "aged" the composites in artificial saliva for up to six weeks, have shown that the toxicity diminishes in some materials but remains high for others [5].

The aim of this study was to evaluate and compare the biological effects of composite resins on human gingival fibroblasts.

MATERIALS AND METHODS

Human gingival fibroblasts are amplified from gingiva waste from dental extraction in a patient who gave his consent and had no periodontal pathology. Biopsies are previously cut with surgical blade. RPMI-1640 medium with 10% of fetal bovine serum, 1% of penicillin/streptomycin and 1% of L-glutamine in Petri dishes stored in a humidified incubator at 37 °C under 5% of CO_2 in air.

The culture medium is changed every two days up to confluence. Cells are then trypsinized (Trypsin 0.25% in EDTA 0.02%) for 3 minutes at 37°C.

Specimen Preparation

Specimens (10 mm in diameter and 1 mm in thickness) were prepared from seven commercially composite resins (Table-1), cured for 20 seconds using BA Optima 10 LED Curing Light and then tested for cytotoxicity.

Table-1: Characteristics of resins used in the study

Product/Lot	Resin matrix	Manufacturer
Clearfil Majesty ES-2/4D0069	Bis-GMA: 5-15% (CAS No. 1565-94-2); Hydrophobic aromatic dimethacrylate; Hydrophobic aliphatic dimethacrylate	Kuraray
Clearfil Majesty ES Flow/A60239	TEGDMA: < 10% (CAS No. 109-16-0); Hydrophobic aromatic dimethacrylate	
Grengloo/6623923	TEGDMA: 5-10% (CAS No. 109-16-0); UDMA: 0.1-1% (CAS No. 72869-86-4); HEMA: 1-5% (CAS No. 868-77-9); Bis-EMA6: 1-5% (CAS No. 41637-38-1); GMA: 0.1-1% (CAS No. 106-91-2); EO-TMPTA: 0.1-1% (CAS No. 28961-43-5); 3-trimethoxysilylpropyl methacrylate: 1-5% (CAS No. 2530-85-0)	Ormco
Blugloo/6556174	UDMA: 1-5% (CAS No. 72869-86-4); Bis-EMA6: 5-10% (CAS No. 41637-38-1); GMA: 1-5% (CAS No. 106-91-2); EO-TMPTA: 1-5% (CAS No. 28961-43-5); 3-trimethoxysilylpropyl methacrylate: 1-5% (CAS No. 2530-85-0)	
Transbond XT/N921496	Bis-GMA: 10-20% (CAS No. 1565-94-2); Bis-MEPP: 5-10% (CAS No. 24448-20-2)	3M
Transbond LR/N919866	Bis-GMA: 5-15% (CAS No. 1565-94-2); TEGDMA: 10% (CAS No. 109-16-0)	
Filtek Supreme XTE/N879475	Bis-GMA: 1-10% (CAS No. 1565-94-2); UDMA: 1-10% (CAS No. 72869-86-4); TEGDMA: < 1% (CAS No. 109-16-0); Bis-EMA6: 1-10% (CAS No. 41637-38-1); PEGDMA: < 5% (CAS No. 25852-47-5)	

Cytotoxicity assay

The cytotoxic effects of the eluated extracts were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded onto 96-well plates and incubated for 24 hours at 37°C in a humidified incubator at 37 °C under 5% of CO_2 in air. The culture medium was then replaced with 100 µL of resin extracts for 24 h incubation.

Cells were also seeded onto 12-well plates in direct contact with resin samples and incubated for 24 hours. Then 100 µL of immersion medium was instilled onto 96-well plates. The culture medium was used as a control.

10 µL (5 mg/mL) of MTT solution was added to each well (96-well plate) and the plates were incubated for 3 hours. The MTT was then removed and 100 µL per well dimethyl sulphoxide (DMSO) was added to each well to dissolve the formazan crystals. Optical densities (OD) were measured at 570 nm in an EPOCH reader and cell viability was calculated using the following formula:

Cell viability = (OD test group/OD control) x100

Experiences are triplicated and repeated for a minimum of three times. The data were presented as mean and SD. The cell viability in types of contact was compared by t-test.

RESULTS

Table-2: Values of optical densities

	Clearfil ES-2		Clearfil Es Flow		Grengloo		Blugloo		Transbond XT		Transbond LR		Filtek Supreme XTE		Control
	D	I	D	I	D	I	D	I	D	I	D	I	D	I	
OD	0.564 ± 0.03	0.55 ± 0.02	0.564 ± 0.05	0.584 ± 0.03	0.565 ± 0.03	0.599 ± 0.04	0.543 ± 0.04	0.552 ± 0.03	0.545 ± 0.03	0.55 ± 0.04	0.573 ± 0.04	0.563 ± 0.03	0.568 ± 0.03	0.578 ± 0.03	0.637 ± 0.02
P value	0.39		0.31		0.07		0.67		0.77		0.63		0.54		

D=Direct; I=Indirect

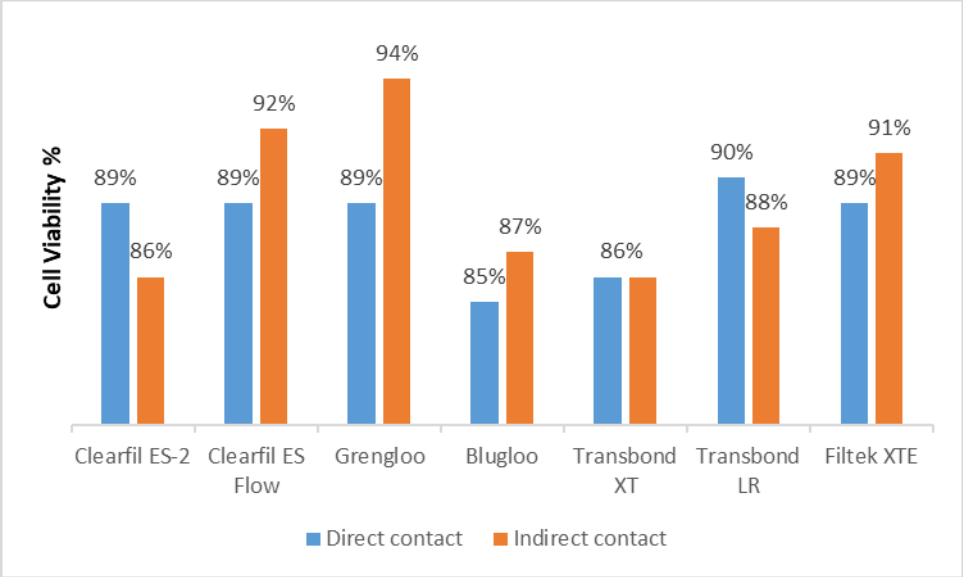

Fig-1: Cell viability in types of contact

DISCUSSION

Human gingival fibroblasts (HGFs) were used for cytotoxicity testing because they are in close proximity with restorative dental materials in the oral cavity and more clinically relevant. Also HGFs are sensitive cells that can be easily isolated and cultured in normal culture medium [6].

In this experiment the exposure time was 24 h, because it has been shown that monomer release from composite resins is complete in 24h [7]. Therefore, most toxic effects from composite resins occur during the first 24 h.

All our composite resins showed reduced values (85 to 94%) of cell viability compared to the control. According to Sjogren *et al*., [8], all tested resins have a slight toxicity (cell viability between 60 and 90%) except Clearfil Majesty ES Flow, Grengloo and Filtek Supreme XTE which are classified non-toxic (cell viability >90%) in indirect contact.

It is evidenced in literature that the polymerization rate can significantly affect the cytotoxicity of a composite material, through the diffusion of a large number of unreacted resin monomers [9-11]. In the current study, several monomers contained in the composite material used (such as Bis-GMA, Bis-EMA, UDMA, TEGDMA declared by the manufacturer) are known to diffuse from partially polymerized composite resins and to be cytotoxic in vitro [12, 13].

Malkoc *et al.*, [14] evaluated the cytotoxic effects of five different light-cured orthodontic composites. There were significant similarities in resin matrixes when evaluating the ingredient of tested materials. However, Transbond XT also contains Bis-EMA. In addition, a Bis-EMA monomer showed a cytotoxic effect analogous to that of TEGDMA [15]. The mechanism of cytotoxicity induced by TEGDMA in human fibroblasts was studied [16]. Hence, cytotoxicity of Transbond XT could be explained by the presence of Bis-EMA in its matrix.

TEGDMA, Bis-GMA and UDMA biological effects on three HGF cell lines and immortalised human keratinocytes were evaluated and compared by Moharamzadeh *et al*., [17]. The three resin monomers showed toxic effects on the HGFs and HaCaT cells. Bis-GMA was the most toxic and UDMA was the least toxic of the monomers tested.

CONCLUSION

Resin monomers contained in the composite material are known to diffuse from partially polymerized composite resins and to be cytotoxic in vitro. Orthodontic and dental composite resins are toxic to human gingival fibroblasts. Dental materials that are used in dentistry should be harmless to oral tissues, so they should not contain any leachable toxic and diffusible substances that can cause some side effects.

ACKNOWLEDGEMENTS

Cell cultures and cytotoxicity assay were carried out to the cellular culture platform of the Laboratory of Applied Biochemistry and Chemistry (LABIOCA) of the University Joseph Ki-Zerbo.

REFERENCES

1. Craig RG. Restorative dental materials. Chap 9. 11th ed.United States: Mosby Inc.; 2002
2. Stein PS, Sullivan J, Haubenreich JE, Osborne PB. Composite resin in medicine and dentistry. J Long Term Eff Med Implants, 2005;15:641-654.

3. Hensten-Pettersen A. Skin and mucosal reactions associated with dental materials. Eur J Oral Sci, 1998;106:707-712
4. Darmani H, Al-Hiyasat AS, Milhem MM, Cytotoxicity of dental composites and their leached components, Quintessence Int, 2007;38(9):789-795
5. Goldberg M, In vitro and in vivo studies on the toxicity of dental resin components: a review. Clin Oral Investig, 2008;12(1):1-8
6. Hensten-Pettersen A, Helgeland K. Sensitivity of different human cell line in the biologic evaluation of dental resin-based restorative materials. Scand J Dent Res, 1981;89:102-107
7. Ferracane JL, Condon JR. Rate of elution of leachable components from composite. Dent Mater, 1990;6:282-287
8. Sjogren G, Sletten G, Dahl J E. Cytotoxicity of dental alloys, metals, and ceramics assessed by millipore filter, agar overlay, and MTT tests. The Journal of Prosthetic Dentistry, 2000;84: 229-236
9. Geurtsen W, Spahl W, Leyhausen G. Residual monomer/additive release and variability in cytotoxicity of light-curing glass-ionomer cements and compomers. J Dent Res, 1998;77(12):2012-2019
10. Ferracane JL, Condon JR. Rate of elution of leachable components from composite. Dent Mater, 1990;6(4):282-287
11. Ferracane JL. Elution of leachable components from composites. J Oral Rehab, 1994;21:441-452
12. Hanks CT, Strawn SE, Wataha JC, Craig RG. Cytotoxic effects of resin components on cultured mammalian fibroblasts. J Dent Res, 1991;70(11):1450-1455
13. 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(3):474-480
14. Malkoc S, Corekci B, Ulker HE, Yalcin M, Sengun A. Cytotoxic effects of orthodontic composites. Angle Orthodontist, 2010;80(4): 759-776.
15. 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
16. Stanislawski L, Lefeuvre M, Bourd K, Soheili-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
17. Moharamzadeh K, Van Noort R, Brook IM, Scutt AM. Cytotoxicity of resin monomers on human gingival fibroblasts and HaCaT keratinocytes. Dent Mat, 2007;23:40-44.