

# Microporous Alendronate-Conjugated Fibrinogen Scaffolds for Bone Tissue Engineering

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**Statement of Purpose:** Fibrin is a natural polymer known to support wound healing by inducing angiogenesis and promoting cell attachment and proliferation, and thus may provide a more conducive environment for accelerated bone regeneration. However, the fibrin gel was found to impede cell invasion due to the properties of fibrin such as dense, low porosity structure and fast degradation. Alendronate (ALN) is a biophosphonate drug used for several bone diseases. ALN not only induces the osteoblasts to secrete inhibitors of osteoclast-mediated resorption but also stimulates the formation of osteoblast precursors and mineralized nodules, thereby promoting early osteoblastogenesis.

In this study, we have developed an alendronate-conjugated fibrinogen (ALN-FN) scaffolds for bone regeneration. The aims of this study were to generate microporous fibrinogen scaffolds with a uniform pore structure and enhanced mechanical strength, characterize the structural properties of these scaffolds and investigate the ability of these scaffolds to mineralization and osteoblast differentiation *in vitro*.

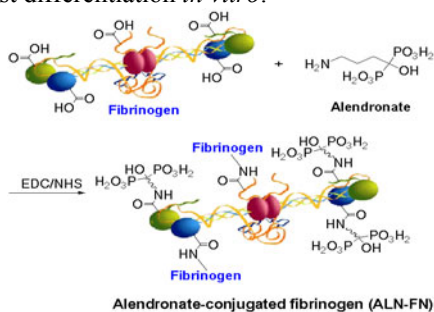


Figure 1. Preparation of ALN-FN scaffolds.

**Methods:** Microporous ALN-FN scaffolds were fabricated by a freeze drying technique. The solution of fibrinogen was homogeneously mixed with difference concentration of ALN (1, 10 and 100  $\mu\text{g}/\text{scaffold}$ ). The ALN-FN mixture was cast into molds and lyophilized for 3 days. Each clot was then soaked in EDC/NHS solution. To remove residual chemical, these clots were washed in ethanol and distilled water several times. All scaffolds were lyophilized for 3 days. ALN-free fibrinogen scaffolds were prepared by same procedure omitting the drug.

The qualitative properties of both scaffolds such as pore size and durability of the scaffold were estimated by SEM analysis and medium immersion test. Cytocompatibility of the fibrinogen scaffolds was evaluated through culturing fibroblasts into the scaffolds *in vitro* for 1day and 7days. The morphology of cells cultured on the scaffolds was evaluated using SEM imaging. Human osteoblasts were cultured either on fibrinogen or ALN-FN scaffolds at 37° C in a humidified atmosphere of 5% CO<sub>2</sub> for 7 and 21 days, respectively. After 21 days cultures

were examined for mineralization by Alizarin Red-S and von Kossa staining and for alkaline phosphatase (ALP) activity by ALP kit.

**Results:** Microporous fibrinogen and ALN-FN scaffolds were successfully prepared and characterized. SEM observation showed that the macro-pore size of the scaffold was about 100  $\mu\text{m}$  with highly inter-connective morphology. The scaffolds maintained original shape after 4 weeks in aqueous medium, suitable for three-dimensional culture of various cells. *In vitro* cell study showed that the fibrinogen-based scaffolds had cell-compatibility and provided a suitable 3-D environment to support the adherence and proliferation of fibroblasts. SEM image depicts the cells are able to adhere to the pore walls and spread (Fig 2). Fig 3. shows that ALN-FN scaffolds promote calcium nodule formation of the osteoblasts in ALN dose-dependent relationship.

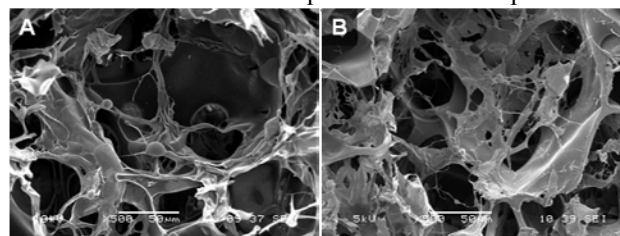


Figure 2. The SEM images of the cultured cells into the scaffolds for 1day (A) and 7days (B).

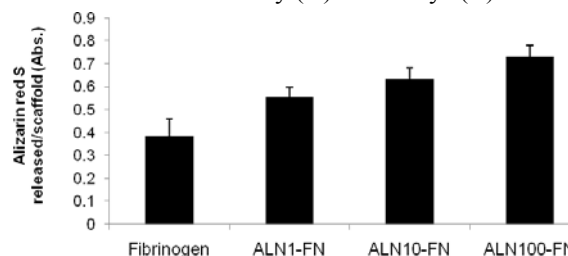


Figure 3. Calcium deposition in the ALN-FN scaffolds with different concentrations of ALN, cultured for 7 days.

**Conclusions:** Microporous ALN-FN scaffolds were prepared and confirmed that they have proper pore size, improved mechanical properties and cytocompatibility for dependable cell delivery vehicle. In addition, the ALN-FN scaffolds promoted calcium nodule formation of the osteoblasts. The favorable properties of these scaffolds make them excellent candidate materials for use in bone tissue engineering.

**References:** 1) Thanaphum Osathanon. *Biomaterials*. 2008;29:4091-4099.

2) Hyon Jong Kim. *J Biol Chem*. 2008;18:10310-10317.

**Acknowledgements:** This work was supported by grants from Nano-biotechnology Project (Regenomics), Ministry of Science & Technology (B020214) and Korea Health 21 R&D Project, Ministry of Health Welfare & Family Affairs, Republic of Korea (A091120).