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Extent of iron pick-up in deforoxamine-coupled polyurethane materials for therapy of chronic wounds

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Abstract

Polyurethane net substrates (PNS) coupled with deferoxamine (DFO) have been studied to determine the extent of Fe^{2+} pick-up for use in chronic wound therapy. A M solution of ferrous sulphate ($FeSO_4$) was used to generate ferrous ions similar to those found in chronic wounds. The concentration of Fe as a function of position through the dressings was evaluated using a variety of techniques. Atomic force microscopy (AFM) and energy-filtered transmission electron microscopy (EFTEM) revealed a rough precipitated layer at the surface of activated PNS exposed to $FeSO_4$ solution. Optical microscopy (OM) and backscattered environmental scanning electron microscopy (ESEM) showed a clear layer of Fe^{3+}-enriched material in the surface regions exposed to DFO. The penetration depth of DFO into activated dressings was found to be 20–30 μm. Energy-dispersive X-ray (EDX) analysis was used to approximate the distribution of bound- and unbound-Fe as a function of position within BPNS and DFO-activated dressings after immersing them in a $FeSO_4$ solution for various times. These studies have shown the activity of iron with respect to ionic state in DFO-activated PNS for potential using as dressing for chronic wounds.

Keywords: Wound dressing; Inflammation; Polyurethane; Bioactivity

1. Introduction

Wound healing is a highly complex biological activity involving numerous biochemical processes. Chronic wounds are those where the healing process is impaired and the wound either does not heal or takes an unacceptably long time to heal (sometimes many years). Longevity or failure to heal is due to a permanent state of inflammation [1]. Developments in the understanding of chronic wounds such as venous leg ulcers, diabetic foot ulcers, and pressure ulcers have recently led to more-effective and less-invasive treatments.

Treatments for chronic wounds vary considerably. Common therapies include exudate management, moist wound healing dressings and compression therapy. These often take long times to acquire minimal results. More aggressive 'hard to heal ulcers' require grafting of tissue-engineered products such as Dermagraft™ or natural skin from the patient. Some success has been found using these procedures; however, a less-invasive treatment that provides rapid results is more desirable.

A number of biochemical factors are thought to contribute to the persistent nature of chronic wounds [2]. Recent research has shown that the level of oxidative stress in chronic wounds differs greatly from those levels observed in acute wounds [3,4]. Oxidative stress in chronic venous ulcers [5] is caused by an imbalance of reactive oxygen species resulting in high levels of

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potentially damaging pro-oxidant free radicals such as hydroxyl radicals (OH) [3]. Iron released from haemoglobin, and other iron–sulphur proteins plays a major role in the facilitation of oxidative stress [6,7]. Hydroxyl radicals formed via the Fenton reaction from hydrogen peroxide exists in the wound environment [8]. The Fenton reaction is shown as follows:

$$O_2 + Fe^{3+} = Fe^{2+} + O_2 \text{ (singlet)},$$

$$H_2O_2 + Fe^{2+} = Fe^{3+} + OH^{\cdot} + OH^{-},$$

Together they form the Haber–Weiss reaction:

$$H_2O_2 + O_2 = O_2(\text{singlet}) + OH^{\cdot} + OH^{-}.$$

These processes rely on the presence of iron ions that redox cycle between Fe^{3+} and Fe^{2+} thus making the reaction self-perpetuating [9]. The singlet oxygen and hydroxyl radicals irreversibly oxidize biological materials such as lipids [10,11], proteins [12,13], nucleic acids and connective tissue [14] thereby causing irreparable damage. This has been evidenced by increased iron concentrations as well as oxidation damage found in patients suffering with venous ulceration. The increased levels of iron ions within the wound environments is thought to contribute to their chronic nature by maintaining the generation of reactive oxygen species [15]. In a healthy environment, iron is safely bound to proteins thus preventing damaging oxidative reactions. However, in chronic wounds, evidence suggests that iron is present in the ionic form or loosely bound to proteins such as albumin.

Recently, medical grade materials have been developed to bind free iron [16], so it may be removed from the wound bed during dressing change [17]. These materials are made up of a polymer substrate such as cellulose [18] or viscose that has an iron chelator covalently bonded to it. The chelate of choice is deferoxamine (DFO), which is a medically approved material used in the treatment of iron overload. The DFO not only binds iron tightly but also prevents it from redox cycling. The use of these substrates as wound dressings is not ideal due to the stiff nature of cellulose.

A polyurethane net substrate (PNS) with DFO covalently bound to its surface [19] was used for the current study. Polyurethane is an attractive matrix because it offers a high degree of conformability to the wound surface and does not shed material into the wound as experienced with cotton and non-woven fibre substrates. The polymer net was formed by reactively extruding an isocyanate functional pre-polymer with a tetra-functional polyether polyol to yield a hydroxyl-rich polyurethane as shown in Fig. 1. DFO was then covalently bonded to the net surface by a two-stage process. The first stage involved the formation of a carbonyl–imidazole complex with the surface hydroxyl groups followed by the second-stage reaction of the complex with DFO.

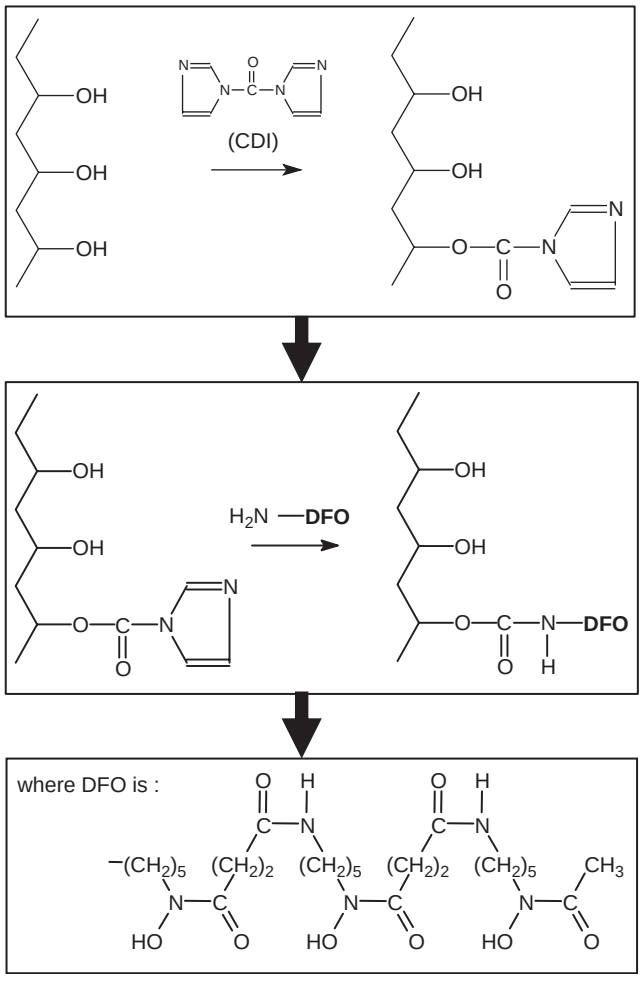

Fig. 1. Chemical schematic showing the binding of DFO to the PNS matrix.

The purpose of this work is to characterize the extent of iron binding within the polyurethane net material with and without exposure to DFO. Exposing the PNS to an aqueous ferrous sulphate ($FeSO_4$) solution produces an environment with high concentrations of Fe^{2+} ions [20]. The location of bound Fe (likely to be Fe^{3+}) and unbound Fe (likely to be Fe^{2+}) will be examined by using an array of characterization techniques.

2. Materials and methods

2.1. Materials

PNSs were synthesized at Smith and Nephew Inc., York. Perforations were made in un-reacted polyurethane gel films to form PNS with high surface area. Activated PNS (APNS) were made for the pick-up of Fe^{2+} by covalently bonding DFO to the surface of both sides of the PNS using the process described in Section 1.