The Response of Rheumatoid Arthritis Synovial Fibroblasts to Therapeutic Ultrasound in 2D and 3D Culture

Christopher A. Close[1], George K Lewis, Jr.[2], Rebecca A. Bader[1]
[1]Department of Biomedical Engineering, Syracuse Biomaterials Institute, Syracuse University, Syracuse, NY 13244
[2]Department of Biomedical Engineering, Cornell University, Ithaca, NY 14853

Statement of Purpose: Although therapeutic ultrasound has been shown to be an effective method of reducing swelling and pain associated with rheumatoid arthritis, no studies have been conducted thus far to provide a mechanism for the apparent reduction in inflammation.[1] The goal of this study was to use 2D and 3D culture systems to evaluate the response of RA synovial fibroblasts (RASFs) to long duration low-intensity therapeutic ultrasound (LITUS).

Methods:

Cell Culture

The MH7A RASF cell line (RIKEN) was cultured in RPMI 1640 supplemented with 10% FBS at 37°C and 5% CO_2. Upon reaching confluence, 40,000 cells in 1 ml of TNF-α supplemented media (1 ng/ml) were seeded into the 20 mm inner well of 35 mm glass bottom culture plates (MatTek Corp.; 2D culture) or collagen gels generated within the inner well of 35 mm glass bottom culture plates (3D culture, see below). After allowing 1 hour for cell adherence, an additional 2 ml of TNF-α supplemented media were added. Experiments with US were begun 24 hours after cell seeding.

Formation of Collagen Gel

For 3D culture systems, collagen gels were prepared from rat tail type I collagen (5 mg/ml) following an established literature protocol utilizing 1-ethyl-3(3-dimethyl aminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS). 5 molar equivalents of EDC and NHS were added relative to the COOH groups of collagen.[2] 400µl of the collagen solution were added to the inner well of 35 mm glass bottom cell culture plates. Incubation at 37°C for 3 hours facilitated gelation. The gels were sterilized by incubation with PBS supplemented with penicillin, streptomycin, and glutamine for 24 hours.

Fig. 1: Application of US to 2D (left) and 3D (right) MH7A cultures.

Application of Ultrasound & Analysis of Cell Response

A battery powered, prototype device for the application of long duration LITUS *in vitro*, developed by George K. Lewis Jr., was used to stimulate the 2D and 3D cultures confined to the glass bottom cell culture plates. Continuous ultrasound at an intensity of 97mW/cm^2 was applied to the cells for 6 hours (Fig. 1). 2D and 3D cultures within glass bottom plates to which LITUS was not applied were used as controls. 24 hours after the initial application of LITUS, the supernatant was removed and analyzed for the following proinflammatory mediators with multiplex immunoassay on the Luminex® platform: IL-6, IL-8, and VEGF.

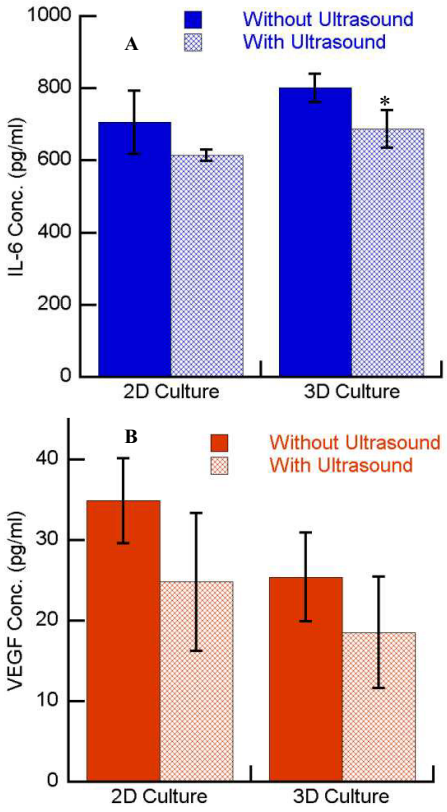

Fig 2. Impact of US on IL-6 (A) and VEGF (B) secretion by MH7A cells. * indicates $p \leq 0.05$ relative to cultures without ultrasound.

Results: As shown in Fig. 2, application of long duration LITUS resulted in a reduction in the secretion of IL-6 by MH7A cells in both 2D and 3D cultures, although the difference was only significant for 3D cultures. A similar trend was observed for VEGF secretion. LITUS did not lead to an alteration in IL-8 secretion (not shown).

Conclusions: The results obtained thus far suggest that long duration low-intensity therapeutic ultrasound can be applied in the treatment of RA to reduce the secretion of proinflammatory mediators by RASFs, the so-called conductors of joint destruction.[3] The results will form the basis of a relationship between basic science and clinical practice.

References:

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