RUNX2-GENETICALLY ENGINEERED STROMAL CELL/POLYMERIC SCAFFOLDS FOR BONE TISSUE ENGINEERING

Benjamin A. Byers (1,2), Robert E. Guldberg (1,2), and Andrés J. García (1,2)

Woodruff School of Mechanical Engineering
Georgia Institute of Technology
Atlanta, GA

Parker Petit Institute of Bioengineering and Bioscience
Georgia Institute of Technology
Atlanta, GA

INTRODUCTION

Bone tissue engineering has emerged a promising strategy to develop bone grafting substrates. However, this approach is limited by inadequate supply of committed osteoprogenitor cells, loss of osteoblastic phenotype expression *in vitro*, and host-construct interactions [1]. To address these cell sourcing limitations, our work focuses on overexpressing of Runx2/Cbfa1 via retroviral gene delivery in target cells for bone tissue engineering applications. Runx2 is an essential transcription factor controlling osteoblast differentiation and bone mineralization [2,3]. We previously demonstrated that overexpression of Runx2 in the MC3T3-E1 immature osteoblast-like cell line upregulates osteoblastic gene and protein expression and 2-D *in vitro* mineralization [4]. In the present study, we examined the effects of exogenous Runx2 expression in bone marrow stromal cells seeded onto 3-D polymeric, biodegradable scaffolds. We demonstrate that sustained Runx2 overexpression in stromal cells enhances osteoblastic phenotype expression and mineralization compared to unmodified cells following *in vitro* and *in vivo* 3-D culture.

MATERIALS AND METHODS

Bone Marrow Stromal Cells

Primary bone marrow stromal cells were harvested from the femora of young adult male Wistar rats in accordance with an IACUC-approved protocol. Passage 1 cells were transduced with Runx2 as previously described [4] or left unmodified (control) and were cultured in α-MEM supplemented with 10% FBS, 1% pen-strep, 3 mM β-glycerophosphate, 50 μg/ml ascorbic acid, and 10 nM dexamethasone. Gene expression was investigated by real-time RT-PCR, alkaline phosphatase (ALP) activity was examined by a biochemical assay, and matrix mineralization was quantified by von Kossa staining.

Stromal Cell-Scaffold Constructs

Runx2-transduced or unmodified stromal cells were trypsinized 1 day post-infection and seeded onto fibronectin-coated Innopol 75/25 PLGA scaffolds (8 mm dia, 5 mm thick, 100-200 micron pore size, 85% porosity) at $4x10^6$ cells/cm^3. Constructs were cultured *in vitro* in α-MEM supplemented with 10% FBS, 1% pen-strep, 3 mM β-glycerophosphate, 50 μg/ml ascorbic acid, and 10 nM dexamethasone. Histological analysis was performed to determine cellular distribution throughout the scaffolds and micro-CT was used to quantify mineralized matrix deposition following 3, 4, 6, and 8 weeks in culture. Additionally, Runx2-modified and unmodified cell-seeded constructs were subcutaneously implanted into syngeneic rats for 8 weeks to evaluate the capacity of Runx2-expressing cells to direct ectopic bone formation in a stringent *in vivo* model. Furthermore, to examine the effect of *in vitro* culture on *in vivo* mineralization, constructs were pre-cultured for 1, 7, or 21 days prior to implantation.

RESULTS

Runx2 Enhances Stromal Cell 2-D In Vitro Mineralization

Infection efficiencies (>50%) were observed in stromal cell transductions by flow cytometric detection of an eGFP co-selectable marker. Quantitative PCR (Figure 1) of 2-D cultures revealed significant upregulation in Runx2 (10-fold) and OCN (5 to 10-fold) gene expression in Runx2-infected cultures compared to controls ($p<0.001$). ALP activity was upregulated two-fold in Runx2-infected cultures compared to controls at 7 days ($p<0.005$). Runx2-expressing stromal cell cultures demonstrated upregulated mineralized area (Figure 1) at 14 (2-fold) and 21 (1.5-fold) days compared to matched controls ($p<0.001$).

Runx2 Enhances Construct 3-D In Vitro and In Vivo Mineralization

Based on 2-D results, we expected that the increased surface area provided by 3-D scaffolds would enhance the up-regulated mineralization capacity of Runx2-expressing stromal cells. Micro-CT evaluation revealed higher levels of mineralization at 3 (25-fold), 4 (2-fold), and 6 and 8 (1.5-fold) weeks in culture for scaffolds seeded with Runx2-expressing cells compared to control cells ($p<0.00001$) (Figure 2). Histological and micro-CT analyses confirmed that cellular

distribution and mineralization were confined to the construct periphery by 21 days.

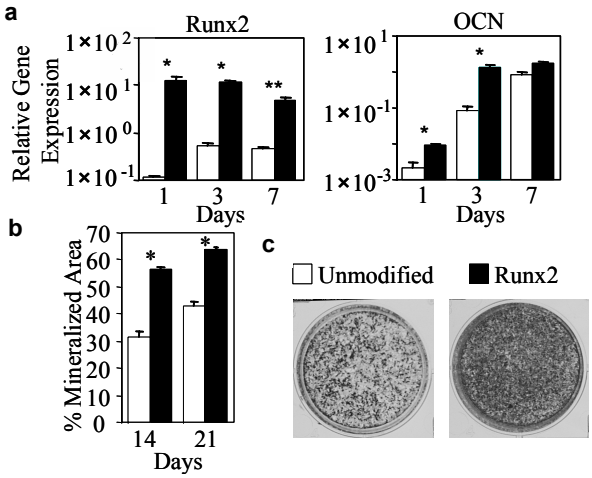

Figure 1. Gene expression and mineralization in 2-D cultures. (a) Quantitative PCR for Runx2 and OCN gene expression in Runx2- and unmodified cells at 1, 3, and 7 days. (b) Mineralized area from Runx2 and unmodified cells at 14 and 21 days. (c) von Kossa stained unmodified and Runx2-infected cultures at 21 days.

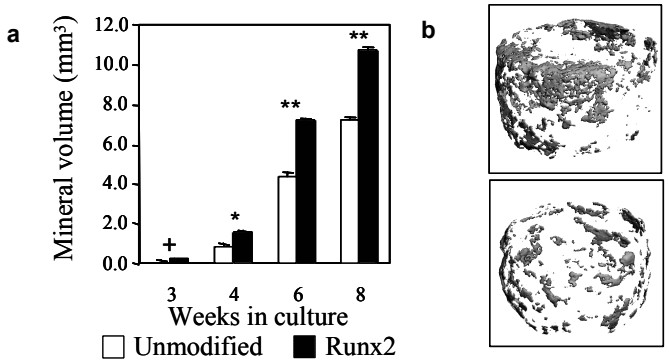

Figure 2. Mineral formation in constructs cultured in vitro. (a) Micro-CT analysis (* vs. control, p < 0.005; ** vs. control, p < 0.0001). (b) Representative images of Runx2-transduced (top) and control (bottom) constructs at 4 weeks.

Micro-CT analysis revealed little (< 0.1 mm^3 mineral volume) in vivo mineralization for constructs containing either Runx2-modified or unmodified cells that were pre-cultured in vitro for 1 and 7 days. In contrast, Runx2-cells/scaffolds which were cultured in vitro for 21 days prior to implantation exhibited significant levels of in vivo mineralization and these levels were 50-fold higher than those observed in scaffolds carrying unmodified cells (Figure 3).

DISCUSSION

Stromal cells engineered to overexpress the osteoblastic transcription factor Runx2 exhibited enhanced *in vitro* and *in vivo* osteoblastic differentiation and matrix mineralization when compared to unmodified stromal cells cultured on 3-D polymeric scaffolds. In addition, the strong dependence of *in vivo* mineralization on *in vitro* construct development suggests that this parameter may be critical in engineering constructs for bone repair. We expect the use of dynamic culture conditions or a more macroporous scaffold will maintain greater differences between treatments at later time points by supporting cellular growth and differentiation throughout the interstitial regions of 3-D constructs. Current work focuses on further evaluation of 3-D culture characteristics of Runx2-modified cells, including osteoblast-specific gene expression and immunohistochemistry. Primary stromal cells overexpressing Runx2 represent a potential alternative to address the clinical need for an osteogenic cell source in the development of tissue-engineered constructs for treatment of damaged or diseased bone.

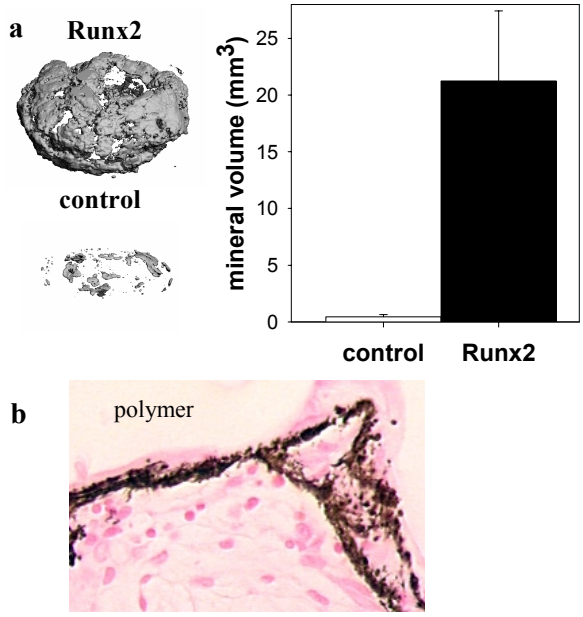

Figure 3. Runx2 enhances mineral formation in constructs implanted subcutaneously for 8 weeks. (a) Micro-CT analysis showing 50-fold enhancement in Runx2 vs. control groups (p < 0.003); (b) von Kossa/nuclear red staining showing mineral deposition (black deposits) associated with cells on the scaffold surface.

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