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ORIGINAL ARTICLE

Combined effects of ageing and type 2 diabetes mellitus on human dental mechanics

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Abstract Dental collagen, a protein subject to essentially zero turnover, is highly susceptible to the accumulation of advanced glycation end-products (AGEs) due to elevated blood glucose in type 2 diabetes mellitus (T2DM). Progressive loss of collagen's structural stability likely increases the risk of tooth decay and fracture complicating the proper fixation of dental restorations. Our study aimed at investigating the structural and mechanical alterations in T2DM-affected teeth by measuring the stiffness and detecting micro-traumas and fractures. By using computed tomography (CT) and static compression tests, caries-free human molars and front teeth from healthy and T2DM patients were analyzed. Our results revealed a significantly reduced enamel/dentin ratio and larger dentin crack sizes in T2DM molars compared to controls, indicating increased susceptibility to mechanical damage. Although statistical analysis showed age as a dominant factor in tooth deterioration, T2DM was associated with a clinically relevant increase in dentin fracture size, suggesting that diabetes aggravates tooth fragility. Our findings underscore the importance of the cooperative effect of age and diabetes in dental biomechanics which bears significant implications in restorative treatments. Altogether, while ageing significantly affects tooth mechanical stability, T2DM contributes to additional structural weakening, particularly in molars, due to altered mechanical properties and higher occurrence of dental wears.

Keywords Diabetes · Mechanics · Dental morphology · Extracellular matrix (ECM) · Collagen

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Introduction

Ageing results in the natural accumulation of chemically modified molecules throughout the human body. Examples for such changes are DNA methylation, posttranslational protein modifications, and accumulation of D-aspartic acid residues (aspartic acid racemization, AAR) and advanced glycation end products (AGEs) [1–3]. AGEs are formed by non-enzymatic glycation, which is an irreversible reaction of reducing sugars with free amino groups on proteins, lipids, or nucleic acids. AGEs typically accumulate in

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relatively long-lived tissue proteins throughout the lifetime, such as in collagen, crystalline of the lens, myosin of skeletal muscle, articular cartilage, rib cartilage or intervertebral disc causing significant structural and mechanical alterations, such as increased matrix rigidity, and impaired repair capacity with aging and metabolic disorders [4–8]. These cumulative alterations align with gerontological concepts of extracellular matrix (ECM) ageing, in which slowly replacing structural proteins are able to record the person's biological history. Furthermore, besides the biochemical alterations of dental collagen, teeth are subjected to characteristic mechanical loads, making them a sensitive indicator of the long-term consequences of ageing, metabolic processes, and local biomechanical effects. Dentin is a calcified tissue that occupies the majority of the human tooth. It serves as an elastic support for the more brittle enamel and provides a protective medium for the inner pulp. Dentin consists of hydroxyapatite-embedded collagen matrix in which dentinal tubules are present, forming a traversed network. It has been reported that in a normal, caries-free tooth the dental collagen is also exposed to glycation by dentinal fluid which is affected by blood sugars, resulting in a brownish discoloration of the teeth and a change in their mechanical properties [9]. Since collagen receives direct nourishment through dentin tubules, AGEs can accumulate in dentin during natural ageing, as a part of the physiological process. AGEs content of dentin can be quantified by various, well-established biochemical, immunological, and spectroscopic methods [9–13].

Conditions of chronically high blood glucose such as type 2 diabetes mellitus (T2DM) may accelerate dental alterations via biophysical effects resulting in the premature stiffening of collagen and fragility of teeth. This characteristic also means that dentin is highly exposed to the accumulation of AGEs in T2DM. Since dentin is a mineralized tissue similar to bone, the most dominant ageing- and metabolism-related effect is the formation and accumulation of AGEs, making the calcified matrix stiffer and more fragile. In other relatively long-lived collagen-containing tissues, such as vessels or tendons, the main differences of the ageing are that, besides the mechanical deteriorations by AGE accumulation, further structural and functional alterations, such as fiber degradation and fragmentation can occur [14, 15]. Furthermore, in these non-mineralized connective tissues the fibroblast activity is also impaired, causing dysfunction in repair and healing mechanisms [16, 17].

According to the International Diabetes Federation (IDF) Diabetes Atlas, 537 million people had T2DM in 2021 and this number is projected to rise steadily to 643 million by 2030, and 783 million by 2045 [18]. In Hungary, the same tendency can be seen. The most recent data show that T2DM affects one in six people and more than one third are over 65 years. Studies and reports indicate that a large portion of the population is affected by a wide range of oral complications such as tooth decay, gum disease, periodontitis, xerostomia, and other dental issues [19].

Fracture resistance of teeth is important in clinical dentistry and has been investigated extensively [20–22]. It has been shown that fracture toughness of aged dentin is significantly lower than that of the young. This age-related deterioration of mechanical stability can be explained by various factors: tubule number and diameter, changes in chemical composition of intertubular dentin, and alterations in the extent of tubular occlusion [20]. Considering that dental collagen displays no protein turnover, it is highly susceptible to progressive accumulation of AGEs caused by elevated blood glucose, which affects its long-term structural stability. Until now, limited data are available regarding the effects of type 2 diabetes on the mechanical properties of dental hard tissues. It has been shown in an in vitro study that T2DM negatively impacts root fracture resistance (RFR), pointing at a higher susceptibility to fracture under vertical forces (such as occlusal) affecting the outcome of root canal treatment [23]. However, fracture tests under static force on diabetic coronal region of extracted tooth samples have not been investigated yet.

We propose that besides the numerous oral complications associated with T2DM [24], the early deterioration of mechanical stability of dental hard tissues is an important factor leading to the loss of teeth. Since tooth fracture is the major cause of tooth loss, a better understanding of the mechanical alterations of teeth in diabetic conditions is crucial. The combination of reduced fracture resistance of the teeth with exposure to mechanical forces, such as chewing or bruxism, may promote the development of micro cracks in enamel, paving the way for bacterial penetration towards dentin [25]. By experimentally

exposing dental samples to static compression, we can reveal the load-bearing capacity of the tooth material. To assess the initial status of extracted tooth samples and detect micro-traumas or fractures caused by mechanical testing, we used high resolution computed tomography (micro-CT). From CT images the volume of enamel and dentin, hence the extent of dental wear was determined, allowing assessment of its association with type 2 diabetes mellitus. Following the compression test, we measured the crack growth direction, size and surface location. In our work we performed a micro-CT imaging and mechanical test of normal and T2DM-affected extracted teeth to reveal the alterations of fracture resistance by diabetic conditions. Our results showed reduced stiffness and lowered maximal force of diabetic molar tooth samples and CT images revealed a remarkably extent of tooth wears in the same specimens. Furthermore, following the mechanical test larger crack sizes were determined in diabetic molar samples, highlighting the compromised mechanical stability of tooth hard tissues by diabetic conditions.

Materials and methods

Samples

Non-carious human teeth were extracted as a part of routine treatment from 20 healthy ($n=27$ mean age: 38.1 years $\pm$ 19.1 years) and 15 T2DM-affected patients ($n=19$ mean age: 60.4 years $\pm$ 14.8 years) at Semmelweis University, Department of Oro-Maxillofacial Surgery and Stomatology (Table 1). All patients included in the study group had been diagnosed with type 2 diabetes mellitus for at least five years. The institutional review board of Semmelweis University Regional and Institutional Committee of Science and Research Ethics approved the study protocol (approval number: 99/2025). According to teeth type, samples were selected into two subgroups: frontal (including incisors and canines) and molar. The control group included 22 molars and 5 frontal teeth, while in the T2DM group 10 molar and 9 front teeth were examined. Samples were cleaned with hypochlorite solution in a sonicator bath for 15 min, then stored in Hank's balanced salt solution (HBSS) at 4 °C until further use.

Computed tomography

To detect microtraumas and fractures, each tooth specimen was scanned both before and after mechanical testing with a nanoScan SPECT/CT system (Mediso Medical Imaging Systems, Hungary). Samples were excluded from the further procedures if any crack was detected which may have formed during the extraction procedure. The scans were performed in helical CT mode with the following acquisition settings: the tube voltage was set to 70 kV peak, and the tube current was 760 µA. Each projection had an exposure time of 300 ms. A 1:1 binning mode was applied to preserve native spatial resolution, and the pitch factor was set to 1.0 to ensure continuous helical coverage. The system operated at maximum zoom to enhance spatial detail, and a total of 720 projections were acquired over a full 360° rotations. The reconstructed images had an isotropic voxel size of $22\times22\times22$ µm, allowing for high-resolution volumetric datasets suitable for detecting fine structural

Table 1 General descriptive statistics of investigated samples

	Control ($N=27$)	T2DM ($N=19$)	Overall ($N=46$)
Age (years)			
Mean (SD)	38.1 (19.1)	60.4 (14.8)	47.3 (20.6)
Median (IQR)	28.0 (31.5)	61.0 (19.0)	48.0 (43.0)
Min, Max	16.0, 73.0	37.0, 78.0	16.0, 78.0
Sex			
Female	16 (59.3%)	10 (52.6%)	26 (56.5%)
Male	11 (40.7%)	9 (47.4%)	20 (43.5%)
Type of teeth			
FRONT	5 (18.5%)	8 (42.1%)	13 (28.3%)
MOLAR	22 (81.5%)	11 (57.9%)	33 (71.7%)

changes such as microcracks and fractures. The location of fractures and tooth material loss was determined manually using vivoQuant 1.22 software (inviCRO-Konica-Minolta Inc., Boston, USA). Prior to mechanical testing, enamel/dentin volume ratio were measured to assess the extent of tooth wear. Following the mechanical testing, the surface location and size of the fractures were identified and measured. The enamel and dentin of the teeth were segmented in successive steps. First, the entire tooth was segmented by using three-dimension volume of interest (3D VOI) with a threshold value. The dentin of the teeth had different densities because it came from different patients and different tooth positions, thus it had to be segmented separately. The density ranges of tooth enamel in Hounsfield units (HU) were determined by sampling (above 4500 HU for molars and above 7000 HU for incisors). After determining the threshold, the selected volume was corrected manually, resulting in the enamel volume in mm^3 (V enamel). The remaining volume of the tooth is dentin in mm^3 (V dentin), but individual pixels not belonging to the surface were corrected in two steps with minimal erosion, followed by minimal but equivalent dilation. This smoothed the surface and corrected imaging errors. To determine fracture size, the largest dimension in the broken part of the tooth was marked in the manner described above. To analyze fractures in selected 2D CT images, ImageJ (National Institutes of Health) software was used. Fracture size in the enamel was measured as the perpendicular distance from the outer enamel surface to the enamel–dentine junction (EDJ). Fracture size in dentin was measured from the enamel–dentine junction (EDJ) to the dentin–pulp junction (DPJ). To accurately identify the surfaces, precise anatomical knowledge of the dental structures was required, supported by the application of Mühlreiter's signs. To differentiate the oral and vestibular surfaces, the curvature of the crown was examined, as well as the size and position of the cusps in the case of molars and premolars. For distinguishing the approximal surfaces, root curvature and angulation indicators were used.

Mechanical test

For the static compression test a ZWICK Z005 computer-controlled tensile tester device was used. Teeth with uncovered coronal region were embedded in epoxy resin (Ipox Chemicals MR3010 + MH 3124, mixing weight ratio 100:33) in a cylindrical plastic container. Coronal regions were compressed perpendicularly to the occlusal surface with 2 mm/min crosshead displacement by using an 8 mm diameter spherical steel indenter (Fig. 1A). The device was set to automatically stop at 50% force drop in all cases (i.e., when the force drops to 50% of the maximum value during the indentation). Thus, by using this limit the complete destruction of the tooth could be prevented, and it was made possible to examine the cracks and fractures following the mechanical test. To characterize the fracture resistance of the samples during the compression test the force (N) was recorded as a function of indentation distance (mm) (Fig. 1B). From the elastic region of these curves when there is a linear relationship between the force and indentation, stiffness of the tooth could be calculated from

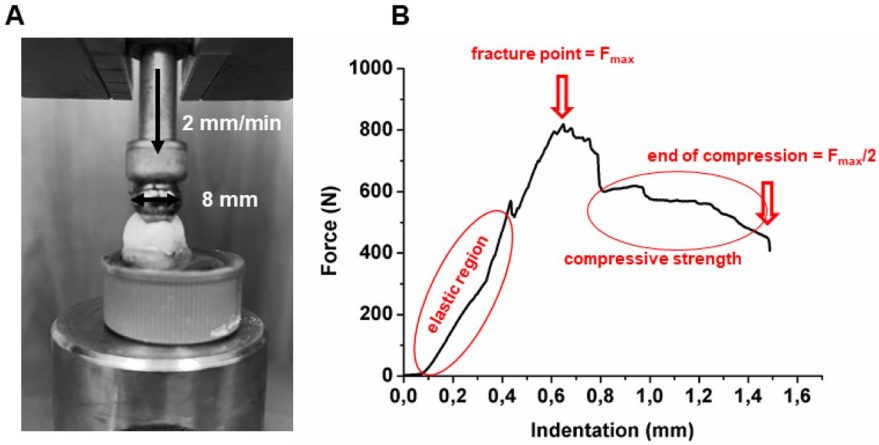

Fig. 1 **A** Measuring setup of static compression test. **B** Representative force versus indentation curve with regions reflecting the mechanical behavior of samples

the slope in N/mm. Furthermore, the maximum point of the curves (F_{max}) also could be determined showing the fracture point during the mechanical testing. Following this point further crack propagation could be seen from the continuously dropping and fluctuating force values, indicating the material separation. At the 50% drop of F_{max} the cycle of the mechanical tester stopped.

Statistical analysis

Throughout the presented graphs, the bars represent the mean, and error bars show the standard deviation (SD). Box plots indicate the median, interquartile ranges, minimum and maximum values, and outliers. We used Origin Pro8 statistical software (Origin Lab Corporation). Groups were compared with a 2-sample *t* test. Statistically significant difference was accepted at 95% confidence level and $p < 0.05$ value. Furthermore, we aimed to estimate the effects of diabetes status (T2DM group vs. control) and tooth type (molar vs. frontal) on the following three outcomes such as breaking force (F_{max}), enamel/dentin volume ratio, and dentin fracture size. It was taken into account, that multiple teeth were measured from the same patient, thus linear mixed-effects models were applied with a random intercept for each patient. This approach allows us to properly account for the correlation of observations within the same individual. All models were adjusted for patient age and tooth type, as these variables are potential confounders regarding the interested effect. For breaking force, the natural logarithm of F_{max} was used as the outcome variable. This transformation was chosen because the distribution of the raw values was skewed, while the log-transformed values were approximately normally distributed, and model diagnostic plots indicated a better fit on the log scale. We evaluated possible two-way interaction effects between predictors (for example, the effect of diabetes on F_{max} may differ on tooth type). Interaction terms were retained in the model only if they were statistically significant and clinically relevant. In the final models, no interaction terms were included. Model assumptions were checked using diagnostic plots, which indicated acceptable fit. All models were fitted by R software (v4.4.3) [26].

Results

CT analysis prior the mechanical test

To detect the origin and size of failures, specimens were scanned before and after mechanical tests. By using CT images that were acquired prior to the mechanical test the enamel/dentin volume ratio was assessed, which revealed the extent of dental wears. In CT images the layer of enamel and dentin could be distinguished very precisely since they have different composition causing different densities. Due to the high mineral content of enamel, it represents the brighter, outermost part of the tooth while the underlying dentin shows lower density, appearing slightly darker on the grayscale (Fig. 2A). Our results showed that the enamel/dentin volume ratio is significantly reduced in diabetes-affected molar samples. In representative 2D slices enamel displayed thinned structure in diabetic molar teeth. This ratio of molar teeth in control groups was 0.406 ± 0.07, while in T2DM group 0.228 ± 0.059 was determined. Front teeth did not show difference in enamel/dentin volume ratio between control and diabetic groups (Fig. 2B).

Mechanical test

In the present work, we examined the mechanical behavior of normal and T2DM-affected teeth by using a compression test. The coronal region of epoxy-embedded samples was exposed to compressive load with 2 mm/min crosshead speed by 8 mm diameter spherical steel indenter. In the recorded force versus indentation curves the x axis represents indenter displacement from touching the tooth surface, while y axis shows the force applied by the indenter during the compression. Results of the fracture tests are shown in Fig. 3. In general, in these force versus indentation curves various regions can be identified reflecting the mechanical resistance of teeth during mechanical testing. At the beginning an elastic region could be determined showing a linear relationship between the force and indentation (Fig. 1B). This ascending part reflects the elastic deformation of the tooth material, supposing that it can return to its original shape when the load would be removed. First, from this linear region the slope was determined, obtaining the stiffness (N/mm) of the tooth material. Our

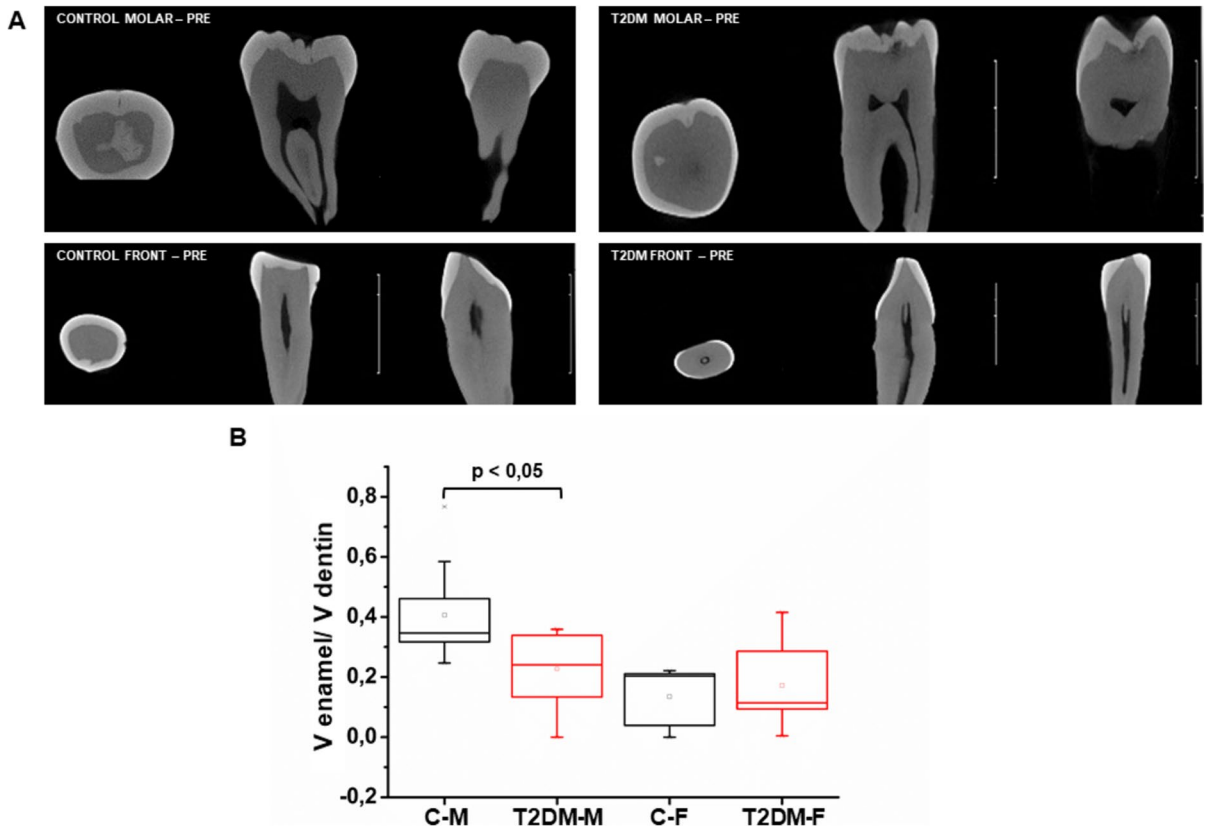

Fig. 2 CT analysis prior the mechanical test. CT image are shown together with transverse, coronal, and sagittal images (from left to right). Scale bar: 1 cm. **A** Representative two-dimensional (2D) slices selected from 3D CT reconstruction of control and T2DM samples. **B** Before the compression test the enamel/dentin volume ratio in mm3 was determined. This ratio of molar teeth in control groups was 0.406 ± 0.07, while in T2DM-M group 0.228 ± 0.059 was determined. The enamel/dentin volume ratio of front samples was slightly different between control (0.135 ± 0.053) and T2DM-F groups (0.172 ± 0.067). C-M, control molar teeth; T2DM-M, type 2 diabetes mellitus molar teeth; C-F, control front teeth; T2DM-F, type 2 diabetes mellitus front teeth

results showed that T2DM molar teeth have significantly lower stiffness (1052.35 N/mm ± 147.58 N/mm) comparing to the control samples (1661.86 N/mm ± 205.89 N/mm) (Fig. 3E). Then, peak of the curves was determined revealing the maximum force (F_{max}) required for creating significant cracks in the tooth material. Figure 3B shows that in T2DM molar teeth reduced force response could be recorded comparing to controls (Fig. 3A), while control and diabetic front teeth have similar tendency in curves (Fig. 3C and D). Figure 3F shows broad distribution of data points with values reaching up to 2000 N. In T2DM-M group the maximal force is decreased comparing to the control molar samples. In the C-M group 1321.33 N ± 365.63 N was measured, while in the T2DM-M group 938.4 N ± 434.038 N maximal force was determined, which means 30% reduction but non-significant difference in force response. Front teeth displayed similar values in both groups with high variability. Following the F_{max} point further crack formation and propagation could be seen from the continuously dropping and fluctuating force values, indicating the separation of material. This part of the curve reflects the compression strength of teeth. In our measurements, this region showed high variability in various tooth types and samples. In those curves that terminated abruptly without a visible decrease in force, the subsequent data point has already dropped below 50% of the maximum force, therefore, the tensile testing machine's software registers this as the end point.

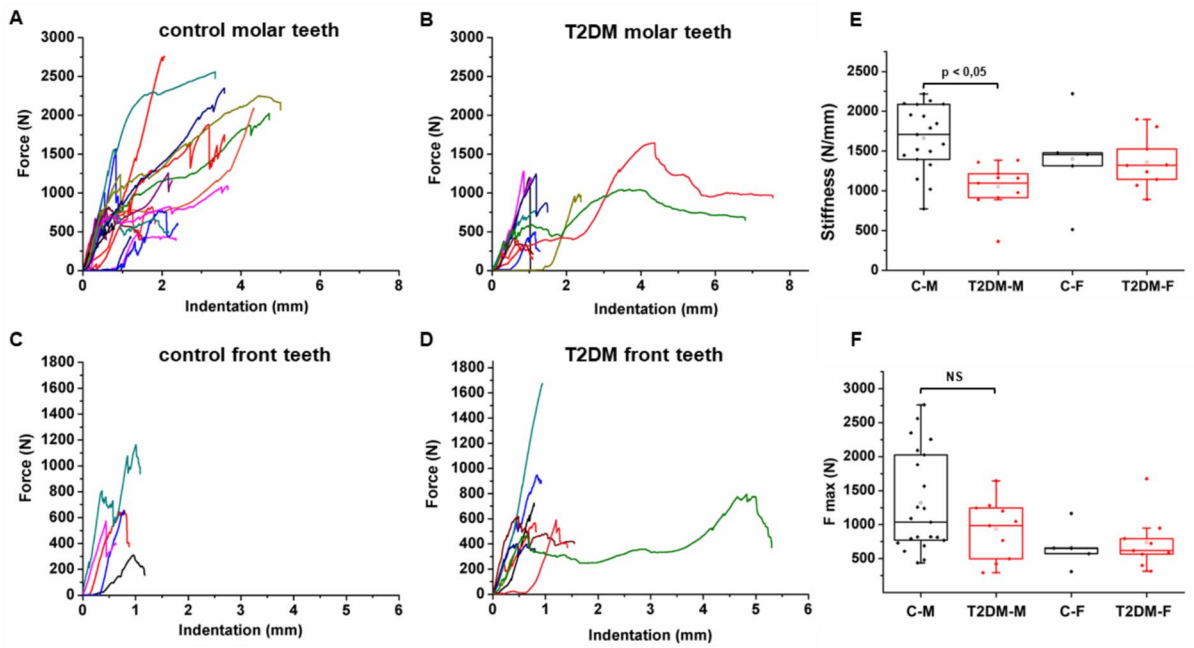

Fig. 3 Results of mechanical tests. **A–D** Recorded force versus indentation curves of mechanical test in various groups. **E** The stiffness of control molar samples was 1661.86 N/mm ± 205.89 N/mm while diabetic molar teeth displayed significantly lower 1052.35 N/mm ± 147.58 N/mm values. **F** The box plot shows that the maximal force is reduced by 30% in T2DM-M group comparing to the controls. In the C-M group, 1321.33 N ± 365.63 N was determined, while the diabetes-affected molar teeth showed 938.4 N on average with a standard deviation of 434.038 N. C-M, control molar teeth; T2DM-M, type 2 diabetes mellitus molar teeth; C-F, control front teeth; T2DM-F, type 2 diabetes mellitus front teeth

CT analysis following the mechanical test

Following the compression test, crack sizes were determined in enamel and dentin respectively from two-dimensional (2D) slice selected from 3D CT reconstruction, in which cracks appeared most prominent (Fig. 4A). Furthermore, the surface location of fractures was also determined. Results indicated that the dentin in diabetes-affected molar teeth is more exposed to fracture formation than in controls. The mean size of failures in T2DM-M group was 3.354 mm ± 1.045 mm while in the control samples, it was only 1.55 mm ± 0.65 mm. In front teeth both the enamel and dentin were equally exposed to fractures, no significant differences were determined between crack sizes (Fig. 4B).

Regarding the location of failures occlusal and incisal surfaces of all control molar teeth were damaged following the mechanical testing ($n = 22$) (Table 2). In 10 molar samples the distal surface and in 7 cases the mesial surface was also affected by fractures (Fig. 5). Diabetic molar teeth showed significant material loss at occlusal surfaces ($n = 8$), however in these samples the damage of vestibular surface was more highlighted. Front teeth were significantly affected by cracks at many surfaces in both groups. In T2DM front group the oral surface was more frequently damaged during the compression.

Statistical analysis

Using linear mixed-effects models, we assessed the effects of diabetes status (T2DM vs. control) and tooth type (molar vs. frontal) on measured physical quantities such as breaking force (F_{max}), enamel/dentin volume ratio, and dentin fracture size, while adjusting them for patient age.

For breaking force, the analysis was performed on the logarithm of F_{max}, however results are presented on the linear scale for the easier interpretation (Fig. 6A). Age had a significant effect on maximal force ($p = 0.0113$). Predicted values indicated a clinically relevant decrease from ~ 1200 N at age 20 to ~ 520 N at age 80, highlighting

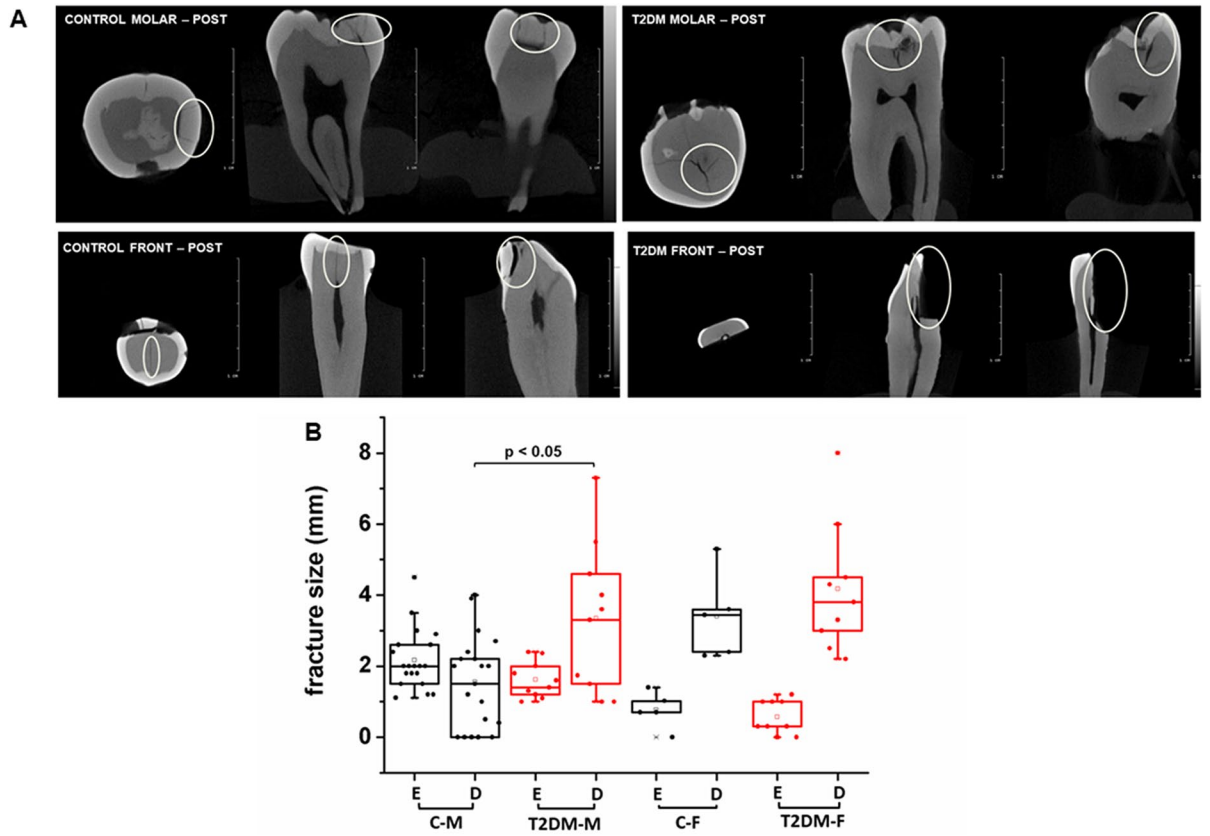

Fig. 4 CT analysis following the mechanical test. CT image are shown together with transverse, coronal, and sagittal images (from left to right). **A** Representative two-dimensional (2D) slices selected from 3D CT reconstruction of control and T2DM samples with larger crack size appearance. White circles indicate the location of cracks. Scale bar: 1 cm. **B** Following the compression test crack sizes were measured. In diabetic molar samples the dentin was more exposed to fractures, significantly larger, 3.354 mm ± 1.045 mm cracks were determined, while in the control molar samples displayed 1.55 mm ± 0.65 mm fractures. C-M, control molar teeth; T2DM-M, type 2 diabetes mellitus molar teeth; C-F, control front teeth; T2DM-F, type 2 diabetes mellitus front teeth; E, enamel; D, dentin

Table 2 Summary table of tooth samples affected by failures following the mechanical test

	Number of affected teeth					
	Occlusal surface	Incisal surface	Mesial surface	Distal surface	Oral surface	Vestibular surface
Control molars	22	22	7	10	3	1
Diabetic molars	8	8	0	0	2	6
Control fronts	5	5	5	5	3	2
Diabetic fronts	11	11	11	11	7	4

age-related weakening of mechanical properties. Diabetes showed no significant, nor relevant association with F_{max} ($p = 0.7900$), with an estimated mean difference of ~ 50 N between groups. Tooth type was also not significant ($p = 0.5491$), however the estimated effect suggested a relevant, slightly

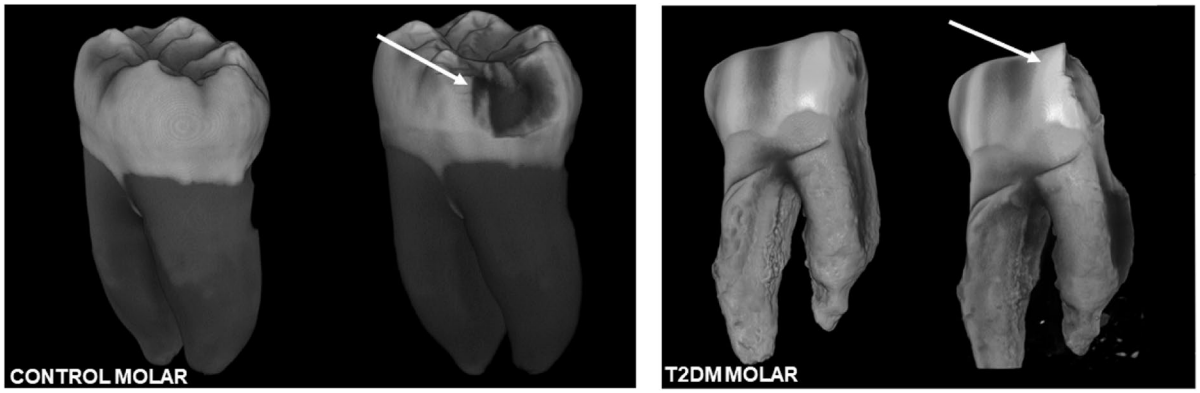

Fig. 5 Representative 3D reconstruction of control and T2DM molar samples. Both cracks affected the proximal mesial surface of teeth (arrows)

higher forces (~ 100 N) in molars compared with frontals.

For the enamel/dentin volume ratio, age showed again a significant effect ($p = 0.0032$). Predicted values indicated an approximately 6% relevant decrease per decade, independent of diabetes status and tooth type (Fig. 6B). Neither diabetes ($p = 0.6282$, estimated –2.3% difference) nor tooth type ($p = 0.9633$, estimated 0.2% difference) had significant or clinically relevant effects on the enamel/dentin volume ratio.

For dentin fracture size, diabetes showed a non-significant effect on fracture size ($p = 0.0521$), with an estimated increase of ~ 1.24 mm compared with controls (Fig. 6C). Although, this result is not statistically significant in this sample, the magnitude of the effect pointing to the potentially important clinical consequence. Age, and tooth type showed no significant effect on dentin crack size.

Discussion

Non-enzymatic glycation severely affects the common collagen-containing connective tissues, and it is already known that the dental collagen is also exposed to this process. In T2DM, due to the permanently high level of blood glucose, dental alterations are accelerated via biophysical effects resulting in the premature stiffening of collagen and tooth fragility [27]. Since dental collagen does not undergo protein turnover, this property makes it an excellent record of the person's biological history, and it can be used for age estimation in forensic cases [28]. However, the potential structural and mechanical alterations of tooth hard tissues in T2DM are not precisely known. We suggested that besides the numerous diabetic complications the early deterioration of the mechanical stability of teeth should not be ignored. Furthermore, the higher level of accumulation of AGEs on long-lived dental collagen in T2DM can be a crucial factor in protein turnover rate, and in age estimation studies [29]. For this reason, in our work we performed a detailed structural, and mechanical analysis of normal and diabetic tooth samples applying unique methods in dental science.

First, micro-CT images were acquired about samples in order to determine the extent of dental wears and excluding samples if any crack was detected which may have formed during the extraction procedure. For this reason, the volume of enamel and dentin was determined respectively, then an enamel/dentin volume ratio was calculated. We observed that in diabetes-affected molar samples significantly reduced enamel volume could be measured, thus enamel/dentin ratio decreased almost by half comparing to control molar teeth (Fig. 2B). This difference may be explained by the higher age of the diabetic individuals, first (Table 1). However, another relevant condition can be mentioned as well. In T2DM periodontal diseases are the main oral complications, and it is already known that periodontitis and hyperglycemia are mutually and bi-directionally related and adversely affect each other [30]. First, it leads to the loss of front teeth, thus chewing load shifts to the molars. Instead of using the small front teeth,

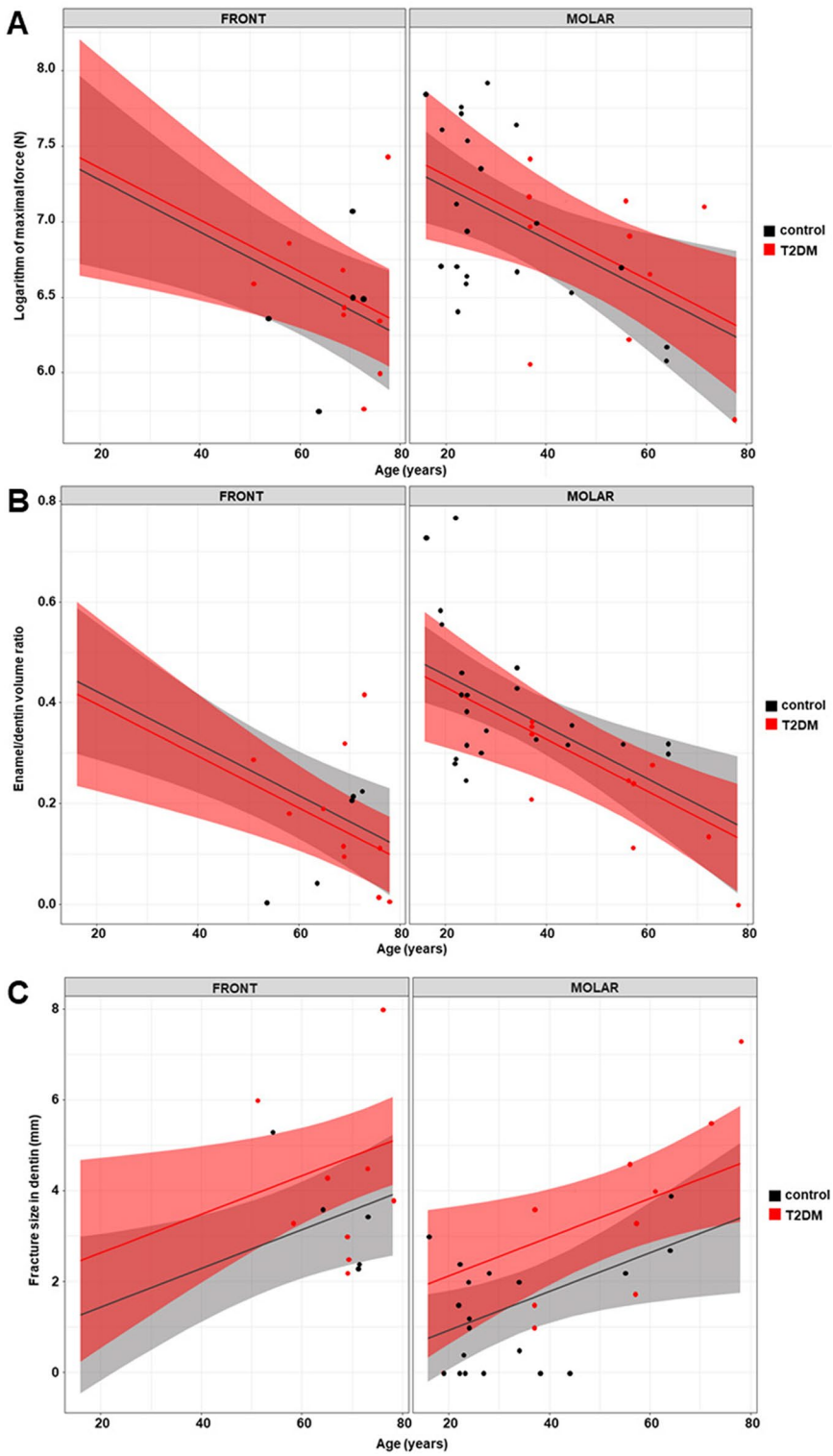

Fig. 6 Prediction plots based on regression models. Solid lines indicate point estimates, while shaded areas represent 95% confidence intervals. Dots correspond to observed values. The *y*-axes display the outcome variables: logarithm of maximal force, enamel/dentin volume ratio, and fracture size in dentin. The association between age and each outcome was stratified by tooth location (molar vs. frontal) and diabetes status (control vs. T2DM: type 2 diabetes mellitus)

all types of biting and chewing are transferred to the back teeth. The conduction of front teeth and canines is lost during lateral jaw movements, unilateral balance develops. Therefore, a person with healthy, normal dentition, when the jaw moves sideways, it glides along the canine. However, in case of lacks of front teeth (e.g., due to periodontitis), unilateral balance occurs. Consequently, the remaining molar teeth are exposed to the masticatory forces, which promotes the formation of attrition resulting the loss of tooth occlusal surface [31].

To assess the mechanical properties of diabetes-affected teeth, static compression test was used. Coronal region of epoxy embedded samples was exposed to 2 mm/min load velocity by using an 8 mm diameter spherical steel indenter, and force versus indentation curves were recorded. First, the slope of the ascending part of the curve was determined, thus the stiffness (N/mm) of tooth material could be calculated. Our results showed that the stiffness of T2DM molar teeth significantly reduced comparing to the control samples. This result can be explained by the extent of the dental wears of molar samples. Since the enamel layer is thinned in diabetic molar samples, we think, that the force answer is originated from the less rigid dentin resulting lower stiffness values. Furthermore, our results also showed, that molar teeth in T2DM group displayed 30% reduction in maximal force comparing to control samples, indicating a lower fracture resistance and diminished structural stability. Front teeth displayed similar values in both groups with high variability. Fracture toughness of human dentin in young and aged individuals has already been extensively studied from many aspects. Nazari et al. showed in Compact Tension specimens under cyclic loading, that in aged dentin the average crack growth resistance significantly reduced, which can be explained by the higher extent of dental sclerosis and by different tubule dimensions and densities [32]. Furthermore, the reduced mechanical strength of aged dentin was investigated by Shinno et al. Accumulation of AGE(s) on dental collagen and high mineral density resulting occlusion of dental tubules are the main reasons which contribute to the low flexural strength and fragility of dentin [33]. However, it is very important to highlight that these kinds of comparative mechanical analyses are unrevealed in T2DM-affected tooth samples. In our study we could observe the same mechanical property of diabetic dentin by using the tensile tester device. We hypothesize that molar teeth may be more exposed to glycation due to their pulp anatomy and complexity resulting the early appearance of diabetic complications and accelerated AGE(s) formation which finally cause reduced fracture resistance and fragility of teeth.

Following the mechanical test, CT analysis was repeated of each samples exploring failures. CT images revealed significant damages in both groups. Dentin of diabetic molar samples was more exposed to fractures, significantly larger, 3.354 mm $\pm$ 1.045 mm crack sizes were determined, while control molar teeth displayed 1.55 mm $\pm$ 0.65 mm fractures. This result shows strong relation with the extent of dental wears which were defined prior the mechanical test. Since diabetic molar teeth have lower enamel/dentin ratio consequently, dentin is more exposed to damages, thin enamel was not able to provide its energy-dissipating role during loading of indenter, cracks could propagate more in the dentin. Mechanical properties and composition of dentin in diabetic mouse models have already been investigated by Tang et al. [34]. Raman Spectroscopy and nanoscale dynamic mechanical analysis revealed significant reduction in hardness, storage modulus and lower mineral-to-organic ratio of intertubular dentin in T2DM as leading causes of tooth fragility. Furthermore, Arola et. al. confirmed that old dentin is less tolerant to damage than young dentin under flexure load, indicating increased both rate of damage initiation and microcracks propagation in dentin [35].

Furthermore, based on CT images we could determine the locations of failures. In molar samples the occlusal surface, and in front teeth, the occlusal/incisal surface, were always affected in both groups since these areas received the greatest load during testing. The applied forces also impacted the proximal surfaces, with the mesial and distal sides were similarly involved, which can be attributed to the thinner enamel layer in these regions. The involvement of the vestibular surface in the T2DM molar group can be explained by the thinner enamel and dentin layers of the cusps, whose already unfavorable mechanical properties are further deteriorated in T2DM [36].

In our study, following the assessment of data we considered it to essential to address the question, whether the mechanical and structural alterations in T2DM group are induced by diabetic complications

or to ageing. Since we did not quantify directly the level of glycation or AGEs accumulation, statistical method was applied to predict the main factor of mechanical alterations of teeth. Linear mixed effect model allowed us to isolate the effect of diabetes from age-related changes to understand better the underlying factors contributing to altered tooth biomechanics in T2DM. Statistical analysis revealed that the age has significant effect on various physical quantities such as breaking force, enamel/dentin volume ratio and dentin fracture size. The effect of T2DM was not significant in these predictions. However, T2DM showed the highest effect (1.24 mm difference) on dentin fracture size, pointing out relevant clinical consequences of diabetes. It is important to note that these analyses were carried out in small sample size groups. Probably in a larger population these predictions would be more accurate to demonstrate the effect of T2DM on various physical quantities. However, the main limitation of our work is the collection of tooth samples from diabetes-affected patients especially those with uncontrolled or poorly controlled diabetes. The extraction procedure can be more difficult and risky due to many factors such as the delayed wound healing, increased risk of infection, brittle or sclerotic alveolar bone resulting the fracture of the tooth already during the extraction [37]. Furthermore, since the dental caries is more common in diabetic patients it was difficult to collect caries-free and non-treated samples in T2DM group.

Conclusion

In summary, we have shown here a detailed mechanical and structural analysis of extracted tooth samples in T2DM by using CT imaging and compression test. Diabetes-affected specimens showed lowered mechanical properties during compression test and CT results indicated reduced enamel thickness before mechanical examination in T2DM group, consequently greater fracture sizes were detected following the compression test. Although statistical analysis showed age as a dominant factor in tooth structural weakening, our study highlights the importance of diabetic complications, which should be considered in planning of various clinical treatments. The structural condition and thickness of dentin influence the choice of dental restoration and the selection of corresponding adhesive system. In patients with T2DM, due to the altered mechanical properties the bonding ability to adhesive system is also impaired. To compensate, the usage of restorative materials with high toughness and shock-absorbing ability (e.g., zirconia) is crucial, and clinical protocols must aim to limit occlusal loading to ensure restorative longevity. Nowadays, the escalating T2DM epidemic is leading to increased overlap in health profiles among diabetic patients of various ages, increasing number of young individuals are affected by diabetic complications. Therefore, our study emphasizes that while ageing significantly affects tooth mechanical stability, T2DM may contributes to additional structural weakening. Furthermore, our findings implicitly model the cumulative damage in dentin due to its essentially zero turnover. Considering this special property from a system biology perspective, dental collagen displays unique ageing mechanisms compared to other collagen-containing tissues in which further structural and mechanical alterations and ability to renewal can occur. Clear evidences of diabetic complications affecting structural and mechanical properties of teeth should be investigated in larger populations in future studies.

Author contribution Sarolta Antal: writing—original draft, formal analysis, methodology, conceptualization; Kata Sára Haba: writing—original draft, investigation, data curation; Gábor Szebényi: methodology, investigation, formal analysis, data curation, resources; Ildikó Horváth: methodology, investigation, formal analysis, visualization; Krisztián Szigeti: methodology, investigation, formal analysis, data curation; Dániel Sándor Veres: formal analysis, visualization, data curation; Miklós Kellermayer: writing—review and editing, validation, supervision, data curation, resources; Dóra Haluszka: writing—review and editing, supervision, conceptualization, investigation

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Data availability All data used during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval The institutional review board of Semmelweis University Regional and Institutional Committee of Science and Research Ethics approved the study protocol (approval number: 99/2025).

Competing interests The authors declare no competing interests.

References

1. Helfman PM, Bada JL. Aspartic-acid racemization in tooth enamel from living humans. Proc Natl Acad Sci U S A. 1975;72(8):2891–4.
2. Dunn JA, Patrick JS, Thorpe SR, Baynes JW. Oxidation of glycated proteins - age-dependent accumulation of N-epsilon-(carboxymethyl)lysine in lens proteins. Biochemistry. 1989;28(24):9464–8.
3. Zbiec-Piekarska R, Spolnicka M, Kupiec T, Makowska Z, Spas A, Parys-Proszek A, et al. Examination of DNA methylation status of the ELOVL2 marker may be useful for human age prediction in forensic science. Forensic Sci Int-Gen. 2015;14:161–7.
4. Paul RG, Bailey AJ. Glycation of collagen: the basis of its central role in the late complications of ageing and diabetes. Int J Biochem Cell Biol. 1996;28(12):1297–310.
5. Verzijl N, DeGroot J, Oldehinkel E, Bank RA, Thorpe SR, Baynes JW, et al. Age-related accumulation of Maillard reaction products in human articular cartilage collagen. Biochem J. 2000;350:381–7.
6. Ramamurthy B, Höök P, Jones AD, Larsson L. Changes in myosin structure and function in response to glycation. FASEB J. 2001;15(13):2415–22.
7. Pilin A, Pudil F, Bencko V. Changes in colour of different human tissues as a marker of age. Int J Legal Med. 2007;121(2):158–62.
8. Nandi SK, Nahomi RB, Rankenberg J, Glomb MA, Nagaraj RH. Glycation-mediated inter-protein cross-linking is promoted by chaperone?client complexes of ?-crystallin: implications for lens aging and presbyopia. J Biol Chem. 2020;295(17):5701–16.
9. Miura J, Nishikawa K, Kubo M, Fukushima S, Hashimoto M, Takeshige F, et al. Accumulation of advanced glycation end-products in human dentine. Arch Oral Biol. 2014;59(2):119–24.
10. Fukushima S, Shimizu M, Miura J, Matsuda Y, Kubo M, Hashimoto M, et al. Decrease in fluorescence lifetime by glycation of collagen and its application in determining advanced glycation end-products in human dentin. Biomed Opt Express. 2015;6(5):1844–56.
11. Matsuda Y, Miura J, Shimizu M, Aoki T, Kubo M, Fukushima S, et al. Influence of nonenzymatic glycation in dentinal collagen on dental caries. J Dent Res. 2016;95(13):1528–34.
12. Schuh C, Leiva-Sabadini C, Huang S, Barrera NP, Bozec L, Aguayo S. Nanomechanical and molecular characterization of aging in dentinal collagen. J Dent Res. 2022;101(7):840–7.
13. Asami R, Sato T, Sakiyama K. Measurement of advanced glycation end products (AGEs) accumulated in dentin collagen. J Oral Biosci. 2025;67(1):100600.
14. Witt NJ, Woessner AE, Herrmann J, Quinn KP, Sander EA. Mechanical models of collagen networks for understanding changes in the failure properties of aging skin. J Biomech Eng. 2024. https://doi.org/10.1115/1.4064406.
15. Panwar P, Butler GS, Jamroz A, Azizi P, Overall CM, Bromme D. Aging-associated modifications of collagen affect its degradation by matrix metalloproteinases. Matrix Biol. 2018;65:30–44.
16. Lemarchand M, Thouin K, De Serres-Berard T, Bellenfant S, Cadau S, Berthod F. In vitro glycation of a tissue-engineered wound healing model to mimic diabetic ulcers. Biotechnol Bioeng. 2023;120(6):1657–66.
17. Guvatova ZG, Kudasheva ER, Efremov YM, Timashev PS, Fedorova MS, Pudova EA, et al. Changes in gene expression patterns in young and senescent fibroblasts in glycated three-dimensional collagen matrices. Int J Mol Sci. 2025. https://doi.org/10.3390/ijms26104769.
18. International Diabetes Federation. IDF Diabetes Atlas: Europe region data [Internet]. 2025 [cited 2026 Mar 16]. Available from: https://diabetesatlas.org/data-by-location/region/europe/.
19. Liptak K, Liptak L, Haba KS, Banyai D, Veres DS, Rozsa NK, et al. Oral health practices and literacy in Hungarian diabetes patients: insights from a pilot-study using a WHO-adapted questionnaire. BMC Oral Health. 2025;25(1):431.
20. Imbeni V, Nalla RK, Bosi C, Kinney JH, Ritchie RO. Fracture toughness of human dentin. J Biomed Mater Res A. 2003;66a(1):1–9.
21. Bajaj D, Sundaram N, Nazari A, Arola D. Age, dehydration and fatigue crack growth in dentin. Biomaterials. 2006;27(11):2507–17.
22. Lin F, Ordinola-Zapata R, Ye N, Xu HP, Fok ASL. Fatigue analysis of restored teeth longitudinally cracked under cyclic loading. Dent Mater. 2022;38(1):204–13.
23. Saghiri MA, Nath D, Rahmani B, Amini S, Karamifar K, Peters OA. The effect of diabetes on fracture resistance of teeth: an study. Aust Endod J. 2021;47(3):499–505.
24. Verhulst MJL, Loos BG, Gerdes VEA, Teeuw WJ. Evaluating all potential oral complications of diabetes mellitus. Front Endocrinol. 2019. https://doi.org/10.3389/fendo.2019.00056.

25. Chen SS, Arola D, Ricucci D, Bergeron BE, Branton JA, Gu LS, et al. Biomechanical perspectives on dentine cracks and fractures: implications in their clinical management. J Dent. 2023. https://doi.org/10.1016/j.jdent.2023.104424.
26. R Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2025. Available from: https://www.r-project.org/. Accessed 16 Mar 2026.
27. Haluszka D, Aminmansour S, Toth P, Aminmansour S, Kellermayer M. Nanomechanical and nonlinear optical properties of glycated dental collagen. J Dent Res. 2022;101(12):1510–6.
28. Ohtani S, Yamamoto K. Age estimation using the racemization of amino acid in human dentin. J Forensic Sci. 1991;36(3):792–800.
29. Meissner C, Ritz-Timme S. Molecular pathology and age estimation. Forensic Sci Int. 2010;203(1–3):34–43.
30. Loe H. Periodontal disease. The sixth complication of diabetes mellitus. Diabetes Care. 1993;16(1):329–34.
31. Srisilapanan P, Jindarat M, Roseman J. The prevalence and severity of tooth wear in type 2 diabetic patients. Int J Dent. 2018;2018:3608158.
32. Nazari A, Bajaj D, Zhang D, Romberg E, Arola D. Aging and the reduction in fracture toughness of human dentin. J Mech Behav Biomed Mater. 2009;2(5):550–9.
33. Shinno Y, Ishimoto T, Saito M, Uemura R, Arino M, Marumo K, et al. Comprehensive analyses of how tubule occlusion and advanced glycation end-products diminish strength of aged dentin. Sci Rep. 2016;6:19849.
34. Tang K, Ceteznik S, Kim M, Bornfeldt KE, Kanter JE, Zhang H, et al. Changes in the composition and mechanical properties of dentin in mouse models of diabetes. Dent Mater. 2024;40(11):2017–24.
35. Arola D, Reprogel RK. Effects of aging on the mechanical behavior of human dentin. Biomaterials. 2005;26(18):4051–61.
36. Suwa G, Kono RT. A micro-CT based study of linear enamel thickness in the mesial cusp section of human molars: reevaluation of methodology and assessment of within-tooth, serial, and individual variation. Anthropol Sci. 2005;113(3):273–89.
37. Yang ST, Li Y, Liu CC, Wu YF, Wan ZX, Shen DN. Pathogenesis and treatment of wound healing in patients with diabetes after tooth extraction. Front Endocrinol. 2022. https://doi.org/10.3389/fendo.2022.949535.