# Hyaluronic Acid Hydrogels that Emulate the Pro-osteogenic Niche Enhance the Osteogenesis of hMSCs

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**Statement of Purpose:** Cell–cell interactions and formation of tight mesenchymal aggregates is the earliest morphogenetic event associated with the development of several musculoskeletal tissues such as cartilage and bone[1]. N-cadherin is a transmembrane protein considered to be the key factor in directing cell–cell interactions during mesenchymal condensation. Blocking cell–cell adhesion using specific peptides or antibodies against N-cadherin in MSCs or osteoblastic cells leads to reduced osteoblast differentiation and bone nodule formation in vitro and in vivo[2]. Hyaluronic acid (HA) hydrogels prove to be a bioactive material with which mesenchymal stem cells (MSCs) can interact through cell surface receptors including CD44 and CD168[3]. We have shown that HA hydrogels conjugated with N-cadherin mimetic peptide promote chondrogenesis of the encapsulated hMSCs[4]. In this study, we functionalized HA hydrogels with N-cadherin mimetic peptide to evaluate the effect on the osteogenic differentiation of hMSCs. We hypothesize that the conjugation of the N-cadherin peptide helps mimic the pro-osteogenic endosteal niche and will enhance the osteogenesis of hMSCs (Figure 1 D)[5].

**Methods:** Methacrylated HA (MeHA) was synthesized as previously reported[6]. N-cadherin mimetic or scrambled sequence peptides and RGD peptides were conjugated on MeHA chains in alkaline phosphate buffer (pH 8.0) by reacting over night at the mole ratio of 10 (methacrylate):1(peptide thiol) (Figure 1 A). 2D hydrogel substrates were fabricated on the methacrylated glass surface under the UV exposure (5mW 30min). 3D porous hydrogels were formed in PVC mold using DTT as crosslinker and PMMA microspheres as porogen (Ø250μm) (Figure 1). Constructs were cultured in osteogenic media for 12 days or 24 days for in vitro studies (Figure 1 B). Real-time PCR was performed using Taqman primers and probe specific for GAPDH (housekeeping gene) and other genes of interest. Alkaline phosphatase was stained using Fast Blue staining. Statistical comparisons were performed via AVOVA with Tukey's HSD post hoc analyses with $p<0.01$.

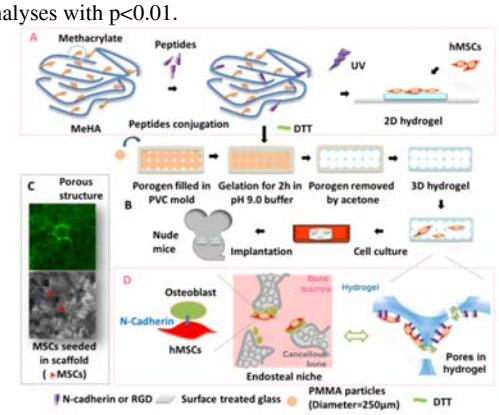


Figure 1.Peptide conjugation and seeding of hMSCs on 2D HA hydrogel substrates (A) and 3D porous HA hydrogels (B) for in vitro culture or subcutaneous implantation in nude mice. (C) Micrographs of the 3D porous hydrogels. (D) Comparison between the endosteal niche and the biomimetic porous hydrogels.

**Results:** After 12 days of osteogenic culture, hMSCs seeded on the 2D hydrogel substrates conjugated with N-cadherin mimetic peptides (Cad+RGD) form significantly larger cell clusters compared to those on the control hydrogel substrates (Scram+RGD, RGD) (Figure 2A). ALP staining shows a significantly higher percentage of stained cells in the Cad+RGD group than that of the control groups (Scram+RGD, RGD) (Figure 2B). The mRNA expression of osteogenic markers is up-regulated in the 3D porous hydrogels conjugated with N-cadherin peptides (Cad+RGD) compared to that in the control hydrogels after 4 days of osteogenic culture (Figure 3). Von Kossa and immunohistochemical staining reveals significantly higher mineralization and type I collagen content, respectively, in the Cad+RGD porous hydrogels compared to the control hydrogels on day 24 of the culture (Figure 4).

**Conclusions:** This study demonstrates that the conjugation of N-cadherin mimetic peptide to HA hydrogels promotes the osteogenesis of the seeded hMSCs. The pro-osteogenic niche recreated by the conjugated N-cadherin peptide likely contributes to the enhanced osteogenesis[5]. Knowledge obtained from this study will help guide the design of HA hydrogels as carrier materials for stem cell-based bone repairs.

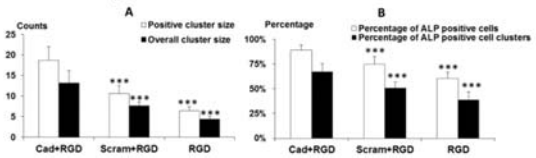


Figure 2.(A) ALP positive and overall cell cluster size and (B) percentage of ALP positive cells cultured on peptide conjugated 2D hydrogel substrates after 12 days of osteogenic culture. ***p<0.001 vs. Cad+RGD.

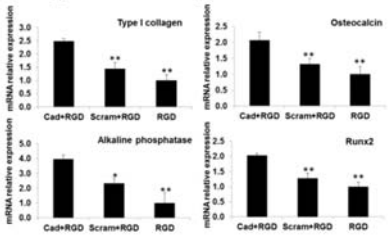


Figure 3.Gene expression of osteogenic markers including type I collagen, osteocalcin, alkaline phosphatase, and Runx2 in hMSCs seeded in 3D porous HA hydrogels after 4 days of osteogenic differentiation. *p value<0.05, **p value<0.01 vs. Cad+RGD (n=4)

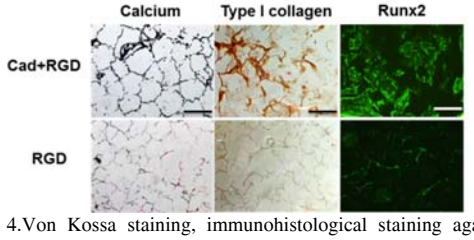


Figure 4.Von Kossa staining, immunohistological staining against type I collagen and Runx2 of the hMSCs-seeded 3D porous HA hydrogels after 24 days of osteogenic culture (Scale bar =500μm).

**Reference:** [1] Gadjanski ll, et al. Stem Cell Rev. 2012 Sep;8(3):63-81. [2] Eric Hay, et al. Journal of cellular physical. 2000, (183):117–128. [3] Pittenger, M. F. Stem Cells. Sci 1999, 284, 143-147. [4] Bian L,et al. PNAS. 2013 June 18,110(20):10117-10122. [5] Mbalaviele, G., et al. Journal of Bone and Mineral Research. [6] Smeds KA, et al. J Biomed Mater Res 2001; 54:15-21.