Overcoming the challenges of topical antibody administration for improving healing outcomes: a review of recent laboratory and clinical approaches

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ABSTRACT

Therapeutic antibodies present numerous opportunities for the treatment of wounds and cutaneous conditions; however, they have not been widely adopted due to the difficulty of administering antibodies through the skin. Local antibody administration to the skin may result in fewer side effects, reduce cost of therapy and be less invasive than systemic methods and recent advances in antibody engineering have addressed many stability and formulation challenges. Penetration of the epidermal barrier is crucial to effective delivery of antibodies and other protein drugs and can be achieved through chemical or physical methods. Chemical penetration enhancement is poorly suited for delivery of large hydrophilic molecules such as antibodies; however, enhancers based on surfactants or terpenes may improve antibody delivery to the dermis and novel cell-penetrating peptides provide opportunities for well-tolerated local antibody delivery. Physical penetration enhancement methods (including electroporation, iontophoresis, microneedles and ultrasound) address many formulation challenges common to chemical penetration enhancers; however, more studies are required to demonstrate effective antibody delivery for clinical translation. While topical antibody administration to the skin remains challenging, advances in antibody engineering and skin penetration enhancement may render antibodies more viable treatment options for improving wound outcomes.

Keywords: Antibodies, wounds, penetration enhancer, topical delivery.

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INTRODUCTION

This narrative review aims to provide an overview of recent advances in the field of antibody delivery to the skin. Methods that have successfully delivered antibodies or large proteins through the epidermal barrier, particularly in a clinical setting, are described and discussed in the context of wound applications including chronic wounds, chronic skin blistering and scar management.

Poor outcomes from wounds represent an increasing health burden in Australia as populations of diabetic, obese and elderly persons who exhibit deficient wound healing increase. Therapeutic antibodies have the potential to treat many kinds of wounds[1]; however, antibody therapies for wounds have not been widely adopted due to the difficulty of formulating antibodies for local administration and penetration through the skin. Antibodies are one of the fastest growing categories of biopharmaceuticals today, with over 60 antibodies approved for use as therapeutics and over 50 candidates currently in phase III clinical studies[2]. Presently, antibodies are employed in the treatment of cancer, inflammatory disease and autoimmune disorders[3] and their success is attributed to their high specificity and binding affinity to their target proteins with reduced side effects compared to small molecules[4]. In recent years, preclinical and clinical research has demonstrated roles for antibodies for a number of cutaneous conditions including psoriasis[5,6], atopic dermatitis[7,8], skin cancers[9] and wound healing[10-14]. However, the development of antibody therapies for skin conditions is challenging as antibodies exhibit poor tissue permeability[15] and are thus not efficiently absorbed through the intact skin barrier. This complicates the use of antibodies for wound applications, as while therapies promoting active healing may be directly administered to open wounds, administration to intact skin would be required for blister or scar management.

Human antibodies, also termed immunoglobulins, are antigen-binding proteins consisting of four polypeptide chains arranged in a Y-shape (Figure 1). Two heavy chains linked by disulfide bridges form the constant (Fc) stem region of the antibody molecule, while a light chain linked with each heavy chain forms two identical antigen binding (Fab) regions. The Fab regions are responsible for the specificity

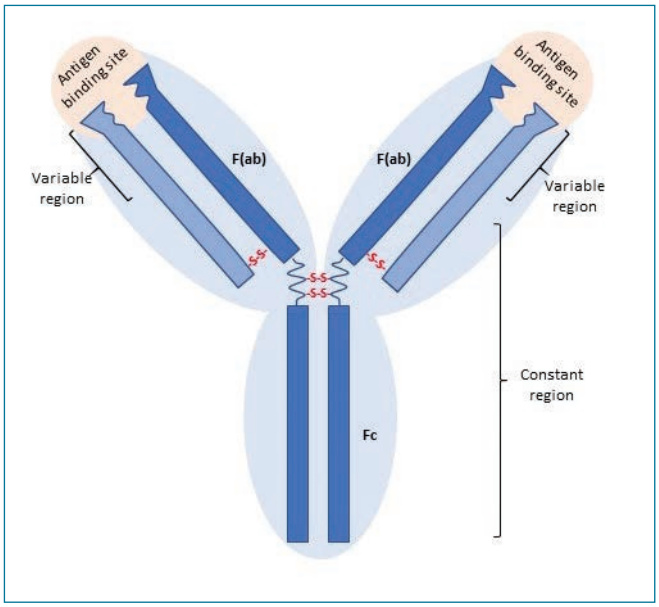

Figure 1: Antibody structure
Human antibodies are composed of four polypeptide chains: two heavy chains (dark blue) and two light chains (light blue). Antigen binding sites are each formed by the variable regions of a light chain and a heavy chain (Fab regions). The constant regions of the heavy chains form the tail (Fc region). The two Fab regions and Fc region are linked by a flexible hinge region that improves the ability of the Ab to bind antigen.

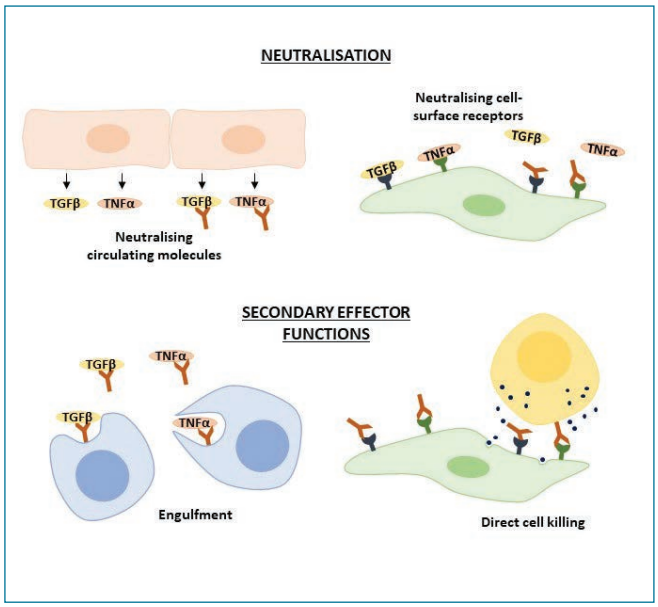

Figure 2: Therapeutic antibody functions
Therapeutic antibodies function through two main routes: direct neutralisation or secondary effector functions. Neutralisation occurs by binding circulating mediators or their receptors and preventing further signalling. Secondary effector functions recruit immune cells to engulf the target or directly kill the target cell.

of the antibody, while the Fc region determines the antibody isotype and influences further immune signalling, termed secondary effector functions. Antibodies can function as therapeutics via direct neutralisation of their targets by blocking active sites on the protein, or by binding to the target and triggering its destruction by secondary effector functions (Figure 2) as in the case of many anti-cancer antibody therapies[9]. The function of antibodies is dependent on their three-dimensional structure, which enables binding to a target antigen; consequently care must be taken when developing antibody therapeutics to ensure antibodies are properly folded and retain binding capacity.

ANTIBODIES FOR TOPICAL ADMINISTRATION

Antibodies have previously been used in the management of multiple cutaneous conditions, including in inflammatory skin diseases, healing wounds or managing scars. Clinical studies have shown improvement in psoriatic lesions after administration of antibodies targeting inflammatory mediators tumour necrosis factor alpha[5], interleukin 23[16] and interleukin 17[17], and atopic dermatitis has been successfully treated with antibodies targeting interleukin 4 receptor alpha[18]. Antibodies against wound mediators including Flightless I[10,19] and tumour necrosis factor alpha[20] have been shown to improve healing in a number of wound types, and antibodies against transforming growth factor beta have been used to reduce scarring[12,21]. Therapeutic antibodies are generally administered by intravenous, subcutaneous or intramuscular injection, as direct oral administration commonly employed for small molecule drugs would result in gastric digestion of the proteins[15]. While injection of antibodies has been used successfully for cutaneous conditions[22-25], direct application to the skin may confer several advantages to systemic methods. Topical administration provides local therapeutic effect while reducing systemic adverse effects[26-28], avoids drug metabolism and dilution thereby reducing the quantity of antibody required[29] and noninvasively delivers therapeutics to patients with simplified dosage requirements[30,31]. Antibodies can be prohibitively expensive to use, thus approaches that use lower dosages via topical administration would reduce costs and allow therapies to be available to more patients[32]. Local administration of antibodies avoids drug metabolism and improves retention within the tissue[33], thus antibodies for treatment of chronic wounds or scarring may only require dosing every 1–4 weeks to maintain effective concentrations within the wound. Topical administration of gel-formulated anti-tumour necrosis factor antibody (Infliximab) has previously been effective in the treatment of open chronic wounds[20]; however, lesions with intact epithelial barriers including blisters, burns, partially re-epithelialised wounds and scars are not readily accessible by antibodies within a gel formulation.

Despite the potential benefits of therapeutic antibodies for cutaneous conditions, protein-based drugs exhibit challenging properties that render their formulation and delivery complex. As correct folding of antibodies is essential to their function, protein stability must be considered

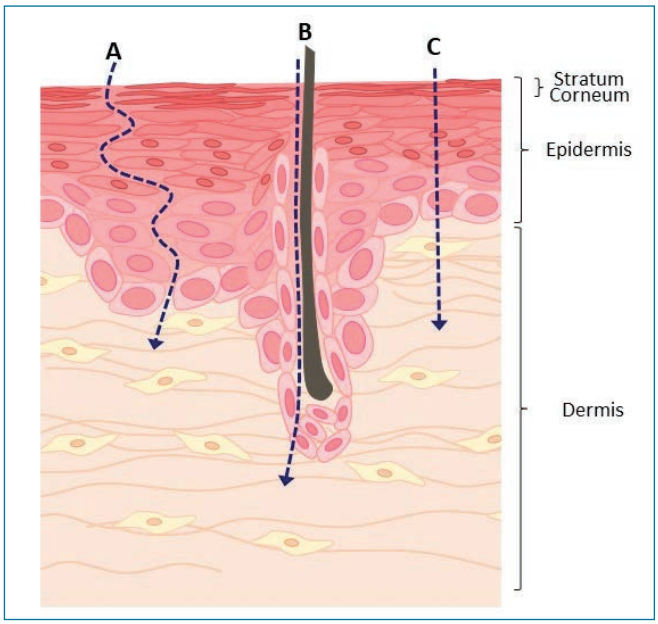

Figure 3: Routes of diffusion through the epidermis
Substances applied on intact skin may reach the dermis through three main routes. (A) Non-polar substances commonly diffuse through the intercellular route via dissolution in lipid-rich ECM. (B) The most efficient transport into the dermis occurs through transappendageal absorption through pores associated with sweat glands, hair follicles and oil glands. However, appendages represent a very small percentage of skin surface area and this reduces the rate of drug penetration. (C) The transcellular pathway through corneocytes represents the shortest distance from the skin surface to the dermis; however, only substances able to diffuse through polar and non-polar environments to efficiently diffuse this way.

when developing antibody-based biotherapeutics. Protein aggregation is the most common stability issue encountered in antibody development[34,35] which influences the potency[36] and safety of antibody therapies[37,38]. Consequently, approaches to alter the aggregation of antibodies through glycosylation[39], altering protein charge[40,41] or the rational design of antibody sequences to remove aggregation-prone regions[41-43] are crucial to the formulation of an effective cutaneous antibody therapy. Fragments of antibodies which comprise the Fab region without the Fc region can still exert neutralising function, and may exhibit greater tissue penetration[44] and stability[45,46] than their whole antibody counterparts. Systemically administered antibodies are generally lyophilised for reconstitution at the bedside; however, topical formulations are often in gel or cream forms, adding additional complexity to maintaining stability of antibodies. Consequently, antibody therapies for skin administration are likely to require multiple re-engineering processes to optimise formulation stability and tissue penetration into the skin.

CHALLENGES OF DELIVERING DRUGS TO WOUNDS

Skin acts as a boundary between the body and the environment and effectively protects internal structures from infection, physical and chemical injury and the loss of water or other valuable compounds. Skin also constitutes a barrier to the topical delivery of antibodies, largely due to the hydrophobic nature of the outer keratinised layer, which prevents delivery of hydrophilic and polar compounds such as proteins[47].

The epidermis represents the outermost layer of the skin and is highly cellular, consisting primarily of keratinocytes. The top layer of the epidermis is termed the stratum corneum and comprises a 10–15 µm thick layer of dead cornified keratinocytes embedded in a lipid-rich extracellular matrix (ECM). Below the epidermis lies the dermis, comprised of fibrous collagen punctuated with blood vessels, hair follicles, nerves and secretory glands. As the dermis is a source of fibroblasts and immune cells which secrete proliferative and inflammatory wound mediators, this is often a desirable site for drug delivery in cutaneous conditions. In open wounds where the wound bed is exposed, direct delivery of antibodies and other biologicals to dermal tissue or wound edges is feasible; however, the amount of therapy administered largely relies on the size of the exposed area. The epidermis may be intact in blister and burn wounds, and treatment of scars also requires penetration through an intact epidermis. Consequently, penetrating the barriers of the stratum corneum and intact epidermis[48] is essential to delivering therapies to certain types of wounds or for managing scarring.

Passive diffusion through the epidermis to the dermis occurs through one or a combination of three main routes: intercellular, transcellular or transappendageal (through dermal appendages, for example, hair follicles, pores; Figure 3). Intercellular diffusion involves diffusion around and between cells, and is the most common transport route for hydrophobic substances as this pathway involves dissolution in lipid-rich ECM. However, transport via this route is slow as substances must diffuse through a convoluted meshwork of ECM which surrounds densely-packed corneocytes — it has been estimated that the path of intercellular diffusion is up to 20 times longer than the thickness of the stratum corneum[49]. Transcellular diffusion that occurs through cells is the most direct route to the dermis which is more commonly exploited by hydrophilic substances, as they diffuse through the keratin-rich cytosol of keratinocytes. However, these substances must still diffuse through the lipophilic cell membrane and some ECM, and thus substances effectively transported via the transcellular route are ideally of low molecular weight (<600 Da) and have hydrophilic and hydrophobic regions[50]. While the transappendageal route allows rapid diffusion of hydrophilic molecules, this method is dependent on the number of pores which comprise under 0.1% of total skin surface, thus transappendageal diffusion is not likely to facilitate delivery of proteins in therapeutic doses[49].

Therapeutic antibodies are poorly suited to passive diffusion as they are too hydrophilic for intercellular diffusion, too large for transcellular diffusion and require higher doses than

can be delivered through transappendageal diffusion alone. Antibodies are also prone to aggregation and denaturation in hydrophobic solvents which reduces their function. However, recent advances in pharmacological methods to penetrate the stratum corneum may provide the solution for topical antibody administration to skin.

CHEMICAL PENETRATION ENHANCERS

Effective delivery of therapeutic antibodies to intact skin is reliant on the use of penetration enhancer (PE) strategies, which aim to reversibly disrupt the structure of the stratum corneum[51]. However, lipophilic PEs may impact the stability of antibodies and cause protein aggregation and denaturation[51,52]. Thus, an ideal chemical PE strategy would exhibit enough hydrophobicity to penetrate the stratum corneum, while protecting protein-based antibodies from denaturation[14,53].

Terpene-based PEs function by associating with lipid ECM and increasing the fluidity of this phase. While it has been demonstrated terpene-based PEs with low lipophilicity may stabilise protein drugs, this effect may be dependent on the physiochemical characteristics of the protein[51] and further studies are required to determine if terpene PEs are suitable for antibody delivery.

Surfactants have the ability to solubilise hydrophilic molecules in a hydrophobic phase and have successfully been used for delivery of drugs such as insulin[54] with no appreciable loss of activity. However, effective doses of surfactants have been demonstrated to cause skin irritation[55], thus it is likely surfactants must be combined with another PEs to provide effective delivery and protein stability with reduced irritation[56].

CELL-PENETRATING PEPTIDES

Peptide-based PEs are of particular interest for the delivery of proteins to tissues, and recent studies have focused on topical application[57,58] which may reduce toxicity compared to chemical PEs. Peptide PEs are short amino acid sequences which enter cells without the use of specific receptors or damaging the cell membrane[59] and are able to cross the stratum corneum. The mechanism by which these peptides enter cells is not well understood; however, endocytosis, keratin interaction and membrane pore formation have been proposed as potential mechanisms[60]. Peptide PEs have been used successfully to topically deliver proteins such as elastin[61], fluorescein[62] and anti-VEGF antibody[63] in preclinical studies and topical cell-penetrating peptides are in clinical development for delivery of botulinum toxin[64], anti-scarring agents[65] and anti-inflammatory cyclosporin A[66]. While topical skin delivery of antibodies using peptide PEs has not been realised in the clinic, this technology provides clear opportunities for well-tolerated topical antibody administration.

PHYSICAL PENETRATION ENHANCERS

Physical methods to disrupt the epithelial barrier use the application of energy to form holes through which therapeutics can enter. In most cases, these methods are used prior to the topical application of aqueous, cream or gel drug formulation; thus formulating antibodies for application using physical PEs may be significantly easier than with chemical-based PEs which involve components that impact protein stability. Some of the most successful physical disruption methods for the delivery of proteins into skin include ultrasound, iontophoresis, electroporation and microneedling[67].

Ultrasound induces transient permeability in the stratum corneum through the formation of small bubbles in the targeted tissue, which allow fluids to pass through as they collapse[68]. This method has successfully been used to deliver molecules up to a size of ~50 kDa, which may make it suitable for the delivery of antibody fragments[69,70]. Ultrasound delivery is capable of delivering molecules to subcutaneous structures such as joint cartilage[71] and transdermally for systemic absorption[70]. Consequently, antibody fragment delivery by ultrasound may be suited to the treatment of deep or tunnelling pressure ulcers or abscesses, or thickened scar tissue.

Transdermal iontophoresis functions by applying charge to drug molecules and applying an external charge to drive molecules through the stratum corneum[72]. It has been adopted clinically for dermal delivery of anti-scarring hormones[73], local anaesthesia[74] and insulin[75]. While delivery of larger proteins has been trialled in some preclinical studies[76] it is unlikely that proteins larger than 10 kDa could be delivered, and delivery of only very small antibody fragments may be achievable using this method. Depth of penetration of proteins using iontophoresis is generally under 150 μm[77]; thus this method is most suitable for delivery of antibody fragments to scars or for the treatment of inflammatory skin conditions, rather than deeper tissue lesions.

Electroporation methods apply high-voltage pulses across the skin to perturb the barrier and cause transient pores to form. Electroporation is currently used in the clinic for delivery of DNA and small molecules into tissues for cancer therapy[78] and vaccination[79] and has successfully delivered peptides and small proteins in preclinical studies[80,81]. While high voltages are required for transdermal protein delivery, dermal delivery may require lower voltages, which may reduce patient discomfort associated with the procedure. Electroporation has also been used preclinically to assist with cosmetic skin regeneration[82] and scar resolution[83], thus this method may have additional benefits for managing scarring following trauma or burns.

Microneedles are thin micro-scale projections which can pierce the skin to access structures below the stratum corneum. Microneedles can be used either to pretreat the skin to improve drug penetrance or deliver drugs directly into the skin, either via hollow bore needles for drug infusion or needle tips coated with or comprised of dissolvable drug formulation[84,85]. Microneedling is considered painless

as the needles do not stimulate nerves within the dermis, and pores formed by microneedling are within micron range and thus close rapidly (generally within 12 hours)[86]. Microneedle pretreatment has improved the penetration of peptides[87], anti-cancer agents[88] and anti-scarring agents[89] and seems amenable to the application of large biologics such as antibodies[90]. Microneedling itself has been shown to initiate healing responses in skin which can reduce scars and thus may serve an additional adjuvant-effect of stimulating wound healing pathways[91]. However, the use of microneedles as skin permeabilisation agents for topical antibody treatment may be limited by the longevity of pores formed[67]. As microneedles form larger pores than other physical skin penetration methods, this method is unlikely to be useful in conditions where epidermal layers are poorly adhered, such as blistering conditions. However, microneedle delivery may be particularly useful for delivering therapeutic quantities of antibodies to surgical wound edges or tissue surrounding chronic wounds, and it had been postulated that microneedles may also be a convenient method for antimicrobial delivery to infected wounds[92].

Recent research efforts have focused on developing coated or dissolvable microneedles for the direct delivery of antibodies through application of a microneedle patch. The challenge of this delivery method lies in using fabrication methods amenable to maintaining the stability of the antibody — antibodies must be desiccated or solidified to form dissolvable microneedles, which may promote aggregation[93]. Selection of a compatible microneedle matrix is also crucial to achieving protein stability and ensuring antibodies can disperse into solution. A preclinical study recently demonstrated dissolvable hyalruonic acid-based microneedles can deliver anti-PD1 antibody transdermally to subcutaneous tumours[94,95]. Clinically, microneedle delivery has been trialled for influenza vaccination and shown to be safe and effective, with few adverse effects at the site[96]. As microneedle delivery relies on local diffusion from the puncture site, it is most suited to areas where the epidermal barrier is intact, including minor burns, scars or intact wound edges and would not be suitable for blisters. While challenges in stability of formulation still exist, microneedles may represent an effective method for dermal delivery of therapeutic antibodies.

CONCLUSION

Poorly healing wounds remain a significant healthcare burden, and antibody therapeutics may fill a clinical niche in treating chronic, non-healing wounds or scars. Local antibody administration has many benefits over systemic methods, including reduced therapeutic cost, higher drug availability at the site and reduced adverse effects associated with local administration. However, dermal delivery of antibodies remains to be realised within the clinic due to the delicate nature of antibody proteins and the formidable barrier of the stratum corneum. Despite these challenges, several new technologies or combinations of these technologies have the potential to render antibody therapeutics a viable treatment option for wound healing.

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