TGFB-1 Release From Radicular Dentin After The Application of Calcium Silicate Based Coronal Barriers in Regenerative Endodontics: A Comparative In-Vitro Study

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Method Article

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Abstract

The aim of this study is to evaluate the TGFB-1 released from radicular dentin after the application of calcium silicate based coronal barriers (MTA ProRoot®, NeoMTA Plus®, ENDOCEM MTA®) in regenerative endodontics.

Introduction

In recent years, the field of dentistry has witnessed remarkable advancements, especially in the realm of endodontics, with the emergence of regenerative endodontic procedures (REPs). Unlike traditional root canal therapies that focus on removing infected or damaged pulp, regenerative endodontic procedures take a pioneering approach by seeking to restore the vitality and function of the dental pulp (Murray et al., 2007). This revolutionary technique holds great promise, particularly in cases involving immature permanent teeth with necrotic pulp tissue resulting from traumatic injuries. However, the fundamental goals of regenerative endodontic procedures revolve around encouraging the natural regeneration of dental tissues. By eliminating the necrotic pulp and disinfecting the root canal space, regenerative materials enriched with growth factors and scaffolding agents are introduced facilitating the ongoing growth of the root and promoting the healing of periapical tissue.(Caviedes-Bucheli et al., 2022; Diogenes and Ruparel, 2017)

However, transforming growth factor-β1 (TGF-β1) is a pleiotropic molecule that is part of the TGF-β family and is involved in various biological processes (Massagué and Xi, 2012). The release of TGF-β1 from dentin is of interest in the context of regenerative endodontic procedures.

This study is grounded on the well-established fact that TGF-β1 modulates a wide range of reparative processes in different tissues. TGF-β1 stimulates odontoblast differentiation (Begue-Kirn et al., 2004) and progenitor cell migration in the dental pulp (Howard et al., 2010). Research has indicated that TGF-β1 is present in dentin, and its release can occur under certain conditions, such as when dentin is demineralized or subjected to specific treatment (Sloan et al., 2000), TGF-β1 has been implicated in promoting the early stage of odontoblastic differentiation of dental pulp stem cells into odontoblasts (Bai et al., 2023). Since TGF-β1 at high concentrations caused a negative effect on APC proliferation, it might not be essential to promote the production of a considerable quantity of TGF-β1. In addition, a certain concentration was needed to promote mineralization. (Srisuwan and Wattanapakkavong, 2022)

Thus, understanding the release of TGF-β1 from dentin as well as the materials used in the regenerative endodontic treatment and their clinical potential applications in enhancing regenerative approaches in dentistry is essential. This includes developing strategies to optimize the release of growth factors to promote tissue repair and regeneration.

Procedure

Description of study sample
Permanent single-rooted premolars extracted due to periodontal, orthodontics, or prosthodontic reasons will be collected from the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Cairo University.

· **Eligibility criteria**

**Inclusion criteria:**

- Single-rooted premolars.
- Mature root apices.
- Teeth length ranges from 18 - 25 mm.

**Exclusion criteria:**

- Root curvature more than 10°.
- Root caries.
- Complex root and root canal anatomy.
- Calcified root canals.
- Internal resorption.
- Cracked teeth.
- Previously root canal treated teeth.

Pre-operative radiographs will be taken from bucco-lingual and mesio-distal aspects to evaluate the internal anatomy and confirm fulfilling eligibility criteria without any complexities or defects.

**Sample preparation:**

- Single-rooted human premolars will be collected from healthy individuals aged 15–25 years, creating 10-mm-long tooth samples.
- The root canals will be Instrumented by using gate-glidden (Mani, Japan) up to size 3 to simulate conditions found in immature teeth.
- All tooth samples will be Standardized by adjusting the outer surface to achieve uniform weight and size.
- The smear layer will be removed by immersing all samples in 17% EDTA (PD, Switzerland) and then 2.5% sodium hypochlorite (CALIX, USA) for 1 minute each. Ultrasonic activation will be done three times
with phosphate-buffered saline (PBS) for 1 minute each.

- The disinfection step will be extended to the regenerative endodontic procedures (REPs) by applying calcium hydroxide paste (UltraCal™ XS, USA) to all root segments. The treated samples will be incubated (Binder, NY, USA) under 95% humidity and 5% CO2 for 1 week at 37°C.

- Subsequently, will be rinsed with 5 mL PBS and Ultrasonic activation will be done with 17% EDTA for 5 minutes.

- The teeth will be longitudinally split, and their external surfaces will be coated with nail varnish

**Intervention for each group**

This intervention aims to simulate conditions related to immature teeth and assess the release of transforming growth factor-beta 1 (TGF-β1) over a designated period.

- Group1: NeoMTA Plus®
- Group2: ENDOCEM MTA®
- Group3: MTA ProRoot®

- Prepared root segments will be randomly assigned into three groups: MTA Neo, MTA EndoCem, and ProRoot MTA (control).

- Each material will be mixed according to the manufacturer’s instructions and will be placed into the coronal 3 mm of the root canal. Then will be exposed to an environment with 95% humidity and 5% carbon dioxide at a temperature of 37°C for a duration of 4 hours.

- The root samples will be separated into polystyrene plates containing 500 µL PBS and stored at 4°C for 14 days.

- For analysis, root canal samples in PBS will be retrieved.

- The quantities of TGF-β1 released will be determined using an enzyme-linked immunosorbent assay kit (Human TGF-β1 Quantikine ELISA Kit).

- The concentration of TGF-β1/mL will be calculated from the total volume of the root canal space.

**Outcome assessment (TGF-β1 release)**

To assess and quantify the concentrations of Transforming Growth Factor Beta 1 (TGF-β1) to be released in response to an experimental intervention imitating conditions to immature teeth, various root canal materials NeoMTA Plus® ENDOCEM MTA® MTA ProRoot® will be utilized. The assessment will employ the Human TGF-β1 by ELISA Kit from R&D Systems, Wiesbaden, Germany, ensuring meticulous
quantification of TGF-β1. Following a designated 14-day storage period in phosphate-buffered saline (PBS), root canal samples will be retrieved for analysis.

The procedural steps will include the preparation of samples by the ELISA kit instructions, ensuring the effective extraction of TGF-β1 from the root canal space. The subsequent adherence to the ELISA protocol will facilitate the establishment of a standardized setup for each sample, encompassing necessary dilutions and controls. A microplate reader will be employed to measure absorbance at a designated wavelength, enabling the determination of TGF-β1 concentration.

Subsequently, a calibration curve will be generated using known concentrations of TGF-β1 standards provided in the ELISA kit. This calibration curve will serve as a reference for the quantification of TGF-β1 concentrations in the root canal samples. Data analysis will involve calculating the mean and standard deviation of TGF-β1 concentrations for each experimental group (ProRoot MTA, MTA Neo, and MTA EndoCem). Statistical analyses, such as t-tests or ANOVA, will then be performed to recognize significant differences in TGF-β1 release among the experimental groups.