Atomic Force Microscopic Analysis of Sundew (*Drosera*) Adhesive for Biomedical Applications

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Statement of Purpose: The goal of this research was to determine the nanostructure of the adhesive from the Sundew, *D. binata* and *D. capensis*. The chemical composition of the adhesive has been characterized, and it was found to contain acids and sugars in varying concentrations. In 1977 it was discovered that the adhesive contained a single macromolecule termed mucin that had a molecular weight of 2×10^6 Daltons [1]. The mucin varied between species and was composed of xylose, mannose, galactose, glucuronic acid, and ester sulfate in the ratio of 1:6:6:6:1 in *D. capensis*, and arabinose, xylose, galactose, mannose, and glucuronic acid in a ratio of 8:1:10:18:17 for *D. binata* [1,2]. Despite the careful chemical analysis of this polysaccharide, no study has been conducted to elucidate the nanostructure of this adhesive. Using atomic force microscopy we have examined the nanostructure of the Sundew adhesive. This study has shed light on the unique properties of the nanoparticle and nanofiber-based scaffolds for potential applications in tissue engineering and regenerative medicine.

Methods: The Sundew species (*Drosera binata* and *Drosera capensis)* were purchased from the Carnivorous Plant Nursery, Derwood, MD and maintained in the lab. Samples were initially prepared for AFM by touching the sample substrate to the Sundew leaf and collected the adhesive drops. Alternatively, we have also removed individual tentacles from the Sundew leaf surface, and streaked the tentacles across the substrate to disperse the adhesive. Samples were prepared on a silicon wafer, glass coverslip, and fresh mica, respectively. After allowing the adhesive to contact the surface, the samples were allowed to dry for 24 hours under a bio-safety cabinet. The samples were then stained with Alcian Blue pH 2.5 (Richard Allen Scientific®) for 30 minutes to confirm the areas coated by the mucin. Agilent 5500 and 6000 AFMs were used for all imaging experiments and all samples were evaluated by two independent investigators. Experiments were also conducted to determine the elasticity of the liquid adhesive, as well as, some material properties through AFM force versus distance curves.

Results: At large scan regions, from 10-50µm, the fibrous nature of the adhesive was observed (Figs. 1A&B, Fig. 2). Smaller scans revealed uniform nanoparticles that were associated in and around the fibrous network (Fig. 1C&D). The nanoparticles can be seen to form a mechanical "bridge" for the polysaccharide to bind, providing the mechanical support for the polysaccharide to be stretched into long thin fibers. The smallest nanofibers observed were about 50 nm. In all tested specimen, a network of interwoven fibers could be observed, although the mesh and density was dependant on the type of sample preparation, streaking or tapping, and the amount of adhesive. In all species of Sundew tested, nanoparticles were found to be closely associated with the development of nanofibers. Fig. 1D clearly shows the association between the nanoparticles and the individual nanofibers. The nanofibers have a "string of pearls" appearance. The network of thin nanofibers was easily observable in the AFM from scans of large areas of 50 um and greater. The network and pattern of fibers, along with the thickness of the fibers, varied between two species observed, *D. binata* (Fig. 2) and *D. capensis* (Fig. 1). The nanofibers formed independent of the surface and could be observed on all tested surfaces, including glass, mica, and silica, etc. With increasingly smaller scans, it was possible to closely examine individual nanofibers and determine their structural makeup. From scans of 2.5 um and smaller, a chain of individual nanoparticles was found to exist within these nanofibers.

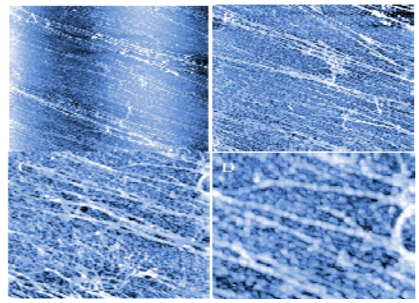

Figure 1. AFM imaging of Sundew (*D. capensis*) adhesive.

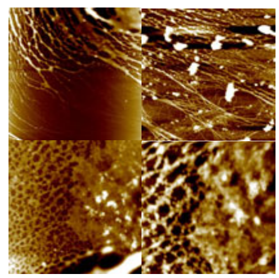

Figure 2. AFM images of the Sundew (*Drosera binata*) adhesive on a Silicon surface.

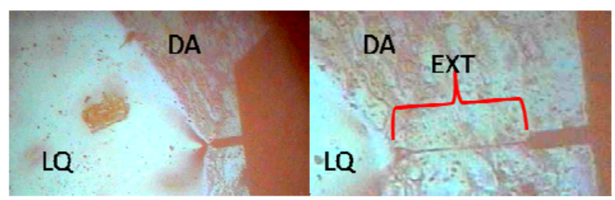

Figure 3. High elasticity of the Sundew adhesive. The cantilever tip is shown as reference. LQ = liquid adhesive, DA= dried adhesive, EXT = extension.

A preliminary setup for determination of the adhesive elasticity is outlined in Figure 3.

Conclusions: From this study we concluded that at the micro-nano scale, the Sundew adhesive formed a complex scaffold consisting of micro and nanofibers. This tightly woven network could have implications in the design of biomaterials for the growth of cells. This material has the advantage of being a naturally occurring sample that may be less toxic to cells. Further studies have been conducted and are reported in the same meeting for potential application to neural cell differentiation.

References:

1.) Rost, K. Phytochemistry. 1977; 16:1365-1368.
2.) Gowda, DC. Phytochemistry. 1982; 21:2297-2300.

* These authors contributed equally to this work.