T Ultrasound stimulation of platelet-like particles to promote wound healing *in vitro* and in a rodent laceration model *in vivo.*

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Background, Motivation and Objective

Platelet-like particles (PLP) are highly deformable hydrogels that bind to fibrin and emulate the behavior of platelets promote wound healing by collapsing fibrin networks[1]. However, the kinetics of synthetic platelets are much slower than those of natural platelets (days VS hours). We propose to enhance the kinetics of synthetic platelets by ultrasound stimulation. As previously demonstrated, exposing PLPs embedded in a gelatin tissue-mimicking phantom to ultrasound led to higher deformations in the phantom compared to ultrasound alone[2]. From these results, we hypothesized that ultrasound stimulation enhances the deformations of PLPs, which in turn will enhance the PLP-induced clot retraction and promote healing. We demonstrate here that the combination of PLP and ultrasound stimulation leads to an enhanced polymerization of a fibrin clot in vitro, and study the effect of ultrasound on wound healing in a rodent laceration model.

Statement of Contribution/Methods

Ultralow crosslinked (ULC) poly(N-isopropylacrylamide) (pNIPAM) microgels were synthesized using precipitation polymerization, and conjugated to a fibrin-specific IgG antibody. They were embedded at a concentration of 0.025 mg/ml in fibrin clots in vitro produced with 2 mg/ml fibrinogen and 0.1 units/ml thrombin. Ultrasound stimulation was performed at 1MHz, with 10 μs bursts every 250 μs for 24 hours. The stiffness of the fibrin clots was evaluated using atomic force microscopy (AFM), and their microstructure was evaluated using Cryogenic Scanning Electron Microscopy (CryoSEM), before and after ultrasound stimulation. For the in vivo experiments, dermal wounds were created in the flank of C57BL/6 mice. The wounds were then treated with 0, 0.1, or 1 mg/ml PLP and exposed to no ultrasound treatment, or to 1 MHz ultrasound with 10 μs bursts every 250 μs for 30 minutes every day. 6 animals were used for each configuration (each concentration, with or without ultrasound). Wound closure was visually imaged every day for 9 days. Epidermal thickness and angiogenesis were assessed 9 days post-injury using Hematoxylin and Eosin staining and immunohistochemical labeling for CD31 positively-labeled areas, respectively.

Results/Discussion

CryoSEM analysis showed enhanced density and connectivity of the fibrin clots treated with both PLP and ultrasound, compared to the clots treated with PLPs alone. A significantly higher epidermal thickness was observed in the dermal wounds treated with a combination of ultrasound and PLPs, for concentrations of 0.1 mg/ml and 1 mg/ml, compared to controls and to PLP alone (At 0.1 mg/ml: 63.4 +/- 37.4 um with US VS 42.1 +/- 21.1 um without US. At 1 mg/ml: 71.9 +/- 21.7 um with US VS 42.4 +/- 13.3 um without US). These results suggest that ultrasound can be used to stimulate synthetic platelets to enhance wound healing, even a low concentrations of PLPs.