Microencapsulated cells expressing VEGF for the treatment of Alzheimer's disease

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Introduction

Alzheimer's disease is a chronic neurodegenerative disorder characterized by a progressive impairment of cognitive functions. Neurofibrillary tangles, β-amyloid plaques, neuron loss, and astrogliosis are major pathologic hallmarks of Alzheimer's disease in the brain. Some recent reports suggest that Alzheimer's disease may have some links with other disorders such as atherosclerosis and cerebral microvascular pathology, indicating that vascular factors might play a pivotal role in the pathogenesis of AD. In fact, it has been hypothesized that Alzheimer's disease could be an angiogenesis-dependent disorder and that abnormal endothelium activation might lead to amyloid deposition and neural death.

Vascular endothelial growth factor (VEGF) is a hypoxia-inducible biological mediator that promotes the proliferation and survival of endothelial cells. It is a physiological regulator of brain angiogenesis and of blood-brain barrier integrity. Recent evidence shows that this angiogenic growth factor also protects against motoneuron loss, confirming the associations between blood vessels and neurons (Storkebaum 2005). In fact, reduced VEGF levels in knock-out mice for this gene caused progressive motor neuron degeneration, whereas treatment with VEGF protected mice against ischemic motoneuron death.

In the last few years, the possible connections between VEGF and Alzheimer's disease have been a matter of much research. A down-regulation of VEGF production has been demonstrated by peripheral immune cells of patients suffering from Alzheimer's disease. Additionally, Del Bo et al, (2005) observed in a preliminary study that a functional polymorphism within the promoter region of VEGF gene (-2578), related with a lower VEGF expression, could be associated with an increased risk of Alzheimer's disease. Although, a subsequent study revealed that this polymorphism did not confer greater risk for the neurodegenerative disease (Chapuis 2006), the possible links between VEGF gene polymorphism and Alzheimer's disease are still on debate. More recently, serum VEGF levels of patients suffering from Alzheimer's disease were compared with control subjects (Llorca 2007). The mean concentration of VEGF in the patient group was significantly lower than that of the controls. From these results authors concluded that decrease in serum levels could contribute to the neurodegenerative process in Alzheimer's disease.

Assuming these considerations, we hypothesized that a local, controlled and continuous delivery of VEGF into the brain could be a novel therapeutic approach to treat Alzheimer's disease by stimulating the development of blood vessels. In the present work, cell microencapsulation technology was employed to provide a continuous release of VEGF. This technology presents some potential advantages including the immunoprotection of the enclosed VEGF producing cells, the possibility to release the angiogenic factor locally and in different anatomic locations and finally the long-term release of the therapeutic product (Orive 2003). In this preliminary work, VEGF

secreting cells were encapsulated and the *in vitro* and *in vivo* effects of the secreted VEGF were evaluated and discussed.

Material and Methods

Cell culture: BHK cells genetically engineered to secrete human VEGF were grown in Dulbecco´s modified Eagle medium (DMEM) containing 2% L-glutamine, 10% foetal bovine serum (FBS) and 1% antibiotic/antimycotic. Cells were passed every 2-3 days and maintained at 37ºC in 5% CO_2. All the components of the culture medium were purchased from Gibco BRL (Invitrogen S.A., Spain).

Cell encapsulation: We encapsulated the VEGF-secreting cells in alginate-poly-L-lysine-alginate (APA) microcapsules prepared using an electrostatic droplet generator. Cells were suspended in 1.5% (w/v) low-viscosity high guluronic acid (LVG) alginate (FMC Biopolymer, Norway) obtaining a cell density of 5 x 10^6 cells per mL alginate. This suspension was extruded into a calcium chloride solution and the resulting alginate beads were successively coated with poly-L-lysine 0.05% (w/v) (MW: 15,000-30,000, Sigma, St. Louis, MO) and alginate 0.1% (w/v). Microcapsules were prepared at room temperature and under sterile conditions and cultured in complete medium. The mean microcapsule diameter was 392 ± 12 μm.

Characterization: The diameter, surface characteristics and overall morphology of the encapsulated cells were characterized using an inverted optical microscope (Nikon TSM) equipped with a camera (Sony CCD-Iris). The *in vitro* viability of the entrapped cells was evaluated by the tetrazolium assay. Morphology of the enclosed cells was also evaluated by confocal microscopy. VEGF levels were determined using a sandwich ELISA kit for human VEGF (Amersham Biosciences, USA).

In vitro proliferation assay: Brain endothelial cells were cultured as described elsewhere (Lopez-Lopez 2004). Cells were cultured with different amounts of stock VEGF (500 ng/mL) and functional VEGF (released by the microencapsulated cells) (33 ng/mL) during 7 and 20 days. Cell proliferation was measured by the tetrazolium assay.

Implantation: C57BL/6 mice were used in this study. A control group (n: 6) and a group receiving approximately 40 cell-loaded microcapsules (n:6) were formed. Microcapsules were carefully implanted in the cortex of the mice. The animals were kept under standard laboratory conditions in accordance with European Communities council guidelines.

Histological analysis. At day 15, 30 and 90, animals were sacrificed and a full immunocytochemistry was performed. New blood vessel formation was determined using a biotinylated tomato lectin and factor VIII. DNA-synthesizing cells were counted using a rat anti-BrdUrd antibody. A double BrdUrd-lectin staining was performed as described elsewhere (Lopez-Lopez 2004).

Results and Discussion

Genetically modified BHK fibroblasts (10^6 cells/mL alginate) secreted approximately 174 ng VEGF/24h. VEGF-secreting cells were immobilized in APA microcapsules as it is illustrated in Figure 1. Cells were successfully adapted to the new microenvironment and released VEGF along the time.

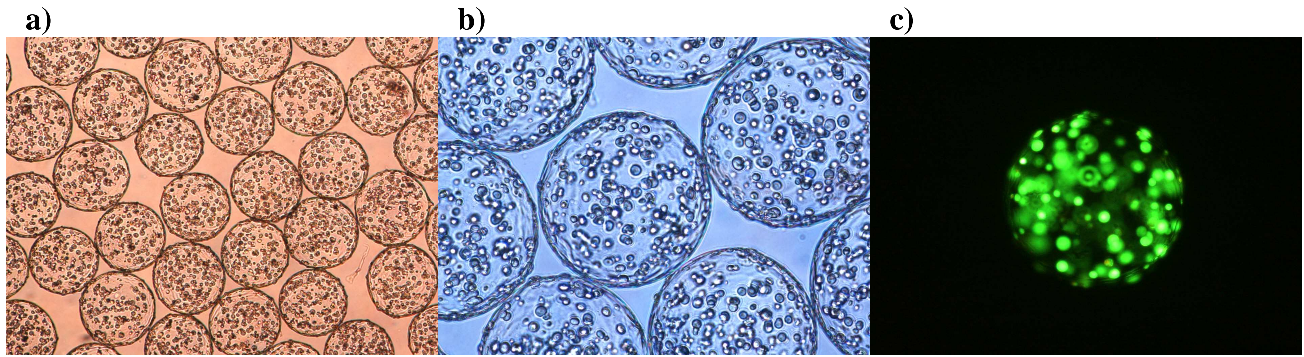

Figure 1. Morphology of microencapsulated VEGF-secreting cells. Optical microscopy images (A, B) and fluorescence microscopy image (C).

In order to assess the functionality of the VEGF released by the encapsulated cells, a proliferation study of brain endothelial cells was performed. A simulated production of 200 cell-loaded microcapsules (33 ng/mL) was compared with a higher VEGF dose (500 ng/mL).

Results show that VEGF released from the microcapsules was functional (Figure 2). In fact, at day 7, both treatments stimulated significantly the proliferation of the cells (compared to controls). However, at day 20, it was clearly observed that lower amounts of VEGF provided even a higher cell proliferation, suggesting that the VEGF dose secreted by the immobilized cells could be enough to induce a proliferation response without major risks of edema.

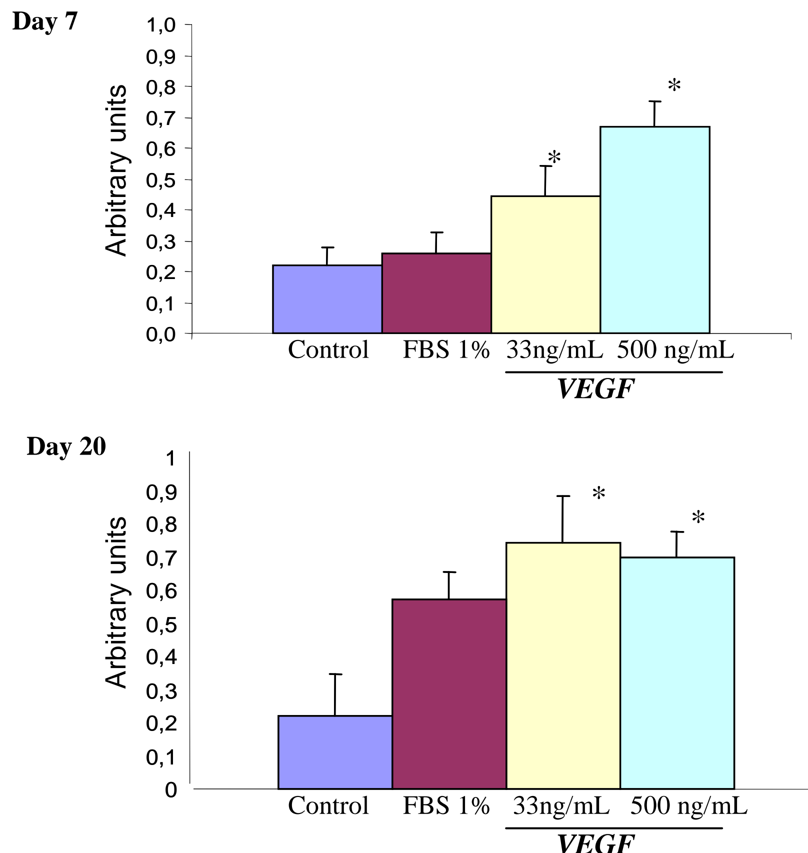

Figure 2. Proliferation of brain endothelial cells cultured with VEGF secreted by the encapsulated cells (33ng/mL) and stock VEGF (500 ng/mL) at day 7 and day 20.

Once the *in vitro* functionality of the VEGF released by the immobilized cells was demonstrated, an *in vivo* assay was carried out to evaluate the *in vivo* effects of the secreted VEGF. Results show that blood vessel density was clearly increased in those animals implanted with capsules loaded with VEGF secreting cells. This angionenic effect was evident at day 15 and persisted at day 30 and day 90, indicating the long-term release of VEGF from the encapsulated cells (Figure 3A).

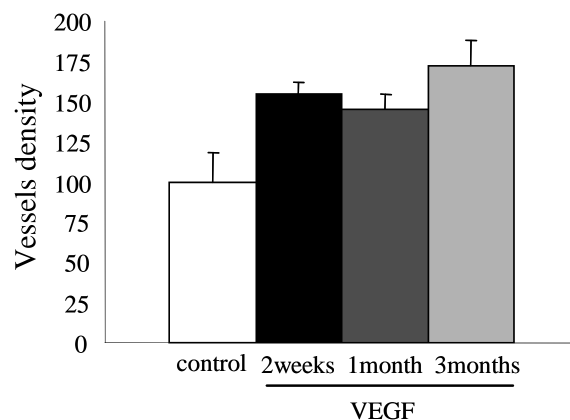

Figure 3. Vessel density stimulated by the release of VEGF from the encapsulated cells

A double BrdUrd-lectin staining was performed to distinguish the VEGF-induced new blood vessels. Figure 4 illustrates the newly formed blood vessels.

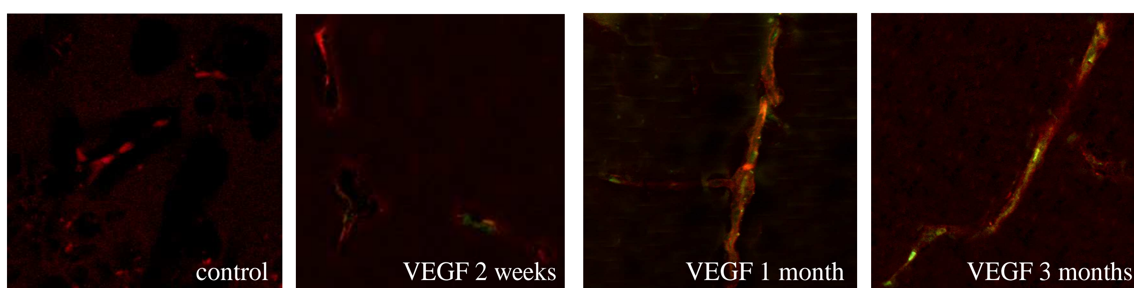

Figure 4. A double BrdUrd-lectin staining the newly formed endothelial cells

Conclusions

This preliminary work demonstrates the *in vitro* and *in vivo* functionality of VEGF secreted by immobilized cells. In a second step, the impact of such neo-vascularization in the memory and learning activities of APP-PS1 mice developing Alzheimer's disease will be evaluated.

Bibliography

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