The effect of nanoparticles on the translucency and antimicrobial activities of experimental resin cements

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ABSTRACT

The purpose of this study was to evaluate the effect of the nanoparticles in experimental resin cements on opacity (contrast ratio) and antibacterial activities. Experimental resin cements were prepared with silica microfiller, silica nanofiller and silver nanoparticles. The opacity and the distribution morphology of the filler particles in the resin matrix were evaluated. Surface characteristics (roughness and free energy) were analyzed and shear bond strength were also estimated. The experimental resin cements with more than 3% nanofiller had significantly lower opacity. The resin cements with 6 wt% nanofiller had opacity 50% lower than the resin cements without nanofiller. The experimental resin cements with 250 ppm silver nano-particles showed significantly slower bacterial growth than the resin cements without silver nanoparticles. This study suggested that experimental resin cements with silver nanoparticles can reduce the demineralization without compromising esthetical and physical properties.

Keywords: nanofiller, silver nanoparticles, resin cement, translucency, antimicrobial activity

1. INTRODUCTION

Dental resin composites and cements continue to evolve with the development of new filler particle systems; better bonding systems, curing refinements, and sealing systems; and increasing focus on the maintenance of existing restorations. Nanocomposites are a new class of materials in which nanoscale particles are finely dispersed within the resin matrixes [1]. In comparison with conventional composites, nanocomposites exhibit markedly improved mechanical properties, chemical and thermal resistance, and optical transparency [2-8]. Among the many characteristics of nano-materials, superior aesthetic properties due to excellent translucency were the most important advantage [8,9,10]. These improvements can be achieved at low concentrations of nanoparticles (1–10 wt.%); in this context, the nanocomposites are more easily processed [4].

Patient demand for esthetic and metal-free restorations has increased recently, and this has resulted in frequent clinical use of all-ceramic restorations. Current dental ceramic materials offer preferred optical properties for highly esthetic restorations. However, the opacity of resin cement may reduce the translucency of restorations, which would be a concern during the cementation procedure of esthetic restorations. To ensure a successful esthetic outcome, the adhesive system would need to be tooth colored reflecting and transmitting light in a manner similar to a natural tooth [10].

The light transmittance characteristic, one of the optical properties of the resin cement, may play an important role in the aesthetic properties of the dental esthetic restorations. Incident light, which transmits through the material with scattering, reflects on the background and the surrounding wall and again transmits through the material to reach the eye of the observer. Therefore, it may be important to understand the light transmission characteristics of the resin cement to consider aesthetic color production. Although many studies on the translucency of microhybrid and hybrid resin composites have been reported there are few studies on the dental resin cements.

Demineralization is a commonly recognized complication of dental treatment with resin cements for an esthetical restoration and fixed prostheses. Preventing these demineralizations is an important concern for esthetic treatments because the demineralizations are unaesthetic, unhealthy and potentially irreversible. The placement of artificial biomaterials creates a favorable environment for the accumulation of microorganisms, which causes tooth structure demineralization or exacerbates the effects of any pre-existing caries. A previous study reported that the resin cements on the tooth structure interface provide sites for the rapid attachment and growth of oral microorganisms due to their rough surfaces [11].

Silver has a long history of use in medicine as an antimicrobial agent [12]. Resin composites containing silver ion-implanted fillers that release silver ions have been found to have antibacterial effects on oral streptococci [13,14]. Several studies have demonstrated that silver ions are selectively toxic for prokaryotic microorganisms with little effect on eukaryotic cells [15,16].

The purpose of this study was to evaluate the effect of the silver nanoparticles in experimental resin cements on opacity (contrast ratio) and antibacterial activities.

2. MATERIALS AND METHODS

The light-cure experimental resin cements were formulated with 29.65 wt.% of Bis-GMA (Bisco, USA), 29.65 wt.% of 2,2-bis[4-(2-hydroxy-3-methacryloyloxy-propoxy)phenyl]-propane (Esstech, USA), 9.75 wt.% of triethylene glycol dimethacrylate (Esstech), 4.90 wt.% of mono-2(methacryloyloxy)ethyl phthalate (Sigma, USA), 5.0 wt.% of 4-META, 0.7 wt.% of di-camphorquinone (Sigma), 0.35 wt.% of 2-(dimethylamino)ethyl methacrylate (Sigma), and uniformly dispersed with three different kinds of fillers: silica microfiller (0.04 μm), silica nanofiller (< 7 nm), and silver nanoparticles (<5 nm). To increase antimicrobial activities, silver nanoparticles (Miji Tech Co, Korea) were added to the resin cements (250 ppm).

Table 1. Experimental resin cements tested in this study

Experimental resin cements	Fillers (wt%)		
	Silica microfiller (0.04 μm)	Silica nanofiller (7 nm)	Silver nanoparticles (< 5 nm)
EXP-1	19	1	0 ppm
EXP-2	17	3	0 ppm
EXP-3	14	6	0 ppm
EXP-4	11	9	0 ppm
EXP-5	11	9	250 ppm

Disk-type (10.0 × 2.0 mm) specimens were used for surface characterization and optical properties. Surface roughness was analyzed using confocal laser scanning microscopy (Axiovert 200M; Carl Zeiss, USA). Surface free energy was measured using a video camera equipped with an image analyzer (Phoenix 300, Surface electro optics, Seoul, Korea) visualized the shape of the drop and gave the contact angle. The opacity (contrast ratio) of specimen was determined as the ratio of reflectance when the specimen is placed over black and white backgrounds using a reflection spectrophotometer (CM-3500d, Minolta, Japan) with SCE (specular component exclusive) geometry. Reflectivity was measured at 20 nm intervals between 400 and 700 nm. Each experiment was repeated five times for three specimens of each material.

Fifty extracted, healthy (without caries and restoration-free) human premolars were cleaned and stored in a 1% aqueous solution of chloramines-T at 4 ^{o}C until further use. The premolars were embedded individually in an acrylic mold and the teeth were randomly assigned to one of five groups. The teeth were prepared for bonding by exposing dentinal surface. Zirconia cores were bonded using experimental resin cements to dentin and the light cured for 20 sec. After core bonding, the specimens were stored in deionized water for 24h at 37 ^{o}C prior to the shear bond strength test. A universal testing machine (Instron 4465, USA) with a crosshead speed of 1 mm/min was used.

Adhesion of cariogenic streptococci to experimental resin cements was performed. *Streptococcus mutans* ATCC 25175 and *Streptococcus sobrinus* ATCC 33478 were used. Disk-type (3.0 × 2.0 mm) specimens were prepared for adhesion assays. Fifteen specimens of each group were incubated in 2 mL of UWS with agitation for 2h at room temperature. For negative control tests, the same procedure was performed with sterile PBS (phosphate-buffered saline). After washing three times in sterile PBS, specimens were incubated with 1×10^{9} tritium labeled bacteria in 2 mL of HBSS containing 0.5% bovine serum albumin (HBSS–BSA) under agitation for either 3 or 6h at 37 ^{o}C. The number of adherent cells was determined using a Beckman LS-5000TA liquid scintillation counter (Beckman Instruments, USA). The radioactive counts were divided by the total counts per minute of the bacterial suspension solution. All test samples were counted in triplicate and each experiment was repeated six times.

The disk diffusion test was designed to test the composites containing an antimicrobial agent that could readily diffuse through agar to produce an inhibition zone. Disk-type (10.0 × 2.0 mm) specimens were used. The test was performed on *mitis salivarius* agar plates. Each plate was spread on the top of the plate with 200 μL of freshly grown cariogenic streptococci (about 2×10^{8} bacteria). Ten 5-mm diameter holes were punched in the agar surface of each plate, and the respective adhesive was introduced and polymerized immediately. The plates were incubated at 37 ^{o}C for 48h and then visually inspected for the presence of inhibition zones in the bacterial lawn. The bacterial inhibition zone halo was measured and expressed in millimeters. The tests were assayed at least three times.

3. RESULTS AND DISCUSSION

Table 2 summarized the results of surface roughness, surface energy, and shear bond strength. There were no significant differences in surface roughness and surface free energy among the experimental resin cements ($p>0.05$). There was no significant difference in the shear bond strength ($p>0.05$), nor in the debond pattern between the resin cements and tooth surface. Results demonstrate that experimental resin cements have a clinically relevant range of physical properties.

Although mechanical properties of a methyl methacrylate based polymer incorporating antibacterial agents could be changed due to the disruption of the physical form of the polymer [17], incorporation of silver nanoparticles seemed to have minimal effect on shear bond strength. This suggests that incorporation of silver nanoparticles may not have an adverse effect on physical properties of experimental resin cements if proper amount of silver nanoparticles is used.

Table 2. Surface characteristics and shear bond strength data of experimental resin cements

Groups	Surface roughness (μm)	Surface energy (mJ/m)	Shear bond strength (MPa)
EXP-1	0.40±0.08	39.2±2.45	23.4±3.25
EXP-2	0.38±0.07	42.7±3.27	21.8±4.01
EXP-3	0.42±0.04	40.5±4.12	22.4±2.99
EXP-4	0.43±0.06	41.6±1.89	20.4±3.07
EXP-5	0.44±0.09	43.1±2.07	21.1±3.89

Table 3. The opacity (contrast ratio) of experimental resin cements at 400 nm as function of aging period.

Groups	0-day	7-days	28-day
EXP-1	0.64±0.03	0.63±0.04	0.63±0.03
EXP-2	0.57±0.02	0.56±0.04	0.56±0.04
EXP-3	0.51±0.03	0.50±0.04	0.49±0.04
EXP-4	0.44±0.03	0.46±0.03	0.45±0.03
EXP-5	0.72±0.04	0.70±0.05	0.70±0.04

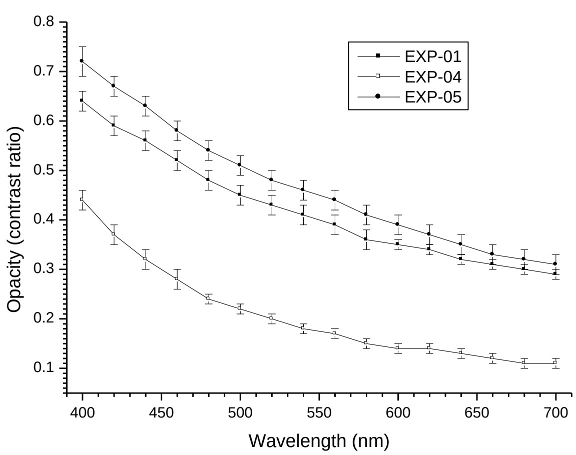

Fig. 1. Effect of naofiller and silver nanoparticles on the opacity (contrast ratio) of experimental resin cements.

EXP-04 (with 11 wt. % microfiller and 9 wt. % nanaofiller) exhibited the lowest contrast ratio. EXP-01 (with 11 wt. % microfiller and 9 wt. % nanaofiller) showed a higher contrast ratio than EXP-04. EXP-05 (with 11 wt. % microfiller, 9 wt. % nanaofiller, and 250 ppm silver nanoparticles) exhibited the highest contrast ratio. In comparison with EXP-01 and EXP-04, an increase in the nanofiller fraction from 1% to 9% resulted in about 20% decrease in the contrast ratios.

The main advantage of nanocomposites is the superior translucency. There are several factors that can influence the translucency of dental resin composite materials. First, the translucency of the component itself of composite materials is important. Dental zinc oxide phosphate cements are nearly 100% opaque because of the opacity of the zinc oxide powder. Glass-ionomer cements with improved aesthetics have translucent characteristics because of the translucent or opalescent glass powder. Thus, the translucency of components plays important role in the translucency of materials. Second, although the filler and matrix of the composite exhibit excellent translucency, the filler will increase light scattering in the resin-filler interface and produce opaque materials if the filler and matrix have mismatched refractive indices. The opacity of the resin composite increases as the refractive index difference between the filler particles and the resin matrix increases. Third, the translucency of the resin composite depends on the sizes of filler particles. When a particle shrinks to a fraction of the wavelength of visible light (400–800 nm), it will not scatter that particular light. In contrast, if the size of the particles is far below the wavelength of light, it will not scatter or absorb the light, resulting in the human eye's inability to detect the particles. Thus, dental resin composites with tiny nanoparticles produce superior translucency and deliver optimal aesthetics [18]. In comparison with EXP-05 and EXP-01, an addition of 250 ppm silver nanoparticles in the resin cements resulted in about 10% decrease in the contrast ratios. But, it could be acceptable to keep proper esthetic properties of dental ceramic restorations.

The amount of cariogenic streptococci adhesion varied according to the experimental resin cements. After 48h incubation with each adhesive disk, no inhibition zones were observed around any of the specimens. The bacterial suspension containing EXP-5 group (resin cement with silver nanoparticles) showed significantly slower bacterial growth than those without silver nano-particles. Although the detailed mechanism of the antimicrobial effect of silver has not been determined, it has been suggested that oxygen is changed into active oxygen (including hydroxyl radicals) by the action of light energy in the air or water as a result of catalytic action of silver, because silver ion can facilitate electron displacement from a molecule. The active oxygen causes structural damage in bacteria, which is called an oligodynamic action [12]. As a result, silver ion can denature proteins and enzymes of bacteria by binding to reactive groups resulting in their inactivation. Because the antimicrobial effect was maintained after saliva coating, the silver was able to penetrate the saliva coating. These results have obvious clinical implications. The surfaces including silver nano-particles rapidly become coated with salivary proteins in the oral cavity and organic-film coating has been cited as one of the main reasons for the clinical failure of silver-coated medical devices [16].

Possible disadvantage of resin cements with silver nanoparticles is the color, which can produce a potential limitation, especially when aesthetic restorations are used. However, finding the appropriate blending proportion of silver nanoparticles (≥250 ppm) to resin cements might solve the color problems. Further studies will be necessary to improve the color problem of the resin cements and to investigate long-term antimicrobial activities and physical properties of the experimental adhesive system.

4. CONCLUSION

The ideal dental resin cements should not only optimize esthetic characteristics of restorations, but at the same time provide sufficient bonding strength and less bacterial adhesion. Though the quality and ease of handling of resin cements have improved greatly, relatively little attention has been directed toward increasing their antimicrobial properties. This study shows that incorporation of silver nanoparticles into resin cements was successful on both esthetical and antimicrobial level. The inhibition of bacterial adhesion and/or growth on the resin cement surfaces may be effective in preventing demineralization around restorations. This study suggests that experimental resin cements containing silver nanoparticles and nanofillers can contribute to preventing demineralization around restorations without significant effects on the physical and esthetical properties.

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