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Improving tissue engineering scaffolds with non-mulberry silk fibroin

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Composite nanofibers of polycaprolactone and Antheraea pernyi silk fibroin exhibit good mechanical properties and promote cell attachment, spreading, and proliferation.

The abundance, excellent biocompatibility, and tunable degradation properties of silk fibroins—insoluble proteins present in silks—means that they are appealing materials for numerous biomedical applications, including drug delivery, tissue engineering, and implantable devices.[1,2] Silks are classified into structurally distinguishable mulberry and non-mulberry types,[3] which are produced by domesticated *Bombyx mori* and wild silkworm species, respectively. *Antheraea pernyi*—commonly known as the Chinese (oak) tussar moth—is a fairly widespread wild silkworm species belonging to the Saturniidae family. *Antheraea pernyi* silk fibroin (ASF) inherently contains arginyl-glycyl-aspartic acid (RGD) tripeptide sequences,[3,4] and as a result has been shown to provide much stronger cell adhesion than mulberry *Bombyx mori* silk fibroin.[5] In their natural form, silk protein fibers have excellent mechanical properties (such as strength and elasticity).[6] However, regenerated ASF materials—in which the fibers have been dissolved and reformed using physical or chemical methods—have poor mechanical properties. The applications of ASF nanofibers as biomaterials are therefore greatly limited.

A common method for producing new biocompatible materials, with improved mechanical properties, is the blending of synthetic and natural polymers.[7] For example, polycaprolactone (PCL) is frequently used in biomedical materials because it provides extraordinary mechanical properties and because it has been approved by the US Food and Drug Administration for in vivo applications.[8] The low cell affinity of PCL, however, makes it less than ideal for tissue engineering scaffolds.

In this work, we show that blending PCL and ASF produces a composite biomaterial that could fulfill the requirements for tissue engineering scaffolds.[5] Indeed, our nanofibers provide suitable microenvironments for cell attachment and migration by mimicking the topographic properties of extracellular matrices.[9] We hypothesize that our electrospun ASF nanofibers can provide physical cues for cell growth, and—as a result of the presence of RGD sequences—offer biochemical signals for cell adhesion (thus providing the potential for use as tissue engineering scaffolds). We have also demonstrated that ASF solutions possess good spinability, and that ASF nanofibers can therefore be easily manufactured using electrospinning methods.[10]

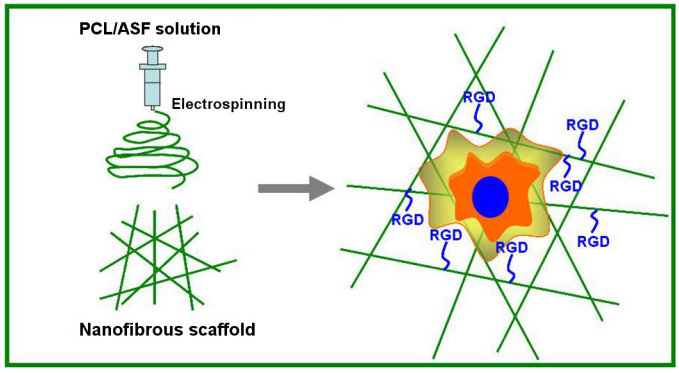

Figure 1. *Schematic illustration of the preparation of electrospun polycaprolactone (PCL)/Antheraea pernyi silk fibroin (ASF) nanofibers for tissue engineering scaffolds that exhibit strong cell adhesion. RGD: Arginyl-glycyl-aspartic acid tripeptide sequence.*

In the initial stage of our nanofiber preparation technique (see Figure 1), we produce electrospinning solutions by dissolving PCL particles (with an average molecular weight of 300,000Da) and freeze-dried regenerated ASF sponge (molecular weight above 9000–14,000Da) in 1,1,1,3,3,3-hexafluoroisopropanol at various blend ratios and concentrations. We then use a high voltage (15kV) to draw these solutions into fibers. We also used scanning electron microscopy (SEM) to characterize the resulting nanofibers.

Our SEM images of pure PCL (from a 6wt% solution)—see Figure 2(a) and (b)—show that this solution produces fibers in a diameter range of 238±47nm. We also observe that the composite nanofibers

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produced from an 8wt% blend solution, i.e., containing 70% PCL and 30% ASF (70PCL/30ASF)—see Figure 2(c) and (d)—have a very similar diameter range (237±43nm). We find that this ratio of PCL to ASF in the (i.e., 70PCL/30ASF) nanofibers gives the optimal results. We thus produce a material that possesses greatly improved mechanical properties, compared with pure regenerated ASF materials, and that promotes cell viability.

To investigate the ability of the nanofibers to promote cell viability, we seeded human umbilical vein endothelial cells onto mats of either pure PCL nanofibers or 70PCL/30ASF composite nanofibers, at a density of 100,000 per sample (see Figure 3). We used confocal microscopy to characterize the morphology of the resulting cells. In addition, we used a CCK-8 analysis (a colorimetric method of cell counting) to quantitatively evaluate the cell viability. After four hours, the number of cells on the 70PCL/30ASF nanofibers was significantly higher than on the pure PCL nanofibers, indicating that the incorporation of ASF facilitates cell attachment on the nanofibers. We also found that over a number of days, the rate of cell proliferation on the 70PCL/30ASF nanofiber mats was significantly higher than on the pure PCL mats. The results of these experiments show that incorporating ASF into the nanofiber mats significantly increases cell viability. Furthermore, the cells cultured on the 70PCL/30ASF nanofiber mats display better spreading and proliferation than those cultured on pure PCL nanofibers.[5]

In summary, we have used electrospinning methods to produce a range of PCL/ASF nanofibers. We have shown that by blending PCL and ASF in these fibers, the mechanical properties are improved

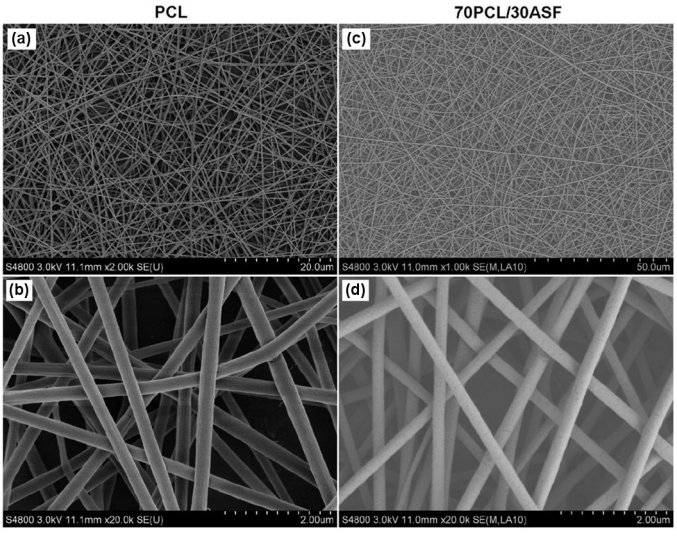

Figure 2. *Scanning electron microscopy images of (a, b) electrospun 100% PCL and (c, d) 70% PCL/30% ASF (70PCL/30ASF) nanofibers.*

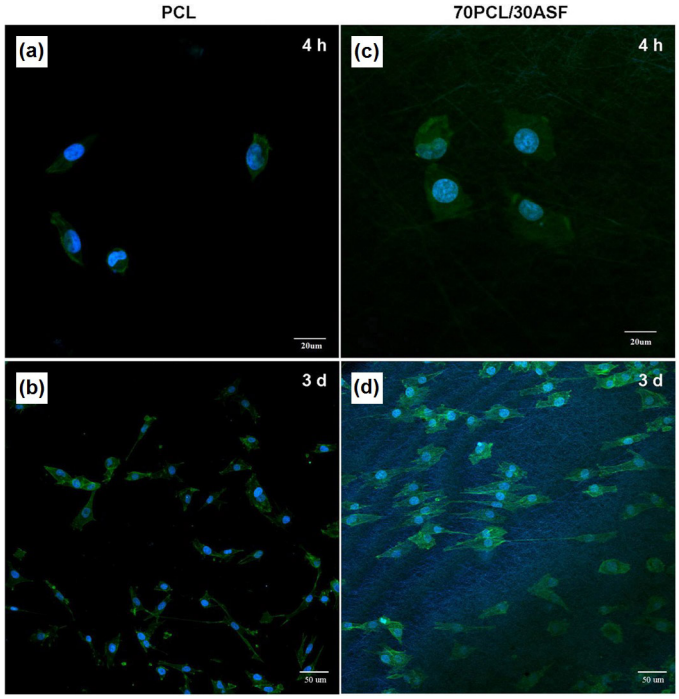

Figure 3. *Confocal microscopy images of human umbilical vein endothelial cells cultured on nanofibrous mats of (a, b) PCL and (c, d) 70PCL/30ASF. Images were obtained after four hours (4h) and three days (3d).*

(compared with regenerated ASF materials), and cell viability is promoted. The optimal blend of 70% PCL with 30% ASF increases cell attachment, spreading, and proliferation when compared with pure PCL nanofiber mats. The results of this investigation therefore show that electrospun composite PCL/ASF nanofibers are potentially useful materials for biomedical applications. Our future work in this area will focus on using these composite nanofibers to fabricate tissue engineering scaffolds (e.g., for blood vessel grafts and nerve guide tubes). We also aim to optimize our method for dissolving ASF fibers, to thus improve the strength of regenerated ASF materials and their composites.

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References

1. A. E. Thurber, F. G. Omenetto, and D. L. Kaplan, *In vivo bioresponses to silk proteins*, **Biomaterials 71**, pp. 145–157, 2015.
2. R. You, Y. Xu, G. Liu, Y. Liu, X. Li, and M. Li, *Regulating the degradation rate of silk fibroin films through changing the genipin crosslinking degree*, **Polym. Degrad. Stab. 109**, pp. 226–232, 2014.
3. S. C. Kundu, B. Kundu, S. Talukdar, S. Bano, S. Nayak, J. Kundu, B. B. Mandal, *et al.*, *Nonmulberry silk biopolymers*, **Biopolymers 97**, pp. 455–467, 2012.
4. R. You, Y. Xu, Y. Liu, X. Li, and M. Li, *Comparison of the in vitro and in vivo degradations of silk fibroin scaffolds from mulberry and nonmulberry silkworms*, **Biomed. Mater. 10**, p. 015003, 2015.
5. X. Li, Q. Zhang, D. Ye, J. Zhang, Y. Guo, R. You, S. Yan, M. Li, and J. Qu, *Fabrication and characterization of electrospun PCL/Antheraea pernyi silk fibroin nanofibrous scaffolds*, **Polym. Eng. Sci.**, 2016. doi:10.1002/pen.24402
6. F. G. Omenetto and D. L. Kaplan, *New opportunities for an ancient material*, **Science 329**, pp. 528–531, 2010.
7. A. Sionkowska, *Current research on the blends of natural and synthetic polymers as new biomaterials: review*, **Prog. Polym. Sci. 36**, pp. 1254–1276, 2011.
8. P. Xiang, K.-C. Wu, Y. Zhu, L. Xiang, C. Li, D.-L. Chen, F. Chen, *et al.*, *A novel Bruch's membrane-mimetic electrospun substrate scaffold for human retinal pigment epithelium cells*, **Biomaterials 35**, pp. 9777–9788, 2014.
9. X. Li, R. You, Z. Luo, G. Chen, and M. Li, *Silk fibroin scaffolds with a micro-/nano-fibrous architecture for dermal regeneration*, **J. Mater. Chem. B 4**, pp. 2903–2912, 2016.
10. X. Li, M. Li, Q. Zhang, S. Yan, and R. You, *Aqueous-based electrospinning of regenerated Antheraea pernyi silk fibroin*, **Fibers Polym. 17**, pp. 1421–1427, 2016.