Original Article

Human ex vivo dentin-pulp complex preservation in a full crown model

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ABSTRACT

Objectives: Currently, there is lack of human in vitro full tooth models that hold the odontoblast layer with pulp tissue in their native environment. The appearance of new in vitro and in vivo models has provided new understanding of the potential of tissue engineering in dental pulp regeneration. However, the development of new in vitro full tooth models will allow us to get closer to in vivo conditions. Thus, the aim of this study is to preserve a living dentin-pulp complex, in a novel in vitro full crown model, after tooth extraction.

Methods: Twenty intact third molars, after preparation, were divided into four groups, with five samples each. We placed the negative control samples (C) in saline, and the tested groups were placed (T) in supplemented DMEM, at two different times: 1 and 7 days. The specimens were processed for light microscopy observation.

Results: Contrary to C-groups, T-groups showed a functional dentin-pulp complex. The treated dentin-pulp complex presents normal histological appearance.

Conclusions: This study showed that it is possible to preserve a living dentin-pulp complex after tooth extraction during 7 days.

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1. Introduction

Odontoblasts are post-mitotic specialized pulp cells, organized in a pseudostratified palisade layer.^{1,2} At the dentin-pulp interface, odontoblasts are connected by junctional complexes, from where each cell projects an odontoblastic process into the pre-dentin/dentin matrix.³ These cells are strategically placed and plays a key role in the first response of the dentin-pulp complex to injury.

Dentin-pulp complex is fundamental to the functional life of the tooth and its complex interaction during development, injury, defence or regeneration ensures the tooth response to growth, damage or aging.⁴ Besides, the pulp cell activity and the signaling processes are crucial to the odontoblasts' behavior and fate.

The main goal of pulp therapies is to maintain vitality of pulp tissue, which is crucial to the functional life of the tooth.⁵ However, in cases of irreversible pulpitis, pulp necrosis and periapical disease, the aim becomes the reestablishment of a vital and organized dentin-pulp complex.⁶

In the past two decades, the pursuing of new endodontic regeneration therapies gave rise to new culture models. Presently, there are in vitro and in vivo models to study dental pulp regeneration. In vitro models can be classified as two-dimensional (2D), three-dimensional (3D), and organ culture models. Organ culture models comprises in vitro full tooth models⁷ and in vitro tooth slice models.^{8–12} These models mimic in vivo conditions, providing an environment to test cells or substances.

Formerly, primary odontoblasts have been cultured pulpless.¹³ These pulpless methods contributed to study odontoblast polarity¹⁴ and the effect of growth factors.¹⁵ Recently, there was enzymatic isolation of viable human odontoblasts.¹⁶ Nevertheless, pulpless tissue cultures leave out the dentin-pulp complex dynamics and the symbiosis between odontoblasts and the pulp tissue.

The methodology used in this study consisted on an in vitro full crown model. The hypothesis was that the dentin-pulp complex

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Fig. 1. Automatic microtome Struers Accutom 50 (A) was used to cut each tooth, immediately after surgery and cleaning (B). The cut was performed at 3200 rpm, 0.350 mm/s speed and irrigation (C), which gave rise to 2 specimens (D).

Fig. 2. Summarized schematic of the in vitro full crown model. After extraction (A), all teeth were prepared (B), and then the specimens were placed with the crown bottom-faced and the dentin-pulp complex exposed, and submerged by the respective medium. The samples from the negative control group were placed in saline (C). The samples from test groups were cultured in supplemented DMEM (D–F).

could be preserved in the pulp chamber. The aim was to preserve a functional crown dentin-pulp complex for, at least, 7 days after extraction.

2. Material and methods

2.1. Specimen collection and preparation

Twenty intact third molars, with root completion and scheduled for extraction because orthodontic reasons were selected from young adult patients (the age ranged from 18 to 25 years). Teeth were clinical and radiological evaluated before and after surgery. Teeth that presented signs of caries, fractures, wear or incomplete root formation or not met all the requirements were excluded.

After reading and receiving all necessary explanations, patients signed the informed consent. The study was approved by the Institutional Ethical Committee and the procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Teeth were immediately prepared after extraction, cleaned of all external soft-tissue remnants and rinsed in 98% ethanol. Then, all teeth were horizontally cut, 2 mm below the cement-enamel junction with an automatic microtome Struers Accutom 50, at 3200 rpm, 0.350 mm/s speed and irrigation (Fig. 1). The cut was made with full water irrigation to prevent frictional heat and dentin-pulp complex damaging.^{17,18} Each tooth gave rise to

Table 1

Group description and number of teeth per group.

Group	Time of treatment				Total
	1 day	n	7 days	n	
Negative control in saline (C)	C1	5	C2	5	10
Treated (DMEM supplemented) (T)	T1	5	T2	5	10

2 specimens (coronal and root pieces). We used the coronal specimens, and the root ones were discarded (Fig. 2).

2.2. Culture groups

Teeth were randomly distributed into four groups (Table 1). All samples were placed in 24-well culture plates with the crown bottom-faced and the dentin-pulp complex exposed¹³ (Fig. 2).

For the negative control, we placed the samples in saline. For the treated groups, we used Dulbecco's Modified Eagle Medium (DMEM, Sigma–Aldrich, St. Louis, USA) supplemented with FBS (100 mL/L), minimal essential medium (MEM, Sigma–Aldrich, St. Louis, USA) (1 mL/L), and antibiotic–antimycotic solution, with a final concentration of 1 µg/1 mg/L penicillin G-streptomycin and 2.5 mg/mL/L. All test groups were incubated at 37 °C in a Memmert INB 500 Incubator (Schwabach, Germany). In group T2, DMEM serum was changed every 2 days using sterilized material.

Fig. 3. We can observe pulp asthenia with the displacement of the odontoblastic layer and signs of pyknosis (*) in C1 (A) and C2 (B). In T1 (C) and T2 (D) there is a functional dentin-pulp complex with typical tissue and cellular architecture. H/E, 400×. D' – dentin, Pd – predentin, O – odontoblast layer.

2.3. Light microscopy

For light microscopy, all the samples were fixed in 4% formaldehyde (pH 7.0) for 48 h. The samples were decalcified with 5% trichloroacetic acid for 45 days.¹⁹ Then, teeth were labio-lingual vertically cut in the middle of the chamber and decalcified with 5% trichloroacetic acid for 2 days. Finally, we made 3 µm paraffin sections for hematoxylin and eosin staining with a Microm HM 355S microtome (Thermo Scientific, Massachusetts, USA).

We used a Leica DMLB light microscope and, for the image capture, the Leica DFC290 HD with Leica Application Suite Software.

3. Results

In group C1 (Fig. 3A), we observed tissue asthenia, with odontoblast layer disruption and separation from the dentinal walls, odontoblast pyknosis, absence of odontoblastic processes, predentin absence and abnormal pulp architecture. We also detected the disorganization of the different layers of the pulp tissue.

In group C2 (Fig. 3B), the signs of pulp asthenia are more obvious, higher level of disruption of the odontoblastic layer, with pyknotic odontoblast nucleus and disorganized pulp.

T-groups showed a functional dentin-pulp complex in contrast with C-groups. T1 group (Fig. 3C) presented normal pulp architecture, typical odontoblast layer, and presence of predentin. In T2 (Fig. 3D), we observe few changes on pulp tissue and the maintenance of predentin, and odontoblast morphology and pulp architecture similar to T1.

4. Discussion

Preservation of the dentin-pulp complex in their native conditions allows the study and understanding of the tissues in their normal environment and three-dimensional space.²⁰

Predentin is an unmineralized organic matrix produced and regulated by vital odontoblasts and is a constant feature in healthy teeth.^{21,22} The observation of predentin supports evidence of an active odontoblastic layer in T-groups. Expectably, in C-groups there were no predentin, since this is a serum without any nutrients. The tissue asthenia observed in these groups was not present in T-groups, confirming the importance and presence of an active odontoblastic layer.

One of the major advantages of this type of model is the natural presence of the so-called tissue engineering's triad: stem cells, scaffold and growth factors.^{5,23} The heterogeneous nature of dental pulp niches, within the central pulp stroma and in perivascular regions, implicates local tissue signals for self-renewal, proliferation, differentiation, mobilization, and homing of cells.^{20,24,25} So, the tooth itself as a preservation system ensures unique features that none of the 2D and 3D in vitro models guarantees.

The only in vitro full tooth model⁷ showed to be useful on the assessment of the hydration of pulp capping materials. However, in spite of maintaining the humidity and temperature, they did not assess the dentin-pulp complex after the time of culture. Also, we believe that the position of the model, with the crown upturned and the dentin-pulp complex exposed bottom-faced, does not facilitate the dispersion of the supplemented serum.

Future studies need to be conducted to understand if there is the possibility to extend this preservation for more days, investigate cellular and molecular changes during this process, and cell's behaviors and responses.

In conclusion, the tested in vitro full crown model has sound histological characteristics, thus, it is possible to preserve a living dentin-pulp complex after tooth extraction for at least 7 days. Our method may prove to be important concerning the preservation of these tissues.

Conflicts of interest

The authors have none to declare.

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