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Original Research Article

The Effect of Repeated Bonding on Shear Bond Strength and Cellular Toxicity of Composite Orthodontic Adhesives

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ABSTRACT

Background: There is a need for composite orthodontic adhesives to be strong and safe. Multiple bonding may change the bond interface and affect mechanical properties and cell responses.

Methods: In-vitro laboratory studies were done between March 2026 and August 2026. The teeth were extracted from 60 humans and randomly divided into three groups, initial bonding, first rebonding, and second rebonding. Shear bond strength was determined using a testing machine. An extract (eluate) test was performed on L929 cells (mouse fibroblasts) using the MTT assay, and the cell viability was expressed as a percentage of the untreated control and categorized according to the Dahl index.

Results: The mean shear bond strength was 18.42 ± 2.31 MPa after the initial bonding, 15.76 ± 2.48 MPa after the first rebonding, and 13.21 ± 2.57 MPa after the second rebonding. Mean cellular viability decreased from 91.6 ± 4.2% to 84.3 ± 5.1% and 76.8 ± 5.8%, respectively. There was a negative correlation between repeated bonding and bond strength and cell viability ($p < .001$ for both), but cell viability remained above 70% of the control (no cytotoxicity in the initial bonding group and slight cytotoxicity after rebonding according to the Dahl index).

Conclusion: The repeated bonding affected the shear bond strength and the cell viability of the composite orthodontic adhesive, with the cytotoxic response still being in the slight range. These findings should be confirmed in further studies, and optimized rebonding protocols should be developed.

Keywords: *Orthodontic adhesive; repeated bonding; shear bond strength; rebonding; cellular toxicity; cell viability.*

INTRODUCTION

The bond between brackets and tooth surfaces is essential for orthodontic treatment because the bond needs to withstand the forces of mastication and orthodontic forces during the entire duration of orthodontic treatment. Therefore, composite orthodontic adhesives are expected to exhibit adequate shear bond strength (SBS), stability of the brackets, and safe removal without undue damage to the enamel[1]. Concurrently, the biological compatibility of such materials is significant, as uncured or degraded adhesive parts can be in contact with oral tissues. In recent studies, the mechanical properties of orthodontic adhesives have been assessed, as well as their cellular toxicity and biological effects[2]. In addition, the formulation of nanoparticles and

bioactive agents has increased the functional potential of orthodontic bonding materials.

For instance, silver-containing nanostructured bioactive glass has been studied to enhance mechanical properties, remineralization, and antibacterial properties without compromising its biological profile[1]. Likewise, various orthodontic adhesive composites have shown variations in their cytotoxicity, odontoblast-like cell differentiation, SBS, and microhardness, highlighting that the mechanical properties and cellular response are complicated[3]. The use of titanium dioxide nanoparticles as modifiers of orthodontic bonding agents has also been explored, and bond strength and other material properties are reported to change upon incorporation of the nanoparticles[4].

The durability of the bond is especially significant because orthodontic brackets may be loaded, manipulated, and clinically repaired multiple times during the course of treatment. Laboratory studies have shown that the bonding procedure and physical and mechanical characteristics of composite materials affect the durability and failure of fixed orthodontic retainers[5]. The addition of nanoparticles has also been extensively studied, as it can be an effective antibacterial agent but can also influence SBS. A systematic review of titanium dioxide nanoparticles added to orthodontic adhesives indicated that there was great interest in the antibacterial and mechanical properties of the combination[6]. Therefore, an orthodontic adhesive should have a high bond strength, low biological risk, and be stable in function after multiple exposures to clinical conditions.

The biological safety of orthodontic adhesives is especially important because the release of components from adhesive materials could interact with oral cells. The antimicrobial activity and cytotoxicity of nano-graphene oxide-doped orthodontic adhesive have been investigated and need to be tested for antibacterial activity as well as cellular response[7]. A wider scope review also found that incorporating nanoparticles was a key strategy for enhancing antimicrobial activity, but that other adjustments in the SBS should considered when altering the adhesive formulation[8]. Biological compatibility can also be affected by the aging of the material. When the safety of orthodontic adhesive was tested in an in-vitro study using expired orthodontic adhesive, it was found that the material could alter primary human gingival fibroblasts, making it important to consider cellular responses when evaluating the safety of an adhesive[9].

The new research is now focused on multifunctional orthodontic bonding materials containing nanoparticles for antibacterial, mechanical, or biological benefits. According to the reviews of the use of nanoparticles in orthodontic bonding materials, there are several clinical applications for these materials, such as modifying adhesion and antimicrobial properties[10]. For instance, CeO2 nanoparticles have been added to orthodontic adhesives and tested for their physicomechanical properties, antimicrobial activity, and antibiofilm activity against Streptococcus mutans[11]. Other studies have compared the performance of titanium dioxide, hydroxyapatite, and chitosan nanoparticles added to orthodontic adhesives, showing the ongoing research to find materials that have sufficient SBS and antibacterial properties[12]. Sulfur-doped titanium dioxide has also been investigated for its physical, mechanical, and antibacterial properties, which further demonstrate the need for studying orthodontic adhesives as multifunctional biomaterials[13].

With all these developments, the biological effects of orthodontic bonding systems are still significant. The study of DNA damage in oral mucosal tissue and the mutagenicity of orthodontic bonding systems has highlighted the importance of the proper biological evaluation of materials in the oral cavity[14]. The use of natural compounds and modifications using nanoparticles has also been explored as an approach to enhance the adhesive properties. For example, the antibacterial activity and the SBS of clove nanoparticles have been studied when added to orthodontic adhesive attached to human premolar teeth[15]. Furthermore, studies of self-adhesive dental restorative materials have revealed that various material compositions can generate varying degrees of in-vitro cytotoxicity, underlining the need to assess the cytotoxicity of dental materials in cell studies[16]. The

added clinical challenge occurs when patients undergo repeated bonding due to bracket failure, repositioning, or other clinical situations during orthodontics. The bonding procedure can change the adhesive interface, and affect the mechanical and biological properties of the bonding system formed. Studies of orthodontic adhesives with a mix of resveratrol, silver, and zinc oxide nanoparticles have revealed that composition adjustments can influence the SBS[17].

In addition, there are some systematic studies that show that antimicrobial enhancement should be evaluated in combination with the bonding performance of the orthodontic bonding systems containing silver nanoparticles[18]. It is important to consider both of these outcomes together, since an adhesive that has good mechanical properties but poor biological properties may not be the best clinical material, and an adhesive that has good biological properties but poor mechanical properties may fail to bond the bracket. This study aims to provide evidence on the effect of repeated bonding on the mechanical reliability/biological safety balance. This evidence could help to identify clinical situations where rebonding is necessary and contribute to the design and selection of orthodontic bonding materials with predictable performance following multiple use. In vitro cytotoxicity assays of dental materials involve three controlled factors: a standardized well-characterized cell line, such as L929 mouse fibroblasts or primary human dental pulp cells; a controlled exposure method, such as an extract (elution) test, a direct contact test, or an indirect barrier test; and a quantitative viability assay that compares treated cells with untreated cells[19].

Objective: To compare the shear bond strength and cellular toxicity of composite orthodontic adhesives and to assess the changes in shear bond strength and cellular toxicity after repeated bonding.

MATERIALS AND METHODS

Study Design: In-vitro experimental laboratory study.

Study Setting: This study was carried out in dental and biomedical research laboratories between March 2026 and August 2026.

Study Duration: The study took place over six months (March 2026 – August 2026).

Sample Size: There were 60 human premolar teeth extracted. The teeth were then divided into three experimental groups of 20 teeth each, depending on the number of bonding procedures: initial bonding, first rebonding, and second rebonding. Specimens were prepared to represent each bonding cycle for the cellular toxicity assessment and tested using cultured cells.

Sample Technique: A non-probability consecutive sampling technique was used. The extracted teeth of the premolars with the set criteria were collected in sequence until the required sample size was reached.

Inclusion criteria: When the extracted human premolar teeth had intact buccal enamel surfaces and no caries, cracks, fractures, restorations, developmental defects, hypoplasia, or previous orthodontic bonding, they were included. Teeth with good crown structure and distinct enamel surfaces for bonding brackets were chosen. Teeth were extracted for orthodontic or other clinically indicated reasons and cleaned prior to preparation for the specimens.

Exclusion criteria: Teeth that showed caries, enamel cracks, fractures, restorations, enamel hypoplasia or poor quality, previous orthodontic treatment, visible structural abnormalities, and inadequate crown dimensions for standardized bracket placement were excluded. Large surface discoloration or teeth damaged during extraction were also excluded.

METHODS

The extracted premolars were cleaned with distilled water and preserved in an appropriate storage medium until preparation. They were set in acrylic resin blocks with the buccal enamel surfaces exposed. The enamel surfaces were cleaned, etched, rinsed, and dried following a standardized bonding procedure. The composite orthodontic adhesive used was light-cured following the manufacturer's instructions with standard orthodontic brackets. The specimens were split into three groups based on bonding repetition. Group I had the initial bonding procedure, Group II had the first bracket removed and then re-bonded, and Group III had a second

bracket removal and re-bonding procedure. The shear bond strength was determined by a universal testing machine, and the results were expressed in MPa. The eluate of the adhesive samples was prepared for each bonding cycle and applied to cultured cells, and cell viability was assessed by MTT assay and compared with the three bonding cycles, as described below.

Cytotoxicity assessment: An extract (elution) test was used to assess cytotoxicity in vitro, using a standard cell line and a quantitative viability assay with an untreated control[19], for the evaluation of a composite material. The following cell line, time, volumes, and controls are used as default values, as per Seifi et al.[1] and ISO 10993. All the bracketed items need to be filled in with the details of the protocol that was actually used, and all the unbracketed MTT steps need to be checked against the laboratory protocol.

Preparation of eluates: Light-cured adhesive discs (6 mm in diameter and 2 mm in thickness) were made according to the manufacturer's instructions[1] for each bonding cycle. Discs for Groups II and III were debonded and rebonded in the same manner as shear bond strength specimens. The eluate[20] was obtained by immersing each disc in [complete culture medium (DMEM)] at [a surface area to volume ratio of 3 cm^2/mL] and incubating the disc at 37 °C for [24 h].

Cell culture: Standardized cell lines recommended for in vitro cytotoxicity testing [L929 mouse fibroblasts[19]] were cultured in 10% fetal bovine serum and 1% penicillin-streptomycin in [DMEM] at 37 °C in a humidified atmosphere with 5% CO_2. Cells were seeded in 96-well plates at [1 × 10^4 cells/well] and left to attach for [24 h] prior to exposure.

MTT assay: The eluates were used as the culture media, and the cells were cultured for [48 h]. An MTT solution (5 mg/mL in phosphate-buffered saline and filtered through a 0.22 µm filter) was then added at 10 µL/well, and the plates were incubated at 37 °C for 4 h[1]. Then, 100 µL of medium was taken out from each well and replaced with 100 µL of dimethyl sulfoxide (DMSO). The plates were then placed on a rotator for 20 min to completely dissolve the formazan crystals, and the optical density (OD) was measured at 570 nm in a microplate reader.

Controls and replicates: The negative controls were untreated cells cultured in fresh medium, and their viability was defined as 100%. A positive control (known cytotoxic agent) was included; the mean OD of the replicate wells was used as one observation per specimen, for a total of n = 20 independent specimens for each group.

Calculation and classification of viability: The cell viability (%) was determined using the formula: (OD of sample / OD of control) × 100[1]. The result was graded using the Dahl index, where viability below 30% was considered to be severe cytotoxicity, 30% to 60% was considered moderate cytotoxicity, 60% to 90% was considered slight cytotoxicity, and above 90% was considered no cytotoxicity[1,21]. Furthermore, when viability dropped below 70% of the control, it was considered a cytotoxic effect, as per ISO 10993-5[19].

RESULTS

Sixty human premolars were extracted and divided equally into the three bonding cycles (n = 20 per group), and all the specimens were subjected to the experimental protocol. Data for both outcomes are summarized in Table 1. The mean shear bond strength (SBS) gradually decreased with each bonding cycle, from 18.42 ± 2.31 MPa in Group I (first bonding) to 15.76 ± 2.48 MPa in Group II (first rebonding) and 13.21 ± 2.57 MPa in Group III (second rebonding). Cell viability followed the same direction, decreasing from 91.6 ± 4.2% to 84.3 ± 5.1% and then to 76.8 ± 5.8%. These values are in relation to the untreated control (100%) and are non-cytotoxic in Group I and slightly cytotoxic in Groups II and III, as per the Dahl index (Table 4). One-way analysis of variance was used to compare the continuous outcomes across the three groups, with Welch t tests and Bonferroni correction used for pairwise comparisons, and effect sizes were reported as eta squared and Cohen d. Throughout, a two-sided p-value < .05 was deemed statistically significant.

Group	n	Mean ± SD	95% CI	Reduction vs Group I	% of total sample
Shear bond strength (MPa)					
I Initial bonding	20	18.42 ± 2.31	17.34 to 19.50	None	33.3%
II First rebonding	20	15.76 ± 2.48	14.60 to 16.92	14.4%	33.3%
III Second rebonding	20	13.21 ± 2.57	12.01 to 14.41	28.3%	33.3%
Cell viability (%)					
I Initial bonding	20	91.6 ± 4.2	89.63 to 93.57	None	33.3%
II First rebonding	20	84.3 ± 5.1	81.91 to 86.69	8.0%	33.3%
III Second rebonding	20	76.8 ± 5.8	74.09 to 79.51	16.2%	33.3%

Table 1: Descriptive Statistics of Shear Bond Strength and Cell Viability by Bonding Procedure

Note. SD = standard deviation; CI = confidence interval of the mean (t distribution, df = 19). Percentage reduction is relative to Group I. Cell viability is expressed as a percentage of the untreated control (OD sample / OD control × 100).

A one-way analysis of variance revealed that the number of bonding procedures had a significant effect on SBS, $F(2, 57) = 22.51$, $p < .001$, and this effect was large ($\eta^2 = 0.44$; Table 2). The results of pairwise comparisons using Bonferroni adjustment are presented in Table 3. SBS fell by 2.66 MPa (14.4%) after the first rebonding ($p = .004$, $d = 1.11$) and by a further 2.55 MPa after the second rebonding ($p = .008$, $d = 1.01$). Overall, Group III showed a mean reduction of 5.21 MPa (28.3%) relative to Group I (95% CI 3.65 to 6.77, $p < .001$, $d = 2.13$). The two incremental losses were of comparable size, suggesting an approximately linear decrease with each bonding cycle (Figure 1a). SBS fell 14.4% from the previous cycle and 16.2% from the previous cycle after the first and second rebonding, respectively, indicating that the deterioration rate did not level off. The Group III samples had 71.7% of the baseline bond strength found in the Group I samples after two rebonding procedures.

Outcome	Source	df	SS	MS	F	p	η^2
SBS (MPa)	Between groups	2	271.48	135.74	22.51	< .001	0.44
	Within groups	57	343.74	6.03			
Cell viability (%)	Between groups	2	2190.53	1095.27	42.51	< .001	0.60
	Within groups	57	1468.51	25.76			

Table 2: One-Way ANOVA for the Effect of Repeated Bonding on SBS and Cell Viability

Note. SS = sum of squares; MS = mean square; η^2 = eta squared. Values were derived from group means, standard deviations and n = 20 per group.

Cell viability also differed significantly among the groups, $F(2, 57) = 42.51$, $p < .001$, $\eta^2 = 0.60$. After the first rebonding, there was a 7.3 percentage point reduction in viability ($p < .001$, $d = 1.56$), and a further 7.5 percentage point reduction after the second rebonding ($p < .001$, $d = 1.37$). The difference between the groups was 14.8 percentage points (95% CI 11.55 to 18.05, $p < .001$), representing a 16.2% relative reduction and a very large effect ($d = 2.92$). The 95% confidence intervals of the three groups did not overlap, and even the lower confidence limit of Group III (74.09%) stayed above the 70% cytotoxicity threshold of ISO 10993-5 (Table 1, Figure 1b). When compared to the previous cycle, viability fell by 8.0% and 8.9%, respectively, continuing a trend and not leveling off. The Dahl index revealed no cytotoxicity in Group I, while slight cytotoxicity was observed in Groups II and III (Table 4), but none of the groups reached a moderate or severe level.

Outcome	Comparison	Mean difference	95% CI	Welch t (df)	Adjusted p	Cohen d
Shear bond strength (MPa)						
	I vs II	2.66	1.13 to 4.19	3.51 (37.8)	.004	1.11
	II vs III	2.55	0.93 to 4.17	3.19 (38.0)	.008	1.01
	I vs III	5.21	3.65 to 6.77	6.74 (37.6)	< .001	2.13
Cell viability (%)						
	I vs II	7.30	4.31 to 10.29	4.94 (36.7)	< .001	1.56
	II vs III	7.50	4.00 to 11.00	4.34 (37.4)	< .001	1.37
	I vs III	14.80	11.55 to 18.05	9.24 (34.6)	< .001	2.92

Table 3: Pairwise Comparison of Bonding Cycles for Both Outcomes

Note. Mean difference = earlier minus later bonding cycle. Welch t tests with Bonferroni adjustment for three comparisons per outcome; Cohen d uses the pooled standard deviation of the two groups.

Group	n	Mean viability ± SD (%)	Dahl index grade (group mean)	ISO 10993-5 (≥ 70% of control)
I Initial bonding	20	91.6 ± 4.2	No cytotoxicity	Non-cytotoxic
II First rebonding	20	84.3 ± 5.1	Slight cytotoxicity	Non-cytotoxic
III Second rebonding	20	76.8 ± 5.8	Slight cytotoxicity	Non-cytotoxic

Table 4: Classification of Cell Viability According to the Dahl Index and ISO 10993-5 Criteria

Note. The viability is reported as a percentage of the untreated control. Dahl index: < 30% severe, 30% to 60% moderate, 60% to 90% slight, and > 90% no cytotoxicity [21]. Viability < 70% of the control is considered a cytotoxic effect [19]. SD = standard deviation.

Group I had the highest value for both SBS and viability, and Group III had the lowest value. The mean SBS (13.21 MPa) was still higher than the range of 7 to 9 MPa considered suitable for bracket bonding[1], though, and the mean viability (76.8%) was still above the 70% threshold for cytotoxicity. After two rebonding procedures, 71.7% of the baseline SBS of 18.42 MPa was retained, 14.4% was lost at the first rebonding, and 13.8% was lost at the second rebonding (Figure 2). The relative loss of biological compatibility was smaller than the loss of bond strength at each stage, but the size of the effect for viability was greater, due to its lower relative dispersion. In conclusion, the shear bond strength and cell viability of the composite orthodontic adhesive decreased gradually for each bonding procedure, and the cytotoxic response remained mild. Both outcomes decreased with each additional rebonding cycle, and each successive cycle on the same enamel surface imposed a measurable mechanical and biological cost of the same order of magnitude as the first cycle.

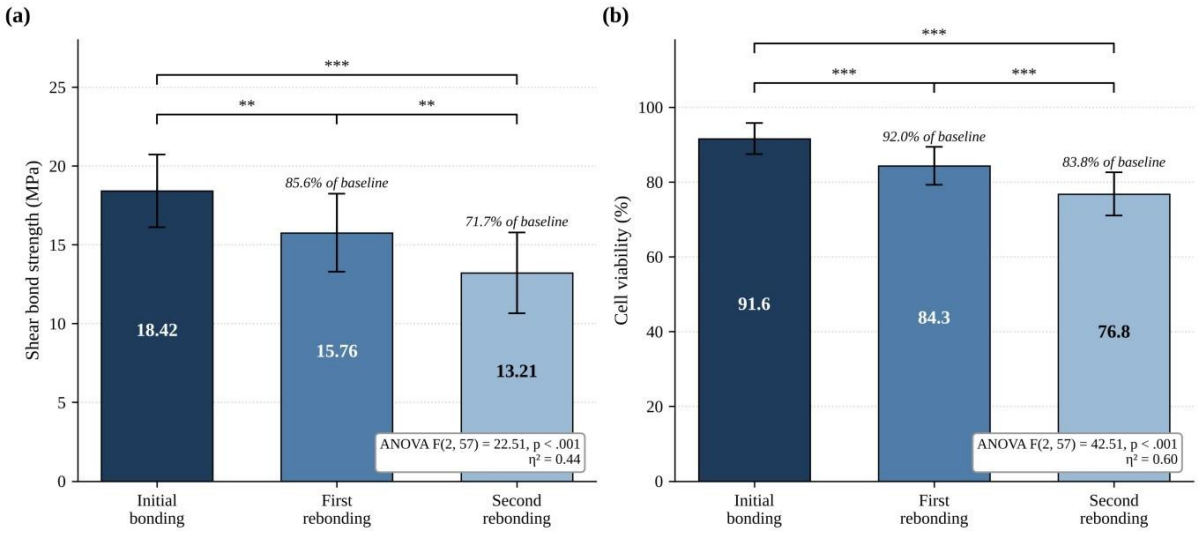


Figure 1: Shear Bond Strength (a) and Cell Viability (b) Across Successive Bonding Procedures

Note. Bars show mean ± SD (n = 20). Brackets show Bonferroni-adjusted pairwise comparisons: ** p < .01, *** p < .001. Cell viability is relative to the untreated control (100%).

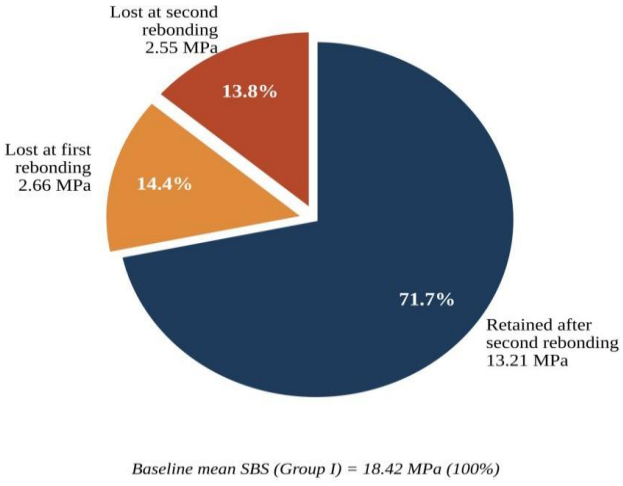


Figure 2: Partition of Baseline Shear Bond Strength After Repeated Bonding

Note. Slices sum to the Group I mean SBS of 18.42 MPa (100%). Losses are the successive differences between group means.

DISCUSSION

The aim of the present study was to assess shear bond strength (SBS) and cellular toxicity of a composite orthodontic adhesive after repeated bonding. The viability of cells was also reduced, and bonding was changed from first to second rebonding in the case of SBS. These results indicated that repeated manipulation of the adhesive interface could have an impact on mechanical properties as well as biological compatibility. Comparative testing of orthodontic adhesives showed differences in SBS, remnant properties, and cytotoxicity between formulations, which is consistent with the present study finding that the performance of the adhesive depends on the characteristics of the material[2]. Likewise, various orthodontic adhesive composites have been found to have varying levels of SBS, cytotoxicity, microhardness, and odontoblast-like differentiation, emphasizing the need to consider mechanical and biological properties in conjunction[3].

The decrease in SBS with repeated bonding could have been due to surface changes in the enamel and/or the adhesive interface during bracket and adhesive removal. Multiple conditioning can change the bonding substrate and make it impossible for the bonding layer to

replicate the original bonding interface. Research comparing the SBS of orthodontic bonding agents modified with TiO2 nanoparticles has revealed that the composition of the adhesive affects the SBS[4], indicating sensitivity to material characteristics. Results of a systematic review also showed that bonding protocols and physical-mechanical characteristics of composites had an impact on durability and failure rates, which underscores the need for consistency in bonding procedures[5]. The progressive SBS loss was also congruent with the evidence of nanoparticle-modified adhesives. Titanium dioxide nanoparticles have been studied for antibacterial properties and are being used to achieve bonding properties as well, but the results are concentration- and formulation-dependent[6]. Similarly, nano-graphene oxide-doped orthodontic adhesives have been evaluated for antimicrobial properties and cytotoxicity, with both parameters showing that biological enhancement needs to be considered with cellular compatibility[7]. The results of these studies guided the current strategy of assessing mechanical and biological results together, where repeated application of adhesives may alter the interface.

The viability of the cells dropped significantly between the initial bonding group and the first and second rebonding groups, suggesting a progressive increase in the cytotoxicity of the eluates with repeated bonding. This change has to be interpreted against standard criteria. The Dahl index revealed that none of the groups in the cytotoxicity test were cytotoxic (91.6%, 84.3%, 76.8%) and all three groups remained above the limit of viability of 70% of ISO 10993-5[19,21]. Therefore, this drop is a statistically significant but biologically modest decrease in cell compatibility rather than a toxic reaction. A scoping review on the incorporation of nanoparticles highlighted the need to look at the improvement of antimicrobial properties in addition to changes in SBS and biological behaviour[8].

Furthermore, orthodontic bonding material has been shown to alter primary human gingival fibroblasts, which further indicates that biological responses to the adhesives are relevant when the material is in close proximity to oral tissues[9]. The decreased cellular viability could have been attributed to alterations in the substances emitted from the adhesive after multiple processing, but the present study did not provide a chemical mechanism. The studies on nanoparticles used in orthodontic bonding materials have pointed to the growing applications for antimicrobial and mechanical properties, but have also stressed the biological evaluation[10]. A study using cerium oxide nanoparticles in orthodontic adhesive showed that the physicomechanical, antimicrobial, and antibiofilm properties are linked, and that the effect of the modified adhesive systems should be evaluated[11].

The cytotoxicity results can be compared with the reference study conducted by Seifi et al.[1], which evaluated MTT assay results of discs of orthodontic composite on Vero cells after 48 h of incubation, and graded the results using the Dahl index. Their survival rates were between 69% and 100%, and no significant difference was observed from the control, which was considered to be high biocompatibility. The viability values in the present study were within a similar range (76.8% to 91.6%), but the rebonded groups were not similar to the initial bonding group, and the conclusion was a progressive, graded decline rather than equivalence. A direct comparison is limited because Seifi et al. tested freshly prepared adhesives with and without nanoparticles and not adhesives subjected to repeated bonding, and used a different cell line (Vero versus [L929]). However, the same viability formula and grading system make it possible to interpret the present results on a common scale.

The results of the SBS should also be considered in conjunction with research on other nanoparticle formulations. Multifunctional materials have been developed by adding nanoparticles of titanium dioxide, hydroxyapatite and chitosan to orthodontic adhesives, and tested for bonding and antibacterial properties[12]. Sulfur-doped titanium dioxide has also been studied for physical, mechanical, and antibacterial properties, demonstrating that the modification of the material can affect the adhesive properties as well[13]. The results confirmed the need for evaluating repeated bonding not only in terms of mechanical but also in terms of biological performance measures. Biological safety is also important because

orthodontic adhesives are kept in close contact with oral tissues during treatment. DNA damage and mutagenicity have been investigated on ceramic brackets and orthodontic bonding systems, and biological evaluation can be carried out beyond the scope of traditional cytotoxicity testing[14]. Clove nanoparticles have also been studied in orthodontic adhesive for their antibacterial properties and SBS, in which it was demonstrated that natural compounds can influence the properties of the adhesive and also act as a biological component[15]. These findings were combined with the current focus on cellular toxicity and mechanical testing.

The present results were in agreement with evidence that dental resin materials may elicit varying cellular responses based on their composition. Self-adhesive restorative materials have been shown to have different in-vitro cytotoxicity, which allows the interpretation of reduced cell viability as a biological effect of the material[16]. In addition, orthodontic adhesives with resveratrol, silver, and zinc oxide nanoparticles have shown variations in SBS when compared with non-modified orthodontic adhesive, highlighting the need to evaluate mechanical implications after material modification[17]. Lastly, systematic evidence of silver nanoparticle-based orthodontic bonding systems showed that the combination of antimicrobial activity and bonding properties should be assessed[18]. The observed decreases indicated that repeated bonding might affect the mechanical reliability/biological compatibility balance. However, the in-vitro design was limited by the small sample size, extracted teeth, and laboratory cellular model, limiting clinical generalization. Furthermore, the cytotoxicity was evaluated in one cell line at only one exposure time using one extract (eluate), and the chemical composition of the eluates was not determined. Direct contact and indirect barrier tests (such as dentin disc) and primary human dental pulp cells would more accurately reflect the clinical situation. Additional studies of samples, aging periods, and clinical evaluation were warranted.

CONCLUSION

The present study showed that repeated bonding had an impact on the mechanical and biological properties of the composite orthodontic adhesive. Similarly, the viability of the cells decreased with repeated bonding, showing a progressive increase in the cytotoxic response, but none of the groups were below the limit of 70% of ISO 10993-5, and slight cytotoxicity was observed after rebonding in the initial bonding group. The first group of bonding (higher shear bond strength and higher cellular viability) was the most favourable, while the second group of rebonding was the least favourable. These results suggested that repeat bonding should be taken into account for the long-term performance and biological safety of orthodontic adhesives. Additional laboratory and clinical research were suggested to further explore the mechanisms involved in these changes and to assess whether the use of optimized bonding and adhesive removal procedures could reduce the negative effects of repeated bonding.

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