The reliability of microleakage studies using dog and bovine primary teeth instead of human primary teeth

ABSTRACT

Aim This was to investigate if dental restorations on human, dog and bovine primary teeth are equatable for microleakage analysis, and the respective marginal adaptation deficiency in in vitro conditions.

Materials and methods In order to determine the level of microleakage in Class V polyacid-modified composite restorations of human, dog and bovine primary teeth, samples were evaluated by dye-leakage method in sections of the inner area of the restorations.

Results We found no leakage in 6 out of 10 (60%) human restorations, in 7 out of 10 (70%) of dog and in 7 out of 10 (70%) of bovine teeth. The levels of dye-microleakage were tested with Kruskal-Wallis one-way variant analysis method. The relative leakage differences were not statistically significant among all species (p>0.05).

Conclusion We concluded that dog and bovine primary teeth might be suitable for in vitro studies instead of human ones.

Keywords Human, Dog, Bovine, Primary teeth; Microleakage.

Introduction

Microleakage may be defined as the passage of bacteria, fluids, molecules or ions between a cavity wall and the restorative material applied to it [Taylor and Lynch, 1992]. With the advances in dentistry, a better understanding of dentin substrate characteristic has provided a significant decrease in marginal leakage of restorations [Pazinatto et al., 2003]. Many methods used for the determination of microleakage have been used to demonstrate that, despite what clinicians may like to think, the margins of restorations allow the active movement of ions and molecules. These techniques include the use of bacteria, compressed air, chemical and radioactive tracers, electrochemical investigations, scanning electron microscopy and perhaps most commonly of all the use of dye penetration studies. Because of certain benefits, the dye-leakage method has been preferred in generally by dentists [David, 1990; Reeves et al., 1995].

Several recent comprehensive reviews of microleakage methods are available. Nearly all microleakage studies suggest that most restorative materials leak. That is, they permit dyes, radioisotopes or bacteria to enter a space between the cavosurface margin of the restoration and the walls of the cavity [David, 1990; Fitchie et al., 1995; Yavuz and Atakul, 1998, 2001, 2000].

Resin-dentin interface sealing is a desirable characteristic of dentin bonding systems, which can be used for preventing the pulp-dentin complex from being exposed to bacteria and their toxins [Ovrebo and Raadal, 1990]. Resin composites still present polymerisation shrinkage and linear thermal expansion coefficient that differ from those of the natural tooth structure. The linear thermal expansion coefficient of a material, thermal and occlusal stresses, and polymerisation shrinkage have been noted as factors influencing microleakage [Nelsen et al., 1952].

Glass ionomer cements, which physical characteristics are constantly improving, can be bounded to mineralised tooth tissue, and also have thermal expansion coefficients similar to tooth tissue. They have been widely used due to their ability to release fluoride. The use of glass ionomer cement in dentistry is mainly as a restorative, luting and lining material [Lopes et al., 2009]. Studies carried out by Hatibovic and Kofman showed increased concentration of fluoride in saliva for as long as 1 year after placement of glass ionomer restorations [Ovrebo and Raadal, 1990]. However, the use of their traditional versions is still subjected to discussion due to their non-resistance to abrasion and sensitivity to humidity.

Newly developed dental adhesive agents proved their validity in in vitro experiments and their usage is recommended [David, 1990; Cooley and Barkmeier, 1991; Kirzioglu and Seven, 1993; Bouschlicher et al., 1996; Kocabalkan, 1993; Fitchie et al., 1995; Yavuz and Atakul, 1998, 2001, 2000; Ovrebo and Raadal, 1990; Nelsen et al., 1952]. For example in some
clinical studies, glass ionomer sealants have shown low retention and correspondingly high microleakage rates compared with resin sealants, and this has limited their use [Lopes et al., 2009; Hatibovic-Kofman and Koch, 1991; Ziskind et al., 1998].

If dental materials sealed dentin as well as enamel or cementum, there would be no measurable microleakage and no clinical problems associated with their use. However, microleakage is a serious clinical problem that requires thorough analysis and discussion because most dental materials exhibit varying degrees of microleakage [Taylor and Lynch, 1992; David, 1990; Cooley and Barkmeier, 1991; Kirzioğlu and Seven, 1993; Bouschlicher et al., 1996; Kocabalkan, 1993; Fitchie et al., 1995]. Extreme microleakage permits the penetration of these materials to the floor of the cavity, through the remaining dentin and to the pulp chamber. In vitro studies of microleakage should be regarded as setting a theoretical maximum amount of leakage that may or may not occur in vivo [David, 1990].

One of the biggest problems of restorative dentistry today is that restorative materials cannot be fully bonded to the enamel. This situation leads to microleakage between restoration and teeth and following this, discoloring of cavity wall, secondary tooth decay, postoperative pain and pulp inflammation occur [David, 1990; Cooley and Barkmeier, 1991; Kirzioğlu and Seven, 1993].

Besides testing the leakage in in vitro restorations, another important problem is the tooth used. Teeth used for these studies should have some standard characteristics like being free of caries and cracks, but it is not always possible to provide all these features in each case [Raadal et al., 1996; Tintoğlu, 1994]. Therefore, teeth of various mammals like cows and dogs are used for in vitro leakage studies. However, there are not enough data concerning which animal teeth exhibit characteristics similar to those of human teeth [Yavuz et al., 1998, 2000, 2001; Holan et al., 1986; Barkmeier, 1992; Zax and Maye, 1991; Saunders, 1988]. The present study was planned to investigate these problems.

**Materials and methods**

For the experimental study, class V cavities were prepared on human, dog, and cow primary teeth. Experimental groups were composed as follows: 10 human primary canine teeth (from 10–11 years old children) without caries and cracks, extracted for various reasons (1st group), 10 dog primary canine teeth (4–6 months old) (2nd group), and 10 primary bovine incisor teeth (7–11 months old) (3rd group). The primary dog canine teeth were obtained for a degree thesis study, while the bovine primary incisors were obtained from Dicle University Faculty of Veterinary Medicine, Department of Anatomy (Fig. 1, 2, 3). Standard class V cavities with mesiodistal length of 3 mm, occluso–cervical width of 2 mm, and depth 1.5 mm were prepared on the bucco-cervical surfaces of the teeth. Samples in each group were restored with paste type polyacid-modified composite resin (F 2000 Compomer, 3M Dental products, USA) with the assistance of 35% orthophosphoric acid and Scotchbond multipurpose adhesive agent (3M Dental Products, France). Polishing of restorations was completed in the same session with Sof-Lex discs (Countouring and Polishing Discs, 3M Dental products, USA).

All teeth underwent thermocycling between 4±2 OC and 60 OC ± 2 for 500 cycles. The dwell times in each bath and the time interval at room temperature between baths were 1 min. After thermocycling, the surface of the teeth, except the restorations and approximately 1.5 mm beyond the margins, were coated with a layer of nail varnish. The coated teeth were immersed in 2% basic fuchsin solution for 24 hrs, to allow dye penetration into any possible existing gaps between the tooth substance and the restorative material. Then, the samples were washed with ordinary water and the nail polish was stripped [Raadal et al., 1996].

All samples were sectioned with low speed diamond discs in the middle of the restoration and were prepared for dye-leakage inspections under water cooling [Kocabalkan, 1993; Saunders, 1988]. Three buccolingual sections were obtained by grinding off the teeth buccolingually parallel to their axes. As each section was exposed, it was polished under running water and examined using a binocular microscope at 25 X magnification (Olympus, Tokyo, Japan) [Taylor and Lynch, 1992; David, 1990; Kirzioğlu and Seven, 1993; Yavuz and Atakul, 2001; Yavuz and Atakul, 2000; Mclean and Wilson, 1974; McKenna and Grundy, 1987; Holan et al., 1986; Zax et al., 1991].

The depth of the dye penetration was evaluated separately for the occlusal and the cervical areas. Scores were assigned to each individual sample in accordance with the following scale:

0 no leakage;
1 leakage in less than 1/3 of the cavity wall;
2 leakage between 1/3 and 2/3 of the cavity wall;
3 leakage in the entire cavity wall;
4 leakage in the cavity base;
5 leakage in all edges of the cavity and leakage from the cavity base toward the pulp, partly or wholly.

Statistical analysis was conducted with SYSTAT statistical package programmes [Fitchie et al., 1995]. Since the variables have been expressed by fractional variables (absolute numbers), and mean and standard deviation are not used, median value has been used as average parameter. The three groups were compared using the Kruskal Wallis non-parametric test.
Results

Microleakage scores and statistical findings (averages) of three groups are shown in Table 1.

As a result, there were no leakage (score 0) in 6 teeth out of 10 (70%) in human, in 7 out of 10 (70%) in dog and bovine teeth. These scores indicate that leakage was found in 4 teeth out of 10 (40%) in human, in 3 out of 10 both in dog and bovine teeth (30%).

The difference in dye leakage values between the three groups, were not statistically significantly (p>0.05). At the end of the analysis, median values of all groups were “0”; and according to these results, there were no significant differences between the groups.

The results were the same for the in vitro process in all species. Photographs of dye leakage in group 1 (human), 2 (dog) and 3 (bovine) are shown in figures 4, 5, 6 respectively.

Discussion

For experimental in vitro microleakage studies, teeth without caries and cracks and similar characteristics should be used, but it is hard to provide this standard every time. Therefore, many studies assessed the utility of animal teeth instead of human teeth in microleakage studies [Reeves et al., 1995; Fitchie et al., 1995; Zax et al., 1991; Tjan and Tan, 1991]. In our study, primary...
Microleakage studies: bovine and dog primary teeth vs. human ones

Incisors of 7-11 months old bovines, primary canines of 4-6 months old dogs and primary canines of 10-11 years old children were used to provide similar and standard characteristics among teeth. This study was planned to examine the feasibility of use of dog and bovine teeth instead of human teeth in in vitro microleakage studies.

In the study of Almeida et al. [2009] it was found that there were statistically significant differences: the highest leakages were found for adhesives placed on both human and bovine teeth. However, no statistically significant differences were detected between the human and bovine substrates for the tested adhesive systems (Prime & Bond 2.1 - \( p = 0.6923 \) and Adhese - \( p = 0.6109 \)).

Various techniques, like dye penetration, radioisotope technique, chemical agents (markers), bacterial studies, pressurised air use, SEM (Scanning Electron Microscopy), neutron activation analysis, thermal cycling were used. For in vitro microleakage studies, the dye leakage technique has been mostly preferred [McLean and Wilson, 1974; Hotta et al., 1991; Yavuz and Aydin, 2011], and therefore for this study we used the same technique.

In in vitro studies another important problem is the limitation of mechanical and thermal stresses arising in the mouth on teeth and filling materials. This problem has been reported for the first time by Nelsen et al. [1952], who placed restored teeth into ice water, then at room temperature and observed small water drops between fillings and cavity walls. Thermal cycling, which we applied in our study, is based on the same logic [Taylor and Lynch, 1992; Bouschlicher et al., 1996; Tjan and Tan, 1991; Altay and Usmen, 1993].

The glass ionomer cement (GIC) was developed by Wilson and Kent in 1972, and it is widely used in dentistry [Barkmeier and Cooley, 1992]. It is suggested to use GIC materials for their ability to release fluoride, and for the low coefficient of polymerisation contraction. In this study, cavities were restored with GIC and paste-type polyacid-modified composite resin (F 2000 Compomer, 3M Dental products USA) [Bulucu et al., 1995; Manhart et al., 2001]. Studies reported that GIC and multipurpose Scotchbond and other adhesive agents can reduce microleakage, especially in class V cavities [Bulucu et al., 1995; Manhart et al., 2001]. The low microleakage levels obtained in this study seem to confirm that.

According to the results obtained in this study, dog and bovine primary teeth exhibit similar characteristics for in vitro microleakage studies and can be used instead of human teeth. Moreover, it has been seen that the use of polyacid-modified composite resin and multipurpose Scotchbond adhesive agent together on primary tooth decreases microleakage.

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