Nanofibrillar scaffold for monitoring induced pluripotent stem cell fate in 3D culture system

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Statement of Purpose: Extracelluar matrix protein (ECMP)-based culture substrate such as Matrigel™ is commonly used for culturing induced pluripotent stem cells. However, drawbacks such as high price and batch-to-batch inconsistency were among the concerns. Many studies have tried to replace ECMP-based substrates, and it has been demonstrated that short amino acid sequences derived from vitronectin and laminin are helpful for adhesion, long-term proliferation and maintenance of pluripotency of induced pluripotent stem cells. Eletrospun nanofibers have been widely applied as scaffolds due to their 3D microenvironment. Fragmented nanofibers called nanofibrils have the advantage of allowing cells to be cultured in three dimensions. Its high surface area allows can immobilize high content of biomolecules. In this study, we suggest ECMP-derived peptide-bound nanofibril scaffold for monitoring cell fate in 3D culture system

Methods: Poly(ε-caprolactone) nanofibers were prepared by electrospinning to prepare ECMP-derived peptide-bound nanofibril scaffolds. Electrospun nanofibers were ground with an analytical grinder to obtain PCL nanofibril (NF). PCL NFs were aminolyzed by a 1 M ethylenediamine (EDA)/methanol mixture to functionalize the amine groups on the surface of PCL NFs (aPCL NFs). The amount of amine groups on the surface of aPCL NFs was quantified by fluorescamine assay using EDA as standard. Peptide conjugation was performed in a two-step method. First, MAL-PEG-NHS was reacted with aPCL and functionalized with a spacer (MAL-PEG-PCL NF). Second, vitronectin-derived peptides (VDP, *CGGKKQRFRHRNRKG*) and laminin-derived peptides (IKVAV, *CGGIKVAV* and YIGSR, *CGGYIGSR*) were conjugated with MAL-PEG-PCL NF. The amount of conjugated peptide was quantified by BCA analysis. Peptide-conjugated PCL NF (PEP-PEG-PCL NF) was characterized by X-ray photoelectron spectroscopy (XPS). PEP-PEG-PCL NFs and induced pluripotent stem cells (iPSCs) were co-cultured to form composite spheroids and cultured for 10 days. iPSC/NF composite spheroids were sliced by cryosection method and fluorescently labeled. The labeled spheroid sections were visualized with a confocal laser scanning microscope. (CLSM).

Result: Figure 1. schematically represents the surface modification process of PCL NFs and the formation of composite spheroids composed of iPSCs and peptide-bound PCL NFs to monitor cell fate. As the aminolysis time increased, the size of PCL NF significantly decreased, and the amine groups on the PCL NF surface increased in proportion to time. For conjugation of the peptide aPCL, NFs were surface modified with PEG spacers. The reaction between the NHS group of the PEG spacer and the amine group of aPCL NF was performed in ethanol to protect the maleimide group of the PEG spacer from hydrolysis. A 10 molar excess of NHS to amine ratio was sufficient to bind 80% of the amine to the PEG spacer.

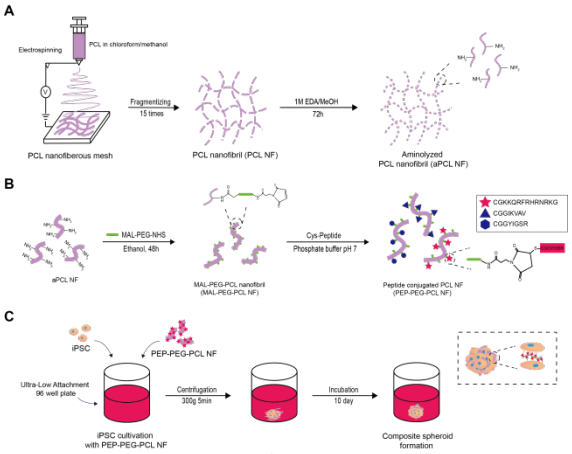
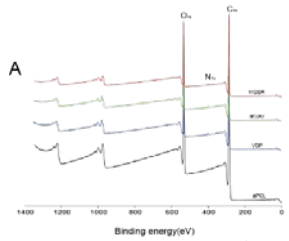


Figure 1. Schematic diagram of preparing peptide conjugated PCL nanofibrils (PEP-PEG-PCL NF) and formation of induced pluripotent stem cell (iPSC) composite spheroid with PEP-PEG-PCL NF. (A) Preparation of aminolyzed PCL nanofibril (aPCL NF) by fragmentizing and aminolysis. (B) PEG spacer and peptide conjugation on aPCL NF; Vitronectin derived pepetide is makred as red and laminine derived pepetides as blue color. (C) Schematic representation of composite iPSC spheroid with PEP-PEG-PCL NF.

After conjugation of the peptide to aPCL NF, PEP-PEG-PCL NF was characterized by XPS (Figure 2A), and two peaks for C_{1S} and O_{1S} were clearly detected in the aPCL NF spectrum at 291.08 eV and 537.08 eV, respectively. After conjugation of vitronectin and laminin-derived peptide, a distinct peak for N_{1S} was detected in PEP-PEG-PCL NF at 402.08 eV, indicating that the vitronectin-derived peptide and laminin-derived peptide were successfully conjugated to aPCL NF. Otherwise, the N_{1S} peak of PCL NF was not detected. The atomic ratio of PEP-PEG-PCL NFs was analyzed based on XPS spectra (Figure 2B). Among PEP-PEG-PCL NFs, IKVAV peptide-bound nitrogen ratio was the highest, followed by VDP and YIGSR peptides.



PCL NF	Atomic ratio (%)		
	C	O	N
aPCL	76.5	23.5	
VDP-PEG-PCL	75.06	23.78	0.66
IKVAV-PEG-PCL	76.02	22.91	0.81
YIGSR-PEG-PCL	76	23.04	0.64

Figure 2. Characterization of PEP-PEG-PCL NF. (A) XPS spectra with O_{1S}, C_{1S}, and N_{1S} peaks for aPCL (black), VDP-PEG-PCL (blue), IKVAV-PEG-PCL (green) and YIGSR-PEG-PCL (red) NF. (B) Atomic ratio on surface of PEP-PEG-PCL NF analyzed by XPS.

To develop composite spheroids, PEP-PEG-PCL NFs were pelleted into iPSCs with the aid of centrifugal force. Spheroids were formed after 24 hours of incubation and maintained their spherical shape until 10 days of incubation time (Figure 3).

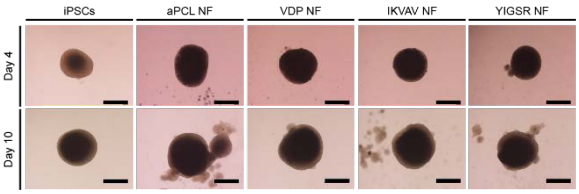


Figure 3. Bright-field microscopy images of iPSCs, VDP-PEG-PCL NFs, IKVAV-PEG-PCL NFs, YIGSR-PEG-PCL NFs and aPCL NFs composite iPSCs spheroid in different time point (scale bar = 500μm)

References:

1. KLIM, JR., et al. Nature methods. 2010, 7.12: 989-994.
2. PARK, HJ., et al. Biomaterials, 2015, 50: 127-139.
3. OVADIA, EM.; COLBY, DW.; KLOXIN, AM. Biomaterials science, 2018, 6.6: 1358-1370.