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ORIGINAL RESEARCH

Evaluation of the pit and fissure system in primary and permanent molars with micro-computed tomography and 3D printing

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Abstract

This study aimed to characterize the anatomical and physiological features of pits and fissures in primary and permanent molars by microtomographic (micro-CT) examination and three-dimensional (3D) printing. The occlusal surfaces of 84 primary molars and 60 permanent third molars were examined. The samples were scanned with micro-CT and the occlusal surface separated. The areas of the crown, its occlusal part, and fissures and pits were calculated. Digital impression of the occlusal surface was created and 3D printed. The frequency of different fissure types was determined by direct observation. Data were subjected to statistical analysis using Mann-Whitney U Test and chi-square test ($p < 0.05$). There was statistically significant difference between the ratio of occlusal surface and the crown area for the molars in primary and permanent dentitions (24.78% and 28.85% respectively, $p < 0.05$). In terms of the percentage ratio of the fissure area to the occlusal surface (24.24% and 22.30%) and the fissure area to the crown (6.02% and 6.52%), no significant difference was observed ($p > 0.05$). V-shaped fissures were predominant in both primary and permanent teeth, with a higher occurrence in primary dentition (59.48%, $p < 0.05$). Permanent molars exhibited a higher prevalence of I-type and U-type fissure configurations compared to primary molars ($p < 0.05$), with I-type fissures being the least common in primary molars. In both dentitions there was no statistically significant difference in the prevalence of IK-configuration ($p > 0.05$). The fissure depth was significantly greater in permanent molars than primary molars ($p < 0.05$). In conclusion, this study revealed remarkable diversity in fissure morphology among primary and permanent molars.

Keywords

Occlusal caries; Pits and fissures; Fissure morphology; Micro-CT

1. Introduction

Dental caries stands as one of the most prevalent oral diseases in childhood [1], impacting a significant proportion of students, adolescents and adults, with estimates ranging from 60% to 90% [2]. Despite the implementation of primary prevention methods and existing recommendations for caries prevention, it remains the leading cause of tooth loss worldwide. This persistent challenge affects the physical and mental well-being of children and has evolved into a global public health concern [3]. Data from 2015 to 2017 reveals a dental caries prevalence of 45.8% among children aged 2 to 19 [4].

Immediately after eruption, the occlusal surfaces of permanent teeth are particularly susceptible to carious lesions and the first permanent molar remains the most frequently affected [5, 6]. These molars emerge with deep fissures and retentive occlusal surface characterized by weaker enamel mineralization [7]. Moreover, the entrance to these deep fissures is often narrow, with an underlying expansion of varying shapes. This specific anatomical arrangement facilitates the entry and retention of food residues, the formation and retention of biofilm, and increases the risk of caries [8, 9]. Studies have demonstrated a close relationship between caries susceptibility and various types of occlusal morphology [8, 9]. In addition, as toothbrush bristles typically struggle to access these narrow spaces, this makes removing the accumulated biofilm challenging [5]. Pediatric dentistry guidelines recommend sealing primary and permanent molars in children and adolescents. Sealant use should be increased along with other preventive interventions, especially in high caries risk patients. Further *in vitro* studies are needed to test the mechanical properties of novel sealing agents [10].

Traditional methods, such as direct observation and standard radiographs, are inadequate for comprehensively assessing the anatomical features and depths of an individual tooth's fissure system. Early investigations into pit and fissure morphology were achieved using serial sections of extracted human teeth. A classification system for occlusal fissures was introduced, which categorized them into five distinct types based on their unique morphological characteristics—V-, U-, I-, IK- and other types, that are not part of the described [11]. Numerous authors have examined the intricate occlusal anatomy of molars through various methods, including clinical observation, photographic documentation with magnification techniques, scanning electron microscopy and intraoral scanner [12–14]. However, not all of these approaches enable comprehensive three-dimensional analysis and reproduction of the molars' occlusal surface anatomy.

Micro-CT has emerged as a non-invasive technique capable of providing high-resolution three-dimensional images and has recently been increasingly used for dental examinations [15–18]. It allows for precise measurements of volume and the accurate delineation of areas of interest for visual analysis. This method permits the differentiation of individual fossae and fissures on the occlusal surface of teeth. Additionally, through image processing, adjustments in color, texture and illumination enhance the comprehension of internal tooth anatomy from various perspectives [15].

3D printing is an advanced manufacturing technique that involves the layer-by-layer application of material to create a three-dimensional (3D) object [19]. It has found successful applications in various areas, including orthognathic surgery, orthodontic treatment for children and the formation of splints for dental and maxillofacial injuries [20, 21]. It can generate and reproduce the complex 3D morphological structure of pits and fissures found on the occlusal surface of both primary and permanent molars. Previous efforts to develop and recreate fissure models have predominantly relied on qualitative assessments through 2D visual analysis using microscopy or X-ray scanning, which are often insufficient to adequately capture the 3D aspects of fissure patterns [22]. Exploration of 3D fissure analysis for this particular application is relatively limited. To fill this gap, the study was designed to perform a thorough 3D examination and comparative assessment of fissure morphology in primary and permanent molars. Based on our current knowledge, there are no similar studies in this domain.

The primary objective of this study is to comprehensively understand the anatomical features of pits and fissures in both primary and permanent molars through micro-CT examination and subsequent 3D printing, which may help clinicians in diagnosing the occlusal surfaces of newly erupted permanent molars and making informed decisions regarding the most suitable prevention or treatment methods. The study null hypothesis is that there are no significant differences between the surface area, depth and distribution of different fissure profiles in fissure systems of primary and permanent teeth.

2. Materials and methods

2.1 Preparation of the samples

In this study, the occlusal surfaces of 87 primary molars and 62 permanent maxillary third molars were examined (Table 1). The teeth were irreversibly anonymized immediately after extraction. Primary teeth were collected from healthy children aged between 8–12 years. Permanent teeth were also collected from healthy patients aged between 14–17 years. The primary molars were extracted shortly before their natural exfoliation occurred. Permanent third molars were extracted for orthodontic indications. After extraction, the tooth crowns were cleaned with hydrogen peroxide and were then preserved in a 10% formalin solution.

Before scanning, the teeth were cleaned with pressurized sodium bicarbonate (PROPHYflex 3, Kavo, Biberach, Germany) and examined using an operating microscope (Semorr 3000E, Semorr Medical Tech Co., Suzhou, Jiangsu, China) to detect the presence of occlusal carious lesions. The inclusion criteria were: (1) absence of fractures, defects or cracks on the crown for both third molars and primary molars; (2) no carious lesions present; and (3) macroscopic similarity between third permanent molars and first permanent molars.

2.2 Three-dimensional reconstruction

Each tooth was positioned in transparent sealed Eppendorf tube with soaked in distilled water cotton on the bottom to prevent the specimens from drying out and formation of cracks in the enamel. The examined samples were scanned using a desktop X-ray microtomograph (SkyScan 1272, Bruker, Billerica, MA, USA) with the following parameters: X-ray tube voltage of 100 kV, current magnitude of 100 μA, and a 0.55 mm copper filter. The X-ray radiation was conical in shape, and each voxel had a size of 12 μm. At this resolution, the entire length of the tooth crown was within the detector field. Subsequently, the acquired images from all samples underwent corrections for radiation spectrum variations, circular perturbations and fine/thermal specimen displacements. A separate 3D reconstruction of the tooth enamel and dentin was then performed by aligning the series of 2D images using the provided software (CTAn, v. 1.18.8.0, Bruker, Billerica, MA, USA). The reconstructions were saved as STL-files.

2.3 Measurement of crown area, occlusal surface and fissure system

The occlusal portion was manually separated from the three-dimensional enamel reconstruction using the Autodesk Meshmixer software (v. 3.5.474, Autodesk, San Rafael, CA, USA). To standardize the measurements, only one person performed the separation. This separation process involved marking and erasing the non-occlusal surfaces, resulting in the isolation of the occlusal component, which was then saved as a distinct STL-file. Subsequently, the morphology of the fissures and pits within the occlusal part was manually characterized and preserved. The resulting images are shown in Fig. 1.

The Autodesk 3ds Max program (Autodesk, San Rafael, California, USA) was used to calculate the dimensions of the crown, its occlusal segment, as well as the fissures and pits from the obtained 3D files by accessing the menu and selecting Utilities > Measure > Surface area.

TABLE 1. Distribution of samples included in the study by teeth type.

Primary	Upper first molar	Upper second molar	Lower first molar	Lower second molar
	12	36	16	23
Permanent	Upper third molars		Lower third molars	
	40		22	

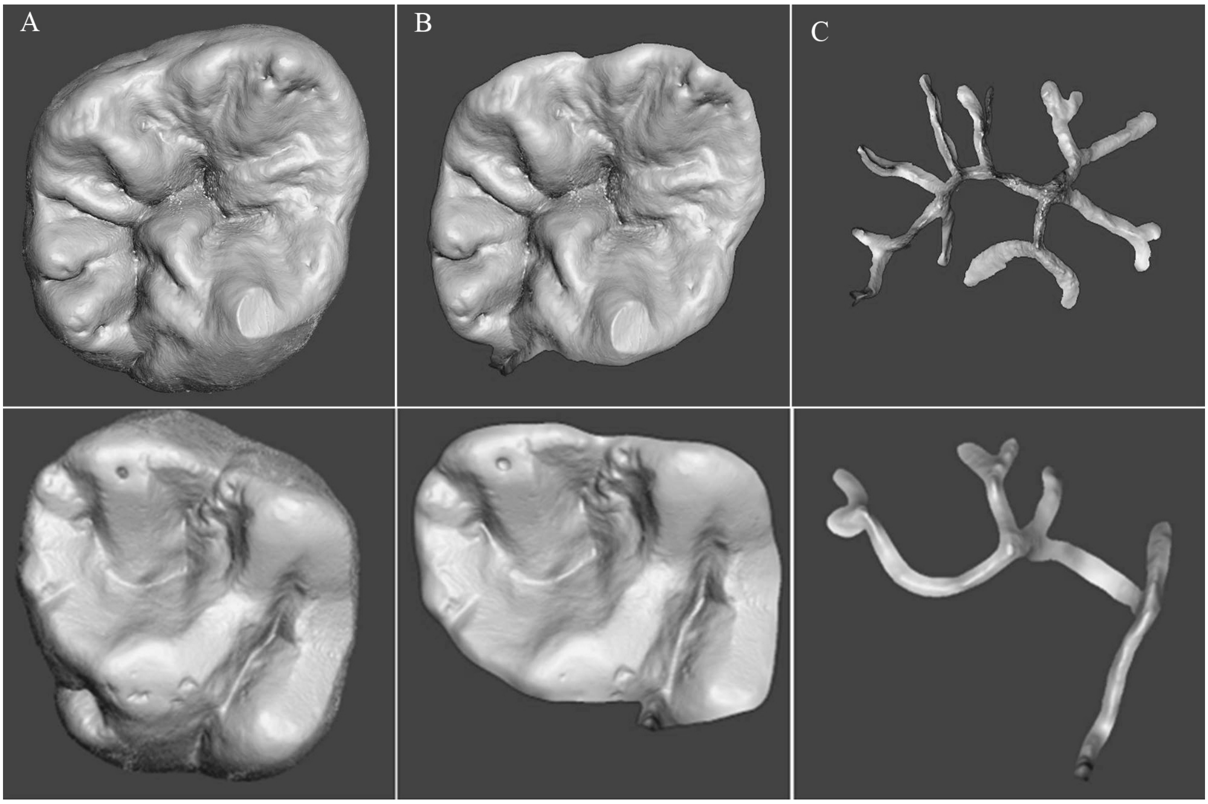

FIGURE 1. Three-dimensional reconstruction. (A) the crown of the tooth; (B) the occlusal part; (C) pits and fissures of permanent (above) and primary (below) tooth.

2.4 Printing 3D models

In Autodesk Meshmixer, a negative image of the isolated occlusal portion of the examined teeth was generated, creating a digital impression of the fissure and pit system. To achieve this, the entire occlusal surface was initially marked and then extruded along the Y-axis. Subsequently, the walls were smoothed, and a model platform was established. The resulting file underwent scaling using Autodesk Meshmixer's scaling function and was then exported in STL format for 3D printing using a Form 3 3D printer (Formlab, Somerville, MA, USA). Following the printing process, the frequency of different fissure types was determined through direct observation. The resultant images are presented in Fig. 2. The 3D printing of the models was done to observe the samples directly thus reducing the risk of digital eye strain and decreasing the possibility of errors [23].

2.5 Determination of average fissure depth

Fissure depths were assessed on two-dimentional images of the micro-CT scan, covering various regions of the crown in a mesial to distal direction using the software provided by the device (CTAn v. 1.18.8.0, Bruker, Billerica, MA, USA).

3. Statistical analysis

Statistical analysis was conducted with a statistics computer software SPSS v.19.0 (SPSS Inc., Chicago, IL, USA). Values of quantitative variables were expressed as mean ± standard deviation (sd). The data were analysed using a non-parametric tests (Mann-Whitney U Test, chi-squared test) because assumption of normality could not be met (Shapiro-Wilk test, $p < 0.05$). The significance level was set at $p = 0.05$.

4. Results

Following microradiography scanning, three primary lower second molars and two permanent lower third molars were excluded from the analysis due to the presence of carious lesions. Consequently, the analysis was conducted on the remaining samples, consisting of 84 primary molars and 60 permanent molars, using the methods previously described.

Fig. 3 provides an overview of the relationships between the fissure area, occlusal surface and crown dimensions of both primary and permanent molars.

The average percentage of the ratio between the occlusal surface and the crown is about 25% for primary teeth and 29%

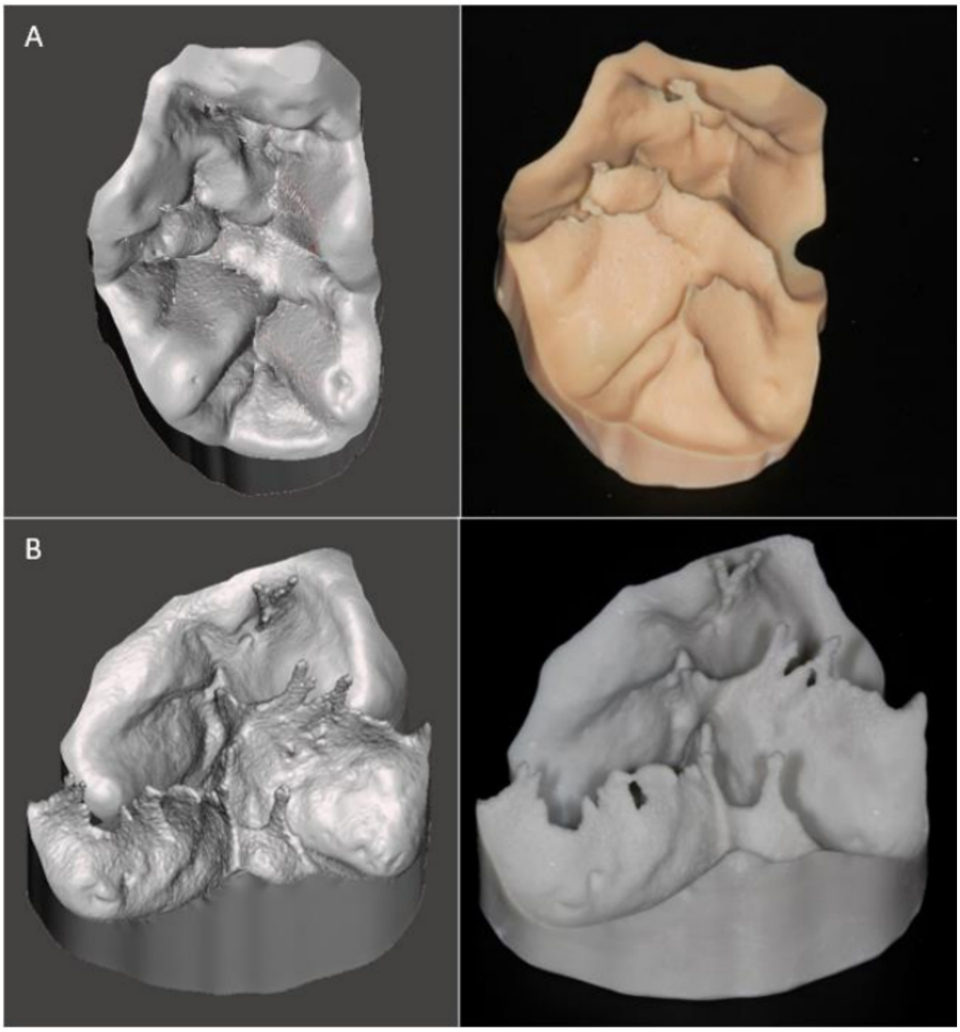

FIGURE 2. Digital impression of occlusal surface and printed 3D model of primary (A) and permanent (B) tooth.

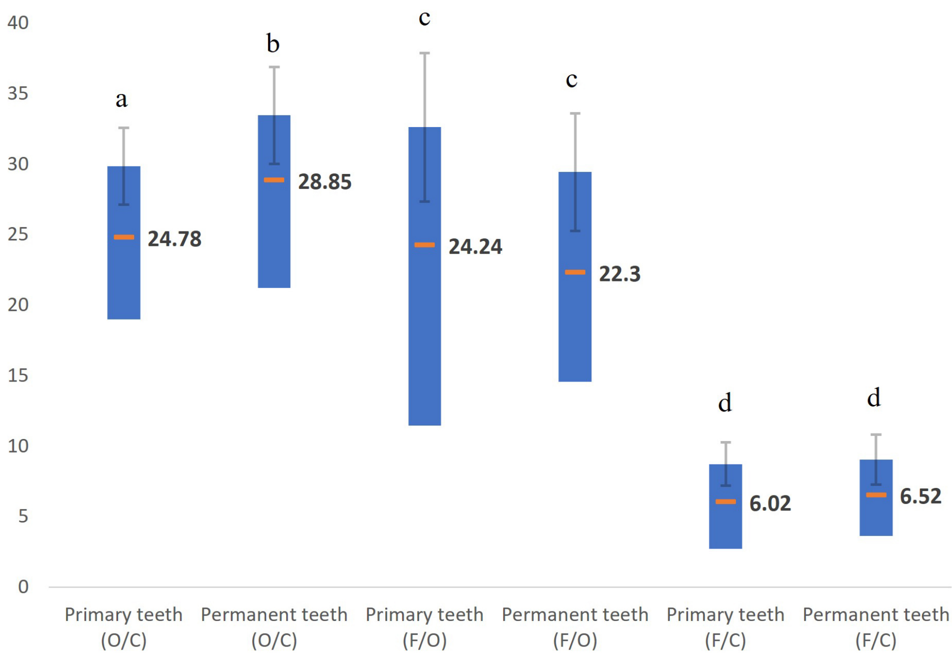

FIGURE 3. Mean and standard deviation of the relationships between the fissure area, occlusal surface and crown area of primary and permanent molars (in percentage). Orange line—mean ratio, different lowercase letters a–d indicate significant difference, C—crown area, O—occlusal surface area, F—pits and fissures area, ($p < 0.05$; Mann-Whitney U Test).

for permanent teeth. Statistical analysis reveals a significant difference between these ratios in both primary and permanent molars ($p < 0.001$). However, in terms of the percentage ratio of the fissure area to the occlusal surface and the fissure area to the crown in primary and permanent teeth, no significant difference was observed ($p = 0.13$, $p = 0.38$ respectively). Pits and fissures occupy roughly 22.30% to 24.24% of the entire occlusal surface or nearly a quarter of it and represent only a small fraction of the dental crown area (6.02% in primary and 6.52% in permanent molars).

Fig. 4 shows different fissure types in primary and permanent molars. Table 2 presents the distribution of fissure types in primary and permanent molars.

Table 2 illustrates that V-shaped fissures are the predominant configuration in both primary and permanent teeth, with a notably higher occurrence in primary dentition (59.48%, $p = 0.02$). Significant differences are observed in the distribution of I-shaped and U-shaped fissure configurations, which are more prevalent in permanent molars compared to primary molars ($p < 0.001$). Type I fissures are the least common in primary molars. In both dentitions, the IK configuration is present at approximately 13%, with no statistically significant differences observed between them ($p = 0.31$).

Fig. 5 shows the fissure depth of the primary and permanent molars in micrometers (μm), indicating that fissures exhibit significantly greater depth in the permanent molars ($p < 0.001$).

5. Discussion

Our study aimed to investigate the morphological and physiological features of the fissure system in both primary and permanent teeth and their implications for the prevention and development of occlusal caries. According to the results, the study null hypothesis was partially accepted. The results indicate that the pits and fissures within primary and permanent molars collectively occupy approximately 23% of the occlusal surface. This finding contrasts with previous studies, where occlusal surfaces accounted for only 13% of a molar's total surface area [24]. The higher ratio observed may be attributed to differences in study methods, as 3D assessments are more accurate compared to serial sectioning. The percentage of carious lesions in children in pits and fissures reaches and even exceeds 60% [2, 24, 25]. Our results show that the riskiest crown zone for development of caries—the pits and fissures—occupies only 6% of the entire tooth surface in primary and around 7% in permanent molars. Early diagnosis of occlusal caries and its active prevention is crucial in childhood for both dentitions.

Based on some studies, the most frequently observed fissure type was V-shaped (34%), followed by IK-shaped (26%), U-shaped (14%), and I-shaped in 19% of cases. Additionally, other types not falling into these categories were observed in 7% of cases [26]. The relationship between fissure shape and depth has been reported, with V-shaped fissures tending to be shallow, U-shaped fissures having a medium depth, and the remaining types primarily deep. Furthermore, the authors established a correlation between the localization of carious lesions and the type of fissure. Carious lesions typically begin at the entrance of the fissure in I and IK-shaped fissures, from the middle in U-shaped fissures, and most frequently from the bottom in V-shaped fissures [26]. Our data reveals that V-shaped fissures are the most prevalent in permanent dentition (38.73%), followed by U-shaped fissures (29.73%). I (18.47%) and IK (13.20%) types of fissures are the least common. The distribution of the fissure morphology in the primary dentition is as follows: V-type—59.48%; U-type—18.67%; I-type—8.14%; IK-type—13.71%. No other fissure morphologies were observed. This is likely due to the unique nature of three-dimensional reconstruction and the opportunity for direct observation following the printing of negative occlusal surface images, which is not achievable with serial sectioning method, where the fissure profile will depend significantly on the section plane. Molars have an increased risk of caries development due to the complex morphology of their fissure system, characterized by both high retentiveness and inaccessibility to mechanical cleaning [27, 28]. The printed three-dimensional models demonstrate this feature of the occlusal surface.

There have been very few attempts to develop qualitative and quantitative pits and fissure model characteristics with two-dimensional visual analysis by microscopy and X-ray scanning without showing the three-dimensional pattern of the fissure [28]. The three-dimensional reconstruction in the current study allows a very accurate assessment of the morphology and anatomy of the occlusal surface of the molars. Our results demonstrate that the generated 3D models of pits and fissures effectively restore missing pieces of occlusal anatomy in detail.

Three-dimensional replicas of occlusal surfaces were created from extracted retained teeth [29]. These surfaces showed significant variability within the molars. On average, molars exhibit about 12 pits, with some having twice that number. Fine details of this morphology can be lost when using serial sections [29]. Our research similarly reveals remarkable diversity in fissure morphology among primary and permanent molars. While the occlusal surface and pit and fissure arrangement in primary molars may not inherently promote plaque accumulation and caries development due to their shallower depth, certain characteristics can be diagnostically challenging in clinical settings, which can make these places risky. Predominantly, U- and V-shaped fissures were observed in primary teeth, but other configurations, such as I- and IK-shaped, appeared in over 20% of cases.

Attempts to assess fissure depth in primary and permanent teeth have yielded varied results [9, 11, 30]. Most authors classified fissures only by sure signs using visual inspection, a photograph of the tooth and under 10× magnification. Exact values have not been measured, but the fissures are distributed according to specific criteria into smooth, shallow, intermediate depth and deep [31]. Other publications state that occlusal fissure depth can vary from 0.1 up to 1 mm [30]. When examining permanent third molars, it has been found that the fissure extends to the enamel-dentine border in nearly 50% of cases [30]. Another recent study revealed that children with deep fissures were over three times more likely to develop dental caries than those with shallow fissures [9]. A micro-CT study of fissures and pits concluded that the internal morphology of the occlusal surface influences the

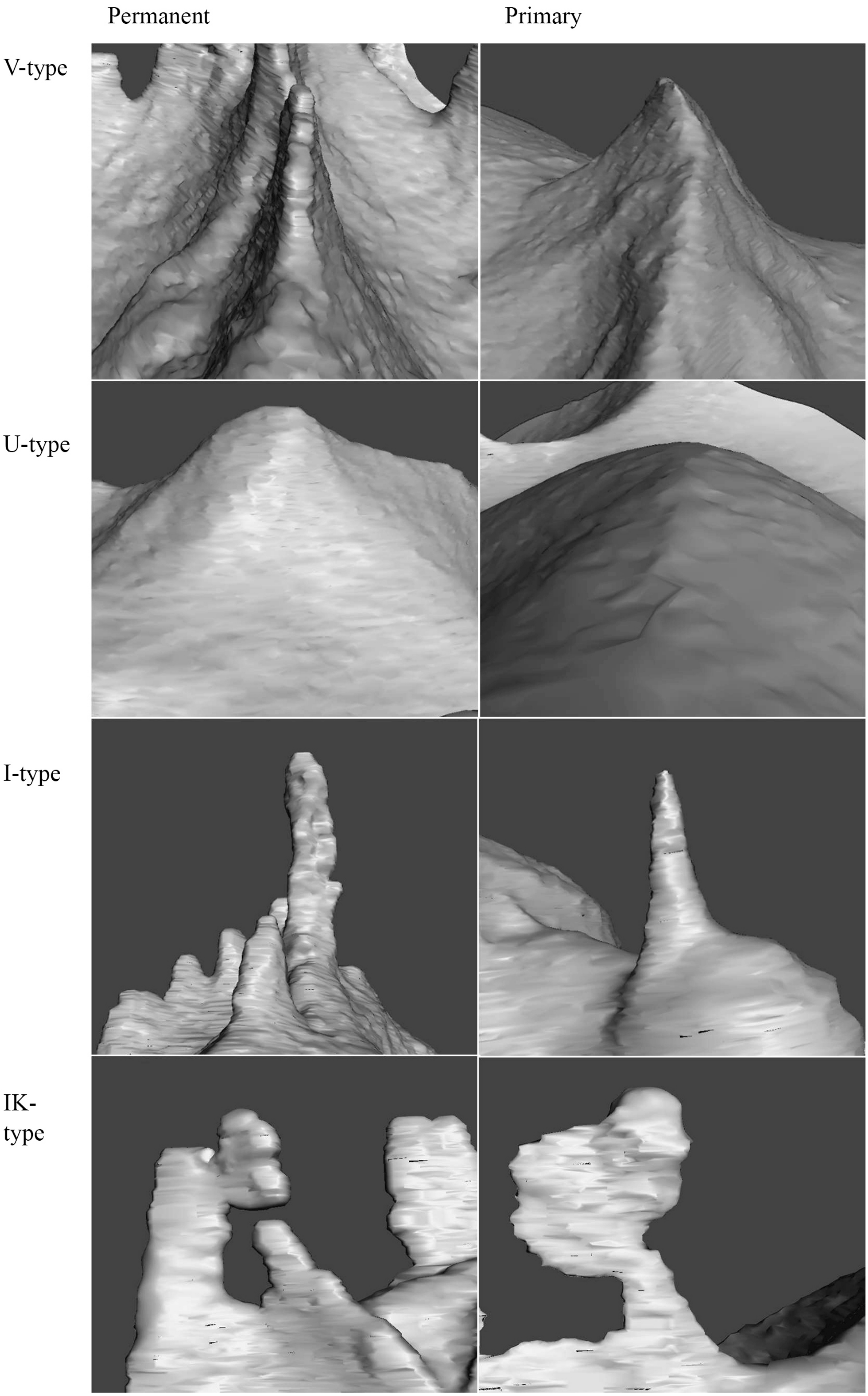

FIGURE 4. Different fissure types in primary and permanent molars.

TABLE 2. The relative share of fissure types in primary and permanent teeth (%).

	Fissure type			
	U-type* (Mean ± sd)	V-type* (Mean ± sd)	I-type* (Mean ± sd)	IK-type (Mean ± sd)
Tooth group				
Primary Teeth (N = 84)	18.67 ± 13.86	59.48 ± 16.81	8.14 ± 6.34	13.71 ± 10.06
Permenent Teeth (N = 60)	29.73 ± 7.94	38.73 ± 7.96	18.47 ± 7.96	13.20 ± 8.06

**Statistically significant difference ($p < 0.05$; chi-square test) in the distribution of identical fissure types between the primary and permanent teeth.*

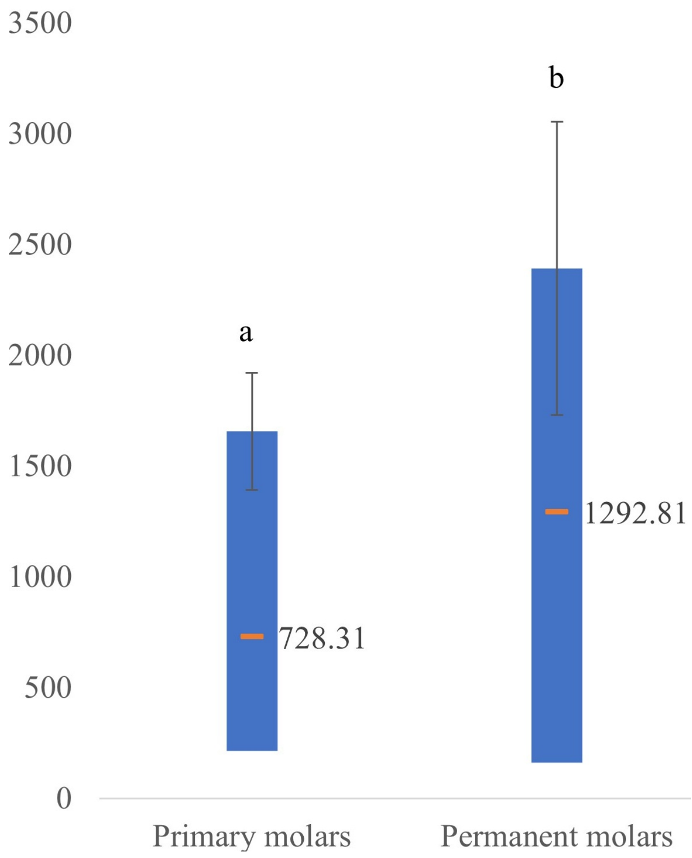

FIGURE 5. Means and standard deviation of fissure depth in primary and permanent molars (μm). Orange line—mean depth, different lowercase letters a,b indicate significant difference, ($p < 0.05$; Mann-Whitney U Test).

conditions for bacterial growth, which determines the location of carious lesion progression in the pits and fissures system [32]. Our data showed that the fissure depth of the permanent teeth was significant, averaging 1292 μm. This area poses challenges for effective plaque removal by toothbrush bristles. Hence, clinicians must be familiar with the complex fissure morphology of both primary and permanent molars to select appropriate prophylactic measures against fissure caries. The shape and depth of occlusal pits and fissures create ideal conditions for food and bacteria retention, emphasizing the importance of sealing for caries prevention. Silanization has been shown to significantly reduce the risk of carious lesion development [33].

The purpose of this study was to characterize the anatomical and physiological features of pits and fissures in primary and permanent molars that would help to understand better the management of occlusal caries in pediatric patients. Sealant application is one of the most commonly used measures for reducing occlusal caries. The future perspective of this study should be to perform trials evaluating the activity of different remineralizing agents such as calcium glycerophosphate and casein phosphopeptide-amorphous calcium fluoride phosphate on pits and fissures, especially for teeth affected by molar-incisor hypomineralization [34, 35]. They would be used as a cheaper kind of preventive treatment with respect to fissure sealants. Some limitations should be mentioned, first one being the unequal sample size when comparing primary and permanent molars. Another important aspect is that the study focused on third permanent molar. The rationale behind this choice was rooted in the frequent extraction of third molars for orthodontic reasons, their intact nature, and their classification within the same tooth group according to the field model of human dentition development [36]. These considerations suggest a degree of similarity in their occlusal morphology.

Combined with the observed visual resemblance to the first molars during the examination, this approach allows for valid conclusions to be drawn regarding the fissure morphology of permanent molars. The results obtained in primary molars may not be very indicative because those with the riskiest occlusal anatomy probably develop carious lesions rapidly and are restored or extracted prior their physiological exfoliation.

6. Conclusions

This study revealed remarkable diversity in fissure morphology among primary and permanent molars. The intricacies and complexity of the fissure and pit system in both dentitions highlight the importance of timely prevention, early diagnosis and treatment of carious lesions in these areas.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

KH—design and implement the research, to perform data extraction and synthesize the results, to write the manuscript. RG—design and implement the research, to perform data extraction and synthesize the results, to write the manuscript. NaG—design and implement the research to write and revise the final manuscript. NM—perform data extraction and synthesize the results. NeG—perform data extraction and synthesize the results. LA—implement the research and to revise the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All parents gave written informed consent for using their children's teeth for research purposes, and all molars were irreversibly anonymized immediately after extraction. Approval from the Ethics Committee of the Medical University of Sofia was also obtained (No 1598/20.05.2022).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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