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Role of ECM/peptide coatings on SDF-1α triggered mesenchymal stromal cell migration from microcarriers for cell therapy

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ABSTRACT

Many cell therapies rely on the ability of mesenchymal stromal cells (MSCs) to diffuse and localize throughout the target tissue – such as tumoral and ischemic tissues-, in response to specific cytokine signals, rather than being concentrated at the site of implantation. Therefore, it is fundamental to engineer biomaterial carriers as reservoirs, from which cells can migrate, possibly in a controlled manner. In this work, microcarriers (μCs) made of polylactic acid are characterized as MSC delivery vehicles capable of modulating key chemotactic pathways. The effect of different functionalization strategies on MSC migratory behavior from the μCs is studied *in vitro* in relation to SDF-1α/CXCR4 axis, – a major actor in MSC recruitment, chemotaxis and homing. Collagen and arginine–glycine–aspartic acid (RGD) peptides were either covalently grafted or physisorbed on μC surface. While stable covalent modifications promoted better cell adhesion and higher proliferation compared to physisorption, the functionalization method of the μCs also affected the cells migratory behavior in response to SDF-1α (CXCL12) stimulation. Less stable coatings (physisorbed) showed sensibly higher number of migrating cells than covalent collagen/RGD coatings. The combination of physic-chemical cues provided by protein/peptide functionalization and stimuli induced by 3D culture on μCs improved MSC expression of CXCR4, and exerted a control over cell migration, a condition suitable to promote cell homing after transplantation *in vivo*. These are key findings to highlight the impact of surface modification approaches on chemokine-triggered cell release, and allow designing biomaterials for efficient and controlled cell delivery to damaged tissues.

1. Introduction

One of the most challenging limitations in cell therapy is poor cell survival upon transplantation. More than 90% of the therapeutic cell population dies in the first days after intravenous or direct injection [1]. Moreover, the limited amount of surviving cells suffers from poor tissue localization, because biological fluids can easily disperse them from the desired site [2]. This massive cell death can occur for mechanical damage during the injection, but also for the environmental stress imposed by the target tissue, since injected cells are usually required to attach, home and survive in injured tissues, that can be ischemic, highly inflamed and even necrotic [3]. The use of biomaterial carriers can dramatically increase anchorage-dependent cell viability and engraftment in host tissues, by providing mechanical support, homing and pro-survival cues. Thus, several injectable biomaterials, such as *in situ*-forming hydrogels [4] or microcarriers [5], have been proposed to deliver differentiated and progenitor cells, including MSCs [6]. However, cell survival and engraftment alone may not be sufficient for clinical applications, since cells are often required to migrate and localize at specific sites throughout the target tissues, in order to express their therapeutic activity.

The use of MSCs for cell therapies has received a lot of attention due to the ability of these cells to differentiate toward several phenotypes, exert immunomodulatory activity and secrete paracrine factors [7]. Furthermore, MSCs can migrate toward damaged tissues in a specific manner, and thus can act as vehicles to deliver therapeutic agents to organs whose surgical treatment is not always possible. For example, MSCs transplanted to treat myocardial infarction, can localize to the ischemic heart, and improve its function via bystander effects [8]. Moreover, MSCs have been used to target tumoral cells in aggressive gliomas. It has been shown that, in the brain, MSCs are recruited to the forming tumor vascular network, and then spread into the main tumor mass. At the same

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time, they can also track satellite glioma cells in process of invasion, and associate with them with great accuracy [9]. To exploit this capability, MSCs have been engineered to deliver tumor-killing agents, able to effectively reduce glioma mass in an animal model [10].

This MSC specific migration occurs in response to chemokines expressed by the pathological tissue. Among these signals, a major role is played by stromal cell-derived factor 1α (SDF-1α, or CXCL12), which is a potent chemotactic agent for MSCs, and is also secreted by ischemic and tumoral tissues [8,11]. Additionally, the recognition of SDF-1α by its receptor, CXCR4, triggers a cascade of pro-survival and homing responses [12], and MSCs (as well as immature osteoblasts) continuously secrete SDF-1, presumably to keep themselves in their niche [13]. CXCR4 and SFD-1α are fundamental in physiological tissues, and their knock outs are lethal, resulting in severe bone marrow failure and abnormal development of the heart and brain [14,15]. In this context, the SDF-1α/CXCR4 axis became a promising target for regenerative medicine approaches aiming cardiac, nervous and osteochondral tissue repair [16]. A variety of studies have been conducted to fabricate SDF-1α controlled delivery devices able to induce progenitor cells recruiting for tissue engineering [17,18], as well to improve CXCR4 expression in MSCs, in order to increase viability, migration and regenerative potential, *e.g.* for cardiac cell therapy [19] and angiogenesis [13]. Along with these strategies, biomaterials are still deemed necessary for cell delivery. Therefore, it is important to design biomaterial carriers not only to retain seeded cells, but also to act as reservoirs from which cells can migrate, possibly in a temporally-controlled manner. Even though the study of cell–material interactions is a fundamental aspect of tissue engineering, it is still unclear how to tune biomaterial properties to control cell responsiveness to cytokine stimulation. Furthermore, there is no report about how common biomaterial engineering strategies, such as surface modification, can influence key cell migratory pathways. A deeper understanding of such relationship would provide fundamental hints to improve cell delivery device design.

The aim of this work is to characterize polylactic acid (PLA) μCs as vehicles for cell homing and delivery, and to evaluate their effect on MSCs migratory potential. μCs design was studied to discern how to target SDF-1a/CXCR4 axis via surface functionalization strategies, and thus exert a control over cell release. μCs allow for efficient expansion of MSCs under 3D culture [20] and have been studied for cell release to a wide range of tissues including bone [21], cartilage [22], adipose tissue [23], heart [24] and brain [25]. PLA is used as a material to produce the μCs, since it is a well-know biocompatible polymer, used in FDA-approved devices, is biodegradable with a tunable degradation kinetics, and can be chemically modified to introduce cues to guide cell behavior [26]. Herein, the effect of several μC functionalization approaches on MSC viability, release and migration in relation to SDF-1α/CXCR4 axis was studied *in vitro*. Collagen and RGD peptide-modified μCs were compared to evaluate coatings having different nature – long extracellular matrix (ECM) protein vs. short functional peptide domain. The biomolecules were introduced either via covalent grafting or simple physisorption, in order to assess also the role of the stability of the coating on cell delivery potential.

2. Materials and methods

2.1. Materials

PLA (Purasorb PLDL 7038, inherent viscosity midpoint 3.8 dL/g−1, Mw ≈ 850,000 Da) was purchased from Purac. (−)-Ethyl L-lactate (purity > 99.0%) was obtained from Fluka and used without further purification. Glass microcarrier beads, polyvinyl alcohol (PVA, 30–70 kDa, 88% hydrolyzed) and all the other reagents were from Sigma–Aldrich, unless specified otherwise.

2.2. μC fabrication

PLA μCs were prepared following a previously described protocol [27]. Briefly, a 3.5% w/v PLA solution in ethyl lactate was loaded into a syringe pump and dispensed at a constant rate through the inner bore (30G) of a dual concentric nozzle (NNC-DN-2230, NanoNC, South Korea), while the outer coaxial bore (22G) was fed with gaseous N_2 (feeding pressure $P \approx 0.5$ bar). The polymer solution was dispensed at 10 mL h^{-1}, and the formed droplets were collected into a coagulation bath, composed by 0.3% w/v PVA in 70 vol% ethanol. μCs were rinsed with deionized water, frozen in liquid nitrogen and lyophilized for 48 h. μCs were visualized with Scanning Electron Microscopy (SEM, Quanta Q200, FEI company) and their size, polydispersity and surface area were measured via electrical sensing zone technique (Coulter Counter Multisizer IIe, Beckman Coulter).

2.3. μC functionalization

Surface modification of the μCs was carried out using a three step procedure [28]. At first, μCs were immersed into a 50 mM NaOH solution for 10 min to induce hydrolysis of the PLA backbone and thus enrich the μCs surface with carboxyl groups. After that, exposed COOH terminals were activated with an ethyl(dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide 100 mM/200 mM (EDC/NHS) solution in 70 vol% ethanol for 2 h. Finally, biomolecule coatings were covalently attached to the μC surface through amide bonds between the activated carboxyles and the amino groups from the target protein or peptide. For this purpose, human recombinant collagen type I (FibroGen Inc., USA) or custom-made linear GGGGGGRGDS peptides (composed by a glycine tail, the arginine–glycine–aspartic acid RGD sequence, and a serine residue, GenScript Inc., USA) were dissolved in phosphate buffered saline (PBS) at a concentration of 100 μg mL^{-1} and incubated with the μCs for 24 h. Functionalization via physisorption was carried out by simply immersing untreated PLA μCs into a 100 μg mL^{-1} solution of the desired biomolecule for 24 h. Bicinchoninic acid assay (BCA assay, Thermo Scientific) allowed for the quantification of the grafted protein, following the protocol described by the manufacturer. The different experimental groups are summarized in Table 1.

2.4. Isolation of mesenchymal stromal cells

MSCs were isolated from long bones of 2–4 week old Lewis rats according to a previously published protocol [29]. Briefly, rats were anesthetized using 5% isoflurane and the sacrifice was performed through CO_2 saturated atmosphere. Bone-marrow was obtained by flushing through the bone M199 medium supplemented with 20% fetal bovine serum (FBS), 1% sodium pyruvate, 1% penicillin/streptomycin, 1% L-glutamine and 22 μg mL^{-1} heparin. The cell fraction was resuspended in the same type of medium and plated

Table 1
μCs modified with the different functionalization strategies analyzed in this study.

Sample	Biomolecule	Type of coating
PLA	None	None
CC	Collagen	Covalent
CP	Collagen	Physisorbed
RC	RGD peptide	Covalent
RP	RGD peptide	Physisorbed

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