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# Characteristics of the number of odontoblasts in human dental pulp post-mortem

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ABSTRACT

Estimation of the time since death is important in forensic medicine, and so far not much is known in employing dental pulp for such purposes. The tooth organ is the hardest organ in the human body, with a loose connective tissue of dental pulp situated within a rigid encasement of mineralized surrounding tissues. Human material was obtained from 31 corpses of people who died in car and train accidents and had healthy oral statuses. Samples were divided into two groups at different environmental temperatures. During the autopsy, the jaws were resected to keep teeth *in situ*, and every day one tooth was extracted. After decalcification, serial thin sections stained with hematoxylin and eosin were cut. Odontoblasts in the dental pulp were counted and data analysed. Statistical analysis showed that the number of odontoblasts drops during the time after death, and no odontoblasts remain in the pulp after 5 days.



## 1. Introduction

The estimation of the time since death has obvious and important implications in the investigation of criminal deaths. The vast amount of labour in this direction has not been rewarded by comparable improvements in accuracy because of permutations of factors that defy exact calculation of the post-mortem interval.

Methods applicable to the early post-mortem period of the first day or two after death include: body temperature; rigor mortis and hypostasis; electrical excitability of muscle; gastric emptying; eye changes—retinal appearance, iris reaction to drugs, vitreous humour chemistry; blood, pericardial and cerebrospinal fluid chemistry; cytological changes in bone marrow [1].

After cessation of vital functions, ischemia causes hypoxia, which interferes with aerobic oxidative respiration. Hypoxia causes cells to pass the nebulous "point of no return" into irreversible injury and cell death. Two phenomena consistently characterize irreversibility: the inability to reverse mitochondrial dysfunction (lack of oxidative phosphorylation and ATP generation) and profound disturbances in membrane function. The injury to lysosomal membranes results in the enzymatic dissolution of the injured cell that is characteristic of necrosis. The events that determine when injury becomes irreversible and progresses to cell death remain poorly understood [2].

The tooth organ is the hardest organ in the human body. It consists of the tooth and its surrounding tissues. Dental pulp is a loose connective tissue situated within a rigid encasement of mineralized dentin, covered with enamel on the crown and cement on the root.

Histologically, beneath the dentin, a layer of odontoblasts circumscribes the outermost part of the pulp. Subjacent to the odontoblast layer, towards the middle of the pulp, the cell-free zone, cell-rich zone and pulp core extend [3].

The odontoblast is considered to be a fixed post-mitotic cell in that once it has fully differentiated, it apparently cannot undergo further cell division. It is responsible for dentinogenesis both during tooth development and in the mature tooth, and is the most characteristic cell of the dentin–pulp complex. It produces a matrix composed of collagen fibrils, non-collagenous proteins and proteoglycans that are capable of undergoing mineralization. The ultrastructural characteristics of the odontoblast exhibit a highly ordered RER, a prominent Golgi complex, secretory granules and numerous mitochondria. In addition, it is rich in RNA, and its nuclei contain one or more prominent nucleoli; these are general characteristics of protein-secreting cells. In contrast to the active odontoblast, the resting or inactive odontoblast has a decreased number of organelles. During dentinogenesis odontoblasts form the dentinal tubules, and their presence within the tubules makes dentin a living responsive tissue [4].

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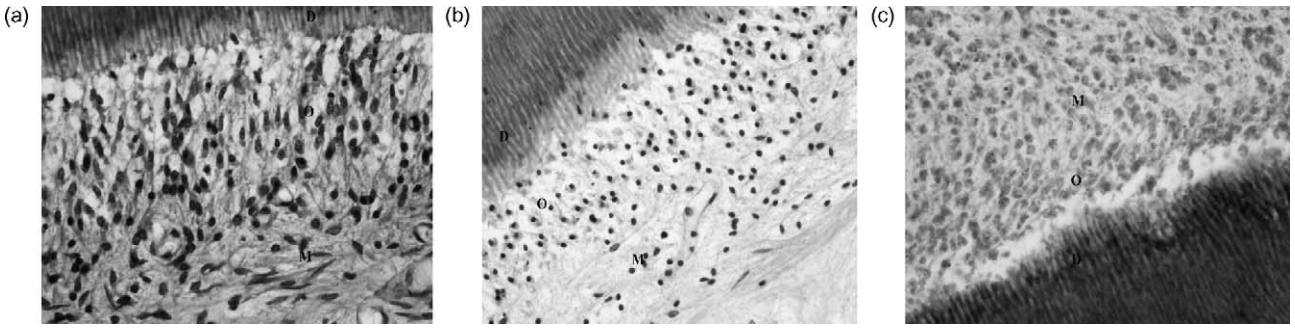


**Fig. 1.** The layer of odontoblasts in human dental pulp 7 h (a), 55 h (b) and 103 h (c) after death at room temperature. D: dentin, O: layer of odontoblasts, M: middle part of the dental pulp. Hematoxylin and eosin, 40×.

Because of the relatively sparse cellular composition of the pulp, the rate of oxygen consumption is low in comparison with that of most other tissues. During active dentinogenesis, metabolic activity is much higher than after the completion of crown development. The greatest metabolic activity is found in the region of the odontoblast layer, and the lowest is found in the central pulp, where most of the nerves and blood vessels are located. Yu et al. in their study established the $O_2$ consumption of odontoblasts in the lower incisor pulp of the rat at the rate of $3.2 \pm 0.2$ ml/min/100 g of pulp tissue [5]. In addition to the usual glycolytic pathway, the pulp has the ability to produce energy through a phosphogluconate (e.g., pentose phosphate) shunt type of carbohydrate metabolism [6], suggesting that the pulp may be able to function under varying degrees of ischemia.

Not much is known about employing dental pulp as a tissue for estimating the time since death. Duffy et al. [7] compared rates of putrefaction of dental pulp in the northwest coastal environment in Canada for extracted human and pig teeth and pig teeth *in situ*. The cells in the pulp were preserved from 96 to 336 h or 4–14 days, according to environmental conditions. Flow cytometric evaluation of dental pulp DNA degradation revealed minimal DNA degradation in dental pulp tissue by 144 h after surgical removal of human third molars; therefore, the pulp tissue was found to be unreliable for determining the early post-mortem interval. Light microscopic examination of the same human dental pulp tissue at 144 h after surgical procedure did not show any morphologic changes of autolysis [8]. The study of Caviedes-Bucheli et al. [9] revealed 41% pulp tissue cell viability 24 h after death.

So far not much has been written about post-mortem changes of human dental pulp in teeth *in situ* in the alveolar bone (jaw). Taking into consideration that odontoblasts are highly differentiated cells, which are usually highly oxygen sensitive, and specific to the dental pulp, and the suggestion of Fisher and Walters [6] that the pulp may be able to function under varying degrees of ischemia, we decided to analyse the basic characteristics of the number of odontoblasts in dental pulp after death.

The article is focused on the dynamics of changes in the number of odontoblasts in human dental pulp *in situ* as being dependent of time. The aim at this point of our research is to check and verify the basic characteristics of this process. The number of cases in our dataset permits these relatively simple analyses, but it is rather low at present to construct a reliable statistical calibration tool.

## 2. Materials and methods

The study was carried out in accordance with the requirements of the Institute of Forensic Medicine at the University of Ljubljana medical faculty regarding manipulation and research experiments carried out on organs or tissue from dead human bodies, and with the approval of the National Medical Ethics Committee of the Republic of Slovenia, No. 114/12/03.

The material was obtained from 32 corpses (cases) of people aged 18–40 years old who died from lethal injuries sustained mostly in car and train accidents, and whose heads were mutilated beyond recognition. Their teeth had to be caries and restoration free; no calculus should be present, and pocket probing depth should not exceed 3 mm. During the autopsy, the jaws were removed and stored in an open plastic bag; thus some airflow was enabled in an attempt to simulate a corpse with a slightly opened mouth. Every day one single-rooted tooth (sample) was extracted from the mandible, starting with a canine as soon as the corpse arrived at the Institute of Forensic Medicine, and followed by premolars every 24 h after the first extraction. Cases were divided into two groups: 15 at room temperature (23 °C) and 17 at a refrigerated temperature of 4 °C.

### 2.1. Control

For orthodontic reasons, intact mandibular first premolars from a young adult were extracted pain-free under local anaesthesia (Ultracain D-S forte, 2 ml ampoule, Hoechst AG) and examined as a control. The sample was prepared for light microscopy investigation. The time from application of anaesthesia to immersion in fixative was optimized to 5 min.

### 2.2. Light microscopy

Immediately after extraction, with elevator and forceps, the apical three quarters of the root was chiseled off to facilitate the penetration of the 10% neutral buffered formalin fixative solution in the dental pulp tissue for 3 days at room temperature. After decalcification and preparation of the samples by the classic method, 4-μm-thin paraffin longitudinal serial sections were cut in the vestibulo-lingual plane through the middle of each tooth. The middle of the tooth was considered to be as the dentinal tubules gave their straight and S-shaped course; the best sections were selected and stained with hematoxylin and eosin stain, and then examined at 10× and 40× objective magnifications with a Nikon Eclipse E600 light microscope. Images were digitized and grabbed with a CCD-1300 CB device camera (VDS Vosskühler, GmbH) and analysed with NIS-Elements AR (Nikon) software. Cells in the odontoblast layer were morphologically evaluated, counted and analysed.

According to stereological calculations, in each sample 10 microscopic examination fields in the coronal part of the dental pulp were investigated at 40× objective magnification. After grabbing the photo of the layer of odontoblasts (Figs. 1 and 2), we measured its area in the microscopic field; we then counted odontoblasts with nuclei either intact, with condensed chromatin or pyknotic. We calculated the average absolute (number of odontoblasts per square micrometre and conversion to number of odontoblasts per square millimetre) and relative (the first sample of every case represented 100% of odontoblasts per square micrometre/millimetre) density of odontoblasts for each sample. To diagnose necrosis with classic light microscopy, several hours must elapse, even more than 12 [10].

We carried out statistical analysis of the data using the SPSS statistical package for MS Windows. The two groups of cases (room and refrigerated temperature) were analysed separately.

## 3. Results and discussion

### 3.1. Density of odontoblasts

By counting the number of odontoblasts in the samples taken in the first 24 h after death, we estimated the average density of odontoblasts per square millimetre in healthy vital teeth of Caucasian adults aged 18–40 years. Our result of 11,764 odontoblasts per square millimetre in the average of all cases corresponds to the approximately 11,000 odontoblasts per square millimetre after cavity preparation with remaining dentin thickness of 0.501–2.993 mm obtained by Murray et al. [11]. In another study he, with co-workers, reported 350 odontoblasts per 1 mm