ECM-mimic coating of biopolymer scaffolds to promote bone regeneration

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Statement of Purpose: Development of scaffolds with biomimetic surfaces favors cellular responses for the repair and regeneration of tissues including bone. Here we propose a novel approach of tailoring biopolymer scaffold surface using the bone ECM mimic protein, namely, collagen linked with osteocalcin-fibronectin (COF). While the collagen supports the structural network, fibronectin and osetocalcin can trigger cells at initial adhesion and later differentiation stage, respectively. We engineered the COF on biopolymer scaffolds through self-assembled approach. The self-assembled structure of COF and the biological roles to be played were examined.

Methods: The biopolymer scaffolds made of polycaprolactone were produced by robocasting. The morphology of the scaffolds was evaluated by SEM. The collagen degradation was investigated by Sirius red assay. TEM imaging of gold nanoparticles conjugated with osteocalcin antibody visualized the dispersion in collagen. The cell adhesion and proliferation were assessed by MTS assay. SEM and CLSM were employed to analyze cell morphologies. RT-PCR and western blot were used to identify expression of genes and proteins, respectively. Calcium deposition was measured by alizarin red S assay. *In vivo* studies were performed in rat calvarium defect model over a 6 week period. The bone formation was investigated by μ-CT imaging and H&E histological staining.

Results: The collagen networks assembled into fibrillar structure were tethered successfully on the scaffolds while maintaining the porous structure (figure 1). The COF networks were shown to preserve morphological stability over a long period (1 month). The osteocalcin-fibronectin proteins were well-dispersed within the fibrillar collagen networks. The rMSCs cultured on the COF-scaffolds showed significantly higher adhesion levels than those on the bare-scaffolds. The adhered cells reached a confluence rapidly on the COF-scaffolds, indicating a promoted switch to a mature cellular status. The gene and protein expressions of cells were substantially higher on the COF-scaffolds when compared to those on bare-scaffolds, implying the cells secreted a great level of bone-associated ECMs. The mineralization was also enhanced on the COF-scaffolds. The bone forming capacity of the COF-scaffolds was significantly higher than that of bare-scaffolds in terms of quality and quantity of newly-formed bone.

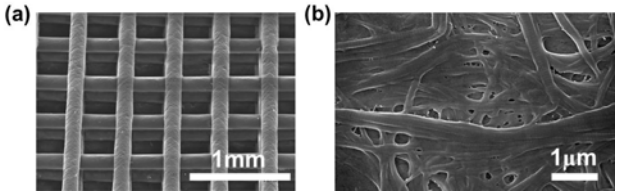

Figure 1. SEM images of the COF-tethered ECM mimic scaffolds at (a) low and (b) high magnification.

Table 1. Expression levels of ALP and BSP proteins in rMSCs after 7 days of culture. Significantly higher levels observed in COF-scaffolds with respect to bare-scaffolds.

	Bare	COF
ALP	1	1.73
OPN	1	3.10

Conclusions: The bone ECM-mimic COF tethering of biopolymer scaffolds was effective in stimulating the initial adhesion and osteogenic differentiation of rMSCs in vitro as well as the consequent bone formation in vivo. The scaffolds are considered to provide promising 3D matrix conditions for bone tissue engineering.

References:

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