Project BioCombs4Nanofibers

D2.4 Adhesion measurements of natural and artificial fibers

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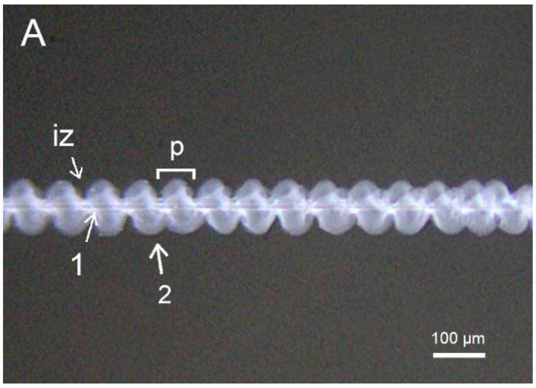
1. Goals and Detailed Description

Overall goal: D2.4 is a public intermediate summary of adhesion measurements of natural and artificial nanofibers on the project website.

Spider silk

Spider webs are an interplay of many different thread types, each with unique mechanical properties. Capture threads, for example, intercept and retain prey. This requires them to be tear-resistant and extensible enough to absorb the prey's impact energy, withstand traction forces of the struggling prey and provide an adhesion force strong enough to hold the prey in the thread, but not the spider itself. These numerous requirements are not fulfilled only by material properties but are also the result of a complex production and processing. Cribellate spiders produce particularly complicated and elaborate threads. Thereto, they interweave several different types of fibers, including supporting axial and sometimes additional undulating fibers. A mat made of thousands of nanometer-thick cribellate fibers gives them a woolly appearance and bears the adhesive properties (**Image 1**).



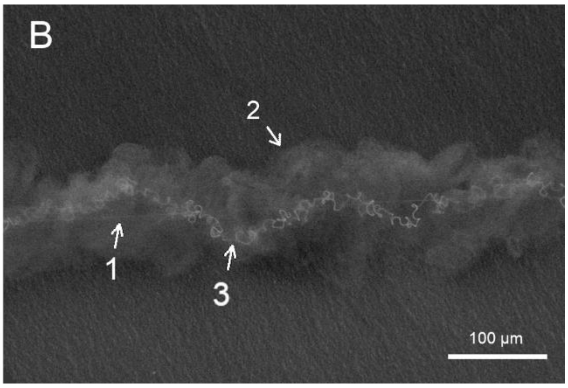


Image 1: The cribellate threads of the feather-legged lace weaver (*Uloborus plumipes*) and the grey house spider (*Badumna longinqua*). **A)** Capture thread of *U. plumipes* under a binocular, whose cribellate mat (2) displays the characteristic puff structure (p), parted at regular intervals by intermediate zones (iz). The construction is supported by two parallel axial fibers (1). **B)** SEM image of the capture thread of *B. longinqua*. In addition to the axial (1) and cribellate fibers (2), an undulating fiber (3) is incorporated.

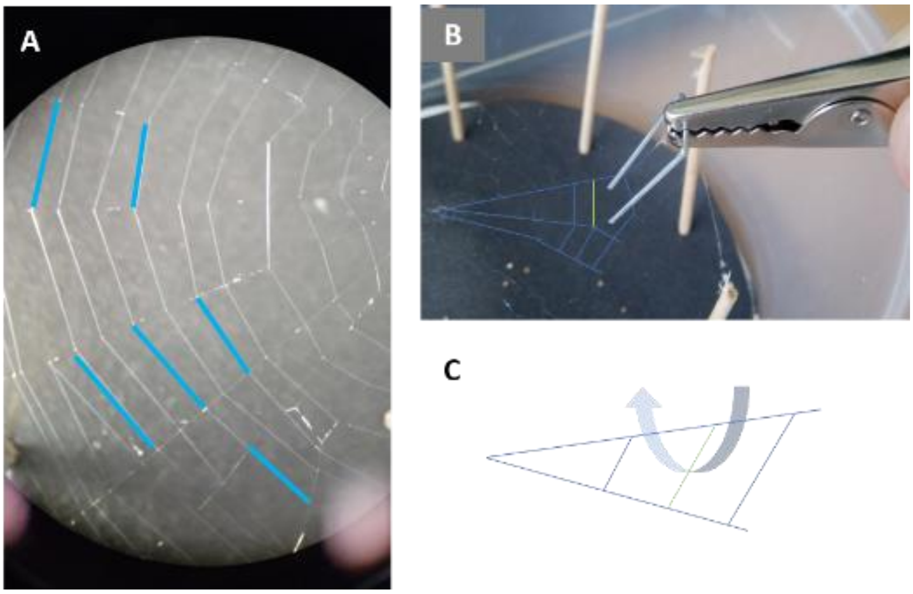


Image 2: Example of spider silk harvesting approach performed at **JKU**. **A)** Exemplary pattern of harvesting to avoid creating artificial tension (or lack of tension); silk sections to be harvested are marked by blue lines. **B)** Typical spider silk harvesting arrangement; part of the web is highlighted in blue for better visualization, the target silk section is highlighted in green; Silk harvesting is performed using a staple fixed at an angle into a small crocodile-clamp in a way that the staple can be inserted between the target silk section and the neighboring thread. **C)** Schematic representation of silk harvesting technique; bent arrow indicated the trajectory of the staple; the target silk section is depicted in green.

Through an interweaving process, a hierarchical structure is created, which may have a beneficial effect on the extensibility of the threads (see for example Piorkowski *et al.* (2020)). When capture threads of *Psechrus* are stretched, the undulating fibers lose their wave-like form, while the axial fibers are elongated. This leads to an extension of ~ 500 % before break, compared to an extension of ~ 100 % when the undulating fiber is cut. In preliminary measurements of our team, the threads of a lace-weaver species (*Amaurobius* sp.) reached a maximum extensibility of 1600 %, those of the feather-legged lace-weaver (*Uloborus plumipes*) of 600 % (with the data of *U. plumipes* matching those found in literature for other Uloboridae (Opell & Bond 2000)). Furthermore, tension experiments showed that threads of *Amaurobius* sp. can carry a load of 37 µN.

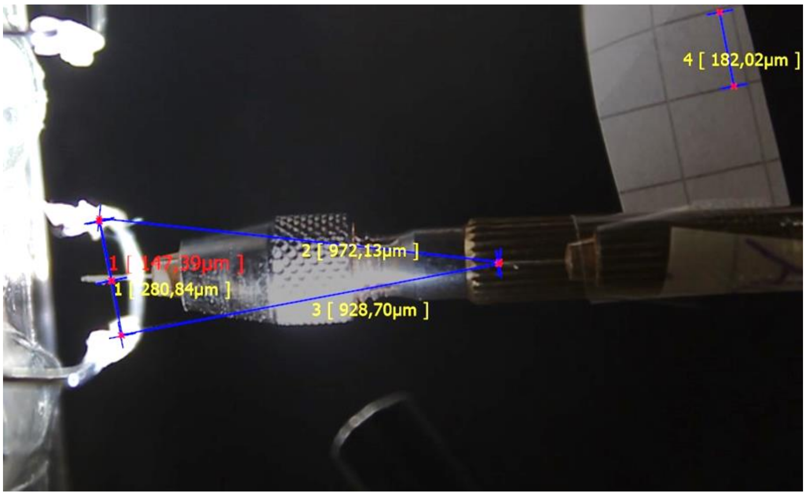


Image 3: Measurement of extensibility and tension. The extensibility was measured by superimposing images of the initial position of the thread and of the thread at maximum stretch before it tore. Shown are the initial position of the thread (1), the maximum elongation of the two halves of the thread (2 & 3) as well as a pre-defined distance that served as reference length.

Measuring antiadhesion

Additionally to the mechanical properties, also adhesive forces affect the success of prey capture. Cribellate spiders capture the prey by interacting with a viscous cuticular coating, covering all insects (see Bott *et al.* (2017)). However, it has been assumed for a long time that the adhesive strength depends primarily on the number of cribellate fibers due to van der Waals forces, because only the adhesion between cribellate threads and artificial surfaces were measured (see Hawthorn and Opell (2003)). Though it is not the primary force retaining insects in the web, it is a universal force between any nanomaterial and surfaces. Cribellate spiders have to overcome this force during processing of nanofibers, utilizing a specialized comb on their hind leg, the calamistrum (see public deliverable D2.1 Capture strategy of spiders). Its nanoscale structure was the model for a nanotopography, which we transferred to artificial surfaces (PET foils; **Image 4**).

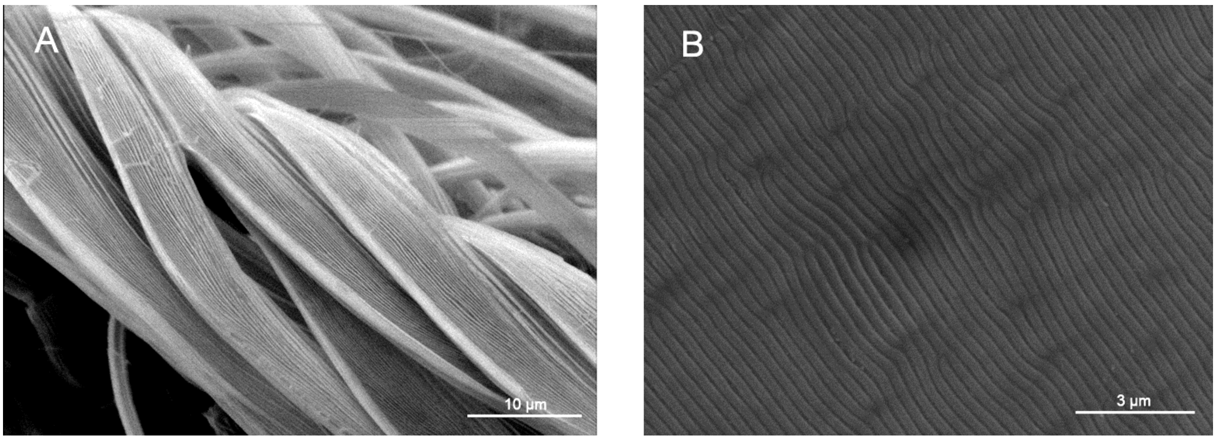


Image 4: Biomimetic transfer of the nanotopography found on the calamistrum. Inspired by the nanostructure of the calamistrum (**A**), laser-induced structures were created on polyethene terephthalate (PET) foils (**B**). SEM images, samples coated with gold.

In order to quantify even these miniscule forces, we use an indirect measurement of adhesion. While the adhesion strength of threads to insects is determined directly, using a microbalance (**Image 5**), we quantify reduced adhesion forces (“antiadhesion”) by measuring the deflection of a thread or single fiber, pulling a potential antiadhesive sample after contact with the thread or fiber slowly away (**Image 7**). However, such an indirect measurement leads to difficulties in terms of comparability between different fiber samples since the deflection depends not only on the adhesion force but also on e.g. the mechanical properties of the thread/fiber and its original length.

Image 5: Adhesion setup in the Spider Silk lab at **RWTH**. In order to measure the adhesion force of a thread, a motorized linear table brings the sample into contact with the thread. A microbalance measures the relief while the sample is withdrawn.

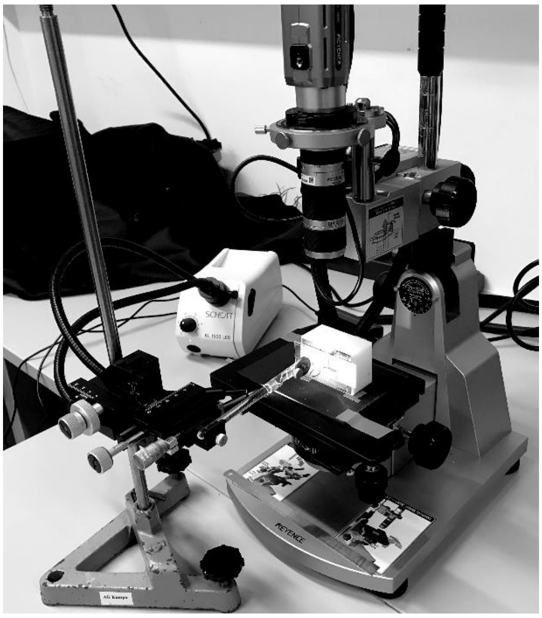


Image 6: Setup of indirect measurement of adhesion at the Spider Silk lab. (Anti)adhesion is measured indirectly using a high-speed video recording microscope.

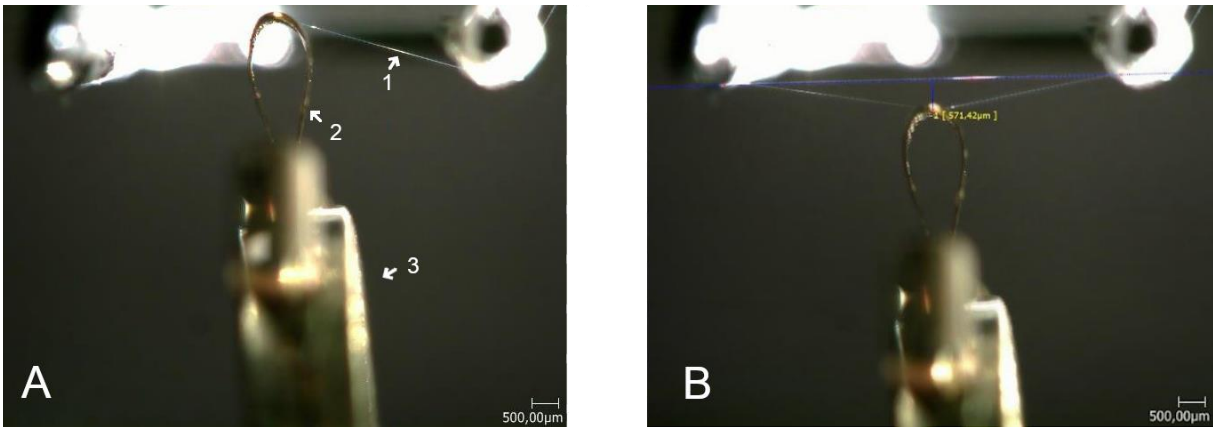


Image 7: A) A sample foil (1) is brought into contact with a cribellate thread (2) via micromanipulator (3). **B**) The adhesion is measured indirectly via the deflection of the thread when a sample is pulled out of it.

Artificial fibers and their mechanical properties

Artificial small-scale single fibers can be produced with a microfluidic wet-spinning technique (see Lölsberg *et al.* (2018)). In this process, a fiber is produced from a dissolved polymer using non-solvent induced phase separation. Through confinement in a channel with diameters on the micrometer scale, the phase separation process can be tightly controlled. For example, regenerated *Bombyx mori* silk fibers were collected from the chip and subjected to tensile tests in order to quantify their mechanical performance (**Image 8**).

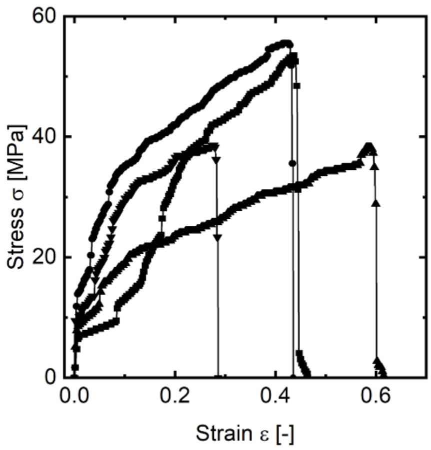


Image 8: Stress-strain diagrams of multiple individual fibers.

Image 8 shows stress-strain diagrams of multiple individual fibers. The fiber morphology was investigated with scanning electron microscopy. As can be seen in **Image 9**, a wide range of different fiber morphologies, ranging from smooth to rough and "sharkskin"-surfaces, can be created by selecting the right solvent, non-solvent and protein-concentration. The influence of these surface morphologies on adhesion properties is the subject of further investigations.

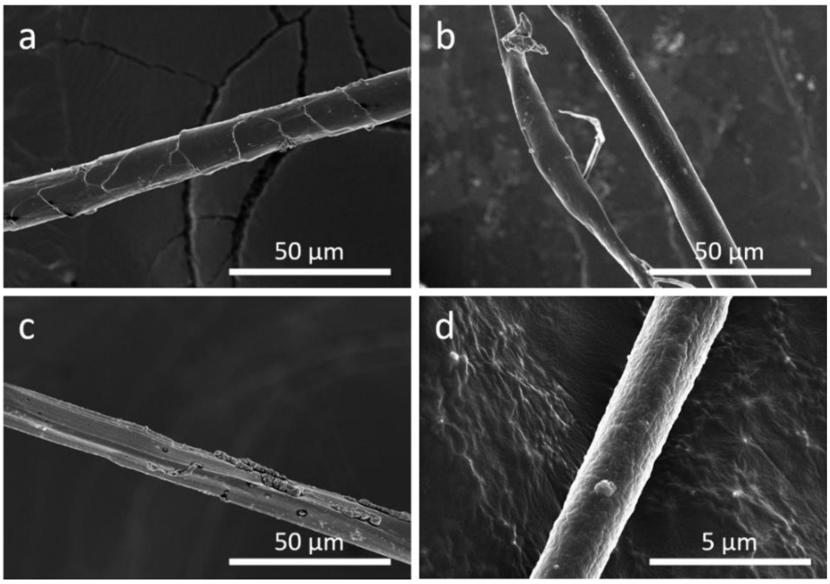


Image 9: Fibers with various morphologies resulting from variations of the spinning parameters (e.g. protein concentration, flow rates). The fiber morphology was investigated with scanning electron microscopy.

The Nanospider™ technology of project partner **ELMARCO** allows the production of even smaller fibers, with distinct diameters from 80 nm to 500 nm or higher and a standard deviation of fiber diameter of 30% or less. In comparison, melt blown or spun bond micro fibers range in the area of 0.9 to several micrometers and, e.g., merino wool fibers range around 12 - 24 micrometers. A human hair has a diameter around 80 micrometers and is about 200 times bigger in diameter than an average nanofiber. The nanofibers are produced from polymers solved in water, acids or bipolar solvents and is suitable for the production of organic high-quality fibers. For this purpose, the Nanospider equipment uses a needle-free high voltage, free liquid surface electrospinning process that can be easily adapted to a variety of process parameters for the optimization of the specific properties of the produced nanofibers. The technology is based upon the discovery, that it is possible to create Taylor Cones and the subsequent flow of material not only from the tip of a capillary, but also from a thin film of a polymer solution.

Due to its dimensions and thus to its unique features, nano-materials show an incremental improvement of final products (for example nanofibers in comparison to micro-materials). In addition to the low density of nanofibers and the large specific surface area, the special properties of non-woven nanofibers include small pore sizes as well as high porosity and thus good breathability. The fibers also have excellent mechanical properties in proportion to their weight and the ability to incorporate different additives.

Though the Nanospider^{TM} technology has a higher technological readiness, the production of non-single fibers but non-woven makes it more difficult to test antiadhesion with our established setup. For non-woven, the setup has to be adapted, e.g. characterize the amount of remnant fibers on a surface after peeling, instead of measuring forces indirectly.

Intermediate summary of our results of adhesion measurements with nanofibers in contact with the calamistrum or a biomimetic foil

With the setup for indirect measurement of adhesion at **RWTH**, we were able to prove that the cribellate threads do not stick to the calamistrum because of a special fingerprint-like nanostructure on the comb. Coating the calamistrum with gold or the removal of a potential surface-coating with *n*-hexane had no influence onto the forces. In a theoretical model, we could demonstrate that this structure most likely prevents the nanofibers from smoothly adapting to the surface of the comb, thus minimizing contact and reducing the adhesive van der Waals forces between the nanofibers and surface. This leads to the spiders' ability of nonsticky processing of nanofibers for their capture threads. The successful mimicry of these structures by laser-induced periodic surface structures (LIPSSs) on PET foils proved that the biological model can be used to optimize future tools in technical areas in which antiadhesive handling of nanofibrous materials is required (see Joel *et al.* (2020)).

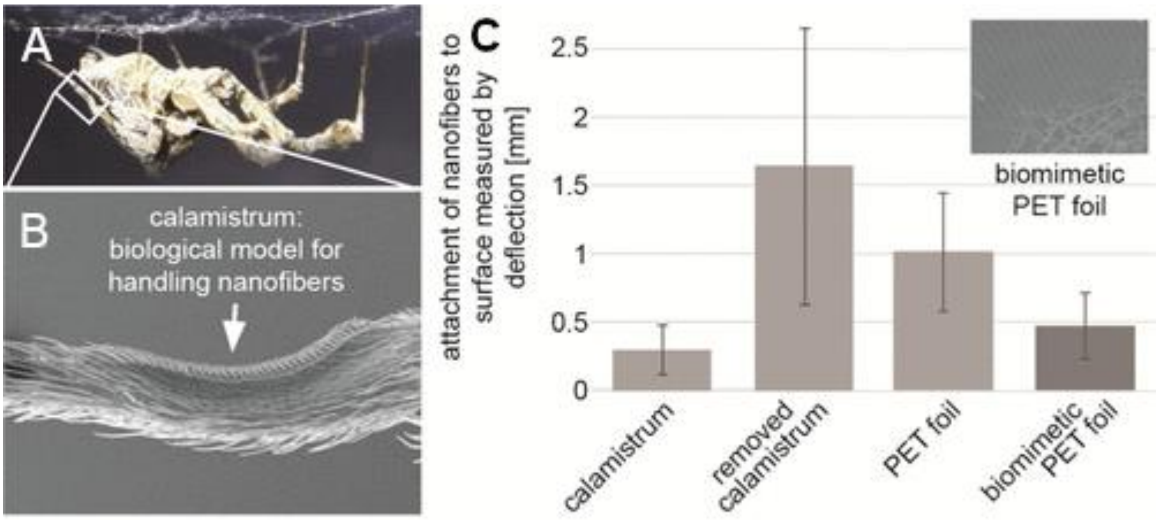


Image 10: The antiadhesive effect was successfully transferred to biomimetic PET foils. Shown is the deflection of the threads (**C**) of *U. plumipes* (**A**) by withdrawing different samples (the calamistrum as a model (B) as well as native PET foils and biomimetically structured PET foils). Figure adapted from (Joel *et al.* (2020)) published under a Creative Commons Non-Commercial No Derivative Works (CC-BY-NC-ND) Attribution License.

Furthermore, Polyamide-6 (PA6) fiber mats of fiber diameters of 150 – 250 nm were electrospun at **JKU** onto a selection of substrates to assess fiber mat's peeling ability from various materials as well as from structured and unstructured surfaces. The peeling ability was assessed so far non-quantitatively by removing the fiber mat with tweezers at an obtuse angle, and the peeled-off area was inspected under the scanning electron microscope after sputter-coating with gold.

Unsurprisingly, in all cases when fibers were spun onto smooth surfaces, it was difficult to peel the fiber mats off, and even if parts of the fiber mat could be removed, obvious reminiscences of more fiber layers were left on the substrate (**Image. 11**). This indicates that fiber mats separate into layers more readily than peel off the substrate.

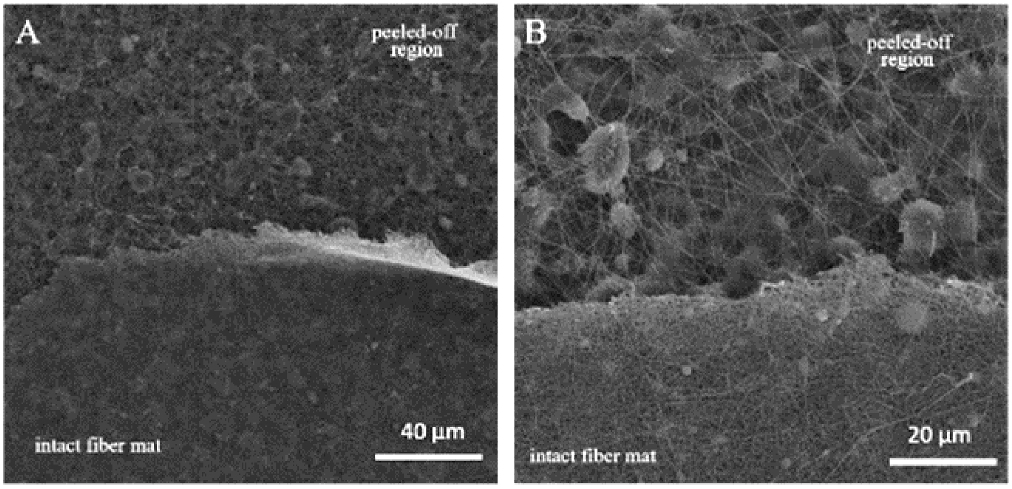


Image 11: Examples of SEM micrographs of polyamide-6 electrospun fiber mats onto smooth surfaces. **A)** Native PU belt sample from **ELMARCO**, **B)** Close-up of fiber mat rupture line from **A.**

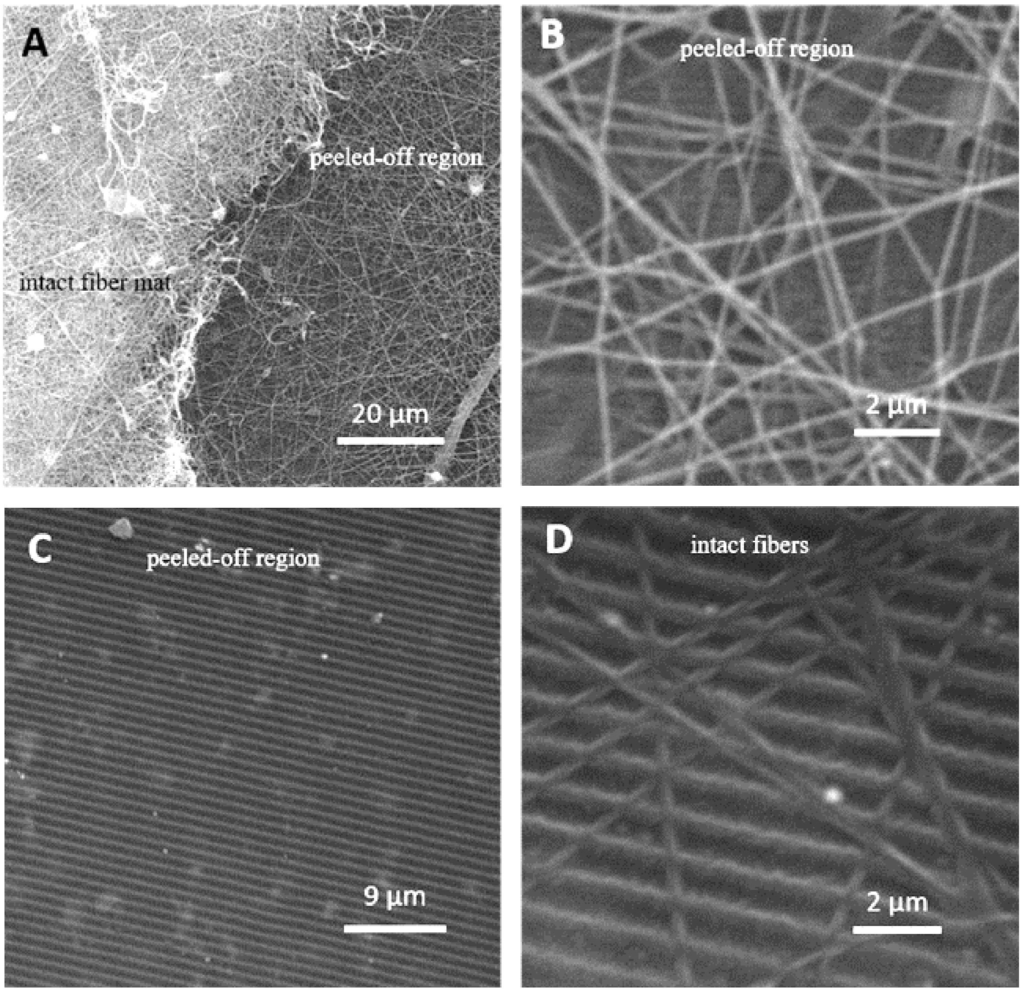


Image 12. Examples of SEM micrographs of polyamide-6 (PA6) electrospun fiber mats onto surfaces with nanoripples (**A, B**) and microripples (**C, D**). **A)** Laser-processed PET foil with nanoripples covered with PA6 electrospun fiber mat; substantial amount of fibers present in the peeled-off region (right of the tear line). **B)** Close-up of **A**; nanoripples can be recognized under the fibers. **C)** Silicone imprint of a 1-µm diffraction foil with all fibers cleanly peeled off. **D)** Several PA6 electrospun fibers on the silicone imprint of the 1-µm diffraction foil; it is noticeable that fibers rest on the topmost features of the substrate.

Most structured substrates facilitated an easier peel-off as compared to smooth ones. Substrates with larger structures (diffraction foil silicone imprint: 1-µm-spaced ripples (**Image 12 C, D**) showed a considerably easier peel-off and clear separation of the whole fiber mat from the substrate noticeable already by bare eye. Little to none of the reminiscent fibers were observed in the peel-off regions of these samples (**Image 12 C**). However, laser-processed PET foil with nanoripples was not noticeably beneficial for peeling ability of the fiber mat, and a substantial amount of fibers was still present in the peeled-off region (**Image 12 A, B**). Nanoripples of the PET foil could be observed through the layer of electrospun fibers (**Image 12 B**).

Literature hints:

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2. Evaluation of Goals and Resulting Actions

This report has been published as a public report (PDF) in the dissemination section of the website of the **BioCombs4Nanofibers** project (http://biocombs4nanofibers.eu) and will be uploaded to Zenodo. Additionally, a reference to this report will be published on twitter and researchgate as project update for our followers.

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