

## Effects of UV Laser Radiation and Soft Lithographic Patterning on the Wettability of Silk Thin Films

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### ABSTRACT

Liquid interaction with solids is crucial to optimize material design and processes across many applications. Therefore, wettability is of significant importance in such interactions. This study investigates the impact of an excimer laser of 193 nm wavelength patterned Silk fibroin (SF) films. Various techniques were employed to characterize the SF films, including AFM, SEM, and WLI. A UV-visible spectrophotometer was utilized to assess the morphological and optical characteristics of the SF films. Laser structuring generated periodic gratings (5–10  $\mu\text{m}$ ) and increased surface roughness, reducing water contact angles by 50%—indicating a dramatic wettability shift from hydrophobic to hydrophilic behavior. The films exhibited strong UV absorption ( $\alpha = 7.3 \times 10^4 \text{ cm}^{-1}$ ) while maintaining optical clarity in visible wavelengths, ideal for light-guiding applications. AFM and SEM revealed that laser patterning softened the SF matrix and introduced micro/nanoscale topography, directly correlating with a 40–50% increase in surface energy (WLI-validated). These modifications enable precise liquid-surface interactions critical for biosensor interfaces, drug-eluting coatings, or cell-adhesive substrates. The combined optical tunability (UV-Vis) and wettability control (contact angle:  $90^\circ \rightarrow 45^\circ$ ) position laser-patterned SF films as a dual-functional platform for lab-on-chip devices or implantable photonic therapeutics.

**Keywords:** Wettability, Contact angle, Surface energy, Silk Fibroin, Laser ablation, 193 nm laser

### 1. INTRODUCTION

Laser modifications of biological materials, such as proteins, is being developed for various bio applications. Lasers were used to synthesise the protein silk fibroin (SF) [1–5]. Moreover, different laser wavelengths have been used to modify the SF and characterize the effect of pulsed laser on the material [6–9]. Silk, also known as fibroin fiber, is a natural protein produced by insects such as spiders' or worms' cocoons. Silk consists of two proteins: the heavy and light fibroin chains, along with sericin, which crosslinks the fibroin to form the cocoon [10–15].

Silk fibroin fibers are among the strongest biomaterials, known for their high toughness, biocompatibility, biodegradability, and excellent optical properties. Fibroin films are transparent across visible wavelengths until the mid-infrared region can be implanted or produced as free-standing films, and remain thermally stable in ambient conditions [16–18].

Due to these high-quality properties, silk fibroin can be used for many applications [19–24], including biophotonic applications that combine biology and optics. It can be fabricated into various structures such as thin films, diffraction grating (high-resolution, fewer than 20 nm), photonic crystals, organic crystals (i.e. bioactive optical filters), waveguides, biosensors, bio-detectors, and

microfluidic chambers [25–29]. It also has an application in medicine and drug delivery [30, 31].

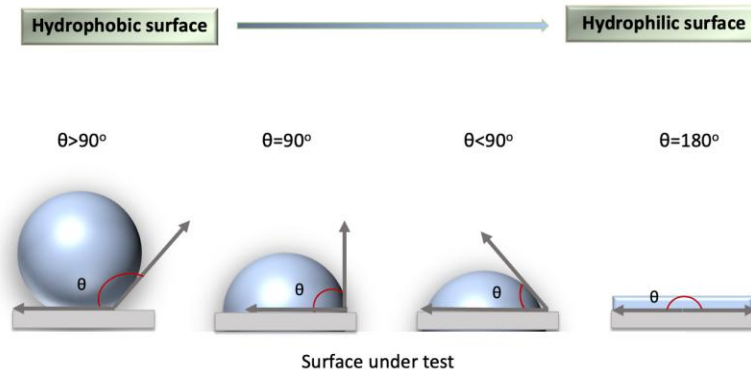
Silk fibroin possesses a unique combination of hydrophobic and hydrophilic properties. The sericin proteins are hydrophilic, whilst hydrophobic sequences dominate the  $\beta$ -sheet-crystalline regions of fibroin [32, 33]. The beta-sheets within silk fibroin make it highly resistant to water, and even solvents, such as formic acid, will not completely dissolve it [34, 35]. Surface wettability has attracted a significant research attention, as it is possible to alter it by controlling the material surface and so allowing the material to be used in different applications [36, 37]. Furthermore, a material's wettability significantly influences its biophotonic properties. Wettability refers to a liquid's ability to spread across a solid surface, typically quantified by measuring the contact angle formed at the intersection of liquid/air and solid/liquid interfaces [38–40].

### 2. THEORETICAL BACKGROUND

On an ideal surface (homogeneous, smooth, flat), the contact between the surface and the liquid can have different shapes and angles of contact Figure 1. Contact angle represents the angle controlled by two interfaces, that of the liquid/solid and liquid/gas. If the contact angle is  $\theta > 90^\circ$ , then it is a hydrophobic surface. A neutral surface

has a contact angle of  $\theta=90^\circ$ . A surface with  $\theta<90^\circ$  is called hydrophilic, and a perfect wetting surface has a  $\theta=180^\circ$  [41-

43]. The contact angle at equilibrium is calculated by the Young equation (Eq 1) [44-47].

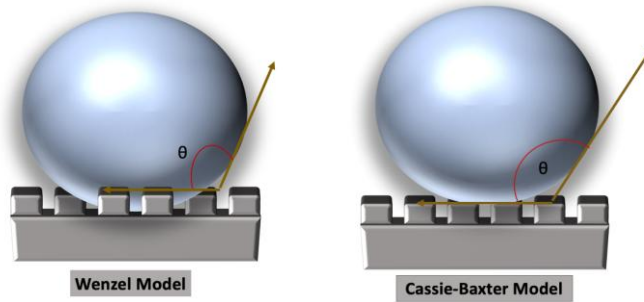


**Figure 1.** Young Model contact angle schematic behaviour of liquid on the surface. Non-wetting behaviour for  $\theta>90^\circ$ , Neutral for  $\theta=90^\circ$ , good wetting for  $\theta<90^\circ$  and perfect wetting for  $\theta<<90^\circ$ .

$$\gamma_{SG} = \gamma_{SL} - \gamma_{LG} \cos \theta \quad (1)$$

Where  $\theta$  is the contact angle and  $\gamma_{SG}$ ,  $\gamma_{SL}$  and  $\gamma_{LG}$  are the interfacial tensions of the liquid vapour phase, solid/vapour phase, and solid/liquid, respectively. For the non-ideal solid surface Figure 2, with higher roughness than the homogeneous, flat surface, the situation is more complicated because the surface area is increased, and sharp edges exist [48, 49]. Therefore, the contact angle has to be evaluated by the Wenzel model (Eq 2) [50, 51]. He

assumed that a surface roughness would increase the liquid-solid surface area. This has more influence on the good wettability surfaces than the poor ones, where the droplets don't go all the way to the surface and instead form air pockets [52, 53]. Wenzel, in 1936, articulated the relation between roughness and wettability. He explained that increasing the surface texture would change the wettability behaviour caused by the surface chemistry (i.e., the hydrophobic surface would increase in hydrophobicity) [54-56].



**Figure 2.** Schematics of wettability models: Wenzel's homogeneous wetting regime (left) and Cassie-Baxter's heterogeneous composite state (right), illustrating how surface roughness influences liquid droplet behavior. Arrows denote contact angle transitions due to microstructural features.

$$\cos \theta_w = R \cos \theta \quad (2)$$

is called the roughness factor (the ratio between the actual surface area and the apparent surface,  $R \geq 1$ ), and  $\theta_w$  is the *apparent contact angle*. If  $R=1$ , that means the apparent contact angle and the actual angle are the same as the surface is smooth. This equation is used for droplets with no trapped air. It is crucial to acknowledge that the Wenzel equation is predicated upon the assumption that the liquid entirely infiltrates the grooves of roughness. The Wenzel equation serves as an approximation that becomes increasingly precise as the droplet dimensions exceed the scale of the surface roughness. Consequently, if the droplet

size surpasses the roughness scale by two to three orders of magnitude, the application of the Wenzel equation is warranted. Furthermore, owing to its elevated band gap, the electronic and optical characteristics are significantly enhanced.

However, when air pockets exist in the roughness texture under the liquid, the wettability Wenzel equation will be derived into the Cassie-Baxter equations [57-59]:

$$\cos \theta_A = R \cdot \Phi_{SL} [\cos \theta + 1] - 1 \quad (3)$$

Where  $\theta_A$  represents the apparent contact angle,  $\Phi_{SL}$  is the solid surface fraction ( $0 \leq \Phi_{SL} \leq 1$ ). Surfaces with superhydrophobicity form air pockets beneath their droplet.

The surface tension could be determined by the contact angle and, thus, the wettability. The surface energy well defines the surface tension of a droplet. Surface tension is defined as the intermolecular forces that control the droplet shape [43, 60].

In this work, both the contact angle and the surface energy of droplets on SF films were studied. Furthermore, a 193 nm excimer laser was employed to alter the surface texture of the SF films to study the effect of roughness on its wettability characterisation. The silk fibroin films were studied before and after irradiation. Silk has been reported to have good wettability with a contact angle of about 60°-70° [61, 62]. Other studies show a change in hydrophobicity when changing a silk fibroin film surface [63, 64].

### 3. METHODOLOGY

BioMatrix's silk solution, containing approximately 50 mg/mL (5% W/V) of solubilized protein with an approximate molecular weight of 100 kDa, was utilized. This silk solution is exclusively composed of 100% fibroin protein sourced from the domesticated *Bombyx mori* silkworm. Careful handling of this solution is needed, as it necessitates storage at temperatures between -60°C and -70°C [65]. This solution must be allowed to attain room temperature gradually over several hours. The fabrication of silk films is achieved by depositing the fibroin solution onto a fused silica substrate utilizing a spin-coating technique at a 250 rpm rotational speed for 30 seconds. Subsequently, the solution was air-dried, allowing the crystallization process to form a film. The resultant thicknesses of the films were engineered to range from a few nanometers to several micrometers. These films exhibit a uniform and smooth morphology, characterised by a surface roughness measuring 3-4 nm due to the controlled drying process.

Characterization of the films was conducted utilizing UV-Vis spectroscopy (Thermo Scientific, Evolution 220) to investigate the synthesized SF film transmittance and absorbance. The absorption coefficient was derived from

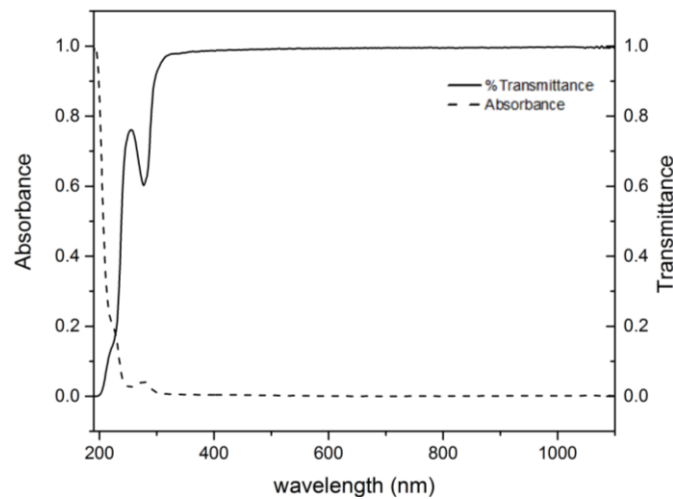
the optical spectra using the equation  $\alpha = 2.303A/t$ , where  $A$  represents absorbance, and  $t$  denotes the film thickness [44]. The roughness and texture of these films were analyzed by Atomic Force Microscopy (AFM) (Bruker edge).

For the determination of contact angle and surface energy, a sessile drop device (goniometer OCA100; DataPhysics Instruments GmbH, Germany) integrated with SCA20 software was utilized. Triple distilled water (TDI) served as the probe liquid, with a volume of 5  $\mu$ L administered onto the fibroin surface. Subsequently, an additional 2-5  $\mu$ L of TDI water was added. Liquid addition resulted in the advance of the triple line, allowing for the measurement of the advanced dynamic contact angle. Five dynamic contact angles were recorded and utilized to compute the average contact angle for the surface. The evaluation of surface free energy was conducted using the SCA20 software following the Equation of State method.

The optical grating was subsequently executed via laser irradiation employing an excimer laser (Lambda Physik LPF 202) of 193 nm wavelength with a 11.5 nm pulse duration. The laser light was reimaged (5-factor demagnification) with a parallel bar grid customised by (Agar Co, 400 Parallel Bar Nickel 3.05 mm diameter: AGG2016N), with a static ablation site of 390  $\mu$ m. The applied laser fluence was 28 mJ/cm<sup>2</sup>, with an Aerotech nano stage (Aerotech Fibre Align) operating at a 0.1 mms<sup>-1</sup> velocity. [66-68] The control of laser fluence was managed by an attenuator (Metrolux, ML2110) and measured utilising a Joulemeter (Moletron). The characterisation of the created grating was performed using Scanning Electron Microscopy (SEM) (SEM, Zeiss EVO60) and a White Light Interferometer (WLI, WYKO NT1100).

### 4. DISCUSSION AND RESULTS

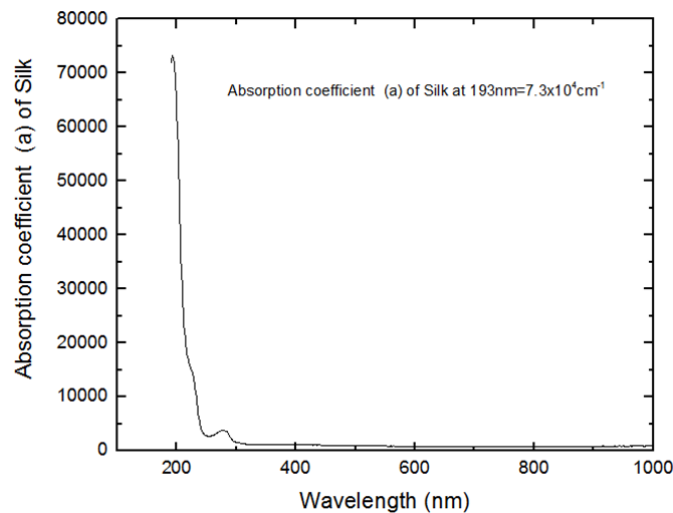
Several characterisations were conducted to investigate the silk surface before the laser texturing. The transmittance and absorbance of the silk fibroin were conducted using a UV-visible spectrometer Figure 3. The SF was found to be transparent (~99%) in the visible spectrum and near-infrared spectra (300–1100 nm), with a dramatic drop in transparency in the UV range (<300 nm), which was the result of protein absorbance.



**Figure 3.** UV-Vis spectra across 200-1000 nm wavelengths. The plot demonstrates high absorbance in the UV region (200-400 nm) and increased transparency in the visible to NIR range (600-1000 nm).

The transmittance of silk fibroin exhibits a direct correlation with the methodology used in its fabrication. In the present study, the observed transmittance was higher than the values documented in previous research [62]. This elevated transmittance within the visible spectrum provides a solid ground for its application in optical

devices, such as gratings. Furthermore, from the above measurements, SF's absorption coefficient at (193 nm) was derived to be approximately  $\alpha \sim 7.3 \times 10^4 \text{ cm}^{-1}$ , as illustrated in Figure 4.

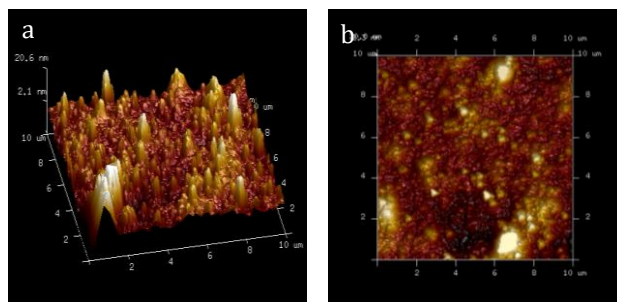


**Figure 4.** Absorption coefficient ( $\alpha$ ) spectrum of silk, showing strong UV absorption with  $\alpha = 7.3 \times 10^4 \text{ cm}^{-1}$  at 193 nm.

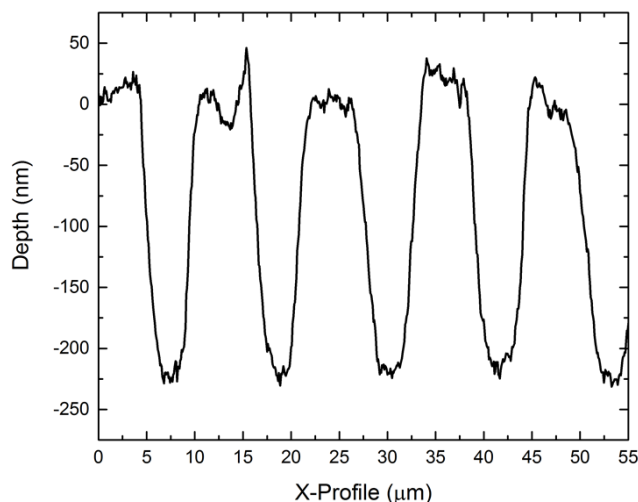
AFM was employed to analyze the topography of the surface after preparation. The AFM was operated in tapping mode utilizing "unmounted high-quality Pyramidal Silicon Tips" (Tespas-V2 with 8 nm radius) to characterize the roughness of the surface within a designated area of  $10 \times 10 \mu\text{m}$ . The roughness is significantly influenced by the grating pitch and depth, controlling the extent of the surface area subjected to interacting liquids. Another crucial aspect of the grating is the periodic patterns that regulate the interval between the grating lines and directly affect how fluids and light interact with the material's surface. A reduced pitch may lead to enhanced capillary phenomena concerning wettability, which can alter the behaviour of fluids in terms of their dispersion or the formation of droplets on the surface. This consideration is

particularly relevant for systems such as microfluidics, where precise fluid management is essential [59].

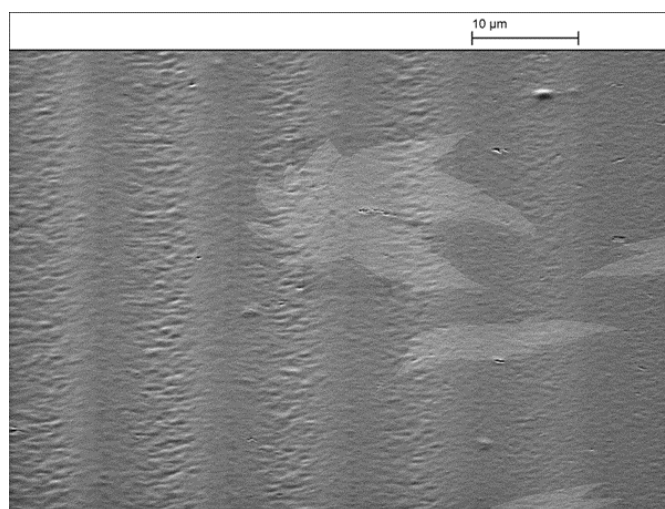
The AFM micrographs exhibit the occurrence of small-scale elevations and columns, with roughness measurements varying between 3-4.5 nm, refer to Figure 5. Furthermore, Figure 6 illustrates the depth of the prepared grating as quantified by White Light Interferometry (WLI). The channel widths were determined via the employed objective mask, ranging between 5-10  $\mu\text{m}$ . The channels were determined to possess a depth of 0.25  $\mu\text{m}$ , corresponding to the mentioned laser operational parameters. These findings were substantiated by scanning electron microscopy images in Figure 7.



**Figure 5.** AFM micrographs of the SF surface a) 3D image for an area of  $10 \times 10 \mu\text{m}$  b) top view of the film for the same area.



**Figure 6.** Cross-sectional 1D stylus profile measurement of the created grating by 193 nm laser of SF. The channel depth was 250 nm and a width of 5-10  $\mu\text{m}$ . The X-profile reveals nanoscale surface roughness characteristic of spin-coated or solvent-cast fibroin layers.



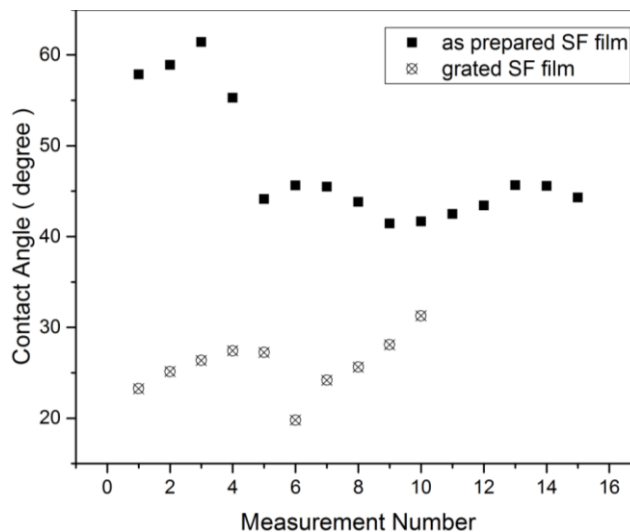
**Figure 7.** SEM micrograph of the grating produced by 193 nm laser ablation of SF. The ablated SF thickness was 475 nm. The film was created with a laser fluence of  $28 \text{ mJcm}^{-2}$ .

Despite the robustness of the film, the rapid thermal excitation from the laser facilitated the ablation of the film. The silk fibroin film has been documented as a resilient and stable protein that does not undergo melting due to thermal exposure. However, it has been demonstrated to melt under specific conditions, as explained by [15]. The lighter regions observed in Figure 7 may be attributed to the fact that silk fibroin exhibits various crystallization phases or the heterogeneity of the film.

Figure 8 demonstrates the measured contact angle for silk fibroin, both in its prepared state and after grating via a 193 nm laser, as determined through the sessile drop technique. The measurement was conducted repetitively. The obtained data indicates that the contact angles of silk fibroin prior to irradiation ranged from  $41^\circ$  to  $61^\circ$ , with an average contact angle of  $47.8^\circ$ . The contact angles recorded post-laser irradiation, following the formation of the grating, were found to range between  $19.7^\circ$  and  $31.2^\circ$ , with

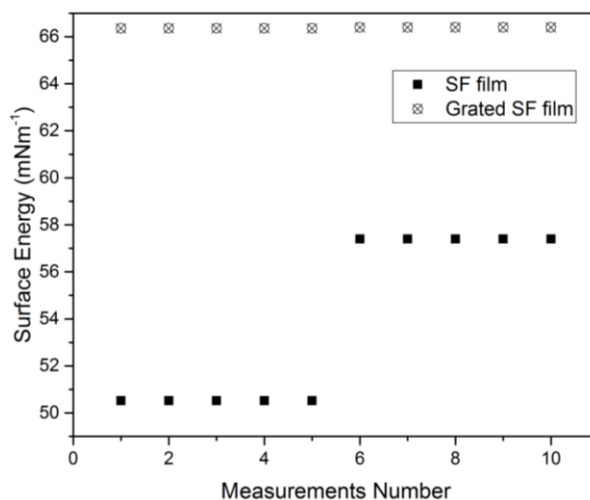


an average contact angle of  $25.8^\circ$ . This observation signifies that the grating structure resulted in a 50% reduction in the contact angle.



**Figure 8.** Average water contact angle of silk fibroin films before ( $47.8^\circ \pm X^\circ$ ) and after ( $25.8^\circ \pm X^\circ$ ) 193 nm laser irradiation, measured using the sessile drop technique ( $n=5$ ).

It is believed that these outcomes are indicative of the Cassie-Baxter model. The same concepts were applied to the surface energy measurements Figure 9.



**Figure 9.** Surface energy measurements of pristine silk fibroin (SF) films (52–56 mN/m) versus grated SF films (58–60 mN/m), showing increased surface energy after physical modification. Data points represent 10 consecutive measurements per sample type.

The free surface energy measurements were conducted twice for the prepared silk fibroin film, valued at  $50.5 \text{ mNm}^{-1}$  and  $57.4 \text{ mNm}^{-1}$ . A singular measurement of the surface energy was recorded following the grating process via the 193 nm laser. It was calculated to be  $66.4 \text{ mNm}^{-1}$ , representing an increase relative to the surface free energy of the non-grated SF. Therefore, it was found that the transition of the surface from a nanometer-scale roughness to a micrometer-scale structure induced by grating resulted in a decreased contact angle and enhanced the hydrophobic character of the surface. Additionally, this modification correlated with an increase in free surface energy. Upon the grated microchannel, the irregularity of

the surface increased, thus reducing wettability. This can be attributed to the elevation of the contact angle at a more textured surface. Wenzel and Cassie–Baxter models exhibit the influence of roughness on wettability phenomena. Following the grating process, the SF surface could have transitioned into a state wherein air pockets are trapped by surface grooves and irregularities, aligning closely with the Cassie–Baxter model. In surfaces subjected to excimer laser treatment, there is an observed increase in surface-free energy due to enlarged surface area, coupled with a potential rise in the number of reactive sites. On softer substrates, a higher surface-free energy typically correlates with improved wettability. Elevated surface energy on a

roughened surface may result in more pronounced air interaction and, consequently, an increased contact angle [69, 70].

## 5. CONCLUSION

It has been established that each surface exhibits a distinct degree of hydrophobicity. By altering the surface roughness from the nanometre scale to the micrometre scale through the application of laser grating techniques, a decrease in hydrophobicity can be observed, indicated by a reduction in the contact angle. In comparison, an enhancement in surface energy was noted.

The roughness of the spin-coated SF film was determined to average 4 nm as assessed by (AFM). This measurement was recognised as the smoothest surface achieved via the spin coating methodology. Within this roughness range, wettability was minimal, and it decreased further when 5-10  $\mu\text{m}$  channels were produced on the surface.

Researchers and technicians possess the capability to control the surface free energy by modifying surface roughness to optimise wettability based on defined criteria. It has been observed here that the wettability of Silk Fibroin films, even at minimal surface roughness values, is subject to alterations due to fluctuations in roughness. The architecture of the SF grating films was determined to reduce the liquid  $\theta$ , thereby signifying an improvement in wettability. Furthermore, it is essential to comprehend how the grating dimensions, including (width, height, shape), and grating periodicity, influence the Wenzel and Cassie-Baxter states of the water contact. Grating dimensions may be employed to customise the wettability of solid interfaces.

Patterned films elevate the surface free energy, thereby emphasising the complex interrelationship between wettability and surface roughness. Consequently, this study offers significant insights into the modification of surface properties of SF films through laser texturing, which possesses prospective applications across a diverse range of fields, such as protective coatings and medical devices.

## ACKNOWLEDGMENTS

Acknowledgments may be inserted at the end of the paper, before the references, not as a footnote to the title. Use the unnumbered Acknowledgements Head style for the Acknowledgments heading

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