Contents lists available at ScienceDirect



Journal of the Mechanical Behavior of Biomedical Materials

journal homepage: www.elsevier.com/locate/jmbbm



The biomechanical effect of root amputation and degree of furcation involvement on intracoronally splinted upper molar teeth – An *in vitro* study^{$\star, \star \star$}

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ARTICLE INFO	A B S T R A C T				
Keywords: Furcation involvement Root amputation Intracoronal splinting Fracture resistance Biomechanical behavior	<i>Objectives</i> : The aim of this study was to evaluate the effect of the amount of periodontal support and the presence or absence of root amputation on the fracture resistance of intracoronally splinted maxillary molar teeth. <i>Materials and methods</i> : 48 extracted human upper first molars and 48 s premolars were included in the study. All teeth underwent standard mesio-occluso-distal (MOD) (molars) and standard occluso-distal (OD) (premolars) cavity preparation. After the preparation, all molars were root canal treated, and 48 molar-premolar units were created by intracoronal splinting. The units were randomly divided into 4 groups (Groups A-D, 12 units per group): in Groups C and D, the disto-buccal (DB) roots of the molars were amputated, while in Groups A and B, no root amputation was performed. All units were embedded in methacrylate resin at different levels: in Groups A and C, at 4 mm apically from the cemento-enamel junction (CEJ), while in Groups B and D, at 6 mm apically from the CEJ, mimicking the different stages of furcation involvement. All units were submitted first to dynamic and then to static, load-to-fracture mechanical testing. Fracture resistance values were recorded fracture mode was analysed.				
	was analysed. <i>Results:</i> During the load-to-facture test, Groups A and B (without root amputation) were characterized by significantly higher fracture resistance values compared to Groups C and D (with root amputation) ($p < 0.05$). Regarding fracture mode, irreparable fracture was more frequent in Group D (with root amputation and advanced furcation involvement) than in any other group ($n = 8$). <i>Conclusions:</i> Root amputation has a negative effect on the fracture resistance of intracoronally splinted upper first molar-second premolar units with modeled furcation involvement.				

1. Introduction

Periodontitis is one of the most common oral health conditions among adults (Eke et al., 2016). According to epidemiological surveys, the incidence of periodontitis shows a quite uniform picture worldwide (Eke et al., 2015). Those types of periodontal disease that affect the supporting tissues usually result in irreversible destruction of the alveolar bone. Simultaneously with bone degradation, the periodontal ligaments are also damaged, which leads to attachment loss that –if left untreated- can result in tooth loss (Kurgan et al., 2014; Graetz et al., 2019). Deterioration of the attachment apparatus is usually a slow process and it shows individual differences in its extent and clinical appearance over time (Dommisch et al., 2020). In the case of multi-rooted teeth, a special situation known as furcation involvement may occur (American Academy of Perio, 2001; Derks et al., 2018). The treatment of this is one of the greatest challenges in periodontal therapy (Dommisch et al., 2020; Salvi et al., 2014; Jepsen et al., 2020). Periodontal disease is treated either in a conservative manner or surgically.

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https://doi.org/10.1016/j.jmbbm.2022.105143

Received 12 January 2022; Received in revised form 18 February 2022; Accepted 23 February 2022 Available online 4 March 2022 1751-6161/© 2022 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

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According to Hermann et al., by the time the furcation has been exposed, more than 30% of the available attachment surface has been lost (Hermann et al., 1983). Also, due to the poor accessibility of the exposed furcal area, molar teeth respond less favorably to non-surgical periodontal treatment than single-rooted teeth (Walter et al., 2011), which may necessitate more invasive interventions (e.g. root amputation, etc.). Root amputation is the surgical procedure by which one or more roots of a multi-rooted tooth are removed at the level of the furcation whilst the crown and remaining roots are left in function (American Academy of Perio, 2001; Schmitz et al., 2020). Restoration and maintenance therapy of teeth that have undergone root amputation poses a serious challenge to dentist and patient alike. Both furcation involvement and root amputation can increase tooth mobility, which can lead to further loss of attachment (Wang et al., 1994), and intracoronal splinting of the affected posterior tooth with fiber-reinforced material may become necessary.

From a clinical perspective, degradation of the periodontal tissues most commonly affects multi-rooted, upper posterior teeth, which are difficult to clean and have complex anatomy (Svärdström and Wennström, 1996; Gonçalves et al., 2021). As a result, the second and third molars are often extracted earlier than the other teeth, and so the upper first molars may become the most distal teeth in the arch, exposed to excessive loading during chewing. It is characteristic of this unfortunate, yet common situation that the pre-existing periodontal bone destruction on the distal surface is further aggravated by the degeneration of the alveolar bone following extraction.

The question arises as to whether the presence or absence of root amputation and the remaining bone level may play a role in the fracture behavior of intracoronally splinted upper maxillary first molar teeth. In this study, our null hypothesis was that neither of the mentioned factors would have a significant effect on the maximal fracture resistance and fracture mode in intracoronally splinted upper maxillary first molars.

2. Materials and methods

2.1. Sample selection

All procedures of the study were approved by the Ethics Committee of the University of Szeged, and the study was designed in accordance with the Declaration of Helsinki.

In this study 48 maxillary molars and 48 maxillary premolars extracted for periodontal or orthodontic reasons were selected for this study. The freshly extracted teeth were immediately placed in 5.25% NaOCl for 5 min and then stored in 0.9% saline solution at room temperature until the soft tissues from the root surface were removed with hand scalers. All teeth were used within 6 months after extraction. Inclusion and exclusion criteria, and standardization regarding coronal and root dimensions were the same as in our previous studies (Szabó et al., 2017, 2019a). The inclusion criteria were visual absence of caries or root cracks, absence of previous endodontic treatment, posts or crown, or resorptions. Regarding the coronal dimensions of the molar teeth, approximately 80% of the specimens ranged 10-10.9 mm in the bucco-palatal dimension, and the rest were between 11 and 12 mm. The mesio-distal dimension of the specimens was also measured; a mean was calculated and specimens that fell within the $\pm 10\%$ range of the mean were included. The height of the specimens was between 8 and 9 mm measured from the cementoenamel-junction (CEJ). Also, root length was also standardized as follows: mesio-buccal: 12-14 mm, distobuccal: 11-13 mm, palatal 12-15 mm. Regarding the coronal dimensions of the premolars, 90% of the teeth ranged between 9 and 10 mm bucco-palatally. The average mesio-distal dimension was between 7 and 7.5 for 90% of the samples. Ten percent maximum deviation was allowed in the remaining 10% of the samples.

2.2. Cavity preparation and coronal restoration

Standardized occluso-distal (OD) (premolars) and mesio-occlusodistal (MOD) (molars) cavities were prepared in all teeth according to Cara et al. (2007), as follows: the bucco-palatal width (BPW) of the approximal box was prepared to two-thirds of the BPW of the tooth, and the occlusal isthmus was prepared to half the BPW. In addition, the cavity depth at the occlusal isthmus was also standardized to 3.5 mm from the tip of the lingual cusp and 1 mm above the CEJ at the cervical aspect of the approximal boxes. After cavity preparation, the molar teeth underwent root canal treatment according to the protocol described by Szabó et al. (2019a). The root canals were instrumented with Pathfiles (1-2-3) and ProTaper (S1-S2-F1-F2-F3) (Dentsply Maillefer, Ballaigues, Switzerland) to the working length. The specimens were irri-NaOCl alternated with gated with 5% 10% EDTA (ethylenediaminetetraacetic acid) with a 2-mL syringe and 25-gauge needle. Root canal filling was performed by matched-single-cone obturation with a master cone (F3 gutta-percha, Dentsply-Maillefer) matching the final instrument used for preparation and sealer (AH plus, Dentsply-Maillefer). Following root canal obturation, a base was applied to the pulp chamber in the form of an approx. 2 mm thick glass-ionomer barrier (Equia Forte, GC Europe, Leuven, Belgium).

After the root canal obturation, all cavity preparations were finalized. The cavosurface margins were prepared perpendicular to the tooth surface at the end of the preparation. The cavity was rinsed with water and air-dried with an air/water syringe. All premolar and molar specimens received the same adhesive treatment. The enamel was acidetched selectively with 37% phosphoric acid for 15 s, rinsed with water and air-dried. The cavity was adhesive-treated with G-Premio Bond (GC Europe) according to the manufacturer's instructions. The adhesive layer was light-cured for 40 s with an Optilux 501 halogen light in standard mode at a light intensity of 740 \pm 36 mWcm². The gingival boxes were levelled till the position of the occlusal cavity with packable composite resin (G-aenial Posterior A3, GC Europe) to aid the positioning of the splinting fibers. Premolar and molar tooth pairs were randomly selected and embedded in silicon material (Elite HD Putty, Zhermack SpA, Badia Polesine, Italy) to stabilize the teeth during the intracoronal splinting procedure (Fig. 1).

Splinting was carried out with long E-glass fibers (everStick Perio, GC Europe). The size of the cavities was measured with a periodontal probe and fiber bundles were cut to the adequate length. After adhesive treatment of the fiber bundle splint according to manufacturer's instructions, the fibers were positioned into a layer of highly filled flow-able composite resin (G-aenial Universal Flo A3, GC Europe) and light cured for 1 min. The remaining part of the cavities were restored with packable composite resin (G-aenial Posterior A3, GC Europe) in an oblique layering manner. Each layer was light cured for 40 s.

The splinted models were randomly divided into 4 groups (n = 12, Group A, B, C and D). In Group C and D each disto-buccal (DB) root was sectioned horizontally at the level of the furcation with a fissure diamond bur (881.31.014 FG – Brasseler USA Dental, Savannah, GA). The sectioned surfaces were smoothened to eliminate any remnants below the sectioning level to have a cleansable non-retentive surface. In Group A and B no root amputation was performed.

The restored specimens were kept wet (Isotonic Saline Solution 0.9%; B. Braun, Melsungen, Germany) in an incubator (37 °C). The root surface of each specimen was coated with two layers of liquid latex separating material (Rubber-Sep, Kerr, Orange, CA, USA) prior to embedding for mimicking the periodontal ligament. To simulate the bone level, the restored teeth were embedded in methacrylate resin (Technovit 4004, Heraeus-Kulzer, Germany). The artificial bone level was set at 4 mm from the cementoenamel junction (CEJ) in Group A and C, simulating mild furcation involvement (Fig. 2 A and C), while in Group B and D 6 mm from the CEJ, simulating more advanced/moderate furcation involvement (Fig. 2 B and D).

Mechanical testing was carried out in two phases. In the first phase

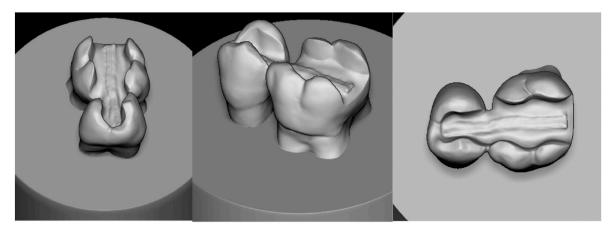


Fig. 1. The images show the positioning of the teeth and the splint during the restorative procedures.

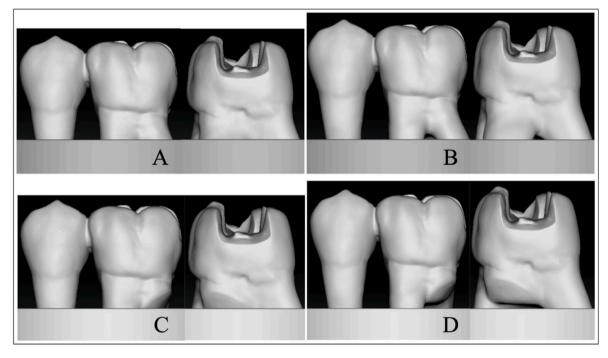


Fig. 2. Three-dimensional virtual reconstructions to illustrate the four study groups: A: Group A, no root amputation and mild furcation involvement (4 mm), B: no root amputation and advanced/moderate furcation involvement (6 mm), C: root amputation and mild furcation involvement (4 mm), D: root amputation and advanced/moderate furcation involvement (6 mm).

(pretesting), the restoration-tooth units were firstly submitted to an accelerated fatigue-testing protocol (Fráter et al., 2021a, 2021b), performed with a hydrodynamic testing machine (Instron ElektroPlus E3000, Norwood, MA, USA). Cyclic isometric loading was applied on the connector part of the splinted teeth units with a 5 mm wide, round ended metallic tip. Cyclic load was applied at 5 Hz, starting with gradually increasing static loading till 100 N in 5 s, followed by cyclic loading in 100 N steps up to 500 N, with 5000 cycles per step. The specimens were loaded until fracture occurred or up to 25,000 cycles. This phase served the purpose of simulating biting forces occurring during normal mastication. In the second phase, the survived specimens underwent static load-to-fracture testing (Lloyd R1000, Lloyd Instruments Ltd., Fareham, UK) at a crosshead speed of 2 mm/min. This phase simulated the occurrence of traumatic forces. A force vs. extension curve was dynamically plotted for each specimen. Fracture threshold, defined as the load at which the tooth-restoration complex exhibited the first fracture (detectable as peak formation on the extension curve), was recorded in Newtons (N).

After completing the loading test, the fracture mode of all specimens was examined both visually and under stereomicroscope (Heerbrugg M3Z, Heerbrugg, Switzerland) at different magnifications (6.5 and 15x) and illumination angles. Fracture mode was classified into two categories according to the extension of the fracture line. A reparable fracture was defined as a fracture of the restoration with or without the fracture of the tooth structure that did not extend below the simulated bone level, while an irreparable fracture was defined as one that extended below the simulated bone level.

Representative fractured specimens were selected and examined by scanning electron microscopy (SEM) (JSM 5500, Jeol Ltd., Tokyo, Japan). Prior to observation, all the specimens were cleaned with alcohol and then coated with a gold layer using a sputter coater in vacuum evaporator (BAL-TEC SCD 050 Sputter Coater, Balzers, Liechtenstein). The analysis started at the upper, load-bearing part and progressed toward the inner surfaces.

Statistical analysis was performed in SPSS 26.0 (IBM, USA). Beside the descriptive analyses, ANOVA and factorial ANOVA were used. For the factorial ANOVA, bone level and amputation were used as factors. The level of significance was p = 0.01 (corrected for multiple comparisons according to Bonferroni).

3. Results

Fig. 3 displays the boxplots of the fracture thresholds by study group. The results of the post-hoc pairwise comparisons (Tukey's HSD) are given in Table 1.

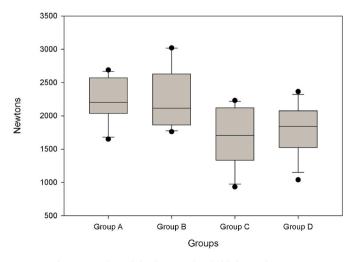
Groups without root amputation (Groups A and B) exhibited significantly higher fracture resistance than groups with root amputation (Groups C and D). Groups without root amputation (Groups A and B) did not show significant difference regarding fracture resistance from each other, irrespective of the degree of furcation involvement. The same applies for groups with root amputation (Groups C and D) when compared to each other. Therefore, the null hypothesis regarding fracture resistance was rejected. Factorial ANOVA was conducted with bone level and amputation (F = 18.99, df = 1, p < 0.001), but neither the effect of bone level (p = 0.694) nor the interaction of amputation and bone level was significant (p = 0.689).

In terms of the fracture mode, groups with mild furcation involvement (Groups A and C) exhibited a reparable fracture mode more frequently (n = 8 & 9, respectively) than groups (Groups B and D) with advanced/moderate furcation involvement (n = 6 & 4, respectively). Therefore, the null hypothesis regarding fracture modes was also rejected.

Optical microscope and SEM images of the tested specimens showed that the crack path propagated from the loading surface (occlusally at connector area) to the inner part of composite restoration (Fig. 4). Fig. 4 A,B & C illustrate fracture propagation through the occlusal composite resin towards the fiber bundle splint and Fig. 4D shows how fracture crack is stopped or redirected by fibers.

4. Discussion

The present study focused on the possible effect of root amputation and remaining bone level on the fracture resistance of splinted upper molar teeth. The goal of root amputation is to remove a root or roots of a multi-rooted molar tooth if the root cannot be treated due to endodontic and/or periodontal reasons. DeSanctis et al., summarized the periodontal indications of root amputation as follows: moderate to advanced furcation involvement, severe bone loss affecting one or more root(s), severe recession or dehiscence of a root or unfavorable root proximity between adjacent teeth (DeSanctis and Murphy, 2000). In our study, we modeled two situations with different degrees of furcation involvement



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Table 1

Results of the post-hoc pairwise comparisons (Tukey's HSD). Significant values are highlighted with red.

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group A	Group B	.750	164.085	1.000	-437.36	438.86
	Group C	552.333 ^a	164.085	.008	114.23	990.44
	Group D	459.667 ^a	164.085	.036	21.56	897.77
Group B	Group A	750	164.085	1.000	-438.86	437.36
	Group C	551.583 ^a	164.085	.008	113.48	989.69
	Group D	458.917 ^a	164.085	.037	20.81	897.02
Group C	Group A	-552.333 ^a	164.085	.008	-990.44	-114.23
	Group B	-551.583^{a}	164.085	.008	-989.69	-113.48
	Group D	-92.667	164.085	.942	-530.77	345.44
Group D	Group A	-459.667 ^a	164.085	.036	-897.77	-21.56
	Group B	-458.917 ^a	164.085	.037	-897.02	-20.81
	Group C	92.667	164.085	.942	-345.44	530.77

^a The mean difference is significant at the 0.05 level.

and bone loss. It is well documented in the literature that, due to specific anatomical features, furcation involvement affects the upper molars three times more often than the lower ones (Ross and Thompson, 1980). Of the upper molars, the first molars (Megarbane et al., 2018) and their distal furcations are the most frequently affected (Svärdström and Wennström, 1996; Al-Shammari et al., 2001; Majzoub and Kon, 1992). Partly because of this, it has been previously shown that of all periodontally compromised teeth, maxillary molars are the most likely to be lost (Hirschfeld and Wasserman, 1978; McFall 1982). Therefore we chose to test maxillary molars.

In the present study, amputation of the DB root (Groups C and D) significantly reduced the fracture resistance of intracoronally splinted upper premolar-molar tooth pair units compared to non-amputated ones (Groups A and B). To our knowledge, this is the first study to show the effect of root amputation alone on the fracture resistance of periodon-tally involved, intracoronally splinted maxillary molar teeth under *in vitro* conditions. Furthermore, our results indicate that the amount of bony support does not play a major role in the fracture resistance of such teeth as the groups did not differ significantly when only the degree of furcation involvement was different (comparing Group A to Group B, and Group C to Group D). This was further confirmed by the factorial variance analysis, which revealed a significant effect for root amputation, but not for furcation involvement or the interaction of the two.

In a previous *in vitro* study (Szabó et al., 2019a), we examined how the level of periodontal support (modeling physiological conditions and furcation involvement) may impact the fracture resistance of root-amputated upper first molars that were restored by either a direct filling or an overlay.

To our knowledge, so far only we have dealt with the fracture resistance of root-amputated and/or furcation-involved teeth, so a direct comparison is possible only with our own studies. Regarding the amount of supporting bone, our current results contradict our previous findings where furcation involvement appeared as a significant factor in weak-ening root-amputated maxillary molars (Szabó et al., 2019a). It is important to note that in the present study, splinted tooth pairs were tested, whereas previously we tested single upper molars, and this might

Fig. 3. Boxplots of the fracture thresholds by study group.

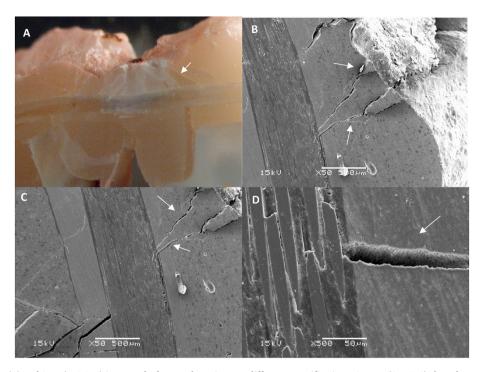


Fig. 4. Stereomicroscopic (A) and SEM (B,C & D) images of a fractured specimen at different magnifications. Arrows (A,B & C) show fracture crack propagation from the load application area (connector) through composite resin towards the fiber bundle splint. Panel D clearly shows the fibers' ability to stop/redirect a crack.

well be the reason for the seemingly opposing results. Our current results also contradict the findings of Soares et al., as in their study the amount of bony support influenced the strain that developed in the splinted teeth (Soares et al., 2011). However, it should be emphasized that Soares et al., examined lower front teeth, which could easily account for the difference in their results.

Regarding the fracture modes, mainly reparable fractures occurred in all groups, except for teeth with root amputation and advanced furcation involvement (Group D). This partly supports our previous results, according to which root-amputated furcation-involved teeth develop predominantly irreparable fractures (Szabó et al., 2017, 2019a). Fig. 4 shows clearly that the splinting fiber bundle has a reinforcing effect because it acts as a crack stopper. Many cracks started from the loading area and stopped at the fiber-composite interface. This could explain why all restorations survived fatigue loading. However, during static loading, a few cracks did pass the fibers and lead to failure. In terms of reinforcement, it is important to highlight the effect of the adhesion between the fiber bundle and the composite resin, and the position of the splinting fibers on the distribution of the occlusal forces. It has been shown that when the fiber bundle is applied to the occlusal third of the crown, it has all the benefits of occlusal splinting. That is, it works as an early stress-redirecting layer and its application results in a shorter working arm under loading (Sáry et al., 2019).

In our study, premolar-molar tooth pairs were splinted with fiberreinforced composite splinting. It is important to emphasize that root amputation alone does not necessarily indicate splinting (Graetz et al., 2019). Tooth mobility should also be considered when assessing the need for splinting in everyday practice. Klavan et al., found no difference in the survival rates of splinted and non-splinted, root-amputated upper first molar teeth, unless included in the anchorage of partial removable prosthesis (Klavan, 1975). Kumbuloglu et al., found good survival rates for periodontally involved teeth splinted with the same fiber-reinforced composite splint (everStick Perio) as we used in our study, however, they splinted lower front teeth (Kumbuloglu et al., 2011). Periodontal disease with bone loss and secondary furcation involvement is one of the most difficult-to-manage conditions in periodontology (Walter et al., 2011), reducing the 10- to 15-year survival rates by about 50% compared to non-furcation-affected teeth (Nibali et al., 2016). Root amputation has both advantages and disadvantages in such a situation. On the one hand, it provides better cleanability, but on the other hand, it has a significant impact on tooth stability and statics. The lifespan of teeth that have undergone root amputation has long been in the focus of research, but the results are diverse: some authors report survival rates above 90% (Derks et al., 2018; Schmitz et al., 2020; Carnevale et al., 1998; Fugazzotto, 2001; El Sayed et al., 2020), while others report significantly less favorable results of only 40–60% (Langer et al., 1981; Alassadi et al., 2020).

In the present *in vitro* study, we modeled a clinically relevant, common periodontal situation, when the upper first molar becomes the most distally located tooth in the upper quadrant, and the bone loss on the distal surface necessitates the amputation of the DB root. In this specific clinical situation, the molar tooth is always furcation-involved to a certain extent. Furcation-involved molars are at a greater risk of further attachment loss than teeth without furcation involvement (Dommisch et al., 2020). Furthermore, furcation involvement has been shown to be among the most serious deteriorating factors regarding the long-term prognosis of multi-rooted teeth (Derks et al., 2018). In these clinical cases, it is important that the teeth that have become mobile (above a certain extent) should be stabilized in some way. Intracoronal splinting with FRC is one of the least invasive procedures that can be applied in the posterior zone in this clinical situation.

In this study, all specimens were pretested in an accelerated fatigue testing protocol under dynamic loading conditions. It is known that cyclic fatigue loading is a better model of the clinical situation than static loading, since cyclically applied forces act in a manner that is closer to what happens during normal mastication (Battancs et al., 2021). The accelerated fatigue protocol was introduced as a middle ground between the classic load-to-fracture test and the more sophisticated, but also time-consuming, fatigue tests (Magne et al., 2014, 2017; Lazari et al., 2018). As all specimens survived the pretesting phase, load-to-fracture testing was also carried out on all specimens. Static load-to fracture testing simulates a sudden traumatic event, with greater forces or loading compared to normal chewing (e.g.: biting on a foreign object, stone, seed, etc.), which is usually a limitation compared to

dynamic loading (Szabó et al., 2019b). Therefore, combining the dynamic and static loading conditions in the same setup makes *in vitro* mechanical testing more reliable and more clinically realistic compared to static loading alone (T Szabó et al., 2021). We would like to point out that, in our opinion, the static load-to-fracture test is almost mandatory in this specific situation as clinically mobile teeth are more prone to sudden facture compared to their sound, periodontally intact counterparts (Szabó et al., 2019a, 2019b). Thus, we consider the application of both dynamic and static load-to-fracture tests a major strength of our study.

On the other hand, cyclic loading was not performed in a fluid chamber, and this weakens the comparability of our results to those of in vivo studies where saliva is always present during the loading cycles. This is a limitation should be addressed in future studies. Furthermore, the extracted teeth used in this study were not standardized according to their age but only according to their dimensions. Aging can alter the mechanical features of dentine, especially in the root canal. This is a known limitation of all current *in vitro* mechanical testing studies, which should be addressed in future. Finally, we would like to point out that the fact that our results are not comparable to independent studies (as studies in the simulation and mechanical testing of periodontally compromised teeth are absolutely lacking) is a clear limitation to the generalizability of our findings.

5. Conclusions

Within the limitations of this study, it can be concluded that root amputation has a negative effect on the fracture resistance of furcationinvolved, intracoronally splinted upper first molar and second premolar units. The degree of furcation involvement, as modeled in this study, does not seem to influence the fracture resistance of such units.

Conflicts of interest

Author Veronika T. Szabó, Balázs Szabó, Balázs Szabó P., Balázs Paczona, Csongor Mészáros, Gábor Braunitzer, Sufyan Garoushi and Márk Fráter declares that they have no conflict of interest.

Funding

This study was supported by the Bolyai János Research Scholarship (BO/701/20/5) and by and by the ÚNKP-21-5-SZTE New National Excellence Program of The Ministry for Innovation and Technology from the Source of National Research, Development and Innovation Fund, Hungary, and by the University of Szeged Open Access Fund (5623).

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

For this type of study, formal consent is not required.

CRediT authorship contribution statement

Veronika T. Szabó: Investigation, Methodology. Balázs Szabó: Conceptualization, Investigation, Methodology, Visualization. Balázs Paczona: Investigation, Project administration. Csongor Mészáros: Investigation, Project administration. Gábor Braunitzer: Data curation, Formal analysis, Software. P. Balázs Szabó: Formal analysis, Methodology, Software, Validation. Sufyan Garoushi: Conceptualization, Supervision, Writing – original draft, Writing – review & editing. Márk Fráter: Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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