Adhesive and Proliferative Response of Primary Mouse Dermal Fibroblasts to Human Hair Keratins: An *in vitro* Wound Healing Evaluation

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STATEMENT OF PURPOSE

Like many structural proteins found in nature, "hard" keratins have shown promise in tissue regenerative studies conducted *in vivo*. Preliminary analyses of 2nd degree thermal burns on pig skin collected by our lab suggest that keratin biomaterials can accelerate the wound healing process by a) restricting post-injury wound growth and b) increasing the rate of healing for 14 days post-injury. Despite these results, little is known about the underlying cellular mechanisms governing the interactions of dermal cells and keratin. The ability of fibroblasts to attach to and proliferate onto provisional fibrin-fibronectin matrices formed during wound inflammation drives the formulation of granulation tissue. Keratin applied as a hydrogel wound dressing or formed into a biodegradable scaffold may accelerate this process.

In this study, primary mouse dermal fibroblasts seeded onto keratin coatings were evaluated for their propensity for attachment using the Calcein AM static adhesion assay. Fibroblasts were also exposed to keratin in growth media to monitor their proliferative activity using the MTS assay method.

METHODS

Primary cell isolation: Fibroblast cells were isolated from the intact skins of CD-1 mouse pups (0-2 days old), based on a procedure adapted from Lichti, *et al.*[1] Cultured cells from passages 2-6 were used in this study.

Keratin preparation: Soluble hair keratins were extracted from manufactured Chinese hair via oxidative methods.[2] Crude extracts of keratin were separated into their alpha and gamma components. The alpha extract was further purified into acidic Type I and basic Type II fractions. Concentrated extracts of each keratin fraction were lyophilized into fine powder form and sterilized via gamma irradiation (Co^{60} source, 1 MRad) prior to experimental use.

Calcein AM adhesion assay: Keratin dissolved in PBS (200 µg/ml) was added to 96-well tissue petri dishes and incubated at 4°C overnight to produce coatings. Rat tail collagen (Type I) and human fibronectin (HFN) coatings served as positive controls; uncoated wells as a negative control. Primary fibroblasts were seeded onto the dishes and allowed to adhere for 2 hrs. Unattached cells were washed away with PBS; the remaining cells were labeled with Calcein AM at 37°C for 30 min. Fluorescence readings at 485/535 nm were taken to determine relative cell numbers per treatment group.

MTS proliferation assay: Fibroblasts were seeded onto 48-well tissue culture plates and allowed to proliferate for 1 day prior to treatment. Keratin was dissolved in fibroblast growth media (high glucose DMEM + 10% fetal bovine serum) to prepare 10 µg/ml treatment solutions. Cells subjected to standard fibroblast growth media (FM) served as a positive control; those pretreated with Mitomycin C (MMC) to inhibit proliferation served as a negative control. The cells were allowed to proliferate in the treatment solutions for 1, 3, 4 and 6 days. At each time point, relative cell numbers for each treatment group was determined by MTS assay, with absorbance readings taken at 490 nm.

RESULTS

Fibroblast adhesion: All keratin fractions, except Type II, appear to enhance cell attachment in comparison to the uncoated wells. Statistically, these fractions appear to behave as well as collagen and fibronectin. The gamma and Type I fractions revealed the highest adhered cell counts.

Fibroblast proliferation: Several days after treatment, all keratin fractions initiated significantly better growth (at least 30%) than standard fibroblast media. On average, the gamma fraction—followed by the acidic and basic components of alpha—showed the greatest growth margin. At later time points, keratin did not appear to enhance proliferation, while standard fibroblast media continued to encourage cell growth.

CONCLUSIONS

Inherently biocompatible, bioactive, readily available and relatively easy to extract, hair keratins may prove to be a suitable therapeutic tool to accelerate the dermal wound healing process. Results from this study suggest that a biomaterial combining properties of gamma, alpha acidic and basic keratins may encourage dermal fibroblast activity. After several days of fibroblast proliferation, keratin bioactivity appears to level off, suggesting possible limits of efficacy on the cellular level. An evaluation of other dermal cell types (e.g., keratinocyte, epithelial) and molecular studies of focal adhesion activation and subsequent cellular signaling pathways may prove useful in further understanding the interactions between keratin and dermal cells.

REFERENCES

1. Lichti U, *et al.* Nature Protocols. 2008;3(5):799-810.
2. Sierpinski P, *et al*. Biomaterials. 2008;29:118-128.