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Solvent-free preparation of porous poly(L-lactide) microcarriers for cell culture

Mirasbek Kuterbekov [a,b], Paul Machillot [c], Pierre Lhuissier [d], Catherine Picart [b,c], Alain M. Jonas [a,*], Karine Glinel [a,*]

[a] Université catholique de Louvain, Institute of Condensed Matter & Nanosciences (Bio & Soft Matter), Croix du Sud 1, box L7.04.02, 1348 Louvain-la-Neuve, Belgium
[b] Communauté Université Grenoble Alpes, LMGP, 3 parvis Louis Néel, 38016 Grenoble, France
[c] CNRS UMR 5628 (LMGP), 3 parvis Louis Néel, 38016 Grenoble, France
[d] Université Grenoble Alpes, CNRS, Grenoble INP, SIMAP, Grenoble, France

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A B S T R A C T

Porous polymeric microcarriers are a versatile class of biomaterial constructs with extensive use in drug delivery, cell culture and tissue engineering. Currently, most methods for their production require potentially toxic organic solvents with complex setups which limit their suitability for biomedical applications and their large-scale production. Herein, we report an organic, solvent-free method for the fabrication of porous poly(L-lactide) (PLLA) microcarriers. The method is based on the spherulitic crystallization of PLLA in its miscible blends with poly(ethylene glycol) (PEG). It is shown that the PLLA spherulites are easily recovered as microcarriers from the blends by a water-based process. Independent control over microcarrier size and porosity is demonstrated, with a higher crystallization temperature leading to a larger size, and a higher PLLA content in the starting blend resulting in a lower microcarrier porosity. Microcarriers are shown to be biocompatible for the culture of murine myoblasts and human adipose stromal/stem cells (hASC). Moreover, they support not only the long-term proliferation of both cell types but also hASC differentiation toward osseous tissues. Furthermore, while no significant differences are observed during cell proliferation on microcarriers of two different porosities, microcarriers of lower porosity induce a stronger hASC osteogenic differentiation, as evidenced by higher ALP enzymatic activity and matrix mineralization. Consequently, the proposed organic-solvent-free method for the fabrication of biocompatible porous PLLA microcarriers represents an innovative methodology for ex vivo cell expansion and its application in stem cell therapy and tissue engineering.

Statement of Significance

We report a new solvent-free method for the preparation of porous polymeric microcarriers for cell culture, based on biocompatible poly(L-lactide), with independently controllable size and porosity. This approach, based on the spherulitic crystallization in polymer blends, offers the advantages of simple implementation, biological and environmental safety, easy adaptability and up-scalablility. The suitability of these microcarriers is demonstrated for long-term culture of both murine myoblasts and human adipose stromal/stem cells (hASCs). We show that prepared microcarriers support the osteogenic differentiation of hASCs, provided microcarriers of properly-tuned porosity are used. Hence, this new method is an important addition to the arsenal of microcarrier fabrication techniques, which will contribute to the adoption, regulatory approval and eventually clinical availability of microcarrier-based treatments and therapies.

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1. Introduction

Microcarriers are an important class of biomedical constructs that have attracted strong research interest due to their high

* Corresponding authors.
E-mail addresses: alain.jonas@uclouvain.be (A.M. Jonas), karine.glinel@uclouvain.be (K. Glinel).

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surface-to-volume ratio [1], compatibility with dynamic cell culture [2] and mimicry of natural extracellular environments [3]. Their micrometer size allows them to circumvent many of the issues facing traditional monolayer cultures, which confine cells to non-native 2D environments, and cultures performed on macro-sized 3D substrates, which suffer from limited nutrient-metabolite exchange and cell colonization at the core [4]. First conceptualized [1] and still in use as substrates for efficient ex vivo cell expansion [5,6], microcarriers found further utility as delivery vehicles for drugs [7], proteins [8], cells [9] and as tissue engineering substrates [10–12]. This versatility extends to their composition and design as well: metals [13], ceramics [10], polymers [14] and their composites [15] have been used to produce either solid-filled, porous or hollow microcarriers depending on the intended application [16].

In the context of cell culture and tissue engineering, porous polymeric microcarriers are particularly attractive [17], in part due to their high porosity which facilitates cell adhesion, colonization and survival [5] but also improves nutrient-metabolite exchange [2]. Moreover, the use of polymers to fabricate microcarriers allows to couple the tunability of their composition and material properties with affordability and reliability in terms of large-scale production. To date, various methods have been developed for the fabrication of polymeric microcarriers such as emulsion solvent evaporation [14,18], solvent evaporation [19], spray-drying [20] and jet milling [21]. Some of them permit control over microcarrier architecture, like the modified double emulsion solvent evaporation method [14]. However, they usually require potentially toxic organic solvents and complex setups with time-consuming drying steps and costly waste management which are detrimental for *in vivo* applications and large-scale developments, respectively [17]. Alternative methods such as the jet break-up solvent displacement method [22] were developed to sidestep these concerns. However, till now, they lack the ability to exercise fine control over microcarrier architecture.

Herein we describe a novel and very simple solvent-free methodology to produce microcarriers, based on the spherulitic crystallization of the biocompatible poly(L-lactide) (PLLA) polymer. To the best of our knowledge, no study has attempted to recover polymeric spherulites as discrete 3D structures for use in biomedical applications. The crystallization of the macromolecular chains leads to the formation of spherulites which are polycrystalline aggregates composed of highly crystalline chain-folded lamellae interconnected by conformationally-disordered tie segments accumulating in interlamellar amorphous regions [23,24]. In this work, the spherulitic crystallization of PLLA [25–29] was performed within a polymer blend containing poly(ethylene glycol) (PEG). PEG has a much lower crystallization temperature than PLLA (close to ambient) [26] and forms miscible blends with molten PLLA [30]. It is rejected into interspherulitic and interfibrillar regions during PLLA crystallization, and can be subsequently dissolved in water [31] in a biocompatible manner to recover isolated porous PLLA spherulites. The commercial availability of PLLA and PEG ensures upscalability, and both polymers are already approved for use in biomedical applications by regulatory bodies. Additionally, PLLA displays an interesting biodegradability profile [32]. Importantly, we demonstrate the ability to independently control microcarrier porosity and size, by varying crystallization temperature and PLLA/PEG blend composition and confirm their suitability for long-term culture of murine myoblasts (C2C12) and human adipose-derived stromal/stem cells (hASCs) in static culture conditions. We further validate these microcarriers as viable 3D tissue engineering substrates for the formation of hASC-derived osseous tissues.

2. Materials and methods

All reagents and products were purchased from Sigma-Aldrich and ThermoFischer Scientific unless stated otherwise.

2.1. Observation of spherulitic crystallization in PLLA/PEG blends

$PLLA_{20}$ (M_n 20,000 g/mol) and PEG (M_n 3350 g/mol) (Fig. 1B) were dissolved in chloroform (40 mg/mL) and mixed at a $PLLA_{20}$/PEG ratio by mass of 2/8. The polymer blend was precipitated with *n*-hexane in excess, filtered and dried under vacuum for 3 h to remove the solvent. The resulting powder was placed between two glass slides inside a microscope hot-stage system (Mettler Toledo HS82, Belgium) mounted on an optical microscope (Olympus AX70, Belgium) fitted with a cross-polarized filter and a camera. The temperature of the hot stage system was first set to 180 °C for 1 min to melt the polymer powder then lowered to 125–140 °C and kept for 30 min to crystallize PLLA. During this time, microscopy images were recorded to observe the formation of spherulites (Fig. 1C).

2.2. Microcarrier fabrication via isothermal spherulitic crystallization

PLLA microcarriers were prepared via isothermal spherulitic crystallization of PLLA/PEG blends at 110–140 °C (Fig. 1). PLLA of three different molar masses ($PLLA_5$ M_n 5000 g/mol; $PLLA_{20}$ M_n 20,000 g/mol; and $PLLA_{59}$ M_n 59,000 g/mol, M_w 101,000 g/mol) were used along with PEG (M_n 3350 g/mol) to achieve three different PLLA/PEG ratios (1/9, 2/8 and 3/7, expressed by mass). The blends were prepared either via solvent-based co-precipitation or solvent-free melt-mixing (Fig. 1A) and isothermally crystallized overnight under inert atmosphere, either in aluminum crucibles placed in a differential scanning calorimeter (DSC, Mettler Toledo DSC821e, Belgium) or in glass tubes fitted with a rubber septum inside an oil bath with a 0.1 °C temperature control. Once crystallized, the blends were repeatedly washed with deionized water to dissolve PEG and collect the insoluble PLLA microcarriers.

2.2.1. Blend preparation via co-precipitation

A PLLA/PEG chloroform solution (40 mg/mL) was prepared with a desired PLLA/PEG ratio (Fig. 1A). Then the polymer blend was precipitated with *n*-hexane in excess, filtered and dried under vacuum for 3 h to remove the solvent. The obtained powder was melted at 230 °C for 10 min under inert atmosphere before decreasing the temperature (between 110 and 140 °C) to perform the isothermal spherulitic crystallization of PLLA.

2.2.2. Blend preparation via melt-mixing

PLLA and PEG in the form of dry powders were combined in a desired PLLA/PEG ratio and melt-mixed at 230 °C for 30 min using a magnetic stir bar before decreasing the temperature to perform the isothermal spherulitic crystallization of PLLA (Fig. 1A). The use of a low molar mass PEG allowed us to achieve a reproducible blending with a simple stirring procedure.

2.3. Characterization of microcarrier size

Microcarriers suspended in deionized water were observed with a stereomicroscope (Leica MZ 6, Germany) equipped with a digital camera (Olympus SC30, France). Their average size for a given crystallization temperature was obtained based on the geometric means between the longest and shortest axes of 30 randomly-selected microcarriers per crystallization temperature.

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