Development of an Engineered Nanoparticle System to Increase Adipose Stem Cell Survival

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Statement of Purpose: Peripheral artery disease (PAD) is a prevalent and increasing problem in our society that has few clinical solutions.[1] PAD can cause ischemic complications, resulting in tissue death due to the decrease in oxygen and nutrient flow. A regenerative therapeutic strategy could help the damaged tissue restore normal structure and function. Adipose-derived stem cells (ASCs) possess excellent regenerative potential due to abundancy, mesenchymal differentiation and angiogenic induction.[2,3] However, there is high ASC death upon implantation, with the majority of stem cells dying within one week.[4] Therefore, this study focuses on increasing ASC viability to maximize their regenerative effects. To achieve this, two strategies are being employed: the first will deliver the gene encoding heat shock protein (HSP70) to improve the ASC stress response, and the second will incorporate perfluorocarbon nanodroplets (PFCnDs) within the ASC delivery vehicle to increase oxygen availability to cells. Additionally, both strategies will have the capability to act as photoacoustic/ultrasound (PA/US) contrast agents for cell tracking and visualization upon implantation. We expect the combination of these strategies to mitigate ASC apoptosis and enhance healing capabilities.

Methods: The studies utilize human ASCs (PT-5006, Lonza). Gold nanorods (AuNRs) were synthesized and coated with silica (Si). NRs were coated in a layer of polylysine (PLL), giving them a positive charge. Negatively charged HSP70 cDNA was electostatically attached to the PLL-Si coated AuNRs. Our lab group's current delivery vehicle for ASC implantation is a PEGylated fibrin gel. PFCnDs were synthesized with a bovine serum albumin shell and encapsulated wth a dye that absorbs at 1064 nm (Epolight 2057, Epolin). A Vevo 2100 system was used for ultrasound and photoacoustic imaging of the AuNRs and PFCnDs. A trypan blue assay assessed ASC membrane integrity following induced phase transition of the PFCnDs incubated with ASCs.

Results: The optimal size and shape of AuNRS for ASC uptake was previously studied and determined to be 10 nm x 35 nm length with a ~18nm silica coating thickness (Fig 1A). Zeta potential measurements confirmed a stable positive charge of NRs with the addition of PLL coating. Gel retardation studies showed that HSP70 readily attaches to PLL-Si coated AuNRs (data not shown). The AuNRs were incubated with ASCs and imaged by photoacoustic techniques. Results indicated that they behave as efficient contrast agents for photoacoustic imaging at the predicted wavelength (Fig B,C). Future ASC transfection studies will determine the optimized parameters for AuNR gene loading concentrations. The PFCnDs incorporated a 1064nm absorbing dye. Synthesized PFCnDs increased dissolved oxygen in cell media. PFCnDs also show a controlled phase-changing contrast mechanism (Fig D,E). By inducing phase-change of the PFCnDs total release of oxygen within the droplets is expected. Trypan blue assays indicated there is no significant ASC membrane damage as a result of induced phase transition of PFCnDs within the vicinity of ASCs. PFCnDs have been successfully incorporated within PEGylated fibrin gels and show an enhanced stability. Future studies will involve placing ASCs and PFCnDs inside the PEGylated fibrin gel and assessing cell viability and oxygen concentration following phase transition of the PFCnDs.

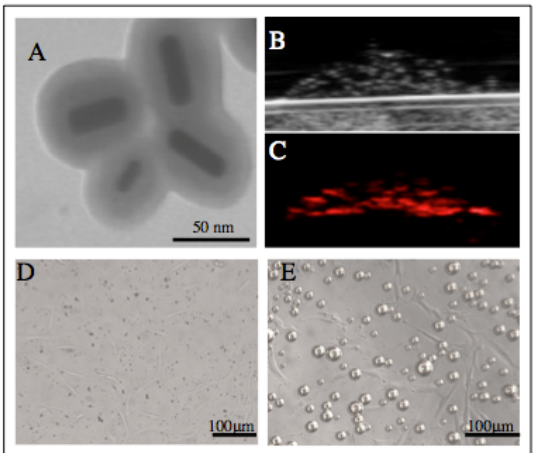

Figure 1. A.TEM image of 2 hr Si coated NRs. **B,C**. US and PA images of ASCs incubated with NRs, respectively. **D,E**. ASCs with PFCnDs before and after phase-transition, respectively.

Conclusions: Optimization of this therapeutic system involving AuNRs and PFCnDs may lead to enhanced levels of ASC survival. Delivering the HSP70 gene to ASCs and increasing the dissolved oxygen within the PEGylated fibrin gel should improve ASC survival. The synthesized NRs are readily taken up by ASCs and act as effective PA imaging contrast agents (Fig 1C). Furthermore, HSP70 has been conjugated to the AuNRs, and transfection studies will determine the optimized AuNR gene loading capacity. PFCnDs have been synthesized and demonstrate the abilty to increase the amount of dissolved oxygen. Inducing phase transition of the PFCnDs has no significant cytotoxic effect on ASCs. Future work will assess the abilty of PFCnDs to enhance oxygen concentration when incorporated within the PEGylated fibrin gel.

References: [1]Moon, M.H., et al. *Cell Phys and Biochem*. 2006, 17: 279-90. [2] S. Natesan, G. Zhang, D.G. Baer, T.J. Walters, R.J. Christy, L.J. Suggs, Tissue Eng Part A, 2011, 17:941-953. [3] R.J.D.a.G.J.D. S. Y. Lim, in: S. Wislet-Gendebien (Ed.) Adv in Reg Med 2011. [4] Boldrin, L. et al. *Tiss Eng.* 13(2), 2007:253-62.