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Microcryogels as injectable 3-D cellular microniches for site-directed and augmented cell delivery

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ABSTRACT

The success of cell therapy for tissue repair and regeneration demands efficient and reliable cell delivery methods. Here we established a novel microengineered cryogel (microcryogel) array chip containing microcryogels with predefined size and shape as injectable cell delivery vehicles. The microscale macroporous cryogels enabled automatic and homogeneous loading of tailored cellular niches (e.g. cells, matrices, bioactive factors) and could be easily harvested from the ready-to-use array chip. In contrast to microscale hydrogels, microcryogels exhibited excellent elasticity and could retain their shape and integrity after injection through the microsyringe routinely used for cell therapy. Human mesenchymal stromal cells loaded within microcryogels could be shielded from the mechanical insult and necrosis caused by during direct cell injection. After subcutaneous injection to the mice, cell-loaded microcryogels exhibited concentrated localization and enhanced retention at the injection site compared to dissociated cells. To demonstrate the potential therapeutic application for ischemic diseases, site-directed induction of angiogenesis was achieved subcutaneously in mice 2 weeks after injection of NIH/3T3 fibroblast-loaded microcryogels, indicating long-term engraftment, accumulative paracrine stimulation and augmented host tissue integration. Our results convincingly showed the great promise of microcryogels as 3-D cellular microniches and injectable cell delivery vehicles to tackle major challenges faced by cell therapy-based regenerative medicine including shear-induced damages, uncontrolled localization, poor retention, limited cellular survival and functionalities in vivo.

1. Introduction

Cell-therapy-based regenerative medicine holds great promise for repair and restoration of damaged tissues or organs. It has been widely investigated in animal models and clinical trials for treating hematological disease, cardiovascular disease, diabetes, Parkinson's disease, cartilage tissue damage and tumors [1]. However, the lack of effective cell delivery methods has been one of the main problems preventing cell therapy from achieving satisfactory clinical outcomes [2]. A common delivery route in clinical cell therapy is based on either systematic administration (e.g. intravenous), relying on cells homing into the lesion sites [3,4], or direct injection of cells into the damaged tissues [5], but the therapeutic benefits of the transplanted cells are limited due to cell loss and cell death. Taking cell therapy for ischemic heart diseases as an example, success in various clinical trials of cell-based cardiac repair (e.g. BOOST, TOPCARE, the MAGIC II, the REPAIR-MI, the ASTAMI) with direct cell injection into the heart and/or coronary arteries have been modest due to poor cell survival [6–9]. Acute (<24 h) cell retention of autologous human mesenchymal stromal cells (MSCs) injected for treating myocardial infarction was normally less than 10%, regardless of the delivery route [10]. For long-term effects, only 7% of skeletal myoblasts were reported to survive for 3 days after grafting into an infarcted mouse heart [11], and ~5% of MSCs survived after being transplanted into an infarcted porcine heart [12]. Mechanical damage during injection, high leakage to surrounding tissues, ischemic and inflammatory in the in vivo microenvironment within the lesion tissues could all contribute to poor cell retention, cell survival and reproducibility of the treatment [7]. As a potential solution, cellular aggregates were preformed and injected as spheres, resulting in enhancement of the cellular retention and survival compared to direct injection of dispersed cells [13,14]. However, additional time was required to perform the aggregates, which usually led to non-uniform size and inevitably mechanical injury to the cells on the superficial layer during injection. Clearly, there is a considerable demand for delivery strategies to attenuate cell loss and death for long-term cell engraftment and functional improvement.

Biomaterial-assisted cell delivery systems are expected to tackle some of the above-mentioned challenges. Various types of biomaterial-based therapeutic cell delivery systems have been developed and could be mainly categorized as implantable and

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injectable. Scaffold-based cell delivery platforms have been widely used in tissue engineering as implantable support for cell growth and function [15,16]. An obvious disadvantage of scaffold-based cell implantation lies in its invasiveness with surgical trauma, which restricts its wide translation from bench to bedside.

Minimally invasive therapeutic approaches, such as injectable treatment, can avoid concerns raised by traumatic surgery and post-operative wound healing, providing a simple, convenient and less painful operation. For mini-invasive cell delivery, in situ crosslinkable hydrogels are one of the most widely applied injectable vehicles for cell therapy. Cells are usually injected along with a hydrogel precursor solution, which gels in situ via thermo- [17], chemical [18] or enzymatic crosslinking [19]. Injectable hydrogels could effectively improve cell retention and provide an amiable microenvironment. However, cells are still exposed to mechanical shear during co-injection with aqueous hydrogel precursors, and in situ gelation of hydrogel might cause damage to the encapsulated cells. In addition, in situ gelation is hard to control, resulting in heterogeneous cell distribution and a bulky 3-D structure with mass transfer issues endangering cell survival.

Recently, self-assembled hollow spheres [20] and microspheres synthesized by the airjet droplet-based method [21] were developed as injectable cell carriers and demonstrated improved cellular growth and tissue repair [22]. The fabrication and cell-seeding procedures were relatively complicated and tedious, resulting in a wide size distribution of the microcarriers and non-homogeneous cell seeding onto the carriers. In addition, the mechanical properties of the peptide or extracellular matrix-constituted carriers were expected to be poor, easily breaking or deforming after injection through a microsyringe with the fine-size needles (e.g. 27-gauge) routinely used for cell therapy [23,24].

An optimal cell delivery system is critical for ultimately making the promise of current cell therapy a widely acknowledged and practiced clinical success. The ideal biomaterial-assisted cell delivery system needs to at least satisfy the following requirements: (i) simple and large-scale manufacture of the delivery vehicles; (ii) efficient and reproducible loading of the tailored cellular niche components (cells, matrix or bioactive factors); (iii) protection of cells from mechanical and biochemical injury during loading and injection; (iv) targeted delivery and long-term retention at the therapeutic site; (v) enhanced integration with the lesion tissue and therapeutic efficacy; (vi) easiness and readiness to be adapted to existing clinical practices for cell therapy. The goal of the present study is to meet these unmet needs in biomaterial-assisted cell delivery by developing novel microengineered cryogels (microcryogels) as injectable 3-D cellular microniches for optimal cell delivery and therapy.

Cryogels are gel matrices formed in moderately frozen solutions of monomeric or polymeric precursors [25]. The application of cryogels in microbiology goes back to the 1980s, when they were applied as matrices for cell immobilization via mechanical entrapment [26]. During cryogelation at subzero temperatures, the reactants concentrate in the unfrozen/semi-frozen phases forming the crosslinked network, while ice crystals nucleated from the aqueous phase (formed by the freezing of water at low temperatures) function as porogens [27]. Cryogels typically exhibit interconnected macroporous structures, allowing unhindered diffusion of molecules, particles and even cells, together with exceptional elasticity, permitting shape recovery after deformation [28]. The pore size of cryogels can be tuned to offer optimum ranges for culturing different types of cells: for example, pore sizes of ~5 μm for neovascularization, 5–15 μm for fibroblast ingrowth, ~20 μm for hepatocyte ingrowth, 20–125 μm for skin regeneration, 70–120 μm for chondrocyte ingrowth, 40–150 μm for fibroblast binding, 45–150 μm for liver tissue regeneration and 60–150 μm for vascular smooth muscle cell binding [29]. The unique properties of cryogels make them attractive matrices for bioseparation (e.g. plasmids, viruses, microbial or mammalian cells) or for immobilization of biomolecules as 3-D scaffolds for cell culture [26,30]. In this work, we prepared poly(ethylene glycol) (PEG)-derived cryogels due to the good biocompatibility and wide applicability of PEG-based materials in tissue engineering [28].

Here, we integrated microfabrication technology with cryogel preparation to develop a microcryogel array chip containing arrayed microscale PEG-derived cryogels (microcryogels) with predefined sizes and shapes as injectable cell delivery vehicles. The microscale and macroporosity of the novel microcryogels allowed automatic and homogeneous loading of cellular niche components (e.g. cells and matrices) on the array chip by a simple scraping approach. Also, the miniaturized size and exceptional elasticity of the microcryogels enabled the desired injectability, cell protection and site-directed retention after injection in vivo. In addition, tailored 3-D cellular niches loaded in the microcryogels provided a biomimetic microenvironment beneficial for cell survival and functions both in vitro and in vivo [31]. To our knowledge, this is the first demonstration of fabrication and application of microscale cryogels as cell carriers and delivery vehicles via injection that meets the operation standards routinely applied for cell therapy.

As an illustration of potential therapeutic effects, we conducted preliminary investigation on angiogenesis induction by microcryogel-delivered NIH/3T3 fibroblasts. Numerous studies, including two Phase I clinical trials, have proved the angiogenic capacity of fibroblasts for treatment of ischemic diseases such as myocardial infarction [7,32,33]. Augmented angiogenesis was induced in a site-directed manner after subcutaneous injection of hundreds of these microniches in BALB/c mice. The microcryogels developed here offer powerful platform technology for cell delivery and are expected to be widely applicable to assist and augment current cell therapy in regenerative medicine.

2. Materials and methods

2.1. Design and fabrication of the microstencil array chip

A microstencil array chip was used as a micromold for the fabrication of PEG diacrylate (PEGDA) microcryogel array chips. Poly(methymethacrylate) (PMMA) microstencil array chips (75 cm × 25 cm, with thickness of 300 μm) were designed by CAD software and fabricated by a laser prototyping technique using the Rayjet laser system (Rayjet, Australia) [34]. The microstencil chip contained arrays of specific geometries, such as circles, ellipses, triangles or clovers, with different sizes (e.g. the circle microstencil had diameters of 100, 200, 400 and 800 μm). To increase the hydrophilicity, the microstencil array chips were treated with Plasma Cleaner (Mycro Technologies, USA) for 1 min.

2.2. Fabrication of the PEGDA microcryogel array chip

PEGDA precursor solution was prepared by dissolving 10% w/v PEGDA, 0.5% w/v of ammonium persulfate and 0.15% w/v of *N,N,N′N′*-tetramethylethylenediamine in cold phosphate-buffered saline (PBS) and maintained on ice [28]. Next, 150 μl of the precursor solution was pipetted onto the upper surface of the microstencil array chip (circle microstencils with diameter 400 μm), and was evenly distributed by manually scraping with a cover-slide back and forth several times. The scraping of the cover-slide facilitated the automatic loading of the precursor solution into the empty microstencil arrays in the chip. The microstencil array chip filled with PEGDA precursor solution was then placed in a refrigerator at −20 °C and underwent cryogelation for 20 h. The array chips were subsequently lyophilized (Boyikang, China) for 30 min,