Functionalized PLGA Scaffolds Embedded with Mesenchymal Stem Cell-encapsulated Alginate Hydrogel Microspheres for Tissue Regeneration

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Introduction: Cell-laden scaffolds have been widely investigated in tissue engineering. Hydrogels are attractive materials for making cell-laden scaffolds due to their high water content and excellent biocompatibility [Kim YB, et al., *J Colloid Interface Sci*, 2016, 461:359-368.]. But they are disadvantaged by their weak mechanical strength, which make them difficult to be used alone for scaffolds in tissue engineering. Furthermore, an important issue for their processability is to obtain complex three-dimensional bulk structures with an internal porous architecture, which is necessary for the transport of metabolic waste and nutrients [Hwang CM, et al., *Biofabrication*, 2010, 2(3): 035003]. To improve the mechanical stability of cell-laden scaffolds, other techniques or materials need to be combined with the cell-laden structure. Improvements in mechanical properties as well as cell embedding ability could be made by using synthetic materials to support cell-laden hydrogels [Yeo MG and Kim GH, *J Mater Chem B*, 2014, 2(39): 6830-6838]. However, a few important issues need to be addressed. For example, when cells are released from hydrogels, they will contact the supporting material. Therefore, the induction property of supporting materials for cells is critical for cell proliferation and differentiation. In the current study, electrospray was used to produce mesenchymal stem cell (MSC)-containing alginate hydrogel microspheres and basic fibroblast growth factor (bFGF) was encapsulated in poly(lactic acid-co-glycolic acid) (PLGA) nanofibers via emulsion electrospinning. The mechanically strong cell-incorporated scaffolds consisted of a bFGF-encapsualted PLGA membrane as the base and MSC-encapsulated alginate microspheres as cell reservoir.

Methods: (1) Preparation of MSC-encapsulated alginate microspheres: An alginate polymer solution (5% alginate concentration) was mixed with an MSC suspension ($1x10^6$ cell density), which was then used to make MSC-encapsulated alginate microspheres via electrospray (20 kV for applied voltage and 2 ml/h for solution feeding rate). (2) Electrospinning of PLGA nanofibers: PLGA (LA:GA at 75:25) was dissolved in a co-solvent (dichloromethane/N,N-dimethyl formamide (DCM/DMF) at 3:1 by volume) to make polymer solutions of 20% PLGA concentration which were electrospun into nanofibers (15 kV for applied voltage and 2 ml/h for solution feeding rate). (3) Preparation of PLGA/bFGF membranes: bFGF (125 μL, 20 μg/mL) was dripped into a PLGA/chloroform solution of 20% PLGA concentration, followed by ultrasonication in an ice bath. The uniform microemulsion was used for electrospinning to form nanofibrous PLGA/bFGF membranes.

Results: PLGA nanofibers and bFGF-encapsulated PLGA nanofibers (PLGA/bFGF) were fabricated using electrospinning and emulsion electrospinning, respectively. The morphology of electropsun PLGA fibers and PLGA/bFGF fibers are shown in Figs.1a and 1b, respectively. Smooth and bead-free nanofibrous membranes could be successfully produced. The fibers were randomly interconnected and stacked up. TEM micrographs of electrospun fibers are shown in Figs.1c and 1d, revealing the fiber structures. The core phase of bFGF and shell phase of PLGA can be distinguished in the core-shell structured PLGA/bFGF fibers (Fig.1d), while the uniform structure of PLGA fibers can be clearly observed for electrospun PLGA fibers (Fig.1c). For MSC encapsulation in alginate microspheres (Fig.2a), cell electrospray and post-spray crosslinking were performed using optimal parameters. Cell viability was assessed by live/dead assay and the result is shown in Fig.2b. MSCs could be encapsulated in alginate microspheres efficiently and they exhibited high cell viability after encapsulation.

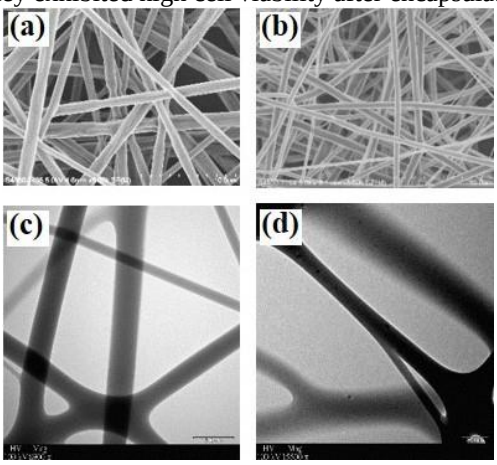

Fig.1. (a, b) SEM images of PLGA fibers and PLGA/bFGF fibers, respectively, (c, d) TEM images of PLGA fibers and PLGA/bFGF fibers, respectively.

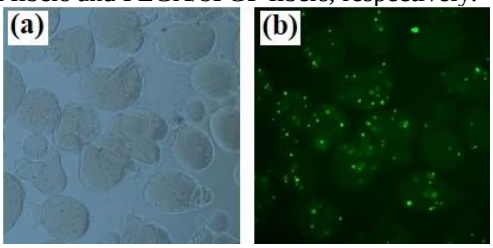

Fig.2. (a) MSC-encapsulated alginate microspheres, (b) cell viability of MSCs encapsulated in alginate microspheres.

Conclusions: Cell-encapsulated alginate hydrogel microspheres for cell delivery and bFGF-incorporated PLGA scaffolds for controlled growth factor delivery could be successfully fabricated by electrospray and emulsion electrospinning. MSCs encapsulated in alginate microspheres maintained high viability. The MSC-laden bFGF-encapsulated scaffolds have high potential for tissue regeneration applications.

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