The Influence of Keratin Biomaterial Treatment on Macrophage Phenotype in Spinal Cord Injury

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Statement of Purpose: Traumatic spinal cord injuries (SCI) affect more than 2.5 million people worldwide with no current treatment options available. SCI elicits a massive inflammatory response, which recent findings suggest is both necessary and detrimental. This is most likely due to the distinct macrophage phenotypes and their functions. Following SCI, M1 macrophages, a proinflammatory phenotype responsible for the release of cytotoxic compounds and tissue destruction, dominate at the site of SCI compared to M2 macrophages, an anti-inflammatory phenotype known to promote angiogenesis and matrix remodeling. A new intervention being studied for SCI is the use of a keratin biomaterial, which has previously been shown to have regenerative properties in a peripheral nerve injury model. Previous data has also demonstrated keratin's ability to significantly improve functional outcomes following hemisection injury in rats. In this study, we examined both *in vivo* and *in vitro* responses to a keratin hydrogel using well-established models in cell culture as well as a lateral thoracic spinal cord hemisection injury. We hypothesize the use of a keratin biomaterial will modulate macrophage phenotype following such an injury to a degree that will facilitate regeneration of the spinal cord.

Methods: Lateral left spinal cord hemisection injuries were surgically inflicted at thoracic vertebral level 9 (T9). Keratin hydrogels, prepared using published methods, were placed on the injury site. Saline vehicle was used as a control. Rats were sacrificed at respective timepoints, transcardially perfused, and spinal cords removed for further histological evaluation. Tissue sections were stained with CD86 (M1 marker) and CD206 (M2 marker), as well as cresyl violet and silver stain. *In vitro* experiments were done using the human monocytic cell line THP-1 from ATCC. THP-1 cells were differentiated into macrophages using the phorbol ester PMA (5ng/ml) and then subsequently incubated on keratin coatings (200µg/ml) or with M1 or M2 macrophage control solutions (LPS/IFNγ (100ng/ml; 20ng/ml) or IL-4 (20ng/ml), respectively). Supernatants were collected for cytokine production using ELISA. Cells were then washed, removed and treated for flow cytometry analysis. M1 macrophages were defined as $CD14^{+}CD206^{-}$ and M2 macrophages were defined as $CD14^{+}CD206^{+}$. A two-way analysis of variance (ANOVA) with a Bonferroni's post-hoc test was used to determine any differences among groups for quantitative data. Differences were considered significant when $p<0.05$.

Results: Histological data of keratin-treated spinal cords shows a greater presence of M2 macrophages at 7 days post-injury at the lesion site, compared to M1 macrophages. Silver staining rostral to the lesion epicenter shows greater degeneration in the saline-treated rats. Cresyl violet stained sections suggest scar formation is limited to the lesioned half of the spinal cord with keratin treatment. Cytokine production measured from THP-1 cells indicates a time-dependent response, where significantly increased levels of TNF-α, IL-6, IL-8, and IL-1α peaked at 24 hours of treatment, with a significant decrease by 72 hours. Flow cytometry analysis shows PMA-induced THP-1 macrophages display significantly higher double positive (M2 phenotype) events following 24 hours of keratin treatment (Figure 1).

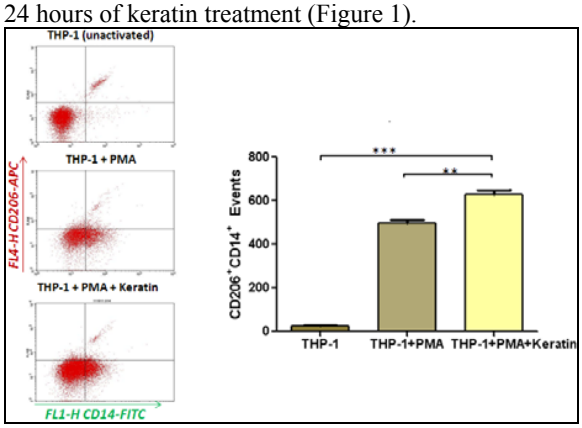

Figure 1. *Keratin treatment promotes an M2-like phenotypic shift after 24 hours.*

Additionally, extended keratin exposure (3 and 5 days) promotes a further M2 macrophage phenotypic shift.

Conclusions: These data suggest that keratin has the ability to influence the macrophage inflammatory response, which may in turn allow for greater recovery following a hemisection injury. Keratin-treated rats show an increased presence of M2 macrophages at 7 days post-injury. There was also a greater degree of degenerating axonal and cell body profiles in the saline-treated rat at 1 week following injury. This correlates with our previous studies showing significant improvement in gait properties as well as bladder function (unpublished data). *In vitro* data suggests keratin has a priming effect, as indicated by a cytokine profile traditionally considered proinflammatory (TNF-α, IL-6, IL-1α) that peaks at 24 hours and appears to resolve by 3 days. As there are increasing levels of M2 macrophages at 24 hours, 3 days, and 5 days, this may explain the initial production of proinflammatory cytokines. This study is both promising and important, since there are currently no biomaterials shown to favor an anti-inflammatory M2 macrophage response and no practical SCI treatments.

References: (Kigerl KA. J Neurosci. 2009;29:13435-44) (Sierpinski P. Biomaterials. 2008;29:118-128)

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