

## Platelet-Derived Growth Factor-Stimulated Migration of Bone Marrow Mesenchymal Stem Cells into Injectable Gelatin-Hyaluronan Hydrogels

Wanting Niu,<sup>1,2</sup> Ding Weng,<sup>1,2</sup> Abdulmonem Alshihri,<sup>1,3</sup> Teck Chuan Lim,<sup>1,4</sup> Motoichi Kurisawa,<sup>5</sup> Myron Spector<sup>1,2,4</sup>  
<sup>1</sup>VA Boston Healthcare System, Boston, MA; <sup>2</sup>Brigham and Women's Hospital, Harvard Medical School, Boston, MA; <sup>3</sup>Harvard School of Dental Medicine, Boston, MA; <sup>4</sup>Massachusetts Institute of Technology, Cambridge, MA; <sup>5</sup>Institute of Bioengineering and Nanotechnology, Singapore

**Statement of Purpose:** For certain regenerative medicine applications, the reparative potential of endogenous mesenchymal stem cells (MSCs) may be tapped by recruitment of the cells into the injury site. In the case of cavitory defects, this will require a defect-filling matrix that incorporates an MSC chemoattractant and is permissive of MSC migration. Gelatin-hydroxyphenyl propionic acid (Gtn-HPA)<sup>1</sup> and hyaluronic acid-tyramine (HA-Tyr)<sup>2</sup> are injectable liquid formulations of natural biopolymer hydrogels capable of being covalently cross-linked *in vivo*. Gtn-HPA and HA-Tyr have been shown to accommodate neural stem cell migration<sup>3</sup> and MSC chondrogenic differentiation,<sup>2</sup> respectively. The objective of this study was to combine Gtn-HPA, which provides cell adhesion ligands, with HA-Tyr, which offers higher modulus and slower degradation rate and the biological benefits of HA, to form a composite gel for several potential applications including the treatment of defects in the intervertebral disc. Bone marrow-derived MSCs (bMSCs) express tyrosine kinase receptors, including platelet-derived growth factor (PDGF) receptors, providing the rationale for incorporating PDGF-BB into the gel to induce bMSC migration.

**Methods:** Gtn-HPA and HA-Tyr were synthesized as previously described.<sup>1,4</sup> The 3D-migration assay (n=4) comprised 10<sup>5</sup> cells/ml goat bMSCs in a 2mg/ml type I collagen annular, tissue-simulating gel (containing 10 ng/ml FGF-2), surrounding a core gel containing 20ng/ml PDGF-BB (Fig. 1A): 2%(w/v) Gtn-HPA; HA-Tyr; 3:1 Gtn:HA (w/w); 1:1 Gtn:HA; and 1:3 Gtn:HA. Inverted microscope images were taken on days 4 and 7 to quantify the number of cells in the core gel. Unconfined compression tests were performed using a Zwick/Roell Z2.5 static material testing machine. Tests were performed at a constant strain rate of 1%/s to a maximum strain of 10% and 0.1%/s back to zero using a 20 N load cell sampling at a frequency of 2 Hz. Rheology of gels was performed using an AR-G2 rheometer with a geometry of 40 mm diameter and 2° angle at 37 °C The oscillation mode was with the constant strain of 1% and the frequency of 1 Hz. The time point at which the crossover between storage modulus (G') and loss modulus (G'') occurred was marked as the gelation time. The time point at which the G' curve reached its plateau was recorded as the time to completion of gelation.

**Results:** On day 4, cell migration was observed in the 1:3 Gtn:HA gel (Fig. 1B), in contrast to the pure HA-Tyr gel which displayed no cells in the gel (data not shown). Gels with greater % of Gtn—1:1, 3:1, and pure Gtn-HPA gels—were more highly permissive of MSC migration (no significant difference among groups; Fig. 1B). On day 7, the number of cells in the 1:3 gels increased 2-fold

from its 4-day value, while no cells were yet found in the pure HA-Tyr gel. Interestingly, the 3:1 gel recruited significantly more cells than the pure Gtn-HPA gel. Cells could be found at distances greater than 0.5 mm in the 1:3 gel and >1 mm into the gels with greater percentages of Gtn (Fig. 1C). The compressive modulus of the gels generally decreased with the increased percentage of Gtn-HPA (Fig. 2A). The gelation time (and time to completion of gelation) also decreased with the addition of Gtn-HPA, from ~90s (1000s) for the pure HA-Tyr to 20s (100s) for the pure Gtn-HPA gel (Fig. 2B).

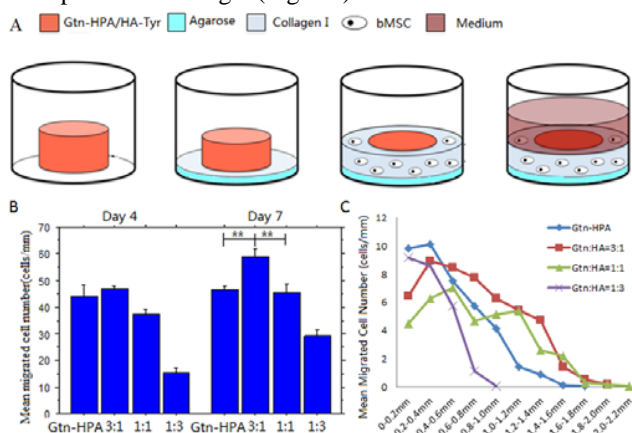


Fig. 1. (A) Annulus-core migration model. (B) Migrated cell numbers/mm of interface in each group. (C) Migration distance on day 7 (\*\*  $P < 0.01$ ).

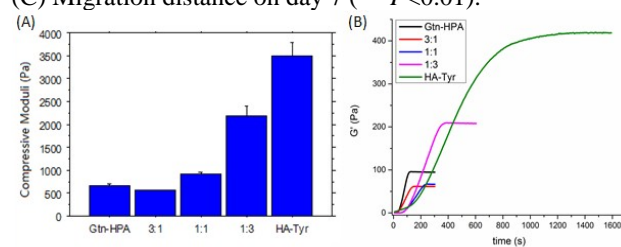


Fig. 2(A) The compressive moduli. (B) Time to gelation.

**Conclusions:** While HA-Tyr holds many benefits as an injectable scaffold for cartilage repair, its inability to accommodate MSC migration is a limitation. Addition of as little as 25% (wt%) of Gtn-HPA to HA-Tyr gels enables bMSC migration. The attendant decrease in modulus and time to gelation may not prove to compromise the performance of the 1:3 injectable gel for indications including the treatment of defects in the IVD.

**References:** (1) Wang LS. *Biomaterials* 2010;31:8608-8616. (2) Toh WS. *Biomaterials* 2012;33:3835-3845 (3) Lim TC. *Biomaterials* 2012;33:3446-3455. (4) Kurisawa M. *Journal of Materials Chemistry* 2010;20:5371-5375

**Acknowledgements:** Dept. of Veterans Affairs and A\*STAR Singapore (TCL).