Bioactive Hybrid Nanomatrix Composed of Electrospun Polycaprolactone and Self-Assembled Peptide Amphiphiles

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Statement of Purpose

Electrospinning has been extensively used to produce fibrous scaffolds for various biomedical applications. This technology fabricates nanofibrous scaffolds using both synthetic and natural polymers in a controlled manner to mimic the structure of the extracellular matrix (ECM). However, the current limitation of electrospinning is its inability to present bioactive surfaces to control cellular behaviors. To address this issue, we have developed a novel strategy to create a bioactive nanomatrix by combining electrospun polycaprolactone (ePCL) with self-assembled, ECM mimicking peptide amphiphiles (PAs). It is hypothesized that these PAs, with inscribed cell adhesive ligands, can be self-assembled onto the surface of ePCL nanofibers and thus endow them with bioactivity. PAs are amphiphilic molecules consisting of a hydrophobic alkyl tail attached to a functional hydrophilic peptide sequence.[1,2] The functional peptide region of the PAs designed in this study consists of a matrix metalloproteinase – 2 (MMP-2) enzyme degradable site[2] coupled to either an RGDS cell adhesive ligand (PA-RGDS) or to no ligand (PA-S). The self-assembly of PAs onto ePCL was characterized with TEM, and cellular behaviors of human mesenchymal stem cells (hMSCs) on uncoated ePCL nanofibers, ePCL nanofibers coated with PA-S (ePCL-PA-S), and ePCL nanofibers coated with PA-RGDS (ePCL-PA-RGDS) were evaluated.

Materials and Methods

All PAs were synthesized by solid-phase synthesis using Fmoc chemistry and their self-assembly into nanofibers was evaluated using TEM. ePCL nanofibers were fabricated by dissolving 22.5 wt% PCL (Mn = 80000) in a solvent system of 1:1 chloroform: methanol, and applying a voltage of +21 kV at a distance of 28 cm between the needle and the collector. The self-assembled coating of PAs onto ePCL nanofibers was also characterized using TEM. Initial attachment of hMSCs on the hybrid scaffolds was evaluated using PicoGreen assay, and their morphologies were determined using Live/Dead assay and rhodamine/phalloidin staining.

Results

ePCL nanofibers were successfully fabricated with typical fiber diameters ranging from 300 nm – 400 nm (*Fig. 1).* This distribution falls within the range of collagen fiber bundles found in native tissue.[3]

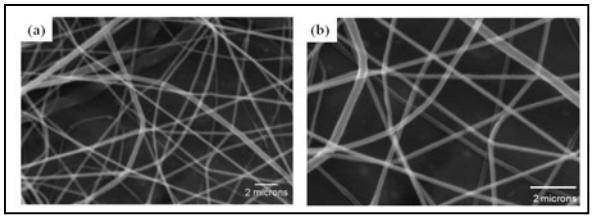

Fig. 1. SEM images of ePCL at (a) 7400X and (b) 14800X. Scale bar = 2 µm.

PAs were successfully synthesized and self-assembled (using solvent evaporation) into nanofibers with diameters of 8 nm onto ePCL nanofibers (*Fig. 2*). *Fig. 2b* shows the same hybrid nanofiber as *Fig. 2a*, with the TEM stage tilted by 42° to demonstrate PA coating all around the ePCL nanofiber.

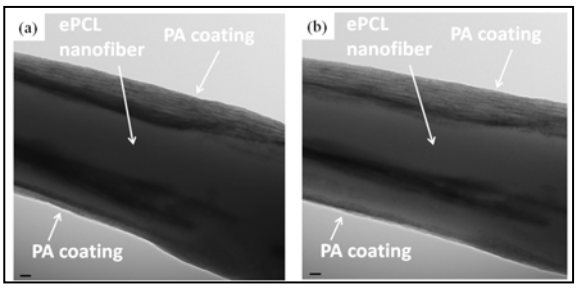

Fig. 2. TEM images show successful self-assembled PA coating on ePCL at (a) 0° and (b) 42° tilt. Scale bar = 20 nm.

Fig. 3 shows that the initial attachment on ePCL-PA-RGDS was significantly greater than on ePCL-PA-S and ePCL, indicating that hMSCs can recognize the RGDS ligand inscribed into the PA.

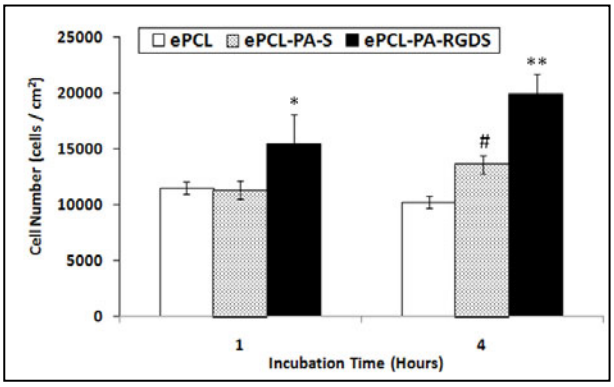

Fig. 3. Initial attachment of hMSCs. ePCL-PA-RGDS shows significantly greater cell attachment than ePCL-PA and ePCL (*,**,#: $p<0.05$, *n=4*).

Conclusion

This study shows a novel strategy to endow electrospun scaffolds with bioactivity using self-assembled ECM mimicking PAs. This natural tissue mimicking hybrid scaffold can be adapted for a variety of tissue regeneration applications.

References

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