Collagen and Wound Healing – The Impact of Single Alpha Chain Collagen

Key words: Wound healing, matrix metalloproteinases (MMPs), animal free collagen, Type I collagen.

Abstract

Collagen plays a central role in wound healing not only in terms of restoring the structure and mechanical properties of the damaged tissue but also in the cascade of molecular events that occur during the tissue repair process. In this white paper we report on how single alpha chain collagen significantly upregulates expression of the interleukins IL-6 and IL-8; molecules that are key to wound healing. This activity of collagen alpha chains can be utilised in dressings and devices to aid in improving the process of wound treatment.

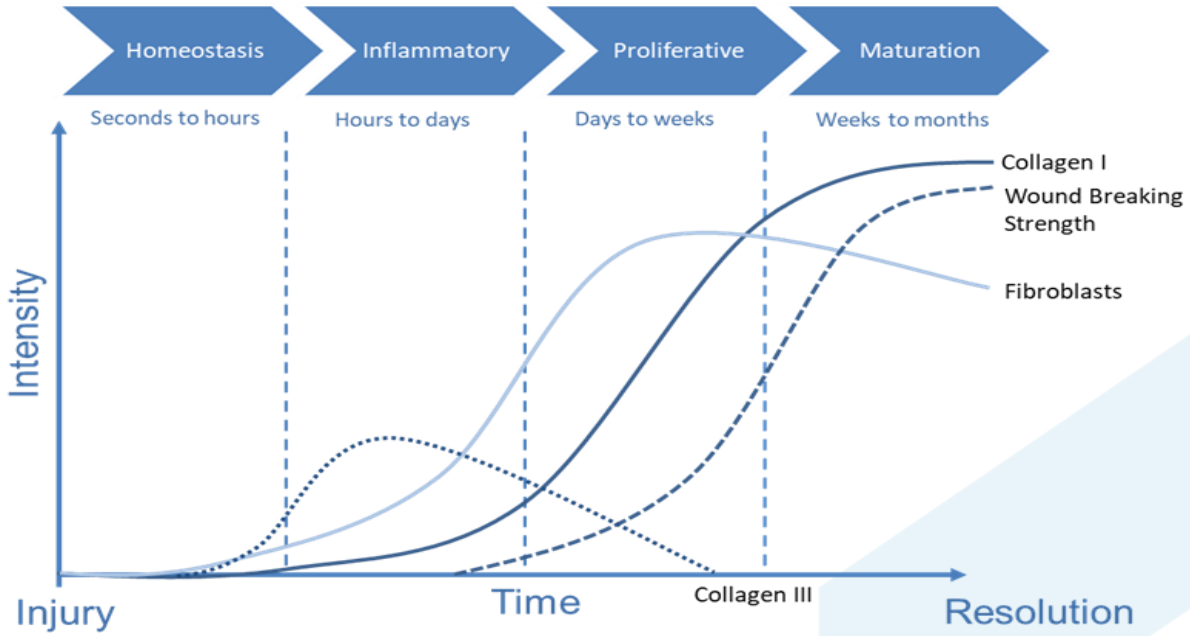

Figure 1. The phases of wound healing; adapted from McElroy and Webb (1)

Introduction

The management of wounds is constantly improving with greater understanding of the healing process. As regenerative medicine better understands how the body orchestrates the multitude of factors that are involved in wound healing there is great opportunity to improve wound treatment, especially important in severe cases such as deep tissue burns or chronic wounds of the elderly. Current treatment of severe or chronic wounds tends to be expensive and still unfortunately unable to achieve ideal healing. However, we are seeing significant improvements in wound care technology and strategies as wound healing materials become smarter with the inclusion of bioactives such as antimicrobials and proteins, that improve the process of wound healing. Collagen, a key protein of tissues is critical for the regulation of the different phases of wound healing (1) either as a fibrillar assembly, triple helical form or as degradation fragments found in the wound bed (Figure 1). Thus, collagen is critical for inclusion in wound treatment materials and there are an increasing number of products that use collagen base materials in the form of gels, fibres, foams, coatings or powder forms.

In this white paper, we discuss how ProColl's single alpha chain collagens (Type I α1 and α2) provide stimulation of wound healing, with great potential for the development of the next generation of wound dressings.

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Single Alpha Chain Collagen

Collagen is a triple helix consisting of three alpha chains, held together by hydrogen bonding and crosslinked into mature tropocollagen. (Type I collagen is made up of two α1 and one α 2 single chains) (2). The insoluble triple helical form is assembled in tissues from procollagen alpha chains that the cells have synthesised. Traditionally, when collagen is extracted from animal tissue the resultant material contains all forms of the collagen, single chains, double chains, and triple helical, as well as some residual fibril stacked collagens. At ProColl, we have scaled up the extraction and purification of single alpha chain collagens, ensuring purity of the collagens we supply. ProColl also use synthetic biology to manufacture animal-free human collagen molecules.

Having a bulk supply of single alpha chain collagen (Type Iα1 Iα2), previously unavailable to the research community, has allowed us to investigate the properties and application of these molecules. Firstly, a point to highlight is the water solubility of the single alpha chain collagen form (98% w/v in PBS and other benign physiological buffers) compared to the triple helical collagen (~1.5% w/v in acetic acid), this means it is easier to use this water-soluble collagen in fabrication of medical devices such as wound dressings. (See our white paper on electrospinning collagen as an example). Secondly, and importantly, single alpha chain collagen has bioactive sites, that would normally not be exposed in the triple helical form of the molecule, which explains how this stimulates such key molecules of the wound healing cascade (3,4). Further, the ability for our single alpha chain collagen to reform into a triple helix in physiological conditions offers opportunities to advanced wound healing.

Collagen and Wound Healing

Collagen is essential in the wound healing process, beginning when platelets aggregate around exposed collagen as part of the body's own process for physically blocking the wound and ceasing blood flow (5). The damage to the tissue and the extracellular matrix during wounding triggers a cascade of events that include the release of collagen fragments that influence the healing processes through molecular mediators of inflammation, angiogenesis, and re-epithelialization (6). Later in the process, as the wound heals, cells such as fibroblasts synthesise collagen to reinstate the tissue architecture with the collagen fibrils that form the extracellular matrix.

In this research we examined the production of interleukins (IL-6 and IL-8), mediators of wound healing, after exposing cells to different collagens. Interleukin IL-6 is produced in response to tissue injuries and contributes to host defence through the stimulation of the acute phase of wound healing. IL-8 is also an important protein related to inflammation and also an integral part of the wound healing process

Methods

The cells studied were human umbilical vein endothelial cells (HUVEC), which are primary cells isolated from the vein of the umbilical cord. They are a model system for studying cell function, with applications including understanding hypoxia, inflammation, oxidative stress, response to infection, and both normal and tumour-associated angiogenesis. HUVECs were seeded on a precoated 96 well plate at a density of 50000 cells/cm^2 or medium alone for 48 hours. IL-6 and IL-8 release (pg/ml) was determined by measuring the absorbance at 450nm. with all assays performed in triplicate. The data is presented as the mean ± standard deviation. Statistical significance is denoted as the following: compared to the tissue culture plastic non-coated control $p<0.01$(*); and compared to Gelatin $p<0.01$(#) (Figure 2).

Results and Discussion

Recombinant single chain collagens (α1 and α2) significantly upregulated IL-6 and IL-8 pathways when compared with either triple helical collagen, gelatin or tissue culture plastic, indicating that single alpha chains have a direct, positive influence on wound healing (Figure 2). The influence of exposed collagen fragments on wound healing has also been observed in previous research (3,4,5,6). During the inflammation phase, the peptide fragments of collagen were shown to have a chemotactic effect in the recruitment of cells essential to wound healing, while the collagen derived peptides stimulate fibroblast proliferation during the proliferation phase (5,6). When migrating cells such as keratinocytes encounter type I collagen in a wound, the cells secrete matrix metalloproteinases (MMPs) which denature the collagen to gelatine in order to expose the active RGD site (Arg-Gly-Asp) sequences, which are responsible for the creation of granulation tissue (5). Previous research has also shown that when the collagen triple helix is unwound, through partial denaturation, elements of the molecule are exposed that up regulate many pathways important in wound healing (3,4).

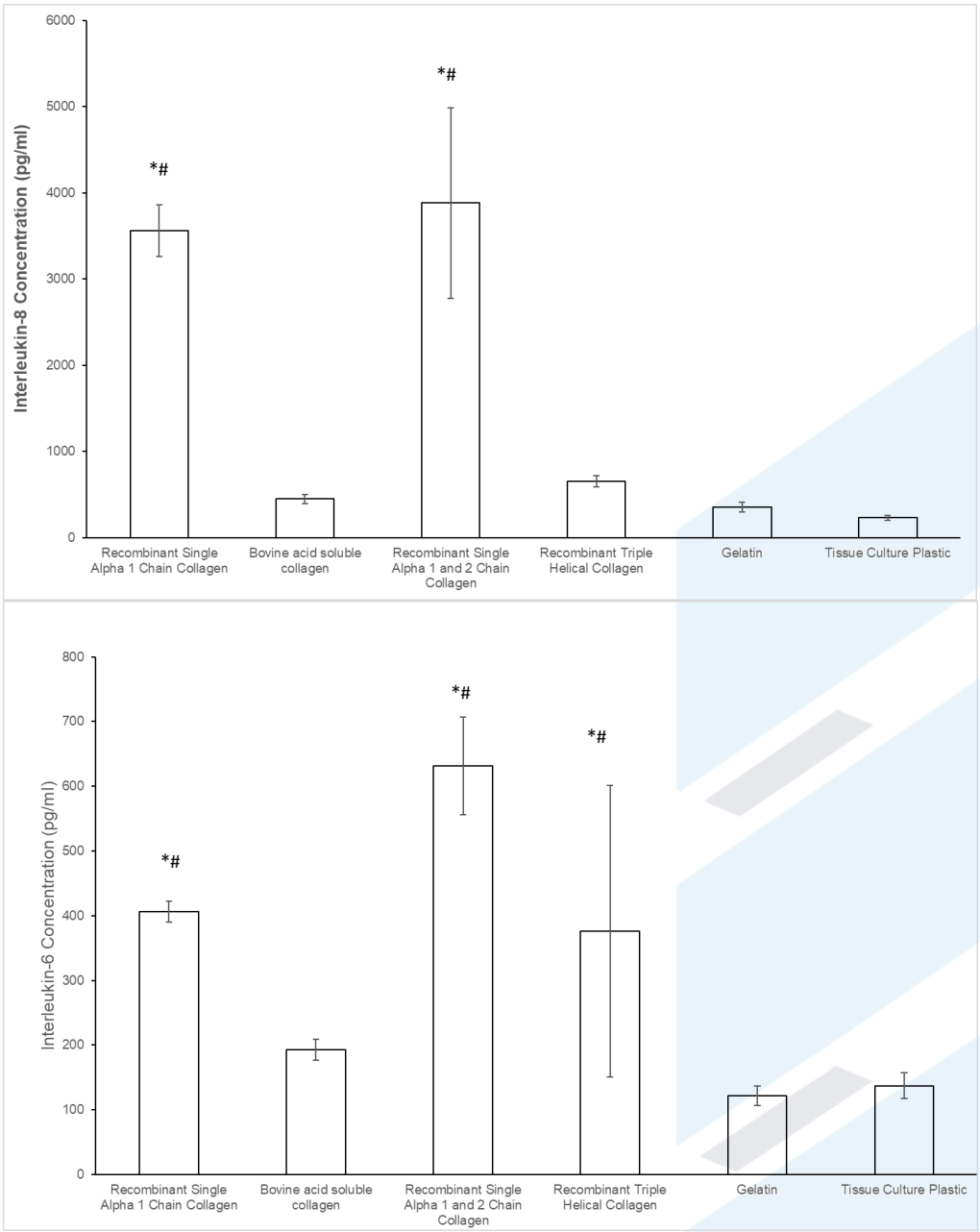

Figure 2. The upregulation of IL-8 (i) and IL-6 (ii) expression by single alpha chain collagen. The data is presented as the mean ±standard deviation. Statistical significance is denoted as the following: compared to the tissue culture plastic non-coated control $p<0.01$(*); and compared to Gelatin $p<0.01$(#)

Conclusion

The research in this white paper has demonstrated that single alpha chain collagen stimulates key pathways in the wound healing cascade. This is due to fragments of the collagen triple helix influencing the wound healing process positively. The ability of ProColl to manufacture bovine and animal-free recombinant single alpha chain collagen at scale now enables the exploitation of the wound healing stimulatory properties of these key molecules.

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If you are interested in purchasing ProColl’s collagen, or joining our growing list of distribution and OEM partners, please use the contact form on our website (www.procoll.co.uk) and one of the team will be in touch. ProColl’s collagen is also available direct for both research & bulk orders.