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Paclitaxel impairs adipose stem cell proliferation and differentiation

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ABSTRACT

Background: Cancer patients with chemotherapy-induced immunosuppression have poor surgical site wound healing. Prior literature supports the use of human adipose–derived stem cell (hASC) lipoinjection to improve wound healing. It has been established that multipotent hASCs facilitate neovascularization, accelerate epithelialization, and quicken wound closure in animal models. Although hASC wound therapy may benefit surgical cancer patients, the chemotherapeutic effects on hASCs are unknown. We hypothesized that paclitaxel, a chemotherapeutic agent, impairs hASC growth, multipotency, and induces apoptosis.

Methods: hASCs were isolated and harvested from consented, chemotherapy and radiation naive patients. Growth curves, MTT (3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide), and EdU (5-ethynyl-2-deoxyguridine) assays measured cytotoxicity and proliferation. Oil Red O stain, Alizarin Red stain, matrigel tube formation assay, and quantitative polymerase chain reaction analyzed hASC differentiation. Annexin V assay measured apoptosis. Immunostaining and Western blot determined tumor necrosis factor α (TNF-α) expression.

Results: hASCs were selectively more sensitive to paclitaxel (0.01–30 μM) than fibroblasts ($P < 0.05$). After 12 d, paclitaxel caused hASC growth arrest, whereas control hASCs proliferated ($P = 0.006$). Paclitaxel caused an 80.6% reduction in new DNA synthesis ($P < 0.001$). Paclitaxel severely inhibited endothelial differentiation and capillary-like tube formation. Differentiation markers, lipoprotein lipase (adipogenic), alkaline phosphatase (osteogenic), CD31, and van Willebrand factor (endothelial), were significantly decreased (all $P < 0.05$) confirming paclitaxel impaired differentiation. Paclitaxel was also found to induce apoptosis and TNF-α was upregulated in paclitaxel-treated hASCs ($P < 0.001$).

Conclusions: Paclitaxel is more cytotoxic to hASCs than fibroblasts. Paclitaxel inhibits hASC proliferation, differentiation, and induces apoptosis, possibly through the TNF-α pathway. Paclitaxel's severe inhibition of endothelial differentiation indicates neovascularization disruption, possibly causing poor wound healing in cancer patients receiving chemotherapy.

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1. Introduction

Nonhealing surgical wounds are a significant problem. Cancer patients with chemotherapy-induced immunosuppression are at a higher risk for poor surgical site wound healing [1–3]. Numerous approaches to improve wound healing have included treatment with transforming growth factor-β, fibroblast growth factor, platelet-derived growth factor, and epidermal growth factor [4–8]. Success with these approaches has been limited secondary to cost, poor efficacy, and the need for continual application of growth factors.

Several studies have revealed advantages of exogenous adult mesenchymal stem cells (MSCs), primarily from bone marrow MSCs, to improve wound healing [9–11]. MSCs use paracrine signaling to increase cell migration, proliferation, surrounding cell survival, and accelerate wound healing [9,12]. However, limitations to MSCs include decreased differentiation potential with increased donor age and donor morbidity secondary to bone marrow isolation for MSC access [13,14].

More recently, the use of human adipose–derived stem cells (hASCs) has emerged as an encouraging alternative to MSCs. An advantage of hASCs is ease of harvest *via* lipoaspiration causing less donor site morbidity [14,15]. Additional advantages include large quantities of available stem cells given adequate adipose tissue donor sites with less patient discomfort and the decreased effect of age [14–17].

Similar to bone marrow MSCs, hASCs are multipotent stem cells that play an important role in wound healing and regenerative medicine [18]. Prior literature demonstrated hASC lipoinjection into wounds increases paracrine cytokine secretion facilitating angiogenesis allowing for neovascularization, accelerated epithelialization, and quickened wound closure in animal models [19–21]. *In vivo*, hASCs have successfully been used as an adjunct to lipoinjection to create an autologous filler to improve soft tissue defects [22–25].

The animal and human studies discussed previously show accelerated wound healing and improved soft tissue defects after hASC therapy, and they reveal considerable potential for hASC therapy in human patients postoperatively to improve surgical wound healing. Figure 1 illustrates the sequence of procedures used to obtain an hASC line usable for clinical applications. Cancer patients can have significant wound healing morbidity secondary to chemotherapy-induced immunosuppression [1–3]. The use of hASCs in these immunosuppressed patients could accelerate nonhealing surgical wounds. Although hASCs may benefit these patients, the chemotherapeutic effects on hASCs are unknown.

Paclitaxel (Fig. 2) is a commonly used chemotherapeutic agent in breast and ovarian cancer [26,27]. Paclitaxel disrupts microtubule organization causing mitotic arrest and induces cancer cell death *via* apoptosis [28–31]. We focused our present study on paclitaxel because it is commonly used to treat breast cancer, which occurs in one of every eight women and accounted for 232,340 new cases in 2013 in the United States [32]. Secondly, prior literature has established the successful use of hASCs in breast tissue reconstruction and augmentation [22]. Therefore, there is a large population of immunosuppressed breast cancer patients treated with paclitaxel who could benefit from hASC surgical wound therapy. Our primary study objective was to evaluate the chemotherapeutic effects of paclitaxel on hASCs *in vitro*. We hypothesized that paclitaxel impairs hASC growth, multipotency, and induces apoptosis.

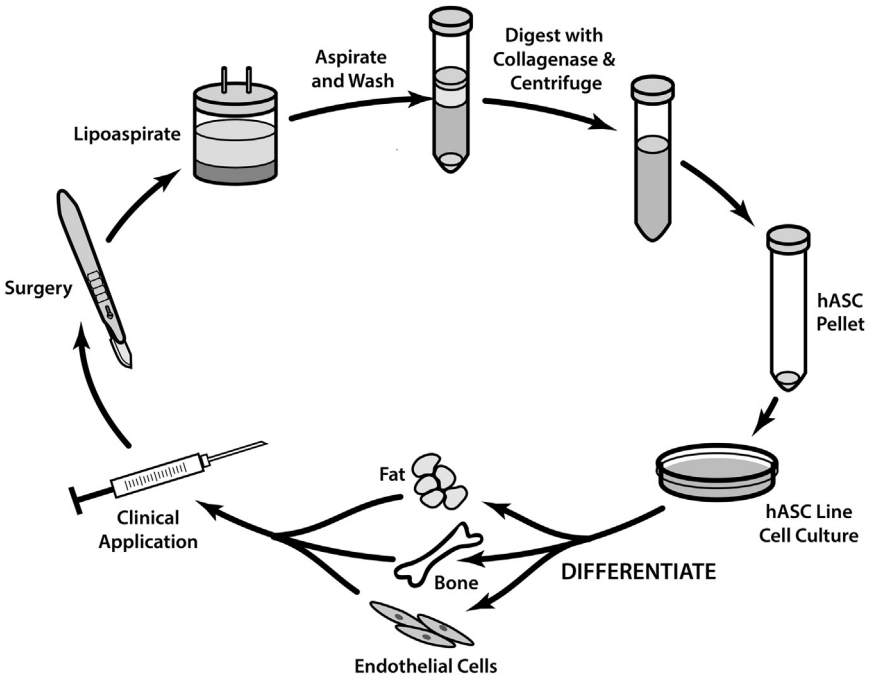

Fig. 1 – Establishing human adipose–derived stem cell (hASC) lines. Starting with patient lipoaspiration, followed by digestion with collagenase and centrifugation to create the stem cell pellet. After hASC incubation, culture in different differentiation mediums results in adipocyte, osteocyte, and endothelial cell development and the possibility for clinical application such as improving wound healing with hASCs.