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

Sue Hyun Lee[1], Young Wook Chun[1], YunKi Lee[3], Desirae Deskins[2], Pampee Young[2], Ki Dong Park[3], Hak-Joon Sung[1,2].
[1]Vanderbilt University School of Engineering, [2]Vanderbilt University 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 is still considered to be challenging, although it is possible[1]. Gelatin, a hydrolyzed form of collagen, 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]. This process enabled rapid gelation of hydrogels with tunable mechanical strengths, and excellent long-term biocompatibility with murine MSCs when encapsulated in 3D culture. Interestingly, preliminary studies revealed that encapsulated MSCs began to form endothelial-like, tubular networks without addition of any biological molecules *in vitro*. In this study, we aimed to further investigate the vasculogenic effect of GHPA on MSCs *in vitro* and confirm its effect *in vivo* using murine subcutaneous implantation model.

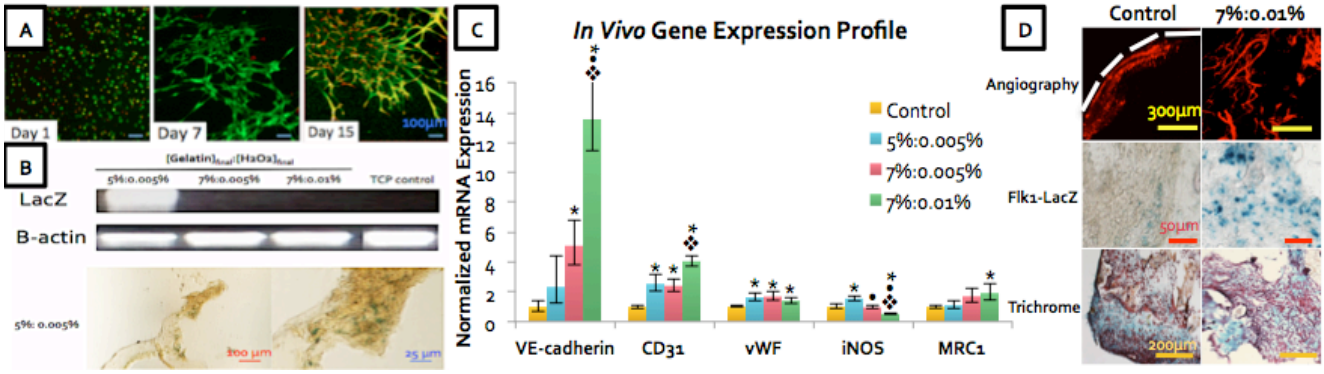
**Methods:** GHPA was synthesized as reported[2]. Test gel solutions contained $10^6$ MSCs/ml (*in vitro*) or $8.3*10^6$ Flk1-LacZ transgenic MSCs/ml (*in vivo*), 5-7% (w/v) GHPA, 0.005-0.01% (w/v) $H_2O_2$, and 2.5 μg HRP/ml. Storage moduli were measured by rheometry. MSCs encapsulated within the gels 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 for *in vivo* studies. PVA sponges allowed for tracking of the injected gel and cells *in vivo*. At the end points, cell viability test, tubulogenesis imaging, qRT-PCR for gene expressions, staining (β-gal, collagen/GHPA, and CD31), and fluorescence microangiography were conducted.

**Results:** The storage modulus could be tuned (0.1~2.5 kPa) by changing GHPA and/or $H_2O_2$ concentrations. Encapsulated murine MSCs culture for 15 days showed over 70% cell viability, compared to tissue culture plate. MSCs attached, spread through the gels, and formed branched networks over time (**Fig. A**). Gene-level expression of several endothelial markers (e.g., CD31, Flk1 and Flt1) was significantly up-regulated (data not shown). Flk1-LacZ MSCs provided a useful reporter system to confirm the vasculogenic effect of GHPA where LacZ+/blue cells are indicative of endothelial differentiation, as Flk1 is an early marker for endothelial cells (*in vitro*; **Fig. B**). When Flk1-LacZ MSCs were delivered with crosslinked GHPA *in vivo*, gene-level expression of endothelial markers and the alternatively-activated macrophage marker, MRC1, were up-regulated; however, iNOS, a pro-inflammatory/classically-activated macrophage marker was down-regulated compared to the non-crosslinked GHPA control (**Fig. C**). The magnitude of such responses correlated positively with the degree of crosslinking in GHPA gels. 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. Lastly, trichrome staining showed the remaining GHPA gels and tissue ingrowth into PVA scaffolds 2 weeks post-implantation (**Fig. D**).

**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 MSC retention and material-guided endothelial differentiation. The results are highly significant as this could be achieved without addition of any bioactive molecules. In summary, its injectability, exemplary biocompatibility, bio-degradability, favorable interaction with host macrophages and pro-vasculogenic effects make GHPA a promising biomaterial for vascular/regenerative applications.

**References:**

1. Oswald J. Stem Cells. 2004; 22(3): 377-384.
2. Park KM. Biomacromolecules. 2012; 13(3): 604-611.




(A) Live/Dead staining of MSCs in 5%:0.005% hydrogels *in vitro*. (B) Flk1-LacZ MSCs expression of LacZ *in vitro* by RT-PCR and beta-gal staining. (C) Gene expression in hydrogel/PVA scaffolds after 2-week implantation with N=4 and error bar = ±1 SEM. *•❖: $p<0.05$ where * is in comparison to control, • to 5%:0.005%, and ❖ to 7%:0.005%. (D) Neovasculature in the scaffolds, Flk1-LacZ expression in delivered MSCs, and trichrome staining are shown.