A cytotoxic evaluation of 7th generation dentin bonding agent on human pulp cells

Salwani A. Ghani,* Sam'an M. Masudi, Norshamsuria Omar*****
*Dental Officer, Ministry of Health
**School of Dental Sciences, Health Campus, Universiti Sains
***School of Dental Sciences, Health Campus, Universiti Sains
Malaysia

ABSTRAK

Dentin bonding adalah bahan berbasis resin yang digunakan dalam kedokteran gigi klinis untuk mencegah celah dan menimbulkan perlekatan antara bahan tambal ke email dan dentin. Meskipun demikian, polimerisasi *dentin bonding* akan melepaskan monomer yang dapat berinteraksi dengan jaringan pulpa. Penelitian *in vitro* ini bertujuan untuk mengevaluasi sitotoksisitas dari *dentin bonding* generasi ketujuh (*G bond*) pada sel stem dari *human exfoliated deciduous teeth* (SHEDs). Sel pulpa manusia yang proliferasi diinkubasi pada suhu 37 ºC selama 48 jam. Dengan kondisi aseptik, spesimen uji yang telah diekstraksi ditempatkan di dalam *well of tissue tray*. SHEDs ditempatkan pada setiap mangkok yang berbeda-beda konsentrasi bahan *dentin bonding*-nya, lalu diinkubasi pada suhu 37 ºC selama 72 jam. Pengaruh sitotoksisitas dicatat dengan menggunakan metode *MTT assay*. Analisis statistik menunjukkan bahwa makin tinggi konsentrasi *dentin bonding* (IC_{50}= 0,035 mg/ml) melepaskan pengaruh toksik yang lebih banyak ke sel pulpa manusia (SHEDs). Dari hasil tersebut disimpulkan bahwa reaksi pulpa terhadap *dentin bonding* tergantung pada jumlah prosedur aplikasinya.

Kata kunci: *dentin bonding* generasi ketujuh, sitotoksisitas, SHEDs

ABSTRACT

Dentin bonding agents are resin based materials that used in clinical dentistry in order to prevent leakage and promote adherent of filling material to the enamel and dentin. However, the polymerization of dentin bonding agents will release residual monomer that may interact with pulp tissue. This in vitro study is aimed to evaluate the cytotoxicity of new 7th generation dentin bonding agents (G Bond) on stem cells from human exfoliated deciduous teeth (SHEDs). The proliferated human pulp cells were incubated at 37î C for 48 hours. Under aseptic conditions, extracted test specimen were plated in 96 well of tissue tray. SHEDs were placed on each well with different concentration of dentin bonding agents, and then incubated at 37î C for 72 hours exposure. The cytotoxic effect was recorded by using MTT assay method. Statistical analysis showed that higher concentration of dentin bonding agents (IC_{50}= 0.035 mg/ml) exerts higher toxic effect to the human pulp cells (SHEDs). This study concluded that pulpal reaction to dentin bonding agent may depend on number of application procedure.

***Keywords**: 7th generation bonding agent, cytotoxicity, SHEDs*

Correspondence: Sam'an Malik Masudi, School of Dental Sciences, USM Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia. Phone: +60129596858, +6097675842. E-mail: sam@kb.usm.mymasudi1904@yahoo.com.

INTRODUCTION

Dental adhesive systems were introduced into clinical dentistry after 1960's. Since then, the development in dentin bonding agents has greatly changed the practice of restorative dentistry. According to Eliades *et al,*[1] dentin bonding agents are resin based materials widely used in clinical dentistry in order to prevent leakage and promote adherent of filling material to the enamel and dentin.

Research is available regarding the *in vitro* and *in vivo* cytotoxic effect of monomers that are present in dentin bonding systems. *In vivo* studies showed that adhesive systems biocompatible with the pulp tissue.[2,3] On the contrary, other studies have demonstrated that resin based materials do not seem appropriate to be used as pulp capping material.[4-6] *In vitro* studies demonstrated that resin components present definite toxic effect on fibroblast cells.[7-9] Data suggest that pulpal reactions to dentin bonding agents may be influenced by a number of factors, such as composition, clinical application procedure and dentin permeability.[10] Based on Demirci. *et al.,*[11] polymerized dental resin materials release residual monomers that may interact with pulp tissues. Dental adhesives might cause cytotoxicity in pulp cells via the generation of reactive oxygen species (ROS), which may also contribute to genotoxic effects *in vitro*.

The 7th generation dentin bonding agent is a new bonding material in the field of dentistry which is recently introduced. As a new material, there are very limited cytotoxic evaluations on the 7th generation dentin bonding agents that have been studied and reported.

Researchers have reported the effect of resin based materials on human pulp cells. Human exfoliated pulp cells contain multipotent stem cells and highly proliferative. Therefore the stem cells from human exfoliated deciduous teeth (SHEDs) is more preferable to the study of cytotoxic effect of dentin bonding agents.[12,13] One of the recommended and appropriate steps for the biological assessment of medical materials is *in vitro* assessment of cytotoxicity of new biomaterials. In this primary screening, we evaluated the cytotoxicity of dentin bonding agent by using MTT assay test on human exfoliated deciduous pulp cell (SHEDs).

This paper reported an in vitro study which is aimed to evaluate the cytotoxicity of new 7th generation dentin bonding agents (G-Bond) on stem cells from human exfoliated deciduous teeth (SHEDs).

MATERIALS AND METHODS

This is a descriptive experimental study using *in vitro* cytotoxicity extract test. The test was conducted in Craniofacial Laboratory at School of Dental Sciences, Universiti Sains, Malaysia from 3rd June to 18th July, 2008.

Extraction method of cytotoxicity was evaluated based on protocol that was reported in International Standard ISO 10993, prepared by Technical Committee ISO/TC 194, Biological evaluation of medical devices. All procedures were done in sterile, chemically inert closed container using aseptic technique in accordance with ISO 10993.

Materials

The 7th generation of dentin bonding agent tested was G Bond (GC Corporation, Japan). The components and manufacturers are listed in Materials Table. Under germ poor conditions, the dentin adhesives to be tested were applied into glass tubes (3mm inner diameter x 1 mm in height), in order to occupy the same volume as the cured bonding agents according to the

manufacturer's instructions with the use of curing light.

Human exfoliated deciduous pulp cell (SHEDs) line

SHEDs was cultured in Alpha Modification of Eagle's Medium supplemented with 20% Fetal Bovine Serum (FBS), 100µM L-ascorbic acid 2-phosphate, 2 mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin as reported by Shi and Gronthos.[13] The culture was incubated at 37ºC in 5% CO^2. The SHEDs at passage 3-5 was be used in this study.

Material preparation

Cured 0.6g/ml G Bond bonding agent was sterilized with UV light provided at Craniofacial Lab of USM.

Material extraction

Test specimens were placed on the centers of culture medium trays. The extraction material was incubated in CO2 incubator at 37°C for 48 hours.

MTT assay

Dentin bonding agents were incubating with the culture medium (0.2g/ml) at 37 ˚C for 48 hours. After 48 hours, they were separated with the culture medium (material extraction). 100 ul of culture medium was pipetted in each well of 96 multi-well plate except row A. 100ul of material extraction was added into row A and row B. Starting from B, the solution was mixed by pipetting and 100ul of solution from row B was aspirated and added into next well. This step was repeated until row G. The excess (100ul) was discarded. Row H was left untouched. Adherent cells form culture flask was harvested by trypsinization. The cells counted and cell suspension 1×10^5 cells/ml was prepared. 100ul of cells suspensions were added into all wells the cells were thoroughly re-suspend before adding into the wells. The plate was incubated in 37 ˚C, 5% CO_2 incubator for 72 hours. After 72 hours incubation, 10ul of MTT solution into all wells and was incubated for 2-4 hours in CO_2 incubator. After 2-4 hours incubation, the culture medium was removed and MTT was excesses by inversion and the plate was blotted carefully on tissue paper. 100ul of DMSO was added into each well and the plate was shaken for 5 minutes. The absorbance was read using ELISA reader at reference wavelength 630 nm, test 570 nm.

The data collected were presented as mean. This was a descriptive analysis using IC_{50} plot. IC_{50} = concentration when only 50% of cells proliferate. Materials concentration more than IC_{50} is cytotoxic to the cells.

$$\% \text{ of cell viability of sample} = \frac{\text{sample mean}}{\text{sample control}} \times 100$$

RESULT

MTT assay

The effects of dentin bonding agents extracts on human exfoliated pulp cells (SHEDs) viability were measured by MTT test as shown in table 1. The table summarizes the overall result of MTT assay of dentin bonding agents on human exfoliated pulp cells (SHEDs). The percentage of viable cells after exposure to various concentrations of dentin bonding agents depends on different concentration of material. Increasing concentration of material caused increasing of cell in most instances throughout the 3 days of the experiment based on the percentage of viable cell referenced to 100% for the control. As shown in figure 1 the IC_{50} is determined at 0.035mg/ml concentration of dentin bonding agents. Therefore, material extractions more than 0.035mg/ml are cytotoxic to human exfoliated pulp cells.

Table 1. Result of MTT assay of 3 replications of dentin bonding agents on human exfoliated pulp cells (SHEDs)

Number of samples with concentration of dentin bonding agents (mg/ml)	Replication 1 (mean) (%)	Replication 2 (mean) (%)	Replication 3 (mean) (%)
1 (0.6)	6	5	10
2 (0.3)	11	8	19
3 (0.15)	15	29	53
4 (0.075)	16	37	71
5 (0.0375)	27	52	77
6 (0.01875)	39	56	76
7 (0.009375)	50	71	77
8 (Control)	100	100	100

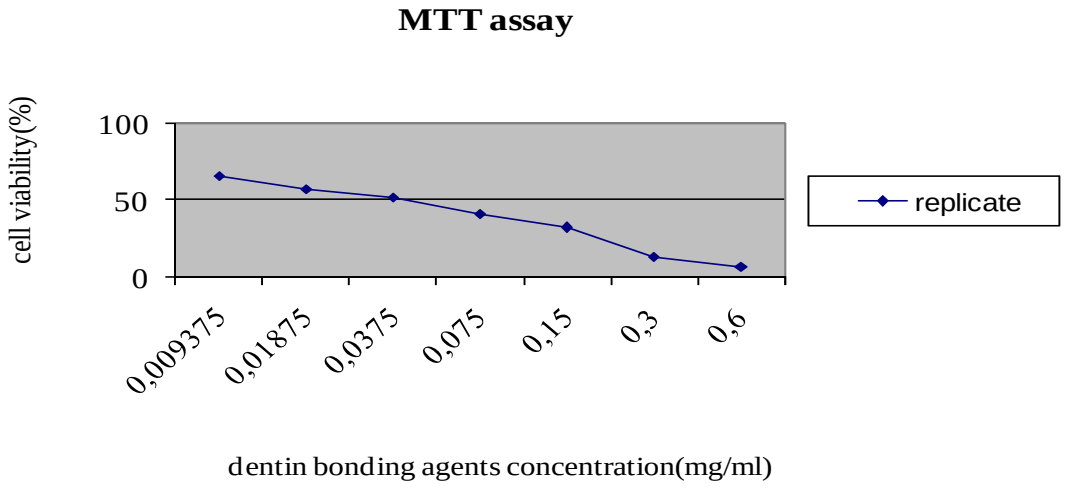

Figure 1. MTT assay result of means of 3 replications of dentin bonding agents on human exfoliated pulp cells (SHEDs).

DISCUSSION

The main purpose of this study was to determine the cytotoxicity effect of the dentin bonding agent (G Bond, 7th generation) on human exfoliated deciduous pulp cells (SHEDs). During the last few years, there was an increase in study of biomaterial cytopathic effect either *in vitro* or *in vivo*. Cytotoxicity is the harmful or noxious unwanted effect induced by a biomaterial in *in vitro* cell culture system. Cytotoxicity testing includes numerous methods, both qualitative and quantitative [ISO 10993-5:1999(E)]. Various methods have their own strengths and weaknesses, easier obtained of densitometry evaluations also preserve monolayer, but it was less precise and time consuming. The visual method provides accurate outcome with minimal use of equipment. However, some trained observers are required in this method. The tetrazolium-based colorimetric assay (MTT) is objective, less time consuming and showed little variations. According Sjogren *et al.*,[14] MTT is appropriate overall estimator of cytotoxicity. Therefore cytotoxicity in this study, was determined using MTT assay at different

dentin bonding agent concentrations. The level of cytotoxicity then was represented by percentage of viable cells compared with controls. MTT is cleaved by all living metabolically active cells that we have tested. The result can be read in a few minutes after the addition of MTT solution, acid-propanolol under incubation at in 37°C and 5% CO_2 incubator in 4 hours. Besides, the results are also apparent visually which is useful in rapid result.

This study is clinically relevant where SHEDs chosen as the tested cells. Previous researchers have found that human pulp cells contain multi-potent stem cells highly replicated and have greater viability. Besides, it is clonogenic cell capable of differentiating into several cell types including neural, adipocytes and odontoblast. Moreover, human pulp cells are readily accessible and capable to fulfill criteria for potential clinical application.[12] Therefore, human pulp cells were chosen to be used in this study for cytotoxic evaluation of dentin bonding agents.

In this study, concentration of dentin bonding agents more than 0.035 mg/ml was found to be cytotoxic to human deciduous pulp cells. The result demonstrated that the concentration of exposure had a strong effect on the toxicity of dentin bonding agents as the higher concentration of extraction material resulted in higher toxicity.

The cytotoxic effect of dentin bonding agents has been assessed by many studies.[15-18] In his *in vitro* studies, Demirci *et al*.[11] suggest that the cytotoxic potency demonstrated by these materials might be of clinical relevance, since all dental adhesives disturbed the cellular redox state of pulp cells in monolayer cultures. In the previous study,[18] DBAs have shown that it exert potential harmful effect on the human pulp cells which was concomitant with marked retraction and rounding of dental pulp cell. Kaga *et al*.[17] reported that clinical exposure of primers and adhesives of dentin bonding agents should be minimized. Besides, Ruey-Song *et al*. suggested that dentin bonding agents exert potential effects in the pulp. They conclude that different toxic effects on pulp cell should be considered during selection of a suitable bonding agent during operative restoration. The results of this study support the re-evaluation of dentin bonding agents as pulp capping material should be considered.[19]

CONCLUSION

In conclusion, different concentration of 7th generation dentin bonding agents (G Bond) exerts a different level of cytotoxicity effect on human pulp cells.

REFERENCES

1. Eliades G, David CW, Eliades T. Dental hard tissues and bonding: interfacial phenomena and related properties. Berlin: Springer Verlag; 2005.
2. Cox CF, Keall CL, Keal HJ, Ostro E. Biocompatibility of surface-sealed dental materials against exposed dental pulps. J Prosthet Dent 1987; 57:1–8.
3. Akimoto N, Momoi Y, Kolno A, Suzuki S, Otsuki M, Suzuki S, et al. Biocompatibility of clearfill liner bond 2 and clearfill AP-X system on nonexposed and exposed primate teeth. Quint Int 1998; 29:177-88.
4. Hebling J, Giro EMA, Costa CAS. Human pulp response after an adhesive system application in deep cavities. J Dent 1999; 38.
5. Pereira JC, Segala AD, Costa CAS. Human pulp response to direct pulp capping with an adhesive system: Histologic study. J Dent Res 1997; 67:180.
6. Nascimento ABL, Fontana U, Costa CAS. Histologic evaluation of human dental pulp in direct contact with different materials. J Dent Res 1998; 77: 690.
7. Hanks CT, Wataha JC, Parsell RR, Straw SE. Delineation of cytotoxic concentrations of two dentin bonding agents in vitro. J Endod 1992; 18: 589–96.

8. Hanks CT, Straw SE, Wataha JC, Craig RG. Cytotoxic effects of resin components on cultured mammalian fibroblasts. J Dent Res 1991; 70: 450–5.
9. Hashimoto M, Kaga M, Oguchi H. Cellular response to dentin-bonding composite resins with dentin disks using the agar overlay method. Pediatric Dent J 1997; 7: 103-7.
10. Soderholm KJ. Correlation in vivo and in vitro performance of adhesive restorative material: a report of the ASC MD 156 Task group on test methods for the adhesion of restorative material. Dent Mater 1991; 7: 74-83.
11. Demirci M, Hiller KA, Bosl C, Galler K, Schmalz G, Schweik H. The induction of oxidative stress, cytotoxicity, and genotoxicity by dental adhesives. Dent Materials 2008; 24: 362-71.
12. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG. SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci 2003; 100: 5807–12.
13. Shi S, Gronthos S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. J Bone Miner Res 2003, 18: 696–704.
14. Sjogren G, Sletten G, Dahl J. Cytotoxicity of dental alloys, metals and ceramics assesed by Milipore filter, agar overlay, and MTT test. J Prosthet Dent 2000; 84: 229-36.
15. Szep S, Kunkel A, Ronge K. Cytotoxicity of modern dentin adhesives – in vitro testing on gingival fibroblast. J Biomad Mater Res 2002, 63: 53-60.
16. Koliniotou-Koubia E, Dioysopoulos P, Koulaouzidou EA. In vitro cytotoxicity of six dentin bonding agents. J Oral Rehabil 2001; 28: 971-5.
17. Kaga M, Noda M, Ferracane JL. The in vitro cytotoxicity of eluates from dentin bonding resins and their effects on tyrosine phosphorylation of L929 cells. Dent Mater 2001; 17: 333-9.
18. Vajrabhaya L, Pasasuk A, Harnirattisai C. Cytotoxic evaluation of single component of single component dentin bonding agents. Oper Dent 2003; 28: 440-4.
19. Costa CAS, Hebling J, Teixeira MF. Estudo preliminar da compatibilidade biológica dos adesivos dentinários all bond 2 e Scotchbond MP. Avaliação histológica de implantes subcutáneos em ratos. Rev Odontol Univ São Paulo 1997; 11**:**11–8.