Evaluation of the Effect of Growth Factors in Combination with Injectable Silicone Elastomer Particles on the Proliferative Activity of Dermal Fibroblasts

J. Robinson B.S.[1], K. Richelsoph M.S.[1], J.D. Bumgardner Ph.D.[1], Y. Yang Ph.D.[2], W.O. Haggard Ph.D.[1]
The University of Memphis[1], The University of Tennessee[2]

Statement of Purpose:

Foot ulcer formation most commonly results from the loss of the naturally occurring protective fat-padding present on the bottom portion of the foot.[1] This loss sometimes occurs during the aging process, but is often observed in patients with diabetic neuropathy. The rationale for this study is to couple the fibroblastic response that takes place with respect to foreign particle implantation with growth factors to produce a fibrous protective pad. The addition of growth factors is to augment wound healing in the diabetic foot.[2]

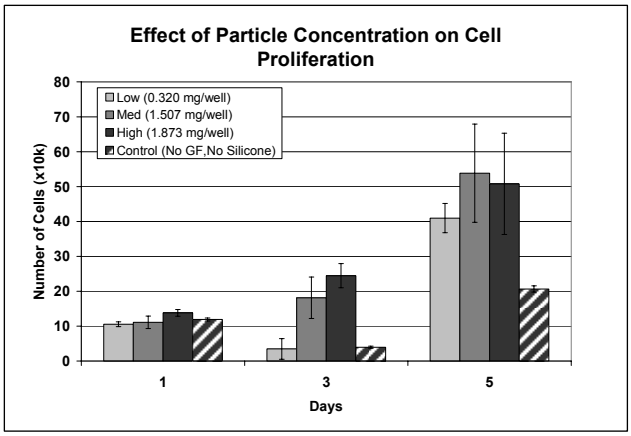
The objective of this study was to investigate the effectiveness of a combination of silicone elastomer particles and growth factors (Basic Fibroblast Growth Factor and Platelet Derived Growth Factor) as a potential treatment for diabetic foot ulcers. The hypothesis is that the injection of growth factors in combination with silicone particles would work to enhance the development of a fibrous protective foot pad in the area.

Materials and Methods:

The 12μm sterilized silicone particles (Tospearl 3120) used in this study were obtained from Toshiba Silicone Company (Ohta-City, Japan). The growth factors were obtained from Peprotech Inc.(Rocky Hill, NJ).

Primary normal human dermal fibroblasts (Cambrex Bio Science; Walkersville, MD) were cultured in polystyrene tissue culture plates (BD Falcon; Franklin Lake, NJ). Culture wells were plated at a density of $1 \cdot 10^4$ cells per cm^2. The fibroblasts in this study were allowed to proliferate for a total of 5 days in standard culture conditions (37°C, 5%CO_2 atmosphere). At each significant time point (Days 1, 3, and 5), the proliferative activity of these cells was assessed utilizing an MTS cell proliferation assay.

Basic fibroblast growth factor (bFGF) and Platelet-derived growth factor BB (PDGF-BB) were added to culture in varied concentrations. Cells were exposed to the growth factors in addition to varying concentrations of injectable silicone particles. High, medium, and low concentrations of these particles were used to determine which particle loading amount would provide the most optimal fibroblastic response when combined with the bFGF and PDGF-BB. bFGF and PDGF-BB were added to the culture in concentrations of 5ng/ml and 20ng/ml. Three solutions containing silicone particles were made. The concentrations of silicone particles were 1.873mg per well (high), 1.507mg per well (medium), and 0.320mg per well (low). Cells were grown in the presence of particles and growth factors (Experimental), presence of particles and absence of growth factors (Control 1), presence of growth factors and absence of particles (Control 2), and absence of both particles and growth factors (Control 3).



Three samples for each treatment were tested.

Figure 1. Graph of cell numbers in response to varying silicone elastomer particle concentrations (bFGF and PDGF-BB at 20ng/ml, no growth factors or particles for control)

Results/Discussion:

A heightened proliferative response was seen with particle addition in combination with bFGF and PDGF-BB(20ng/ml) when compared to the wells in which no particles or growth factors were included (Fig.1). The medium and high concentrations of particles were more effective at increasing cell proliferation than the low concentration. This effect is more notable on days 3 and 5. Additional assays on extracellular matrix proteins from various groups are being conducted.

Conclusions:

Fibroblast proliferation was enhanced in response to the particle and growth factor additions when compared to the control. The combination of growth factors with injectable silicone particles could be viewed as a potential treatment and preventative therapy for diabetic foot ulcers.

References:

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[2] Enoch, S and Leaper, D.J. Basic Science, 2005:23:37- 42

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