Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biomaterials 26 (2005) 3727–3737
Biomaterials
www.elsevier.com/locate/biomaterials

Pharmacologically active microcarriers: a tool for cell therapy

V.M. Tatard[a], M.C. Venier-Julienne[a], P. Saulnier[a], E. Prechter[a], J.P. Benoit[a], P. Menei[a,b], C.N. Montero-Menei[a,*]

[a]*INSERM U 646, Laboratoire d'ingénierie de la Vectorisation Particulaire, 10 rue André Boquel, 49100 Angers, France*
[b]*Service de Neurochirurgie, Centre Hospitalier Universitaire, 4 rue Larrey, 40033 Angers, France*

Received 15 June 2004; accepted 21 September 2004

Abstract

To overcome certain problems encountered in cell therapy, particularly cell survival, lack of cell differentiation and integration in the host tissue, we developed pharmacologically active microcarriers (PAM). These biodegradable particles made with poly(D,L-lactic-*co*-glycolic acid) (PLGA) and coated with adhesion molecules may serve as a support for cell culture and may be used as cell carriers presenting a controlled delivery of active protein. They can thus support the survival and differentiation of the transported cells as well as their microenvironment. To develop this tool, nerve growth factor (NGF)—releasing PAM, conveying PC12 cells, were produced and characterized. Indeed, these cells have the ability to differentiate into sympathetic—like neurons after adhering to a substrate, in the presence of NGF, and can then release large amounts of dopamine. Certain parameters such as the size of the microcarriers, the conditions enabling the coating of the microparticles and the subsequent adhesion of cells were thus studied to produce optimized PAM.

Keywords: Microcarriers; Cell adhesion; Nerve growth factor; PC12; Cell therapy

1. Introduction

Cell therapy by grafting autologous or non-autologous cells is a promising strategy to repair diseased organs. Indeed, cell transplants are commonly used in bone marrow reconstitution [1,2] and clinical trials for the treatment of neurological disorders, such as Huntington and Parkinson's diseases, have been undertaken [3–5]. Moreover, the recent development of stem cell biology has provided further excitement for cell-based therapy. Another emerging clinical application is the implantation of cell-derived constructs with polymeric devices. In this regard, clinical pilot studies for skeletal tissue repair using this therapeutic approach have given promising results [6]. However, for all these therapies, the short but also long-term survival and functional state of the cells after transplantation, still need to be ameliorated.

Growth and differentiating factors may improve survival and differentiation of the cells, and may also affect the immediate environment, thus allowing better graft integration. Nevertheless, the administration of these factors still remains a technological challenge, due to their short half-life, pleïotropic actions and their limited passage through certain biological barriers like the blood-brain-barrier. To overcome these difficulties, a few groups have developed a site-specific delivery approach, using intracerebral implantation of biodegradable microparticles made of poly(D,L-lactic-*co*-glycolic acid) (PLGA) and allowing a controlled and sustained release of a growth factor [7–9]. We have also studied this approach and demonstrated its efficacy in animal models of neurodegenerative studies [10–12]. Furthermore, this strategy has been successfully used for the release of antimitotic agent in cancer therapy [13].

Another strategy, which uses matrices as transplant vehicles, could also be envisaged. Indeed, some reports

*Corresponding author. Fax: 33 2 41 73 58 53.
E-mail address: claudia.montero-menei@angers.inserm.fr (C.N. Montero-Menei).

0142-9612/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2004.09.042

have suggested that Cytodex® or glass microcarriers enhance the survival of rat adrenal chromaffin cells, rat and human fetal mesencephalic cells and human retinal pigmental epithelial cells grafted in the striatum of hemiparkinsonian rats [14–17]. Moreover, some studies demonstrated that PLGA, which is a commercially available product currently used for human application [18], could be employed as a cell scaffold [19–23]. It thus seems interesting to associate those two strategies to improve graft integration in the host tissue. In this way, Mahoney and Saltzman have demonstrated the interest of a similar approach in animals, using a transplantation system that allows control of fetal brain cell survival and differentiation by pre-assembly of neo-tissues containing cells and nerve growth factor (NGF)-releasing synthetic particles [24].

By combining our expertise in cell therapy and controlled-release technology, we have produced and characterized pharmacologically active microcarriers (PAM) (Fig. 1). These biodegradable PAM are made of PLGA and with an adapted size and a special coating, can serve as a support for cell culture, cell sorting and/or cell administration. Their microcarrier role and the programmed delivery of an appropriate growth factor can stimulate the grafted cells survival and differentiation, and also modify the host microenvironment (promote angiogenesis, local immunosuppression…). Moreover, the coating of their surface with specific bioadhesive components, combined or not with the growth factor can enhance cell adhesion and differentiation and affect stem cells lineage restriction. Finally, as they degrade in approximately 1–2 months after their implantation, they allow a complete integration of the grafted cells in the host tissue.

To develop this tool, we first produced and characterized NGF-releasing PAM conveying PC12 cells. We chose this combination as a model of PAM, since PC12 cells are non-adhering cells that need to attach to a substrate to stop proliferating and thus differentiate into dopamine secreting sympathetic—like neurons, in response to NGF [25,26]. The efficacy of this system may be thus tested in vivo in a Parkinson's disease paradigm. We studied some parameters which seemed to be essential to optimize the PAM. First, we produced microparticles with an adapted size, convenient for their microcarrier role as we envisage their transplantation in the rat brain. Moreover, due to the poor adhesion of the PC12 cells, we tested specific bioadhesive components which could not only enhance cell adhesion but also promote PC12 cell differentiation and survival. We also determined the conditions enabling the coating of the microparticles and the subsequent adhesion of the cells. Finally, the coated PAM were characterized.

2. Materials and methods

2.1. Preparation of microparticles

PLGA composed of 75% of lactic units and 25% of glycolic units ($\bar{M}w = 25,000$ Da ($I = 1.8$)) was supplied by Phusis, Saint-Ismier, France. NGF (NGF 2.5 S murine, 28 KDa) was purchased from Promega, Charbonnière, France and poly(vinyl alcohol) (PVA) from Prolabo, Paris, France.

The microparticles were prepared by a water-in-oil-in-water emulsion–extraction–evaporation process as previously described [27]. Briefly, a 60 μL internal aqueous phase (16 mM citrate buffer pH 6, 2.56 mg of human serum albumin) containing 200 μg of NGF was mixed with 90 μL of polyethylene glycol (PEG 400). This aqueous solution was emulsified by sonication in an organic solution (75% of methylene chloride and 25% of acetone, 2 mL) containing 50 or 75 mg of PLGA. This primary emulsion was obtained at 4 °C in a polytetrafluoroethylene vial to limit the protein adsorption. The resulting emulsion was added to a 4–8 °C external aqueous solution of 0.5–4.5% (w/v) PVA

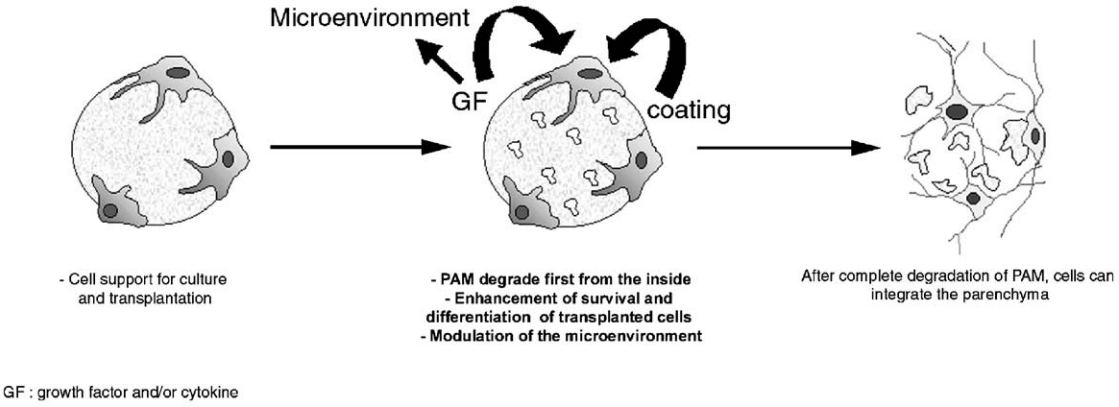

Fig. 1. Drawing illustrating the interest of the PAM's concept. Microspheres coated with synthetic adhesion molecules and releasing growth factors and/or cytokines are used as cell carriers which can then be grafted. Trophic factors and adhesion peptides influence the survival and differentiation of transported cells and the microenvironment. After complete degradation of the microparticles the cells can integrate the parenchyma.