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Biodegradable ECM-coated PCL microcarriers support scalable human early MSC expansion and *in vivo* bone formation

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Abstract

Background aims. Human mesenchymal stromal cells or marrow stromal cells (MSCs) are of great interest for bone healing due to their multi-potency and trophic effects. However, traditional MSC expansion methods using 2-dimensional monolayer (MNL) flasks or cell stacks are limited by labor-intensive handling, lack of scalability, the need for enzymatic cell harvesting and the need for attachment to a scaffold before *in vivo* delivery. Here, we present a biodegradable microcarrier and MSC bioprocessing system that may overcome the abovementioned challenges. *Methods.* We cultured human early MSCs (heMSCs) on biodegradable polycaprolactone microcarriers (PCL MCs) coated with extracellular matrix (ECM) and evaluated the *in vitro* osteogenic differentiation and *in vivo* bone formation capacity of ECM-coated PCL MC-bound heMSCs compared with conventional MNL-cultured cells. *Results.* We found that heMSCs proliferate well on PCL MCs coated with a fibronectin, poly-l-lysine, and fibronectin (FN+PLL+FN) coating (cPCL MCs). During *in vitro* osteogenic induction, heMSCs cultured on cPCL MCs displayed a 68% increase in specific calcium deposition compared with cultures on MNL. In a mouse ectopic mineralization model, bone mass was equivalent for MNL-expanded and cPCL MC-bound heMSC implants but higher in both cases when compared with cell-free cPCL MC implants at 16 weeks post-implantation. In summary, compared with MNL cultures, biodegradable MC MSC cultures provide the benefits of large-scale expansion of cells and can be delivered *in vivo*, thereby eliminating the need for cell harvesting and use of scaffolds for cell delivery. These results highlight the promise of delivering heMSCs cultured on cPCL MCs for bone applications.

Key Words: *Human mesenchymal stromal cells, PCL, microcarriers, bone formation, osteogenic*

Introduction

Human mesenchymal stromal cells or marrow stromal cells (MSCs) are a heterogeneous population of multipotent cells, defined by their ability to differentiate into cells of the osteogenic, chondrogenic and adipogenic [1] lineages. There has been considerable interest in the therapeutic potential of MSCs because of their ability for self-renewal, differentiation and secretion of cytokines [2]. Human early bone marrow–derived MSCs (heMSCs), which are derived from fetal bone marrow, may be especially well suited for bone-healing applications because they can be maintained for many passages, have a faster doubling time and greater osteogenic capacity and support better in vivo bone formation, compared with MSCs from other sources, such as human umbilical cord–derived and human adipose tissue–derived MSCs [3–5].

Although MSCs show promise for multiple therapeutic applications, the translation of these therapies is hindered by challenges in scalable and reproducible manufacturing of MSCs at volumes that can meet clinical demand [6,7] and the lack of integrative bioprocesses for the expansion and delivery of MSCs. Scalable and efficient ex vivo expansion is an important challenge because clinical applications require

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sizeable MSC doses (up to 10^9 cells per treatment [7]. Classical methods of expanding MSCs for industrial applications in two-dimensional (2D) monolayer flasks (usually in cell stacks) engender modest cell productivity, are less suited to culture monitoring and require laborious and time-consuming handling. In contrast, microcarriers (MCs), which are generally 150- to 200-μm diameter microspheres, provide a high surface-to-volume ratio for adherent cell attachment and can be cultured in controlled stirred bioreactors. Therefore, MSC expansion in microcarrier/bioreactor systems provides advantages of scalability, automation and improved monitoring [7].

We have shown previously that heMSCs can grow on Cytodex 3 dextran MCs [8]. Moreover when cell-covered MCs are directly differentiated into bone, they show enhanced osteogenic differentiation efficiency compared with cells harvested from MCs or from 2D monolayer cultures [8,9]. However, crosslinked dextran MCs cannot be used for bone healing because they are not suitable for bioimplantation and biodegradation.

Therefore, the development of biodegradable/bioimplantable MCs for MSC expansion may potentially provide important benefits for the translation of MSC therapies for bone healing, such as improved differentiation and a streamlined bioprocess that eliminates the need for enzymatic cell harvesting and scaffold seeding. Many biocompatible/bioimplantable microspheres or microparticles have been developed for biomolecule release and defect filling in tissue engineering applications. Although fewer biodegradable MCs have been explored specifically for MSC culture for regenerative medicine applications, those explored include poly(lactic-co-glycolic acid [10], polylactic acid [11], gelatin [12], polycaprolactone (PCL) [13] and composite materials such as PCL-hydroxyapatite [14]. We previously developed spherical PCL biodegradable MCs with a uniform size distribution using an oil-in-water emulsion method that employs microfluidic technology and demonstrates their capability to support hESC growth [10]. In this study, we further investigate their use for expansion of heMSCs for bone healing.

Current methods of bone tissue engineering use complicated bioprocesses in which expanded MSCs are seeded onto porous scaffolds and optionally cultured in perfusion bioreactors to ensure homogeneous cell attachment throughout the scaffold and sometimes ex vivo osteogenic differentiation before implantation [5]. The use of implantable and biodegradable MCs for MSC implantation may offer a simpler approach because biodegradable MCs covered with cells can be implanted directly in vivo and may eliminate the need for scaffolds for MSC delivery or for the culture of macro-scale MSC-scaffold in specialized bioreactors [5].

Previous studies using biodegradable MCs for MSC culture [11–15] have established that these MCs support growth and maintain the cells differentiation potential in vitro. However, to our knowledge, no studies have explored both the in vitro expansion and in vivo bone-healing capacity of MSCs that are expanded on and delivered while still attached to biodegradable MCs, including comparisons to 2D monolayer-expanded MSC controls. In this study, we examine the in vitro osteogenic differentiation and in vivo bone formation of biodegradable PCL MCs with a multilayer extracellular matrix (ECM) coating for heMSC culture and investigate the ability of cell laden MCs to support osteogenesis in a mouse subcutaneous ectopic mineralization model.

Methods

Reagents and chemicals

PCL (average 80 KDa), polyvinyl alcohol and poly(tetrafluoroethylene) (PTFE) tubing was sourced from Sigma-Aldrich. Dichloromethane (DCM, ACS grade) was purchased from J.T. Baker, and 27-G needles were purchased from BD Biosciences. Polypropylene (PP) syringes, with 20-mL volume, were acquired from Braun Medical. A dual syringe rate pump (Gemini 88) was purchased from KD Scientific. Cytodex 3 MCs were purchased from GE HealthCare. Human plasma purified fibronectin (FN; catalogue #33016-015) and all culture media/supplements were purchased from Life Technologies. All chemicals reagents and poly-l-lysine PLL (MW 70–150 kDa, catalogue #P6282) were purchased from Sigma-Aldrich. All culture ware other than T-25 (Corning) ultra-low attachment flasks were purchased from BD Biosciences.

Microcarrier fabrication

PCL MCs were fabricated by using a needle/tubing microfluidic device, as described by Li *et al.* [10]. Briefly, two syringes were mounted on a dual syringe rate pump, for feeding the dispersed-phase (3% w/v PCL in DCM) and the continuous-phase (3% w/v PVA in distilled water) into PTFE tubing and a 27-G needle, at flow rates of 350 and 150 uL/min, respectively. The PCL/DCM droplets were collected in 3% aqueous PVA solution. PVA solution was decanted, and DCM solvent was evaporated for 3–8 days. MCs were washed with distilled water and dried in a vacuum oven overnight, treated with 5 mol/L NaOH for 3 h, incubated in 70% ethanol for 30 min, rinsed with distilled water and stored in phosphate-buffered saline (PBS) before coating.