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Biophysical and Fluoride Release Properties of a Resin Modified Glass Ionomer Cement Enriched with Bioactive Glasses

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Abstract: The aim of this study was to evaluate the bond strength, microleakage, cytotoxicity, cell migration and fluoride ion release over time from a resin-modified glass-ionomer cement (RMGIC) enriched with bioactive glasses (BAGs) and a nanohybrid restorative polymer resin agent used as adhesion material in the cemented brackets. One hundred and twenty bovine lower incisors were divided into three groups: (Transbond Plus Self Etching Primer (TSEP)/Transbond XT (TXT), TSEP/ACTIVA, orthophosphoric acid gel/ACTIVA) and brackets were bonded. A bond strength test and microleakage test were applied. A fluoride release test was applied after 60 days for the TXT and ACTIVA group. To evaluate cytotoxicity and cell migration, a cell viability and scratch migration assay were done for each group. p values < 0.05 were considered significant. Regarding bond strength and microleakage test, no significant differences were found between TSEP/TXT and TSEP/ACTIVA. At 6.4 pH, ACTIVA showed a higher degree of fluoride ion release, which increased with acid pH (3.5), with a maximum fluoride secretion at 30 days. MTT assay revealed that TXT reduces the viability of gingival cells with significant differences ($p < 0.001$) compared to the untreated cells (control group). ACTIVA provides optimal adhesive and microfiltration properties, releases substantial amounts of fluoride ions in both acid and neutral media, and its biocompatibility is greater than that of traditional composite resin adhesive systems.

Keywords: fluoride release; microleakage; enamel bond strength; cell viability; cell migration; BAG; RMGIC

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1. Introduction

The appearance of white spots on tooth enamel is one of the undesirable effects of orthodontic treatment, and comprehensive hygiene is essential for its prevention. However, this requires the patient's collaboration, which is not always forthcoming [1]. For this reason, it helps if orthodontists use dental materials that release calcium, phosphate and fluoride in order to prevent demineralisation and/or promote remineralisation of the enamel [2]. In this respect, bioactive materials are receiving growing attention in dentistry [3], and several authors have evaluated the incorporation of bioactive glasses (BAGs) in composite resins for use in orthodontics [2–4] or resin-modified glass ionomers cements (RMGICs) [5], in order to obtain an adhesion material capable of preventing demineralisation around the brackets.

ACTIVA™BioACTIVE-RESTORATIVE™ (Pulpdent Corporation, Watertown, MA, USA) (ACTIVA) is a hydrophilic RMGIC enriched with bioglass particles [6] and fortified with a rubberised polymer resin that does not contain Bisphenol A, Bis-GMA, or BPA derivatives. It is claimed to be a more biocompatible material than other resinous materials [7], favouring the healing of tissues [8]. It shows smart material behaviour since it reacts to changes in pH by releasing and taking up calcium, phosphate and fluoride ions, favouring the formation of apatite crystals at the material-tooth interface. Saunders et al. [9] observed that ACTIVA inhibited the loss of Ca and P ions from the enamel adjacent to brackets.

The flexural strength or diametral tensile strength of ACTIVA is comparable to that of composite resins [10]. However, no important properties have been evaluated with respect to its adhesion to brackets, such as adhesive strength, and the possibility of microfiltration, although it is known that microfiltration between the adhesive and tooth can favour the appearance of white spots on this interface, while the adhesive–bracket interface can play a part in bracket failure caused by bond degradation [11].

It has been described that the effect of fluoride release by GICs and RMGICs follows a general pattern: an "initial burst" during the first 24 h followed by a substantial reduction in the following 2–3 days, reaching a stable plateau at 2–4 weeks. However, the release of fluoride from materials containing bioactive glass begins to fall in the first few hours and then remains constant or increases, depending on the concentration of the bioactive glass in the material and the physical-chemical characteristics of the medium [2].

Many factors influence the release of fluoride from materials containing fluoride including the quantity and composition of fillers, storage medium (saliva, distilled water) and the frequency of any changes in the tooth environment, pH values, the presence of biofilm, the powder–liquid ratio in two-phase systems, the mixing process, curing time, exposed area, etc. [12]. In glass ionomers, fluoride is released through the hydrolysis of Al-O-Si links, particularly below pH < 6. In bioactive glasses, its release results from the degradation of bioactive glass particles that not only release fluoride but also the calcium and phosphorus necessary for the formation of hydroxyapatite and fluorhydroxypatite. Such apatite formation is the fundamental difference between a glass ionomer and bioactive glass [13].

The aim of this study was to evaluate the bond strength, microleakage, cytotoxicity, cell migration and fluoride ion release over time from an RMGIC enriched with BAG and a nanohybrid restorative polymer resin agent used as adhesion material in the cemented brackets. The null hypothesis was that there was no difference between the groups.

2. Materials and Methods

2.1. Bond Strength and Microleakage Testing

Bovine lower incisors (n = 120) were divided into three groups (n = 40), and brackets (Victory Series, 3M Unitek Dental Products, Monrovia, CA, USA) were bonded onto the vestibular surfaces. Three bonding procedures were evaluated: Group 1, Transbond Plus Self Etching Primer (TSEP)/Transbond XT (TXT) (as control group); Group 2, TSEP/ACTIVA; Group 3, Etched with a 37% orthophosphoric acid gel/ACTIVA. The composition of the materials is shown in Table 1.

The buccal surfaces of the teeth from the different groups were polished using a rubber cup and polishing paste (Détartrine, Septodont, Saint-Maur, France). The buccal surface of the teeth from groups 1 and 2 were treated with TSEP, which was gently rubbed onto the enamel for 5 s. A moisture-free air source was used to deliver a gentle burst of air to the primer. In group 3, the vestibular enamel of the teeth was etched with a 37% orthophosphoric acid gel for thirty seconds, and then the enamel was thoroughly washed and dried. In the case of group 1, the brackets were bonded with TXT, which was light-cured with a SmartLite LED lamp (Dentsply®, York, PA, USA) at 1250 W/cm^2 for 10 s on each interproximal side. In groups 2 and 3, the brackets were bonded using ACTIVA, which was light-cured in the same way as TXT.

Table 1. Material composition.

Product	Composition	%/wt
3M UnitekTransbond XT Light Cure Adhesive (MSDS)	Silane Treated Quartz	70–80
	Bisphenol A Diglycidyl Ether Dimethacrylate (BISGMA)	10–20
	Bisphenol A bis(2-Hydroxyethyl Ether) Dimethacrylate	5–10
	Silane Treated Silica	<2
	Diphenyliodonium Hexafluorophosphate	<1
ACTIVA Bioactive Restorative [7]	Blend of diurethane and other methacrylates with modified polyacrylic acid	44.6
	Reactive glass filler	21.8
	Inorganic filler	56
	patented rubberised resin (Embrace)	
	Water	
	Silica, amorphous	6.7
	Sodium fluoride	0.75

Specimens from all groups were subjected to thermocycling, which consisted of 500 cycles in distilled water between 5 °C and 55 °C, after storing the specimens for 24 h in water kept at 37 °C. The teeth were immersed for 20 s each time, while the time between baths was between 5 s and 10 s [14].

2.2. Bond Strength Test

The root of twenty specimens of each group were embedded in type IV stone/gypsum (Zhermack, Badia Polesine, Italy) and left to set. Then, the specimens were placed in 4 cm-long plastic cylinders with an internal diameter of 3 cm. Shear bond strength was measured by means of a universal test machine (Autograph AGS-1KND, Shimadzu, Kyoto, Japan) with a 1 kN-load cell connected to a metal rod and one end angled at 30°. The crosshead speed was 1 mm/min [14]. The shear force was exerted between the base and the wings of the brackets by a metal rod (end angle at 30°) connected to the load cell.

2.3. Microleakage Testing

Twenty specimens from each group were painted with a nail varnish (Resist and Shine, L'Oréal, Paris, France), leaving 1 mm uncovered around the bracket. Then they were immersed for 24 h in a methylene blue (1%) solution (Sigma Aldrich, St. Louis, MI, USA). The specimens were set vertically and the solution only covered the crown and the first third of the root.

Afterwards, three sections per tooth were obtained by sectioning the teeth longitudinally (inciso-cervical direction) using a diamond saw (Horico, Berlin, Germany). Both sides of each section were examined, therefore six observations were obtained per tooth.

The microleakage was determined with image analysis equipment (Sony dxc 151-ap video camera, connected to an Olympus SZ11 microscope) and MIP 4 software (Microm Image Processing Software, Digital Image Systems, Barcelona, Spain). The microleakage was measured at ×100 magnification on enamel-adhesive and adhesive-bracket interfaces, on both the gingival and incisal edge. The total microleakage percentage for each interface was calculated by summing the microleakage percentages measured at the incisal and gingival edges of each interface.

The same researcher made all observations (MJ). Twenty-five measurements were repeated after 1 month. The Wilcoxon test did not find significant differences between the

first and second examinations for total microleakage at both interfaces (enamel-adhesive $p = 0.11$ and adhesive-bracket $p = 0.8$).

2.4. Fluoride Release Test

Fluoride ion release was determined with an ion-specific electrode (Orion 9609 BNWP, Thermo Fisher Scientific Inc., Waltham, MA, USA), coupled to an ion analyser (Orion EA-940 Thermo Fisher Scientific Inc., Waltham, MA, USA).

Prior to each determination, the electrode was calibrated with standard fluoride solutions from 0.125 to 8.0 ppm F, mixing 1 mL of each standard solution with 1 mL of ionic strength adjustable buffer, TISAB II (Hanna Instruments, Woonsocket, RI, USA). The results of the readings were collected in mV and converted to µgF/mL (ppm F).

ACTIVA (n = 20; 0.050 g weight) and TXT (n = 20; 0.064 g weight) tablets were made using 3 mm internal diameter ×3 mm height polyethylene cylinders and polymerised for 20 s with a SmartLite LED lamp (Dentsply®, USA) at 1250 W/cm^2. Each tablet was inserted into a tube containing 10 mL of artificial saliva at pH 6.4 (n = 10) or pH 3.5 (n = 10), and maintained at 37 °C. Measurements were made at 1, 2, 7, 14, 30 and 60 days.

For each determination, 1 mL of each tube was extracted and mixed with 1 mL of TISAB II. To measure the accumulated release, the material remained immersed in the remaining saliva and the release of fluoride ions per gram of material was calculated (1 µg F/g = 1 ppm).

The artificial saliva as composed of 1% carmellose sodium, 3% sorbitol, 0.012% potassium chloride, 0.084% sodium chloride, 0.005% magnesium chloride hexahydrate, 0.015% anhydrous calcium chloride, 0.017% dibasic potassium phosphate and 0.1% Nigapin® sodium, distilled water. The saliva pH was adjusted and maintained at 6.5 [15]. To obtain saliva at pH 3.5, a citric acid buffer (0.84%) and sodium citrate (0.94%) adjusted to pH 3.5 with 1N HCl were added. To test the effect of acidic soft drinks on the shear bond strength of the orthodontic brackets, the saliva pH was taken to pH 3.5 by adding a 0.84% citric acid 0.94% sodium citrate buffer with 1N HCl. The enamel was then evaluated by scanning electron microscopy.

2.5. In Vitro Study—Cell Viability Assay

Discs of the materials (n = 20) were moulded in a sterile cylindrical polyethylene tube (diameter, 5 mm; height, 3 mm), and sterilised by ultraviolet irradiation for 1 h. Evaluation of the materials was based on the results of previous studies and according to the ISO [16]. Material extracts were prepared, and three different dilutions (1:1, 1:2, 1:4) were used. The study protocol was previously approved by the Ethics Committee (Institutional Review Board for Human Subjects Research) of the University of Murcia (ID: 2199/2018). Briefly, fibroblasts from gingival tissues (hGFs) were plated (2×10^3 cells/well) and exposed to several dilutions (1:1, 1:2, 1:4 extracts) for 72 h. Cells without specimens were used as control group. After this treatment, the medium was removed, and cells were incubated with MTT under standard culture conditions for 4 h. Mitochondrial viability was marked by converting the tetrazolium salt MTT to a colored formazan by mitochondrial dehydrogenases. The absorbance was recorded at 570 nm using a spectrophotometer (Synergy H1, BioTek, Winooski, VT, USA) at 570 nm (Abs570).

2.6. Scratch Migration Assay

Cell migration in the presence of specimen extracts was performed using an in vitro scratch assay. To do so, hGFs at a concentration of 2×10^5 cells/well were seeded onto six-well plates (n = 3) and left to proliferate in normal growth medium for 24 h. A wound was created using a sterilised pipette tip, and the cells were exposed to several previously prepared extracts. Images were captured using an inverted phase contrast microscope at the initial time (0 h), 24, 48 and 72 h and analysed using Image J software (National Institutes of Health, Bethesda, MD, USA).

2.7. Statistical Analysis

SPSS 19.0 was used to carry out the statistical tests. Bond strength and biological data complied with the criteria for normality and homogeneity of variance, so the existence of significant differences was analysed by means of one factor variance analysis (ANOVA) and a Tukey test ($p < 0.05$). For microleakage data that did not fulfil the criteria for normality and homogeneity of variance, a Kruskal–Wallis test ($p < 0.05$) was applied. Fluoride release values did not fulfill the criteria for normality. To determine if the acidity of saliva produced different rates of fluoride release in the same material, the Mann–Whitney test was used. Friedman repeated measures analysis of variance and Tukey test was performed to detect differences in fluoride release values and daily fluoride release rate in the same material at different times. To determine differences between the different materials at the same time, a Kruskal–Wallis test was used together with a Dunn test. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Bond Strength and Microleakage Test

Table 2 shows shear bond strength values for each of the evaluated groups.

Table 2. Shear bond strength (MPa).

Group	Mean	Median	Standard Deviation	95% C.I.	
TSEP/TXT	9.41	9.56	2.86	8.06, 10.75	A
TSEP/ACTIVA	8.72	8.39	1.34	8.09, 9.35	A
Phosphoric acid/ACTIVA	11.89	11.29	2.41	10.76, 13.02	B

Groups marked with different superscribed letters showed significant differences from one another ($p < 0.05$).

Mean shear bond strength for Phosphoric acid/ACTIVA was significantly ($p < 0.0001$) higher than for TSEP/TXT ($p < 0.0001$) and TSEP/ACTIVA, while no significant differences were found between TSEP/TXT and TSEP/ACTIVA ($p = 0.61$).

Moreover, no significant differences were found between groups for the total microleakage percentage at the enamel-adhesive ($p = 0.18$) or adhesive-bracket ($p = 0.58$) interfaces (Table 3). Figure 1 shows microleakage at the enamel-adhesive and adhesive-bracket interfaces.

Table 3. Percentage of microleakage.

Group	Enamel-Adhesive			Adhesive-Bracket		
	Mean ± SD	**Median**	**Range**	**Mean ± SD**	**Median**	**Range**
TSEP/TXT	0.26 ± 0.38	0	1.19	0.07 ± 0.22	0	1
TSEP/ACTIVA	0.32 ± 0.75	0	2.60	0.02 ± 0.09	0	0.4
Phosphoric acid/ACTIVA	0.25 ± 0.67	0	2.60	0.00 ± 0.00	0	0

Significant differences were not found between groups for the enamel-adhesive and adhesive-bracket interfaces ($p > 0.05$).

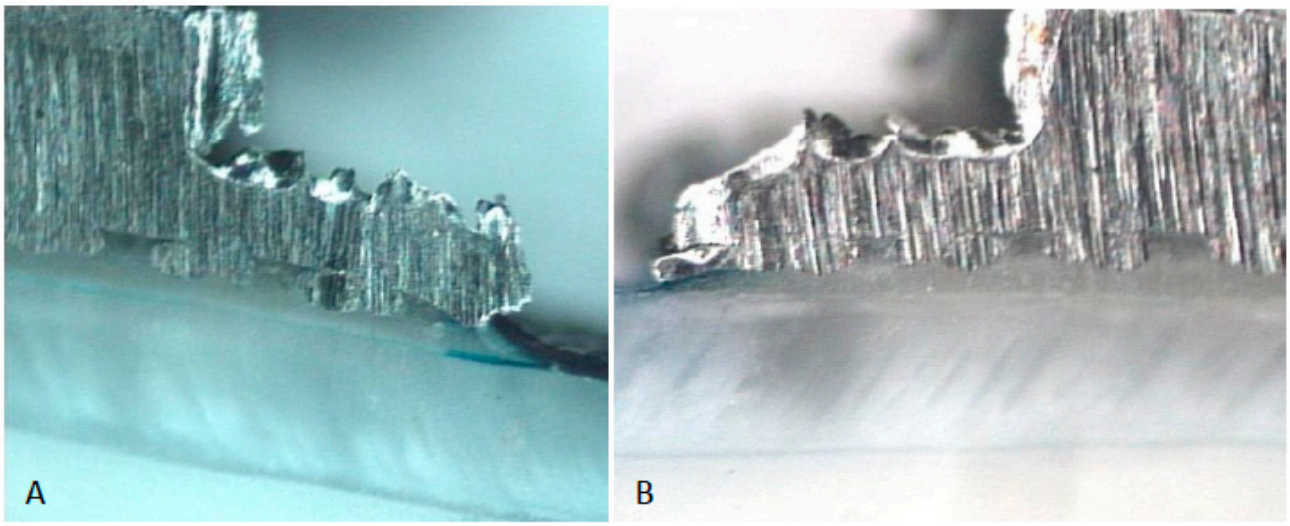

Figure 1. Microleakage Testing. (**A**) Microleakage at the enamel-adhesive and (**B**) adhesive-bracket interfaces.

3.2. Fluoride Release

At pH 6.4, ACTIVA showed a higher degree of fluoride ion release than TXT (Table 4) (9.96 ± 5.45 ppm compared with 0.35 ± 0.47ppm at 24 h). This release increased ten-fold when the pH was 3.5. Thus, at 24 h the fluoride concentration was 108.09 ± 76.85 ppm for ACTIVA and 3.90 ± 6.06ppm for TXT. In the case of ACTIVA at pH 6.4, a cumulative effect of maximum fluoride in saliva was observed at 30 and 60 days, with maximum calculated secretion rates at 1, 7 and 30 days (Table 5; Figure 2) at pH 3.5 the maximum cumulative effect was observed at 30 days, with concentrations of 2399.01 ± 988.26 ppm. The calculated maximum secretion rate was observed at 1 and 30 days (108.09 ± 76.85 and 125.46 ± 57.74 ppm/day, respectively). TXT showed maximum accumulation of fluoride at 7 days for both pH values.

Table 4. Fluoride release (ppm = μg F/g).

	24 h χ (±SD)	**48 h** χ (±SD)	**7 days** χ (±SD)	**14 days** χ (±SD)	**30 days** χ (±SD)	**60 days** χ (±SD)
ACTIVA pH 3.5	108.09 ± 76.85 a	130.01 ± 72.28 a	150.97 ± 26.13 a	440.57 ± 115.67 a	2399.01 ± 988.26	483.88 ± 180.21 a
ACTIVA pH 6.4	9.96 ± 5.45 a,b	14.48 ± 29.58 a,b,c	72.28 ± 21.40	49.04 ± 17.00 a,b	202.87 ± 111.16	191.77 ± 43.25
p value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
TXT pH 3.5	3.90 ± 6.06	0.15 ± 0.22 b,c	15.95 ± 1.11	0.51 ± 0.60 b,c	7.97 ± 20.99	11.51 ± 9.86
TXT pH 6.4	0.35 ± 0.47 c	1.91 ± 4.78 c	16.80 ± 2.60	0.14 ± 0.31 b,c	0.11 ± 0.60 b,c	7.80 ± 3.32
p value	=0.006	=0.054	=0.427	=0.007	=0.031	=0.275

Statistical differences produced by the acidity of saliva are indicated in the *p* value row. Statistical differences of fluoride release values in the same material at different times are indicated by lowercase letters (a: $p < 0.05$ vs. 30 days; b: $p < 0.05$ vs. 60 days; c: $p < 0.05$ vs. 7 days).

Table 5. Calculated rate of fluoride release per day (ppm/day).

Material	**1 day** χ (±SD)	**2 days** χ (±SD)	**7 days** χ (±SD)	**14 days** χ (±SD)	**30 days** χ (±SD)	**60 days** χ (±SD)
ACTIVA pH 3.5	108.09 ± 76.85 b	24.08 ± 127.01	5.92 ± 13.24 a	40.91 ± 16.28	125.46 ± 57.74	−63.89 ± 29.47 a
ACTIVA pH 6.4	9.96 ± 5.45 +	4.51 ± 32,40 a,c,d	11.56 ±7.45	−3.32 ± 1.28 a,c,d,+	9.61± 7.52 +	−0.37 ± 4.05 +
TXT pH 3.5	3.90 ± 6.06 +	−3.75 ± 6.15 c,d	3.16 ± 0.22	−2.21 ± 0.16 b,c,d,+	0.47 ± 1.30 +	0.12 ± 0.70 +
TXT pH 6.4	0.35 ± 0.47 +,#	1.56 ± 4.88 c	2.98 ± 1.14	−2.42 ± 0.40 b,c,d,+	0.002 ± 0.05 c,+,#	0.26 ± 0.11 +

Statistical differences of diary fluoride release rate in the same material at different times are indicated by lowercase letters (a: $p < 0.05$ vs. 30 days; b: $p < 0.05$ vs. 60 days; c: $p < 0.05$ vs. 7 days; d: $p < 0.05$ vs. 1 days). Statistical differences between the different materials at the same time are indicated by symbols (+: $p < 0.05$ vs. active pH 3.5; #: $p < 0.05$ vs. active pH 6.4).

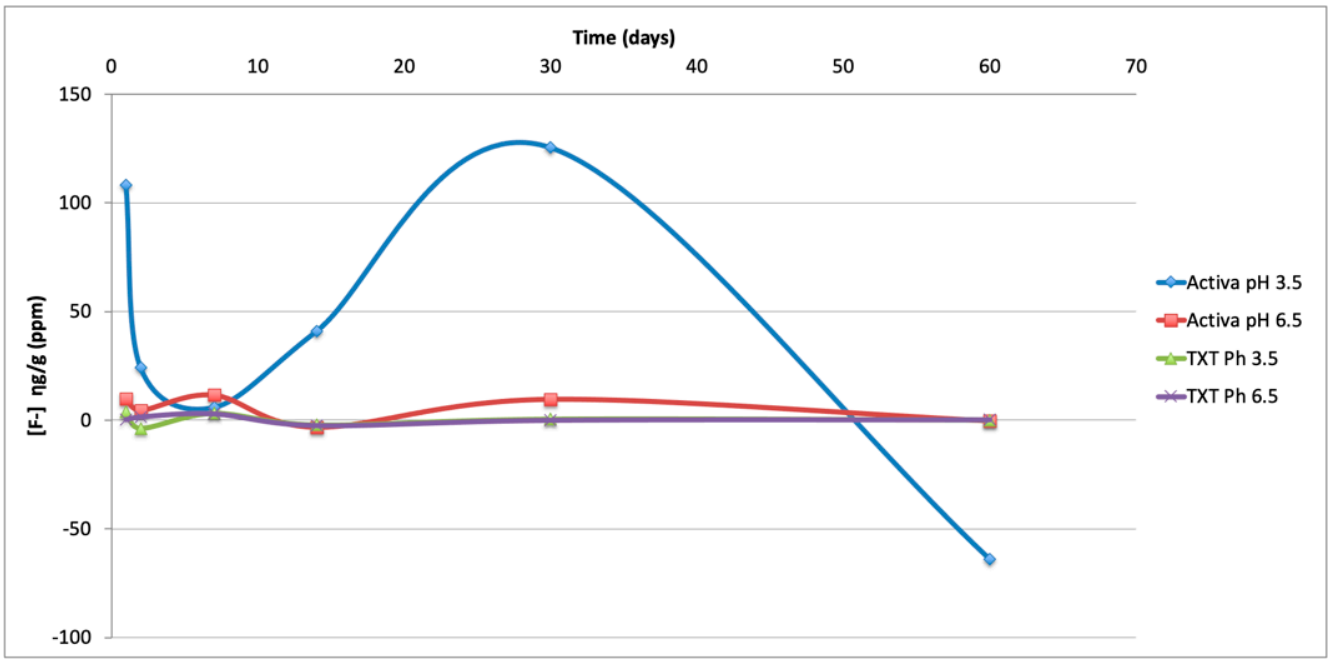

Figure 2. Calculated rate of fluoride release per day (ppm/day).

3.3. Cell Viability Assay

The MTT assay revealed significant differences between undiluted materials and the control group after 3 days ($p < 0.001$; Figure 3), while a notable degree of metabolic cell activity was observed with ACTIVA 1:2 and ACTIVA 1:4 at 72 h. There was a significant difference between the ACTIVA group 1:4 and the control ($p < 0.01$). TXT reduced the viability of gingival cells, which differed significantly from the viability of untreated cells (control) ($p < 0.001$).

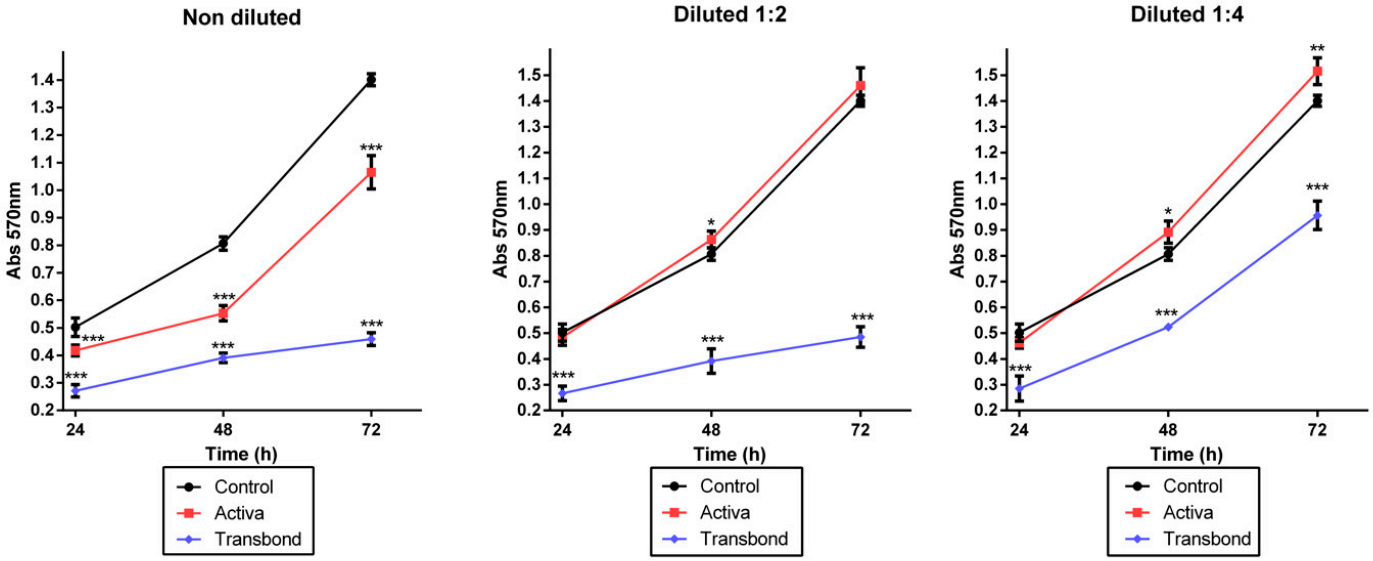

Figure 3. MTT assay. Evaluation of gingival cells treated with ACTIVA and Transbond eluates. Data shown are the mean (±SD) of three different experiments. Cytotoxic differences observed are shown as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, respectively.

3.4. Scratch Migration Assay

In the ACTIVA group, significant differences were found both at 24 h (** $p < 0.01$) and 48 h (* $p < 0.05$), while no significant differences were observed at 72 h for any of the dilutions when compared with the wound healing ability of the control group.

On the other hand, 1:1 and 1:2 dilutions of TXT group showed significantly lower wound closure rates at 72 h when compared to the control group (*** $p < 0.001$). These results indicate that the ACTIVA group exhibited greater migration potential than the TXT group (Figure 4).

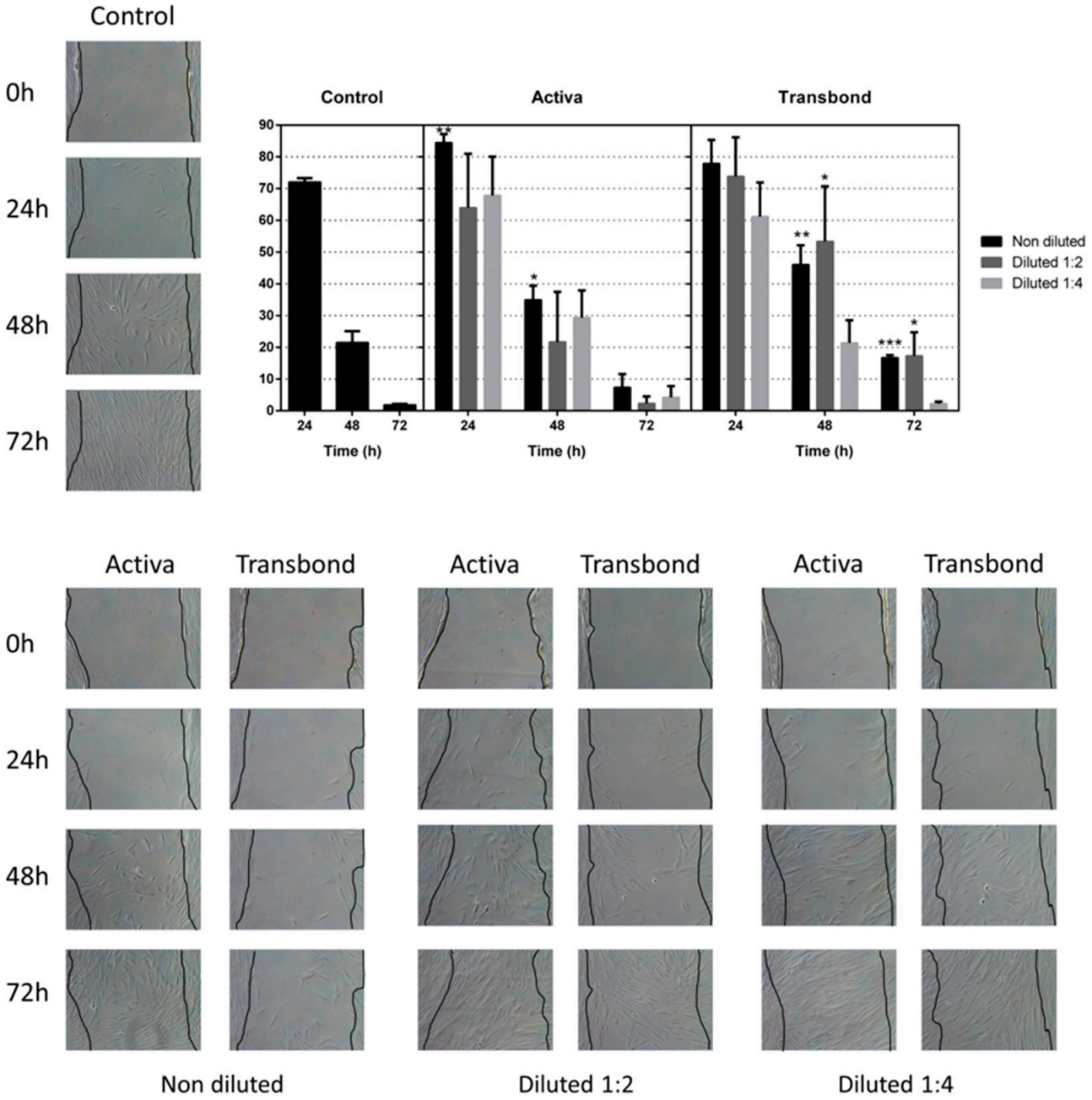

Figure 4. In vitro cell migration assay. Cell migration in the presence of specimen extracts was evaluated at 0 h (initial time), 24, 48 and 72 h. * $p < 0.005$; ** $p < 0.01$; *** $p < 0.001$.

4. Discussion

The objective of this study was to determine whether ACTIVA™BioACTIVE-RESTORATIVE™, an RMGIC enriched with BAG, is suitable for cementing brackets and for inhibiting enamel demineralisation. To this end, we studied its physical (adhesion force and microfiltration), biological (biocompatibility) and preventive (potential for the release of fluoride ions) properties.

For the adhesive force test, it was decided to establish a control group consisting of brackets cemented with the self-etching TSEP and a composite resin (Transbond XT), since the use of self-etching adhesives is increasingly common in orthodontics. Studies show that the adhesive force [17,18] and bond failure rate [19,20] of the self-etching TSEP is similar to that of conventional acid etching.

Our results showed that the shear bond strength (SBS) of the control group was significantly lower than that of the group consisting of brackets cemented with ACTIVA before acid etching of the enamel. To the best of our knowledge, no studies have evaluated bracket adhesion using ACTIVA, so we cannot compare our results. However, some authors, who evaluated the adhesion of brackets using resin systems that incorporated BAG in different proportions found no significant differences from the adhesion obtained with composite resin but without BAG [4,21], although one study found that the bond strength of the brackets decreased significantly when the BAG content was 50% [22].

The manufacturer of ACTIVA recommends acid etching prior to use, and although retention may be compromised, to apply an adhesive agent (Pulpdent®) [23]. In the absence of any such recommendations for cementing brackets, we decided to apply ACTIVA after etching with acid and to compare the results obtained from another group using a self-etching adhesion system. It was found that the adhesive strength of the TSEP/ACTIVA-Restorative group was significantly lower than that of the group consisting of brackets cemented with ACTIVA before acid etching of the enamel and similar to that of the control group. According to the manufacturer, ACTIVA self-adheres to dental tissue, and after contact with water, hydrogen ions of the ionic resins' phosphate groups bind to dental tissue calcium (Pulpdent®). The adhesive agent containing TSEP may interfere in some way with this chemical adhesion between the dental tissue and the material, and therefore, with the formation of a resin-hydroxyapatite complex.

Although a lower degree of adhesive force of ACTIVA compared with a composite has been observed in dentin when no adhesive agent is used [24,25], according to our results, the use of adhesive for cementing brackets can be dispensed with since this led to significantly better results.

Meanwhile, in a study evaluating the adhesive strength of ACTIVA to dentin, without the use of an intermediate adhesive agent, it was observed that flowable and fibre-reinforced composites had superior adhesive strength [24]. In a randomised controlled prospective clinical trial, Van Dijken et al., found that the use of ACTIVA in posterior teeth restorations, after etching but without the application of a bonding agent, resulted in an unacceptable frequency of adhesive failures, suggesting too weak a bonding between ACTIVA and the walls of the cavity [25]. Enamel has a higher mineral content than dentin and so a bonding agent may be necessary in dentin, while our results suggest that during cementing brackets to intact enamel brackets the bonding agent is not necessary.

As regards microfiltration, no significant differences were detected between the three evaluated groups. As in the case of adhesive force, no studies have evaluated microfiltration in brackets using ACTIVA. However, some authors have evaluated this problem in class II cavities in temporary molars and concluded, like us, that microfiltration of ACTIVA, without an adhesive agent [26,27] and with an intermediate adhesive, was comparable to that observed with composites. In addition, ACTIVA microfiltration was similar regardless of whether or not the adhesive agent was applied [27]. On the other hand, in a study evaluating microfiltration in class II cavities in premolars, it was observed that composite materials had better microfiltration properties than ACTIVA without adhesive agent [24]. The difference in the results obtained in the different studies could be due to the fact

that in deciduous molars [26,27], the cervical margin of the cavity is above the cement-enamel junction and so this wall contains enamel, while in the premolar study [24] the gingival wall is located below the cement-enamel junction. As this area does not contain enamel, adhesion without adhesive agent may have favoured the occurrence of greater microfiltration in the restorations with ACTIVA.

The release of fluoride by the GIC could have a preventive effect on the demineralisation of the enamel, and therefore, on the appearance of white spots around the brackets. GICs have optimal properties, which supports their use in clinical dentistry, such as excellent biocompatibility, they provide a direct bonding to tooth structure, and have the ability to release fluoride, which is important as an anticariogenic agent to prevent tooth decay [28]. The incorporation of a BAG into the ionomer, as is the case with ACTIVA [6], conditions the release not only of fluoride but also of other ions, such as calcium and phosphorus, thus preventing demineralisation [2]. In our work, fluoride release from ACTIVA was higher than when TXT was used and was affected by saliva pH. Thus, at a 3.5 pH, ACTIVA released more fluoride than at pH 6.4, while TXT did not release as much fluoride as ACTIVA and was not as affected by pH. The TXT secretion pattern did not follow a cumulative model, with increases and decreases observed at different time points at both and pH 3.5 and pH 6.4. Although it is not a component specifically introduced to achieve the net release of fluoride ions, diphenyliodonium hexafluorophosphate is probably responsible for releasing fluoride into saliva. Rather, it is used to increase the efficiency of polymerisation systems based on camphorquinone by increasing the conversion of resin monomers and improving the physical and chemical properties of the resulting polymers [29]. However, when in contact with aqueous phases it undergoes a slow hydrolysis with the consequent release of fluoride [30].

Fluoride in ACTIVA comes from sodium fluoride and the filler material, which was reactive glass filler (21.8 wt.%) in some studies [7] and bioactive glass (BAG) (55.4 wt.%) in others [6]. Fluoride release followed a cumulative pattern for the first 30 days. However, unlike RMGICs, which have a pattern of strong release for the first 24 h (the "initial burst") followed by a substantial reduction during the following 2–3 days to reach a plateau at 2–4 weeks, the pattern followed by ACTIVA in our study was typical of bioactive glasses: an initial fall in the first few hours, followed by a plateau or increase, depending on the concentration of bioactive glass in the material and the physico-chemical characteristics of the medium [2].

The effect of acid pH on the release of fluoride from ACTIVA was significantly stronger at all time points, involving a ten-fold increase at some time points. A 3–4-fold increase in the amount of fluoride ions released by RMGICs at pH 4 has been described, with the initial burst period depending directly on the pH value [31], while the total time depends on the surface degradation caused by acid pH [13,32]. This would be an important mechanism in ACTIVA for preventing demineralisation lesions (white spots) in vulnerable areas such as those around brackets, where the pH values of the biofilm are very low.

Cytotoxic studies are a useful tool for evaluating the impact of new materials on stem cell viability and their possible influence on healing tissues [33,34]. Furthermore, mesenchymal stem cells are commonly used in experimental studies due to immunomodulatory properties, and are sensitive to mechanical and physical stimuli [35,36]. The results obtained in the present study point to the better cytocompatibility of ACTIVA compared with TXT in terms of cell viability and migration capacity. Supporting our findings, Toy et al., demonstrated the cytotoxic effect of TXT on buccal epithelial cells due to residual monomers [37], which may have been directly related to unreacted residual monomers, such as Bis-GMA, the primary component of TXT. Previous studies have shown that inefficient polymerisation may result in numerous residual monomers, which are liable to dissolve out and cause significant cytotoxicity, in addition to weakening the mechanical properties [38,39]. Nevertheless, ACTIVA showed good cytocompatibility and cell migration capacity, which can be attributed to the bioactivity of this material. In the same line, Abou ElReash et al. [40] demonstrated that ACTIVA favoured tissue healing and exhibited

biocompatibility in vivo. The analysis described above enables the null hypothesis (that there was no difference between the groups) to be rejected and suggest that ACTIVA can be considered as suitable material for cementing brackets.

Finally, the current study's limitation is that in vitro biophysical or ion release studies cannot accurately represent local inflammation conditions and the influence of bone remodelling. More in vivo studies are needed to examine bioactive compounds' safety and efficacy when applied in clinical orthodontics.

Within the limitations of the present study, it can be concluded that ACTIVA provides optimal adhesive and microfiltration properties, releases substantial amounts of fluoride ions in both acid and neutral media, and its biocompatibility is greater than that of traditional resin composite adhesive systems.

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