Delivery of neuroprotective peptides from encapsulated cells in a mouse model for neurodegenerative disease

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Introduction

Delivery of therapeutic compounds to the diseased brain is a challenge for the treatment of most brain diseases, including brain tumours and neurodegenerative diseases. The efficacy of systemically applied drugs is hampered by their limited passage through the blood brain barrier. A short half-life and the need for repeated operating procedures are major obstacles for locally injected/infused therapeutic molecules. Cell-based delivery systems that provide continuous delivery of the biologically active compound *in situ* represent a promising strategy for therapeutic applications in the brain (Visted et al. 2001). The secretion of bioactive agents from alginate-encapsulated cells has already proven its efficacy in treating brain tumors in rodents (Read et al. 2001). The technique is particularly promising for the treatment of chronic and progressive neurodegenerative disorders. Alzheimer's disease (AD) is a devastating progressive neurodegenerative disease whose incidence is rising in the Western countries due to increased life expectancy. The neuropathological hallmarks of AD are the deposition of amyloid plaques and neurofibrillary tangles. Progressive neuronal loss is implicated in the development of AD-associated dementia. Recent data supports the idea that soluble oligomers of amyloid-β peptide (Aβ) are highly neurotoxic and represent a key factor in the early stages of neurodegeneration leading to AD (Pillet et al. 1999, Walsh et al. 2002, Wang et al. 2002, Sponne et al. 2003). We have already shown that endogenous neuroprotective peptides efficiently prevent soluble Aβ-induced neuronal apoptosis in primary cultures (Youssef et al. 2007). We therefore hypothesize that *in vivo* expression of these peptides will prevent neurodegeneration and subsequent cognitive impairment in mice models of AD. Our project aims at evaluating the efficacy of neurotrophic factors including ciliary neurotrophic factor (CNTF) and humanin in AD mice models after stereotaxic implantation of alginate-encapsulated producer cells, thus providing local and continuous delivery of the therapeutic agent in the diseased brain.

Material and Methods

Production of alginate beads. Sodium alginate (ultrapure, low viscosity, high guluronic acid content; PRONOVA (TM) UP LVG) was dissolved in saline solution (0.8% NaCl, 0.1% D-glucose, pH 7.2–7.4) to a concentration of 2%. Producer cells (C_2C_{12} or BHK) were grown to confluency, trypsinized and pelleted. Cells were mixed with the alginate to a concentration of 10×10^6 cells/ml. Beads were generated with an electrostatic bead generator. Sharpened nozzles with diameter of 0.09 and 0.17 mm were used (Nisco Engineering AG, Switzerland). The gelling bath was composed of 0.1M $CaCl_2$ in 0.9% NaCl pH 7.4.

Generation of fluorescently-labeled cells. Lentiviral vectors expressing either enhanced green fluorescent protein (eGFP) or red fluorescent protein (DsRed) were produced as described (Ruitenberg et al. 2002) and applied to BHK and C2C12 cells. Transduced cells with high fluorescence were sorted using a cell sorter (BD FACS ARIA) to obtain a homogenous cell

population.

Animal model. The laboratory of T. Pillot has established a new mouse model for AD by a single intra-cerebroventricular stereotaxic injection of sAβ1-42 in the C57Bl/6 mouse brain. This acute exposure to sAβ results in cognitive impairment within two weeks after injection as measured by two validated behavioral tests: the *Y Maze* and the *Morris Water Maze*. In the *Y Maze* the subject can freely explore and the number of arm entries without repetition is determined. High rates of alternation in the groups indicate that the animals can remember which arm was entered last (measure for short term memory). In the *Morris Water Maze* the rodent is placed in a pool of water where it must use visual cues to remember the location of a hidden platform just below the water's surface. A high score indicates visual recognition and memory (measure for spatial learning and memory) (see Fig. 2).

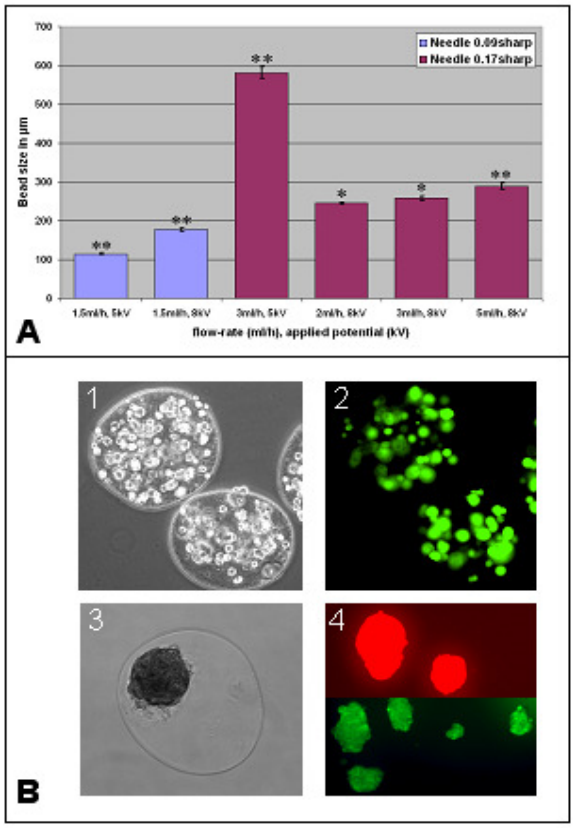

Figure 1: A. Bead size is a function of the nozzle diameter, alginate flow-rate and applied electrostatic potential. Columns marked (**) are significantly different (p value <0.01). B. Alginate beads containing eGFP-expressing BHK cells under phase contrast (1) and UV light (2). Alginate bead encapsulating a spheroid of C_2C_{12} cells (3). Generation of C_2C_{12} cell spheroids showing lentiviral vector-derived eGFP or DsRed expression (4).

Secretion of CNTF from alginate beads and neuroprotective effects. Production and secretion of CNTF from encapsulated cells was monitored by immunoblotting using an anti-CNTF antibody (C3960, Sigma). (Cells stably expressing CNTF were a gift from P. Aebischer, EPFL Lausanne, CH). Conditioned media from these cells were applied to primary cortical neurons in the presence of sAβ. Neuronal cell death through apoptosis was evaluated by counting fragmented nuclei as indicated by DAPI staining.

Results and Discussion

*Optimization of small sized beads***.** Regular sized alginate beads are in the range of 500µm which is too large for implantation in the mouse brain. We therefore tried to optimize the procedure to generate smaller beads (100-200µm). Bead size is dependent on the inner diameter of the nozzle and on the flow-rate and the applied electric potential of the electrostatic bead generator (Klokk & Melvik 2002). When using a sharpened nozzle with a diameter of 0.09 mm we were able to produce cell-containing alginate beads in the desired range (Fig. 1). A problem faced with these small beads is that the number of incorporated cells is limited. Increasing the cell concentration in the alginate from 10 to 50 × 10^6 cells/ml can solve this problem, however this leads to immediate clogging of the 0.09mm nozzle. Using the 0.17mm nozzle the bead size ranged from 245µm to 590µm depending on the flow rate and the electrostatic potential (Fig. 1).

Generation of spheroid containing fluorescently-labeled beads. Proliferation of cells within the bead and migration to the surface leads to escape of cells from the bead to the surrounding tissue. Earlier studies have shown that cell proliferation inside beads differs between cell types and that the C_2C_{12} cells are almost non-proliferative inside beads (Rokstad et al. 2006). Subsequent experiments were therefore performed with the C_2C_{12} cell line. To monitor cell survival inside the beads, we generated cells expressing a fluorescent protein (GFP or DsRed) through lentiviral transduction (Fig. 1B). This will also allow for better monitoring of the beads in the animal *in situ* and for the detection of escaping cells into the surrounding tissue. Despite poor proliferation of C_2C_{12} cells in the beads, cells that are on the bead surface will easily migrate away from the capsule. We therefore generated spheroids from the producer cells prior embedding in alginate (Fig. 1B). The efficacy of secretion from spheroid-containing beads still needs to be addressed.

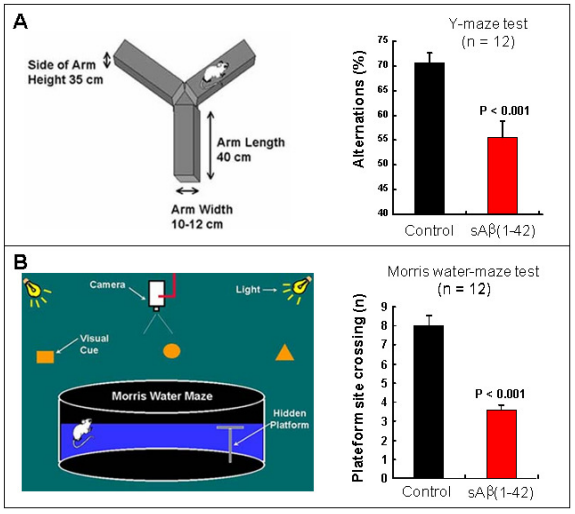

Figure 2: Mouse model for Alzheimer's disease. Intraventricular injection of sAβ in the brain of mice leads to cognitive deficits as measured in the Y Maze (A) and Morris Water Maze (B) behavioural tests.

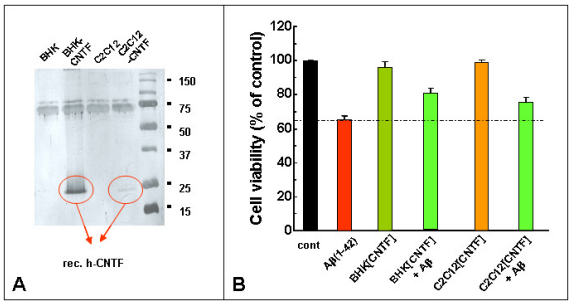

Figure 3: Stable CNTF-producing cell lines (BHK and C_2C_{12}) were encapsulated in alginate beads. CNTF release from the beads was verified by Western blot analysis (A). Application of CNTF-containing conditioned medium provided neuroprotection against soluble Aβ induced neuronal cell death (B).

Alginate-encapsulated cells in a mouse model for AD. Application of soluble Aβ (sAβ) to cortical and hippocampal neurons in culture leads to neuronal cell death through apoptosis (Pillot et al. 1999). The molecular mechanisms leading to apoptotic cell death have been largely elucidated *in vitro* (Sponne *et al.*, 2003, Fifre *et al.*, 2006, Kriem *et al.*, 2005). To evaluate *in vivo* effects of acute exposure to soluble Aβ, a new mouse model for AD has been established (Youssef et al. 2007). Two weeks after a single intra-cerebroventricular injection of sAβ1-42 in the mouse brain altered cognitive functions were detected in the Y maze and Morris Water Maze behavioural tests (Fig. 2). This model will be used for implantation of alginate encapsulated cells secreting neurotrophic factors such as CNTF. Expression of CNTF from alginate beads containing BHK or C_2C_{12} producer cell lines has been confirmed by Western blot analysis (Fig. 2A). Conditioned medium of these cells was found to rescue cultured cortical neurons from sAβ-induced apoptotic cell death (Fig. 2B).

Conclusion

We show that cells producing neuroprotective peptides can be encapsulated in Alginate beads and that conditioned medium from such beads provides neuroprotection against soluble Aß induced neuronal cell death. Moreover, we show that C_2C_{12} cells engineered to express GFP, ds-Red as well as CNTF can be encapsulated in alginate beads that are small enough to be transplanted in the

mouse brain. We show that both single cells, and multicellular spheroids can be encapsulated in such beads.

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