Supporting Information

Avidin Adsorption to Silk Fibroin Films as a Facile Method for Functionalization

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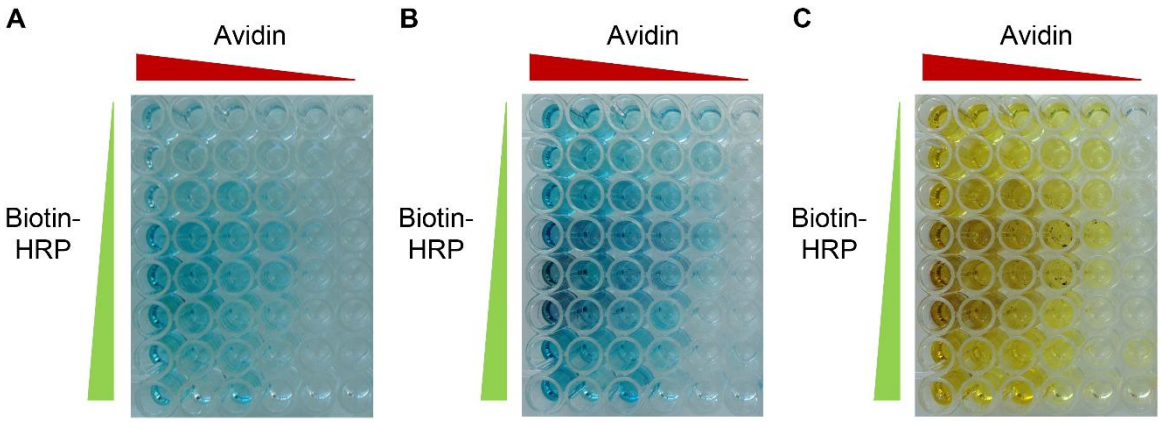

Figure S1. Colorimetric characterization of avidin and B-HRP adsorption. Avidin and B-HRP concentrations were varied to determine the optimal combination of concentrations. HRP was detected via the addition of a clear substrate (TMB) that is cleaved by HRP, forming a blue color. (A) Image taken immediately after adding TMB substrate to the wells. (B) Second image taken six minutes after the image in (A). (C) Image taken after adding 2 M sulfuric acid to stop the enzymatic reaction.

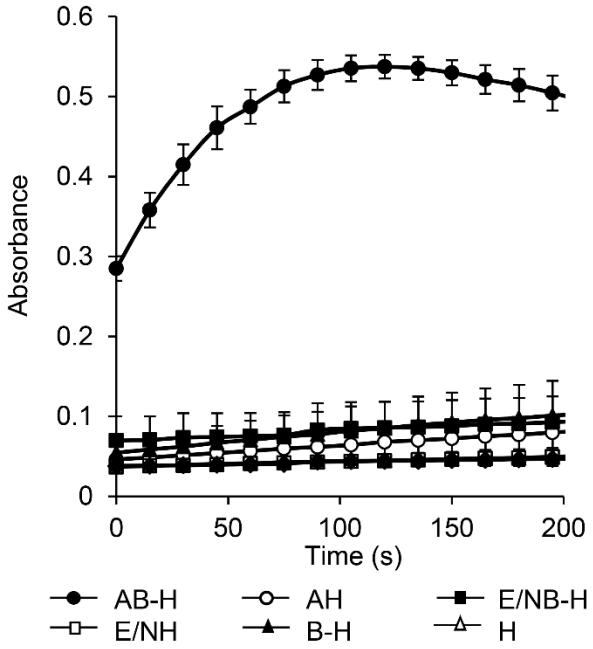

Figure S2. Raw data of kinetic curves of adsorbed or covalently coupled HRP moiety functionalized silk films. Biotin-HRP or HRP (5 μg/mL) were adsorbed onto silk films modified with avidin or carboxylate activation. HRP activity was monitored via TMB cleavage forming a blue chromophore detected at 650 nm (15 s between each measurement). A faster rate of change (higher slope) indicates greater HRP activity. AB-H modified silk films had the highest HRP activity. Data are presented as mean ± SEM of three independent experiments with 3-6 samples each. Key: **AB-H**: Avidin with Biotin-HRP; **AH**: Avidin with HRP; **E/NB-H**: EDC/NHS with Biotin-HRP; **E/NH**: EDC/NHS with HRP; **B-H**: Biotin-HRP background control; **H**: HRP background control well.

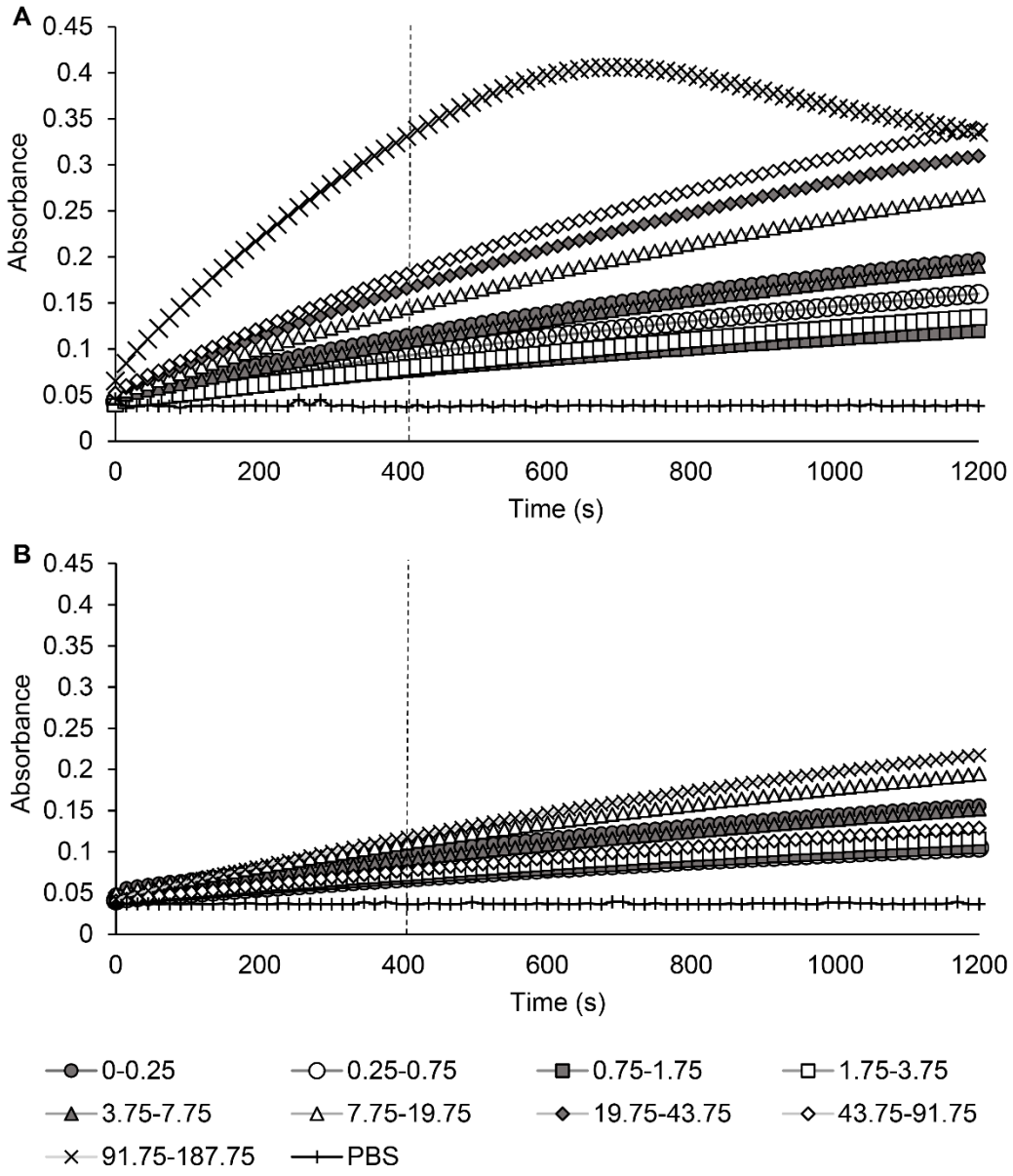

Figure S3. Raw data of kinetic curves of A-HRP released from surface of silk films. Kinetic activity of A-HRP released from silk films at labelled time delta (in h), measured through TMB colorimetric assay from (A) overnight adsorption and (B) 1-hour adsorption. Higher absorbance values indicate greater enzyme activity. Downward slope indicates exhaustion of TMB substrate and formation of precipitate by A-HRP. Dashed line indicates values used to determine release data found in Figure 4. Graph of kinetic activity is representative of three experiments with 8 samples each.

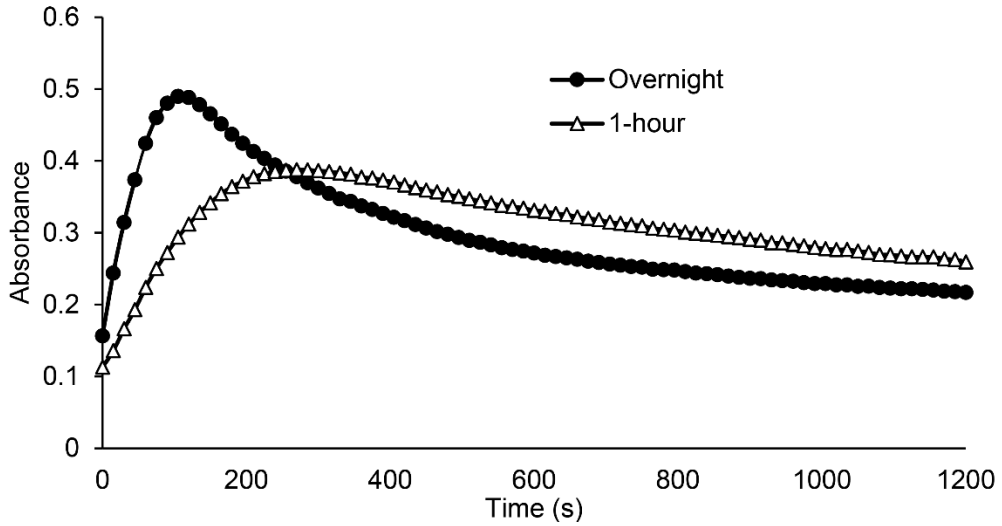

Figure S4. Raw data of kinetic curves of A-HRP remaining on surface of silk films. Kinetic activity of A-HRP present on silk films at release experiment end-point measured through TMB colorimetric assay. A faster rate of change (higher slope) indicates greater A-HRP activity. The downward slope indicates exhaustion of TMB substrate and formation of precipitate by A-HRP. Graph of kinetic activity is representative of three experiments with 8 samples.

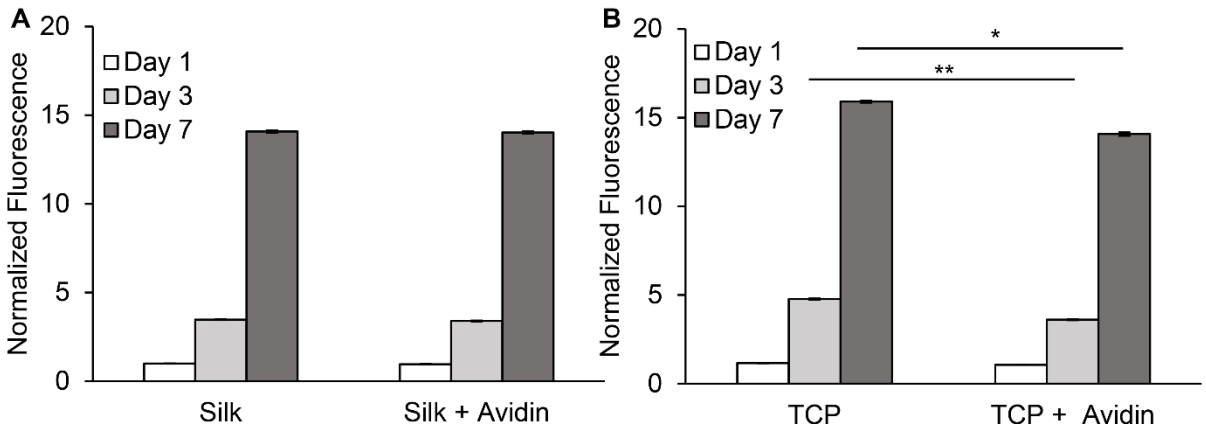

Figure S5. Metabolic activity of hFFs on avidin-modified surfaces. Data is presented as the normalized fluorescence of resazurin incubated with hFFs after 1, 3, and 7 d of culture. Data shown as average with SEM (N = 4 with 12 samples each). All values normalized to average fluorescence value for day 1 silk-only wells (*$p < 0.05$, **$p < 0.01$ as compared to silk only controls). H indicates 10^{-7} M. L indicates 10^{-8} M. + indicates inclusion, - indicates exclusion. TCP is tissue culture-treated plastic.

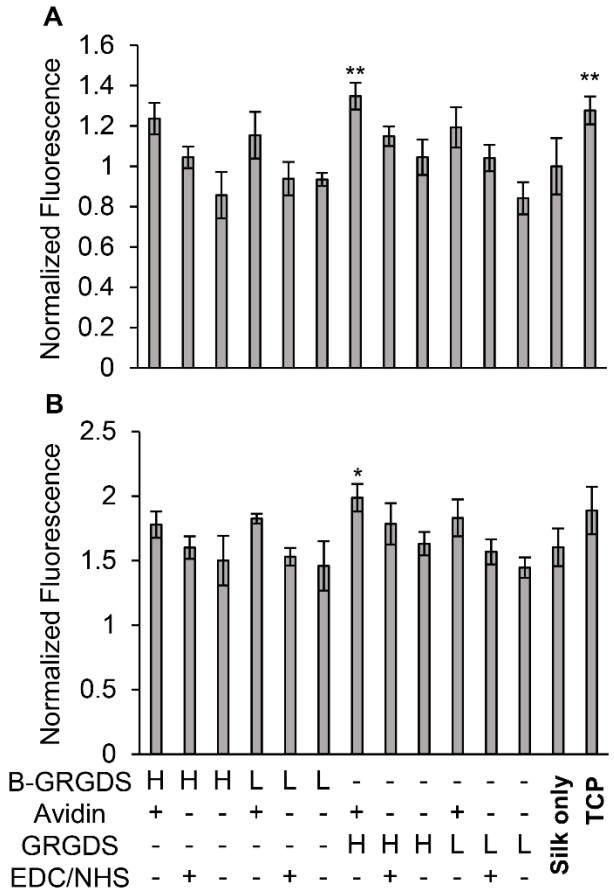

Figure S6. Metabolic activity of hMSCs on modified silk films at Day 1 and 3 of culture. Normalized fluorescence of resazurin incubated with hMSCs after (A) 1 d and (B) 3 d of culture. Data shown as average with standard deviation. All values are normalized to the average fluorescence for hMSCs cultured for 1 d on silk only wells (*$p < 0.05$, **$p < 0.01$ as compared to silk only controls). H indicates 10^{-7} M. L indicates 10^{-8} M. + indicates inclusion, - indicates exclusion. TCP is tissue culture-treated plastic.

Table S1. Slope of linear region of A-HRP remaining on surface of silk films after release studies

Adsorption Time	Slope (AU/s)
Overnight	$4.44 \times 10^{-3} \pm 8.48 \times 10^{-4}$**
1-hour	$1.86 \times 10^{-3} \pm 1.84 \times 10^{-4}$

Data presented as mean ± SEM. Slope of overnight group is significantly greater than slope of 1-hour group ($p < 0.01$). Data is generated from three independent experiments with 8 samples each (** $p < 0.01$).