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Effect of Polymeric Microparticles Loaded With Catechin on the Physicochemical Properties of an Adhesive System

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Clinical Relevance

Adhesive systems doped with epigalocatechin-3-gallate, directly or in poly(D-L lactide-co-glycolide) acid microparticles, do not have a detrimental effect on restorative procedures.

SUMMARY

The objective of this study was to synthesize and characterize epigallocatechin-3-gallate (EGCG)–loaded/poly(D-L lactide-co-glycolide) acid (PLGA) microparticles, evaluate their effects on degree of conversion and release assay of adhesives, and subsequently to examine the resin-dentin bond strength of two EGCG formulations (free EGCG or loaded into PLGA microparticles) applied as a pretreatment or incorporated into an adhesive system. The formulations were prepared according to a PLGA:EGCG ratio of 16:1 using the spray-drying technique. The size and polydispersity index were determined by light scattering in aqueous dispersion. The degree of conversion (%DC) and release assay were assessed by Fourier transform infrared spectroscopy and ultraviolet-visible spectrophotometer, respectively. Subsequently, 45 third molars were divided into five groups (n=9) according to the different EGCG application modes and prepared for bond strength testing in a universal testing machine. Results demonstrated no statistically significant difference among the DC means after the PLGA microparticles were loaded with EGCG. For the release assay, the 1.0% PLGA/EGCG group presented better results after being elected for use in the bond strength test. The resin-dentin bond strengths of the experimental groups after 12 months of

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water storage were significantly higher than in the control group. EGCG could improve the durability of the resin-dentin bond over time and promote a new era for adhesive dentistry with the concept of controlled release.

INTRODUCTION

Studies have shown that adhesive systems lose their bond to dentin over time; plus, there is a consensus that the hybrid layer created by current adhesive systems is imperfect, susceptible to degradation, and could negatively affect bond strength.[1] This decrease in bond strength is related to a hydrolytic degradation of polymers of the adhesive systems and proteolysis of the collagen matrix of the hybrid layer.[2] Host-derived proteases, matrix metalloproteinases (MMPs), and cysteine cathepsins, with collagenolytic activity in the hybrid layers, are primarily responsible for degradation of the collagen fibrils, thus reducing the longevity of clinically applied resin-based restorations.[1,3]

Therefore, studies have focused on the modification of dental adhesives to improve the durability of bonding to dentin of resin-based restorations.[1] The use of protease inhibitors on the dentin surface after acid etching or their incorporation into the adhesive system has been well accepted.[4-7] Chlorhexidine (CHX) was the first protease inhibitor proposed for preserving the hybrid layer through the inhibition of MMPs[8] and cysteine cathepsins[9]; however, recently, researchers have given increased attention to other inhibitors.

Epigallocatechin-3-gallate (EGCG) is a natural substance and is the main polyphenol found in green tea (*Camellia sinensis*); it is a potential MMP inhibitor with low toxicity and anti-inflammatory properties.[10,11] EGCG can reduce the gelatinolytic activity through two mechanisms: irreversible degradation of MMP molecules or binding on the catalytic region of MMPs, with either producing reversible inhibition.[12] In addition, it has been shown that EGCG can increase collagen cross-linking and prevent the free access of collagenase to the active sites on the collagen chains.[13] Besides that, the green tea catechins appear to have a protective effect on dentin loss, which may be promising for the prevention and treatment of noncarious cervical lesions.[14] Du and others[6] incorporated EGCG in different concentrations into etch-and-rinse adhesive systems and evaluated the antimicrobial effect and the physicochemical properties of dentin bonding over time. Similarly, Neri and others[15] evaluated the influence of incorporating EGCG onto the physicochemical properties of a self-etch dental adhesive and observed that the addition of EGCG to the adhesive reduced solubility without affecting the degree of conversion (DC) and flexural strength. Macedo and others[16] assessed the physicochemical properties of experimental etch-and-rinse and self-etch adhesives with EGCG at 0.1% and 0.5%. With etch-and-rinse adhesives doped with 0.5% EGCG, the resin-dentin bond strength was increased after six months. Santiago and others[7] evaluated the effects of dentin pretreatment with EGCG solutions in the preservation of the adhesive interface with etch-and-rinse adhesive systems. All of the studies showed that the use of EGCG is effective in improving the longevity of adhesive procedures.

In an attempt to achieve release of EGCG in a more controlled, prolonged way, the authors of this study sought the use of polymeric microparticles produced by polymers, such as poly(D-L lactide-co-glycolide) acid (PLGA), which is one of the main polymers used in the development of release systems. This polymer is considered the gold standard in controlled drug delivery, mostly because of its superior biocompatibility.[17,18] Thus, the use of polymeric microparticles loaded with EGCG can be an effective strategy, compared with free EGCG, because of the protection of the drug from the physiological degradation and bioadsorption, thus avoiding the loss of pharmacological activity. In addition, controlled drug-delivery systems could contribute to a prolonged effect of the drug in the specific therapeutic site, maintaining effective therapeutic concentrations of the drug for prolonged periods,[19,20] which could result in its being an effective source of drug release for relatively longer time periods. Recently, Fawzy and others[21] demonstrated this new therapeutic strategy in terms of delivery of CHX-loaded PLGA nanoparticles through the dentinal tubules of demineralized dentin substrates for potential applications in restorative and adhesive dentistry. They demonstrated the potential significance of delivering collagen cross-linkers loaded into biodegradable polymer nanoparticles through the dentinal tubules of demineralized dentin on the biodegradation resistance of the dentin collagen matrix.[21]

Therefore, the aim of this study was to synthesize and characterize EGCG-loaded/PLGA microparticles, evaluate the performance of polymeric microparticles loaded with EGCG on the DC and release assay of adhesives, and evaluate the resin-dentin bond strength with two EGCG formulations (free

Table 1: *Materials and Chemicals Used in the Study*

Material (Manufacturer)	Batch No.	Basic Formulation
Adper Single Bond 2[a] (3M/ESPE, St. Paul, MN, USA)	1312201025	Etchant: 35% phosphoric acid (batch 1219600378)
		Bis-GMA, HEMA, dimethacrylates, silica nanofiller (5 nm), polyalquenoic acid copolymer, initiators, water, and ethanol
Resin Filtek Z250 XT (3M/ESPE, St. Paul, MN, USA)	37277	Bis-EMA, Bis-GMA, and UDMA; filled to 60% by volume with zirconia silica filler, average particle size = 0.6 μm
Poly(D-L lactide-co-glycolide acid) Resomer RG502H/Sigma Aldrich, St. Louis, MO, USA	STBD2887V	—
Poly(D-L lactide-co-glycolide acid) Resomer RG756S/Sigma Aldrich, St. Louis, MO, USA	STBC6378V	—
Epigallocatechin-3-gallate (≥80%, Sigma Aldrich, St. Louis, MO, USA)	SLBL1959V	—

Abbreviations: Bis-EMA, ethoxylated bisphenol-A-dimethacrylate; Bis-GMA, bisphenol-A-diglycidyl ether dimethacryalate; HEMA, 2-hydroxyethyl methacrylate; UDMA, urethane dimethacrylate.
[a] *This brand name is the same product as Adper Scotchbond 1XT, Adper Single Bond Plus, and Adper Single Bond 1 XT.*

EGCG or loaded into PLGA microparticles) applied as pretreatment or incorporated into an etch-and-rinse adhesive system.

METHODS AND MATERIALS

The materials and chemicals used in this study are described in Table 1. PLGA: Resomer RG502H (PLGA 50:50, batch STBD2887V) was purchased from Sigma Aldrich (St. Louis, MO, USA). EGCG (batch SLBL1959V) and ethyl acetate (batch DCBB6676) were purchased from Sigma Aldrich. Dichloromethane (batch 65456) was obtained from Dinâmica (Diadema, São Paulo, Brazil). Adper Scotchbond Etchant (35% phosphoric acid gel), Adper Single Bond 2 dentin adhesive system, and restorative resin composite (Filtek Z350 XT A3) were purchased from 3M ESPE (St. Paul, MN, USA).

Microparticle Preparation and Characterization

The microparticle formulations were prepared according to the ratio PLGA 50:50 Resomer 502H (mol wt: 30,000-60,000): EGCG 16:1. Because of the different types of solubility among EGCG and PLGA, an emulsification process was employed. Briefly, PLGA 5.12% w/v was dissolved in dichloromethane, while EGCG 0.64% was dissolved in ethyl acetate. The solutions were vigorously mixed at 25°C using a high shear mixer (Ultraturrax IKA T10B; IKA/Works Inc, Wilmington, NC, USA) at 19,000 rpm for five minutes.

The resulting PLGA/EGCG emulsion was immediately spray dried using a Büchi B-290 Mini Spray drier (Büchi Labortechnik AG, Flawil, Switzerland), according to the methodology used by Sousa and others.[22] The obtained EGCG-loaded PLGA microparticles were immediately collected from the glass containers and stored at 4° ± 2°C for further characterization. A blank formulation (PLGA only) for each branch was obtained in the same manner and used as a control.

Microparticle size and polydispersity index (PdI) were determined by light scattering (Zetasizer Nano ZS, Malvern, Worcestershire, UK) in Milli-Q water. The measurements were performed in triplicate. The amount of EGCG entrapped within the microparticles was determined using the solvent-separation method. The yield of production of the microparticles was determined according to the ratio (in percentage) between the obtained mass and the theoretical mass of the material (polymer and drug) used. Encapsulation efficiency and drug loading were conducted according to a previous study.[22]

Preparation of Adhesive Formulations

The adhesive formulations were prepared by incorporating PLGA-loaded microparticles containing EGCG into the Adper Single Bond 2 adhesive system (3M/ESPE) by manual blending. The amount of microparticles incorporated into the adhesives was assayed at 0.5%, 1.0%, and 2.0% (w/w). The best-performing concentration was used for the subsequent bond strength test.

Each adhesive formulation was briefly mixed in a vortex (Biomixer QL-901, SP, Brazil) for one minute at reduced ambient light.

To determine the maximum amount of microparticles possible for loading into the adhesive system, the DC and release assay of adhesives containing

Table 2: *Description of the Experimental Groups' Degree of Conversion and Release Assay of Adhesives Containing Microencapsulated EGCG*[a]

Group	Material	Microencapsulated Forms of EGCG
Control	Adper Single Bond 2	Without EGCG
0.5% PLGA/EGCG		0.5% (w/w) of microparticles of EGCG
1.0% PLGA/EGCG		1.0% (w/w) of microparticles of EGCG
2.0% PLGA/EGCG		2.0% (w/w) of microparticles of EGCG

Abbreviations: EGCG, epigallocatechin-3-gallate; PLGA, poly(D-L lactide-co-glycolide) acid.
[a] *Groups: control, Single Bond 2; 0.5% PLGA/EGCG, 0.5% microparticles EGCG/PLGA incorporated in Single Bond adhesive; 1.0% PLGA/EGCG, 1.0% microparticles EGCG/PLGA incorporated in Single Bond adhesive; 2.0% PLGA/EGCG, 2.0% microparticles EGCG/PLGA incorporated in Single Bond adhesive.*

different proportions of EGCG-loaded PLGA microparticles were determined (Table 2).

DC—The DC of the adhesive resins was assessed by Fourier transform infrared spectroscopy (FTIR; Perkin-Elmer Spectrum 100, Perkin Elmer, Shelton, CT, USA). Each adhesive system was dispensed into a small agate mortar and thoroughly mixed with potassium bromide (KBr) at a ratio of 4:100. The pellets of the KBr/adhesive solution were prepared with a hand press (Hand Press Kit 161-1100, PIKE Technologies, Madison, WI, USA). The FTIR spectrum of the uncured adhesive was obtained from each sample using 32 scans in a range of 4000-400 cm^{-1} at 4 cm^{-1} resolution in transmission mode.

The adhesive system was light activated for 40 seconds using the light source (Elipar Freelight 2, 3M ESPE). Additional FTIR spectra were obtained immediately after light curing. The analyses were performed at 25°C with 70% relative humidity. Three specimens per group (n=3) were tested. The rate of unreacted carbon-carbon double bonds (C=C) was determined from the ratio of absorbance intensities of aliphatic C=C (peak at 1638 cm^{-1}) against an internal standard (aromatic carbon-carbon bond peak at 1608 cm^{-1}) before and after curing. The DC was determined using the formula $GC\% = (1 - RP/RN) \cdot 100$.

Release Assay of Adhesives Containing Microencapsulated EGCG—From the results obtained in the DC, it was possible to ensure the incorporation of the PLGA microparticles within the adhesive system. The release assay was performed to observe the microparticles' release behavior when incorporated into the adhesive system (Adper Single Bond 2). A Teflon matrix (6.0 mm diameter · 1.0 mm thick) was used to enclose the adhesive formulations, which were prepared in triplicate. The matrix was filled with each adhesive formulation (control group and 0.5%, 1%, or 2% w/w incorporated microparticles), a Mylar strip was placed in the top, and a glass slide was placed to perform the light-curing process. The material was light cured (Elipar Freelight 2, 3M ESPE) for 40 seconds at 600 mW/cm^2. Samples were stored in individual vials containing 1 mL of Milli-Q water at 37°C stored over a four-month period. Aliquots were collected, substituted with fresh release medium, and measured immediately at presettled times.

A calibration curve was used to quantify the drug from a series of reference solutions, ranging from 2.5 to 40 ppm, resulting in a linear relationship between absorbance peak height and drug concentration. An ultraviolet-visible spectrophotometer (DU-730, Beckman Coulter, Fullerton, CA, USA) was used to evaluate and confirm the absorbance peak of EGCG at 275 nm.

Statistical analyses were performed using the statistical software Sigmastat 3.5 (Systat Software Inc, San Jose, CA, USA) for Windows. A Shapiro-Wilk test was applied to all groups to verify the normal distribution of errors, along with the Bartlett test for homoscedasticity. To analyze EGCG cumulative release after 24 and 2952 hours, the two-way analysis of variance (ANOVA) on ranks (independent factors: PLGA branches and storage time) was used. Post hoc comparisons were analyzed by the Holm-Sidak method. The level of significance was set at $\alpha = 0.05$.

Microtensile Bond Strength Test

Forty-five extracted, caries-free human third molars were used. The teeth were collected after obtaining the patient's informed consent under a protocol reviewed and approved by the local Research and Ethics Committee. The selected teeth were stored for about one month after extraction in 0.01% (w/v) thymol solution.

The occlusal enamel of each tooth was removed using a slow-speed diamond saw (IsoMet, Buehler,

Table 3: *Description of Experimental Groups for the Bond Strength Test*[a]

Group	Dentin Pretreatment	Adhesive System
Control	Distilled water	Adper Single Bond 2
SB+EGCG	Distilled water	SB+0.1% (w/w) of free EGCG
SB+PLGA/EGCG	Distilled water	SB+1.0% (w/w) of microparticles (PLGA/EGCG)
RS-EGCG	EGCG aqueous solutions (0.1% EGCG)	Adper Single Bond 2
RS-PLGA/EGCG	Microparticles aqueous solution (1.0% PLGA/EGCG)	Adper Single Bond 2

Abbreviations: EGCG, epigallocatechin-3-gallate; PLGA, poly(D-L lactide-co-glycolide) acid; RS, rewetting solution; SB, single bond.
[a] *Groups: control, Single Bond 2; SB+EGCG, 0.1% EGCG incorporated in Single Bond adhesive; SB+PLGA/EGCG, 1.0% microparticles EGCG/PLGA incorporated in Single Bond adhesive; RS-EGCG, 0.1% EGCG rewetting solution; RS-PLGA/EGCG, 1.0% microparticles EGCG/PLGA rewetting solution.*

Lake Bluff, IL, USA) under water cooling to expose a flat coronal dentin surface. The enamel-free exposed dentin surfaces were further polished on wet #600-grit silicon carbide paper for 60 seconds to standardize the smear layer.

The teeth were divided into five groups (n=9) according to the rewetting solution (RS) or incorporated adhesive system used. The concentration of 1.0% microparticles EGCG/PLGA established in the release test showed the best results. The concentration of free EGCG was in accordance with previous studies.[7,15] The exposed dentin surfaces of all teeth were etched with 35% phosphoric acid gel (Scotchbond Phosphoric Acid Etchant; 3M ESPE) for 15 seconds, rinsed for 30 seconds with distilled water, and dried with oil-water free air for 30 seconds. The teeth were then treated with 20 μL of one of the following RSs: distilled water, 0.1% EGCG aqueous solutions (EGCG), or 1.0% microparticles aqueous solution (PLGA/EGCG; Table 3). The solutions were left in contact with the tooth surface for 60 seconds, and the excess was removed with absorbent paper, leaving the dentin surface visibly moist.

The etch-and-rinse adhesive system Adper Single Bond 2 (3M ESPE) was then applied according to the manufacturer's instructions. After light curing the adhesive (Elipar Freelight 2, 3M ESPE), five 1-mm-thick increments of resin composite were built up (Filtek Z250 XT, 3M/ESPE). Each increment was light cured (Elipar Freelight 2, 3M ESPE) for 40 seconds at a power density of 600 mW/cm^2. The bonded teeth were stored in distilled water at 37°C for 24 hours.

After storage, the teeth were longitudinally sectioned in both the x and y direction across the bonded interface using a diamond saw (Isomet) under water cooling to obtain bonded sticks with a cross-sectional area of approximately 1.0 mm^2. The cross-sectional area of each stick was measured with a digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan) to the nearest 0.01 mm and recorded for subsequent calculation of the bond strength. The bonded sticks were tested immediately and six and 12 months, respectively. For testing, each bonded stick was attached to a jig in the universal testing machine (Emic, São José dos Pinhais, PR, Brazil) with cyanoacrylate resin (SuperBonder flex gel, Loctite, Itapevi, SP, Brazil) and subjected to a tensile force at 0.5 mm/min. The load of fracture, expressed in MPa, was used to calculate the bond strength (μTBS).

The failure modes were evaluated at 80· magnification (StereoZoom Leica S8 APO, Leica Microsystems, Wetzlar, Hesse, Germany) and classified as cohesive (failure exclusively within the dentin or composite [C]); adhesive (A) when failure occurred at the dentin/adhesive interface; or mixed (M), when two modes of failure occurred simultaneously.

Two representative samples of each group were examined using a field-emission scanning electron microscope (SEM; Quanta FEG 450, FEI, Amsterdam, the Netherlands) in backscattered electron mode with 5000· and 10,000· standardized magnifications. SEM photomicrographs of each group are shown in Figure 1.

Statistical Analysis

The DC and μTBS values were submitted to a Shapiro-Wilk test to analyze the normal distribution of errors. As normal distribution was confirmed, the data were analyzed using a statistical one-way ANOVA (DC). Bond strength values were statistically analyzed with two-way ANOVA (treatment and storage), and the Holm-Sidak method was used for post hoc comparisons. Statistical procedures were performed using the SigmaStat 3.5 for Windows statistical program software (Systat Software, San Jose, CA, USA). The significance level was set at $\alpha = 0.05$ for all tests.

Table 4: *Main Characteristics of EGCG Loaded-PLGA Microparticles*

Microparticles	PLGA/EGCG Ratio	Yield of Production, %	Particle Size, μm (PdI)	Encapsulation Efficiency, %	Drug Loading, %
PLGA	8	44.91	1.010 (0.219)	—	—
PLGA/EGCG	8/1	55.38	0.780 (0.687)	42.35	2.49

Abbreviations: EGCG, epigallocatechin-3-gallate; PdI, polydispersity index; PLGA, poly(D-L lactide-co-glycolide) acid.

RESULTS

EGCG-Loaded PLGA Microparticle Characterization

The mean size of the PLGA and PLGA/EGCG microparticles was 1.010 and 0.780 μm, respectively. They were shown to be homogeneous, with a PdI of 0.219 and 0.687 for the blank and loaded microparticles, respectively (Table 4). The percentage of yield of production, encapsulation efficiency, and drug loading are summarized in Table 4. The yield of production was higher for loaded PLGA microparticles compared with blank ones.

DC and Release Assay of the Adhesives

Table 5 shows the DC means and standard deviations for the dental adhesives. There was no statistically significant difference between the DC means after the PLGA microparticles loaded with EGCG were incorporated ($p>0.05$).

Figure 2 shows the EGCG microparticle release profile from the adhesive system. Among all groups, a sustained release pattern was observed throughout the experiment. The groups of 0.5% PLGA/EGCG and 1.0% PLGA/EGCG presented a more pronounced release, reaching the total release (100%) over the assayed period. It can be noted that, except for the 0.5% PLGA/EGCG group, the release started at 96 hours and gradually increased over the assayed period.

Regarding the overall release, the 1.0% PLGA/EGCG group demonstrated greater efficiency, reaching the highest drug amount (77.30 μg) released at the end of 90 days. Accordingly, this group unites two important aspects of its therapeutic application: long-lasting release and the highest drug content achieved.

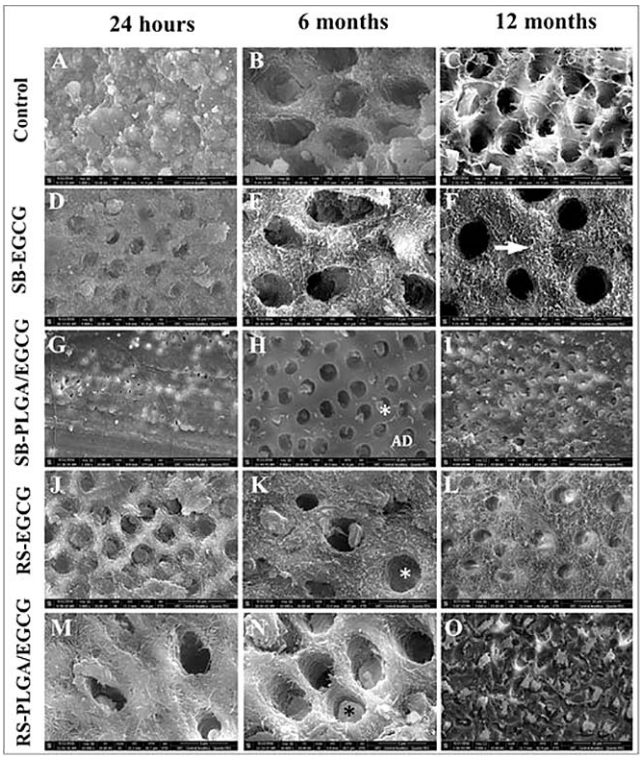

Figure 1. *Representative scanning electron micrographs of the dentin fracture region after bond strength test. White arrows indicate the presence of exposed collagen fibrils in intertubular dentin. AD indicates the presence of the adhesive system. The presence of resin tags is indicated by asterisks (*).*

Bond Strength Test

The mean μTBS values and standard deviations of microtensile bond strength values (MPa) of the experimental and control groups were calculated and are expressed in Table 6. After 24 hours of storage, there was no statistically significant difference between the mean bond strength values of the tested groups ($p>0.05$). At six months, the μTBS of the SB+EGCG and RS-EGCG were significantly higher than in the control group ($p<0.05$). A significant reduction in μTBS was observed for the

Table 5: *Degree of Conversion Mean and Standard Deviation (%) of the Tested Groups*[a]

EGCG Microencapsulated Forms Incorporated in SB	Mean (SD%)
Control	58.32 (0.38)
0.5% PLGA/EGCG	57.29 (0.73)
1.0% PLGA/EGCG	58.11 (0.32)
2.0% PLGA/EGCG	59.07 (0.57)

Abbreviations: EGCG, epigallocatechin-3-gallate; PLGA, poly(D-L lactide-co-glycolide) acid.
[a] Groups: control, Single Bond 2; 0.5% PLGA/EGCG, 0.5% microparticles EGCG/PLGA incorporated in Single Bond adhesive; 1.0% PLGA/EGCG, 1.0% microparticles EGCG/PLGA incorporated in Single Bond adhesive; 2.0% PLGA/EGCG, 2.0% microparticles EGCG/PLGA incorporated in Single Bond adhesive.

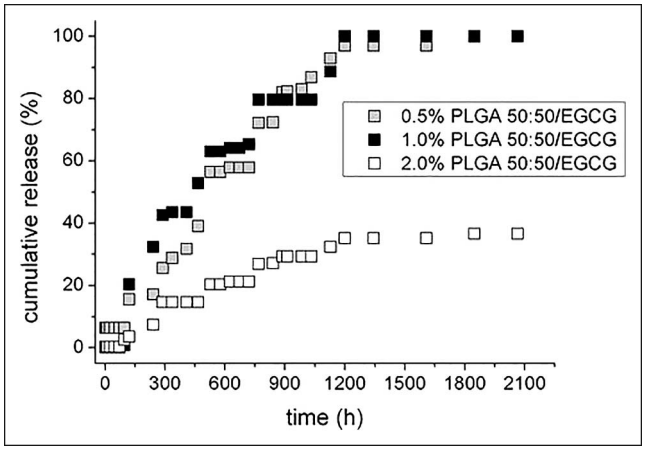

Figure 2. *Cumulative epigallocatechin-3-gallate microparticle release (%) from the adhesive system over a 90-day period.*

control group ($p<0.05$), while no significant reduction was observed when EGCG was used independent of the application mode. The resin-dentin bond strength of the experimental groups after 12 months of water storage was significantly higher than in the control group ($p<0.05$). Table 7 summarizes distribution of the mode of fracture of the debonded specimens. Most failures were mixed in all of the tested groups and in all periods. The number of adhesive failures increased with aging of the specimens directly exposed to water in all groups.

DISCUSSION

Several studies have reported that EGCG has a dose-dependent effect on the stability of the dentin collagen matrix to resist collagenase degradation[10,23,24]; this differs from other inhibitors in that it is a natural product extracted from green tea (*Camellia sinensis*). Thus, it can be used in cavities of any depth because of its low toxicity and anti-inflammatory properties.[10]

Dentin treatment using EGCG significantly improved the mechanical properties of demineralized dentin, which suggests potential collagen cross-linking.[10-11,23] These positive data support the introduction of EGCG in dental practice. However, drugs are released more quickly when uncoated[22,25]; for that reason, polymeric microparticles produced by polymers such as PLGA have been extensively used in several applications pertaining to the controlled release of drugs,[26] including in endodontics, periodontics,[22] and restorative dentistry.[20,21]

The association between EGCG and polymeric materials is aimed at obtaining a long-lasting drug delivery system that can be applied in dental therapeutics. EGCG has not yet been studied in that manner, especially as it pertains to the physical and/or chemical properties of molecules and the resulting effect on release ability from the material. The present study compared the effects on resin-dentin bond strength of EGCG free and microencapsulated, applied as a dentin pretreatment or incorporated into the etch-and-rinse adhesive system. Initially, the microparticles were synthesized using the spray-drying technique according to the methodology used by Sousa and others.[22] Other studies encapsulated CHX into biodegradable drug-delivery systems using different methods,[27,28] but particle size distribution and particle shape, together with some selected chemical properties, usually constitute critical variables of a pharmaceutical manufacturing process, and spray-dried particles are commonly spherical and uniform in size.[29]

The microparticle size is directly related to the dissolution rate and pattern and, as a result, to the bioavailability of the active ingredient.[30] The mean size of the PLGA/EGCG microparticles was 0.780 μm (Table 4), while its blank formulation presented a greater size, likely to be related to the agglomeration observed, mainly because of its high hydrophobicity and/or some particles that were not of average size. The yield of production has been higher for loaded PLGA microparticles compared with blank ones. This result can be due to the higher heterogeneity observed in the blank nanoparticles, and as such, aerodynamics

Table 6: *Bond Strength Mean μTBS Values and Standard Deviation (MPa) of the Tested Groups*[a]

Group	24 h	6 mo	12 mo
Control	35.12 (7.80)A,a	26.36 (6.30)A,b	23.80 (2.07)A,b
SB+EGCG	38.97 (5.41)A,a	36.02 (6.14)B,a	32.75 (5.44)B,a
SB+PLGA/EGCG	35.92 (5.45)A,a	28.98 (9.78)AB,a	30.23 (6.18)B,a
RS-EGCG	33.15 (6.93)A,a	35.63 (5.68)B,a	33.96 (7.96)B,a
RS-PLGA/EGCG	36.93 (5.25)A,a	29.98 (8.66)AB,a	33.51 (6.70)B,a

Abbreviations: EGCG, epigallocatechin-3-gallate; PLGA, poly(D-L lactide-co-glycolide) acid; RS, rewetting solution; SB, single bond.
[a] *Groups: control, single bond 2; SB+EGCG, 0.1% EGCG incorporated in Single Bond adhesive; SB+PLGA/EGCG, 1.0% microparticles EGCG/PLGA incorporated in Single Bond adhesive; RS-EGCG, 0.1% EGCG rewetting solution; RS-PLGA/EGCG, 1.0% microparticles EGCG/PLGA rewetting solution. Identical superscript letters indicate no statistical significance between values. Uppercase letters compare treatments, and lowercase letters compare storage time.*

Table 7: *Distribution of Percentages (%) of Mode of Fracture According to Group*[a]

Group	24 h					6 mo					12 mo				
	A	M	CR	CD	PF	A	M	CR	CD	PF	A	M	CR	CD	PF
Control	10.9	71.2	2.7	0	15	22.5	60.5	5.6	4.2	7.0	41.4	2.8	8.5	15.7	5.7
SB+EGCG	3.6	75.6	13.4	4.8	2.4	9.8	74.6	22.5	4.2	9.8	15.7	45	21.3	12.3	0
SB+PLGA/EGCG	3.8	82.6	1.9	3.8	7.6	29.1	56.2	10.4	2	2	12.7	53.1	8.5	2.1	23.4
RS-EGCG	1.6	86.4	0	3.3	8.4	7.4	82	2.9	1.4	5.9	20.8	62.6	7.4	2.9	5.9
RS-PLGA/EGCG	9.8	84.3	3.9	1.6	1.6	32.6	65.3	1.5	0	0	1.5	62.2	0	8.8	2.2

Abbreviations: A, adhesive failure; CD, cohesive failure in dentin; CR, cohesive failure in resin; EGCG, epigallocatechin-3-gallate; PF, premature failure; M: mixed failure; PLGA, poly(D-L lactide-co-glycolide) acid; RS, rewetting solution; SB, Single Bond.
[a] Groups: control, Single Bond 2; SB+EGCG, 0.1% EGCG incorporated in Single Bond adhesive; SB+PLGA/EGCG, 1.0% microparticles EGCG/PLGA incorporated in Single Bond adhesive; RS-EGCG, 0.1% EGCG rewetting solution; RS-PLGA/EGCG, 1.0% microparticles EGCG/PLGA rewetting solution.

may have a direct effect on the recovery of the spray-dried microparticles. One limitation of the spray-drying technique is the difficulty in recovering the powder content because of its adherence to glass parts, which can also produce agglomeration of the resulting material.[30] In addition, the encapsulation efficacy of the microparticles depends on different factors, such as polymer concentration and solubility as well as the chemical interaction between the drug and polymer.[31]

FTIR analysis showed no significant differences between the degrees of conversion among the different concentrations of microencapsulated EGCG incorporated into the adhesive system. Du and others[6] speculated that higher concentrations of EGCG (0.5%) could interfere with the formation of linear polymer chains. Furthermore, the free radical scavenging effect of a high EGCG concentration may disrupt adhesive polymerization,[7] but in the present study, there was no interference, since the higher EGCG concentration used was 0.08% (2.0% PLGA/EGCG). Neri and others[15] also demonstrated that incorporation of EGCG in low concentrations (0.01% and 0.1%) into a specific one-step self-etch adhesive did not cause any detrimental effect on the DC.

Regarding EGCG release, the results showed different cumulative release profiles between the groups (Figure 2). A sustainable release pattern among the formulations was observed, reaching a total release (100%) over the three-month study period. This behavior can be explained by the hydrophilicity of the EGCG, which, in this way, presents a facilitated release from the hydrophobic matrix beyond the already expected degradation of PLGA. Furthermore, in all groups, a sustained-release profile was characterized by forming a transient plateau, immediately followed by a new arising. This behavior can be explained by the obstacles found in the drug to release from the inner microparticles, crossing the adhesive layer to finally achieve the releasing medium and be quantified. As such, the release is more evident at 96 hours, when the drug started to overcome this route. In this study, the major goal was to evaluate the *in situ* controlled release of EGCG using polymeric micro-encapsulation to protect its efficacy over time. Sustained release enabled higher doses to be reached in individual and gradual moments, encouraging the effects related to enzymatic inhibition. Therefore, considering all factors, the 1.0% PLGA/EGCG group showed superior results and was employed for the subsequent bond strength test. A recent study demonstrated the feasibility of using PLGA nanoparticles for that purpose, whose presented attractive physicochemical properties, low cytotoxicity, slow degradation, and gradual release profiles related to the entrance and entrapment within the collagen fibers of the dentin.[20]

The results of this study revealed that EGCG presented a positive effect on bond strength after 12 months of water storage, independent of the incorporation mode once there was a statistically significant difference between the mean bond strength values of the tested groups as compared with the control ($p<0.05$). The SEM images revealed degradation of the collagen fibrils over time and exposure of dentin in the control group (Figure 1B,C), while in the SB-EGCG and RS-EGCG groups, there was greater preservation of the collagen fibrils up to 12 months (Figure 1D-F and J-L, respectively). The current results confirm those from previous studies, which have shown that, after incorporation of EGCG, the immediate mean bond strength of dental adhesives presented a satisfactory outcome.[6] Analyzing the PLGA groups, they presented less dentin degradation (Figure 1G-I and Figure 1M-O). It can be speculated that the mechanism of slow drug delivery can contribute to this outcome. However, a long-term evaluation is needed to confirm this speculation. Khamverdi and others[24] observed that 50 and 100 μM EGCG could preserve the bond

strength of the Clearfil SE Bond groups after six months, whereas EGCG, especially in high concentrations, could not preserve the bond strength of the Filtek Silorane System samples after six months. In relation to dentin pretreatment with free EGCG, Santiago and others[7] showed that a 0.1% EGCG solution was effective in preserving bond strength after six months of water storage, and Yang and others[32] showed that the combined use of 0.02% EGCG/ethanol solution as a primer during adhesive restoration can effectively improve the immediate dentin bond strength and bond stability.

Sustained drug delivery systems are of great interest,[33] particularly for treatment of certain diseases that depend on therapeutic concentrations during longer periods, according to the onset of the disease or syndrome.[34] Some researchers have experimentally used EGCG associated with polymers to treat atherosclerosis in rabbits[35] to prevent postsurgical adhesions[36] and caries.[37] In this context, PLGA/EGCG microparticles could be a promising alternative for different biomedical applications, where its release patterns can last up to four months and could adjust to the therapeutic condition to be treated.

In the present study, we speculate that aging for 12 months may not have been sufficient to detect the effects of hydrolytic degradation of the adhesive interface by bond strength testing. Also, any impact from PLGA degradation has not been noted. A study by Kiyomura[38] reported that storage times between two and four years were required to detect the effects of hydrolytic degradation. Therefore, complementary studies are being conducted to confirm the potential of this catechin to preserve collagen and maintain bond strength, related to the influence of EGCG-loaded PLGA microparticles on the physicochemical properties of a commercial etch-and-rinse adhesive system.

CONCLUSION

This study proposed a new strategy in terms of EGCG delivery loaded to PLGA microparticles. This overall evaluation indicates that EGCG could improve the durability of the dentin-resin bond over time and promote a new era for adhesive dentistry with the concept of controlled release.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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