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Dewetting based fabrication of fibrous micro-scaffolds as potential injectable cell carriers

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ABSTRACT

Although regenerative medicine utilizing tissue scaffolds has made enormous strides in recent years, many constraints still hamper their effectiveness. A limitation of many scaffolds is that they form surface patches, which are not particularly effective for some types of "wounds" that are deep within tissues, e.g., stroke and myocardial infarction. In this study, we reported the generation of fibrous micro-scaffolds feasible for delivering cells by injection into the tissue parenchyma. The micro-scaffolds (widths < 100 μm) were made by dewetting of poly(lactic-co-glycolic acid) thin films containing parallel strips, and cells were seeded to form cell/polymer micro-constructs during or post the micro-scaffold fabrication process. Five types of cells including rat induced vascular progenitor cells were assessed for the formation of the micro-constructs. Critical factors in forming fibrous micro-scaffolds via dewetting of polymer thin films were found to be properties of polymers and supporting substrates, temperature, and proteins in the culture medium. Also, the ability of cells to attach to the micro-scaffolds was essential in forming cell/polymer micro-constructs. Both *in vitro* and *in vivo* assessments of injecting these micro-scaffolding constructs showed, as compared to free cells, enhanced cell retention at the injected site, which could lead to improved tissue engineering and regeneration.

1. Introduction

Optimal scaffold design/fabrication is an important element in tissue engineering strategies [1], especially to realize the full potential of regenerative medical therapies. Many techniques have been developed for fabricating tissue engineering scaffolds including solution casting [2–4], particulate leaching [2,3,5], thermally induced phase separation (TIPS) [2–5], and electric spinning [2,3,6–9]. They are generally aimed at generating large scaffolds (mm or larger) for applications that would be confined to easily accessible locations, such as the surface of an organ, and they could not be injected or used for a deep wound such as a myocardial infarct or a stroke. For injection based therapy in tissue regeneration, smaller scaffolds are more desirable.

The injection based approach is clinically preferred due to its minimally invasive nature. To this end, the development of biomaterial-assisted cell micro-carriers for effective injection is essential. These carriers not only provide supports for cell attachment/proliferation prior to injection, but also, as compared to a biomaterial without cells or cells without a biomaterial, enhance cell retention at the injection sites and have a greater ability to repair damaged tissues [10]. Moreover, it has also been reported that porous injectable cell carriers are superior to the non-porous counterparts in terms of cell adhesion rate and tissue regeneration [11,12].

Currently, the most common micro-carriers include: (1) hydrogel based cell encapsulation systems [13–18] — with gelation either prior or post-injection; (2) micro-particles, micro-beads or micro-gels, either porous or non-porous, made out of natural and synthetic polymers [5,10–13,17,19,20] or decellularized matrix [21]; and (3) the combinations of (1) & (2). For hydrogel based micro-carriers [i.e. (1) above], the potential lack of sufficient supply of oxygen or nutrient to the entrapped cells that might lead to massive cell death, the non-uniform cell distribution, and the limitation of cell migration and engraftment into tissue are major concerns [10,11,18]. For non-porous spherical carriers — beads or particles, cell infiltration is impossible and modification of these beads/particles is generally needed to enhance cell attachment and matrix–cell interactions [12,22–24]. To enhance cell loading capacity, highly porous microspheres that allowed efficient cell infiltration have been produced. While enhanced cell loading and effective mass transfer of oxygen and nutrients have been achieved using these highly porous microspheres, the process of incorporating cells into the microspheres is tedious [10]. It requires incubation of individual microspheres to avoid aggregation prior to injection. Also, the surface of the scaffold is normally very different from the fibrous structures of native tissue for good cell attachment. To mimic the cellular matrix of native tissue, attempts have been made to generate highly porous hollow microspheres with fibrous structures, but most pores from these attempts are too

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small for cells to infiltrate. In addition to the above requirements, migration and engraftment of cells from these structures into surrounding tissues *in vivo* are essential. Therefore, suitable techniques for generating fibrous porous carriers that can enhance cell attachment/proliferation prior to injection and serve as effective injection carriers for cell retention/migration/engraftment post-injection are still needed.

In this study, we reported a dewetting based approach for generating loosely packed (i.e. highly porous) fibrous scaffolds and subsequently forming micro-constructs as an alternative injectable cell microcarrier (schematic of the process is shown in Fig. 1). A model polymer, biodegradable poly(lactic-co-glycolic acid) (PLGA), and several cell types were used to illustrate the feasibility of the approach. Also, the fibrous micro-constructs were employed to demonstrate that they could be promising for injection based cell therapy.

2. Materials and methods

2.1. Preparation of supporting substrates, PDMS stamps, and imprinted polymer films

Glass slides (cut to 1 cm × 2 cm pieces; Fisher Scientific, Waltham, MA) were cleaned using a freshly prepared piranha solution [i.e. 70/30 (v/v) of 98% H_2SO_4 (Fisher) and 30% H_2O_2 (Fisher)] followed with thorough rinsing using DI water (purified in house with a conductivity value of 0.1 μS or less). Some cleaned slides were modified with 2-[methoxy poly(ethyleneoxy) propyltrimethoxysilane ($CH_3O(CH_2CH_2O)_{6-9}(CH_2)_3Si(OCH_3)_3$, PEG-silane; Gelest, Morrisville, PA) according to the previous procedure [25–27], while others were coated with a layer (200–300 μm) of agarose (UltraPure™ agarose, Life Technologies, Carlsbad, CA) gel by spreading a hot (60–80 °C) 1% (w/w) agarose solution in DI water over the slide and then cooled to room temperature.

Polydimethylsiloxane (PDMS) stamps were fabricated from Sylgard® 184 (Dow Corning, Midland, MI). A piece (12 mm × 10 mm) of silicon wafer (Silicon Quest International, Reno, NV) containing parallel strips fabricated using the fracture-induced structuring method we developed [28] was used as the mold. The mold was first modified with a non-adhesion agent, octadecyltrichlorosilane (Gelest), and the Sylgard® 184 pre-polymer mixture was poured over the mold, de-gassed, cured at ~60 °C for 4 h, and then separated from the mold.

For generating an imprinted PLGA (Sigma-Aldrich, Mw = 35–60 kg/mol, St Louis, MO) film, a drop of 2.5% (w/w) of PLGA solution in acetone (Fisher) was spread on a slide, the PDMS stamp was pressed against the spread drop until the solution dried, and then the stamp was removed (Fig. 2A). Imprinted films of poly(D-lactic acid) (PDLA, Polysciences Inc., M_W = 15 kg/mol, Warrington, PA) and 1% (w/w) of 3-aminopropyltriethoxysilane (APTES, Sigma-Aldrich) in PLGA were also prepared by using 2.5% (w/w) of the corresponding solution in acetone. The resulting imprinted films were examined using an optical microscope (Olympus IX71, B&B Microscope, Pittsburg, PA) and an atomic force microscope (Multimode NanoScope V, Veeco, Plainview, NY).

2.2. Formation of fibrous micro-scaffolds and cell/polymer micro-constructs

In the first approach to form fibrous cell/polymer micro-constructs, polymer films imprinted on glass slides and on the PEG-silane modified glass slides were directly seeded with cells and incubated for a period of 1 to 3 days (bottom row of Fig. 1). Five different cell types used in this study were: mouse embryonic fibroblast (MEF, Millipore, Billerica, MA), rat induced vascular progenitor cells (iVPCs, details can be found in an earlier publication, [29]), human embryonic kidney 293A cell line (293A, ATCC, Manassas, VA), human liver cell line (HepG2, ATCC, Manassas, VA), and rat endothelial cells (ECs, Cell Applications, San Diego, CA). Of the five different cell types, MEFs and iVPCs were used for detailed assessments. The morphology of cells on the films was examined using the optical microscope, and the films, if still remained or formed fibrous constructs, were then separated from glass or the PEG-silane modified glass to be sectioned.

In a second approach, imprinted PLGA films on glass slides were incubated in DI water at ~50 °C for ~2 h to only dewet the thin PLGA strips, and to form fibrous scaffolds (top row of Fig. 1). Then the entire film was floated off and picked up onto a glass slide, a PEG-saline modified glass slide or an agarose coated glass slide. Since the stamp size (12 mm × 10 mm) was slightly larger than the area (~10 mm × 8 mm) containing strips, the strips were connected to the rest of the film by their ends. Four edges of the picked up film were then secured down to the slide by using a small amount of 10% (w/w) PLGA solution as glue.

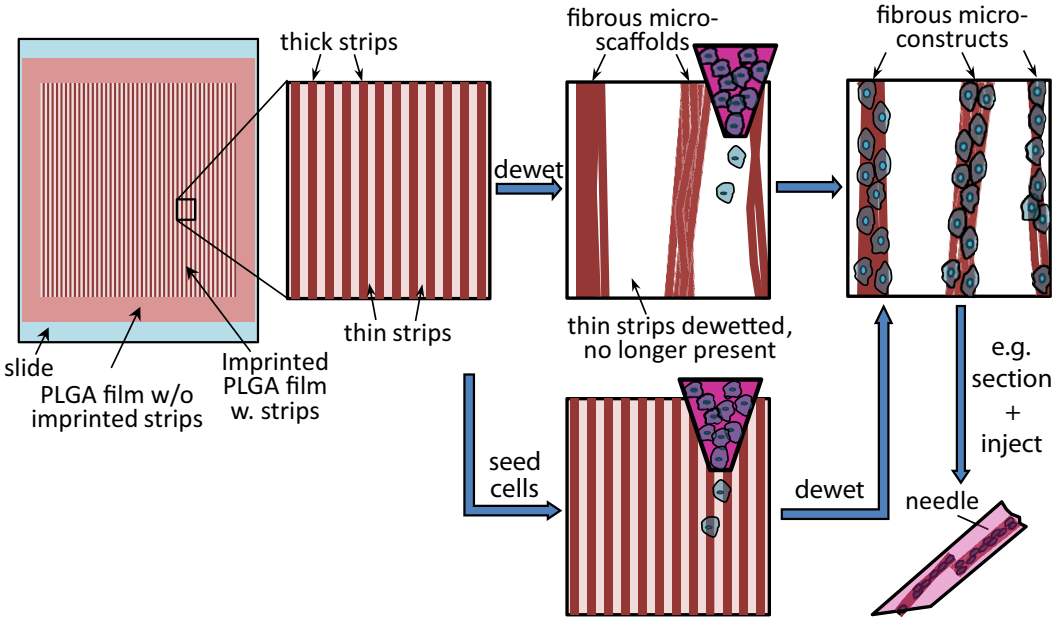

Fig. 1. The schematic illustrates the formation of cell/polymer fibrous micro-constructs from the imprinted films containing strips and utilizing a dewetting process. The polymer fibrous micro-scaffolds might be first formed by dewetting, and then cells are seeded on these micro-scaffolds to form the micro-constructs (top row). Alternatively, the cells might be seeded directly to the imprinted polymer films, which might dewet during cell incubation, leading to the formation of cell/polymer micro-constructs.