# Biological influence of nanofibrous hydroxyapatite-polycaprolactone membrane on human periodontal ligament cell

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## ABSTRACT

Biological effect of the hydroxyapatite-coated nanofibrous membrane was mainly investigated on the proliferation and differentiation of human periodontal ligament fibroblast. SEM revealed favorable cell attachment and spreading appearance on 1 day and MTS assay showed increased cell proliferation on the hydroxyapatite-coated nanofiber membrane during 14 days. From 7 to 14 days, alkaline phosphatase activity of the hydroxyapatite-coated nanofiber was significantly increased when compared to that of control group. Hydroxyapatite-coated nanofibrous membrane showed prominent mineral formation on day 14. This study revealed that the developed hydroxyapatite-coated nanofibrous membrane had favorable effects on the proliferation and differentiation of human periodontal ligament fibroblast and might be a good candidate material for periodontal tissue regeneration.

***Keywords***: hydroxyapatite, membrane, periodontal ligament fibroblast, periodontal tissue regeneration

## 1 BACKGROUNDS

Ultimate goal of periodontal therapy is to regenerate periodontal tissues. The concept is based on preventing the apical downgrowth of the gingival epithelium into a bony defect and creating a secluded space that can be repopulated by regenerative potential cells, such as periodontal ligament (PDL) fibroblasts [1]. Early developed membrane for this purpose was mainly non-resorbable, and required a second surgery to be removed. Therefore, resorbable membranes have been preferred and suggested as effective for GTR treatments.

Although the histological and clinical outcomes of GTR have been well documented, the biological effects of membranes remain a question yet [2]. Few studies have been conducted, such as cell proliferation and differentiation, by which membranes influence the cells adjacent to the periodontium. Polycaprolactone (PCL) has been reported as a comparable substrate suitable for supporting cell growth resulting from two-dimensional bone-marrow stromal cell culture [3]. And preliminary study on hydroxyapatite (HA) included in PCL scaffolds demonstrated that the presence of HA on PCL substrates enhanced osteoblast function and growth [4].

Considering the biological effects of osteoblastic cells on PCL or HA, we hereby study the biological effect of PDL cells on HA-coated PCL.

## 2 MATERIALS

HA-coated PCL (PHA) membrane were fabricated and loaded onto cell culture plates as test groups. The polystyrene multi-well culture dish (CD) surface, without membrane, served as a control group. Human PDL fibroblasts were seeded on each group and cultured for 14 days. The morphology of cell attachment was observed by scanning electron microscopy (SEM) at 1 and 7 days. MTS assay was performed for the cell proliferation analysis at 1, 4, 7 and 14 days. Cell differentiation was determined by alkaline phosphatase (ALP) activity analysis at 7 and 14 days. Mineralization deposition was histochemically examined by Alizarin red S staining at 7 and 14 days. Statistical analysis was performed using a Mann-Whitney U test with 95 % confidence interval.

## 3 RESULTS

SEM revealed good human PDL fibroblast attachment on PHA membranes at 1 day, as well as preferable cell spreading on the PHA membrane at 7 days (Fig 1).

MTS assay showed increased human PDL fibroblast proliferation on PHA membranes within 14 days. The PHA membrane presented a significant cell proliferation rate of day 7/day 4 and day 14/day 7 (Fig 2).

From day 7 to day 14, the ALP activity of PHA membrane groups was significantly increased compared to that of the control group (Fig 3).

Mineral formation appeared on PHA membrane at 14 days showed more abundant mineral foci (Fig 4).

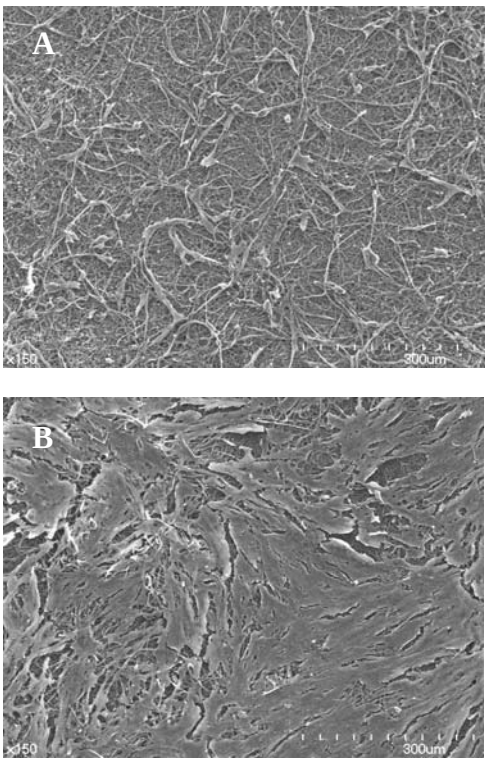


Fig 1. SEM of PDL fibroblast on PHA membrane at day 1 (A) and 7 (B). (150x)

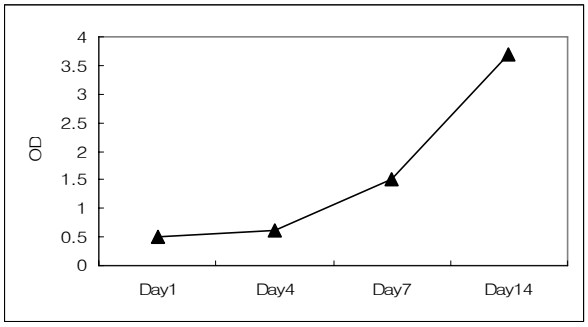


Fig 2. MTS assay showed that PHA membrane ( ▲ ) significantly increase the proliferation rate at the designated time.

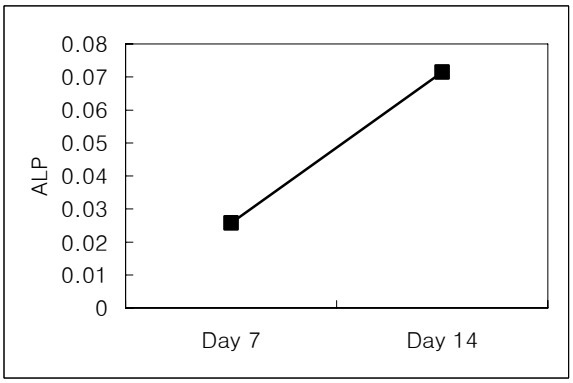


Fig 3. PHA membrane ( ■ ) showed a significant ALP activity rate of day 14/day 7.

Fig 4. Mineralization at day 14 on PHA membrane. Abundant mineral foci observed through Alizarin red S staining (100x).

## 4 SUMMARY

Within the limitation of this study, we can conclude that HA-coated PCL membrane has favorable effects on the proliferation and differentiation of human PDL up to 14 days.

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