Artificial Niche Combining Elastomeric Substrate and Platelets Guides Vascular Differentiation of Bone Marrow Mononuclear Cells

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Introduction
Platelets rich plasma plays an important role in promoting wound healing and tissue regeneration. The elastomeric poly(glycerol sebacate) (PGS) are designed as a vascular biomaterials and proved to increase the compliance and elastin expression of the engineered blood vessels[1]. In this study, we hypothesized that adhered platelets in PGS scaffold would provide a microenvironment which is rich in tissue repairing signals and promote vasculogenesis of bone marrow derived progenitor cells.

Materials and Methods
PGS scaffolds were prepared and coated by platelet poor plasma (PPP), or double coated by PPP and platelet suspension. Rat bone marrow mononuclear cells (BMNCs) were isolated and seeded directly into the scaffolds without Petri dish-culture. All the constructs underwent 21 days’ dynamic incubation in a modified “spinning culturing system”. The cell seeding efficiency, cell proliferation rate and cell morphology were analyzed after certain incubation period. Extracellular matrix formation, cell differentiation and tissue formation were characterized by H&E staining, Masson’s trichrome staining (MTS), immunofluorescent staining for smooth muscle actin (α-SMA), calponin-1, collagen-III and elastin, and western blotting as well as biochemical tests.

Results
Both precoated and uncoated scaffolds maintained good porosity and pore interconnectivity, platelets attached well and were activated in the scaffolds (Fig 1). After 21 days’ incubation, BMNCs showed significant proliferation and tissue formation in PI-P-PGS constructs. Calponin-1and α-SMA expression were observed in large number of cells in the “interstitium” of the constructs, and much more cells with smooth muscle phenotypes were present in PI-P-PGS constructs.

Biochemical assays demonstrated that PI-P-PGS constructs contained significantly higher amounts of insoluble elastin and collagen (Fig. 2). The highest contents of insoluble elastin and collagen were 28.54±4.34 μg per mg construct wet weight and 4.15±0.33μg/mg in PI-P-PGS constructs. These results indicated that platelets significantly enhanced cell proliferation and ECM production.

Discussion and Conclusions
Platelets and the elastomeric substrate[2] provide effective biological and mechanical signals for the proliferation and differentiation of attached BMNCs. The SMC-like cells were positive for calponin-I and SMA; furthermore they synthesized collagen III and elastin.

References

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