BIODEGRADABLE POLYMERIC PARTICLES FOR GDNF DELIVERY INTO THE BRAIN: PREPARATION AND *IN VITRO* CHARACTERIZATION

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Numerous studies have shown the neuroprotective and regenerative benefits of glial cell line-derived neurothropic factor (GDNF) in animal models of Parkinson´s disease (PD) (1). Although several approaches to deliver this protein into the brain have been described (2-4) a new and promising strategy would be the implantation by stereotaxic surgery of drug delivery systems (DDS) containing GDNF in the dopamine-depleted brain areas. This DDS would protect GDNF for degradation and furthermore, would slowly deliver the neurotrophic factor in the central nervous system. Particles would be formulated using biodegradable polymers such as, poly(lactic acid), poly(lactic-co-glycolic acid) which is biocompatible to the brain (5). Several drugs, especially therapeutic proteins like nerve growth factor (NGF) (6) or ciliary neurotrophic factor (CNTF) (7) have been encapsulated in this type of brain delivery system. Unfortunately, proteins are extremely unstable, consequently, their encapsulation and release from biodegradable particles in an active form remain a challenge.

The aim of this study was to develop biodegradable and biocompatible carriers encapsulating recombinant GDNF. These systems were characterized for particle size, morphology, entrapment efficacy and *in vitro* drug release. The relevance of this work was increased by the fact that the GDNF loaded into the particles was purified in our department, and has a higher purity than the commercial (See abstract by E. Ansorena *et al*).

PLGA particles were prepared by the solvent evaporation method after the formation of a multiple emulsion $W_1/O/W_2$ by TROMS (Total Recirculation One Machine System). The mean particle size was analyzed by laser diffractometry, the morphology was studied by SEM and by fluorescence microscopy and the encapsulating efficiency was measured by ELISA (GDNF Emax Immunoassay System Promega). Release studies were performed at 37ºC under horizontal agitation during 7 days. For this purpose, the particles were dispersed in phosphate buffer and at different times, samples were centrifuged and the concentration of the protein in the supernatants was determined by ELISA.

All the formulations displayed a high homogeneous size of about 28 ± 4 µm (Table 1). SEM and fluorescence microscopy showed that the microparticles were spherical in shape and had smooth surface as shown in figure 1. The size range of the particles was found to be consistent with that deduced from the particle size experiments. For all the formulations prepared the encapsulation efficiency was higher than 70%. The *in vitro* release profiles are shown in figure 2. For all the tested formulations, the amount of GDNF released within one week was lower than 20%. *In vitro* release studies will be prolonged during 2 months.

Conclusions from this work are as follows:

- GDNF-PLGA particles were successfully developed by TROMS.
- Particles showed a high protein loading.
- Particles had a suitable size for their administration into the brain and showed a controlled release of the neurotrophic factor.
- GDNF particles could be a promising strategy for treating Parkinson´s disease.

References:
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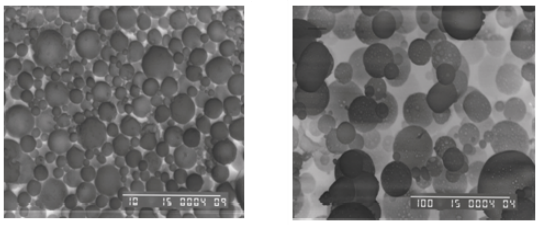
Figures:
Fig. 1: Particles morphology
A) Scanning electronic microscopy (SEM) analysis



B) Fluorescence microscopy analysis

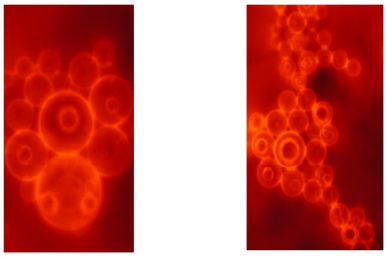
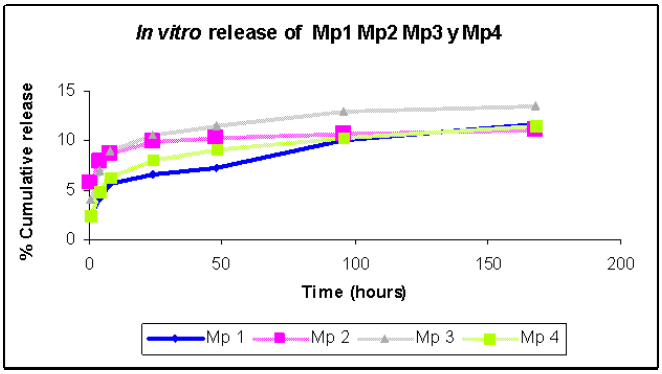


Fig. 2: *In vitro* GDNF release



Tables:
Table 1: Particle size and efficacy of encapsulation

Formulation	Size (µm)	Efficacy of encapsulation
Mp 1	21.52	78.2%
Mp 2	30.90	79.77%
Mp 3	33.18	100%
Mp 4	26.38	69.7%

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