Runx2 immobilization on poly (ε-caprolactone) enhances osteoblast differentiation of bone marrow stromal cells *in vitro*

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Statement of Purpose: *In vivo* regenerative gene therapy is a promising approach for bone regeneration and can help to address cell-source limitations through surgical implantation of osteoinductive materials and subsequent recruitment of host-derived cells. Localized viral delivery may reduce the risk of virus dispersion, enhance transduction efficiency, and reduce administration/injection dosing, which subsequently increases patient safety. Here, we present a custom-tailored strategy to immobilize adenovirus expressing runt-related transcription factor 2 (AdRunx2) [1] by using reactive polymer coatings to enhance *in vitro* osteoblast differentiation of bone marrow stromal cells (BMSCs).

Methods: A thin polymer film of poly[*p*-xylylene carboxylic acid pentafluorophenol ester-*co*-*p*-xylylene] equipped with amine-reactive active ester groups was deposited on the surface of poly (ε-caprolactone) (PCL) using the Chemical Vapor Deposition (CVD) polymerization technique [2] and then goat anti-adenovirus antibody was conjugated on the material with an amide chemical bond. Following antibody conjugation, AdRunx2 was conjugated to the PCL surface through antibody-antigen interaction. Next, primary BMSCs were seeded at a density of 2.5×10^4 cells/cm^2 in 12-well plates with AdRunx2 conjugated to the CVD coating at multiplicity of infection (MOI) ranging from 400 to 1000 pfu/cell. Osteoblast differentiation of BMSCs was induced by incubation in osteogenic medium (growth medium supplemented with 50 μg/ml of ascorbic acid and 10mM of β-glycerolphosphate). Alkaline phosphatase (ALP) activity, calcium deposition, and matrix mineralization were confirmed as markers of osteoblast formation at different time points.

Results: To reduce the need of expensive antibodies, we optimized the conditions for antibody conjugation. A minimum 2.5 μg/cm^2 of antibodies can saturate functional groups on the material surface. Seven and a half micrograms per cm^2 of antibodies can bind almost 100% virus. Immobilized AdRunx2 enhanced osteogenesis by bone marrow stromal cells *in vitro*. We investigated ALP activity in the AdRunx2 treatment groups and the AdLacZ control groups using the p-nitrophenol assay during in vitro osteogenic differentiation. Overexpression of Runx2 promoted a 6.5-fold increase in ALP activity as early as seven days post-differentiation when compared to controls (Figure 1a). ALP activity increased most rapidly with 700 MOI AdRunx2 transduction (Figure 1b). However, both AdRunx2 groups showed peak ALP activity at day 10 post-transduction with a similar maximum value (Figure 1a). These results indicate that overexpression of Runx2 enhances early osteoblast differentiation and demonstrate that increased Runx2 immobilization leads to faster stimulation of ALP activity. As a second marker of in vitro osteoblast differentiation and function, we quantified calcium deposition in the extracellular matrix at time points up to 21 days. All data were normalized to DNA content in the same well (Figure 2a). Calcium content increased significantly at day 10 post-transduction and increased with extended incubation time until the end of the experiment. The amount of calcium in Runx2 groups was significantly higher than in the LacZ control group after 10 days. Alizarin Red staining is used for visualization of matrix mineralization and mineralization was observed at day 14 in both Runx2 groups, while, no mineralization was observed in the LacZ group (Figure 2b). Following an incubation time of 21 days, matrix mineralization could be visualized in all groups (Figure 2b). This finding provides additional evidence to demonstrate that immobilized AdRunx2 increases the rate of osteoblast differentiation of bone marrow stromal cells in vitro.

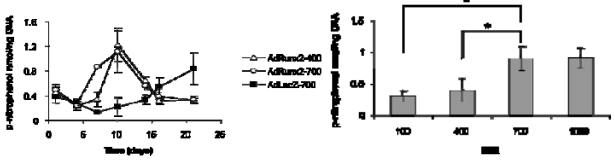

Figure 1. (a) (b)

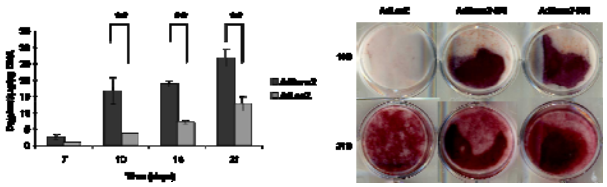

Figure 2. (a) (b)

Conclusions: In this study, we developed a simple and widely applicable strategy to immobilize cell-signaling adenovirus on inert biomaterials using a functionalized CVD coating method. Our application of this strategy demonstrated successful immobilization of AdRunx2 and enhancement of osteoblast differentiation of BMSCs *in vitro*. Our strategy could be widely applied in other systems using different antibody-antigen interactions. Combined with custom-tailored properties [2] of the CVD technique, our method may be used to localize multiple bioactive factors with specific patterns for tissue regeneration.

References:

1. Franceschi RT. Mol Ther 2005;12(2):247-253.
2. Lahann J. Polym International 2006;55(12):1361-1370.