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Acellular dermal matrix-based gene therapy augments graft incorporation

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ABSTRACT

Background: Acellular dermal matrix (ADM) is widely used for structural or dermal replacement purposes. Given its innate biocompatibility and its potential to vascularize, we explored the possibility of ADM to function as a small interfering RNA (siRNA) delivery system. Specifically, we sought to improve ADM vascularization by siRNA-mediated inhibition of prolyl hydroxylase domain-2 (PHD2), a cytoplasmic protein that regulates hypoxia inducible factor-1α, and improve neovascularization.

Materials and methods: Fluorescently labeled siRNA was used to rehydrate thin implantable ADM. Pharmacokinetic release of siRNA was determined. Twelve millimeter sections of ADM reconstituted with PHD2 siRNA (nonsense siRNA as control) and applied to dorsal wounds of 40 FVB mice. Grafts were sewn in, bolstered, and covered with occlusive dressings. Photographs were taken at 0, 7, and 14 d. Wounds were harvested at 7 and 14 d and analyzed (messenger RNA, protein, histology, and immunohistochemistry).

Results: Release kinetics was first-order with 80% release by 12 h. By day 14, PHD2-containing ADM appeared viable and adherent, whereas controls appeared nonviable and nonadherent. Real-time reverse transcription-polymerase chain reaction demonstrated near-complete knockdown of PHD2, whereas vascular endothelial growth factor and FGF-2 were increased 2.3- and 4.7-fold. On enzyme-linked immunosorbent assay, vascular endothelial growth factor was increased more than fourfold and stromal cell-derived factor doubled. Histology demonstrated improved graft incorporation in treated groups. Immunohistochemical demonstrated increased vascularity measured by CD31 staining and increased new cell proliferation by denser proliferating cell nuclear antigen staining in treated *versus* controls.

Conclusions: We concluded that ADM is an effective matrix for local delivery of siRNA. Strategies to improve the matrix and/or genetically alter the local tissue environment can be envisioned.

1. Introduction

Acellular dermal matrix (ADM) is commonly used for breast, abdominal, and head and neck reconstruction [1–7]. A commonly cited advantage of ADM and other biologic dermal substrates is incorporation into the host tissue, which ultimately confers host tissue-like properties thought to result from vascularization and subsequent recellularization by

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local tissues [8]. Moreover, rapid ADM vascularization is critical when ADM is used as a dermal replacement to support split-thickness skin grafts. Additionally, although ADM has become widely used in implant-based breast reconstruction in single and two-stage procedures, there are increasing reports of higher seroma, infection, and explantation rates with the use of ADM [9,10]. Interestingly, radiation, which plays a central role in the treatment of breast cancer and which yields characteristic diminishment in tissue vascularity, has been identified as an independent predictor of adverse outcome in the setting of ADM associated implant-based breast reconstruction [11]. Improving ADM vascularization may improve the performance characteristics of ADM and may allow for new and higher volume applications of ADM. In all these circumstances, attempts to vascularize this pocket may prove beneficial in decreasing complications and improving take in complex wound beds.

Hypoxia inducible factor (HIF)-1α is the master switch that regulates new blood vessel formation, or angiogenesis, in response to hypoxic conditions [12,13]. In fact, the stabilization of HIF-1α is critical to wound healing [14]. Under homeostatic conditions, that is unwounded skin, prolyl hydroxylase domain (PHD)2 protein binds to HIF-1α and causes its degradation by the von Hippel Lindau protein [15,16], thereby turning off this switch. Several investigators have blocked PHD-2 protein expression, creating constitutive HIF-1α expression [15–17]. If one applied this technique to an area with low angiogenic potential, theoretically, angiogenesis could be augmented.

The discovery of silent interfering RNA has spawned a wealth of studies ranging from improvement in delivery systems for cellular uptake, the provision of new therapeutics to combat human diseases, the design of anticancer agents, and to applications in agriculture to advance plant science [18]. Studies have shown that small interfering RNAs (siRNAs) can be used to suppress the transcription of specific gene sequences, thereby inhibiting the production of a specific protein product [17]. Moreover, silencing is transient, eliminating the potential for uncontrolled cell proliferation [19].

Subsequently, a growing need has emerged to create a delivery system that can specifically target the subcutaneous tissue for local gene modulation and therapy. Because ADM is already widely used for structural or dermal replacement purposes owing to its innate biocompatibility and potential to vascularize, we explored the possibility of using ADM as a siRNA delivery system. We therefore hypothesized that this mode of gene therapy delivery could modify the wound bed to improve neovascularization. Second, if treated ADM could create new vessels in a wound, it might also increase the vascular cell ingrowth into the matrix itself.

2. Methods

2.1. Reconstitution of ADM with siRNA

Preliminary studies to assess the adequacy of siRNA uptake and delivery by ADM were performed. In a petri dish, a 12-mm section of ADM was placed in 40 μL of a solution containing 20-pmol fluorescently labeled siRNA (Qiagen, Valencia, CA, Alexa Fluor 488). Once reconstituted, the samples were formalin fixed, paraffin embedded, and sectioned. Slides were viewed under an epifluorescent microscope and digitized; Sigma Scan software (Systat Software, Inc., San Jose, CA) was used to quantify the relative intensity of fluorescence within the matrix of normal saline-reconstituted ADM *versus* siRNA reconstituted samples to determine the presence of autofluorescence.

2.2. Measuring siRNA release from ADM

Release kinetics was determined using a 96-well plate with a semipermeable membrane. ADM reconstituted with fluorescently labeled siRNA in saline was placed on the membrane with diffusion of fluorescent compound into the lower well containing saline. Concentrations of the treated wells were measured at various time points (hourly for 1–6 h, then at 12 and 24 h) and compared against standard dilutions by a spectrophotometer at 490 nm. Values were divided by maximum concentration of 2.5 pmol/uL (500 pmol of fluorescent compound per 200 μL of saline) to calculate percent of elution and then graphed against time of elution to determine *in vitro* kinetics of siRNA release from ADM.

2.3. Application of siRNA-containing ADM to cutaneous wounds

After approval from The New York University Medical Center Animal Care Committee (IACUC #061104-01), 40 adult male FVB mice were obtained from Jackson Laboratories (Bar Harbor, ME) and housed in our animal care facility in accordance with the Division of Laboratory Animal Resources bylaws. Investigative experimental arms included ADM reconstituted with PHD-2 siRNA ($n = 20$) or reconstituted with nonsense siRNA ($n = 20$). Ten animals from each group were sacrificed for the various data analyses on days 7 and 14. A novel model of ADM grafting onto the dorsal surface of the mouse was used. Briefly, a singular wound (12-mm) was created on the dorsum of the mouse. Thin graftable ADM was reconstituted in nonsense siRNA (control) or solution containing siPHD2 (treated) as per manufacturer's protocol [20]. Specifically, 20 pmol of PHD2 siRNA was incorporated into the treated ADM. The ADM was then sewn into the wound bed with interrupted 5-0 nylon sutures, bolstered into place with a petroleum gauze and dry sterile dressing, and covered with an occlusive bandage (3M, St Paul, MN). At 7 and 14 d, the dressing was removed, and the wounds were photographed, assessed, and harvested for further evaluation as described in the following.

2.4. Messenger RNA and protein quantification of ADM-grafted wound

Wounds were harvested on days 7 and 14 after grafting. Real-time reverse transcription-polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) of the wound homogenate were performed. Specific genes