ImmunoTools *special* Award 2015

Sonntana Seang, Ph.D student

Advisor: Chalida Nakalekha Limjeerajarus, DDS., Ph.D.
Co-advisor: Professor Prasit Pavasant, DDS., Ph.D.

Oral Biology Program, Research Unit of Mineral Tissue, Faculty of Dentistry, Chulalongkorn University, Bangkok 10330, Thailand

The Regeneration and Revascularization of Dental Pulp Tissue in 3D Organ Culture System

Tooth is a vital organ for mastication, it also contributes to the body overall function. The dental pulp resides in a highly calcified chamber, which provides strong protection to the delicate pulp tissue. Dental pulp bears the source of nutritious support to the tooth and serves as sensory organ responding to noxious pathogenic stimuli and eventually leads to the production of tertiary dentin. Therefore, pulp vitality is crucial to the homeostasis of dental tissues and surrounding structure.

Dental caries is known to be one of the most prevalent chronic diseases of the population worldwide. Traditionally, the restoration of carious tooth depends on restorative materials. Unfortunately, none of them share the same characteristics to the natural tooth. In many cases where the carious lesion is deep and the pulp is irreversibly inflamed or non-vital, the conventional endodontic therapy is indicated. Despite the satisfactory result following the endodontic treatment in most cases (in terms of tooth's life span), there are significant drawbacks. For example, the endodontic treatment causes the large loss amount of dentin, which contributes to the weakening of the tooth structure. The tooth is therefore more susceptible to fracture. To overcome these drawbacks of conventional root canal therapy, pulp/dentin engineering has become an important area of research in recent years. It has the potential to enhance the success of operative and endodontic therapy, as it provides an opportunity to employ growth factors to enhance the vascularization of the tooth and promote the pulpal healing process [1,2].

Recent findings from our lab (*Limjeerajarus et al., 2014)* have demonstrated that the administration of iloprost, a prostacyclin (PGI_2) analogue, leads to the up-regulation of vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF)-2, and platelet-derived growth factor (PDGF) mRNA expression *in vitro* and increased pulpal blood flow *in vivo* [3]. In addition, we found that iloprost could stimulate the odontogenic and osteogenic response in human dental pulp stem cells (hDPSCs) by up-regulation of DMP-1, OSX, RUNX-2, ALP, Col1, ALP, and BMP-4. Moreover, iloprost's induction potency affected the formation of tertiary dentin *in vivo* [4]. These results corresponded to our previous finding that iloprost up-regulated mRNA expression of FGF-2. This suggested that iloprost is associated with tooth morphogenesis by contributing to the mineralization of the dentin matrix.

For pulp/dentin tissue engineering, vascularization, growth-factor incorporation, and mineralization are the crucial keys to the success. Our previous study design aimed to increase the effectiveness of drug and growth factor delivery to the pulp. More recently, we have been seeking methods of improving the controlled-release of better growth factors and system of targeting delivery. In this study, we aim to investigate the effect of several candidates of growth factors on pulp revascularization, using tooth slice 3D organ culture. In addition, we will also examine the angio-/odonto-/osteogenic response of hDPSCs following the growth-factors treatment, using scaffold cooperates for prolong controlled-release system. The results from this study will be a significant step towards the long-term goal of vital tooth therapy and highlighting the clinical usefulness of growth factors in pulp/dentin regeneration. Ultimately, this 3D organ culture system may be adapted to bone and whole tooth regeneration in the future.

With kindly support from ImmunoTools, the series of our study will be carried out with the request of the reagent as followed:

(i) Primary cultures of hDPSCs will be selected by immunoreativity in the negative expression of CD45, CD34, and CD14.

(ii) To investigate the effect of each induction agent, DPSCs will be treated with the chosen growth factors from ImmunoTools as listed in the request below. After each treatment, angio-/odonto-/osteogenic gene expression will be analyzed and used to compare all groups to each other. CD31, CD34, and CD105 will be used for immunocytochemistry to confirm the differentiation of DPSCs to endothelial cells (EC).

(iii) To challenge the results from our previous findings, recombinant human cytokines will be used as the induction agent in comparison with the effect of iloprost on angio-/odont-/osteogenic gene expression level. Additionally, cytokines production will be essayed using ELISA to detect the present of IL-4, which is known as mineralizing and collagen-stimulating factor.

(iv) In 3D organ culture, tooth slices of 2 mm thickness will be obtained and cultured in the present of the selected growth factors. After each experimental time point, paraffin sections will be obtained from the tooth slice. CD31, CD34, and CD105 will be used for immunohistochemistry against EC.

References:

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3. Limjeerajarus, C. N., Osathanon, T., Manokawinchoke, J., & Pavasant, P. (2014). Iloprost Up-regulates Vascular Endothelial Growth Factor Expression in Human Dental Pulp Cells In Vitro and Enhances Pulpal Blood Flow In Vivo. *Journal of Endodontics*.
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