# Crosstalk between Integrin-β1 and BMPR1A mediate matrix regulated MSC osteogenesis

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**Key words: polyurethane; osteoblast differentiation; mechanical moduli; integrin-growth factor cross-talk**

**Statement of Purpose:** In order to design cell carriers for the application of cell therapy in tissue regeneration, there is a compelling need to understand the mechanisms by which regenerative stem cells sense and respond to the physical properties. It is well-known that osteogenic differentiation of mesenchymal stem cells (MSCs) is regulated by the modulus of the ECM [1] through integrin-mediated signaling [2], yet the mechanism remains unclear. Hydrogels have been extensively investigated as carriers for stem cells, and matrix rigidity has been shown to regulate stem cell differentiation for elastic moduli up to 1 MPa[3]. However, the effects of matrix rigidity on osteogenic differentiation in scaffolds with moduli exceeding 1 MPa, which is representative of the mineralized extracellular matrix in bone, has been investigated in only a limited number of studies. Polyurethane scaffolds, which are biodegradable, and biocompatible, have been reported to support the migration of cells and ingrowth of new tissue *in vitro* and as well as in bone models, with non-toxic degradation product [4]. Moreover, the mechanical properties can be modified by changing the structures of hard and soft segments, which can be easily achieved by controlling the chain length of polyol and isocyanate in the reaction [5].

**Method:** In this study, we have synthesized PUR films from polyester triol, iron acetylacetonate catalyst, and hexamethylene diisocyanate trimer (HDIt) to ensure the same surface chemistry. Different chain lengths of polyester triol (e.g., from 300 Da to 3000 Da) were used to regulate the elastic moduli of the synthesized polyurethanes. Longer chain (higher molecular weight) of polyester triol yielded scaffolds with lower mechanical moduli (noted as "compliant"), while lower molecular weight polyesters yielded "rigid" scaffolds. MSC osteogenic differentiation was then studied on the PUR substrates *in vitro*. BMSCs from rats were utilized in this study, and total protein from cell lysate was used as representative of cell proliferation in this study. Real-time PCR of bone differentiation markers including ALP, collagen I and Runx2 were also measured to further compare the differentiation level MSCs on polyurethane materials. Immunoprecipitation (IP) and FRET were utilized to study the crosstalk between integrin subunits and BMP receptors. Alizarin red S staining was applied after 21 days of osteoinduction for mineralization comparison.

**Results:** The elastic moduli of the PUR substrates ranged from 20 to 3800 MPa. MSCs plated on PUR substrates and cultured in complete medium showed increasing *integrin β1* (*Iβ1*) expression with increasing substrate modulus in a dose-responsive manner (Fig. A). Total protein from harvested cell lysates indicated that MSCs attached to and proliferated on the PUR substrates for up to 3 weeks. With osteoblast induction for 3 days, expression of transcriptional factor *Runx2* by MSCs also increased with substrate modulus (Fig. B). Therefore, the association between Iβ1 and BMP receptor IA (BMPRIA) were analyzed by IP and FRET. Cell lysates were immunoprecipitated with BMP receptor antibodies and blot with integrin antibodies. Complexation between Iβ1and BMPRIA was observed and it was up-regulated on the rigid PUR. FRET between the two receptors showed a similar trend as IP (Fig. C). Alizarin Red S staining of MSCs 21 days after osteoinduction showed increased mineralization on rigid PUR substrates (Fig. D).

**Conclusions:** PUR substrates with tunable mechanical properties (20 to 3800 MPa) were investigated as a potential scaffold for bone regeneration. Osteogenic differentiation of BMSCs increased with PUR elastic modulus due to association of Iβ1 and BMPRIA. Future work will investigate the effects of 3D scaffold mechanical and topological properties on osteogenic differentiation, as well as the application of this tunable polymer carrier in bone regeneration.

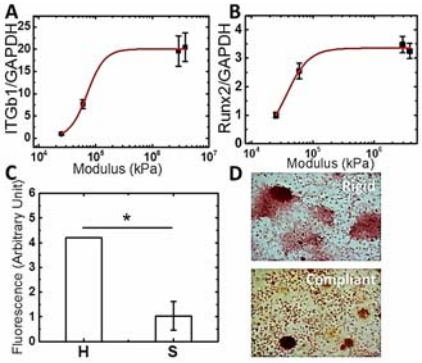


**Fig**. Dose response of integrin-β1 (A) and Runx2 (B) gene expression of cells plated on PUR films of different moduli. (C) FRET readout of integrin-β1 and BMPRIA complex of cells on rigid (H) and compliant (S) PUR films. (D) Mineralization of osteoblast differentiation on rigid and compliant PUR films.

**Acknowledgments:** Funding from and NIH grants AR056138 and CA163499 is acknowledged.

1 MA Wozniak, CS Chen, *Nat Rev Mol Cell Biol.* 2009 January.
2 MA Schwartz, *Cold Spring Harb Perspect Biol* 2010
3 N Huebsch, PR Arany, AS Mao, D Shvartsman, OA Ali, SA . Bencherif, J Rivera-Feliciano, DJ Mooney, Nature Materials, April 2010.
4 B Li, JM Davidson, SA Guelcher; *Biomaterials* 30: 3486-3494, 2009.
5 SA Guelcher, *TISSUE ENGINEERING: Part B Volume 14*, Number 1, (2008).