

ORIGINAL PAPER



Pointing on the early stages of maxillary bone and tooth development – histological findings

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Abstract

Although the morphological stages of tooth development, in parallel with maxillary bone construction, are known for decades, the intimate mechanisms of early development of the oral cavity structures and tooth's proper and associated tissues are still incompletely elucidated. Nowadays, the research in embryology was shifted from the morphological to the molecular and genetic approach. This new approach is accomplished by using *in vivo* and *in vitro* experimental studies performed on animal models and cell lines. The interest in the knowledge of these events at gene and molecular level is still current, aiming to sustain the progress in the endorsement of novel regenerative and restorative therapies. However, the morphological standpoint maintains its interest, because the extrapolation of the results of experimental studies in humans requires a strong confirmation. Within this context, our work aims to analyze the histological characteristics of the maxillary bone and integrated tooth germs during the early stages of embryonic development. The study group consisted in mandible fragments obtained by dissection of the cephalic extremities collected from fetuses aged from 10 to 24 weeks, after medical or spontaneous abortions. The tissue specimens were processed for the histological exam. The histoarchitectonic traits of the initial stages of mandibular bone tissue and tooth development were assessed. The results revealed the dynamics of the ossification stages, from stages of early-dispersed intramembranous ossification to the organization of the dental alveoli, incorporated step-by-step in the maxillary body, and the simultaneous presence of tooth germs with different sizes and shapes, in accordance with the development stage. Our study complements the existing data regarding the embryonic period, bringing an important contribution for the enlargement of existing morphological, visual information for maxillary bone and tooth development.

Keywords: maxillary bone, tooth germ, development, histology.

✉ Introduction

The morphogenesis of the maxillary bone and tooth is implicitly integrated in the embryological development of the individual, in direct correlation with the formation of the cephalic extremity. Classical data given in embryological, anatomical and histological course books are constantly updated by the research focused on these topics, published in the mainstream. However, the intimate mechanisms of early development of the oral cavity structures (in general), and of the tooth's proper and associated tissues (in particular) are still incompletely elucidated.

The development of jaw cannot be separated from the tooth development, due to their common origin in the first pharyngeal arch and their structural interdependency [1–5]. The pharyngeal arches are transient structures with a deciding role in the embryo-fetal development of head and neck. The first pharyngeal arch is divided into the maxillary process (cranial) and mandibular process (caudal) which will contain the Meckel's cartilage [6, 7]. Premaxillary, maxillary, zygomatic bone, and part of the temporal bone are developed from the maxillary process, whereas mandible – from the mandibular process [6, 7]. Migration of the cells of neural crests is a critical event,

ensuring the formation of the ectomesenchymal tissue necessary for the craniofacial evolution [2, 4, 6, 7].

The growth of the maxillary and mandibular processes, approximately in the 6th week of development, leads to the narrowing of the primitive oral cavity, named stomodeum. The stomodeal epithelium begins to proliferate, forming the odontogenic epithelium [6, 7]. On the 37th day of development, two continuous, thick epithelial bands appear on the site of the future dental arches, and each band is rapidly subdivided into a vestibular lamina and a dental lamina [6, 7]. In the underlying ectomesenchyme, the vestibular lamina creates a free space that is the oral vestibule [6, 7]. The dental lamina presents several areas of proliferative activity, recognizable in the regions of the future alveolar processes as epithelial outgrowths oriented inwards, towards ectomesenchyme [6, 7]. The epithelial outgrowths, called epithelial bud [6], are separated from the mesenchymal/ectomesenchymal cells by a distinct basal membrane. Thus, in the 6–8 weeks of development, on the places corresponding to the future deciduous teeth, 20 tooth germs are formed [6]. In the following period, the 32 tooth germs for permanent dentition will also appear [6]. The histological components specific to the tooth should be integrated in the structure of the maxillary bone through the architecture of the deep

periodontium [1, 6]. The result is a unique, individual morphological system that brings together epithelial and connective tissue components in specific variants.

Odontogenesis occurs due to a sequence of processes whose coordination, at a molecular level, is reflected in the events that determine morphogenesis, histogenesis and cell differentiation [1, 4, 6, 8] of the tooth germs. In parallel, tooth and supporting tissue regional development involves a phenomenon of spatiotemporal modeling, based on the existing interrelationship of the induction–competence sequence [1, 4, 6, 8]. Although tooth development stages and phases are succeeding dynamically, the strict delimitation of one stage from another is practically impossible; moreover, at certain times, there is a partial superposition [6].

Nowadays, the research in embryology was shifted from the morphological to the molecular and genetic approach. This new approach is accomplished by using *in vivo* and *in vitro* experimental studies performed on animal models and cell lines. However, the morphological standpoint maintains its interest, because the extrapolation of the results of experimental studies in humans requires a strong confirmation. Within this context, our study is justified by the small number of recent papers that report data on microscopic embryological research in humans – research burdened by current ethical restrictions and, concomitantly, by technical difficulties.

Our interest in the investigation of the maxillary bone and tooth development is part of the general knowledge drive, adding informative value and completing our previous reports on dental histology [9–12].

Aim

The study aims to analyze the histological characteristics of the maxillary bone and integrated tooth germs during the early stages of embryonic development.

Materials and Methods

The analyzed material consists in tissue fragments of mandibles obtained by dissection of the cephalic extremities collected from fetuses aged from 10, 14, 18, 22 and 24 weeks, after medical or spontaneous abortions. The necropsy was done at Vaslui County Forensic Service and Laboratory

of Pathology, Municipal Hospital of Bârlad, Romania. The legal conditions for harvesting, handling and conservation of embryonic human material were respected, based on the written consent of the family, certified by an informed consent protocol. The study was approved by the Ethics Committee of the Grigore T. Popa University of Medicine and Pharmacy, Iași, Romania (Ethics Agreement No. 1/13.10.2009), in accordance with the Helsinki Declaration. The tissue specimens were decalcified (between three days and three weeks) and processed for paraffin-embedding technique. The paraffin blocks were sectioned at 4 μ m. The sections were stained with Hematoxylin–Eosin (HE) for standard histological examination, as well as with special stainings [Masson's trichrome, Szekely trichrome, Periodic Acid–Schiff (PAS)]. The microscopic assessment aimed the identification and characterization of histological and histoarchitectonic elements of the initial stages of mandibular bone tissue and tooth development.

Results

The microscopic exam showed morphological traits that correspond to the dynamics of the ossification stages, from stages of early dispersed intramembranous ossification to the organization of the dental alveoli, incorporated step-by-step in the mandibular body.

In the mesenchymal tissue, we identified elements specific to the formation of the embryonic bone with non-lamellar fascicular histology. Mesenchymal cell proliferation and condensation resulted in the formation of bone spicules growing by fusion into thin bone plates or trabeculae covered on their outer surface by osteoblasts and on the inner layer by osteocytes. The embryonic bone trabeculae were initially separated by mesenchymal tissue (Figure 1), later developing large, areolar spaces occupied by connective tissue and blood vessels (Figures 2 and 3). Microscopic analysis revealed the particular structure of the embryonic bone, due to the young rough collagen fibers oriented randomly and crosswise, rich in cells (Figures 4–6).

The microscopic exam revealed tooth germs corresponding to incisors 1 and 2, canine, molars 1 and 2 in various development stages, located in the forming mandibular structures in relation to the gestational age (Table 1).

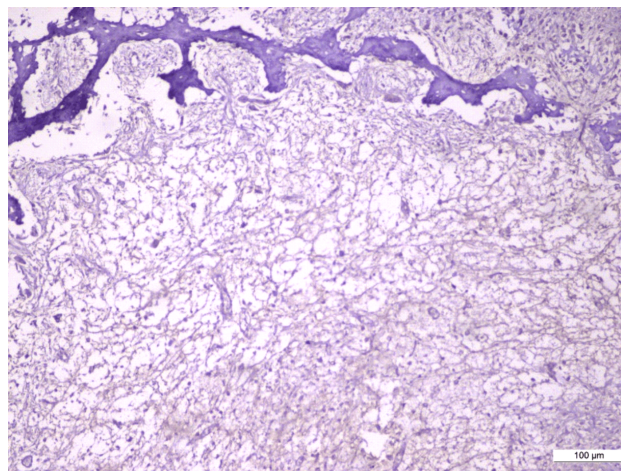


Figure 1 – Embryonic bone trabeculae within the mesenchymal tissue (HE staining, $\times 100$).

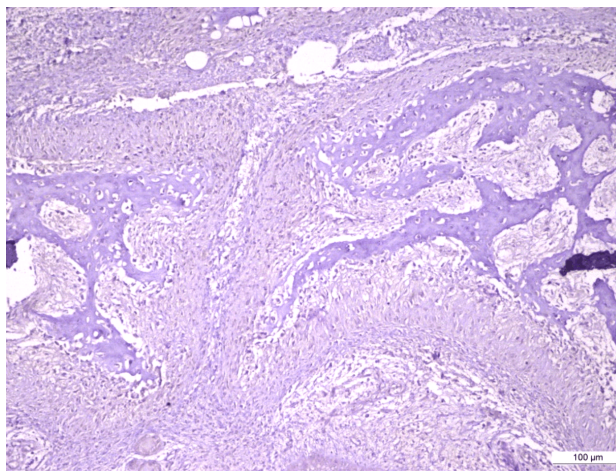


Figure 2 – Anastomosed embryonic bone trabeculae separated by areolar spaces (HE staining, $\times 100$).

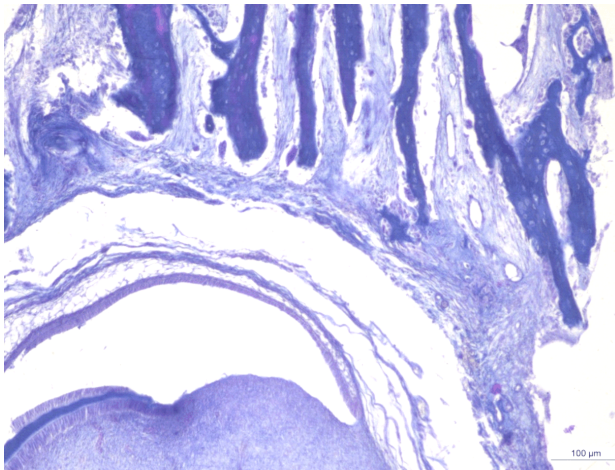


Figure 3 – Embryonic bone trabeculae adjacent to a tooth germ (Masson's trichrome staining, $\times 100$).

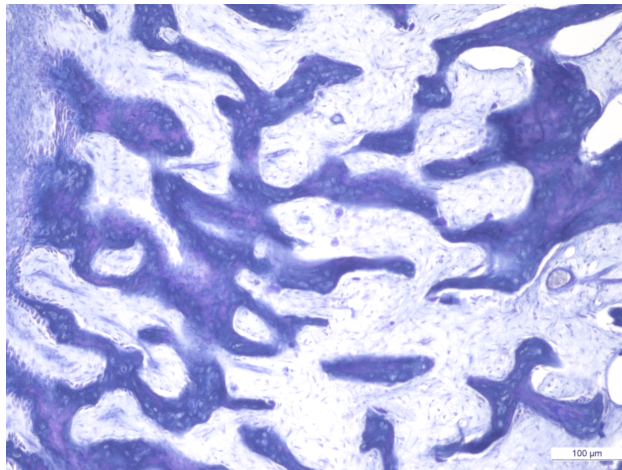


Figure 4 – Embryonic bone trabeculae with different degrees of mineralization (Masson's trichrome staining, $\times 100$).

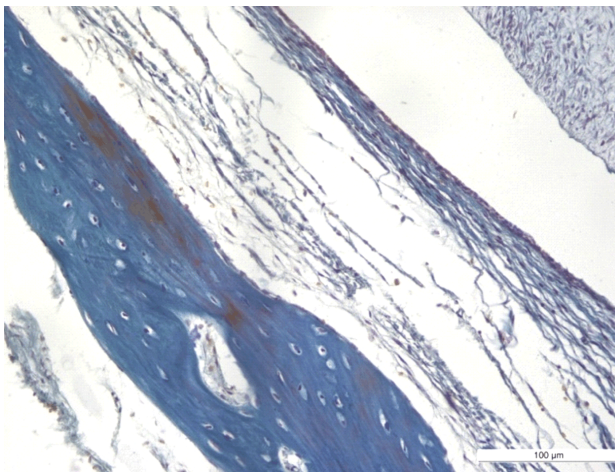


Figure 5 – Dental sac area in continuity with embryonic bone (Masson's trichrome staining, $\times 200$).

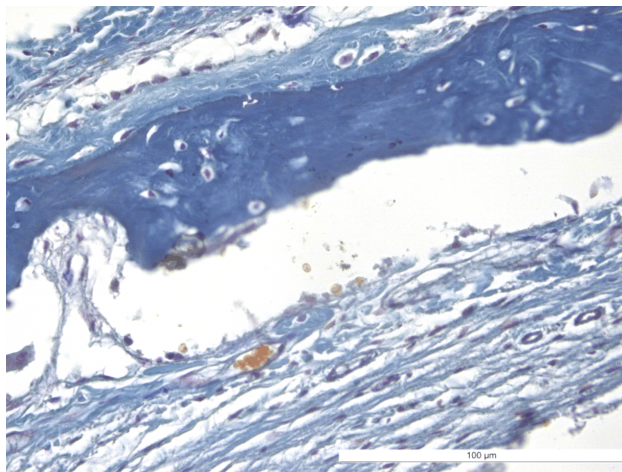


Figure 6 – Embryonic bone lined by osteoblasts, containing numerous active osteocytes among the collagen fibers, partially mineralized (Masson's trichrome staining, $\times 400$).

Table 1 – Development stages of tooth germs

| Case No. | Development age [weeks] | Tooth – development stages | | | | |
|----------|-------------------------|----------------------------|----------------------|------------|--------------------|--------------------|
| | | Incisor ₁ | Incisor ₂ | Canine | Molar ₁ | Molar ₂ |
| 1. | 10 | cap | cap | cap | cap | absent |
| 2. | 14 | early bell | cap | cap | cap | absent |
| 3. | 18 | late bell | early bell | early bell | cap | cap |
| 4. | 22 | late bell | late bell | late bell | late bell | early bell |
| 5. | 24 | late bell | late bell | late bell | late bell | late bell |

The tooth germs in cap stage showed the morphological components characteristic to this development stage: an enamel organ, a dental papilla with mesenchymal and ectomesenchymal cells and a loose dental sac. Adjacent, we noted morphological elements specific to the early moments of the dental alveoli formation in the mandibular body, namely very thin walls of the crypts, with “eggshell” appearance (Figure 7).

Progressive events occurring during the transition from cap stage to early bell stage were reflected by the crowding tendency of ectomesenchymal and mesenchymal

cells near the basal membrane of the inner epithelium, resulting in the successive formation of preodontoblasts and odontoblasts that induce and complete the emergence of ameloblasts (Figure 8).

The tooth germs in later bell stage showed all the histological traits characteristic to that development stage, such as enamel and dentin synthesis in variable amounts (Figures 9–10). The dental sac was denser because of cell maturity. Around the dental germs in the bell stage, the forming crypt had larger sizes, reflecting bone deposits on the inner edge. Due to the low gestational age of the cases included in the study group, the initial phase of root formation was not noticed. Therefore, the obvious histological elements that support the initiation of cementum and periodontal ligament formation were absent.

Discussions

The first studies on the development of maxillary bone and tooth, as part of embryonic human development, are published in the late 19th and early 20th centuries [13–24]. The theoretical substrate of knowledge is founded on morphological studies of normal craniofacial development [4].

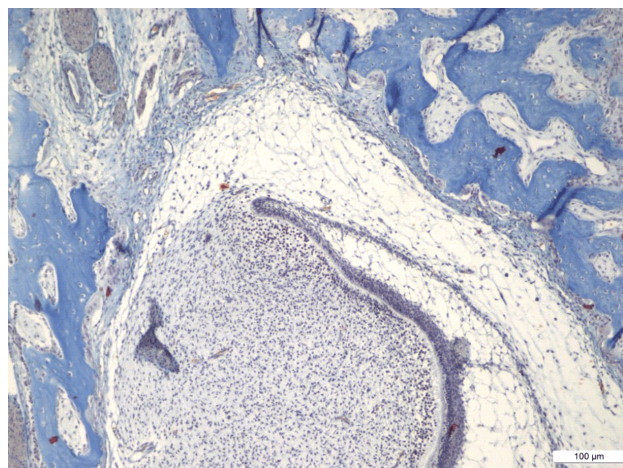


Figure 7 – Tooth germ: cap stage surrounded by the dental sac and developing embryonic bone of the mandible (Masson's trichrome staining, $\times 100$).

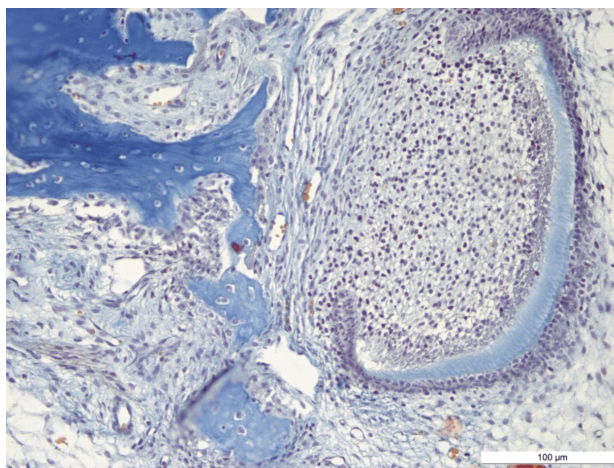


Figure 8 – Tooth germ: early bell stage presenting the first band of dentin; large embryonic bone trabeculae forming the embryonic bone (Masson's trichrome staining, $\times 200$).

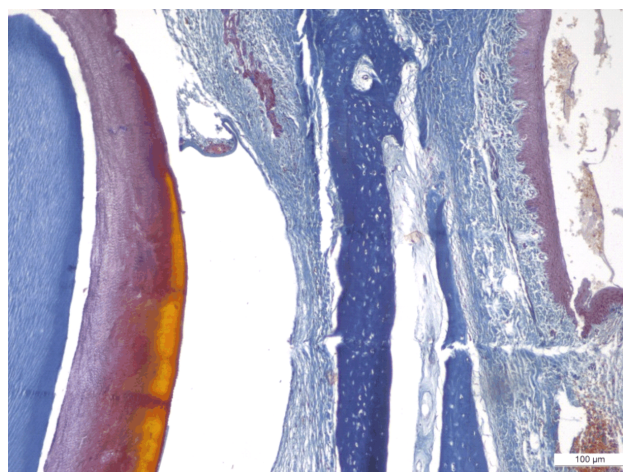


Figure 9 – Tooth germ: advanced bell stage, separated by the dental sac from the maxillary embryonic bone; stomodeal epithelium on the surface (Masson's trichrome staining, $\times 100$).

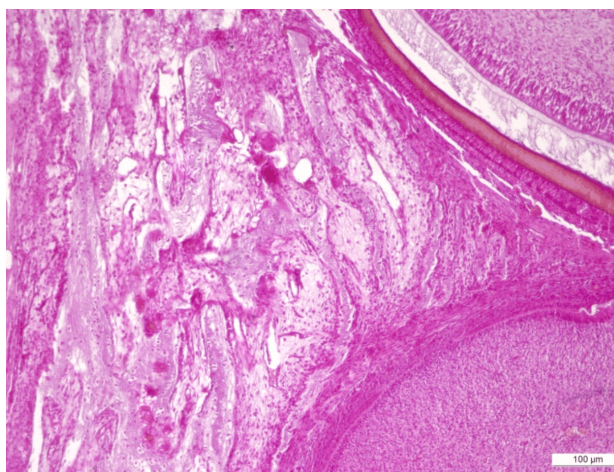


Figure 10 – Tooth germs: advanced bell stage, surrounded by the mesenchymal tissue characteristic of dental sacs and developing maxillary embryonic bone (PAS staining, $\times 100$). PAS: Periodic Acid–Schiff.

Since the '80s, important contributions in this field were brought by the studies on human embryos performed by Merida Velasco's and Radlanski's groups. Merida Velasco's group reports focuses on the development of the mandibulae and proves details on the role of Meckel's cartilage and the differences in the ossification process of the mandibular *corpus* and *ramus* [25–27]; another issue addressed is the tooth development, pointing on the evolution of the dental crest and the growth of first molars [28, 29]. The relationship between Meckel's cartilage and the morphogenesis of the mandible was also investigated by other groups of researchers [30–32]. By using the computer-assisted facilities, Radlanski's groups work is based on three and four-dimensional reconstruction of embryological structures from histological serial sections of human embryos which belong to the valuable Radlanski's collection, Charité – Berlin University of Medicine [33–38]. Relying on the analysis of digitized images of the microscopic structures, associated with radiological and cephalometric evaluations, the results

allow a deeper insight into the prenatal craniofacial and tooth morphogenesis and a better comprehension of the formal development and reciprocal influences that govern this complex process.

In the last years, the understanding of these phenomena and events has been deepened, through targeted research at the cellular, molecular and gene levels. This translation was made possible due to the technical advances registered in the medical research, in parallel with the extension of experimental studies on developmental biology, *in vivo* and *in vitro*, by using animal models and cell lines [1, 3, 5, 39–42]. It is worth mentioning that the experimental approach is justified by the ethical consideration regarding the use of the human material [43, 44], their compliance limiting the research on human samples. Therefore, we consider of interest a brief overview of the gene and molecular substrate that govern the jaw and tooth development.

Recent data based on extended animal studies demonstrate the role of development genes and molecular pathways in the formation of jaw [2, 5], including the regulation

of different stages in bone deposition. A first step in this process is the migration of the neural crests cells into the first pharyngeal arch, where none of the *Hox* genes is expressed [5, 45, 46]. In the body of mandible arch, the migration is regulated by the *OTX* gene, transcription factors of the *MSX* and *DLX* families and several signaling factors [2]. After migration, the cells of neural crests originate chondroblasts and osteoblasts. The development of the basal and alveolar bone by intramembranous ossification starts around the Merkel's cartilage and is controlled by *MSX*, *DLX* and *Gsc* genes and signaling factors like bone morphogenetic proteins (BMPs) [2, 5–7].

The similarities and differences between the development of maxilla and mandible could be explained by the “hinge and caps” predictive model [47], based on the potential of different genes to influence different territories. Thus, *Satb2* gene [32] and *Six1* gene [48] coordinate the development of both maxilla and mandible, *Endothelin 1* gene induces mandible identity [49], whereas Nr2f nuclear receptor induces maxilla individuality [50], and *Dlx5/6* gene expression is inhibited in mandible [51]. Nevertheless, the gene expression is strongly influenced by signaling pathways [*i.e.*, Bmp, fibroblast growth factor (Fgf), Sonic hedgehog (Shh), Wnt pathways], their action being demonstrated in studies focused on the development of the mandible [52, 53].

Old and new concepts on tooth development are brought together in an excellent recent review [4] that offers an integrative insight in the early evolution of human dentition.

Experimental studies have demonstrated the role of epithelial–mesenchymal interactions in the initiation of tooth development. It is proven that tooth development is based on a mutually inductive molecular dialogue between the oral epithelium and the underlying mesenchyme [54, 55], dialogue in which the formation and degradation of dental basement membrane are important events [56]. These functional interrelations ripple through the process of tooth development, so that specific structural transformations, irrespective of the dentition type, remain identical.

Nowadays, the mechanisms involved in tooth development are analyzed by reference to genes, molecules and signaling cascades responsible for mediating or governing this process.

Tooth development is initiated by an orchestration of various genetic and epigenetic factors [1, 57–60]. The shape of the tooth is genetically determined through the homeobox genes [61]. These homeobox genes have been identified in mice; because mice do not have canines, the obtained code is considered “speculative”; however, there is a possible overlap of genes for a presumed canine region. Combinations of different expressions of homeobox genes could be: for incisors, *MSX-1*, *MSX-2*; for canines, *MSX-1*, *MSX-2*, *DLX-2*; for molars, *BARX-1*, *DLX-2* [62]. There are currently known approximately 300 tooth-related genes (*i.e.*, *Gli*, *Lef*, *Pax*, *Fgf*, *Msx*, *Dlx*, *Wnt*, *Lhx*, *Bmp*, *Shh*, *Hgf*, *Ptc*, *Smo*, *Pitx*, *Slit*, *Barx*, *Otlx*) and approximately 100 molecules (FGF8 and BMP4 are considered master

molecules), which intervene in this complex process conjointly to the Shh and Wnt signaling pathways [1, 6, 63–65]. Moreover, recent studies demonstrate the role of the stem cells that ensure the odontogenic potential for tooth development, with possible appliance in the tooth bioengineering research [66–69].

Nevertheless, according to existing data, no direct link has been identified between one gene and the ontogenesis of a specific tooth, most molecules do not act specifically at tooth level and the information of sent signal regulation is limited [61].

With respect to the national and international regulations imposed for the research performed on the human material resulting from abortion, the present study complements our previous works on this topic [9–12].

Despite the alone histological approach, our descriptive data sustained by valuable images can be added to the general repository of information on maxillary bone and tooth development.

Our study reveals early events in the embryonic bone and tooth germ formation. We illustrated the dynamics of the ossification stages, from stages of early-dispersed intramembranous ossification to the organization of the dental alveoli, incorporated step-by-step in the maxillary body, and the simultaneous presence of tooth germs with different sizes and shapes, in accordance with the development stage.

The most important aspects identified in our study target the intramembranous ossification model present in the maxillary bone. Microscopic examination revealed the beginning of bone development within the well-vascularized mesenchymal tissue, by condensing the proliferating mesenchymal cells into a “membrane-type” structure. As the vascularization increases in the areas of condensed mesenchyme, the mesenchymal cells were transformed into osteoblasts. The process was identified in multiple areas within the future intramembranous bone, the regions of initial osteogenesis representing primary ossification centers, in which bone spicules are formed. In relation to the gestational age, we were able to evaluate the formation of the embryonic bone by the progressive fusion of the bone spikes into thin plates or trabeculae, and the fusion of the plates to form a thicker, trabecular-type embryonic bone. It should be noted that the initial plates of intramembranous bone are structurally unstable, not only due to the random orientation of the fibers and the low degree of mineralization, but also because many islands of mesenchymal tissue, extremely loose, remain inside the plates. This embryonic bone (fibrillar, fibrous, or reticular), characteristic of the early embryo and fetus, is a non-lamellar bone. Morphologically, the dominant feature is conferred by the coarse collagen fibers that interlay or intersect to form a meshwork, which is why it is also called “woven bone”. It is richer in cells, which are randomly placed, and the matrix has more ground substance than the mature bone.

In parallel, the microscopic traits characteristic for the developing tooth germs allow the correlation of the histological features with the evolution in relation to the

gestational age. A noteworthy element is the adaptability of the tooth germs to the intraosseous localization, by the variability of size and shape in accordance with the stage of development and the type of tooth. The cellular complexity of the enamel organ epithelia ensures the synthesis and deposition of the enamel, whereas the differentiation of the odontoblasts ensures the synthesis and deposition of dentin. However, the histological architecture of these structures, easily recognizable by light microscopy, concentrates a complex molecular substrate.

From this perspective, the present paper should be considered a preliminary report. As a perspective, the used human material has the potential to be exploited by extending the study on the molecular mechanisms involved in maxillary bone and tooth development.

Last but not least, we reiterated an assertion formulated in a previous paper [9] regarding the small number of available images illustrating the maxillary bone and tooth development stages in humans [25–27, 33–38], because of the ethical and technical impediments in accessing suitable material [9, 44]. Therefore, in addition to our already published data [9], we consider our results an important contribution for the enlargement of existing visual information for maxillary bone and tooth development.

Conclusions

Although the morphological stages of tooth development, in parallel with maxillary bone construction, are known for decades, the interest in the knowledge of these events at the molecular level is still current, aiming to sustain the progress in the endorsement of novel regenerative and restorative therapies. Nowadays, the mechanisms involved in the maxillary bone and tooth development are studied mainly by using experimental models. Therefore, a study conducted on human material, respecting the ethical regulations of the research, maintains its value. Our study complements the existing data regarding the embryonic period, by identification of the morphological elements, which characterize the early stages of the maxillary alveolar bone and tooth development.

Conflict of interests

The authors declare that they have no conflict of interests.

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Received: January 28, 2020

Accepted: July 21, 2020