Effects of cured dentin bonding materials on human monocyte viability

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Objectives. Dentin Bonding Agents (DBAs) have been proposed as root-end filling materials. This study examined the effect of polymerized DBAs on human monocyte viability.

Study design. Monocytes were directly isolated from peripheral blood and being exposed to cured Scotch bond I (Single Bond) and Prime & Bond in different time intervals (36 and 72 hours). The viability of monocytes was determined by MTT assay.

Results. Viability of the cells was time dependent. There was no significant difference between the effect of 2 DBAs on monocytes.

Conclusion. Results indicate that DBAs in polymerized form can alter the viability of monocytes and decrease it within time.


Dentin bonding agents (DBAs) have been proposed as root-end filling material in endodontic surgical procedures since 1990. These resin-based materials have the ability to seal the root end completely in vitro and have shown promise in vivo as root-end filling. DBAs have been reported to reduce apical leakage when applied directly on the beveled root end surface. Since root-end cavity preparation is a difficult clinical procedure and often can be time consuming, it would be valuable if we can eliminate this step from surgical endodontic procedures. This would also enable the exposed dentinal tubules of the beveled root end, which may contribute to apical leakage, to be sealed. Dentin bonding agents contain resin, some of which may remain in unpolymerized form even after thorough curing and can be released from the resin matrix. The biocompatibility of DBAs in periapical tissues has not been adequately defined, but unpolymerized monomeric resins of DBAs are known as cellular toxins. The effect of DBAs on macrophages is of particular interest because of the well documented role of macrophages in directing the inflammatory and healing process.

The first step in examining biocompatibility of materials is to determine their effect on basic cellular function. Previous studies have shown that the activity of mitochondrial succinate dehydrogenase (SDH) is a good indicator for cell function. The components of DBA have been shown to alter SDH activity of fibroblasts at high concentration. Resin components suppressed the mitochondrial activity of macrophages at different concentrations. In this case these substances may have some influence on inflammatory response, by altering macrophage functions. Previous studies have determined the concentration of DBAs that could alter macrophage activity in vitro. Because these materials are used in clinical practice in polymerized form, the current study is designed to determine the effect of polymerized DBAs on human peripheral blood monocyte (PBM) viability.

MATERIALS AND METHODS

Monocyte isolation

Peripheral human blood monocytes were separated from heparinized blood samples. Briefly, blood samples were diluted in calcium- and magnesium-free Hanks balanced salt solution (Sigma Chemical Co, St Louis, Mo). The diluted blood was layered over Ficoll Hypaque (Biotest Diagnostic, Dreieich, Germany) in a 2:1 ratio and centrifuged at 400 g for 20 min at 20°C. The mononuclear cell interface was removed and washed by repeated centrifugations to remove ficoll contamination, platelets, and residual erythrocytes. Mononuclear cells were counted and adjusted to 2.5 × 10⁶ cell/mL and suspended into 96-well microplates. The monocytes were isolated by their adherent capacity, followed by

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1 hour incubation at 37°C in humidified 95% O₂ and 5% CO₂. Isolated monocytes were cultured in RPMI-1640 (Sigma Chemical) supplemented with 10% pooled human AB serum, 100 units/mL penicillin (Sigma Chemical), 100 μg/mL streptomycin (Sigma Chemical), and 2 mmol/L glutamine (Sigma Chemical). For each group (samples and controls) 8 wells of the microplates were used.

Test material
Two commercially available DBAs, Scotch Bond (Single Bond) (3M Dental Products, St. Paul, Minn) and Prime & Bond (Dentsply International, Milford, Del), were selected for this study. The material samples were placed on sterile plastic discs (6 mm diameter) with a dispenser and light cured (Coltolux 75, Coltene/Whaledent, Mahwah, NJ) (power 400 W). To protect from the effect of oxygen, the surface of the agent was covered with glycerin and cured again for 20 seconds. Then the discs were washed in sterile deionized water. The control discs were used without DBA. Preliminary experiments showed that there was not any difference between negative (test without disc) and disc control. The prepared discs were transferred to the microplates and incubated for 36 and 72 hours.

MTT assay of monocyte viability
Monocyte MTT viability assay was performed. After 36 and 72 hours, 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl-2-tetrazolium bromide (MTT) (5 mg/mL) was added to the test wells and incubated at 37°C for 4 hours in 5% CO₂. Four hours after addition of isopropanol and shaking for 30 minutes, the resulting crystalline formazone deposits were dissolved. Optical density was measured at 570 nm by spectrophotometer analysis and viability was calculated as sample optical density divided by control optical density.

The results were analyzed with ANOVA (confidence level 95%) and Bonferroni post hoc tests.

RESULTS
Both Prime & Bond and Scotch bond in polymerized form caused significant decreases in viability of cultured monocytes in 36 and 72 hours (P < .001) (Fig 1). The effect of Scotch bond on viability of monocytes was greater than that of Prime & Bond, but this difference was not statistically significant. Analysis of data showed that the viability of cells decreased with the time of exposure. The major decrease in viability, however, occurred during the first 36 hours and there was no significant change in viability after 72 hours.

DISCUSSION
In the current study the effect of polymerized form of 2 dentin bonding agents on human monocytes was investigated. The time periods were selected based on an earlier study, which reported that monomers are released during the first 6 days after DBA polymerization and the main part of release occurs after 24 hour.²

Our results showed that the viability of monocytes was reduced during exposure times. This finding is in agreement with other results that showed the cytotoxic effect of these materials on different cells such as fibroblasts and monocytes.¹,⁵,⁶ However, most experiments regarding the effects of these materials have been performed with monomeric constituents of the agents. Different concentrations of Bis-GMA, HEMA, 4-META, and UDMA monomers can suppress cellular metabolic activities on the macrophage cell line (THP-1).⁶ The major reduction of cell viability in our study was observed after 36 hours exposure and this may indicate that the main part of monomers are released during several hours after polymerization. Scotch bond and Prime & Bond caused 61.5% and 65% decrease in cell viability after 36 hours, respectively. It has been shown that 10⁻³ dilution of Scotch Bond in unpolymerized form caused 100% mortality of the L929 fibroblastic cell line and 10⁻⁸ dilution of this agent caused 70% mortality.¹⁰ Although inhibitory effects of unpolymerized forms of DBA are more prominent than those of polymerized forms, the effect of leachable components of cured DBAs is not negligible.

Sublethal components of HEMA can inhibit the proliferation of macrophages.¹¹ It has also been reported that a 5-week sublethal concentration exposure to TEGDMA and Bis-GMA has significant long-term effects on monocytes in vitro.¹² Sublethal concentration of HEMA and TEGDMA on THP-1 human monocytes can
modulate the heat shock protein stress response without altering cellular metabolic activity.\textsuperscript{13}

In the current study the major part of the toxic effects of polymerized DBAs was seen during the first 36 hours (61.5\%-65\%). Monomers are released in the first 24 hours after polymerization, although further leaching may occur with time.\textsuperscript{2} Long-term residual effect on cell metabolism of some monomer components on macrophages may continue after removal of the material.\textsuperscript{6}

Thus, the sublethal and the residual long-term effects should both be taken into account when judging the impact of DBAs on cells. In some of previous studies monocytic cell lines such as THP-1 cell line have been used extensively. But there are important differences between PBM and THP monocytes.\textsuperscript{14} PBM are 5-10 times less sensitive than the THP-1 monocytic cell line to DBA components (HEMA, Bis-GMA, TDGMA).\textsuperscript{14} Therefore, in the present study human blood monocytes rather than monocytic cell lines were evaluated.

The current study indicates that clinical use of DBAs as root-end filling material must be done with caution. The components of these materials, which are released after polymerization, can alter normal function of macrophages in inflammatory and healing processes. Further studies in vitro and clinical studies are necessary to obtain more clinically relevant information.

REFERENCES

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