Effect of Growth Factors in Combination with Injectable Silicone Elastomer Particles on The Biological Activity of Dermal Fibroblasts: An *In Vitro* Study

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Statement of Purpose: The degeneration of the natural fat pad of the foot is a key component in the pathogenesis of the diabetic foot ulcer. Ulcer formation on the skin of the diabetic foot is the most common cause of non-traumatic lower extremity amputations.[1] A minimally invasive procedure involving the subdermal injection of liquid silicone has been used clinically for several years to mitigate high pressures and prevent ulceration.[2] However, some implant migration has been detected in long term clinical studies, perhaps owing to impaired healing responses in diabetes patients. Silicone elastomer particles (Si), which can be injected in a similar fashion in a matrix of carboxymethylcellulose (CMC) gel that may have an advantage over liquid silicone in that the spherical design of the Si particles could enable a concentric encapsulation by fibroblasts which would lesson potential migration. The addition of growth factors with the implant particles may also enhance wound response and fibrous tissue encapsulation to lesson migration.

In this preliminary *in vitro* study, fibroblast cell cultures were exposed to Si in conjunction with growth factor combinations. The objective of this study was to measure the effects of growth factors with silicone particles on fibroblast proliferation and matrix production.

Methods: Normal human dermal fibroblasts (Cambrex Bio Science; Walkersville, MD) at passage 5 were plated in polystyrene tissue culture plates (BD Falcon; Franklin Lake, NJ) at a density of $1 \cdot 10^4$ cells per cm^2. Supporting media was Dulbecco's Modified Eagle's Medium (DMEM) with antibiotic mixture (Gentamicin /Amphotericin B) and 10% fetal bovine serum (FBS). Cells were allowed to attach overnight and then starved over night by replacing culture media with serum free DMEM. Starved cell cultures were then treated with growth factors and Si.

Basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF) were used in conjunction with Si. The growth factor combinations used for this study were bFGF+PDGF-BB, bFGF+EGF, and EGF+PDGF-BB. Basic FGF, PDGF, and EGF were obtained from Peprotech Inc. (Rocky Hill, NJ). From preliminary experiments, optimal growth factor concentrations were determined as follows: PDGF at 20ng/ml, EGF at 5ng/ml, and bFGF at 10ng/ml. Si particles (Tospearl 3120, Toshiba Silicone Company, Ohta-City, Japan) with an average diameter of 12µm were sterilized using an autoclave. Si particles at a concentration of 0.25mg/ml were mixed thoroughly in DMEM. The control group for these experiments were cells cultured in serum free DMEM. Two days after growth factor/particle treatment, proliferation and collagen production were measured. Cell proliferation was measured using the Cell Titer-Glo Luminescent Cell Viability Assay (Promega Corp., Madison, WI). Collagen content was determined using a modified hydroxyproline assay.[3] Quantitative data were expressed as the mean ± SEM. Statistical analyses were carried out using One Factor ANOVA followed by the Bonferroni posttest for multiple comparison purposes. Differences with p-value less than 0.05 were considered statistically significant.

Results: The proliferative activity was enhanced in response to the addition of bFGF in combination with PDGF both with and without Si (Figure 1). No other statistical differences in proliferation were detected with respect to Si addition.

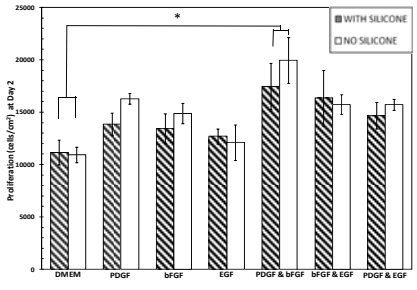

Figure 1. Fibroblast Proliferation

Collagen production was enhanced with the addition of bFGF with Si compared to bFGF only and the control group. Collagen production in the PDGF groups was inhibited compared to the control group. No other statistical differences in collagen were detected with respect to particle addition.

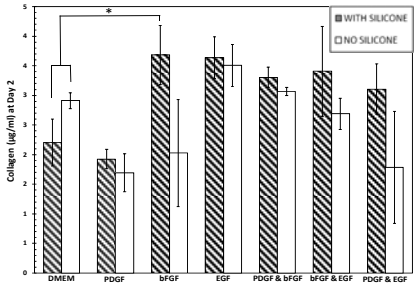

Figure 2. Fibroblast Collagen Production

Conclusions: The combination of PDGF and bFGF significantly increased proliferation compared to all other groups. The addition of Si had no significant effects on cell proliferation. When exposed to bFGF, the presence of particles stimulated a significant increase in collagen production compared to growth factors alone.

Combinations of bFGF and PDGF had a synergistic effect on promoting fibroblast proliferation and collagen production *in vitro*. The use of bFGF in conjunction with Si could boost fibrous capsule formation and reduce implant migration. Future studies will include Si particle characterization for implant optimization and an *in vitro* scratch assay to measure cell migration in the presence of growth factors and Si particles.

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3. Reddy G. Clin Biochem. 1996; 29:225-29.