Keratin Proteins for antibiotic release and bone regeneration

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Introduction

Keratins are a ubiquitous family of structural proteins found in the protective tissues of vertebrates and comprise more than 90% of the hair fiber. Distinct fractions of keratins can be extracted from human hair and used for development of novel biomaterials. In this study, we efficiently extracted useful keratin proteins along with active growth factors from end-cut human hair and formulated them into a prototype bone graft substitute. A controlled release system was added to the formulation that can be useful for infection control by facilitating sustained release of antibiotics.

Materials and Methods

Human hair was processed by oxidation and the free protein extracted with a denaturing solvent. An α-keratose fraction was isolated from the oxidized fibers by isoelectric precipitation and a γ-keratose fraction by addition to a non-solvent of the crude extract. The fractions were re-precipitated and dialyzed against deionized water. Keratose samples were processed to a dry powder by lyophilization, and rehydrated with phosphate buffered saline (PBS) to form hydrogels. Keratose hydrogels were used as a base material to create a bioceramic putty ("KBAP" or keratin bioceramic antibiotic putty) that included a bioceramic filler and encapsulated antibiotics. This mixture formed a viscous paste that could be molded into self-supporting shapes. Several KBAP formulations were structurally characterized by SEM. Antibiotic release kinetics were determined *in vitro*, and cell compatibility and chemotaxis was investigated with osteoblasts using a cell migration assay.

Results and Discussion

Keratin biomaterials have a unique capability of molecular self-assembly, a process by which they reconstruct some semblance of their original tertiary structure (Fig. 1). The structures produced by this self-assembly process are highly porous and conducive to host cell infiltration. KBAP was formulated by blending cefazolin, α+γ-keratose, hydroxyapatite (HA), chitosan lactate and sodium alginate and enzymatically cross-linked by transglutaminase. Keratin biomaterials containing antibiotics were used to generate kill curves for suspensions of *Staphylococcus aureus*. The release kinetic profile from a keratin-based antibiotic drug delivery system (DDS) revealed a controlled release of cefazolin for a week at near zero order release rate (Fig. 2). We have shown that KBAP formulations are highly compatible with osteoblasts. Extracts of KBAP were shown to osteoblast migration, suggesting the scaffold has chemotactic potential (Fig. 3).

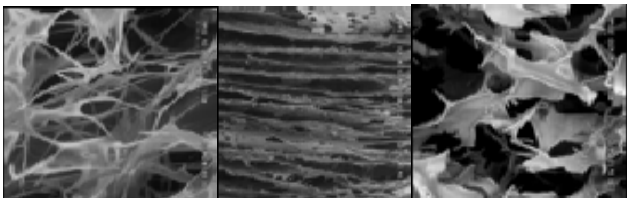

Figure 1. Keratin biomaterial scaffolds formed spontaneously by a self-assembly mechanism. Keratin biomaterial can generate a variety of microstructures by controlling their composition.

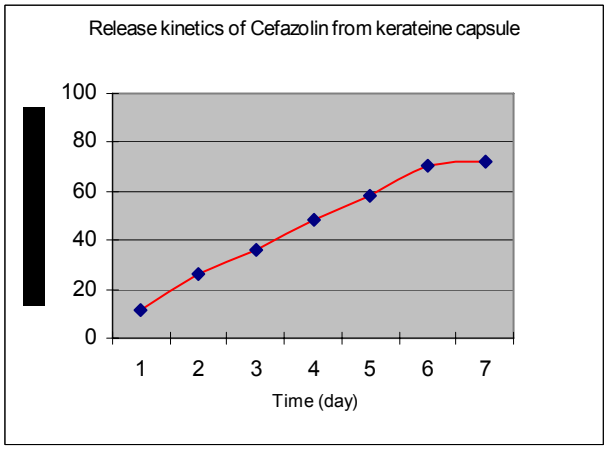

Figure 2. Release kinetics of keratin matrix particles maintained in PBS at 37°C. This DDS displayed near zero-order release kinetics over a period of seven days.

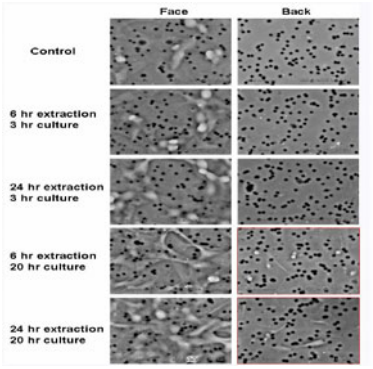

Figure 3. Chemotactic assay of KBAP formulation. Osteoblasts migrate in response to a concentration gradient formed by slowly dissolving KBAP pellets containing intrinsic growth factors.

Conclusions

Keratin bioceramic antibiotic putty (KBAP) is a prototype bone graft substitute that combines the characteristics of biocompatibility, osteoconductivity, chemotaxis, as well as controlled and sustained antibiotic release into an easy to use and inexpensive biomaterial. Self-assembly capability, combined with a remarkable biological activity, affords unique properties that may be conducive to host cell infiltration and rapid regeneration of bone tissue.