Supporting Information

Optimization of Glutaraldehyde Vapor Treatment for Electrospun Collagen/Silk Tissue Engineering Scaffolds

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Table S1. Optimization of electrospinning conditions. Optimized solution concentration and electrospinning parameters are shown in Column 1. Variation of any parameter leads to changes in fiber diameter, density and alignment. Changes induced by the increase of each parameter, while other parameters remain constant, are indicated by arrows (↑ for increase or ↓ for decrease) in the table.

Parameter	Fiber Diameter	Fiber Density	Fiber Alignment
↑ Solution Concentration (100 mg/ml)	↑	< 100 mg/ml ↑ > 100 mg/ml ↓	↑
↑ Electric Potential (25 kV)	↓	↑	↓
↑ Air Gap Distance (150 mm)		↓	↑
↑ Delivery Rate (5 ml/h)	↑	↑	↓
↑ Gap of Collector (8 mm)		↓	↑
↑ Collection Time (30 s)		↑	
↑ Needle Gauge (18 G)	↑		

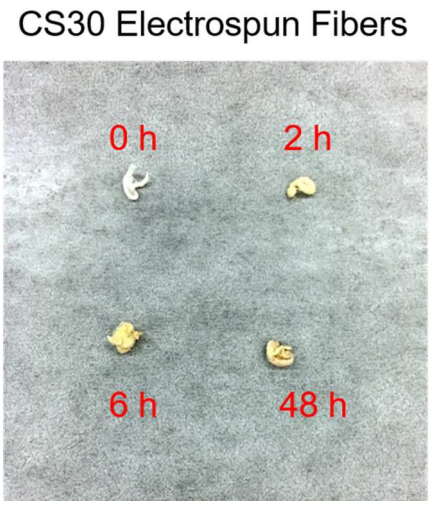

Figure S1. The color change of CS30 fibers with GA treatment time, from white for untreated fibers (0 h) to light yellow for lightly treated fibers (2 h and 6 h) and dark brown for fibers after 48 h treatment. Accompanied with the color change, the sample became harder and less resilient. Similar color change was observed in pure collagen fibers but not in silk fibers. Thus, the color change is relevant to GA induced collagen crosslinking. The color change is likely due to the Schiff base formation when glutaraldehyde reacts with amine groups of collagen or/and the polymerization of excessive GA molecules during the crosslinking reaction [1].

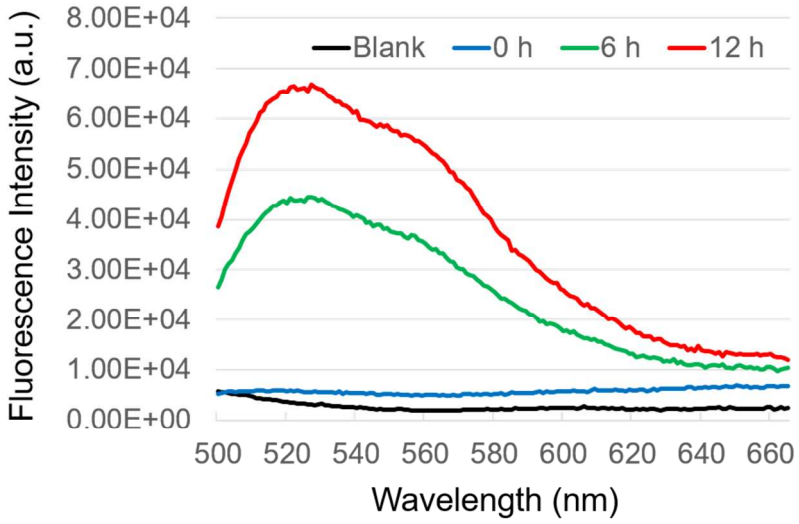

Figure S2. Fluorescence spectra of E-spun collagen fibers after GA treatment for 0h, 6h and 12h, with blank as the control (λ_{ex}: 470 nm). As shown in the figure, collagen without GA treatment shows little fluorescence, consistent with the dark contrast in the fluorescent images (Figure 1D). With the increase of GA treatment time, fluorescence intensity in the range of 520-560 nm increases dramatically, consistent with the increased green fluorescence observed in Figure 1E,F. We ascribe the fluorescence to the formation of Schiff base (C=N) between collagen and polymerized GA molecules[2] as well as the increased fiber stiffness which restricts intramolecular rotation leading to the enhancement of fluorescence emission.[3]

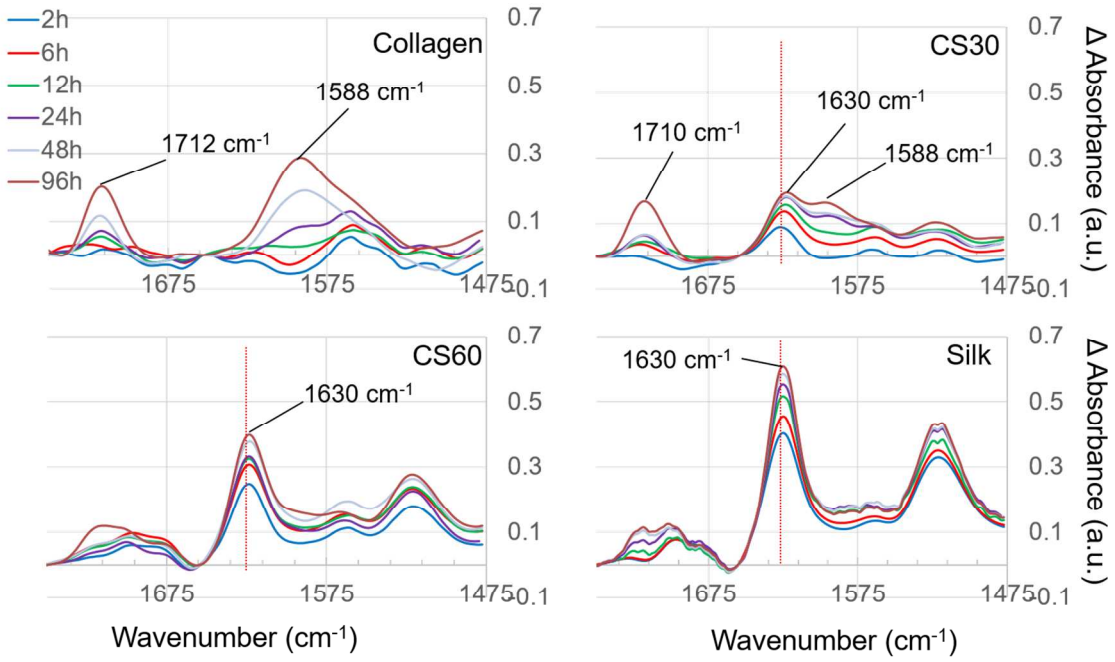

Figure S3. Differential FTIR spectra of collagen, CS30, CS60 and silk fibers after various time of GA treatment. The spectra illustrate changes with respect to the spectrum of untreated fibers. To obtain the differential FTIR spectra, original FTIR spectra in the range of 1480-1720 cm^{-1} were normalized into 0-1 scale, and the normalized spectrum of untreated fibers was subtracted from the normalized spectrum of each treated fibers. As shown in the Figure, with increased GA treatment time, the intensity of peaks at 1712 cm^{-1} and 1588 cm^{-1} for collagen dominant fibers (Collagen and CS30) increased, and the intensity of peak at 1630 cm^{-1} increased in fibers containing silk protein (CS30, CS60 and Silk). The 1630 cm^{-1} is assigned to β-sheet conformation, which is marked with the vertical red line in the figures.

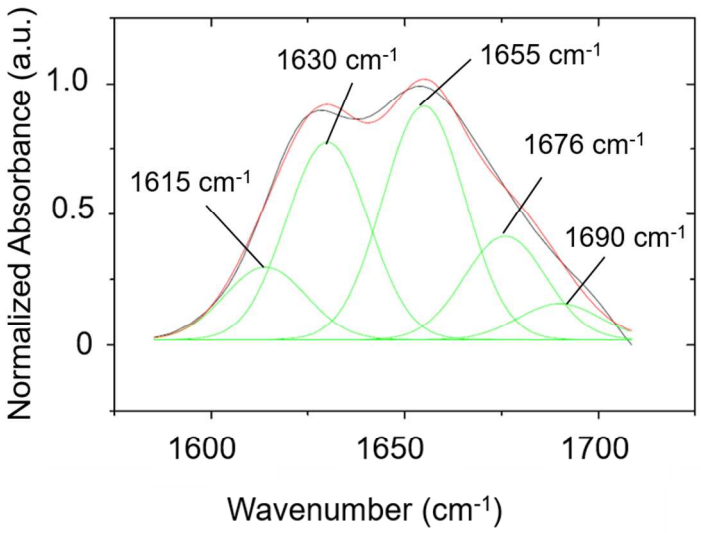

Figure S4. A typical normalized FTIR spectrum of CS30 fibers after 6 h GA treatment in amide I region (black) and the corresponding computational spectrum reconstructed by deconvolution method to produce the best fit (red); 5 individual Gaussian components are also shown (green).

In the deconvolution process, the amide I band was fitted into five Gaussian peaks centered at 1615 cm^{-1} (turns), 1630 cm^{-1} (beta-sheet), 1655 cm^{-1} (coils and helix structure), 1676 cm^{-1} (beta-turns) and 1690 cm^{-1} (beta-sheet), respectively [4, 5]. The choice of using the amide I band for structural analysis is due to its greater intensity than the other amide modes and the low contribution of the amino acid side-chain absorptions present in this region [6, 7]. In the curve-fitting procedure, the band position and band width (25 cm^{-1}) were fixed, whereas the peak intensity was varied to achieve the best fitting ($R^2 > 0.98$). The percentages of integrated intensity of each peak was used to assess the proportion of protein in the corresponding configuration. The peak deconvolution process was done by using "peak analyzer" of Origin Software.

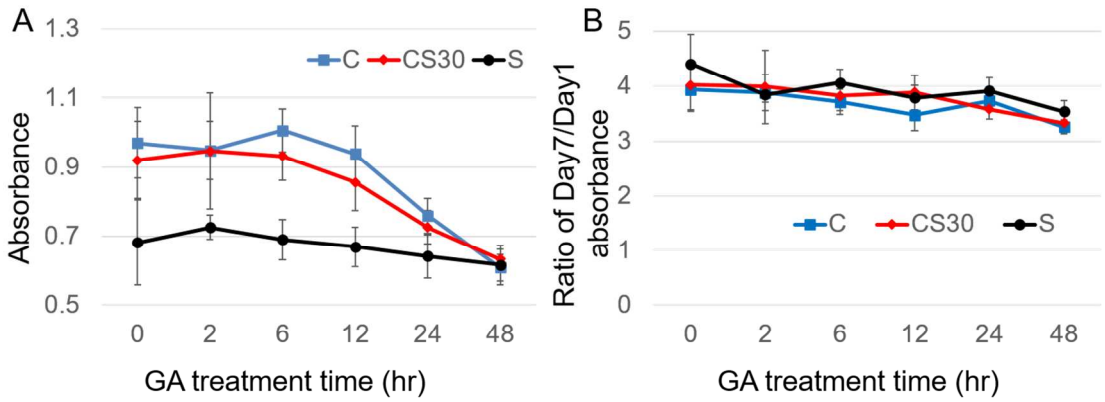

Figure S5. (A) Comparison of normalized MTT absorbances at Day 1 on collagen (C), CS30 and silk (S) matrices with various GA treatment time. The MTT absorbance was normalized with respect to Day 1 MTT absorbance on plastic substrates. The decreased absorbance on silk, collagen and CS30 with long GA treatment (>12 h) suggested decreased initial cell attachment on these substrates. (B) The absorbance ratio of cells at Day 7 and Day 1 grown on collagen, CS30 and silk matrices with GA treatment time. The result indicates that the proliferation rate on various substrates was in the same level. In general, the doubling time of the cells on various substrates is around 3 days.

References

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