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FORMATION OF A THIN-WALLED SPIDER SILK TUBE ON A MICROMACHINED SCAFFOLD

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ABSTRACT

This paper reports on the first formation of a thin bio-functionalized spider silk tube, supported by an internal micromachined scaffold, in which both the inside and outside of the tube wall are freely accessible. The silk tube could potentially be used as an artificial blood vessel in an in vitro tissue scaffold, where endothelial cells and tissue cells can grow on both sides of the silk tube.

BACKGROUND

Within tissue engineering there is a high demand for tubular structures that can be used for development of blood vessels, as it is the barrier between blood and tissues as well as the channel for exchange of oxygen and nutrients. One of the more recent approaches involves creating full tubular tubes in silk, as this is a robust and biocompatible biopolymer [1]. Free standing silk tubes of thicknesses between 150 – 400 μm have been generated using dip coating [2], gel spinning [1], and by electrospinning silk onto a rotating mandrel [3]. While these structures work as replacements for blood vessels in cells scaffold, their thickness makes them non-ideal for applications requiring interaction and transport between cells grown on both the inside and outside of the tube. Recent work describes the formation of down to 2 μm thin silk tubes, but these tubes are either fully supported on the inside or the outside, i.e. one side of the tube wall is not freely accessible [4].

It has recently been shown that it is possible to generate spider silk coatings, nanowires, and sheets on superhydrophobic pillar arrays by taking advantage of the spontaneous silk assembly that occurs at hydrophobic interfaces [5]. However, silk self-assembly on 3D superhydrophobic scaffolds has not been shown previously.

EXPERIMENTS

Figure 1: The experimental process, consisting of three parts: (a-e) scaffold manufacturing, (f-h) silk tube self-assembly, and (i-j) silk functionalization.

Scaffold manufacturing

16 mm long, 5 mm wide, and 1 mm high scaffolds of slanted interlocked pillars (pillar diameter 50 μm , pitch 200 μm) in off-stoichiometry thiol-ene-epoxy polymer (OSTE+) [6] were manufactured (Figure 2a-c). Unlike previous work [7], the scaffold pillars were released from the bottom substrate. The fabrication process is shown in detail in the ‘Scaffold manufacturing’ part of Figure 1. In step: (a) OSTE+ (OSTEMER 322, Mercene Labs, Sweden) is exposed to UV light for 150 s (intensity: $\sim 8.0 \text{ mW/cm}^2$; OAI, Milpitas, US) using a chromium mask and a custom built mirror setup [6], where the bottom substrate is release liner (ScotchPak9775 Release Liner, 3M, USA); (b) the scaffold is developed in acetone by ultrasonication for 300 seconds; (c) the scaffold is incubated in an oven at 75°C for 1 hour; (d) the scaffold is fully cured under UV light (intensity: $\sim 8.0 \text{ mW/cm}^2$) for 600 s; (e) the release liner is peeled off from the scaffold using a scalpel.

The pillar surfaces are naturally hydrophobic; hence the outer scaffold surface constitutes a superhydrophobic surface (Figure 2d).

Figure 2: a) Photograph of the micromachined scaffold; b) bright field and c) SEM magnifications of a); d) droplets of dyed water on the micromachined scaffold, illustrating its hydrophobic nature.

Silk tube self-assembly

Silk encapsulation of the scaffold was achieved using recombinantly produced miniaturized spider silk fusion proteins containing either a domain capable of binding IgG (Z) [8] or an integrin-binding motif from fibronectin (FN) [9] (Z-4RepCT and FN-4RepCT, Spiber Technologies, Sweden). The encapsulation process is shown in detail in the ‘Silk self-assembly’ part of Figure 1. In step: (f) the micromachined scaffold is placed in 100

μL of spidroin solution and set to evaporate in ambient conditions; (g) silk sheets are formed at the two hydrophobic liquid:air interfaces: i) at the free top and ii) inside the scaffold; (h) a dried silk tube has formed around the scaffold.

Figure 3: a) Silk tube formed on the surface of micromachined scaffold, viewed by SEM; b) magnification of a) showing a broken sheet, revealing the thickness of the sheets; c) magnification of a) showing an uneven surface caused by silk conglomerates between the two sheets.

Silk functionalization

The retained biofunctionality of the silk is demonstrated by binding Alexa-488 labeled IgG to the Z-silk tube (Figure 4a-b, and Figure 1j). The FN-silk can be visualized by incubation in Rhodamine-B (Figure 4c, and Figure 1i).

Figure 4: a) Encapsulation of Z-silk visualized by binding Alexa 488 coupled to IgG to the silk; b) magnification of a) showing silk conglomerates between the sheets; c) encapsulation of FN-silk visualized by binding Rhodamine B to the silk.

RESULTS

Bright field, fluorescence microscopy, and SEM were used to visualize the resulting silk tube formation. Figure 3 shows that complete encapsulation of the micro scaffold has occurred and that the thickness of the sheet is approximately 2 μm . Binding fluorescently labeled IgG to the Z-silk tube shows the retained biofunctionality of the

silk. It is possible to make the supporting micromachined scaffold hydrophilic for liquid filling of the silk tube.

CONCLUSIONS AND OUTLOOK

Envisaged applications include the formation of artificial blood vessels for in vitro studies. By using silk functionalized with the integrin-binding fibronectin motif (FN), cells could be grown on both sides of the sheet, and still be able to interact, thus creating a free standing cellular tube of similar proportions as blood vessels.

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