Nanocomposite Gold-Silk Nanofibers

The MIT Faculty has made this article openly available. Please share how this access benefits you. Your story matters.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>As Published</td>
<td><a href="http://dx.doi.org/10.1021/nl302810c">http://dx.doi.org/10.1021/nl302810c</a></td>
</tr>
<tr>
<td>Publisher</td>
<td>American Chemical Society (ACS)</td>
</tr>
<tr>
<td>Version</td>
<td>Author's final manuscript</td>
</tr>
<tr>
<td>Citable Link</td>
<td><a href="http://hdl.handle.net/1721.1/91498">http://hdl.handle.net/1721.1/91498</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>Article is made available in accordance with the publisher's policy and may be subject to US copyright law. Please refer to the publisher's site for terms of use.</td>
</tr>
<tr>
<td>Detailed Terms</td>
<td></td>
</tr>
</tbody>
</table>
Nanocomposite gold-silk nanofibers

Tzahi Cohen-Karni§,†, Kyung Jae Jeong§,†, Jonathan H. Tsui§,†, Gally Reznor†, Mirela Mustata‡, Meni Wanunu⊥, Adam Graham#, Carolyn Marks#, David C. Bell#,‡, Robert S. Langer□, and Daniel S. Kohane§,*
§David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology (MIT), Cambridge, Massachusetts 02139, USA.
†Laboratory for Biomaterials and Drug Delivery, Department of Anesthesiology, Division of Critical Care Medicine, Boston Children’s Hospital, Harvard Medical School, 300 Longwood Avenue, Boston, Massachusetts 02115, USA.
□Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.
⊥Department of Physics, Dana Research Center, Northeastern University, Boston, Massachusetts 02115, USA.
‡School of Engineering and Applied Science, Harvard University, Cambridge, Massachusetts 02138, USA.
#Center for Nanoscale Systems, Harvard University, Cambridge, Massachusetts 02138

Abstract

Cell-biomaterial interactions can be controlled by modifying the surface chemistry or nanotopography of the material, to induce cell proliferation and differentiation if desired. Here we combine both approaches in forming silk nanofibers (SNFs) containing gold nanoparticles (AuNPs) and subsequently chemically modifying the fibers. Silk fibroin mixed with gold seed nanoparticles was electrospun to form SNFs doped with gold seed nanoparticles (SNFseed). Following gold reduction, there was a two-fold increase in particle diameter confirmed by the appearance of a strong absorption peak at 525 nm. AuNPs were dispersed throughout the AuNP-doped silk nanofibers (SNFSAu). The Young’s modulus of the SNFSAu was almost 70% higher than that of SNFs. SNFSAu were modified with the arginine-glycine-aspartic acid (RGD) peptide. Human mesenchymal stem cells that were cultured on RGD-modified SNFSAu had a more than two-fold larger cell area compared to the cells cultured on bare SNFs; SNFSAu also increase cell size. We suggest that this approach can be used to alter the cell-material interface in tissue engineering and other applications.

Keywords
silk; nanofibers; gold nanoparticles; cellular adhesion; tissue engineering; mesenchymal stem cells

A common challenge in engineering tissues is designing the structural biomaterials so as to mimic the natural microenvironment. One approach to addressing this issue has been to modify the chemistry of the substrate on which cells would grow to enhance cell adhesion, proliferation, and differentiation. Such modification has been achieved with biological structural motifs such as extracellular matrix proteins (e.g. fibronectin, collagen and

*Corresponding authors Daniel.Kohane@childrens.harvard.edu.
elastin\(^3\)) or with synthetic polymers or naturally-occurring structural materials whose surface and chemical properties have been altered to impart biological functionality.\(^4\)

Another approach has been to alter the nanotopography of the substrate so as to influence cell behavior.

Studies of cell adhesion to surfaces have focused on two-dimensional (2D) geometries where surface ligands were immobilized in a controlled manner,\(^5\) and their effect on cellular adhesion was explored as a function of ligand island size and separation.\(^6\) Application of these ideas in tissue engineering and other fields will necessitate their adaptation to three-dimensional (3D) nanostructured materials with well-defined surface chemistries, topographies and mechanical properties.

Here, we combine these approaches by creating a 3D silk nanofiber matrix by electrospinning, incorporating gold nanoparticles (AuNPs) on and within the resulting fibers, and subsequently chemically modifying the AuNP surfaces to immobilize molecules on the nanofiber, specifically an integrin-binding cell adhesive peptide, arginine-glycine-aspartic acid motif (RGD).\(^7\) The nanotopography imparted to the silk fibers by incorporation of gold particles is itself intended to enhance cell-material interactions: nanostructures and nanostructured surfaces have been shown to enhance cellular adhesion, proliferation and differentiation.\(^8\)\(^-\)\(^10\) We use silk as a model material because of its desirable mechanical properties, biocompatibility,\(^11\) and possible uses as a scaffold in engineered tissue.\(^12\) A straightforward method of altering the surface and chemical properties of silk to control the interface between cells and scaffold, as we provide here, has not been achieved to date.

Our approach to preparing gold nanoparticle (AuNP)-doped silk nanofibers (SNF\(_{Au}\)) is illustrated in Figure 1 (see also Materials and Methods in Supporting Information). Silk fibroin was extracted from Bombyx mori silkworm silk.\(^13\) Silk fibroin solutions with or without gold seed nanoparticles\(^14\) were electrospun\(^13\) to form silk nanofibers (SNFs) and SNFs containing seed particles (SNFseed respectively) (Figure S1 in Supporting Information). SNFs doped with seed particles were reduced at room temperature in Au\(^+\) solution for 3-4 days, producing SNFs with AuNPs on the surface and within the bulk of the material (SNF\(_{Au}\)) (Figure 1B). For cell adhesion studies, the fibers were modified with RGD peptide\(^15\) (Figure 1C).

The SNF and SNF\(_{Au}\) mats were maroon while the SNF were white (Figure 2A insets). To further confirm this observation, a UV/VIS spectrum was acquired (Figure 2A) While the SNF had no characteristic absorption peaks on UV/Vis spectroscopy, the SNF\(_{Au}\) had a strong absorption peak at 525 nm that is characteristic of AuNPs in the size range from 5 to 10 nm.\(^16\) The UV/VIS spectrum of SNFseed did not show this absorption peak (Figure S2 A). Moreover, the UV/VIS spectrum of a SNF incubated in Au\(^+\) solution for 6 days showed no absorption peaks, indicating that the gold seed nanoparticles are needed to form the AuNPs (Figure S2 B). The gold contents of SNF\(_{Au}\) and SNF\(_{seed}\) as determined by thermogravimetric analysis (TGA) (Figure 2B, Figure S1 C) were 10.4 ± 0.4 % and 5.1 ± 0.1 % respectively; these results further confirmed our hypothesis that the gold content of the fibers increased due to the reduction of Au\(^+\) ions at the surface of Au seeds during incubation.

SNF and SNF\(_{Au}\) were 428 ± 45 nm and 401 ± 58nm in diameter respectively (Figure 3; n = 30). The surface of the SNF (Figure 3 A I and II) was relatively smooth while the surface of the SNF\(_{Au}\) (Figure 3 B I and II, Figure S3) was rough. Elemental mapping using energy dispersive spectroscopy (EDS) confirmed that the fibers contained gold (Figure S4). Cross-sectional transmission electron microscopy (TEM; Figure 3) imaging showed that the gold was reduced and formed nanoparticles throughout the fiber diameter and length (Figure 3C).
The resultant gold nanoparticles had an average diameter of 6.6 ± 1.0 nm (n = 479), a more than two-fold increase in particle size compared to the original seed diameter of 3.2 ± 0.7 nm (n = 135) (Figure S1).

Recently it was demonstrated that incorporation of nanoparticles in silk thin films altered their mechanical properties, specifically their Young’s modulus. To investigate the effect of AuNPs on SNF mechanical properties, a single layer of fibers on a Si/600 nm SiO$_2$ substrate was prepared (see Materials and Methods Supporting Information). The Peak Force Quantitative Nano Mechanics scanning method (PFQNM) was used to map the elastic moduli of the SNF and SNF$_{Au}$. (Figure 4 A I and II, see also Figure S5 for Z height plots of the same fibers). The average elastic modulus of SNF was 11.7 ± 2.3 GPa, which is consistent with the published mechanical properties of silk fibers. The average elastic modulus of SNF$_{Au}$ was 17.7 ± 2.1 GPa, almost 70% higher than that of SNF. While we only used AuNPs, different materials could be loaded in silk in the same manner with potentially differing effects on mechanical properties. The ease of dispersion and homogeneity of nanoparticles throughout the material could allow the facile tailoring of material properties to specific applications.

Modification with AuNP could affect a wide range of cell behaviors by altering the nanotopography of the material. Furthermore, the AuNP enable straightforward chemical surface modifications that could further affect cell interaction. Preliminary to exploring the interaction between cells and the SNF-based substrates we studied the cytotoxicity of SNFs using PC12 cells, a cell line commonly used in toxicology. Neither SNF$_{seed}$ nor SNF$_{Au}$ showed cytotoxicity for 96 hours after initial exposure (see Materials and Methods in Supporting Information, Figure S6).

Adhesion of human mesenchymal stem cells (hMSCs) on SNF, SNF$_{Au}$, and either one after incubation with the integrin-binding peptide RGD (SNF+RGD and SNF$_{Au}$+RGD respectively) was observed 24 hours after the initial seeding (see Materials and Methods in Supporting Information). The peptide readily forms covalent bonds to the AuNPs through the free thiol on cysteine residues, whereas its binding to SNF is through weak physisorption to the fiber surface. We measured the size of the individual cultured cells (termed individual cell area) and the number of cells per unit area of substrate (termed cell density). Both SNF$_{Au}$ and SNF$_{Au}$+RGD showed a marked increase in cell size compared to both SNF and SNF+RGD (Figure 5 A). In addition, SNF$_{Au}$+RGD showed an increase in cell density compared to both SNF and SNF+RGD. These two findings indicate that cellular spreading was enhanced by surface nanostructure and – in combination with RGD – cell adhesion and/or proliferation, consistent with previous reports that RGD modification coupled with nanotopography enhances cell adhesion.

The effects of the various SNF fiber types on the cytoskeletal development and cell morphology of hMSCs were examined. Well-developed cytoskeletal structures, as indicated by the presence and density of actin fibers, were observed in cells exposed to SNF$_{Au}$ or SNF$_{Au}$+RGD (Figure 5 C II, IV and Figure S7), whereas cells cultured on SNFs or SNF+RGD exhibited a faint signal of unstructured actin (Figure 5 C I, II and Figure S7).

Nanostructured surfaces increase the formation of focal adhesions that eventually lead to well-developed cytoskeleton. We observed a close interaction between the cell membrane and the AuNPs on SNF$_{Au}$, as can be seen in Figure 5 D (I and II). The cells extend broad protrusions onto the fibers (Figure 5 D II, marked with white arrows); such were not seen in unmodified SNPs, where only sparse filamentous connections were seen (Figure S8). The broad protrusions seen in gold-containing fibers may correspond with focal interactions with AuNPs; this view is consistent with previous reports.
We have demonstrated the synthesis of SNFs doped with AuNPs dispersed throughout the fiber cross-section (radially and longitudinally). The AuNPs had a strong absorption at 525 nm, an absorption wavelength that corresponds to AuNPs as small as 5-10 nm in size. Doping with AuNPs improved the mechanical properties of silk significantly. The SNF\textsubscript{Au} were not cytotoxic. The AuNPs coating the surface of the nanofibers were readily accessible for chemical surface modification. The SNF\textsubscript{Au} showed an increase in the number of adhered cells compared to bare SNFs (irrespective of modification with RGD).

This nanofiber preparation approach can be used to prepare fibers that are easily chemically modified to include chemical cues important to cellular proliferation and differentiation.\textsuperscript{27, 28} Moreover this platform allows a straightforward method to control cell microenvironment through a single-step chemical modification, with potential uses in tissue engineering. These materials could also be used as smart (e.g. light-triggered) or optically active substrates.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

### Acknowledgments

TCK would like to thank the Juvenile Diabetes Research Foundation (JDRF), RL would like to acknowledge funding from NIH grants R37-EB000244 and R01-EB006365, and DSK would like to acknowledge funding from NIH grant GM073626.

### References


Figure 1.
Synthesis and fabrication of gold nanoparticle (AuNPs) -doped silk nanofibers (SNF). A. The building blocks: silk fibroin and gold seed nanoparticles. B. A SNF doped with AuNPs (SNF$_{Au}$) on the surface and throughout the fiber cross section. C. An illustration of surface modification of the SNF$_{Au}$. In this case the surface of the AuNPs was chemically modified with RGD motif peptide.
Figure 2.
SNF basic investigations. A. UV/VIS optical properties of SNF and SNF$_{Au}$. B. TGA analysis of SNF and SNF$_{Au}$. Each trace is representative of 4 samples. Insets are images of mats of the respective materials.
Figure 3.
SNF surface and morphology investigation. A. I. SEM image of SNFs. Scale bar is 2.5 μm. II. Higher power view of the dashed white box. Scale bar is 500 nm. B. I. SEM image of SNF\textsubscript{Au}. Scale bar is 2.5 μm. II Higher power view of the dashed white box. Scale bar is 500 nm. C. I. Cross-sectional TEM image of SNF\textsubscript{Au}. Scale bar is 400 nm. II. Higher power view of the dotted black box. Scale bar is 60 nm.
Figure 4. 
SNF mechanical properties. A. I. DMT elastic modulus map of SNF. Scale bar is 250nm. II. DMT elastic modulus map of SNF\textsubscript{Au}. Scale bar is 250nm. B. Box cross-section curves of both SNF (blue open squares) and SNF\textsubscript{Au} (red open circles), calculated from the DMT modulus (E) for the white dashed boxes. C. Summary of the DMT modulus for both SNF and SNF\textsubscript{Au}; the presented data are mean ± SD (n=12).
Figure 5.
SNF cell interactions. A. Area of individual hMSCs cultured on SNF, SNF<sub>Au</sub>, SNF<sub>Au</sub>+RGD, or SNF+RGD (n=4). B. Density of adhered hMSCs (Cell density) cultured on SNF, SNF<sub>Au</sub>, SNF<sub>Au</sub>+RGD, or SNF+RGD (n=4). (For both panels A and B, data are mean ± SD). C. Confoc imaging of (I) SNF, (II) SNF<sub>Au</sub>, (III) SNF+RGD, and (IV) SNF<sub>Au</sub>+RGD (II). Stains: nucleus (blue), actin filaments (red) and vinculin (green). Scale bar is 25 μm. D. (I) Morphology of hMSC cultured on SNF<sub>Au</sub>+RGD. Scale bar is 20 μm. (II) Expanded view of the white dashed box, white arrow mark adhesion points. Scale bar is 400 nm.