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Therapeutic intradermal delivery of tumor necrosis factor-alpha antibodies using tip-loaded dissolvable microneedle arrays

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ABSTRACT

Tumor necrosis factor-alpha (TNF-α) specific antibodies (anti-TNF-α Ab) have been shown to be potent TNF inhibitors and effective therapeutics for a range of inflammatory diseases. Typically, these drugs are administered systemically, but systemic dosing sufficient to achieve locally effective concentrations in peripheral tissues has been associated with systemic immunosuppression and related adverse events. Here, we evaluated the use of tip-loaded dissolvable microneedle arrays (MNAs) for localized intradermal delivery of anti-TNF-α Ab. MNAs with obelisk shape microneedles that incorporate the antibody cargo in the needle tips were created from carboxymethylcellulose (CMC) using a micromilling/spin-casting fabrication method. We found that anti-TNF-α Ab integrated into MNAs using this room temperature fabrication process maintained conformationally dependent TNF-α binding activity. Further, these MNAs efficiently delivered anti-TNF-α antibodies to the dermis of human skin with clinically applicable release profiles. To evaluate MNA delivered anti-TNF-α Ab function, we applied anti-TNF-α Ab containing MNAs to established psoriasiform lesions on the skin of mice. MNA anti-TNF-α Ab treatment reduced key biomarkers of psoriasiform inflammation including epidermal thickness and IL-1β expression. Taken together, these results demonstrate efficient and biologically effective MNA delivery of anti-TNF-α Ab to the intradermal microenvironment of the skin in mice and humans, and support the development of MNA mediated antibody delivery for clinical applications.

Statement of Significance

Tumor necrosis factor-alpha (TNF-α) specific antibodies (anti-TNF-α Ab) have been shown to be potent TNF inhibitors and effective therapeutics for a range of inflammatory diseases. Typically, these drugs are administered systemically, but systemic dosing sufficient to achieve locally effective concentrations in peripheral tissues has been associated with systemic immunosuppression and related adverse events. Here we demonstrate efficient and biologically effective MNA delivery of anti-TNF-α Ab to the intradermal microenvironment of the skin in mice and humans. These results support the development of MNA mediated antibody delivery of therapeutic antibodies for clinical applications.

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1. Introduction

A variety of autoimmune mechanisms result in localized inflammatory skin disease characterized by dysregulated cytokine expression in the cutaneous microenvironment. In particular, localized overexpression of tumor necrosis factor-alpha (TNF-α), a central mediator of inflammation, has been associated with a broad range of autoinflammatory dermatosis and is a rational target for therapeutic inhibition [1–4]. TNF-α specific neutralizing antibodies have been particularly effective in treating inflammatory diseases by selectively binding to soluble TNF-α and thereby reducing TNF-α receptor activation. While subcutaneous and intramuscular injection of TNF-α-blockers have been shown to effectively mitigate skin inflammation, administration of TNF-α inhibitors by these systemic routes requires relatively high doses to achieve locally effective concentrations in the skin [5–9]. These relatively high systemic doses of TNF-α inhibitors can result in non-specific immune suppression that has been associated with increased infection rates and a variety of adverse events [10,11]. This trade off in efficacy versus off-target immunosuppression limits both efficacy and the broader applicability of TNF-α inhibitors [12].

Topical administration of immunosuppressive agents has considerable advantages over systemic delivery. However, effective topical administration requires penetration of the stratum corneum, the thick outer barrier of the skin, and localization of the therapeutic agent in the intradermal region. Topically applied corticosteroids can be used to effectively treat inflammatory skin diseases. With molecular weights between 200–500 Da, corticosteroids penetrate the stratum corneum which is generally considered to be permissive to molecules of less than 500 Da [13]. However, due to their low molecular weight, corticosteroids have a short residence time in the skin and quickly diffuse into the blood stream. This necessitates repeated applications and frequently long-term use that can lead to undesirable sequelae including loss of skin tone, deterioration of skin cells, and increased risk of infection. On the other hand, antibody therapeutics, typically in the 150 kDa range, are not effective when administered topically as skin penetration is poor due to their high molecular weight. Interestingly, results from topical application of anti-TNF-α Ab in the setting of an already breached skin barrier demonstrate their potential effectiveness if penetration limitations can be overcome. For example, topical application of infliximab was shown to be a promising strategy to improve healing in diabetic skin ulcers [14].

Over the last decade, a number of approaches have been developed for transdermal delivery of therapeutics [15–19]. Most are being developed for transdermal delivery for systemic dosing and are geared towards small molecule drugs and macromolecules smaller than an antibody. Of these techniques, thermal ablation and microneedles have been most successful in the delivery of larger macromolecules. Thermal ablation is a technique in which the skin is heated up to or above 100 °C for microseconds to milliseconds to selectively disrupt the stratum corneum. Thus far, although this technique has demonstrated some success in animal models, general variability in skin thickness and integrity will likely limit the broad application of this technique [17].

Dissolvable microneedle arrays (MNAs) are transdermal delivery systems designed to mechanically penetrate the stratum corneum [20]. A number of micro-fabrication techniques has been developed to create polymer MNAs that can incorporate drug and fully dissolve in skin to deliver therapeutics in a minimally invasive manner [21,22]. *In vivo* and *in vitro* studies of MNAs loaded with biologics greater than 500 Da supported effectiveness and safety for intradermal drug delivery [23,24]. Several laboratories, including our own, have demonstrated the use of dissolvable MNAs to deliver vaccines with improved efficiencies, enabling far lower required antigen doses compared to traditional intradermal needle injections [25–27]. We have previously described the use of micromilling/spin-casting technique to develop microneedle arrays with unique microneedle and array geometries designed for precise and specific drug delivery to human skin [27]. The unique advantages of dissolvable polymer MNAs suggest that they could be used to effectively deliver anti-TNF-α Ab intradermally for localized treatment of inflammatory skin diseases.

In this paper, we describe the fabrication of MNAs with anti-TNF-α Abs integrated into obelisk-shaped microneedles designed for optimal human skin penetration. Importantly, different from our earlier work where the entire microneedle body and the backing layer was filled with the cargo, in the present work, the fabrication process is modified to integrate the cargo only in the apex (tip) of the obelisk microneedles, enabling efficient, more controlled, and cost effective drug delivery. These MNAs delivered anti-TNF-α antibodies to the dermis of human skin with clinically applicable release profiles, and anti-TNF-α Ab MNA treatment reduced key indicators of inflammation in a murine model of psoriasiform dermatitis. Taken together, our results support the clinical development of MNA delivered TNF inhibitors for the treatment of localized inflammatory skin diseases.

2. Materials and methods

2.1. Fabrication of tip-loaded dissolvable microneedle arrays

Our previous study demonstrated that dissolvable MNAs with obelisk shape microneedles have considerably better insertion and cargo delivery characteristics than those with traditional microneedle geometries, such as pyramidal microneedles [27]. In this work, the MNA design utilized obelisk microneedle geometry to deliver TNF-α inhibitors. A critical departure from previously demonstrated fabrication approach is that the microneedles of MNAs used in this study are tip loaded with the bioactive cargo (anti-TNF-α Ab) for delivering them to the targeted skin sites. The overall approach used for fabrication of tip-loaded dissolvable MNAs is graphically presented in Fig. 1. The approach involves three steps: (a) creation of mastermolds from a wear resistant and easily machinable polymer using the mechanical micromilling process; (b) fabrication of production molds using mastermolds through elastomer molding; and (c) fabrication of tip loaded dissolvable MNAs from production molds using two-step spin-casting technique: (c.1) the sufficient amount of bioactive cargo is loaded into the elastomer mold, and centrifuged at the appropriate temperature and speed into the microneedles. After removal of excess cargo, centrifuging was continued until (only) the tip portions of the microneedles of production molds contain the dry antibody cargo. Next, (c.2) the structural material of MNAs in hydrogel form is loaded into the elastomer molds, and centrifuged at prescribed temperature and speed until the full density, dry, tip-loaded MNAs are obtained. Currently in our laboratories we are scaled to fabricate 500+ microneedle arrays in a 6 h day. The fabrication process is readily scalable using industrial grade equipment and automation to dramatically increase output for clinic applications. The fabrication process facilitates easy and rapid changes in geometric and material parameters so that application-specific optimized microneedle array designs can be achieved.

2.1.1. Fabrication of mastermolds and elastomer production molds

The mastermold geometry, shown in Fig. 1, includes four 10×10 MNAs with obelisk geometry microneedles as well as channels that surround each array. The channels are intended to