Biomechanical behaviour and modelling of periodontally compromised teeth restored with fibreglass restorations

PhD Thesis

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2. List of the publications providing the basis of and related to the topic of the thesis

I. **T Szabó V**, Szabó B, Paczona B, Mészáros Cs, Braunitzer G, Szabó P B, Garoushi S, Fráter M. The biomechanical effect of root amputation and degree of furcation involvement on intracoronally splinted upper molar teeth – An in vitro study. Journal of the Mechanical Behavior of Biomedical Materials 2022 May;129:105143. doi:10.1016/j.jmbbm.2022.105143.

II. **T Szabó V**, Szabó B, Tarjányi T, Trenyik Sz E, Szabó P B, Fráter M. Analog and digital modelling of sound and impaired periodontal supporting tissues during mechanical testing. Analecta Technica Szegedinensia 2021;15:2. doi:10.14232/analecta.2021.2.84-97.

III. **T Szabó V**, Szabó B, Braunitzer G, Szabó P B, Fráter M. A gyökéramputáció hatásának vizsgálata intrakoronálisan sínezett, furkációérintett felső moláris fogak törési ellenállására – előzetes tanulmány. Fogorvosi Szemle 2022; *közlésre elfogadva, megjelenés alatt* (igazolás csatolva)

IV. Szabó B, **T Szabó V**, Fráter M. Digitális technika alkalmazása parodontális betegségek diagnózisában és a sebészi megoldás tervezésében. Orvosi Hetilap 2022; doi:10.1556/650.2022.32495. *közlésre elfogadva, megjelenés alatt* (igazolás csatolva)

3. List of abbreviations

OD: occluso-distal

CBCT: cone-beam computed tomography

3D: three-dimensional

FEM: finite element method

FEA: finite element analysis

CEJ: cemento-enamel junction

NaOCl: sodium-hypochlorite

MOD: mesio-occluso-distal

DB: disto-buccal

BPW: bucco-palatinal width

N: Newton

SEM: scanning electron microscopy

4. Introduction

Clinical background

Periodontitis is one of the most common conditions affecting oral health among adults accounting for severe social and health problems [1]. Although individual differences can occur, similar results from epidemiological surveys are available worldwide on disease incidence [2,3,4,5,6]. Kassebaum et al. reported that severe periodontitis (defined as having a probing pocket depths of more than 6 mm) is the sixth most prevalent disease in the world affecting nearly 800 million people by 2016 [7]. 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 an attachment loss that, if left untreated, can result in tooth loss [8,9].

In case of a periodontal disease, the impairment of the periodontal supporting structures means the destruction of the anatomical and functional unit between the alveolar bone, the periodontal ligaments and the cementum. In multirooted teeth, as the disease progresses, the degeneration of the alveolar bone results in the development of furcation involvement [10]. The deterioration of the attachment apparatus is usually a slow process, and it shows individual differences in its extent and clinical appearance over time [11]. Several morphological factors (including furcation entrance width, root trunk length, root concavities, cervical enamel projections, bifurcation ridges and enamel pearls) related to furcations, roots and the poor accessibility of the posterior teeth during cleaning contribute to the aetiology and the compromised prognosis of furcation involved molars [10]. Furcation involvement affects maxillary molars more frequently than mandibular molars [12,13], probably due to the fact that maxillary molars have more furcation entrances/sites at risk than mandibular molars.

In periodontally affected patients, roughly one-third of all molars and almost one-fifth of all furcation sites exhibit degree II and III furcation involvement [12,14]. Albandar et al. found that the prevalence of furcation involved teeth (all/through-and-through) increases with age (60–69 years: 27%; 70–79: 31% and 80–89: 37%) and is higher in males (17%) than in females (11%) [15]. Taking into consideration that in developed countries teeth are retained for longer and in parallel, populations are significantly ageing, the absolute number of teeth at risk for periodontitis is increasing. Schwendicke et al. estimated that in Germany, the current 65 yearolds and older age groups comprise of 22% of the population (17.9 of 81.2 million); in 2030, this number will be 28% (21.8 of 79.2 million) and in 2040, even 31% (23.2 of 76.0 million) [6]. Long-term molar retention is not only beneficial at the individual level (masticatory, phonetic, aesthetic and social concerns), but the substantial direct and indirect costs of periodontitis make it a global economic issue [16], thus furcation involved molar retention is becoming particularly important and increasingly topical. For these reasons we focused on this particular topic.

Setting up the diagnosis of furcation involvement can be difficult due to the anatomical features of the area. Clinical examination methods, such as the probing of the furcation, do not provide reliable information on bone support in most cases [17]. Imaging techniques play a primary role in the assessment of the severity of periodontal diseases. One of the most difficult questions to decide is the exact shape and size of the bony defect, as it undoubtedly possesses a major impact on our choice of therapy. CBCT (cone beam computed tomography) images show the nature of the defect with higher accuracy compared to x-rays (see Figure 1/A, B) [18,19].

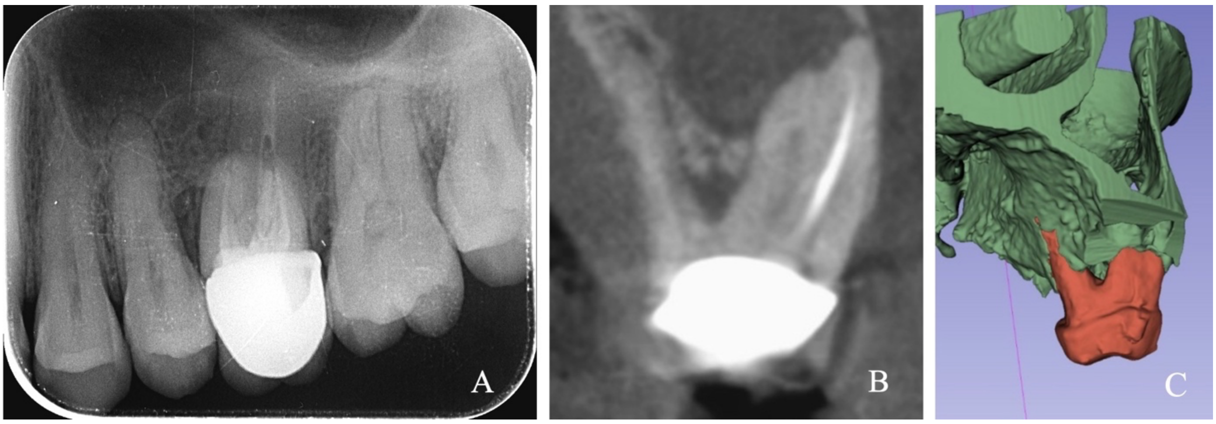

Figure 1: CBCT images are able to show the lesion more accurately than x-rays. Periapical x-ray (A), CBCT image (B) and segmented model (C).

The three-dimensional (3D) view allows us to examine the slices corresponding to the planes; however, their quality and usability is often unsatisfactory. One option for better visualization and analysis is the 3D model, which is created with the help of CBCT and a special technique called segmentation (Figure 1/C). Here, the segmented elements (alveolar bone, teeth, defect and other anatomical structures, etc.) appear as real 3D objects and their size, shape and relative position can be examined much better than with the aforementioned diagnostic tools (Figure 2). It gives a preliminary view of the defect, the number of existing bony walls, and allows us to select the most appropriate treatment.

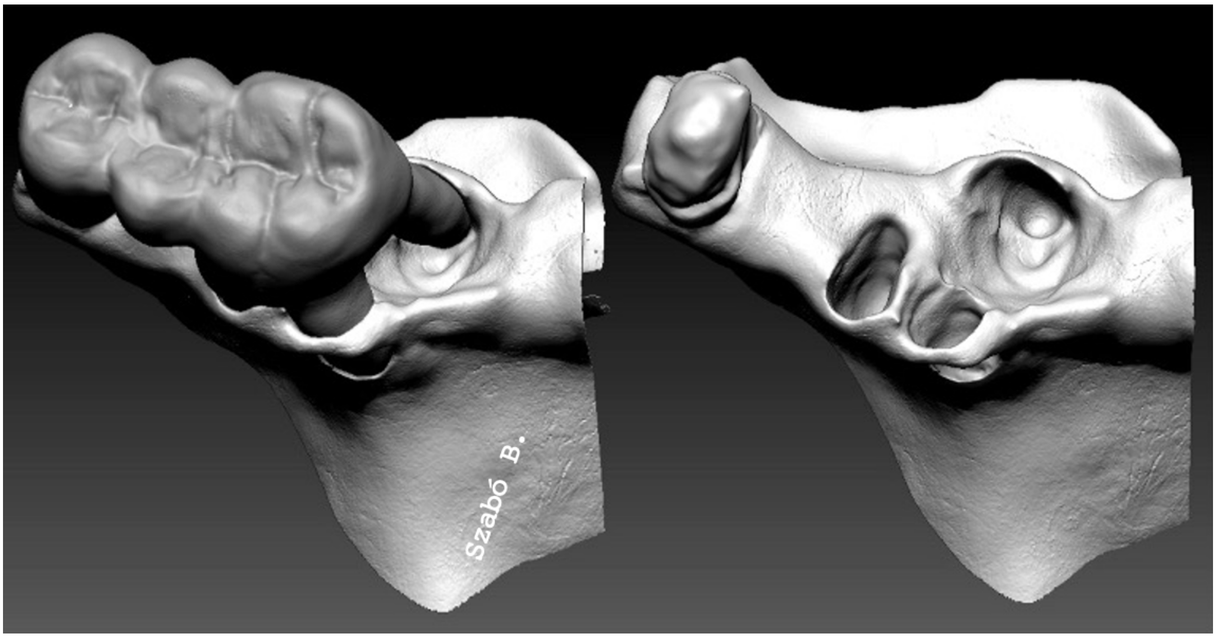

Figure 2: The segmented elements (alveolar bone, teeth, defect and other anatomical structures, etc.) appear as real 3D objects; their size, shape and relative position can be examined much better.

Figure 3-5 demonstrate the role of this method (segmentation) in periodontal surgery through the case of a middle-aged female patient who was referred to the clinic. In her case, periapical radiographs were not able to provide a realistic picture of the extent and location of the attachment loss of the maxillary right first molar. On the CBCT (Figure 3) and the segmented model derived from it (Figure 4), the palatal root and the adjacent periradicular lesion, the involvement of the furcation, the bony support of the buccal roots that remained almost imperceptible during the clinical examination and on the X-rays became well observable.

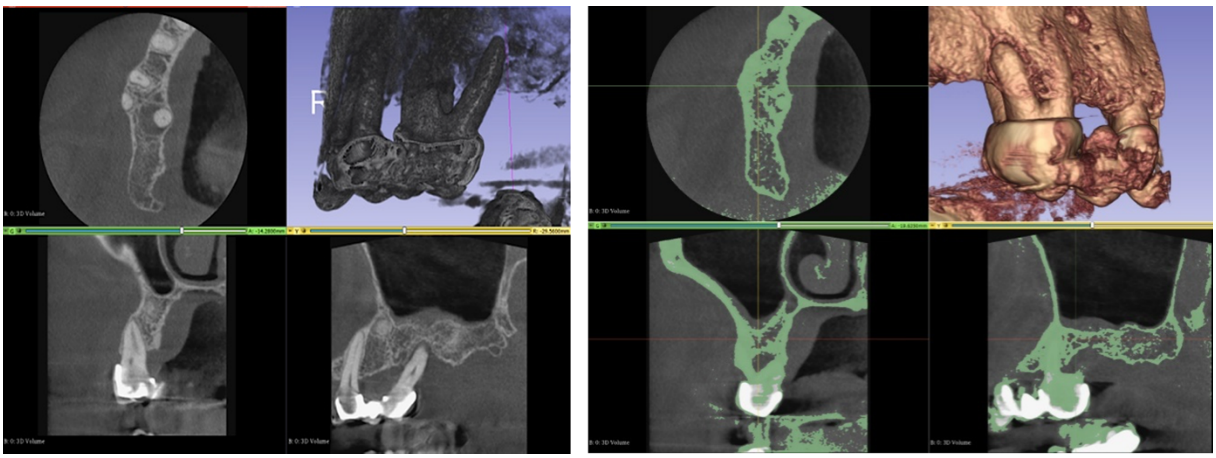

Figure 3: CBCT images show the nature of the defect with high accuracy. The palatal root and the adjacent periradicular lesion are well observable.

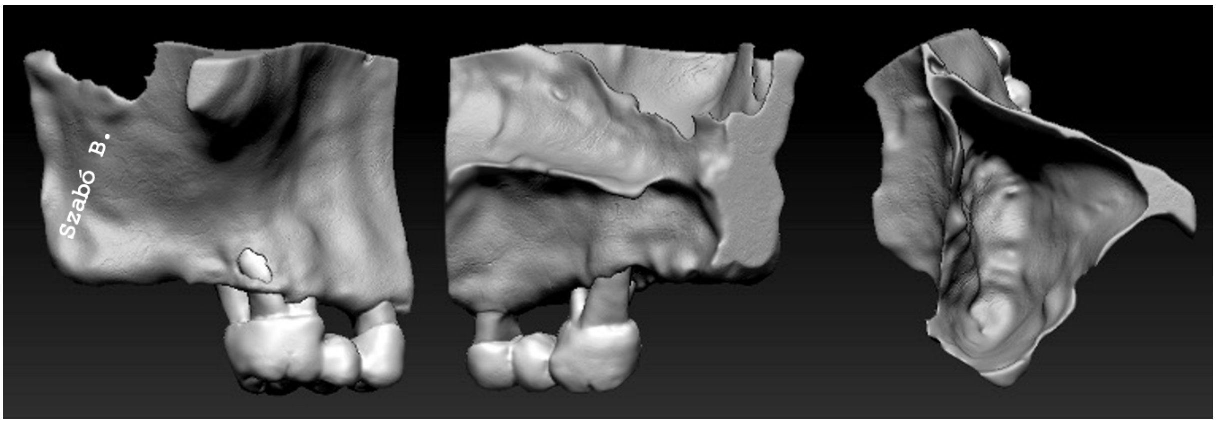

Figure 4: Segmented model of Figure 3 provides better visualization and analysis of the same palatal root and the adjacent periradicular lesion.

Treatment and case presentation

Furcation involvement poses an extreme challenge to both patient and dentist in the elimination of microbial plaque from the exposed surfaces of the root to maintain good individual and professional oral hygiene [20,21]. The main reason for this is that bone loss creates a complex area in terms of shape, which is extremely difficult to access, and the chance of re-infection is also probable [22].

Furcation involvement is treated either in a conservative manner or surgically [10]. According to Hermann et al., by the time the furcation has been exposed, more than 30% of the available attachment surface had been lost [23]. The extent and appearance of the defect influence the choice of therapy. It is generally stated that more extensive defects are more likely to be treated surgically [24]. Also, due to the poor accessibility of the exposed furcal area, molar teeth respond less favourably to non-surgical periodontal treatment than single-rooted teeth [25,26,27], which may necessitate more invasive interventions. The two main trends of surgical treatment are resective [28] and regenerative [29] periodontal therapy, which intend to meet the same objectives with different means: resective interventions aim to create a stable, sustainable state by the further reduction of the remaining tissues, while regenerative surgical interventions restore the form and function of the original structures. A type of resective surgical intervention is root amputation or root resection. 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 (see Figure 5) [30,31].

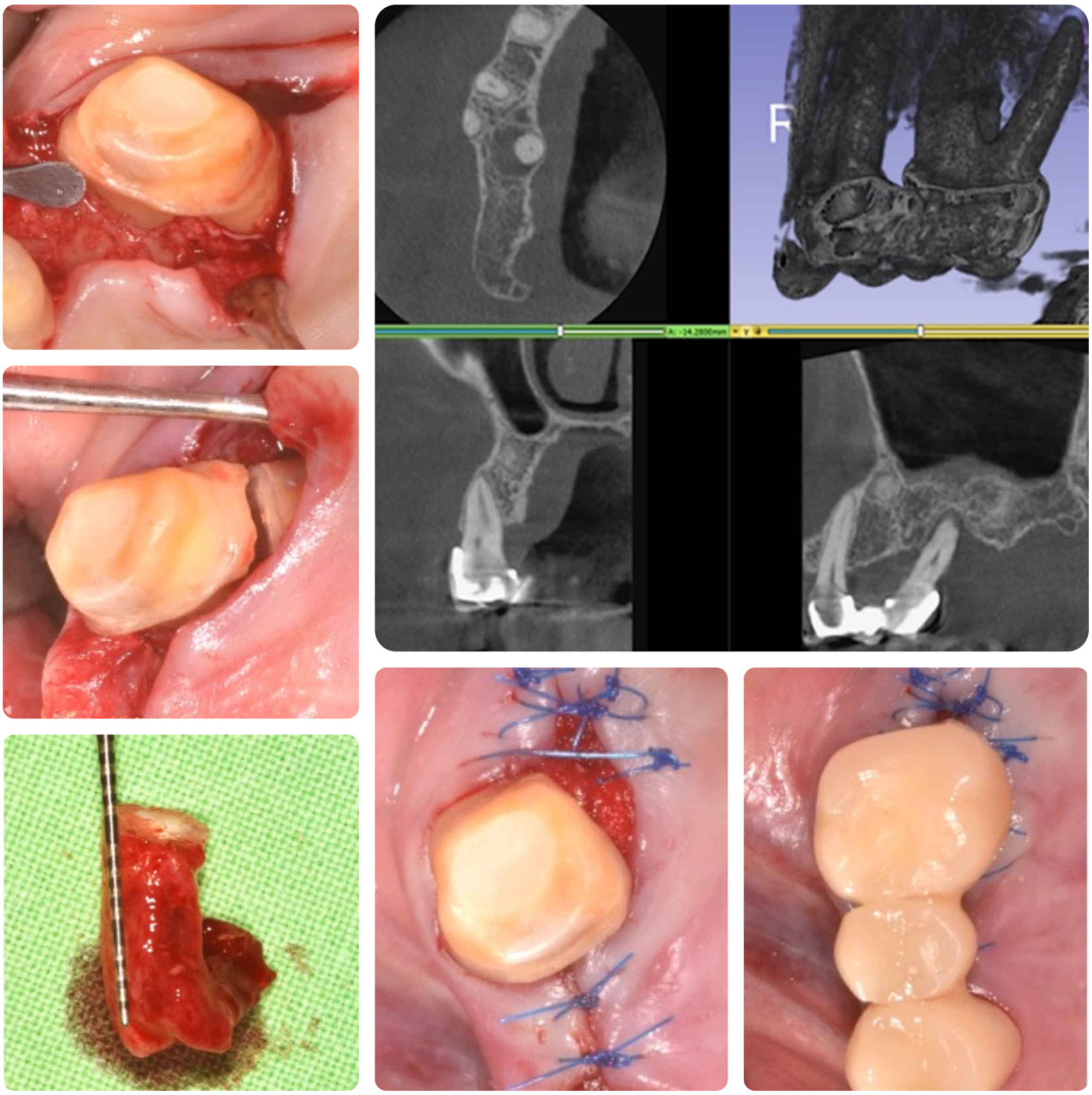

Figure 5: root amputation of the palatal root of the upper right maxillary first molar.

The pictures of Figure 5 demonstrate the surgical procedure of root amputation of the palatal root of the upper right maxillary first molar. As only the palatal root of the three roots was significantly affected, amputation of the palatal root was planned accordingly, preceded by the endodontic treatment of the tooth. As we could previously examine it in the segmented model, after the elevation of a full-thickness flap in the mesial and distal directions from the first molar, the mild involvement of the mesial furcation entrance and an advanced distal involvement became visible. The palatal root was then sectioned horizontally at the level of the furcation with a fissure diamond bur. The root was removed and the corresponding coronal part was subsequently modified. The significant amount of granulation tissue adapted to the root was eliminated, the lesion around the former palatal root was also cleaned and the defect was filled with bone replacement material (Geistlich Bio-Oss, Wolhusen, Switzerland). Because the defect could not be closed primarily, the area between the flap and the tooth was covered with a collagen sponge (Ethicon Spongostan Dental , Lidingö, Sweden) and the soft tissue flaps were secured with non-absorbable sutures (Ethicon Prolene 5-0) at the level of the bone crest. The temporary bridge was relined with adhesive and paste composite filling material to adopt to the new form. The temporary bridge was then cemented onto the abutment teeth. The tooth is still worth preserving even for a shorter period, which can mean years for the patient wearing fixed prosthesis.

Degradation of periodontal tissues most commonly affects multi-rooted, upper posterior teeth with anatomies that are complex and difficult to clean [13,32,33]. As a result, the second and third molars are often removed before the other teeth, and thus the upper first molar teeth may become the most distally located molars in the quadrant, as in the case presented above. It is characteristic of this situation that the pre-existing periodontal bone destruction on the distal surface of the upper first molar is further enhanced by the resorption of the alveolar bone following the extraction. Partly because of this, it has been previously shown that of all periodontally compromised teeth, maxillary molars are the most likely to be lost [34,35].

Irrespective of the above, in terms of furcation involvement the upper molars are affected three times often than the lower molars due to their specific anatomical features [36]. Even among the upper molars, the first molars [37,38], and their distal furcation are the most frequently affected [10,13]. This may explain the high percentage (45.5%) of root resections involving the disto-buccal (DB) root in maxillary molars [39].

This explains why our attention was focused on furcation involved upper first molars in our studies, as we wanted to simulate a common clinical periodontal situation *in vitro* and test it according to different variables that have clinical relevance.

Restorative concerns

Restoration and maintenance therapy of teeth with furcation involvement and/or teeth that have undergone root amputation poses a serious challenge to dentist and patient alike [40]. Both furcation involvement and root amputation can increase tooth mobility, which can lead to further attachment loss [41]. Thus, creating a tight interproximal contact with the adjacent teeth [42,43] or even the splinting of the affected posterior tooth may become necessary. Lee et al. found that among other influencing factors such as age, <50% of pre-operative radiographic bone height of the remaining root(s), pre-treatment mobility II or above, not being splinted to neighbouring teeth nor the incorporation as a bridge abutment were significantly associated with shorter survival rate of teeth subjected to resective therapy [44]. In the posterior zone, direct and indirect splinting methods are available: intracoronal splinting with composite/glass fibre reinforced material (see Figure 6) or indirect splinted crowns or bridge. As root amputation by definition does not affect the coronal part, in case of normal mobility and tight interproximal contact with the adjacent teeth, the choice of coronal restoration type should depend on the amount of existing walls/tooth structure following the rationale of restoring endodontically treated molars [43].

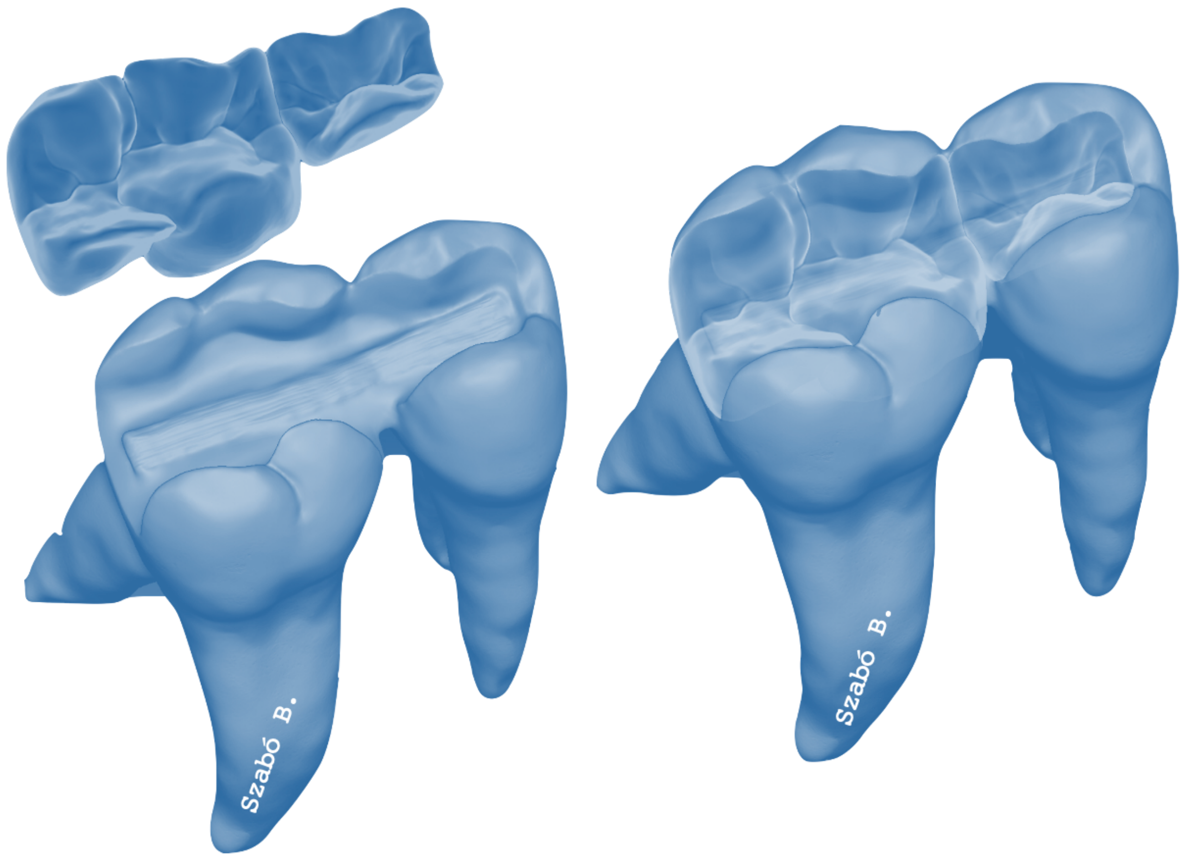

Figure 6: Schematic model showing intracoronal splinting with fibre-reinforced composite in the posterior zone.

However, questions might arise whether root-amputated, intracoronally splinted molars resist occlusal load the same way as non-amputated counterparts or how the bone level would affect their fracture resistance. To answer these questions, we decided to create an *in vitro* modelling protocol described in details in the following.

Analogous and digital methods in modelling the mechanical behaviour of periodontally affected teeth

In severe stages of periodontitis, the deterioration of the supporting tissues, namely the periodontal ligaments and the bone, possibly affect the biomechanical behaviour, and therefore the longevity and survival of the affected teeth. In order to be able to plan both periodontal and subsequent restorative treatment properly, the valid modelling of the current clinical situation is necessary. There are analogous and digital methods for modelling the behaviour of periodontally affected teeth and the surrounding periodontal structures. During the analogous method, real models (e.g.: extracted teeth), at first a general prototype are created that allows us to perform the steps of the planned test and if it succeeds, tests with a larger sample size could be processed. As a next step, the samples are embedded into a medium that simulate the physical properties of the alveolar bone, and then different mechanical tests are carried out by a testing machine. With the continuous improvements of new, up-to-date materials, techniques, equipment and devices, better and better valid physical study models can be prepared, and the same can be observed in the digital world. The digital, finite element method (FEM) does not require the production of real models and the tests can be performed on virtually created digital models with the use of a special software (see Figure 7) [45,46].

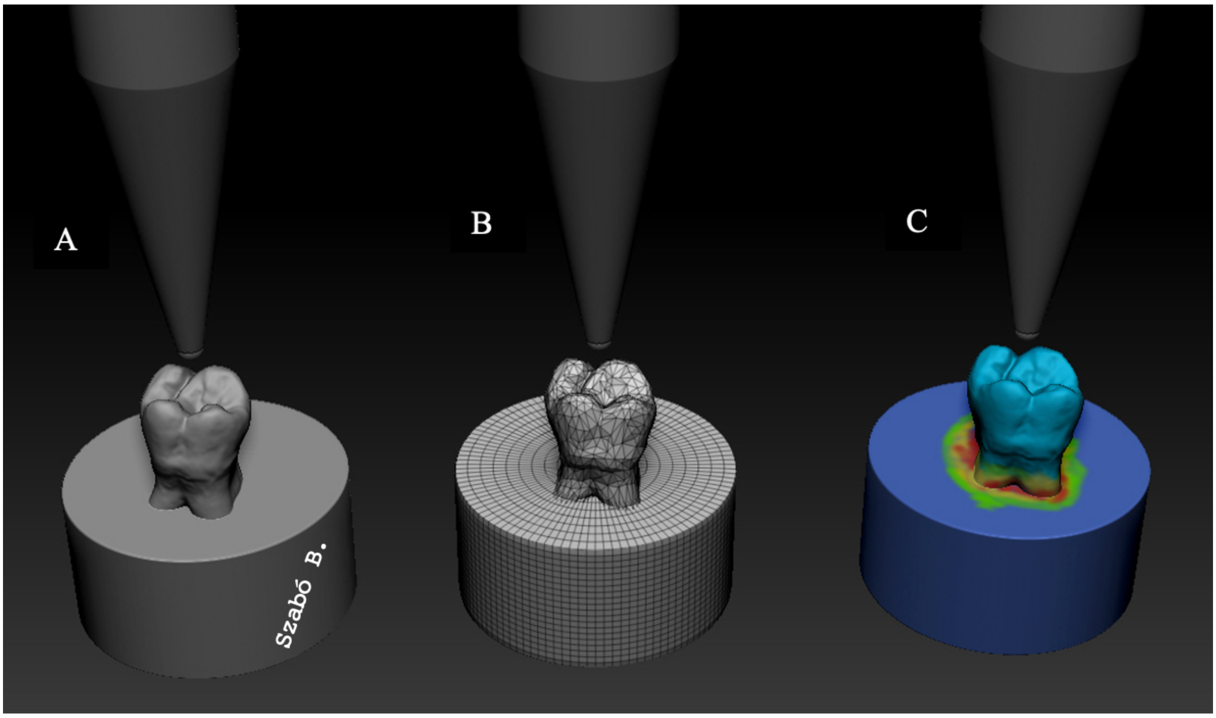

Figure 7: Digital workflow in FEM: creating a geometry (A), meshing (B), solving a system of equations and analysing the results; colour code shows the mechanical stress values (C).

Its operational concept is to break down the created geometries into finite number of smaller elements (hence the name 'finite element'), thus simplifying it. This means that the dimensions of spatial elements are discretized, and the equations describing the problem can be solved algebraically. Analysis performed by FEM methods is called Finite Element Analysis (FEA). It is extremely promising that with the help of a more complex software and faster computers, real physical experiments can be simulated, but they cannot be replaced yet, maybe they never would be. In our studies, we chose the analogous approach, so in the following section, only this method will be presented in details.

Embedding

Based on the available literature, the embedding material is mainly acrylate resin in *in vitro* studies. In our studies, we used the powder and liquid form Technovit 4004 (Heraeus-Kulzer) (see Figure 8/A) as it was already used in the studies of the Biomechanical Research Group in Szeged [47,48,49,50,51,52]. After mixing (Figure 8/C), it is poured into plastic cylinders (Figure 8/D) and the teeth are embedded to the appropriate levels (Figure 8/E). Because the embedding material easily creeps up on the inserted object, areas that are not intended to be embedded should be blocked out with modelling wax. This is a particularly important step when modelling teeth with furcation involvement. During embedding, the occlusal surface is parallel to the top of the table and is set in this position. If we want to simulate physiological periodontal support, the embedding material should be located 2 mm apically from the cementoenamel junction (CEJ) in line with the alveolar bone in a normal situation. Increasing the distance in between the CEJ and the embedding material will simulate horizontal bone destruction, which corresponds to the clinical picture of chronic periodontitis of furcation involvement in multi-rooted teeth.

Realistic modelling also requires the simulation of periodontal ligaments. Periodontal ligaments play a crucial role in load transition in between the tooth and the bone, enabling the teeth to move physiologically approximately 0.05 mm in the alveolus, thus establishing a functional connection between the alveolar bone and the cementum covering the root surface. In their absence, the physiological mobility of teeth, the force transmission, the local remodelling of the alveolar bone and the thickness of the cementum would change. A model without ligaments would simulate an ankylotic condition, which is more relevant when examining implants. In our studies, the surfaces of the roots are coated with a rubber separating agent (Rubber Sep, Kerr, Orange, CA) till the level of embedding [43,53]. The separating material is applied in a single thin layer to the root surface with a factory-packaged nail polish brush (Figure 8/B) in order to standardize the thickness of the separator as much as possible.

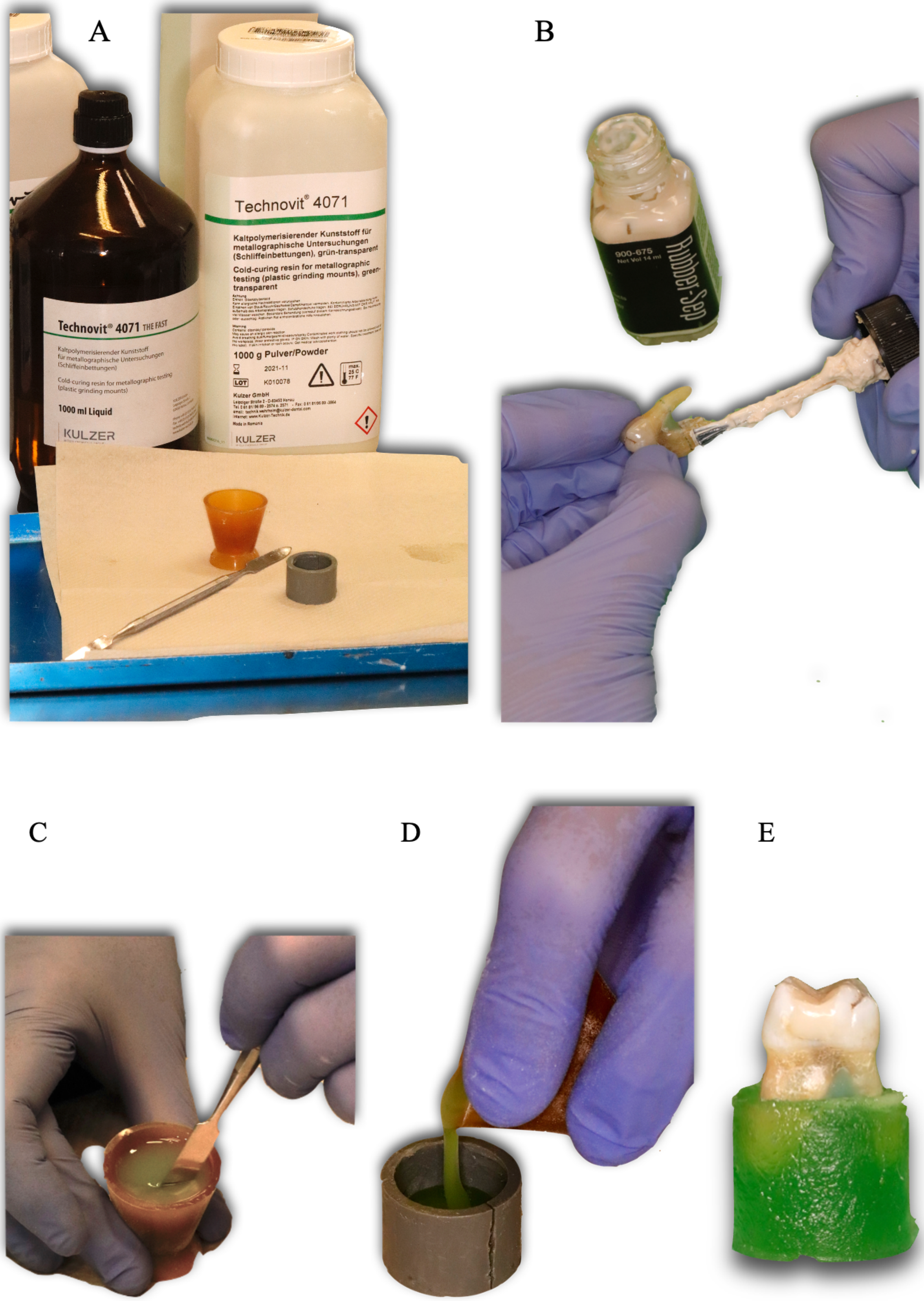

Figure 8: Embedding the samples: the embedding material is an acrylate resin (A), the rubber separating material is applied onto the root surfaces (B), after mixing the embedding material (C), it is poured into plastic cylinders (D) and the teeth are embedded up to the appropriate levels (E).

Mechanical testing

If available, it is always worth performing dynamic loading tests on analogous samples as it enables us to reproduce the oral conditions of chewing to a certain extent. The classical dynamic loading test represents one of the most valid mechanical loading methods in the literature. Relatively small, repetitive forces are exerted onto the teeth through endless cycles, simulating chewing periods and time spent in oral function up to several years [54,55,56]. It is also possible to test samples under moist conditions as found in the oral cavity if the samples are loaded in a fluid chamber. However, from a practical point of view, the classical dynamic loading is extremely time-consuming (loading 1 sample can last up to approximately 1 week 24 hours a day), so it does not only make it almost impossible to perform tests and comparisons with larger sample sizes and group numbers, but also requires shift work from the staff.

Due to the above mentioned limitations, static load-to-fracture tests by which the consequences of a sudden, greater force (e.g.: traumatic injury) can be simulated are performed the most frequently. Although acceptable even on its own, as it only simulates an extreme condition, dynamic loading would still be beneficial in mechanical testing. So far, the accelerated dynamic loading test has been a realistic compromise between the two extremes [57,58,59,60,61]. In this particular test, although cyclic loading occurs, the magnitude of the force is not constant (only within a given cycle) but increases after a given number of cycles for the duration of the next cycle. In our opinion it is recommended to increase the applied force only until the reach of the maximum chewing force, the characteristic of the given tooth group or the oral region. As the samples are subjected to dynamic loading, we will not be able to obtain their fracture resistance values, but rather their fatigue and survival rates, which are clinically more informative data. In addition, the great advantage of this test is that the samples can be tested within a reasonable time (hours), so in terms of time consumption and also clinical relevance, it is positioned between the static load-to-fracture test and the classical dynamic loading test [62,63].

In case of periodontally affected teeth, it is of paramount importance that a dynamic loading test (classical or accelerated) should be performed first, and after that the survived samples should undergo static load-to-fracture test as well. Even if dynamic loading is always the best, static load-to-fracture test should also be carried out in this special situation, because due to either the reduced periodontal support (furcation involvement, etc.) or root amputation, clinically more mobile teeth – are more prone to sudden facture compared to their sound, periodontally not compromised counterparts [43,64].

The aim of our investigations

Taking into consideration the aforementioned evidence and experiences, our aim was to examine the role of DB root amputation on the mechanical behaviour of intracoronally splinted, periodontally compromised maxillary molar teeth and how the different bone levels can influence their performance.

Our null hypotheses were the following:

1. the presence or absence of DB root amputation and the level of embedding do not result in significantly different fracture resistance, and 2. the presence or absence of DB root amputation and the level of embedding do not affect the fracture pattern of intracoronally splinted, periodontally compromised maxillary molar teeth during mechanical *in vitro* tests.

5. Material and method

Pilot study

Sample selection

All procedures of the pilot study were approved by the Ethics Committee of the University of Szeged (ethical approval: 4029), and the study was designed in accordance with the Declaration of Helsinki.

20 maxillary molars and 20 maxillary premolars extracted for periodontal or orthodontic reasons were included in the pilot study. The freshly extracted teeth were immediately placed in 5.25% sodium-hypochlorite (NaOCl) for 5 minutes and then stored in 0.9% saline solution at room temperature until the soft tissues from the root surface were removed with hand scalers (see Figure 9). Inclusion and exclusion criteria, and also standardization regarding coronal and root dimensions were according to Szabó et al.. 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 to 10.9 mm in the bucco-palatal dimension, and the rest were between 11 to 12 mm. The mesio-distal dimension of the specimens was also measured; a mean was calculated and specimens that fell within the ±10% range of the mean were included. The height of the specimens was between 8 and 9 mm measured from the CEJ. Also, root length was also standardized as follows: mesio-buccal: 12–14 mm, disto-buccal: 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. The teeth were used within 6 months of removal and stored in 0.9% saline solution (Isotonic Saline Solution 0.9%; B. Braun, Melsungen, Germany) at room temperature throughout the study.

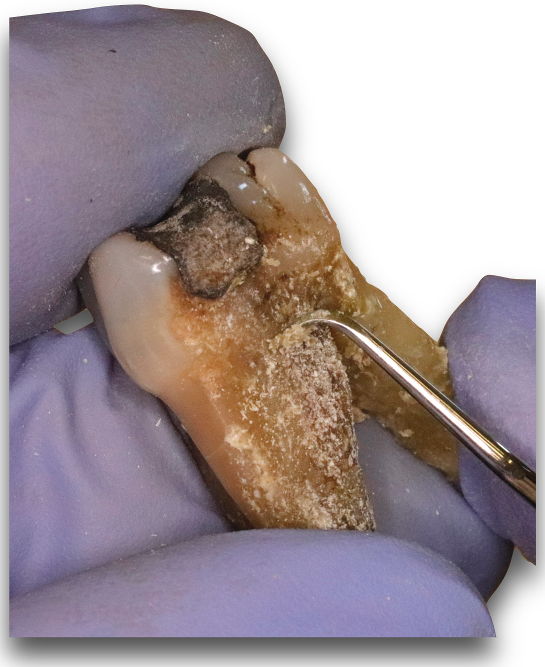

Figure 9: Periodontal ligaments and other soft and hard tissues were removed with hand scalers.

Cavity preparation and endodontic treatment

Standardized occluso-distal (OD) (premolars) and mesio-occluso-distal (MOD) (molars) cavities were prepared according to Cara et al. in all teeth (see Figure 10) [65].

Figure 10: Standardized OD (premolars) and MOD (molars) cavities were prepared.

The bucco-palatinal width (BPW) of the approximal box of each cavity was two-thirds of the BPW of the tooth, and the occlusal isthmus was half the BPW. In addition, the cavity depth at the occlusal isthmus was standardized to 3.5 mm from the tip of the palatal cusp and 1 mm above the CEJ at the cervical aspect of the approximal boxes.

As adequate root canal treatment is a prerequisite of root amputation, and in order to standardize the samples, all molar teeth underwent root canal treatment according to the protocol described by Szabó et al. (Figure 11) [43].

Figure 11: All molar teeth underwent root canal treatment.

The root canals were preflared with No 2-3-4 Gates Glidden burs and instrumented with Pathfiles (1-2-3) and ProTaper (S1-S2-F1-F2-F3) (Dentsply Maillefer, Ballaigues, Switzerland) to the working length. The specimens were irrigated with 5% NaOCl 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, the root canal filling was cut back 2 mm under the orifices and a base was applied to the pulp chamber in the form of an approximately 2-3 mm thick glass-ionomer barrier (Equia Forte, GC Europe, Leuven, Belgium).

Intracoronal splinting and coronal restoration

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 acid-etched selectively with 37% phosphoric acid for 15 seconds, rinsed with water and air-dried. The cavity was adhesively treated with G-Premio Bond (GC Europe) according to the manufacturer’s instructions. The adhesive layer was light-cured for 40 seconds with an Optilux 501 halogen light in standard mode at a light intensity of 740+/- 36 mWcm^2. The gingival boxes of all teeth and the pulp chambers of the molars were elevated till the level of the occlusopulpal wall with packable composite resin (G-aenial Posterior A3, GC Europe) to aid the positioning of the splinting fibres (Figure 12). Premolar and molar tooth pairs were randomly paired and positioned in silicon material (Elite HD Putty, Zhermack SpA, Badia Polesine, Italy) to stabilize the teeth during the intracoronal splinting procedure.

Figure 12: The gingival boxes of all teeth and the pulp chambers of the molars were elevated until the level of the occlusopulpal wall with packable composite resin.

Splinting was carried out with long E-glass fibres (everStick Perio, GC Europe). The size of the cavities was measured with a periodontal probe and the fibre bundles were cut to the adequate length (see Figure 13). After adhesive treatment of the fibre bundle splint according to manufacturer's instructions, the fibres were positioned into a layer of highly filled flowable composite resin (G-aenial Universal Flo A3, GC Europe) and light cured for 1 minute (Figure 14). The remaining part of the cavities were restored with packable composite resin (G-aenial Posterior A3, GC Europe) in an oblique layering manner (Figure 15). Each layer was light cured for 40 seconds.

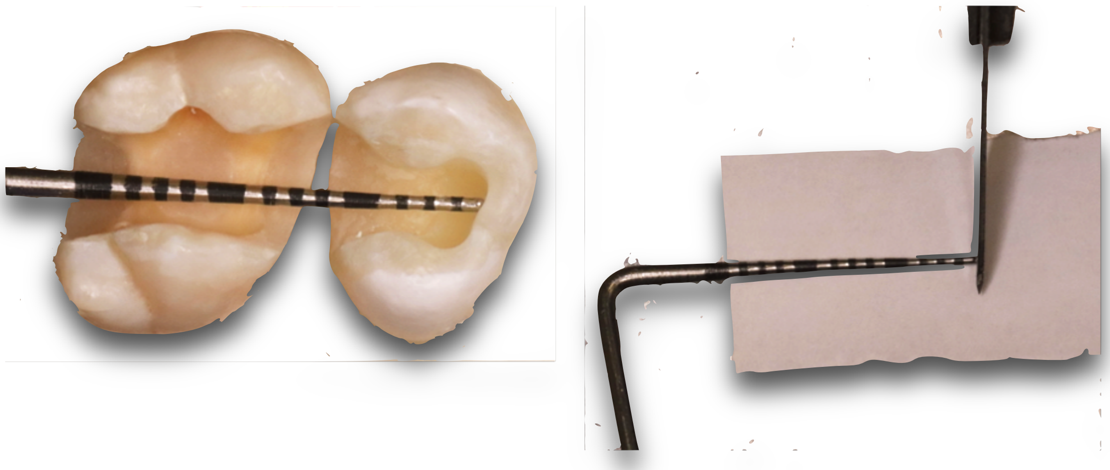

Figure 13: The size of the cavities was measured with a periodontal probe and fibre bundle were cut to the adequate length.

Figure 14: The fibres were positioned into a layer of highly filled flowable composite resin.

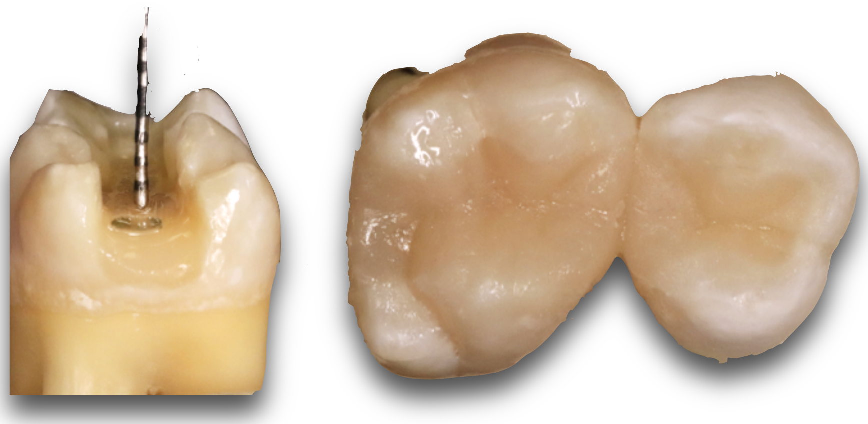

Figure 15: The remaining part of the cavities were restored with packable composite resin.

Root amputation

The splinted models were randomly divided into 2 groups (n=10, Group 1 and 2). In Group 2 each DB root of the molars 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 in order to have a cleansable, non-retentive surface. In Group 1, no root amputation was performed.

Embedding the samples

The tooth pairs were embedded according to the protocol described previously in the introduction. Prior to embedding, the furcation areas were blocked out with wax to prevent creeping of the embedding material into the furcation area. All samples were embedded in methacrylate resin (Technovit 4004, Heraeus-Kulzer, Germany) 6 mm apical from the CEJ to simulate the clinical appearance of horizontal bone loss and more advanced/moderate furcation involvement (Figure 16, 17, 18).

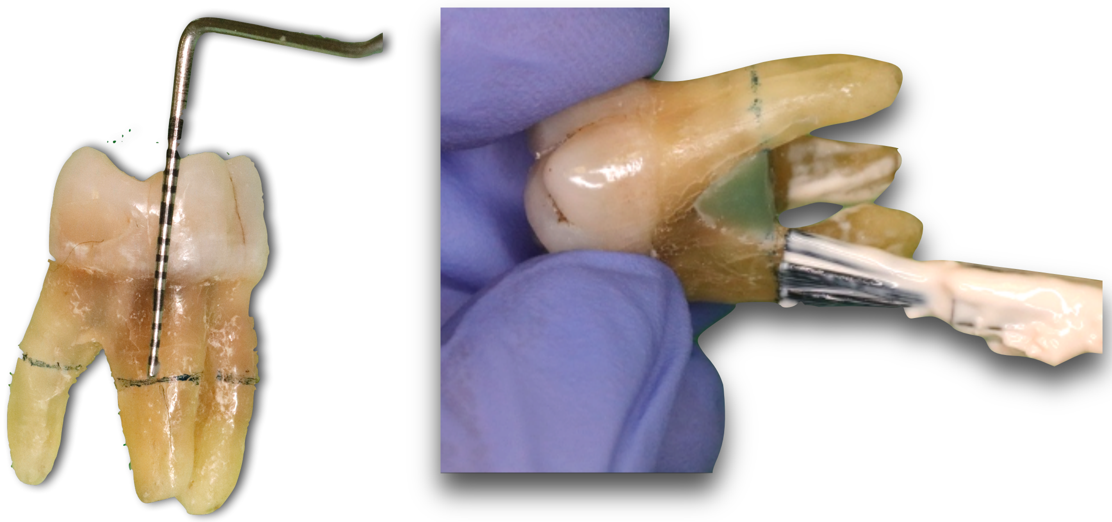

Figure 16: All samples were embedded 6 mm apical from the CEJ. The furcation areas were blocked out with wax and the root surfaces of each specimen were coated with latex separating material to mimic the periodontal ligaments.

Figure 17: The restored and embedded units with which we simulated the clinical appearance of horizontal bone loss and more advanced/moderate furcation involvement.

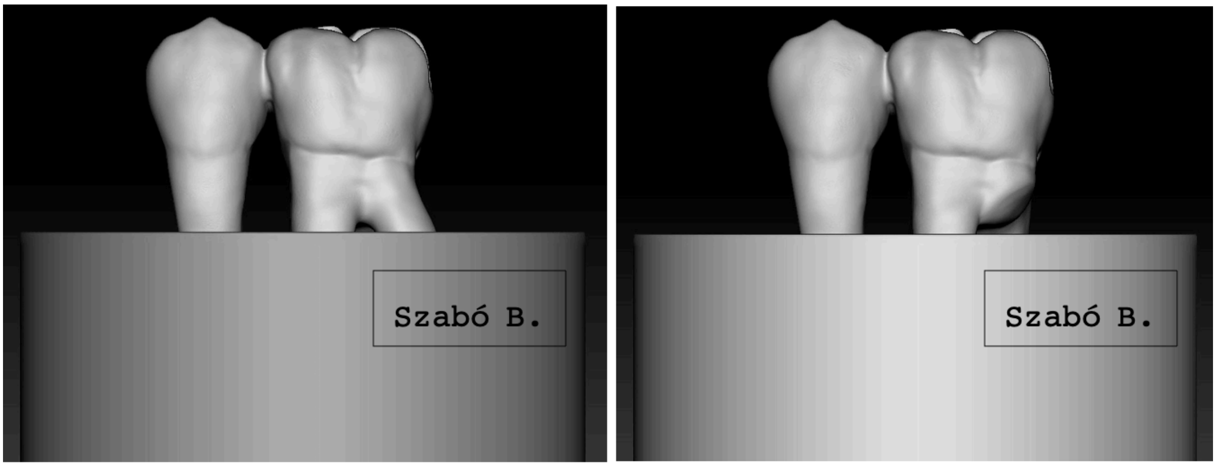

Figure 18: Schematic models representing Group 1 without root amputation and Group 2 with DB root amputation. Both groups are embedded to simulate more advanced/moderate furcation involvement (6 mm apical from the CEJ).

Mechanical testing

Mechanical testing was carried out in two phases. In the first phase (pretesting), the restoration-tooth units were firstly submitted to an accelerated fatigue-testing protocol [62,63,66,67,68] performed with a dynamic testing machine (Instron ElektroPlus E3000, Norwood, MA, USA) (Figure 19). 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 seconds, 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.

Figure 19: The samples were firstly submitted to an accelerated fatigue-testing protocol performed with a dynamic testing machine (Instron ElektroPlus E3000, Norwood, MA, USA).

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).

Second study

Sample selection

All procedures of the second study were approved by the Ethics Committee of the University of Szeged (ethical approval: 4029), and the study was designed in accordance with the Declaration of Helsinki.

48 maxillary molars and 48 maxillary premolars extracted for periodontal or orthodontic reasons were included in this study. Inclusion and exclusion criteria, and also standardization regarding coronal and root dimensions were the same as in the pilot study, described in details above. All teeth were placed in 5.25% NaOCl solution for 5 minutes immediately after extraction. Periodontal ligaments and other soft and hard tissues were removed with hand scalers. The teeth were used within 6 months of removal and stored in 0.9% saline solution at room temperature throughout the study.

Cavity preparation, endodontic treatment, intracoronal splinting and coronal restoration

Cavity preparation was carried out the same way as in the pilot study: standardized OD (premolars) and MOD (molars) cavities were prepared according to Cara et al. in all teeth (Figure 10) [65].

Similarly to the pilot study, all molar teeth underwent root canal treatment according to the protocol described by Szabó et al. (Figure 11) [43], and intracoronally splinted premolar-molar units were created with glass-fibre reinforcement and direct composite coronal restorations were carried out the same way as in the pilot study, described in details above (Figure 12-15).

Root amputation

The splinted models were randomly divided into 4 groups (n=12, Group A, B, C and D). In Group A and B, no root amputation was performed. In Group C and D each DB root of the molars 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 in order to have a cleansable, non-retentive surface.

Embedding the samples

The restored specimens were kept wet (Isotonic Saline Solution 0.9%; B. Braun, Melsungen, Germany) in an incubator (37°C). The tooth pairs were embedded according to the protocol described previously in the introduction. Prior to embedding, in Group B and D, the furcation areas were blocked out with wax to prevent the creep of embedding material into the furcation area. In Group A and C, samples were embedded in methacrylate resin (Technovit 4004, Heraeus-Kulzer, Germany) 4 mm apical from the CEJ to simulate the clinical appearance of horizontal bone loss and mild furcation involvement (see Table 1). In Group B and D, samples were embedded in methacrylate resin (Technovit 4004, Heraeus-Kulzer, Germany) 6 mm apical from the CEJ to simulate the clinical appearance of horizontal bone loss and more advanced/moderate furcation involvement (see Table 1).

Group A	Group B	Group C	Group D
No amputation	No amputation	DB amputation	DB amputation
CEJ-4 mm	CEJ-6 mm	CEJ-4 mm	CEJ-6 mm

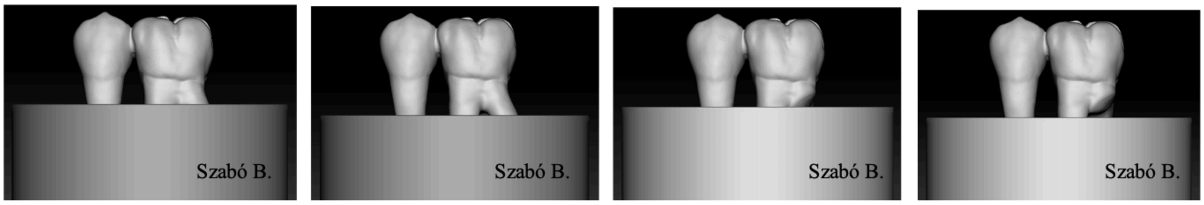

Table 1: Schematic figure representing the test groups in the second study.

Mechanical testing

All units were submitted first to dynamic and then to static, load-to-fracture mechanical testing as described in the pilot study.

After completing the loading test, the fracture mode of all specimens was observed both visually and under stereomicroscope (Heerbrugg M3Z, Heerbrugg, Switzerland) with different magnifications (6.5 and 15x) and illumination angles. Fracture modes were classified into two typical behaviours according to the extending of fracture line. A favourable fracture mode was documented, if composite restoration fractured with/without tooth structure coronally to the simulated bone level (as it could be still reparable under clinical circumstances), whereas an unfavourable fracture, if the fracture line extended apically to the simulated bone level (as it might not be restorable under clinical circumstances).

The 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 by 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 was started from the upper loading part to 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).

6. Results

Pilot study

In the pilot study, during the dynamic loading (preloading), all the samples resisted the force applied to them, no fracture occurred. In the static loading test, the average fracture toughness of Group 1 was 2184.90 N (n = 10, SD = ± 462.133 N), while that of Group 2 was 1752.50 N (n = 10, SD = ± 364.932 N). (see Table 2, 3, and Figure 20). The difference between the two groups was significant in terms of fracture resistance (p = 0.032) (see Table 3). Regarding the fracture pattern, favourable and unfavourable fractures occurred in equal proportions in Group 1, while mostly unfavourable fractures occurred in Group 2 (see Table 4).

				95% Confidence Interval			
	N	Mean	SD	Lower Bound	Upper Bound	Min	Max
Group 1	10	2184,90	462,133	1854,31	2515,49	1762	3021
Group 2	10	1752,50	364,932	1491,44	2013,56	1040	2223

Table 2: Fracture resistance values and related descriptive statistics in the tested groups. Mean values were given in N.

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	934848,800	1	934848,800	5,392	,032
Within Groups	3120679,400	18	173371,078		
Total	4055528,200	19			

Table 3: Results of the ANOVA test. Significant value is highlighted with red.

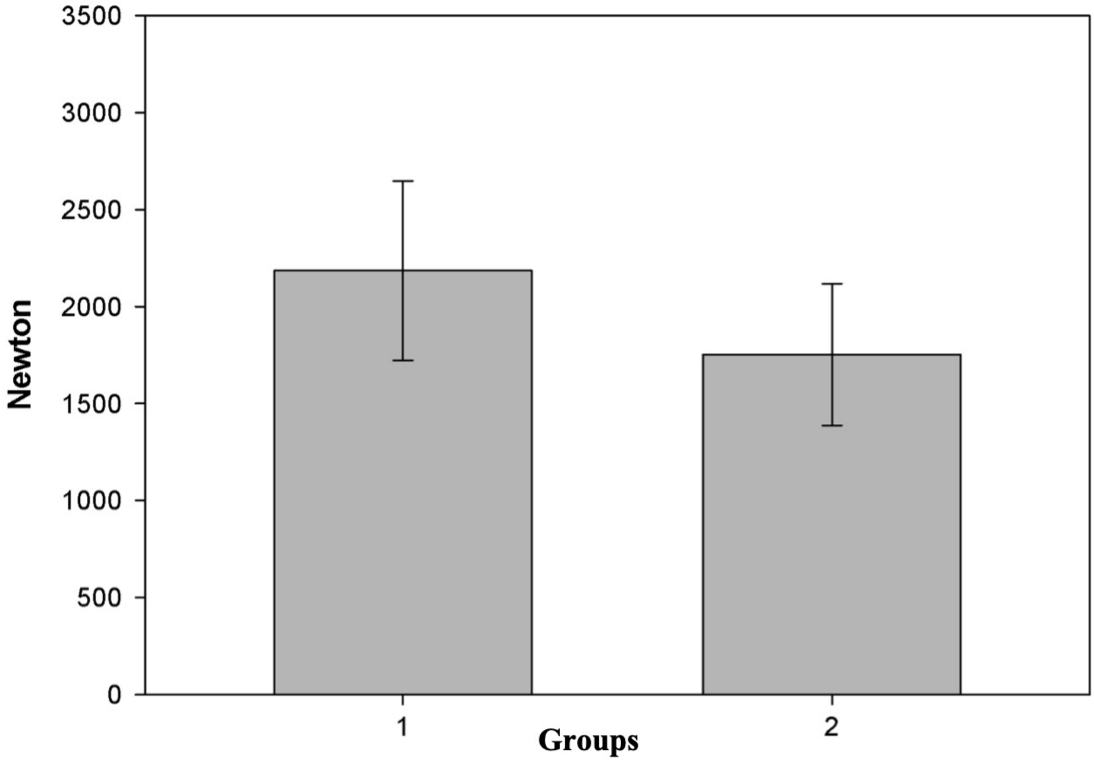

Figure 20: Fracture resistance values and related standard deviation for Group 1 and 2 in the pilot study.

	favourable fracture pattern	unfavourable fracture pattern
Group 1	5	5
Group 2	3	7

Table 4: Fracture patterns by group.

Second study

In the second study, similarly to the pilot study, no fracture occurred during the preloading, so all samples underwent static load-to-fracture test as well.

Figure 21 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 5.

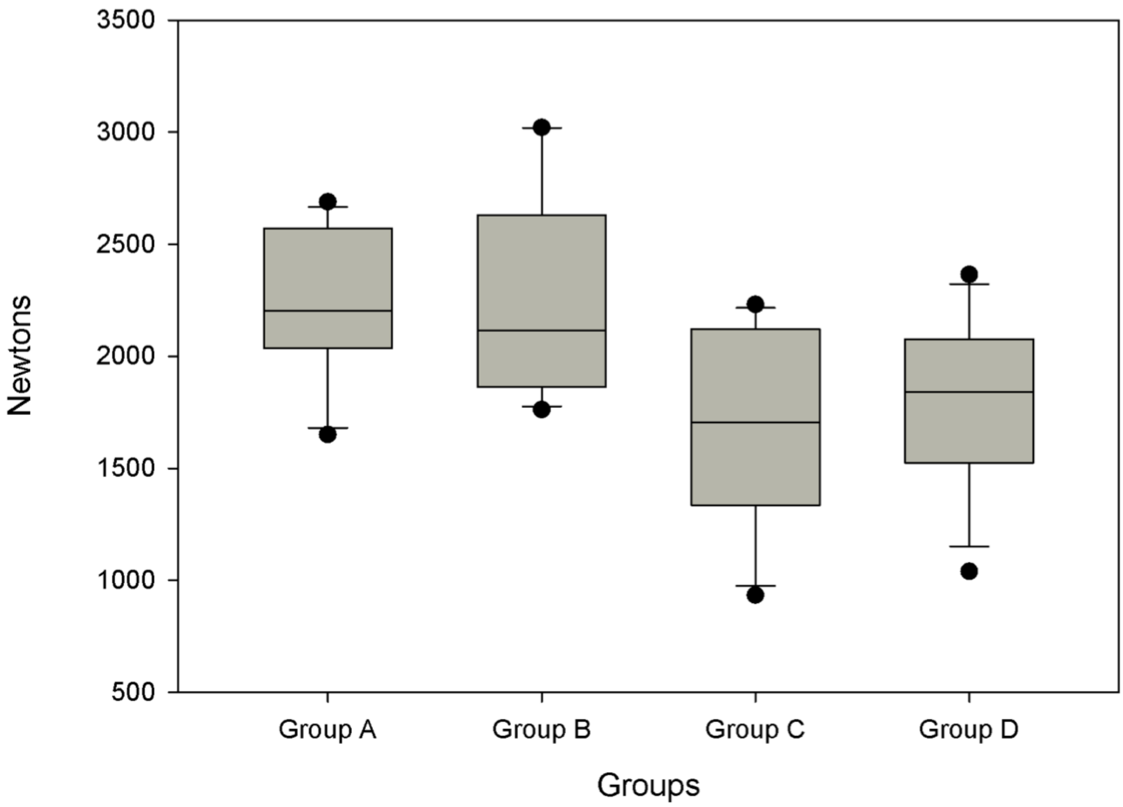

Figure 21: Boxplots of the fracture thresholds by study group.

Mean Difference (I) Group (J) Group (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*	164,085	,008	114,23	990,44
	Group D	459,667*	164,085	,036	21,56	897,77
Group B	Group A	-,750	164,085	1,000	-438,86	437,36
	Group C	551,583*	164,085	,008	113,48	989,69

	Group D	458,917*	164,085	,037	20,81	897,02
Group C	Group A	-552,333*	164,085	,008	-990,44	-114,23
	Group B	-551,583*	164,085	,008	-989,69	-113,48
	Group D	-92,667	164,085	,942	-530,77	345,44
Group D	Group A	-459,667*	164,085	,036	-897,77	-21,56
	Group B	-458,917*	164,085	,037	-897,02	-20,81
	Group C	92,667	164,085	,942	-345,44	530,77
*. The mean difference is significant at the 0.05 level.						

Table 5. Results of the post-hoc pairwise comparisons (Tukey's HSD). Significant values are highlighted with red.

Groups without root amputation (Group A and B) exhibited significantly higher fracture resistance than groups with root amputation (Group C and D). Groups without root amputation (Group A and B) did not show significant difference regarding fracture resistance from each other, irrespectively from the level of embedment. The same applies for groups with root amputation (Group 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 as variable factors. The analysis indicated a significant effect for 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 ($p= 0.689$) (see Table 6).

Between-Subjects Factors			
		Value Label	N
AMP num	1	A	24
	2	NA	24
LEVEL	4		24
	6		24

Tests of Between-Subjects Effects					
Dependent Variable: NEWTONS					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
AMP	3067879,688	1	3067879,688	18,991	**,000**
LEVEL	25346,021	1	25346,021	,157	,694
AMP * LEVEL	26180,021	1	26180,021	,162	,689
Error	7107890,250	44	161542,960		
Total	202255297,000	48			
Corrected Total	10227295,979	47			
a. R Squared = ,305 (Adjusted R Squared = ,258)					

Table 6: Results of factorial ANOVA with bone level and amputation as variable factors.

In terms of the fracture mode, groups with mild furcation involvement (Group A and C) showed more repairable fracture mode (n=8 and 9, respectively) than groups (Group B and D) with advanced/moderate furcation involvement (n=6 and 4, respectively) (see Table 7). Therefore, the null hypothesis regarding fracture modes was also rejected.

	favourable fracture pattern	**unfavourable fracture pattern**
Group A	8	4
Group B	6	6
Group C	9	3
Group D	4	8

Table 7: Fracture patterns by group.

Optical microscope and SEM images of tested specimens showed that the crack path propagated from loading surface (occlusally at connector area) to the inner part of composite restoration (see Figure 22). Figure 22/B and C showed fracture propagation through the occlusal composite resin towards the fibre bundle splint and Figure 22/D showed fracture crack stopped or redirected by fibres.

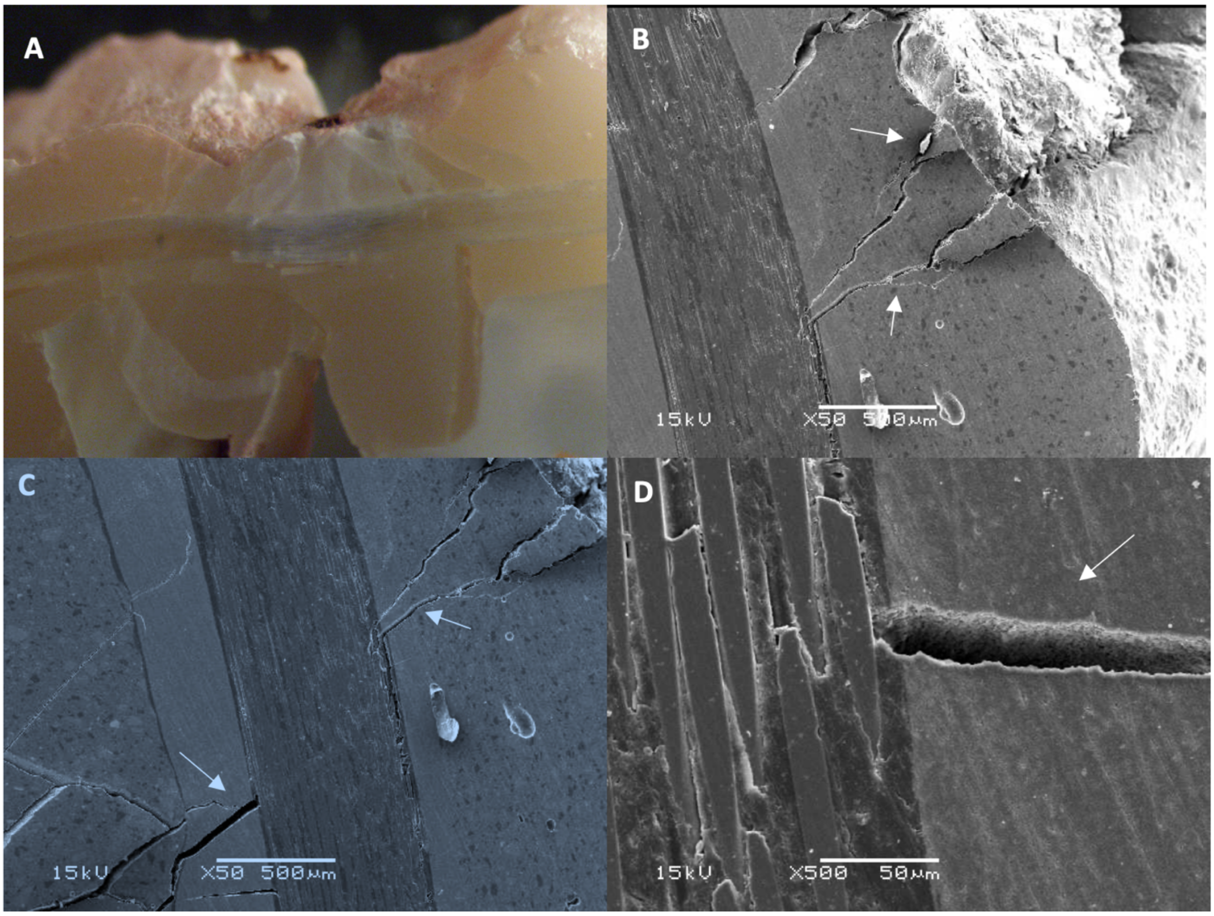

Figure 22. Images with various magnifications showing a crack (arrow) propagated from the load application area (connector) through composite resin to the inner part where fibres redirect or stop the crack propagation.

7. Discussion

In periodontal therapy, long-term retention of periodontally affected natural dentition is often only possible at the cost of compromises. In the above presented studies, we focused on such typical and difficult-to-manage, but common periodontal clinical situations. It is extremely important to reasonably assess the severity and the possible progression of inflammatory processes in the periodontium and to select appropriate therapy: whether a particular tooth is still worth retention or not [27]. In the past decades, the popularity and availability of implant-retained restorations escalated, however, the number of complications (peri-implantitis, peri-implant mucositis) associated with their use also increased. It is known that the success rate of implants in periodontal patients lags behind that of seen in non-periodontally affected patients [69], which seems to make resective surgical approach, such as root amputation, a still nowadays worth-considering, clinically relevant treatment option versus extraction and implant placement [38,70]. This explains why our attention was drawn on this particular procedure type.

The presented studies focused on the possible effect of root amputation and remaining bone level on the fracture resistance of splinted upper molar teeth. During root amputation one or more roots of a multi-rooted tooth is surgically removed at the level of the furcation, while the crown of the tooth and the remaining roots are kept intact [30]. 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 unfavourable root proximity between adjacent teeth [71]. In our study design, the DB root of maxillary first molars was amputated. As already stated above, it is well documented in the literature that due to specific anatomical features and poor accessibility for individual and professional oral hygiene, furcation involvement affects the upper molars three times more often, than the lower ones [36]. Of the upper molars, the first molars [38] and their distal furcation are the most frequently affected ones [10,13,40].

In our **pilot study**, we evaluated the effect of the presence or absence of DB root amputation on the fracture resistance of intracoronally splinted maxillary molar teeth embedded 6 mm apically from the CEJ, in 2 groups (Group 1 and 2, n=10). Our results suggest that amputation of the DB root (Group 2) significantly reduced the fracture resistance compared to non-amputated ones (Group 1). To the best of our knowledge, only Szabó et al. studied the fracture resistance of root-amputated molar teeth so far, so direct comparison is only possible with their results [43]. In their *in vitro* study, they examined the fracture resistance of root-amputated maxillary molars restored with either a direct filling or an overlay simulating sound or impaired periodontal support. Although the variable in our pilot study was the presence or absence of root amputation, whereas in the study of Szabó et al. the bone level was the variable, our results may partially support their observations that the fracture resistance of root-amputated, furcation involved teeth is significantly reduced. However, it is important to note that in the present pilot study, splinted tooth pairs were tested, whereas Szabó et al. tested single upper molars.

In terms of fracture pattern, the root-amputated group (Group 2) showed dominantly unrestorable fractures, while in the non-amputated group (Group 1), restorable and unrestorable fractures occurred equally (see Table 4). This is in accordance with previous findings of Szabó et al., who found that root-amputated, furcation-affected teeth are more likely to produce unfavourable fractures, than non-amputated ones [43,53].

Nieri et al. suggested that the initial amount of bone surrounding the remaining roots at the time of surgery is the most significant prognostic factor in the estimation of the survival rate of molars with periodontal problems [72]. In order to examine the possible effect of different bone levels, we repeated the pilot study with greater sample size and 2 additional groups (n=12, Group A, B, C and D), by introducing 2 different embedding levels to simulate the clinical appearance of a moderate and a more severe horizontal bone loss as a second variant. Furcation involvement can develop in varying extent and severity, however, among these conditions only the ones indicating the amputation of the DB root with still retaining the tooth in such a clinical setting were modelled in our study setup. Samples were embedded to 4 and 6 mm from the CEJ, because on the one hand, they represent 2 separate, well-distinguishable, clinically relevant situations, and on the other hand, the attachment loss and the furcation involvement would indicate the amputation of the DB root, and maintaining the tooth is still realistic.

In our **second study**, the amputation of the DB root (Group C and D) significantly reduced the fracture resistance of intracoronally splinted upper premolar-molar tooth pair units compared to non-amputated ones (Group A and B). To our knowledge, this is the first study to show the effect of root amputation alone on the fracture resistance of periodontally 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.

Regarding the bone level, our current results contradict previous findings of Szabó et al., who found that furcation involvement appeared as a significant factor in weakening root-amputated maxillary molars [43]. As already stated, it is important to note that in the present study, splinted tooth pairs were tested, whereas Szabó et al. tested single upper molars, and this might 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 [73]. 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 previous results of Szabó et al., according to which root-amputated furcation involved teeth develop predominantly irreparable fractures [43,53]. Figure 22 shows how the splinting fibre bundle acts as a crack stopper thus reinforcing the restoration-tooth unit. Many cracks started from the loading area and stopped at the fibre-composite interface. This could explain why all restorations survived fatigue loading. However, during static loading, a few cracks did pass the fibres and lead to failure. In terms of reinforcement, it is important to highlight the effect of the adhesion between the fibre bundle and the composite resin, and the position of the splinting fibres on the distribution of the occlusal forces. It has been shown that when the fibre 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 [52].

In order to prevent further attachment loss due to the mobility increase caused by the both degeneration of periodontal tissues and root amputation [41], intracoronal splinting of posterior teeth with fibre-reinforced material may be necessary [74,75]. Even if in our studies, all samples received fibre-reinforced composite splinting, it is important to emphasize that the extent of mobility should be considered always individually, and root amputation alone does not necessitate splinting [8]. 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 [37]. Kumbuloglu et al. found good survival rates for periodontally involved teeth splinted with the same fibre-reinforced composite splint (everStick Perio) as we used in our study, however, they splinted lower front teeth [76]. Periodontal disease with bone loss and secondary furcation involvement is one of the most difficult-to-manage conditions in periodontology [27], reducing the 10- to 15-year survival rates by about 50% compared to nonfurcation-affected teeth [77]. We are not able to cure the developed condition, but we can slow down the progression if the patient shows proper cooperation during the maintenance of the individual and the professional oral hygiene. 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% [78,79,80,81], while others report significantly less favourable results of only 40-60% [82,83].

In the present *in vitro* study, we modelled 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 [11]. Furthermore, furcation involvement has been shown to be among the most serious deteriorating factors regarding the long-term prognosis of multi-rooted teeth [81]. 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 [47]. 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 [84,60,58]. 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 [64]. 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. 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 [43,64]. 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, we tested extracted teeth, which holds some possible limitations. One disadvantage when using human teeth for testing is the large variation among individual teeth (e.g., in mechanical and physical properties), and existing microcracks in the dentine may not always be seen before testing. In general, this may lead to large standard deviations. Despite the mentioned shortcomings, the use of natural human teeth is still a reliable method for *in vitro* fracture tests [85]. When utilizing human teeth, the establishment of strict exclusion and inclusion criteria is mandatory. Within our study designs, both the coronal part and the roots of maxillary molars were carefully standardized. Another limitation might be that the extracted teeth used in both studies were not standardized according to their age. 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 only comparable to a limited number of 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.

8. Summary

The studies described in the thesis sought to evaluate how the presence or lack of DB root amputation and the severity of horizontal bone loss can influence the fracture resistance and the fracture pattern of intracoronally splinted maxillary molar teeth under *in vitro* conditions. To our knowledge, this is the first study to show root amputation alone as a significant factor affecting the fracture resistance. Within the limitations of this study, it can be concluded that root amputation has a negative effect on the fracture resistance of furcation involved, intracoronally splinted upper first molar and second premolar units. The degree of furcation involvement, as modelled in the studies, does not seem to influence the fracture resistance of such units.

In the thesis, one possible way of analogous modelling method of periodontally affected teeth and the surrounding periodontal structures surrounding is also presented comprehensively that can serve later as the basis of mechanical load studies.

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