Long Range Planning Update

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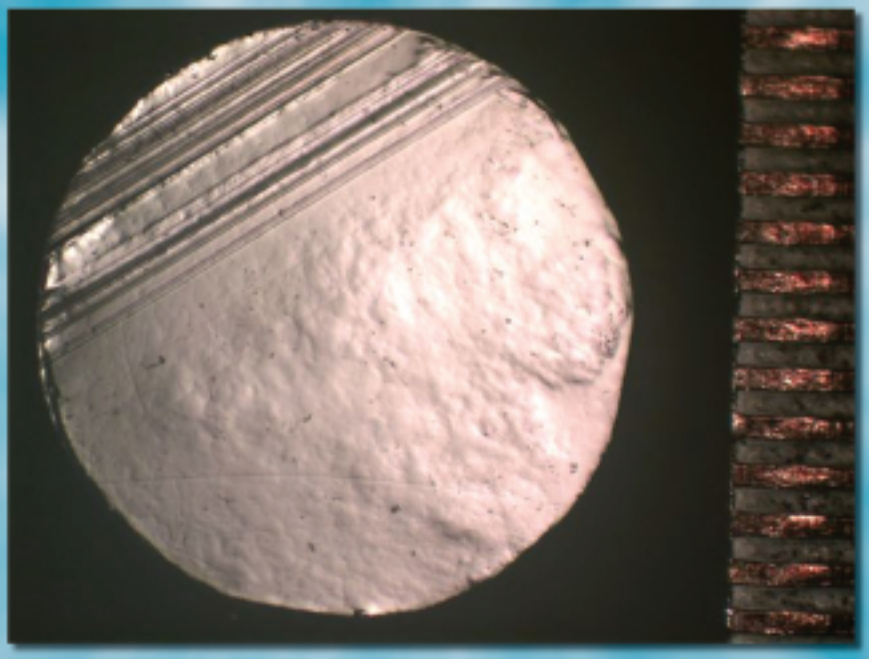

ß-Sheet Formation and RGD-Presentation Effects on Osteoblast Differentiation

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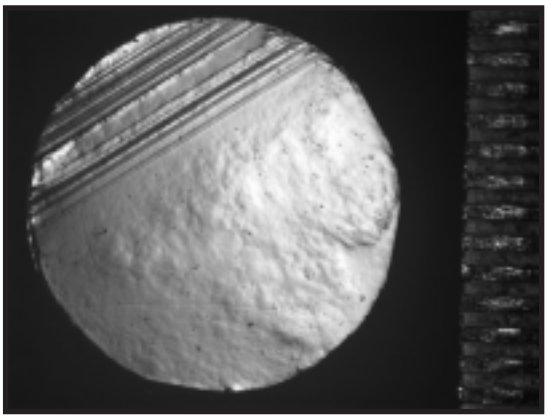
Features

7 **ß-Sheet Formation and RGD-Presentation Effects on Osteoblast Differentiation**

This article highlights the recent investigation into ß-sheet formation and RGD epitope presentation (arginine-glycine-aspartate) on the surface of blended silk films. Surface characterization of biomaterials for tissue-engineering applications is of great importance in understanding how properties drive cellular response. Polymer crystallinity and surface modification with bioactive motifs, such as arginine-glycine-aspartate peptide (RGD), alter cell attachment. In protein-based materials, crystallinity is driven by the transition of α-helix to ß-sheet, as is seen in silk.



Stereometric image of a pin-on-disk polyethylene wear surface at after 20Km of multi-directional sliding

Photograph courtesy of Dr. John Desjardins, Assistant Professor of Bioengineering, Clemson University

Departments

The Torch

2 From the Editor
2 From the President
3 Staff Update from Headquarters
5 Long Range Planning Update
5 Update from Member-at-Large
6 The Tale of Two Engineers

Member News

9 Members in the News

Government News

10 News on NIH Programs

Book Reviews

11 Tissue Engineering

Industry News

12 BioInk

Biomaterials Community

12 Community Calendar

ß-Sheet Formation and RGD-Presentation Effects on Osteoblast Differentiation

Feature

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Government News
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This article highlights our recent investigation into ß-sheet formation and RGD epitope presentation (arginine-glycine-aspartate) on the surface of blended silk films.[1] Surface characterization of biomaterials for tissue engineering applications is of great importance in understanding how properties drive cellular response. Polymer crystallinity and surface modification with bioactive motifs, such as arginine-glycine-aspartate peptide (RGD), alter cell attachment.[2,3] In protein-based materials, crystallinity is driven by the transition of a-helix to ß-sheet, as is seen in silk.[4]

ß-sheet formation was modulated through blending of regenerated *Bombyx mori* silk (fibroin) with engineered dragline spider silk (spidroin). The engineered silk was expressed through E. coli and contains two RGD sequences that the natural silk lacks.[5] We fabricated discrete silk blends (90:10, 70:30, 50:50, 30:70 fibroin:spidroin) from solution by spin-coating onto glass coverslips. Briefly, a self-assembled monolayer of n-octyldimethylchlorosilane was vapor deposited on glass coverslips overnight. Forty microliters of silk solution (3 percent by mass in hexafluoroisopropanol) was deposited onto the coverslip while spinning at 2,000 rpm for 90 seconds. Pure fibroin and pure spidroin films were also fabricated as controls. The films were characterized with Fourier transform infrared spectroscopy (FTIR), film stability studies (delamination), atomic force microscopy, and peptide staining. Cellular attachment to the silk films was also investigated and the effects of ß-sheet formation on cell spreading and differentiation were assessed.

FTIR revealed increased ß-sheet formation with increased RGD-spidroin content after annealing (Figure 1).[1] Annealing increased the ß-sheet content over the unannealed in all cases except the pure fibroin. When the films were incubated for 21 days in aqueous cell culture conditions, the increased ß-sheet content improved film stability. The unannealed samples with less than 30 percent RGD-spidroin dissolved after seven days while the higher-content RGD-spidroin remained for 14 days. Annealing increased film stability through 21 days with only the pure fibroin dissolving after 14 days.

RGD presentation on the surfaces of the films was visualized with a novel approach using an integrin mimicking peptide (CWDDGWLC-biotin).[6] Briefly, films were blocked with a solution of bovine serum albumin, incubated with CWDDGWLC-biotin (0.1 mg/mL), incubated with streptavidin-colloidal gold (10 nm, 3 ng/mL) and enhanced with silver staining. This technique enables detection of RGD on the surface of a biomaterial using light microscopy.

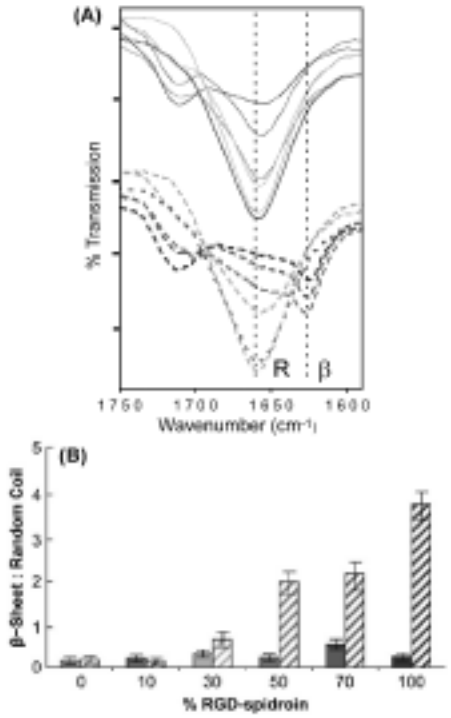

Figure 1. FTIR spectra of fibroin films containing 100% (purple), 70% (blue), 50% (turquoise), 30% (olive green), 10% (red), or 0% (orange) RGD-spidroin before (solid) and after (dashed) annealing. (A) Random coil regions (R: 1660 cm-1) apparent in the unannealed samples were diminished with annealing and increasing RGD-spidroin content. Increasing ß-sheet formation (,: 1640 cm-1) was observed after annealing with increasing RGD-spidroin content. (B) The ratio between the ß-sheet peak (ß) and the random coil peak (R) reveals increasing ß-sheet content after annealing in films with high RGD-spidroin content.

Appearing as dark spots against the grey background of the silk (Figure 2), the RGD epitope was visible on the pure spidroin prior to annealing. For most compositions, the unannealed blends showed increasing staining with increased RGD-spidroin content. The pure fibroin lacks the RGD epitope and therefore displayed no staining before or after annealing. After annealing, most of the blends and the pure spidroin showed decreased RGD presentation on the film surfaces.

Cell adhesion, spreading, and differentiation were assessed on the films using a model osteoblast cell line, MC3T3-E1. Cell adhesion at four hours was statistically similar for all compositions and annealing conditions. By four days, cells proliferated as expected with approximately two doublings for all specimens, with no significant difference in cell number for all compositions and annealing conditions. After four hours,

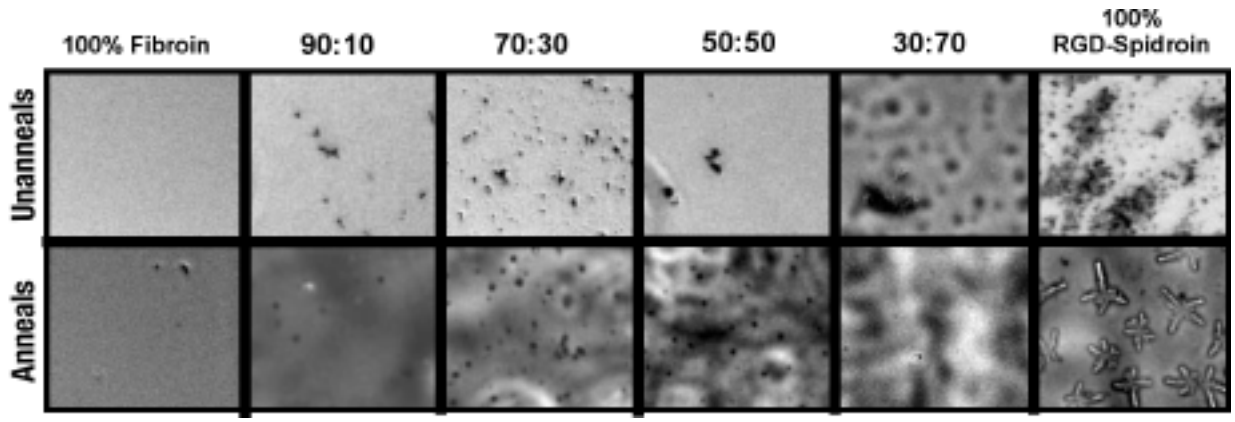

Figure 2. Representative light micrographs of RGD-epitope presentation visualized with phage peptide-biotin/strepavidin-gold nanoparticle enhanced with silver. Dark clusters of the RGD-epitope are present with increasing amounts of RGD-spidroin. The amount of epitope on the surface decreased after annealing. Little to no staining was observed on the pure fibroin where the RGD sequence is not present. 400x magnification.

unannealed blended samples showed an unexpected decrease in cell spreading with increased RGD-spidroin content. Cells on the annealed samples displayed no obvious trend in cell area as a function of the RGD-spidroin content at four hours. By four days, all cells decreased in cell area irrespective of the amount of RGD-spidroin and heat processing. Surface roughness determined by atomic force microscopy increased after annealing suggesting chain rearrangement was involved in the formation of ß-sheet, which "hid" the RGD after annealing.

Due to film stability, only annealed samples were investigated for cellular differentiation. Cells reached confluence on most specimens, including glass controls by seven days (Figure 3). Osteoblastic differentiation was monitored by osteopontin expression after 14 days. Although silk samples produced more osteopontin than the glass controls, no difference in osteopontin expression was detected between silk films.

In this study,[1] the addition of synthetic RGD-spidroin led to increased ß-sheet formation in the silk-blend films. Higher ß-sheet content led to greater film stability under culture conditions, allowing for pre-osteoblasts to differentiate into mature osteoblasts, although no differentiation differences were noted among the blends. Therefore, it was concluded the 90:10 fibroin:RGD-spidroin blend (by mass) was the optimal composition for supporting osteoblastic cells in this study. This blend offered film stability for cell attachment at the lowest RGD-spidroin content, making it less expensive to fabricate. Finally, a novel technique was developed to examine RGD presentation on silk film surfaces and the results were in good agreement with the cellular response studies.

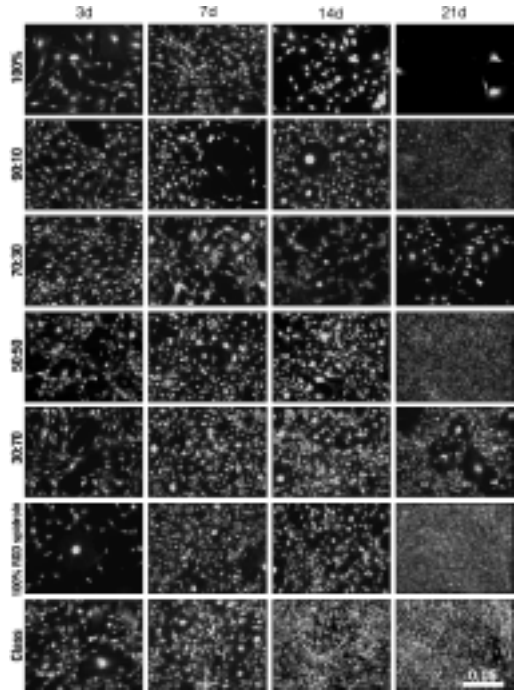

Figure 3. Fluorescent light micrographs of MC3T3-E1 cultured for 3 d, 7 d, 14 d or 21 d in differentiation media on annealed silk blends. Clean glass coverslips were used for the control. For most of the blends, cell number appears to increase with time as RGD-spidroin content is increased.

Acknowledgement

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