Biocompatibility of Keratin Biomaterials Derived from Human Hair

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Introduction

Keratins are a family of structural proteins found in the protective tissues of vertebrates. Hair fiber is made up of greater than 90% keratins. These proteins represent a readily available source of human-derived tissue that can be harvested with no injury or trauma to the donor. Keratins possess several unique characteristics that make them useful as biomaterials such as molecular self-assembly, biological activity, and labile chemistry. Recently, it has been suggested that keratins can be used as scaffolds for tissue engineering.[1] Moreover, we have shown that keratins have a remarkable ability to mediate cell migration and enhance proliferation.[2] However, to date, the basic biocompatibility of keratin biomaterials has not been demonstrated. In the present study, we have explored *in vitro* biodegradation, cell compatibility, *in vivo* biodegradation and host immune response using three different keratin biomaterials.

Materials and Methods

Human hair was obtained from a local salon, washed with warm water and mild detergent, degreased with organic solvent and air dried. The clean dry hair was processed to afford three different types of hydrogels.

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.

Hydrolytic stability: 500mg of each hydrogel was exposed to 3mL of PBS at pH 7.2 at 37°C. Samples of the PBS were taken at several time intervals and analyzed spectroscopically for total protein content.

Structural analysis: Small discs of each hydrogel were prepared by lyophilization and examined in cross section for morphology and architecture by SEM and histology.

Cell compatibility: Keratose powder was dissolved in culture media with and without serum at several concentrations and used to culture amniotic fluid-derived stem cells. 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).

Host response: Lyophilized discs of each hydrogel were implanted into the subcutaneous space of mice. At several time points, the scaffolds and surrounding tissue were retrieved and analyzed histologically.

Results and Discussion

Oxidation of human hair fibers results in almost complete scission of disulfide bonds within the cortex. This chemistry results in highly water soluble proteins that are readily extractable in buffer solution. Consequently, hydrogels formed from these materials are susceptible to hydrolytic degradation. A keratose IF scaffold readily re-hydrates and degrades in PBS at pH 7.2 and 37°C.

Structural characterization of keratose, keratose IF, and kerateine lyophilized discs revealed a highly porous architecture. Keratose IF was the most fibrous, while kerateine formed unique self-assembled architectures. Fiber diameters and pore sizes in the keratose IF hydrogel were on the order of 15 and 200 microns, respectively. This architecture was shown to be conducive to homogenous cell seeding. Cell compatibility was demonstrated using the MTS assay in which proliferation of cells in keratin treated cultures was higher than that of the control (media alone) at several doses. Finally, sub-Q implantation experiments showed robust host cell infiltration, initially at the periphery of the scaffolds, followed by population throughout the construct at later time points. Angiogenesis was evident by H&E staining.

Conclusions

Keratins are potentially one of the most biocompatible materials for regenerative applications in that they are one of the few human tissues that can be collected from donors with no injury or trauma. The biodegradation can be controlled depending on the processing used to obtain keratin extracts and the post-extraction chemistry performed on labile amino acid groups. Properties such as architecture and hydrolytic susceptibility can be modulated using a variety of techniques. The cell compatibility and host response to the materials investigated in the present study was excellent with slight mitogenic activity and good host response observed.

References

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