Magnetic Nanoparticle Drug Delivery System for the Promotion of Vascularization in Tissue Regeneration

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Introduction: Tissue regeneration is most commonly approached by introducing scaffold constructs comprised of natural or synthetic polymers which contain signaling factor(s) and/or cells. A significant challenge with these approaches is that they may not completely regenerate the entire damaged tissue. Inadequate vascularization is one of the most common contributing factors to this problem. The goal of this study is to develop a stimuli-responsive drug delivery system which is stable and inert in vivo, until activated to release a pro-angiogenic drug, vascular endothelial growth factor (VEGF). To accomplish this, we functionalized a poly (acrylic acid) (PAA)-coated iron oxide magnetic nanoparticle (MNP) platform with VEGF using a heat-sensitive linker, 2,2'-Azobis(2,4-dimethylvaleronitrile) (ADVN). The MNPs generate heat only under a specific alternating magnetic field (AMF), which causes release of the attached VEGF.

Materials and Methods: PAA coated $MnFe_2O_4$ MNPs were generated by thermal decomposition of metal-oleate compounds. MNPs (approximately 35 nm core) are then mixed with VEGF and ADVN. The nitrile bonds of ADVN spontaneously react with the VEGF and PAA, and acts as an intermediary linker between the two. The ability of the ADVN to link proteins to the PAA shell was tested initially with a model protein, BSA labeled with Rhodamine B (Figure 1A,B) The release properties of the system were tested by exposure to an alternating magnetic field of a set frequency and power for 20 minutes using a purpose-built magnetic coil system. The resultant supernatant solution was used for quantitative analysis of the concentration of released VEGF using an ELISA kit (R&D Biosystems). The stimulated MNPs were also tested to see how much surface VEGF was left unreleased. The functionality of released VEGF was tested by assessing its effect on the proliferation of HUVEC cells using the Celltiter Glo viability assay (Promega). The next aim was to determine if released VEGF can be delivered from a hydrogel scaffold to nearby cells and remain active. First, particles were added to FBS/VEGF-poor EGM-2 media (Lonza), released, then removed from the media using a magnet. Cells cultured in this media were tested for viability using the same viability assay. Finally, to simulate the *in vivo* delivery system, loaded particles were embedded in a PEG-DA hydrogel, inserted into a Transwell cell culture system, and exposed to a magnetic field to release VEGF into HUVECs. The resultant viability

of the cells was measured to determine if the VEGF was effective. A longitudinal study of gels incubated at 37°C prior to stimulation was also performed to assess the system's stability in *in vitro* conditions.

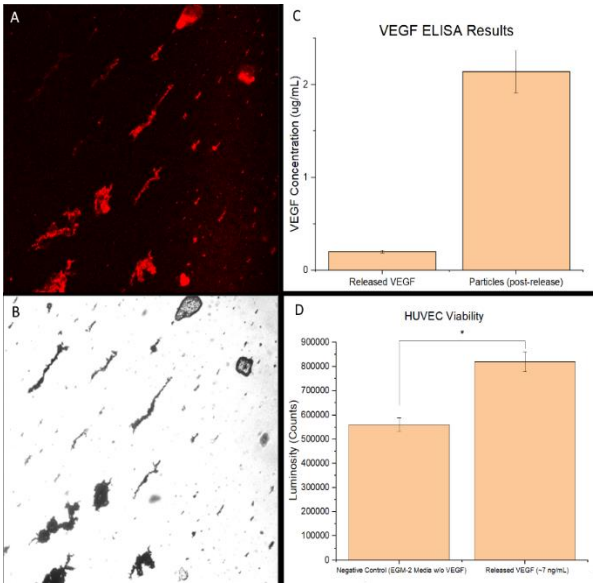

Figure 1. A) Confocal fluorescent image of the MNPs with labeled BSA protein attached. B) Transmission image showing MNPs C) Released VEGF detected using ELISA. D) Bioactivity of VEGF detected using Celltiter Glo test.

Results and Discussion: Approximately 38% (2.3 µg/mL) of the starting 6 µg/mL of VEGF was attached to the MNPs. After a single exposure to the magnetic field, 8.6% (.2 ug/mL) was released. (Figure 1C) The released VEGF maintained its biological activity, increasing cell viability relative to media without VEGF (Figure 1D). Directly releasing VEGF into cell media from particles increased cell viability by 57%. This indicates that VEGF can be attached to this MNP platform and maintains biologic activity after release. Magnetic stimulation of hydrogels containing VEGF loaded particles increased cell viability by 97% relative to unloaded particles. When incubated at 37°C, the effectiveness of VEGF released from gels decreases over time, by up to 76% after 6 days. This indicates that for the delivery system to work effectively *in vivo*, further work will need to be done to develop methods to protect the loaded VEGF. Future studies will also address the function of the system *in vivo*. This system represents a flexible tool which can be applied to a broad range of tissue engineering constructs to enhance their regenerative ability without negative interaction.