Multifunctional Scaffold for the Delivery of Neural Stem Cells to Promote Regeneration after Traumatic Brain Injury

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Statement of Purpose: The brain is arguably the most difficult organ to repair after an injury due to the complexity of the central nervous system (CNS) and its limited capacity to regenerate on its own. Stem cell therapies are a promising field for tissue regeneration but direct transplantation has shown limited success in repairing the brain (Harting MT. J Surg Res. 2009;153:188-94). An effective paradigm for transplanting these cells has yet to be established. In this study, we engineered a chitosan based biomaterial scaffold to promote CNS regeneration from neural stem cells. We optimized a method to manufacture injectable multifunctional microsphere scaffolds and subsequently modified the surface chemistry of the biomaterial in order to achieve a scaffold highly suitable as a vehicle for cell transplantation in traumatic brain injury.

Methods: Chitosan powder (1.5g) was dispersed in 50 mL of water containing 3.0% v/v acetic acid to create 3% chitosan solutions. Microspheres were formed by feeding and passing the chitosan solution through a 23 Gauge syringe and dispersed in a coagulation solution of 2M NaOH and methanol. The syringe was connected to an electrical current to reduce sphere size. The resulting microspheres were rinsed in deionized water and incubated in heparin and genipin. Genipin is a natural cross-linking agent that can covalently bind heparin to chitosan. In turn, heparin has high binding affinity to fibroblast growth factor-2 (FGF-2), a known mitogen and survival factor for neural stem cells. Heparin binding was assessed using toluidine blue stain and FTIR. An ELISA quantified the amount of FGF-2 binding at 100, 500 and 1000 ng/ml concentrations. Cell growth on FGF-2 immobilized chitosan scaffolds was analyzed using an MTT assay. A neural stem cell line, RG3.6, was seeded at a density of 6.25 x 10^4 cells/cm^2 in 96-well plates in B27 supplemented Neurobasal medium onto either chitosan coated with fibronectin with FGF-2 in the medium (Control) or onto the complex film with freshly bound FGF-2 (Bound, Day 0), or onto the complex film where the FGF-2 had been bound 3 days earlier and incubated at 37°C for three days (Bound Day 3) or onto the complex film without added FGF-2 in the medium. The MTT assay was performed after two days *in vitro* as an index for the numbers of viable cells.

Results: Chitosan microspheres between 20-100 µm were generated using electrospray technique (Fig.1A,B). Genipin cross-linking prevented ionic removal of heparin as observed in toluidine blue staining (Fig.1C). 1,000 ng/ml FGF-2 showed the highest percentage of binding at 82% on genipin crosslinked chitosan-heparin complex film. The bioactivity of the FGF-2 was maintained for at least three days at physiological conditions. The number of viable cells was also higher on the scaffold with tethered FGF-2 than standard culture conditions receiving soluble FGF-2 daily (Fig.2A). The benefit of using genipin cross-linked chitosan-heparin-FGF-2 complex is that this process will not require stringent washing after attaching the growth factor, unlike the techniques used for growth factor encapsulation. FGF-2 when bound to heparin remains biologically active. The functionalized substrate maintains the stem cells in a proliferative state. The FGF-2 when attached to the scaffold is more readily available to cells than if it were floating in media interacting only randomly. Heparin on the substrate also facilitates the binding of FGF-2 to its receptors (FGFR-1, FGFR-3, and FGFR-4). The biocompatibility of chitosan and the chitosan-heparin-genipin complex was also verified using RG3.6 cells. These cells attached and proliferated well on the microspheres (Fig.2B). Adherence on the surface of the microspheres allows for easy migration of cells between spheres or into the native tissue to participate in cell replacement.

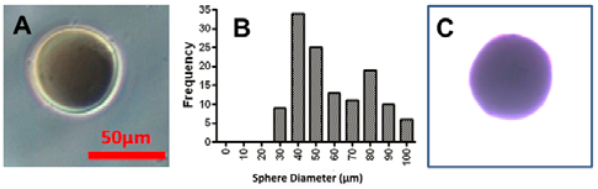

Figure 1. (A-B) Morphology and diameter distribution of chitosan microspheres using electrospray technique. (C) Heparin cross-linked chitosan microspheres using genipin cross-linker. Heparin was stained with toluidine blue.

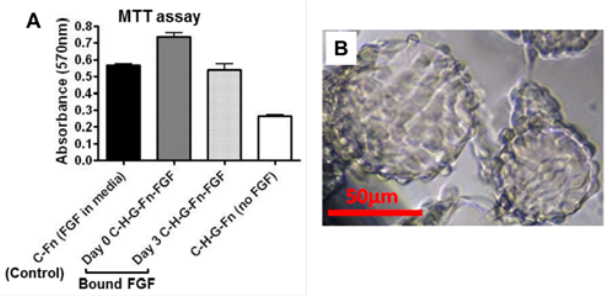

Figure 2. (A) Results of an MTT assay demonstrating FGF-2 bound to the genipin cross-linked chitosan-heparin complex film retains its biological activity. (B) Neural stem cells (RG3.6) grown on electrosprayed chitosan microspheres modified with heparin-genipin-FGF-2 and adsorbed with fibronectin.

Conclusions: In this study, we have designed and optimized 3D multifunctional microspheres using natural biopolymers for the delivery of neural stem cells and growth factors into the injured CNS. Heparin was stably cross-linked onto the chitosan scaffolds using genipin. The cross-linked chitosan-heparin complex was shown to have a high binding affinity for FGF-2 and supported the growth of neural stem cells.