Structural and Functional Characterization of ATP nanoliposomes with potential for accelerating delayed diabetic wound healing and injured spinal cord regeneration

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Delayed wound healing is a major complication of chronic diabetes. Spinal cord injury (SCI) can result in permanent loss of limb function. Our hypothesis has been that ATP being the energy currency of the cell, increasing delivery could enhance cellular processes. We have have been developing ATP encapsulated nanoliposomes for intracellular delivery for a long time to accelerate wound healing in a rabbit ear punch model [1] and recently to regenerate SCI. Investigation of intracellular ATP delivery has resulted in a better understanding of the cellular [2,3,4] and molecular mechanisms [5]. In order to characterize the structure and function of the ATP nanoliposomes several approaches have been developed. To get an idea of the structure, transmission electron microscopy (TEM) with and without negative staining was performed. To evaluate the efficiency of delivery of ATP by ATP nanoliposomes, cells in culture were treated with the ATP nanoliposomes or S–ATP nanoliposomes (control). And the level of ATP compared to control was measured by a NGS Biorad separation system. Also tested was the effect of changes in the composition of biomaterial encapsulating the ATP on the efficiency of delivery and physical stability. Our TEM results have shown that ATP nanoliposomes have a range of sizes and layers surrounding the ATP. Treatment of cells in culture results in a significant increase in intracellular ATP which then causes the upregulation of critical mediators of splicing which then presumably increases the mRNA [5]. In conclusion ATP nanoliposomes have unlimited potential in accelerating healing and regeneration.

References 1: Howard JD, Sarojini H, Wan R, Chien S. Rapid granulation tissue regeneration by intracellular ATP delivery--a comparison with Regranex. **PLoS One.** 2014 Mar 17;9(3):e91787. doi: 10.1371/journal.pone.0091787. eCollection 2: Kotwal GJ, Sarojini H, Chien S. Pivotal role of ATP in macrophages fasttracking wound repair and regeneration. **Wound Repair Regen**. 2015 Sep;23(5):724-7.doi: 10.1111/wrr.12323. Epub 2015 Sep 14. Review. 3: Kotwal GJ, Chien S. Macrophage Differentiation in Normal and Accelerated Wound Healing. **Results Probl Cell Differ**. 2017;62:353-364. doi:10.1007/978-3-319-54090-0_14. Review. Springer NATURE publishers. 4: Sarojini H, Billeter AT, Eichenberger S, Druen D, Barnett R, Gardner SA,Galbraith NJ, Polk HC Jr, Chien S. Rapid tissue regeneration induced by intracellular ATP delivery-A preliminary mechanistic study. **PLoS One.** 2017 Apr5;12(4):e0174899. doi: 10.1371/journal.pone.0174899. eCollection 2017. 5: Kotwal GJ, Martin MD, Chien S. Significant upregulation of U1 and U4 spliceosomal snRNAs by ATP nanoliposomes explains acceleration of wound healing, due to increased pre-mRNA processing to functional mRNA. **Nanomedicine.** 2018 Apr5;14(4):1289-1299. doi: 10.1016/j.nano.2018.03.003.

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