

F Glynn, J Tan, Y Wang, F Caruso, RK Shepherd (2010). A novel therapeutic approach encapsulating brain-derived neurotrophic factor in nanoporous protein particles: implications for clinical neurosciences. Sydney 2010 International Medicine Conference, 30 Jun – 2 Jul 2010. Selected oral presentation by J Tan.

Abstract:

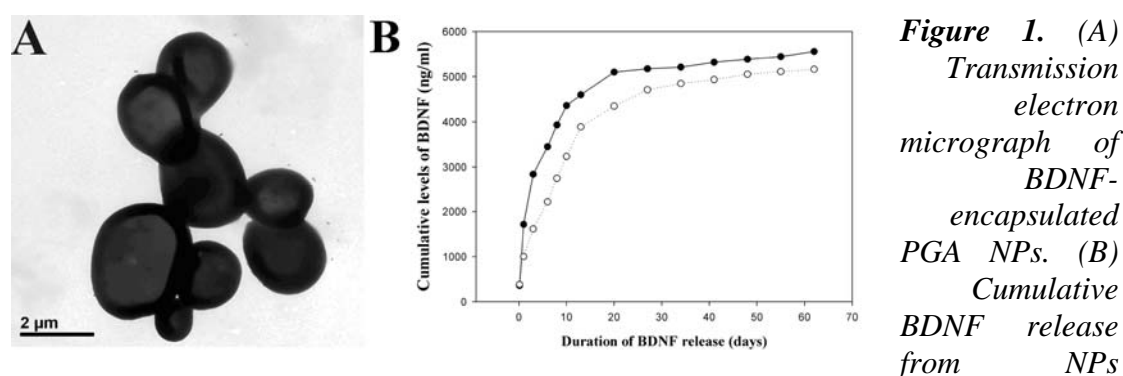
Neurotrophins play an important role in the development and function of the nervous system. In particular, the brain-derived neurotrophic factor (BDNF) is essential for refining connections between neurons and their target cells, activity-driven neuronal plasticity and memory consolidation¹. Complete knock-down of BDNF in mice impairs the function of the hippocampus, a structure of the brain responsible for learning and memory². In the inner ear, reducing BDNF levels or activity prevents effective contacts between the nerve endings of primary auditory neurons and their target sensory cells in the organ of Corti³. We have shown that inner hair cells and tightly-associated supporting cells within the organ of Corti are sources of BDNF⁴. Therefore, destroying these cells with ototoxic drugs diminishes the levels of BDNF available in the cochlea, causing a progressive degeneration of primary auditory neurons leading to nerve deafness⁴. Significantly, delivery of exogenous BDNF after an ototoxic trauma has been shown to increase the survival of these neurons⁵ but they die rapidly once BDNF delivery stops⁶, emphasising a need to devise a strategy for sustainable BDNF release.

Members in this group have successfully used nanoporous protein particles (NPPs) to encapsulate enzymes without compromising its activity^{7,8}. In this study, we have adapted this technique to produce NPPs which are subsequently loaded with BDNF. Poly-glutamic acid was firstly infiltrated into mesoporous silica particles – which acts as template during the assembly – before they are covalently cross-linked with cystamine by the catalysis of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride. The silica template is then dissolved using buffered hydrofluoric acid. Next, the polysaccharide heparin sulphate is infiltrated into the NPPs to provide a highly negatively-charged interface. BDNF is then adsorbed into the particle through electrostatic attractions between residual amine groups on the protein and the highly-sulphated glycosaminoglycan moieties of heparin.

For effective BDNF-NPPs synthesis, it is important that both chemical structure and biological activity of BDNF are significantly preserved. BDNF release from these particles is triggered at 37°C in a physiological pH solution. Using BDNF-specific antibodies, we used an enzyme-linked immunosorbent assay (ELISA) to identify and quantify BDNF. In 2 separate experiments, we were able to detect BDNF release from these particles over a period close to 2 months (Fig. 1). In addition to establishing release rate, we measured the biological activity of BDNF released from these particles using cell cultures. SH-SY5Y retinoblastoma cells differentiate into neurons with the addition of 10 µM retinoic acid in the presence of 10% foetal calf serum. Upon serum withdrawal, the differentiated neurons undergo apoptosis – regulated cell death - which can be measured with TUNEL-staining. However, apoptosis is reduced if recombinant BDNF is added into the serum-free medium. We showed that NPPs-derived BDNF also had a significant effect in rescuing differentiated SH-SY5Y cells from apoptotic death and that this activity was similar to that of recombinant BDNF,

demonstrating that encapsulating BDNF in PGA-based NPs did not adversely affect its biological activity *in vitro*.

In addition to these *in vitro* data, we recently developed a surgical technique for safely delivering BDNF-NPPs into the rat cochlea. A single volume of BDNF-NPPs was delivered into the scala tympani of a deafened animal. Both treated (BDNF-NP) and the contralateral deafened control cochleae were examined 2 months following delivery of the BDNF-NPPs. Importantly, preliminary results are encouraging as they provide evidence of both increased survival of primary auditory neurons and enhanced neurite outgrowth from these neurons. These findings will be discussed in the context of ameliorating cochlear implant functions with drug-based therapies.



References:

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This work was supported by the NIH (HHS-N-263-2007-00053-C), the Royal Victorian Eye & Ear Hospital, the University of Melbourne and the Garnett Passe and Rodney Williams memorial foundation.