# Alginate-graft-Poly(Ethylene Glycol) Microspheres for Intracellular Growth Factor Delivery

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**State of Purpose:** Osteoporosis is a disorder in which mineral density decreases, bones weaken, and risk of fracture increases; one out in two women and one in five men will experience an osteoporosis-related fracture in their lifetime.[1] Intracellular delivery of vascular endothelial growth factor A (VEGFA) has been shown to enhance osteogenesis of mesenchymal stem cells (MSCs), a possible pathway to accelerate mineralization.[2] The overall goal of this study was to formulate a drug delivery vehicle for intracellular transport to primary human MSCs. VEGFA-encapsulating alginate-graft-poly(ethylene glycol) (Alg-g-PEG) microspheres were synthesized and characterized by scanning electron microscopy (SEM), drug release kinetics, cytotoxicity, and differentiation assays. In addition, arginine-glycine-aspartic acid (RGD) was covalently conjugated onto the surface of microspheres to promote cell-material communication.

**Methods:** 2,2'-dithiodipyridine was conjugated onto Amine-Poly(ethylene glycol)-thiol ($NH_2$-PEG-SH, $M_W$=1000g/mol).[3] Alg-g-PEG copolymers were synthesized via carbodiimide chemistry. Alg and Alg-g-PEG solutions were mixed with DyLight 550-labeled VEGFA then formed into microspheres using a water/oil emulsion and calcium chloride. Cysteine-RGD was conjugated on the pyridine end of Alg-g-PEG and covalently conjugated onto the surface of microspheres via a disulfide bond. An Enzyme-Linked Immunosorbent Assay (ELISA) was performed to quantify encapsulation efficiencies and drug release rates of VEGFA encapsulated microspheres; a 14 day release test was performed in PBS at pH=7.4, 37ºC. Microsphere cytotoxicity was determined using a MTT assay after 24h of hMSC culture with microspheres. Confocal laser scanning (CLS) microscopy was used to visualize intracellular transport of VEGFA-encapsulated microspheres into GFP-labeled hMSCs after 24h of culture. Finally, adipogenic and osteogenic differentiation assays were performed; hMSCs were cultured with VEGFA-encapsulated microspheres for 48h in standard medium, followed by 14 days of culture in differentiation media. Adipogensis and osteogenesis were quantified via AdipoRed™ and Alizarin red staining, respectively. Hoechst fluorescent intensity was used to normalize for cell population.

**Results and Discussion:** SEM images confirmed the formation of spherical Alg, Alg-g-PEG and Alg-g-RGD microspheres approximately 1µm in diameter (**Fig.1A,B,C**). hMSC viability was maintained (>75%) up to 500µg/mL for all sample groups (**Fig.1D.**). CLS images of hMSC suggested the microspheres containing VEGFA were endocytosed by the cells, confirming the intracellular delivery of VEGFA (**Fig.2.**). VEGFA encapsulation values were 52, 22 and 35%, respectively. All three groups of microspheres sustained VEGFA release for 2 weeks (**Fig.3.**). The adipogenesis differentiation assay showed no significant differences between microsphere and control groups while the osteogenesis assay showed significant difference ($p<0.04$), suggesting the intracellularly delivered VEGFA guided hMSCs to differentiate into osteoblasts (**Fig.4.**).

**Conclusion:** A novel microsphere-based delivery system has been successfully designed for intracellular delivery of VEGFA to primary hMSCs. The data suggests that additional drugs and cell-targeting ligands may be utilized to control stem cell fate for various applications.

**References:** 1. Clinician's Guide to Prevention and Treatment of Osteoporosis, National Osteoporosis Foundation 2013. 2. Liu *et. al* J Clin Invest 2012. 3. Huang *et. al* Bioconjugate Chem. 1998.

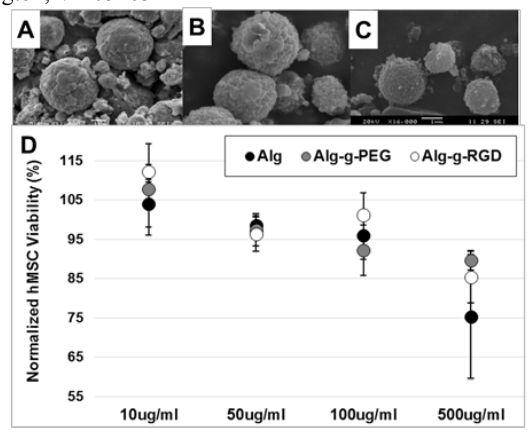


**Fig.1.** SEM images of Alg (A), Alg-g-PEG (B) and Alg-g-RGD (C) microspheres; 16,000x, scale bar=1µm. Effect of microsphere concentration on hMSC viability (D).

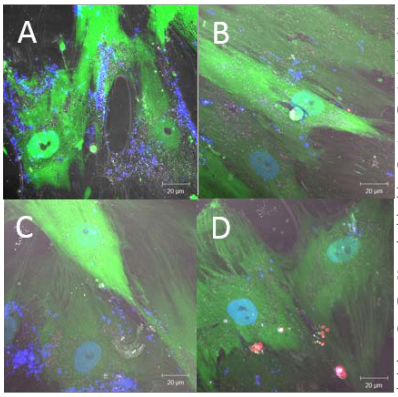


**Fig.2.** Z-stack CLS images of GFP-labeled hMSCs (green) with DyLight 550 labeled-VEGFA-encapsulated microspheres (red); nuclei were stained with Hoechst nuclear stain (blue). (A), (B), (C)&(D) stands for empty cells, Alg, Alg-g-PEG & Alg-g-RGD.

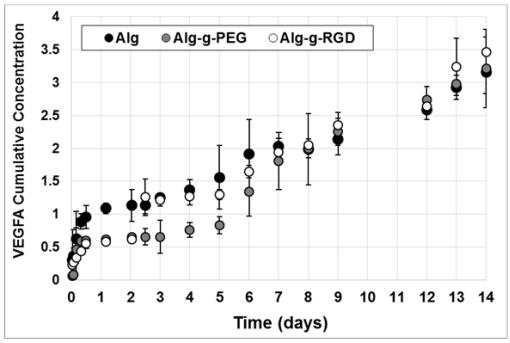


**Fig.3.** Cumulative VEGFA release from microspheres in PBS at pH 7.4, 37°C over a 14 day period.

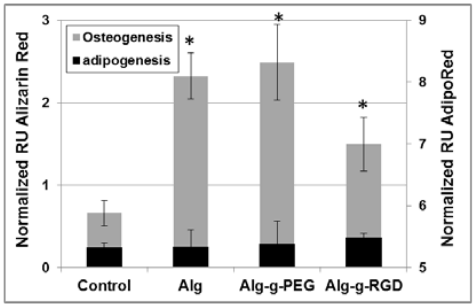


**Fig.4.** Fluorescence reflective units (RU) for differentiation assays (Alizarin red stain for osteogenesis and AdipoRed for adipogensis) (average ± standard deviation, n=3). Values normalized with Hoechst live cell nuclear stain RU.