

# Preparation of cell-specific membranes for bone regeneration by peptide grafting

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## Abstract

In the present work, cell-specific peptides were immobilized on chitosan and PLA scaffolds. The amount of immobilization, determined by HPLC, was confirmed to be in the effective range. Results from cultures of human fetal skin fibroblasts, rat osteosarcoma, and periodontal fibroblasts demonstrated that the immobilization of cell-specific peptides could effectively enhance the attachment and mineralization of osteoblastic cells on scaffolds. The results indicate that, with immobilization of osteoblast-specific peptides, scaffolds with specificity to osteoblastic cells can be obtained which are believed to be of practical use for generation of bone-like tissues.

*Keywords:* Scaffold; Cell-specific; Peptides; Chitosan; PLA

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## 1. Introduction

Tissue engineering, a technique to create new tissue from cultured cells, has now been considered as a potential alternative to organ or tissue transplantation [1]. Biocompatible porous membranes (scaffolds) play an important role

in transforming the cultured cells to a new tissue [2]. It has been shown that modifications of material surface with extracellular matrix (ECM) components can improve material biocompatibility. It is believed that the improvement stems from the presence of certain sequences on the proteins in ECM that could be bound to integrin receptors. In the present work, peptides with specificity to osteoblastic cells were

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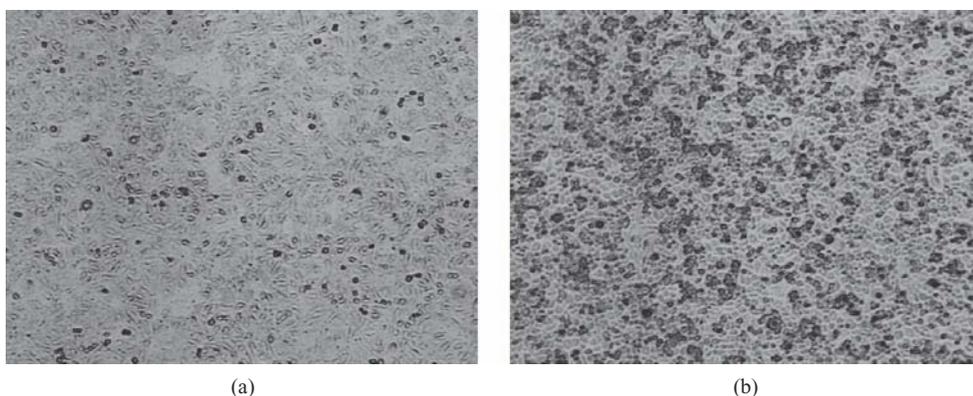


Fig. 1. von Kossa staining for PF cells cultured on the chitosan films grafted with RGDS (a) and with an osteoblastic cell specific peptide (b). The calcium depositions are marked with black color. More black spots indicate more cells being able to form bone-like tissue.

grafted to chitosan and PLA (poly-lactic acid) to prepare cell-specific membranes (scaffolds). The prepared membranes (scaffolds) would have practical applications to bone regeneration.

## 2. Results and discussion

Chitosan porous membranes (scaffolds) were modified with peptides, RGDS(Arg-Gly-Asp-Ser) and an osteoblastic-cell specific peptide, via an amide-bond forming reaction between amino groups on chitosan and carboxyl groups on peptides. Successful immobilization was verified with FTIR spectroscopy, and the immobilized amount was determined with an amino acid analyzer to be on the order of  $10^{-10}$ – $10^{-12}$  mol/cm<sup>2</sup>. Cell culture experiments were performed to evaluate the effect of peptide modification on the material affinity for the cultured cells, and in-vitro mineralization was carried out to investigate the tendency of the cultured cells to form bone-like tissue.

The immobilization of RGDS enhanced the affinity of the chitosan scaffolds for both fibroblasts and osteoblastic cells, evidenced by the observation of more cells on the RGDS-modified

scaffold than on the unmodified scaffold. On the other hand, the immobilization of the osteoblastic cell-specific peptide made chitosan scaffolds have specific affinity for osteoblastic cells, promoting the attachment of ROS (rat osteosarcoma) cells but being ineffective on human fetal skin fibroblasts. For PF (periodontal fibroblast) cells, the graft of the osteoblastic cell-specific peptide also increased the density of the attached cells, although less effectively than the graft of RGDS did. However, the PF cells cultured on the chitosan scaffolds modified with the osteoblastic cell-specific peptide expressed more significant markers in osteoconduction, as shown in Fig. 1. The modification with the osteoblastic cell-specific peptide might induce the attachment of the osteoblastic subgroup in the PF cells or make the non-osteoblastic subgroup in PF cells transform to osteoblastic cells.

The peptides were also grafted on PLA scaffolds with the plasma grafting technique. Similar behaviors to the modified chitosan scaffolds were also observed for the modified PLA scaffolds, further confirming that cell-specific scaffolds can be prepared by grafting the cell-specific peptide to the material.

### 3. Conclusions

The results presented here provide evidence showing that cell-specific scaffolds can be prepared by the graft of cell-specific peptides. With such a technique, we successfully prepared osteoblastic-cell specific chitosan and PLA scaffolds, which would have important applications in bone regeneration.

### References

- [1] R. Langer and J.P. Vacanti, Tissue engineering, *Science*, 260 (1993) 920–926.
- [2] Y. Cao, J.P. Vacanti, K.T. Paige, J. Upton and C.A. Vacanti, Transplantation of chondrocytes utilizing a polymer-cell construct to produce tissue-engineering cartilage in the shape of a human ear, *Plast. Reconstr. Surg.*, 100 (1997) 297–302.