In Situ Cross-linkable Gelatin Hydrogels for Vasculogenic Delivery of Mesenchymal Stem Cells

Sue Hyun Lee[1], YunKi Lee[3], Pampee Young[2], Ki Dong Park[3], Hak-Joon Sung[1,2].
[1]Vanderbilt University School of Engineering and [2] Medical Center, Nashville, TN, USA, [3]Ajou University, Suwon, S. Korea

Statement of Purpose: Directing robust differentiation of mesenchymal stem cells (MSCs) to endothelial cells for regenerative medicine remains challenging, although not impossible[1]. Gelatin is highly biocompatible, biodegradable, adhesive and non-immuno/antigenic, thus possessing desirable characteristics for tissue engineering. However, its application has been limited due to low melting temperature < 37°C. We recently developed injectable gelatin-based hydrogels by conjugating hydroxyphenyl propionic acid to gelatin (GHPA) that crosslinks *in situ* via a horseradish peroxidase (HRP)-mediated reaction[2]. Interestingly, when encapsulated in GHPA, MSCs began to undergo extensive tubulogenesis and express distinctive endothelial cell markers without biological molecules supplementation in *in vitro* 3D culture and an *in vivo* murine subcutaneous implantation model[2]. Studies to identify and elucidate a mechanism involved in this purely material-driven MSC differentiation to endothelial cells are currently under way. Specific candidates include various integrins, possible interplay with VEGF signaling, as well as their downstream signaling cascades.

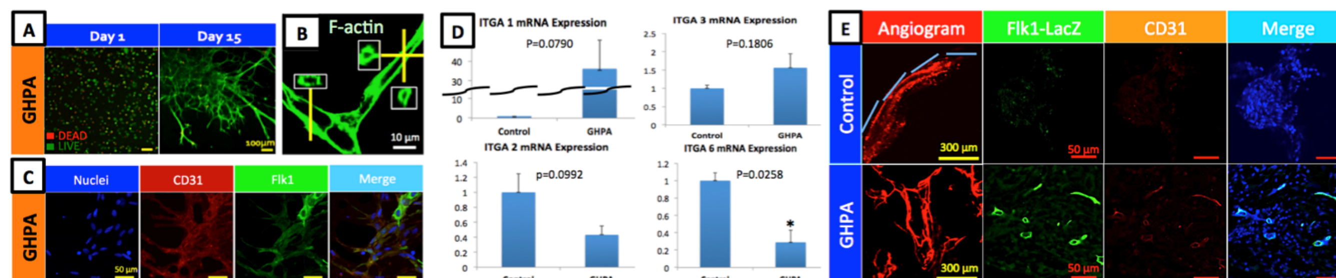
Methods: GHPA was synthesized as reported[2]. Test gel solutions contained 10^6 MSCs/ml (*in vitro*) or $8.3*10^6$ Flk1-LacZ transgenic MSCs/ml (*in vivo*), 5-7% (w/v) GHPA, 0.005-0.01% (w/v) H_2O_2, and 2.5 µg HRP/ml. MSCs encapsulated within the gels were cultured for 15 day for *in vitro* studies, or injected onto polyvinyl alcohol (PVA) sponges, followed by ventral subcutaneous implantation in CL53/Bl6 mice for 2 weeks. PVA sponges allowed for tracking of the injected gel and cells *in vivo*. At the end points, cell viability tests, RT-PCR for gene expression levels, staining (F-actin, β-gal, collagen/GHPA, Flk1/Flk1-LacZ and CD31), and fluorescence microangiography were conducted. For mechanistic studies, additional RT-PCR, western blotting, and molecular inhibitors will be used.

Results: Encapsulated murine MSCs showed over 70% cell viability, compared to tissue culture plate. More importantly, MSCs spontaneously underwent tubulogenesis, forming branched networks over time (**Fig. A and B**). Expression of endothelial markers (e.g., CD31, Flk1, ANGPT 1, Tie2, VE-cadherin, vWF) was significantly up-regulated (data not shown[2]), and MSCs cultured in GHPA stained positive for CD31 and Flk1 (**Fig. C**). Preliminary investigation into integrin-α1/2/3/6 expression in MSCs cultured in GHPA showed significant changes (**Fig. D**). Flk1-LacZ MSCs provided a useful reporter system to confirm the vasculogenic effect of GHPA *in vivo*. When Flk1-LacZ MSCs were grown in GHPA *in vivo* for 2 weeks, expression of endothelial markers (Flk1, CD31, VE-cadherin), and the alternatively activated macrophage marker, MRC1, were up-regulated, while a pro-inflammatory macrophage marker iNOS was down-regulated (data not shown[2]). Angiography revealed that the non-crosslinked control gels had limited vascularization that was localized at the perimeter with only few Flk1-LacZ+ MSCs. In contrast, robust neovasculature was seen throughout the crosslinked GHPA gels, with abundant Flk1-LacZ+ MSCs that incorporated into CD31+ neovasculature, confirming the vasculogenic effect of GHPA on MSCs *in vivo* (**Fig. E**).

Conclusions: The pro-vasculogenic effects of GHPA on MSCs were demonstrated *in vitro* and *in vivo*. In particular, *in vivo* results showed that vasculogenesis was significantly enhanced with crosslinked GHPA gels, suggesting a causative role of the gelatin stability in retention and material-guided endothelial differentiation of delivered MSCs. The results are highly significant as these desirable effects were achieved without addition of any bioactive molecules. The preliminary results indicate a mechanistic role of integrin expression in the vasculogenic effect and necessitate further investigation into potential interplay of integrins with VEGF signaling, and downstream integrin signaling.

References:

1. Oswald J. Stem Cells. 2004; 22(3): 377-384.
2. Lee SH. Advanced Functional Materials. 2014(Accepted).



GHPA directs mesenchymal stem cell differentiation to endothelial cells *in vitro* and *in vivo*. A&B: MSCs embedded 3D in GHPA gels *in vitro* remain viable and undergo tubulogenesis with clear lumen formation as seen in the insets of orthogonal views. **C**: On day 15, MSCs in GHPA gels express endothelial cell markers CD31 and Flk1 *in vitro*. **D**: On day 15, MSCs in GHPA gels exhibits significant changes in the expression of several integrins which mediate cell-material interactions and may direct cell differentiation. MSCs grown on tissue culture plate were used as control. (N=2) **E**: After 2 weeks of subcutaneous implantation, Flk1-LacZ MSC loaded GHPA gel forms extensive perfusable vasculature, retain delivered Flk1-LacZ MSCs that incorporated into newly formed CD31+ vasculature, indicating MSC differentiation into endothelial cells *in vivo*.