The effects of functionalized self-assembling peptide scaffolds on osteoblast proliferation and differentiation.

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Statement of Purpose:

We previously reported a self-assembling peptide nanofiber scaffold that provides a good support material for cell culture[1]. Recently, the application of a self-assembling peptide nanofiber scaffold, especially RAD16-I (also called PuraMatrix ™) toward bone tissue regeneration was studied and enhancement of osteoblastic differentiation was shown [2]. The vascular regeneration enhancement in vivo was also reported [3]. The self-assembling peptide can be modified with functional peptide motifs from proteins which enhance cell proliferation and differentiation .

We tested the potentiality of two functional motifs, OGP: Osteogenic growth peptide and OPN: Osteopontin cell adhesion domain for bone regeneration. We used the mouse preosteoblast cell line (MC3T3-E1) to compare the proliferation and differentiation effects of the motifs with PuraMatrix and Collagen-I hydrogels as controls. The proliferation was measured by DNA contents and the differentiation effects were evaluated using Alkaline phosphatase (ALP) activity and secreted Osteocalcin.

Methods:

Hydrogels:

1% RAD16-I solution was obtained as PuraMatrix™ (3DM Inc./ BD Bioscience). The functionalized peptides were obtained from Synpep Corporation and dissolved in water at final concentration of 1%(v/w). The functionalized peptide solutions were then mixed with 1% PuraMatrix solution at a ratio of 1:1.

The each peptide solutions was loaded in the Culture plate inserts, (10mm diameter, Millicell-CM, Millipore). The maintenance medium described later was added to induce hydrogel formation.

Collagen I gel from rat tail (BD Bioscience) was loaded in the Culture plate inserts as control.

Cell culture and evaluation:

MC3T3-E1 cells were obtained from ATCC. The cells were maintained by the maintenance medium. Cells were plated at $2x10^4$ cells on the hydrogels in the inserts. The cells were cultured in the maintenance medium Day 0 through Day 2 and then converted into the differentiation media containing L-Ascorbic acid (Sigma) 50 ug/ml and β-glycerophosphate (Sigma) 10mM. The medium was changed every three days. The gel, cell lysis and culture medium were harvested after 14 days of culturing for analysis.

Cell proliferation was evaluated using DNA contents in the hydrogel using PicoGreen dsDNA Kits (Molecular Probes). ALP activity was quantitatively measured from cell lysis using Alkaline Phosphatase Fluorescence Assay Kit (Sigma). Osteocalcin was measured from cell culture medium using Mouse Osteocalcin EIA Kit (Biomedical Technologies Inc.).

Results / Discussion:

Cell numbers from DNA content in the hydrogels show that the functional peptides in combination with PuraMatrix promote greater cell proliferation compared to PuraMatrix alone and Collagen-1 gels (Fig.1)

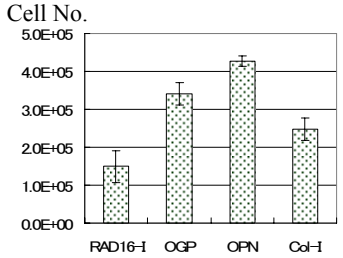

Fig.1 Cell numbers calculated from DNA content in gel

OGP: 1% RAD16-I + 1% RAD16-I-OGP peptide
OPN: 1% RAD16-I + 1% RAD16-I-OPN peptide
Col-1: Collagen-1 gel

Alkaline phosphatase (ALP) activity of the functional peptides (OGP, OPN) is larger or similar compared to controls (RAD16-I, Collagen -I).

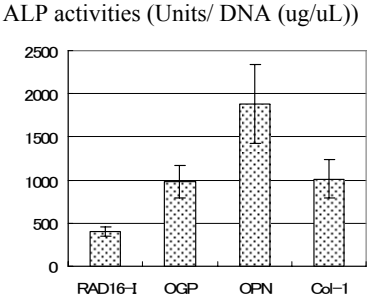

Fig.2 ALP activity normalized by DNA amount

For the results of Osteocalcin, the functionalized peptides (OGP: 3.72 ±0.61, OPN:23.0±9.29 (ng/ml)) had higher concentration compared to controls (RAD16-I: 0.92±0.21, Col-1: 1.91±0.55(ng/ml)).

These results shows that self-assembling peptides functionalized with OGP and OPN have the potential to promote differentiation of osteoblasts.

Conclusions:

We have developed functionalized self-assembling peptide scaffolds with potential to enhance osteoblast proliferation and osteoblastic differentiation. They may be useful as scaffolds for bone tissue regeneration and bone metabolism studies.

References:

[1] Zhang S, Fabrication of novel materials though molecular self-assembly, Nature Biotechnol. 21, 1171-1178 (2003)
[2] Bokhari et al, The enhancement of osteoblast growth and differentiation in vitro on a peptide hydrogel-polyHIPE polymer hybrid material, Biomaterials 26, 5198–5208 (2005)
[3] Davis, et al, Injectable self-assembling peptide nanofibers create intramyocardial microenvironments for endothelial cells. Circulation 111, 442-450 (2005).