Traceable chitosan implants with controllable protein release

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I. Abstract

An ideal nerve regeneration implant should provide complex assistance, including physical guidance for axon outgrowth; be a reservoir for the factors attracting/inducing stem cells as well as the tissue-forming factors; provide a matrix for cell attachment; be traceable; and disintegrate when the regeneration is complete. Here we are demonstrating the assembly from the set of the functional materials with defined nano- and macro-properties into the tubular implant structures. These structures are well tolerated on both cell and tissue levels, slowly releasing their macromolecular cargo, traceable by MRI, and mechanically sound.

II. Results

Chitosan was chemically modified by adding diethylenetriaminepentaacetic acid (DTPA) residues to some of its primary amine groups with target molar ratio of 10% using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxylsuccinimide. Modified polymer was separated from excess of free DTPA, EDC and hydroxylsuccinimide by dialysis via benzoylated membranes with molecular cutoff around 2000 Da. After lyophilization from 50mM acetic acid solution DTPA-chitosan was mixed with unmodified chitosan with ratio 1:100, 1:10 and 1:1, dissolved (4% w/v) in 100mM acetic acid and pH adjusted to 6.3 with 1M tris(hydroxymethyl)aminomethane. Resulting viscous solution was casted into the stainless steel mold, frozen at -80°C and displaced into 9M solution of ammonia. Resulting tubular structure was immersed overnight in 10uM, 100uM, 1mM, or 10mM $GdCl_3$ washed until no detectable Gd^{3+} released and used for MRI *in-vitro* tests.

The highest MRI contrast on T1 weighted image between DTPA-chitosan gels and surrounding oil and agarose gel was achieved for 1:10 mixture soaked in 10mM $GdCl_3$. This gel composition was used for further biochemical experiments.

Murine Nerve Growth Factor (NGF, supplied by Promega) is slightly basic protein with isoelectric point (pI) around 8.0, which makes bounding of it to the slightly basic unmodified chitosan low. However, surface DTPA residues of modified gels were expected to provide anchor points to the NGF molecules. To assess it, the DTPA-chitosan containing gels treated or not treated with NGF were aseptically transferred into culture flasks with murine neuroblast cells, PC12 pheochromocytoma cells and transformed rat Schwann cells. All cultures showed good tolerance with no statistically significant viability change over 7 days period for both unloaded and NGF-loaded gels as measured by LIVE/DEAD® Viability/Cytotoxicity Kit and active Caspase-3 Western Blot; while neuroblast cells, PC12 pheochromocytoma cells have responded to NGF-loaded gels with specific morphological rearrangement. Release of NGF into culture media was detected during first 48 hr, while surfaces of the NGF-treated gels remain immunochemically reactive up to 5 days in media.

HSC-70 cognate heat shock protein has an estimated pI of 5.37, which makes it significantly more acidic than NGF as well as expectedly increases its binding to the unmodified chitosan. Chitosan nanoparticles containing purified HSC-70 were prepared by ionotropic gelation in presence of pentasodium tripolyphosphate (PSTPP). Resulting particles displayed a broad size distribution in range 200-500nm and were stable for weeks in presence of 1mM PSTPP. After dialysis and lyophilization the dry particles were incorporated into DTPA-chitosan/chitosan solution and cast into the tubular structures. The same as above set of the cells have tolerated HSC-70/Gd/ DTPA-chitosan/chitosan structures well with no statistically significant viability change over 7 days period. Protein release into the culture media has declined gradually over the duration of the experiment. We did not register any significant release of the complexed Gd into water from dialyzed culture media.

III. Conclusion

Chitosan-based materials are practical, well tolerated and could be adapted to carry desired protein payload. Complex design including structural, functional and traceable materials combination is able to provide a mechanical support, controllable release of macromolecular cargo, cell attachment as well as surrounding tissue tolerance for the duration of material`s life.

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