Biologically Active Wound Dressings Derived from Human Hair

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Introduction

There are two theories for the biological activity of human hair extracts. The first is that the human hair keratins themselves are biologically active. Over 70 human hair keratins are known and their cDNA-derived sequences published. However, the full compliment of human hair keratins is unknown and estimates of over 100 have been proposed.[1] Within the complete range of HHKs are a small number that have been shown to participate in wound contracture and cell migration.[2] In particular, keratins K-6 and K-16 are expressed in the epidermis during wound healing and are also found in the outer root sheath of the hair follicle.[3] The presence of these human hair keratins in extracts of human hair, and their subsequent dosing directly into a wound bed, may be responsible for "shortcutting" the otherwise lengthy process of differentiation, migration, and proliferation, or for alleviating some biochemical deficiency, thereby accelerating the tissue repair and regeneration process.

A second theory is that a number of growth factors are present in end-cut human hair, and that the keratins may be acting as a highly effective delivery matrix. It has been known for more than a decade that growth factors such as bone morphogenetic protein-4 (BMP-4) and other members of the transforming growth factor-β (TGF-β) superfamily are present in developing hair follicles.[4,5,6] In fact, more than 30 growth factors and cytokines are involved in the growth of a cycling hair follicle.[7] Many of these molecules have a pivotal role in the regeneration of a variety of tissues.[8] It is highly probable that a number of growth factors become entrained within human hair when cytokines bind to stem cells residing in the bulge region of the hair follicle.[9] These growth factors would most certainly be extracted along with the keratins from end-cut human hair.

Materials and Methods

Several *in vitro* and *in vivo* studies were conducted to demonstrate the biological activity of keratin biomaterials. They involved the use of keratin proteins derived from human hair using oxidation and reduction reactions to break down the tertiary structure of the cortex and extract soluble proteins according to the following methods.

Keratose: Clean, dry hair was cut into small fibers and oxidized with peracetic acid. Free proteins were extracted using a denaturing solution, neutralized, purified by dialysis, concentrated, and isolated by lyophilization. A hydrogel was formed by re-hydration with phosphate buffered saline (PBS).

Keratose intermediate filament (IF): During the process of isolating keratose as described above, a fraction of gelatinous material is isolated by centrifugation. This fraction consists of intermediate filament keratins as a result of incomplete oxidation of the cortical region of the hair fibers. Keratose IF gel was neutralized and tested without further purification.

Kerateine: Clean, dry hair was cut into small fibers and reduced with thioglycolic acid. Free proteins were extracted using a denaturing solution, dialyzed, neutralized, and concentrated. Upon concentration, a viscous hydrogel formed upon exposure to air.

Cell proliferation: Keratose powder was dissolved in culture media with and without serum at several concentrations and used to culture human dermal fibroblasts and keratinocytes. The cells had been grown to ca. 50% confluency in serum-containing media and serum starved for 24 hours prior to exposure to the keratin-containing solutions. After 24 hours of culture with the keratin-containing media, cell proliferation was evaluated using a mitochondria metabolic assay (MTT assay).

Wound healing: Immune competent mice were de-haired and a chemical burn induced between the shoulders using phenol. The wounds were treated after 20 minutes with a keratin hydrogel and occlusive bandage. Dressings were changed daily for up to 10 days. Digital photos of the wounds were taken and animals sacrificed at various time points so that the wound area could be excised for histological examination.

Results and Discussion

Cell proliferation assays using keratinocytes and fibroblasts showed statistically significant increases in the keratose treated groups (Figure 1). Wound healing studies in mice demonstrated an interesting passivation of the chemical burn. Figure 2 shows the normal course of wound progression in a wound treated only with an occlusive dressing as initially increasing in wound area. This is due to destruction of vascular support of the peripheral tissue followed thereafter by necrosis at the wound margins. The result is a characteristic growth of the wound area. In the keratin treated groups, however, the trend was toward stabilization of the wound area at the onset of injury. This is thought to be due either to a protective mechanism that limits morbidity, or rapid induction of angiogenesis that counteracts the initial loss of vascular support.

Conclusions

Keratin biomaterials derived from human hair contain intrinsic growth factors that mediate the biological activity of these unique biomaterials. In cell culture experiments, certain types of keratins are strong mitogens of skin component cell types. Keratin-based hydrogels are capable of passivating chemical burns such that the characteristic growth of the wounds is not seen in a mouse model. Keratin biomaterials may have tremendous potential as wound dressings as they are capable of not only growth factor mediated wound healing, but absorption of exudate and drug delivery.

Acknowledgements

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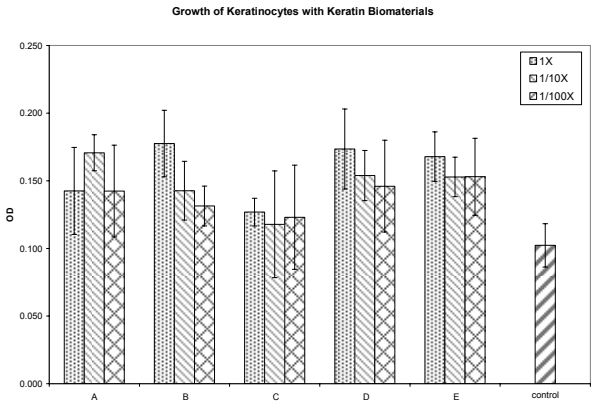
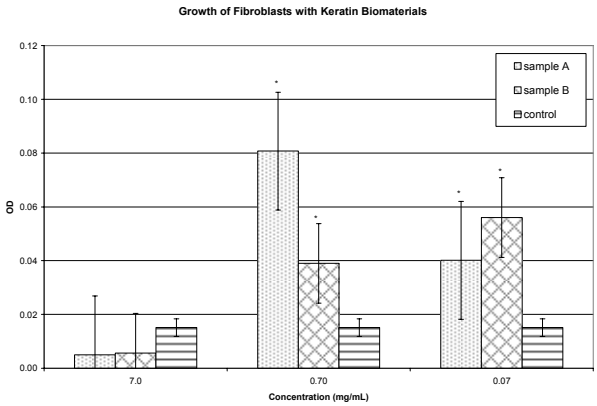


(a)



(b)

Figure 1. Skin component cell proliferation. Keratinocytes (a) and fibroblasts (b) treated with soluble keratin biomaterials proliferate more than do cells treated with media alone.

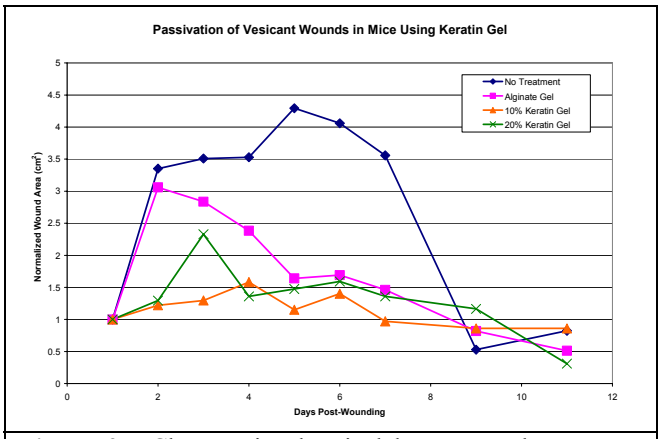


Figure 2. Changes in chemical burn wound area over time. Mice treated with phenol to induce a chemical burn experience a passivation of the wound site such that the normal course of wound growth does not occur. This is thought to be mediated by growth factors found in the keratin biomaterial.

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