The effects of degradation on the nanostructure surface in chitosan scaffolds

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Statement of Purpose: Chitosan has shown very promising results in various applications in controlled drug delivery and tissue engineering. One application of interest is the use of chitosan nerve guides for peripheral nerve regeneration. Studying the interactions between the Schwann cells and chitosan is vital for implant success. Recently, chitosan has been found biocompatible with Schwann cells, however this investigation did not consider differences in surface nanostructure[1]. The degradation of chitosan plays an important role on the interaction of cells with the material scaffold. Degradation kinetics affects cell growth, tissue regeneration and host response mainly via changes in surface characteristics. In vitro degradation techniques mainly focus on the use of lysozyme, which commonly exist in humans. Previous studies have shown degradation of chitosan in terms of molecular weight changes. The aim of this study was to investigate how the degradation kinetics of chitosan scaffold changes the nanostructure of the scaffold surface. Secondly, we examined how this change in surface texture affects cell attachment and growth. The morphological characterization of the degraded scaffolds was done using SEM and AFM. Cell response was characterized in terms of Schwann cells attachment, spreading and proliferation. Determining how the nanostructure of the material surface controls cell response will improve its performance as a biomedical material.

Methods: Chitosan scaffolds were fabricated from 2% chitosan solution in acetic acid freezing and lyophilizing overnight. The thickness of the membrane averaged 0.01mm. Scaffolds were cut into $1cm^2$, weighed and then immersed in 1X PBS with and without lysozyme $(4mg/ml)^2$. The samples were then placed in an incubator-shaker at 37^oC. Weight loss and pH changes were noted for the time period of 18 hrs, 24 hrs, 48 hrs, 1 week, 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks and 12 weeks. The degraded samples were analyzed using SEM to check for the porosity and surface nanostructure was evaluated using AFM. Histological cell response was examined using Schwann cell line, which was cultured on the degraded scaffolds, for seven days. Cell proliferation and viability were tested using the MTT assay.

Results / Discussion: The data displays that a reduction in chitosan scaffold in terms of material/weight in scaffolds incubated in lysozyme as time increased. However, no significant loss was observed until one week. Additionally, the pH of the media containing the PBS with lysozyme showed more acidic conditions with time, which was due to degraded chitosan. SEM analysis of the microarchitecture showed some marginal difference in the porous structure of scaffolds with and without lysozyme (Fig 1A and B). However, AFM testing determined that nanostructural changes began to occur by the two weeks time point. This change in nanostructure corresponded with changes in Schwann cell attachment and spreading as scaffold degradation occurred. As degradation increased, cell spreading and proliferation increased.

Conclusions: Overall, the results display that surface nanostructure changes with chitosan degradation has a dramatic effect on cell/material interactions. It is well known that Schwann cells play a key role in nerve regeneration. Thus, further evaluation of the cell/material interactions will provide the vital information needed for improving scaffold biocompatibility as a nerve guide.

References:

1. Yuan, Y., et. al. (2004). The interaction of Schwann cells with chitosan membranes and fibers in vitro. Biomaterials, 25, 4273-4278.
2. Tomihata, K., Ikada, Yoshito. (1997). In vitro and in vivo degradation of films of chitin and its deacetylated derivatives. Biomaterials, 18, 567-575.

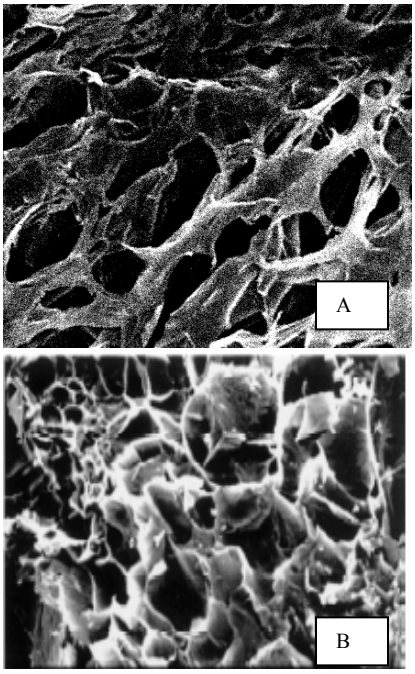

Fig 1A and B show SEM pictures of degraded chitosan scaffold before degradation and 4 weeks after degradation with lysozyme respectively at x30k and 20kV acc. voltage