Human Hair Derived Keratins Mediate Schwann Cell Behavior *in* vitro and Facilitate Rapid Peripheral Nerve Regeneration *in vivo*

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Statement of Purpose:

Nerve defects are a common result of peripheral nerve injury and present a significant challenge. Clinically the gold standard for nerve repair is an autograft. However, full functional regeneration is rarely achieved with this technique. Nerve conduits provide another option to this challenging problem but are currently limited to gaps of 3cm or less. It has been shown that the insertion of tissue engineering scaffolds into the conduit can enhance regeneration. However, no optimal material has been identified and clinically translated.

Keratins extracted from human hair fiber are a novel group of biomaterials with superior biocompatibility to many synthetic and naturally derived scaffolds. Human hair keratins can be processed into tissue engineering scaffolds with remarkable regenerative properties. It has recently been suggested that cell binding to porous keratin scaffolds is facilitated by fibronectin-like binding domains.[1] Hair has also been identified as a "depo" of growth factors involved in normal follicle cycling, many of which are implicated in nerve regeneration.[2] Recently, we have discovered a remarkable ability for certain keratin preparations to self-assemble into complex morphologies that are amenable to cell infiltration.[3]

In a recent pilot study, we demonstrated the ability for a keratin conduit filler to accelerate functional peripheral nerve regeneration in a mouse model. We hypothesize that this rapid regeneration is mediated by keratin's ability to facilitate Schwann cell migration, attachment, and proliferation. The specific aims of this study were to investigate the putative mechanisms by which keratins act on Schwann cells, and how these mechanisms might enhance peripheral nerve repair.

Methods:

Human hair was obtained from a local barber shop and cut into small length fibers, washed, and degreased. Keratose fractions were prepared by treating hair fibers with peracetic acid, followed by extraction with aqueous tris base and deionized (DI) water. The extracts were combined and dialyzed against DI water. The dialyzate was concentrated, neutralized, lyophilized, and the resulting keratose solid ground into a fine powder. Hydrogels were prepared by re-hydrating the keratin powder. Micro-architecture of the self-assembled gels was assessed by scanning electron microscopy (SEM) of lyophilized samples. Schwann cell proliferation in the presence of keratose was assessed using an *in vitro* MTS assay. Schwann cell adhesion to keratin biomaterials was assessed using a parallel flow chamber system (Glycotech). Migration was investigated using a modified Boyden chamber (Chemicon).

To determine the effects of keratin on nerve regeneration *in vivo*, a tibial nerve axotomy model was used. The tibial nerve of CD1 mice was transected and a 4 mm defect was formed by severing the nerve under a microscope. Each animal underwent repair using either 1) empty silicone conduit, 2) keratose filled conduit or 3) autograft. Six weeks following injury and repair, electrophysiology (amplitude and latency) and muscle force generation testing (single twitch and tetanus) were conducted to assess functional recovery. Nerve tissues were sectioned, stained with toluidine blue and examined histologically for myelination and vascularization. Regenerated nerve fibers were further assessed by transmission electron microscopy (TEM).

Results/Discussion:

Examination of lyophilized hydrogels by SEM showed a fibrous and highly porous architecture. Schwann cell biocompatibility upon exposure to keratose containing solutions was excellent. Keratose was not cytotoxic and increased cell proliferation at concentrations ranging from 0.1µg/mL to 1 mg/mL over serum containing media control. Schwann cell adhesion on keratose coatings was higher than on uncoated and fibronectin coated slides. Migration appeared to be only slightly enhanced in the presence of the keratose preparation.

Six weeks following nerve transaction and repair, regeneration was apparent in empty, keratose-hydrogel filled conduits and autograft. Regeneration through the keratose gel resulted in the development of blood vessels grossly visible within the conduit. The latency (in msec) was found to be 1.76 in the keratose treated group vs. 2.10 in the empty and 2.26 in the autograft group. The amplitude of the nerve impulse (in mV) was 9.5 in the keratose group vs. 4.0 and 7.5 in the empty and autograft groups, respectively. Therefore, both the conduction delay and the magnitude of the nerve impulse were improved in the keratose group in comparison to empty and autograft controls. Muscle force testing (single twitch and tetanus) demonstrated comparable muscle reinnervation in all treatment groups.

Histological analysis confirmed the presence of increased vasculature in nerves that regenerated through the keratose hydrogel. Histomorphometric analysis of toluidine blue cross sections showed a significant increase in the number and size of newly formed blood vessels in the keratose group over both empty and autograft controls.

Conclusions:

This data suggests that human hair keratose extracts facilitate Schwann cell proliferation and adhesion *in vitro* and may induce the recruitment of Schwann cells *in vivo*. The insertion of keratose hydrogels into a nerve guidance conduit demonstrated a high degree of regeneration in a mouse model as confirmed by functional testing and histological analysis.

References

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