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ORIGINAL ARTICLE

Levels of mutans streptococci and lactobacilli in plaque on aged restorations of an ion-releasing and a universal hybrid composite resin

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Abstract
The purpose of this *in vivo* study was to evaluate the cariogenic microflora of plaque on aged restorations of a hydroxyl, fluoride, and calcium ion-releasing composite resin (IRCR) (Ariston pHc), and to compare it intra-individually with a universal hybrid composite resin and enamel. Each of 19 subjects received one proximal restoration of the IRCR, one proximal universal hybrid composite resin restoration (CR) and each subject had one non-filled proximal enamel control surface to make intra-individual comparisons possible. To avoid peak ion releases from the materials, aged restorations were studied. Plaque was collected from 57 surfaces using sterile applicator tips. Samples were cultured to determine the numbers of mutans streptococci, lactobacilli, and total microorganisms. The relative numbers for mutans streptococci (% of total bacteria) were: IRCR 0.59%, CR 0.40%, enamel 0.22%. Two outliers were found in the IRCR group. Excluding these outliers resulted in a relative number of 0.33%. Lactobacilli were detected in the plaque from only 9 surfaces and at very low relative proportions for all three surfaces: 0.01%. The enamel surfaces showed the lowest relative numbers of mutans streptococci and lactobacilli, but the differences were not significant. It can be concluded that the ion release of the IRCR did not influence the growth of cariogenic microorganisms in dental plaque.

Key Words: *Antibacterial activity, caries, clinical, dental materials, microorganisms*

Introduction

Tooth decay is perhaps the most common and expensive bacterial disease in humans. Caries, secondary as well as primary, occurs due to disturbance of the demineralization–remineralization balance after frequent intake of fermentable carbohydrates, which are metabolized by cariogenic bacteria into organic acids – resulting in a plaque pH fall [1–3]. The salivary buffer systems protectively influence the pH drop and time of acidity [4]. Secondary caries is the main reason for replacement or repair of restorative materials reported in cross-sectional studies and today make up an important part of restorative dental treatment [5–7].

The use of posterior composite resin restorations has increased during recent years owing to a growing demand for esthetics and concern about the biocompatibility of amalgam [8–11]. The main disadvantage associated with composite resins is their shrinkage during conversion of the monomer molecules into a polymer network. The resulting high shrinkage stress may cause debonding from the cavity walls and increase the risk for microleakage [12–14].

Since the introduction of silicate cements, fluoride release from restorative materials has been advocated to prevent secondary caries and to enhance the rate of remineralization. When fluoride ions are released from dental materials, they result in precipitation of CaF_2 crystals which keep the liquid phase in and around the restoration-tooth surface saturated before a pH drop [15]. This mechanism reduces the degree of demineralization and accelerates the process of remineralization [16]. Fluoride ions can also inhibit the growth of oral bacteria or interfere with bacterial acid production and acidurance [17–19]. Several investigations have demonstrated that fluoride impairs the growth of mutans streptococci by inhibiting acid production and electrolyte metabolism within these bacteria [20,21]. Conventional and resin-modified glass ionomers are the main ion-releasing materials used today. The amount of fluoride released is high during the first day after setting of the material, but declines fairly rapidly

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over the next weeks to finally stabilize at a lower level. The release may increase under acidic conditions and by hydrolysis in saliva [22,23]. Glass ionomer cements inhibited the growth of mutans streptococci *in vitro*, which have been explained by their fluoride release [24–26]. Two studies *in vivo* showed that glass ionomer cement reduced the levels of mutans streptococci in human plaque samples [27,28]. However, the distribution of mutans streptococci and lactobacilli did not differ significantly among the surfaces of aged resin modified glass ionomer cement, compomer, and composite resin restorations [29].

Recently, a new posterior ion-releasing composite resin (IRCR) was developed which contains, apart from conventional glass particles, additional alkaline (calcium silicate) glass fillers. It was suggested that secondary caries formation could be inhibited by the pH-dependent release of fluoride, calcium, and hydroxyl ions from the new type of alkaline filler [30]. After contact with water, hydroxyl ions are formed on the surface of the material. Hydroxyl ions may neutralize all or part of the organic acids produced by the cariogenic bacteria. An *in vitro* study showed that these ions were still being released after 2 years [30]. A buffering effect of the IRCR was recently shown *in vivo* with the micro-touch method [31]. After rinsing with a sucrose solution, the proximal plaque pH on IRCR was more stable than the proximal pH on non-filled or hybrid composite resin surfaces. The total fluoride release from the IRCR has been shown to reach almost double that from glass ionomer cements [32,33]. It has been speculated that the pH-dependent release of fluoride and hydroxyl ions from the IRCR may provide a caries-protective effect by both prevention of enamel demineralization and inhibition of bacterial growth [30,34].

The hypothesis to be tested was that the plaque on the IRCR harboured lower numbers of mutans streptococci and lactobacilli than plaque on a universal hybrid composite resin. The aim of this study was to study the cariogenic microflora in plaque of aged restorations of the IRCR and to compare it intra-individually with a universal hybrid composite resin and non-filled enamel surfaces.

Material and methods

Subjects

Individuals with proximal posterior restorations of the IRCR material (Ariston pHc; Vivadent, Schaan, Liechtenstein), who also participated in a longitudinal follow-up of the material [35], were asked to participate in the present study. Nineteen adults (13 M, 6 F; mean age 64, range 49–86) attending the clinic at the Dental School, University of Umeå, Sweden participated. All participants had received one proximal restoration of the IRCR material and one proximal universal composite resin restoration (CR). The Class II restorations had subgingival located cervical margins and approximately the same size and surface area. A similarly located restoration-free enamel control surface in the same jaw as the IRCR and CR restorations was identified in each patient in order to make an intra-individual comparison possible. The IRCR was applied in accordance with the instructions of the manufacturer as previously described [35]; consequently the cavity was not conditioned with an acid. A resinous dentin liner (Ariston Liner) was applied for 20 s, air-thinned and light-cured for 20 s. The liner contained a methacrylate modified polyacrylic acid, HEMA, ethanol, and maleic acid. The IRCR contains Bis-GMA, urethane dimethacrylate and dimethacrylate monomers. Filler types included are ytterbium trifluoride, Ba-Al-fluorosilicate glass, highly dispersed silica and 48 wt% silanized alkaline glass filler. To avoid peak ion releases from the materials, only aged restorations were studied, with a mean age of 30 months (range 13–59) [35]. Prior to sampling, the participants refrained from oral hygiene for 3 days. The ethics committee of the University of Umeå approved the study.

Bacteriological procedures

At day 4, supragingival plaque was collected from each subject from the cervical area of the two proximal restorations and the non-filled enamel surface. Plaque was collected using the tip of a sterile applicator tip (Applicator Tips; Dentsply/De Trey, Konstanz, Germany). After use, each applicator tip was immersed in a 0.5 ml salt buffer (4.3 g NaCl, 0.42 g KCl, 1.0 g $Na_2PO_4 \times 2H_2O$, 1.0 g KH_2PO_4, sodium glycerophosphate, and 0.1 g $MgCl_2 \times 6H_2O$ per liter; pH 7.2) and transported within 1 h to the laboratory. To obtain homogeneous suspensions, each sample was treated by pulsed ultrasonic oscillation (ten 1-s pulses) (Sonifer B-30; Branson Ultrasonic Corp., Danbury, Conn., USA). The suspensions were then diluted serially in the salt buffer. To determine the total number of bacteria (total bacteria), aliquots of the samples were cultured on blood agar plates. Mitis salivararius agar (Difco; Becton, Dickinson and Company, Sparks, Md., USA) supplemented with bacitracin [36] and Rogosa selective lactobacilli agar (Merck, Darmstadt, Germany) were used to estimate the numbers of mutans streptococci and lactobacilli, respectively. All plates were incubated in 5% CO_2 and 95% air at 37°C for 2 days. Subsequently, the numbers of bacteria on the plates were counted as colony forming units (CFU) and the relative numbers (% of total bacteria) of mutans streptococci and lactobacilli were calculated.

Statistical analysis

The data were processed in SPSS (Statistical Package for the Social Sciences, version 10.0; Chicago, Ill., USA). Frequency distributions of the total bacteria and relative numbers of mutans streptococci and lactobacilli, respectively, were described. The relative frequencies of cariogenic microorganisms, in relation to the total number of microrganisms on the three surfaces were ranked intra-individually and the rank sums for the different surfaces were compared. The data were analyzed with Kolmogorov-Smirnov goodness-of-fit tests for normality. Wilcoxon's signed-rank test was used for the intra-individual comparisons. P-values < 0.05 were considered statistically significant.

Results

Nineteen subjects participated in this study. Plaque samples were collected from 57 surfaces (3×19). The amount of total bacteria recovered from the three surfaces and the relative numbers of mutans streptococci and lactobacilli, respectively, are given in Table 1. The total numbers of bacteria in the plaque samples varied between 4×10^5 and 5.7×10^8. No significant differences were observed between the three surfaces. Mutans streptococci were detected on all surfaces. The relative numbers of mutans streptococci bacteria were: IRCR 0.59%, CR 0.40%, and enamel 0.22%. No significant differences were found in the relative numbers of mutans streptococci among the different surfaces. IRCR showed two outlier surfaces with high mutans streptococci (relative numbers 17% and 24%). Excluding the outliers resulted in a relative number of 0.33%. Lactobacilli were detected in the plaque from only nine surfaces and at very low proportions. The relative numbers were <0.01% for all three surfaces. The total numbers of bacteria from the restored surfaces were not different from those from enamel.

Discussion

Bacterial adhesion to dental restorative materials is an important component in the etiology of secondary caries formation [37]. Growth-inhibitory effects of dental materials are considered to be beneficial in preventing bacterial colonization of marginal gaps [12]. In several studies, an antibacterial effect of different restorative materials has been shown, and an inhibition of the growth of bacteria contiguous with the restorative material has been observed. Dental restorative materials containing zinc-oxide eugenol [34] and copper ions have shown an inhibiting effect on mutans streptocci growth [38]. The antibacterial potential of glass ionomer cements has been shown in several *in vitro* studies [24–27]. Growth inhibition of mutans streptococci on glass cermet restorations was reported *in vivo* by Svanberg et al. [28]. The caries-preventive mechanisms of fluoride include increased resistance of tooth substance to demineralization, promotion of remineralization and antibacterial effects [39]. The growth inhibitory effect of the glass ionomer cements has been attributed to the significantly high fluoride release of the cements, with the highest release during the first 24 h. However, the initial "burst" release drops substantially within the first few weeks to a relatively low level. The release can increase significantly following refluoridation for 2 days but drops quickly and stabilizes after 3 days [33]. Growth inhibition can probably only be obtained with high concentrations in the order of 0.16–0.3 mol/l, which are higher than those usually found in dental plaque [17]. *In vivo* studies of 1 and 3-year-old glass ionomer cement restorations have shown that the fluoride content in plaque on these aged restorations is not significantly higher than that on composite resins or non-filled enamel surfaces [29,40]. Moreover, the presence of mutans streptococci and lactobacilli on these surfaces does not differ significantly.

It was concluded in a recent study that a sucrose rinse induces a lower pH fall in plaque on IRCR than in plaque on a universal hybrid composite resin and non-filled enamel surfaces [31]. This caries-preventive effect may have been obtained by the pH-dependent fluoride ion release from the material, i.e. resulting in a growth inhibitory effect, or by the neutralizing effect of the released hydroxyl ions. The IRCR showed the highest cumulative fluoride release among materials, including several conventional and resin-modified glass ionomer cements [32]. In a novel assay, where bacterial suspensions were placed in narrow 20 μl conical cavities within ion-releasing materials, Boeckh et al. [34] showed that the ion release might have contributed to a reduction in the number of living bacteria, at least with respect to S mutans. After up to 1 week of incubation, the suspensions were removed from the restorations and the number of viable bacteria was determined. However, in a liquid culture assay, where eluates of the IRCR material were incubated with suspensions of mutans streptococci, they failed to show any significant antibacterial activity.

In the present study, no significant differences were observed in the relative numbers of mutans streptococci and lactobacilli between aged restorations of the IRCR, the non-ion releasing universal hybrid composite resin, and the non-filled enamel surfaces. The hypothesis was therefore not accepted. The relative numbers of mutans streptococci were highest on the

Table I. The median (range) of the relative numbers of mutans streptococci and lactobacilli (% of total bacteria) on the three surfaces. Enamel, IRCR (ion-releasing composite resin), CR (composite resin)

	Enamel ($n = 19$)	IRCR ($n = 19$)	CR ($n = 19$)
Total bacteria $\times 10^8$	0.92 (0.09–5.7)	1.12 (0.19–4.9)	1.33 (0.004–3.8)
Mutans streptococci (% of total bacteria)	0.22 (0.00–2.10)	0.59 (0.00–23.6)	0.40 (0.00–4.95)
Lactobacilli (% of total bacteria)	<0.01 (0.00–0.055)	<0.01 (0.00–0.11)	<0.01 (0.00–3.11)

IRCR, but when we excluded outliers of these surfaces the proportions were equal to the CR restorations. Mutans streptococci were presented on all surfaces, while lactobacilli were observed in low relative numbers and in only half of the restorations, which concurs with earlier *in vivo* plaque studies of resin composite materials [29,40].

The intra-individual comparison of the relative numbers of mutans streptococci and lactobacilli showed that the ion-release of the aged IRCR restorations was too low to inhibit their growth. It can be assumed, as for most dental materials, that the release of fluoride ions in aged restorations has decreased to such a low level that it has no growth inhibitory properties [29,40]. The recently observed pH neutralizing effect of the restorative material is therefore probably caused by the pH-dependent release of the hydroxyl ions [31]. Its effect may be reinforced by an interference of the lower fluoride release from the composite resin on acid production. It has been shown earlier that lower fluoride concentrations (approximately 1 mmol/l) may interfere with bacterial acid production and aciduance [18,19].

It can be concluded that this intra-individual comparison did not show statistically different numbers of bacteria recovered from the aged surfaces of the ion-releasing composite resin compared to universal composite resin and non-filled enamel surfaces.

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