Pulpal Reaction to Pulp Capping Material In Human Tooth Culture Model

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Abstract

The study evaluated the influence of Biodentine, TheraCal LC, and Tideglusib on pulpal morphology and dentin mineralization for direct pulp capping material in the tooth culture model. Thirty-six human immature third molars freshly extract from patients aged 15-19 years were used to study the pulpal reaction after the pulpal medication with different pulp capping materials. Six experimental material groups were determined; three different concentrations of Tideglusib (10, 50, and 100 nM), two commercially pulp capping materials (Biodentine and TheraCal LC), and control as a scaffold (Polycarprolactone) soak with $DMSO_4$ in DMEM media. The materials' interactions with the vital pulp were investigated using the tooth culture model for 14 and 28 days (n = 3). The mineralized foci and the mineralization potential were evaluated by histology. The direct pulp capping with Tideglusib showed biocompatibility to dental pulp cells. The positive control Biodentine and TheraCal LC showed the mineralization nodule at 14 and 28 days. The tooth culture model was a valuable technique for biocompatibility tests in dental pulp cells. Biodentine and TheraCal LC have shown a promising mineralize potential. Tideglusib was biocompatible and promote inflammatory healing to pulp cells, however, the mineralized potential was absent.

***Keywords**: Histological study, Pulp capping materials, Tideglusib, Tooth culture model, Vital pulp therapy*

1. Introduction

Vital pulp therapy is a treatment for preserving and maintaining pulpal tissue in teeth that have been exposed to trauma, dental caries, and restorative procedure. The purpose of vital pulp therapy is to promote tertiary reparative dentin or dentin bridge formation (Bogen et al., 2016). In this treatment, the direct pulp capping materials are usually used as medication at the exposure site.

Several biomaterials medications are used in vital pulp therapies. Calcium hydroxide has been a gold standard for pulp capping, which the initial effect is the development of superficial necrosis and tunnel defect (Karthikeson and Jayalakshmi 2016). Mineral Trioxide Aggregate (MTA) has benefited on sealing ability, biocompatibility, and capacity to promote mineralized tissue formation. However, the drawback is a long setting time and coronal tooth discoloration. Biodentine has been introduced as a new calcium silicate-based restorative cement with a setting time improvement, which can be used as a dentin substitute (Laurent et al., 2012). TheraCal LC has lately been proposed as resin-modified calcium silicate, however, the resin monomers may cause the inflammatory to pulp tissue (Camilleri et al. 2014). Recently, A study has introduced a Glycogen synthase kinase 3 (GSK-3) antagonists, which promoted the natural processes of dentin regeneration relating to the Wnt-signaling pathways (Neves et al., 2017). Besides, the Wnt-signaling pathways, including the GSK-3 activity, are inhibited β-catenin passes through the nucleus. The β-catenin regulates the expression gene that included Axin2. The Axin2 can stimulate reparative dentine formation and replacement the damaged dentin in pulpal tissue (Neves et al. 2017). Therefore, Tideglusib is an Alzheimer's drug that influences GSK-3 antagonists in the Wnt-signaling pathways.

The tooth culture model has been introduced to study the activation of pulp progenitor/stem cells and their migration after deep cavity preparation, which perivascular progenitor/stem cells can proliferate in response to an odontoblast after the injury (Tecles et al. 2005). Nevertheless, the studies demonstrated that the tooth culture model could reproduce the early stage of dentin regeneration, which is appropriate for studying the effects of biomaterials on the pulp tissue (Téclès et al. 2008). Therefore, the purpose of this study

was to evaluate the influence of Biodentine, TheraCal LC, and Tideglusib on pulpal morphology and dentin mineralization in the human tooth culture model.

2. Objectives

The study evaluated the influence of Biodentine, TheraCal LC, and Tideglusib on pulpal morphology and dentin mineralization for direct pulp capping material in the tooth culture model.

3. Materials and Methods

Thirty-six human immature third molar teeth were freshly extracted from patients aged 15-19 years from the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Mahidol University, in compliance with a protocol reviewed and approved by the Ethical Reinforcement for Human Research of Mahidol University (COA.No.MU-DT/PY-IRB 2019/044.0807). The teeth were used to study the pulpal reaction after the pulpal exposure and medication with different pulp capping materials. Only teeth with one to two-thirds root formations were used and confirmed with a radiograph. Six experimental material groups were determined; two commercially pulp capping materials (Biodentine and TheraCal LC), three different concentrations of Tideglusib (10, 50, and 100 nM), and a scaffold (Polycarprolactone) soak with $Dmso_4$ in DMEM media as control. Tideglusib was dissolved and diluted in dimethyl sulfoxide ($Dmso_4$, Sigma-Aldrich, St Louis MO) to the expected concentration and associated with a Polycarprolactone scaffold. The composition of experimental materials was shown in table 1.

The extracted tooth was immediately transported to the laboratory in Dulbecco's Modified Eagle medium (DMEM) supplement with 300 UI/ml penicillin, 300 µg/ml streptomycin 0.75 µg/ml amphotericin B. One tooth per tube was kept at 4°C, not more than 4 hours. The tooth was cleaned with sterilized blade no.15 after eliminating the periodontal ligament and the dental sac, and then the tooth was soaked in 0.2% Chlorhexidine solution and PBS for 10 and 30 seconds, respectively. A cavity was prepared with round diamond burs (no.010) using a high-speed airotor under water spray. The pulp was exposed with a round carbide bur under a slow-speed micromotor. The pulp was irrigated with normal saline twice and dried with cotton piler. The tooth was filled with the pulp capping materials on a random: Biodentine, TheraCal LC according to the manufacturers' instructions, and Polycaprolactone soak with Tideglusib in a concentration of 10nM, 50nM, 100nM, and Polycaprolactone soak with DMEM media. The cavity was sealed with GIC (EQUIA Forte™, GC, Tokyo, Japan). The crown was fixed to a metallic wire with sealant (3M ESPE, St.Pual, MN, USA), and the wire was suspended on the two adjacent plates. The apical root was dipped and untouched the plate's bottom in 2 ml of culture medium. The tooth was cultured in 24-well plates in DMEM supplemented with 10% fetal bovine serum, 200 UI/ml penicillin, 200 µg/ml streptomycin, and 0.5 µg/ml amphotericin B. The medium was daily changed for 14 and 28 days.

At the end of the cultural period, the tooth was rinsed in phosphate-buffered saline and fixed in 4% formalin solution (AppliChem GmbH, Darmstadt, Germany) for 7 days. Teeth enamel was removed with D8 bur and rinsed in phosphate-buffered saline. The tooth was demineralized in 10% formic acid at room temperature, changing the demineralized solution twice a week until fully decalcified for histological process and finally embedded in the paraffin (Tecles et al. 2005).

The histological slide was cut in 5 µm thickness. The slide was deparaffinized with xylene and serial dehydration with ethanol. Ten slides per tooth were stained with hematoxylin and eosin and washed in tap water. The slide was dehydrated with ethanol, cleared with xylene, and mounted with a coverslip. The mineralize foci and the mineralization potential were observed under a microscope.

4. Results and Discussion

Histology of tooth culture model stained with hematoxylin and eosin staining was shown in Figures 1 and 2. Histological change in reaction zone closed to pulp capping material, and the rest of underlying pulp tissue was observed. For the control, a thin reaction zone was found with no inflammation, vascularity, and mineralization nodules (Figure1H, 2I).

The histological specimen on Biodentine in both at 14 and 28 days was demonstrated a reaction zone of amorphous eosinophilic next to the medication material. A non-inflammation pulpal tissue was evident with several nodules resembling mineralization nodules were observed (arrow) (Figure 1E-F, 2E-F). On the medication with TheraCal LC, the interface's reaction zone was absent, with several small mineralization nodules were recognized near the interface (arrow). Besides, increasing vascularity and inflammation were absent (Figure 2G, 2H).

For the medication with Tideglusib, the 10 nM Tideglusib at 14 days, a thin reaction zone of an acellular eosinophilic band of the interface was observed. Mild vascularity near the interface was present (arrow), and no mineralization nodule was noted (Figure 1A, 1B). The 10 nM Tideglusib at 28 days, a focal reaction zone was detected, which neither increasing vascularity nor mineral nodule was present in the pulpal tissue (Figure 2A). The 50 nM Tideglusib at 14 and 28 days, the pulpal tissue showed a small reaction zone, which neither increasing vascularity nor mineralization nodule was detected (Figure 1C,2C). The 100 nM Tideglusib, no reaction zone was found, which neither increasing vascularity nor mineralization nodule was found. Nevertheless, for the Tideglusib, the pulpal tissue was not shown a sign of an inflammatory reaction, and the Odontoblastic layer was found under the material, which the odontoblastic layer was more remarkable at 28 days to 14 days (Figure 1D, 2D)

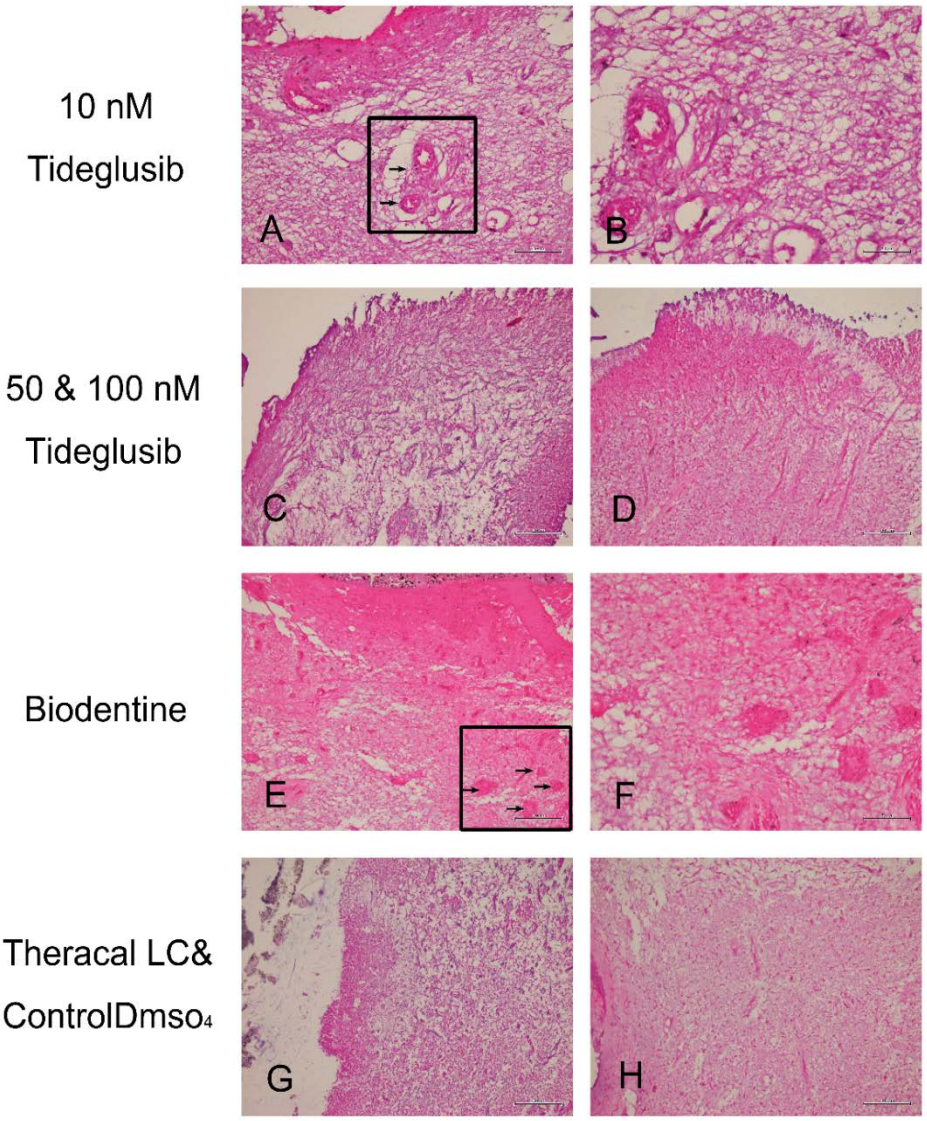

Figure 1 Histology of tooth culture model for 14 days (H&E staining). A: 10nM Tideglusib has a thin reaction zone of the acellular eosinophilic band and increases vascularity (Arrow), B: A vessel was promoted when applying 10nM Tideglusib at a higher magnification, C: 50nM Tideglusib; the pulpal tissue showed a small reaction zone at the interface, D: 100nM Tideglusib; no reaction zone was found at the interface, E: Biodentine; the reaction zone of amorphous eosinophilic, several nodules resembling mineralization nodules (Arrow), F: mineralization nodules were observed in the pulp tissue at a higher magnification, G: TheraCal LC; there was no the reaction zone at the interface, and H: Control $DMSO_4$; a very thin reaction zone was found. Vital pulp tissue with no inflammation was seen under the materials. Scale bars: A, C-E, G-H = 100 µm, B and F = 50 µm

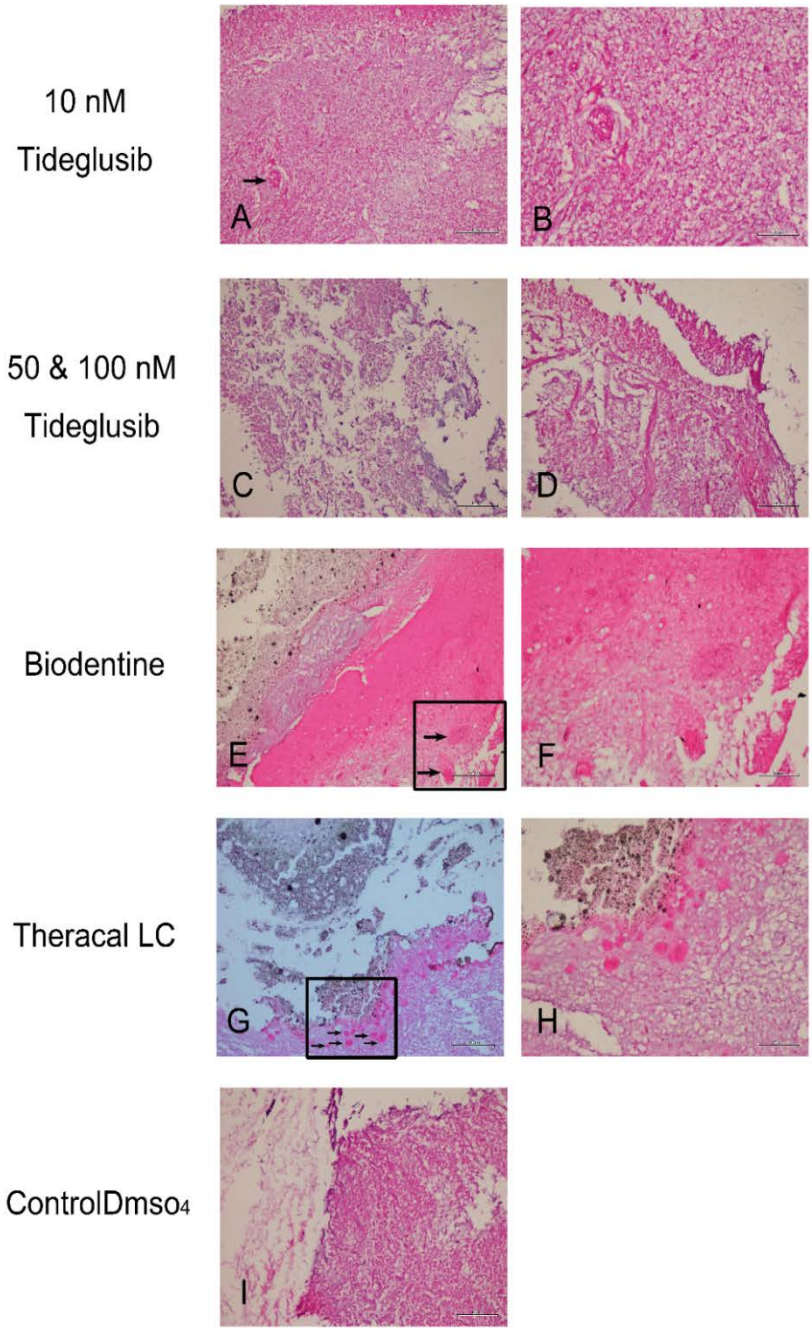

Figure 2 Histology of tooth culture model for 28 days (H&E staining). A: 10nM Tideglusib; Slightly increased vascularity (Arrow) was observed, however, there is no mineral nodule presented, B: 10nM Tideglusib; mild vascularity was increased, C: 50nM Tideglusib; small reaction zone was found at the interface, D: 100nM Tideglusib; there was no reaction zone at the interface, E: Biodentine; The inflammation pulpal tissue was not found, while several nodules resembling mineralization were observed (arrow), F: Biodentine; mineralization nodules were shown near the reaction zone at a higher magnification, G: TheraCal LC; several small mineralization nodules were recognized near the interface (arrow), H: TheraCal LC; mineralization nodules were presented near the reaction zone at a higher magnification, and I: Control $DMSO_4$; vital pulp tissue with no inflammation was seen under the materials. Scale bars: A, C-E, G, and I = 100 µm, B, F, and H =50µm

Table 1 Pulp capping composition used in this study

Material	Company	Composition
Tideglusib	Sigma-Aldrich, St Louis, MO, USA	4-Benzyl-2-(naphthalen-1-yl)-1,2,4-thiadiazolidine-3,5-dione
Biodentine	Septodont, Lancaster, PA, USA	Tricalcium silicate, dicalcium silicate, calcium carbonate, and oxide filler, iron oxide shade, and zirconium oxide
TheraCal LC	Bisco Dental, Schaumburg, IL, USA	45% Type III Portland cement, 10% Radiopaque content, 5% hydrophilic thickening agent (fumed silica), 45% resin (Urethane Dimethacrylate (UDMA), isphenol A-Glycidyl Methacrylate (BisGMA), riethylene Glycol Dimethacrylate (TEGDMA), Hydroxyethyl methacrylate (HEMA), Polyethylene glycol dimethacrylate (PEGDMA))

Discussion

The tooth culture model showed the reaction between pulp capping material and the pulp cell for up to 28 days. The mineralization nodule was observed in Biodentine at 14 and 28 days, while the mineralization nodule was observed in TheraCal LC at 28 days underneath the pulp (Laurent et al. 2012). The results showed the difference in pulpal morphology and mineralization on the tooth culture model to the different concentrations of Tideglusib (10, 50, and 100 nM). These concentrations of Tideglusib were related to the previous study, in which the Tideglusib in 50 nM concentration was suitable for promoting the mineralization nodule in the mouse (Neves et al., 2017).

For Tideglusib, blood vessels were presented next to the interface, however, the mineralization nodule was not detected in histopathological features. Tideglusib at 10 nM concentration condition showed a better biocompatible to dental pulp cell and represented the sign of healing than other concentrations (50 and 100nM). The previous study on Tideglusib shown a 50 nM Tideglusib had shown a good result in promoting reparative dentin in the mouse model in 6 weeks (Neves et al. 2017). However, in the present study, the mineralization signal was invisible, which could be different in the testing method between in-vivo and ex-vivo studies. In this ex-vivo study, the human tooth culture model lacked blood circulation, the immune system, and the source of mineralization. On the other hand, Biodentine and TheraCal LC can release calcium ions to the dental pulp cells (Camilleri et al., 2014).

Therefore, the tooth culture model can be used to represent an early stage of dentin regeneration. However, it has shown a limitation, in which the tooth culture model can be used only for a short period within 28 days (Pedano et al., 2019). Besides, a dentin bridge is generally formed in 3 months (Iwamoto et al. 2006). The tooth culture model was lack of blood circulation resulting in lacking CO_2 and O_2 exchange, which could be stressful to the dental pulp cell in a low pH environment. In summary, Tideglusib can promote pulp cell healing, which the new blood vessel was found in the pulp tissue. However, further study on dentin regeneration needs to be investigated in vivo study.

5. Conclusion

The tooth culture model was a valuable technique for biocompatibility tests in dental pulp cells. Biodentine and TheraCal LC showed a promising mineralize potential. Tideglusib was biocompatible and promote inflammatory healing to pulp cells, however, the mineralized potential was absent.

6. Acknowledgements

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