Structure-Function Relationship of Meta-Kerateine Biomaterials Derived from Human Hair

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STATEMENT OF PURPOSE

Keratins have been explored as a biomaterial system for more than two decades. Recently, several potential medical applications have been identified and explored in large animal models. Most of this work has been done with films and gels made from a heterogeneous mixture of different keratin sub-types which has resulted in biomaterials of limited mechanical strength. Several investigators have attempted to engineer other structures such as foams, sponges, and fibers with limited success, primarily due to a lack of fundamental understanding of the molecular superstructure of these materials. The purpose of this work was to establish a predictable relationship between the composition of kerateine biomaterials and their physical and biological function. Control over the composition of kerateine materials was achieved through separation and purification of protein sub-types, and their de novo recombination into meta-keratins. The structure-function relationship was established using physical, mechanical, and biological characterizations.

METHODS

Protein Extraction: Human hair fibers were reduced with thioglycolic acid. Free proteins were extracted using a denaturing solution, and isoelectric precipitation was used to separate the crude extract into alpha (rod-like) and gamma (globular coils) sub-types. Kerateine extracts were analyzed by SDS-PAGE and quantitative amino acid analysis. Free thiol amount was measured using the Ellman's reagent assay.

Fabrication of Meta-Kerateine Sponges: Meta-kerateines were created by combining alpha and gamma fractions at varying ratios, and viscous hydrogels were formed by exposing the concentrated protein to air to affect disulfide crosslinking. Meta-kerateine sponges were formed by freezing the hydrogels at -80˚C followed by lyophilization.

Physical Characterization: The effect of alpha/gamma ratio on hydrogel formation was assessed, and SEM was used to examine the microstructure of the lyophilized materials. *In vitro* degradation was assessed over a six month time period using the Bradford-Lowry method to measure the amount of protein released into solution in relation to the initial mass of the sample. The swelling behavior of the materials was assessed and used to determine differences in the crosslink density of the meta-kerateines structures.

Mechanical Characterization: Confined compression tests were conducted using an Instron 5544. The compressive yield strength and compressibility of the meta-kerateine sponges were determined and used to establish a relationship between mechanical integrity and meta-kerateine composition.

Biological Characterization: Cellular compatibility was assessed using the CyQuant® assay to quantify cell adhesion and proliferation. Cell attachment was also visualized using SEM.

RESULTS

Characterization of kerateine extracts revealed the presence of high molecular weight alpha proteins and lower molecular weight gamma proteins. These proteins showed amino acid sequences and thiol content consistent with those values reported in literature. The structural architecture, degradation, and swelling behavior of the meta-kerateine sponges were shown to vary with the ratio of alpha and gamma kerateines. The compressive strength of the meta-kerateines was not dependent on composition, however, the stiffness of the sponges decreased with increasing gamma content (Figure 1). All materials supported cellular attachment (Figure 2) and proliferation.

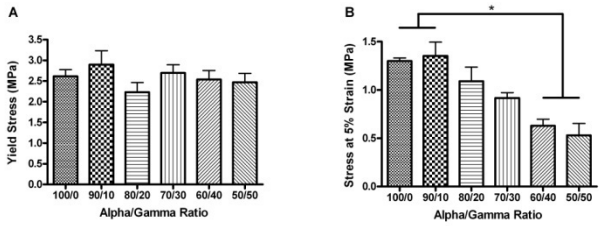

Figure 1. (A) Yield stress and (B) compressive stress at 5% strain of meta-kerateine sponges in confined compression. (*p<0.01; n=3)

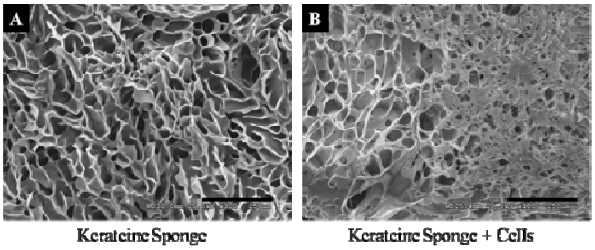

Figure 2. SEM images of (A) kerateine sponge and (B) cell-seeded sponge after 3 days of cultivation. Scale bar is 250μm.

CONCLUSIONS

The ability to formulate natural biomaterials to have controlled physical and biological characteristics is advantageous for the development of a number of biomaterial applications. This study has shown that the structure and mechanical characteristics of kerateine biomaterials may be modulated by altering the ratio of alpha- and gamma-kerateines to create materials with predictable physical and mechanical properties, while preserving the innate biological properties.