Botulinum Toxin Type A Attenuates Hypertrophic Scars Formation by Preventing Macrophage M1 Polarization

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**BACKGROUND:** Botulinum toxin type A has been shown to be a promising therapy for hypertrophic scars by alleviating muscle tension, inhibiting fibroblasts proliferation, and influencing transforming growth factor-β1 expression. However, persistent inflammation in wound healing is one of the main causes of hypertrophic scars. The severity of the inflammatory response is determined in large part by pro-inflammatory M1 macrophages. Studies have reported that botulinum toxin type A had an anti-inflammatory effect and could induce innate immune cell activation such as macrophages. Therefore, we hypothesized that botulinum toxin type A may reduce inflammation and inhibited the hypertrophic scars formation by preventing macrophage M1 polarization.

**MATERIALS AND METHODS:** Murine macrophage RAW264.7 cells were cultured and treated with LPS (to induce M1 polarization) and botulinum toxin type A for 24 hours. M1-type markers such as iNOS, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 were determined by reverse transcription-quantitative polymerase chain reaction. The mouse model of hypertrophic scars was prepared by a mechanical stretch device and treated with botulinum toxin type A. Histologic studies were performed to evaluate scar hypertrophy by hematoxylin and eosin and Masson’s trichrome staining. The pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 were observed by immunohistochemistry. The M1 macrophages (F4/80+ iNOS+ cells) in vivo were further evaluated by immunofluorescence staining.

**RESULTS:** The mRNA levels of iNOS, TNF-α, IL-1β, and IL-6 of RAW264.7 cells were significantly decreased in the botulinum toxin type A treated group than in the controls. In addition, histologic studies and immunohistochemistry showed that local administration of botulinum toxin type A significantly inhibited hypertrophic scars formation and reduced inflammatory response with decreased expression of TNF-α, IL-1β, and IL-6. Besides, botulinum toxin type A administration led to a decrease in the percentage of M1 macrophages in the scar tissue.

**CONCLUSION:** Botulinum toxin type A inhibits macrophage M1 polarization during wound healing and attenuates hypertrophic scars formation.

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The Use of Vitamin D3 (Calcitriol) for Improving Autologous Fat Graft Retention

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**BACKGROUND:** Autologous fat grafting is a powerful technique for replacing soft tissue but is limited by unpredictable long-term tissue retention and considerable interpatient variability. After injection, fat grafts typically experience extreme ischemia causing adipocytes to necrotize and release factors inducing macrophage recruitment and inflammation. This process continues until tissue revascularization occurs. We hypothesize that reducing tissue inflammation and the rate of necrotic tissue clearance will increase graft retention during the revascularization period, ultimately improving tissue replacement by stem
and progenitor cell remodeling. Calcitriol, the active form of vitamin D3, significantly inhibits apoptosis through reduction of oxidative stress and is a potential key stimulator of triglyceride accumulation. This study investigates the novel use of calcitriol for improving adipose tissue survival by reducing inflammation and phagocytic tissue clearance.

**METHODS:** In vitro, adipose tissue from 3 human donors was cultured for 48 hours in 1% oxygen and 0, 15.6, 62.5, and 250 nM calcitriol. Tissue viability was assessed, and quantitative reverse transcriptase polymerase chain reaction was performed to measure genes related to hypoxia or inflammation. In vivo, an immunocompromised mouse model was used to evaluate the impact of calcitriol on fat graft outcomes. Lipoaspirate tissue (0.3 ml) from 3 human donors was implanted bilaterally on the mouse dorsum and assessed at multiple time points out to 12 weeks. Study groups included lipoaspirate incubated with calcitriol for 60 minutes before injection or thrice weekly intraperitoneal calcitriol injections. Study outcomes included residual graft volume (%) and graft injury as observed through histology.

**RESULTS:** Under hypoxic culture conditions, calcitriol did not significantly impact adipocyte viability in vitro but did decrease expression of inflammatory cytokines including SOD1, IFNγ, and interleukin-6. In vivo, lipoaspirate submersion before grafting increased graft retention at 1 week ($P = 0.081$, not statistically significant) and 4 weeks ($P < 0.05$), whereas intraperitoneal calcitriol injections significantly increased fat graft volume retention at both 1 and 4 weeks ($P < 0.01$). Results from 12-week data are pending.

**CONCLUSION:** Calcitriol, an Food and Drug Administration–approved drug with known immunomodulatory properties, seems to be a promising drug for improving long-term fat grafting outcomes. In vitro, calcitriol exhibited anti-inflammatory properties and hypoxic tissue had decreased expression of inflammatory cytokines SOD1, IFNγ, and interleukin-6. In vivo, calcitriol submersion and intraperitoneal injection both significantly increased fat graft volume retention by 4 weeks. Used in tumescent fluid, calcitriol has potential as a simple, economical means of increasing fat graft retention.

Quality- and Quantity-cultured Peripheral Blood Mononuclear Cell Improve the Fat Graft Vascularization and Survival

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**INTRODUCTION:** Fat grafting is a valuable technique in soft-tissue reconstruction. However, ischemia of the grafted tissue with subsequent necrosis and tissue loss impede us from having satisfying long-term results. Recently, the quality and quantity (QQ) culture has been established to increase the vasculogenic potential of endothelial progenitor cells in peripheral blood-derived mononuclear cells (MNCs). Our experiment was designed to test whether QQ-cultured MNC (MNC-QQ) can contribute to vasculogenesis in the human fat graft and decrease the tissue loss.

**METHODS:** Adipose tissue and peripheral blood were harvested from healthy subjects. Fat grafts were created with peripheral blood-derived MNC ($N = 16$), MNC-QQ ($N = 16$), and stromal vascular fraction ($N = 16$) before grafting in BALB/c nude mice, and compared to nonenriched control fat grafts ($N = 16$). Grafts were explanted after 1 and 7 weeks and analyzed by weight persistence, immunohistochemistry, and quantitative polymerase chain reaction.

**RESULTS:** Weight persistence after 7 weeks was significantly higher in the MNC-QQ group (89.8% ± 3.5%) and SVF group (90.1 ± 4.2) compared to control (70.4% ± 6.3%). With 96.6 ± 6.5 vessels/mm², grafts in the MNC-QQ group had the most dense vessel network and scored significantly better than control (70.4 ± 5.6 vessels/mm²). MNC-QQ exerted a direct effect on vasculogenesis by integrating in vessels, and a paracrine VEGF-mediated effect. Tissue consisting of fibrosis and perilipin-positive adipocytes was unchanged among all groups.

**CONCLUSIONS:** QQ-cultured MNC containing endothelial progenitor cell stimulates the formation of a blood vessel network in the fat graft and enhances the graft survival, indicating its potential for clinical fat grafting.

**Nanofiber System for Sustained Release of Insulin-like Growth Factor 1 Nanoparticles to Nerve and Muscle**

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