基于液相AFM的植物黏液功能界面超微结构及其机械特性研究

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摘要 生物黏液在生命活动过程中起着重要的调控作用, 然而由于缺少合适的观测手段, 目前对于天然状态下生物黏液功能界面超微结构的认知还很不足. 原子力显微镜(atomic force microscopy, AFM)的出现为原位研究生物材料的结构和特性提供了新的强大工具, 但现有的研究主要在空气环境下进行观测, 研究结果难以完全反映溶液状态下的生物材料结构. 本文以食虫植物茅膏菜分泌的黏液为研究对象, 利用AFM直接在液相环境下实现了对生物黏液纳米结构的高分辨率成像及分析. 分别将茅膏菜黏液平铺至载玻片和云母表面, 在溶液环境下的AFM成像结果显示茅膏菜黏液中含有大量纳米颗粒. 作为对照, 在空气中进行的AFM成像结果显示干燥后的茅膏菜黏液中含有大量纳米纤维结构, 表明了茅膏菜黏液在空气环境下与溶液环境下的结构差异. 进一步利用AFM多参数成像方法对溶液环境下茅膏菜黏液纳米颗粒和纳米纤维的机械特性进行了可视化表征并揭示了纳米颗粒和纳米纤维机械特性的显著差异. 研究结果为生物黏液功能界面超微结构原位成像及机械特性研究提供了新的方法和思路, 对于生物材料研究具有广泛的基础意义.

关键词 原子力显微镜, 生物黏液, 功能界面, 纳米颗粒, 纳米纤维, 机械特性

1 引言

生物黏液在生命有机体的生理活动过程中起着重要的调控作用. 黏液(mucus)是动物或植物分泌的黏附于体内或体表(外)各种表面的湿滑液体^[1]. 人体内所有非角质化的表面均覆盖有黏液, 包括呼吸道、胃肠道

和泌尿生殖道等^[2]. 这些黏液是人体先天免疫的第一道防线, 阻挡微生物对组织的入侵并为上皮细胞提供润滑、机械应力缓冲等功能^[3]. 蜗牛和蛞蝓在爬行过程中会分泌大量黏液, 这些黏液有助于蜗牛和蛞蝓在水平表面和垂直表面的运动及固定, 还能帮助其定位猎物位置以及交配等^[4]. 贻贝能分泌一种富含多种黏

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附蛋白的黏液, 这种黏液使得贻贝能牢固地附着在水下几乎所有的有机和无机表面并抵挡水流的冲击^[5]. 不仅动物分泌黏液, 植物在生长及生理活动过程中也大量分泌黏液. 许多植物的种子在发生水合作用时会在种子表面形成特殊的纤维胶状黏液以包裹种子^[6]. 这些黏液有助于增强植物种子与土壤之间的黏附, 还能阻止氧气扩散以调节种子发芽, 并可以通过将种子附着在动物表面以促进种子传播^[7]. 除了种子外, 植物根部也能分泌黏液, 这些黏液有助于润滑植物根部并促进植物根系与土壤中离子和微生物之间的相互作用^[8], 还能促进土壤稳固并帮助植物的根穿过紧致的土壤^[9]. 植物黏液还直接参与植物生理活动过程, 如常春藤分泌黏液以攀爬生长, 食虫植物分泌黏液以诱捕昆虫等^[10]. 这些动植物分泌的生物黏液具有显著的医用、药用与食用价值, 如可用于手术缝合、药物递送、伤口愈合、消炎抗菌, 以及作为食品添加剂^[11~13]等. 因此开展生物黏液功能界面^[14]相关研究对于生命及健康科学具有重要的基础意义.

原子力显微镜(atomic force microscopy, AFM)的出现为生物黏液研究提供了新的强大技术手段. 生物黏液成像表征的传统手段主要包括荧光染色光学成像^[15,16]、扫描电子显微镜(scanning electron microscopy, SEM)^[17,18]和透射电子显微镜(transmission electron microscopy, TEM)^[19]. 光学成像的缺点是空间分辨率低, 虽然基于荧光标记的超分辨光学成像技术显著提升了空间分辨率^[20], 但荧光标记有可能影响生物样本生理活动, 给测量结果解释带来困难. 电子显微镜成像可以观测生物样本的精细形貌结构, 但是需要对样本进行固定、干燥、脱水等处理, 无法直接对活体状态的生物样本进行成像^[21]. 虽然环境扫描电子显微镜(environmental SEM, ESEM)可以对含有一定水分的样本进行观察, 但是仍然存在分辨率低、电子束对样本造成不可逆损伤等不足^[22]. 与传统表征手段相比, AFM具有独特的优势. AFM可以直接在溶液环境下对活体状态的生物样本以纳米级的空间分辨率进行观测, 且样本无需固定、染色、标记等预处理, 还能够对生物样本的力学特性进行定量测量, 因而为研究生物界面的结构和特性提供了新的可能^[23]. 研究人员利用AFM对生物黏液进行了成像研究(如人宫颈黏液^[24]、蜗牛黏液^[25]、植物分泌的水凝胶黏液^[26]等), 但这些研究主要在空气中进行, 目前对于溶液环境下

生物黏液功能界面超微结构的认知还很不足. 针对这个问题, 本文以食虫植物茅膏菜(*Drosera capensis*)分泌的黏液为研究对象, 实现了溶液环境下生物黏液纳米结构的AFM成像, 并通过与空气中的AFM成像结果进行对比揭示了茅膏菜黏液在空气中和溶液中的结构差异, 展示了AFM多参数成像方法应用于生物黏液纳米结构机械特性研究的潜力, 研究结果加深了对茅膏菜黏液超微结构及其机械特性的认识, 为功能生物界面研究提供了新的方法和思路.

2 材料与方法

2.1 植物培养

本文选取两种茅膏菜进行研究, 分别为红色好望角茅膏菜(*Drosera capensis red*)和白色好望角茅膏菜(*Drosera capensis alba*), 如图1所示. 两种茅膏菜均购自山东贵翔光电有限公司(潍坊). 茅膏菜为喜光植物, 将购买的茅膏菜盆栽置于阳台接收光照并采用去离子水进行培养. 茅膏菜培养的环境温度为20℃~30℃, 环境湿度为50%~70%. 培养一周后即可在茅膏菜叶子表面大量分布的触须(tentacle)末端观察到晶莹透亮的黏液(图1(II)箭头指示). 这些黏液在茅膏菜捕食过程中起着重要作用^[27]. 小型昆虫被茅膏菜黏液粘住后往往难以挣脱, 进而被茅膏菜分泌的消化酶消化, 为茅膏菜提供生理活动所需的养分^[28]. 图1(III)虚线椭圆指示了被本文茅膏菜黏液所捕获的小型昆虫, 显示了茅膏菜黏液的重要生物学功能.

2.2 样本制作

本文选取两种基底用于制作茅膏菜黏液AFM成像样本, 分别为载玻片和云母, 如图2所示. 待观察到茅膏菜叶子表面分泌黏液后, 将干净的载玻片(图2(a))表面轻轻靠近并接触茅膏菜黏液, 茅膏菜黏液即可覆盖在载玻片表面. 制作云母样本时, 首先利用双面胶将揭去表层的云母片固定在载玻片上, 随后控制云母表面接触茅膏菜黏液, 即可得到覆盖了黏液的云母样本. 在溶液下成像时, 将覆盖有茅膏菜黏液的载玻片样本/云母样本置于含超纯水(Milli-Q, 德国)的培养皿(图2(c))中, 随后将培养皿置于AFM载物台上进行溶液下的AFM成像. 在空气中成像时, 将覆盖有茅膏菜黏液的载玻片样本/云母样本直接置于AFM载物台上进

图 1 (网络版彩图)茅膏菜黏液及其生物学功能. (a) 红色好望角茅膏菜黏液; (b) 白色好望角茅膏菜黏液. (I) 盆栽茅膏菜整体光学图. (II) 单个茅膏菜叶子光学图. 叶子表面分布有大量的触须, 触须的末端分泌露珠样的黏液(箭头指示). (III) 茅膏菜黏液诱捕小型昆虫(虚线椭圆指示)

Figure 1 (Color online) Sundew mucilage and its biological functions. (a) Mucilage secreted by *Drosera capensis* red; (b) mucilage secreted by *Drosera capensis* alba. (I) Overall optical images of the potted sundews. (II) Optical images of the individual leaves of sundews. There are many tentacles on the leaves and the mucilage dewdrop (typically denoted by arrows) is secreted at the end of the tentacles. (III) Small insects (denoted by the dashed ellipses) captured by the sundew mucilage.

图 2 (网络版彩图)茅膏菜黏液AFM成像样本制作及AFM实验平台. (a) 控制载玻片接触茅膏菜黏液以使黏液覆盖在载玻片表面. (b) 控制固定在载玻片表面的云母片接触茅膏菜黏液制作覆盖有黏液的云母样本. (c) 本文所使用的AFM实物图. 插图在溶液中工作的AFM探针

Figure 2 (Color online) Sundew mucilage sample preparation and AFM experimental platform. (a) The glass slide is controlled to touch sundew mucilage to form a layer of sundew mucilage on the surface of the glass slide. (b) Touching sundew mucilage with the mica (mica is immobilized on the glass slide) to coat mica surface with mucilage. (c) Actual photograph of the AFM used in this study. The inset is the AFM probe at work in solution.

行空气中AFM成像.

2.3 AFM成像

利用美国Bruker公司生产的Dimension Icon AFM (图2(c))开展本研究. 分别在水溶液(Milli-Q, 德国)和空

气中对制作的载玻片茅膏菜黏液样本和云母茅膏菜黏液样本进行成像. 溶液中和空气中的AFM成像模式均为峰值力轻敲模式(peak force tapping, PFT). 在PFT模式下, 垂直方向振动的探针在水平面对样本进行光栅扫描, 在每个采样点进行垂直方向的逼近-回退运动以

获取力曲线^[29]. 在逼近过程中, 悬臂梁最大偏转量对应的力即为峰值力. 通过控制探针在垂直方向位置运动维持峰值力恒定以获得样本表面形貌图. 在PFT成像过程中, 通过分析获取的力曲线可同步得到样本力学特性图, 包括形变图、硬度图、黏附力图^[30]. 溶液中成像的探针(图2(c)插图所示)材料为氮化硅, 悬臂梁弹性系数为0.7 N/m, 针尖曲率半径为20 nm; 空气中成像的探针悬臂梁弹性系数为0.4 N/m, 针尖曲率半径为2 nm. 具体来说, 在AFM光学显微镜引导下, 首先控制探针在基底表面空白区域获取力曲线校正悬臂梁的偏转灵敏度, 并进而利用AFM热噪声方法对悬臂梁的弹性系数进行校正. 随后控制AFM探针在样本表面不同区域进行扫描成像. 扫描成像参数如下: 探针驱动频率2 kHz, 扫描线数为512条, 每条扫描线的采样点数为512, 扫描频率为1 Hz.

3 结果与讨论

AFM形貌成像结果显示了茅膏菜黏液在空气中与溶液中的显著结构差异. 首先分别在空气中和纯水

溶液中对覆盖有红色好望角茅膏菜黏液的载玻片样本进行了AFM成像, 如图3所示. 在空气中的成像结果显示茅膏菜黏液含有大量纳米纤维结构(图3(a)). 纳米纤维纵向截面曲线上有很多锯齿状突起峰(图3(a)IV黑色箭头指示), 表明了纳米纤维结构在纵向上并非均匀分布, 而是具有异质性. 事实上从AFM图像上可以看到纳米纤维呈现螺旋状结构(图3(a)III黄色箭头指示). 当在溶液中成像时, 可以看到茅膏菜黏液含有大量纳米颗粒(图3(b)). 截面曲线上的平滑突起峰对应于茅膏菜黏液纳米颗粒(图3(b)IV绿色箭头指示). 需要指出的是, 在溶液中成像时仍能观察到极少数的纳米纤维结构(图3(b)I红色箭头指示). 进一步将覆盖有黏液的载玻片样本进行干燥处理后置于溶液中进行AFM成像, 如图4所示. AFM成像结果(图4(a)和(b))以及截面曲线平滑突起峰(图4(c)中绿色箭头指示)清晰地显示了茅膏菜黏液的纳米颗粒结构. 此外, AFM成像还直观显示了溶液中纳米纤维到纳米颗粒的动态变化, 如图5所示. 从图5(a)上可以明显看到两根纳米纤维(红色箭头指示), 而在纳米纤维上可以看到分布有大量纳米颗粒. 纵向截面曲线的平滑突起峰(图5(b)绿色箭头指

图 3 分别在空气和溶液中对覆盖有红色好望角茅膏菜黏液的载玻片样本进行AFM形貌成像的实验结果. (a) 空气中成像结果; (b) 纯水溶液中成像结果. (I)~(III) 不同扫描范围下的AFM图像. (IV) 从AFM图像中获取的纳米纤维和纳米颗粒截面曲线. (IV)对应(III)中的红色线条

Figure 3 Experimental results of AFM morphological imaging of *Drosera capensis* red mucilage-coated glass slides in air and in liquids, respectively. (a) AFM images obtained in air; (b) AFM images obtained in pure water. (I)~(III) AFM images recorded at different scan ranges. (IV) Section profile curves of nanofibers and nanoparticles taken along the AFM images. (IV) corresponds to the red lines in (III).

图 4 在溶液中对覆盖有红色好望角茅膏菜黏液的载玻片样本进行的AFM成像实验结果. 将覆盖有黏液的载玻片样本置于空气中干燥后再将其置于纯水中进行AFM成像. 大范围(a)和小范围(b)AFM扫描成像结果. (c) 截面曲线(对应(b)中的红色线条)
Figure 4 Experimental results of imaging *Drosera capensis* red mucilage-coated glass slides in liquids by AFM. Mucilage-coated glass slides were placed in air to dry. After air-drying, the samples were imaged by AFM in pure water. Large-size (a) and small-size (b) imaging results. (c) Section profile curve (corresponding to the red line in (b)) taken along the AFM image.

图 5 溶液中AFM成像显示红色好望角茅膏菜黏液纳米纤维转变为纳米颗粒的动态变化. (a) AFM形貌图; 纵向(b)和横向(c)截面曲线. (b)和(c)分别对应(a)中的红色线条和蓝色线条
Figure 5 AFM imaging in liquids revealing the dynamic transformations of nanofibers to nanoparticles for the mucilage secreted by *Drosera capensis* red. (a) AFM morphological image. Longitudinal (b) and transverse (c) section profile curves taken along the AFM image. (b) and (c) correspond to the red line and blue line in (a) respectively.

示)对应于纳米纤维上的纳米颗粒. 横向截面曲线显示该纳米纤维高度约为30 nm(图5(c)). 考虑到载玻片表面并非完全光滑, 为了消除基底表面起伏形貌对成像结果的影响, 分别在空气和纯水溶液中对覆盖有红色好望角茅膏菜黏液的云母样本进行了AFM成像, 如图6所示. 揭去表层的云母片表面光滑平整, 因而可有效消除基底表面起伏对成像的影响. 可以看到在云母表面获取的AFM成像结果与在载玻片表面获取的AFM成像结果类似. 空气中成像时可明显观察到纳米纤维结构(图6(a)I, II). 截面曲线显示了不同尺寸的纳米纤维(图6(a)III). 溶液中成像时可明显观察到纳米颗粒结构(图6(b)I, II). 截面曲线显示三个纳米颗粒的高度分别为8, 10和12 nm(图6(b)III). 随后分别在空气中和溶

液中对白色好望角茅膏菜黏液覆盖的载玻片样本和云母样本进行了成像(图7), 结果显示空气中成像时白色好望角茅膏菜黏液中包含大量纳米纤维结构, 而溶液中成像时可以观察到大量纳米颗粒结构, 与红色好望角茅膏菜黏液实验结果一致(图3~6).

在溶液中对生物黏液进行AFM成像有助于深入理解生物黏液的超微结构. 研究人员利用SEM和TEM等电子显微镜技术对植物种子黏液进行了大量研究, 结果显示植物种子黏液中包含大量纳米纤维结构^[31~34]. 2014年, Hakala等人^[35]利用AFM在空气中对木梨(*Cydonia oblonga*)种子黏液进行了成像, 显示木梨种子黏液中含有大量纳米纤维结构. 这些研究结果^[31~35]与本文利用AFM在空气中得到的茅膏菜黏液

图6 (网络版彩图)分别在空气和溶液中对覆盖有红色好望角茅膏菜黏液的云母样本进行AFM形貌成像的实验结果。(a)空气中成像结果;(b)纯水溶液中成像结果。AFM大范围(I)和小范围(II)成像结果。(III)从AFM图像中获取的纳米纤维和纳米颗粒截面曲线。(III)对应(II)中的红色线条

Figure 6 (Color online) Experimental results of AFM morphological imaging of *Drosera capensis* red mucilage-coated mica in air and in liquids, respectively. (a) AFM images obtained in air; (b) AFM images obtained in pure water. Large-size (I) and small-size (II) imaging results. (III) Section profile curves of nanofibers and nanoparticles taken along the AFM images. (III) corresponds to the red lines in (II).

图7 (网络版彩图)白色好望角茅膏菜黏液的AFM成像。分别在空气和水溶液中对覆盖有白色好望角茅膏菜黏液的载玻片/云母样本进行AFM成像。(a), (c) 载玻片样本; (b), (d) 云母样本。(a), (b) 空气中成像结果; (c), (d) 溶液中成像结果

Figure 7 (Color online) AFM imaging results of mucilage secreted by *Drosera capensis* alba. AFM images of mucilage-coated glass slides (or mucilage-coated mica) were obtained in air and in pure water, respectively. (a), (c) AFM images of mucilage-coated glass slide; (b), (d) AFM images of mucilage-coated mica. (a), (b) AFM images obtained in air; (c), (d) AFM images obtained in pure water.

形貌成像结果一致。然而, 需要指出的是已有的研究^[31~35]均需要对植物黏液进行干燥处理, 忽略了黏液在空气中的干燥过程是否影响黏液自身微观结构这一重要科学问题。此外, 植物黏液的天然状态为溶液状

态, 在空气中对干燥形态的植物黏液进行的成像表征无法完全反映溶液状态下的植物黏液真实微观结构。本文实现了直接在溶液中对红色好望角茅膏菜黏液和白色好望角茅膏菜黏液进行的AFM成像, 实验结果显

示溶液状态下红色好望角茅膏菜黏液和白色好望角茅膏菜黏液均呈现显著的纳米颗粒状结构(图3~7), 表明了空气干燥过程对植物黏液微观结构的重要影响, 同时还显示了在溶液状态下茅膏菜黏液纳米纤维结构转化为纳米颗粒结构的动态过程(图5), 为茅膏菜黏液的超微结构提供了新的认识. 由于已有的研究在空气中进行导致研究焦点集中在植物黏液的纳米纤维结构^[36,37], 本文在溶液中进行的AFM成像观察到了前人

所未能观察到的植物黏液纳米颗粒结构, 给植物黏液纳米结构带来了新的认识. 本文所建立的方法还可直接应用于其他类型的植物黏液, 如植物种子黏液, 这对于全面研究不同状态下(溶液状态、干燥状态)植物黏液的微观结构具有重要的基础科学意义.

AFM多参数成像结果显示了溶液环境下茅膏菜黏液纳米纤维和纳米颗粒之间机械特性的差异, 如图8所示. PFT成像模式下探针在样本表面每个采样点获取力

图 8 AFM多参数成像方法揭示溶液环境下茅膏菜黏液纳米颗粒和纳米纤维机械特性的差异. (a) AFM多参数成像原理. (I) 通过分析力曲线不同部分得到多个反映样本机械特性的参数, 包括形变、杨氏模量和黏附力. (II) 溶液下在茅膏菜黏液纳米颗粒上获取的典型力曲线. (b), (c) 溶液下茅膏菜黏液纳米颗粒和纳米纤维的AFM多参数同步成像结果. (b) 大范围扫描成像. (c) 小范围扫描成像. (I) 形貌图. (II) 形变图. (III) 杨氏模量图. (IV) 黏附力图

Figure 8 AFM multiparametric imaging revealing the different mechanics between nanoparticles and nanofibers in sundew mucilage. (a) Principle of AFM multiparametric imaging. (I) Various mechanical parameters are obtained by analyzing the different parts of the force curves, including deformation, Young's modulus and adhesion force. (II) A typical force curve obtained on the nanoparticles in pure water. (b), (c) AFM multiparametric imaging results of nanoparticles and nanofibers in pure water. (b) Large-size scan; (c) small-size scan. (I) AFM morphological images; (II) Deformation images; (III) Young's modulus images; (IV) adhesion force images.

曲线, 通过实时分析这些力曲线可以得到与样本形貌图对应的多种机械特性图像, 如形变图、杨氏模量图和黏附力图等(图8(a)I), 因此这种模式又被称为多参数AFM成像^[38-40]。需要指出的是, 本文使用的商用AFM利用Derjaguin-Muller-Toporove (DMT)模型^[41,42]从力曲线上计算样本的杨氏模量。DMT模型考虑了探针与样本之间的黏附力^[43], 因此DMT模型采用回退曲线(图8(a)I)计算样本杨氏模量, 与Hertz-Sneddon模型计算样本杨氏模量时采用逼近曲线进行计算^[44,45]有所不同。图8(a)II显示了一条在溶液环境下茅膏菜黏液表面获取的典型力曲线。基于AFM多参数成像技术, 我们获取了溶液状态下茅膏菜黏液纳米纤维和纳米颗粒的形貌及多种机械特性图(图8(b)和(c))。图8(b)和(c)分别对应大范围(图8(b))成像结果和小范围(图8(c))成像结果, 我们可以看到形貌图(I)清晰地显示了纳米纤维(红色箭头指示)和纳米颗粒(红色星号指示)结构。在机械特性图(II~IV)中, 图像颜色明暗亮度直接反映了机械特性(形变、杨氏模量、黏附力)指标的大小。我们可以看到与纳米颗粒相比, 纳米纤维更易发生形变(II), 杨氏模量更小(III), 且黏附力更大(IV)。图3~7的溶液中AFM成像结果显示了纳米纤维由纳米颗粒构成的。从图8的多参数成像结果可以看到在纳米颗粒组装形成纳米纤维的过程中, 会发生显著的机械特性变化, 为茅膏菜黏液纳米结构重构过程中的动态特性变化提供了新的认识。

此外, 现有的AFM多参数成像研究主要集中在细胞^[46]、病毒^[47]和蛋白质^[48]等生物样本。本文的研究结果(图8)证明了AFM多参数成像方法应用于溶液环境下生物黏液纳米结构机械特性研究的能力, 拓展了AFM多参数成像技术的应用领域, 对于直接研究生物材料的结构和特性之间的关联具有重要的基础意义。

4 结论

生物黏液功能界面在生命活动过程中起着重要的调控作用并在生物医药领域得到了广泛的应用, 然而受观测手段的限制, 现有关于生物黏液的研究集中在空气中进行, 导致目前对于溶液状态下生物黏液精细结构及其机械特性的认知还很缺乏。本文利用AFM直接在溶液环境下对两种茅膏菜黏液进行了成像表征分析, 结果显示在空气中茅膏菜黏液呈现明显的纳米纤维结构, 而在溶液中则呈现明显的纳米颗粒结构, 揭示了茅膏菜黏液在不同环境下(溶液、空气)微观结构的显著差异。进一步利用AFM多参数成像方法在溶液环境下揭示了茅膏菜黏液纳米颗粒组装形成纳米纤维过程中机械特性的动态变化, 加深了人们对植物分泌的生物黏液的认识。研究结果展示了AFM应用于液相环境下生物黏液超微结构及其机械特性原位研究的巨大潜力, 对于生物材料研究具有积极意义。

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Ultra-microstructures and mechanics of plant mucilage functional interfaces investigated via atomic force microscopy in liquid

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Mucus plays an important role in regulating biological activities. Accordingly, investigating the structures and properties of mucus can be beneficial for providing a better understanding of physiological and pathological changes. Due to a lack of adequate observation tools, the ultra-microstructures of native mucus functional interfaces are not yet fully understood. Atomic force microscopy (AFM) provides a novel and powerful tool for investigating the structures and properties of biomaterials in their native states. Nevertheless, to date, studies of biomaterials using AFM are commonly performed in air, which cannot faithfully reflect the structures of biomaterials in liquids. In this paper, by adopting the mucilage secreted by the carnivorous sundew plant as an example, high-resolution AFM imaging and analysis of mucous nanostructures in aqueous conditions were achieved. Sundew mucilage was coated onto the surface of a glass slide or mica, and the subsequent AFM imaging of the mucilage-coated substrates in pure water revealed that a large number of nanoparticles were contained in the sundew mucilage. In contrast, AFM imaging of sundew mucilage-coated substrates in air showed that dried mucilage contained a large number of nanofibers. This underscored the structural differences in sundew mucilage in the air and in a solution. Furthermore, AFM multiparametric imaging was employed to visually characterize the mechanical properties of sundew mucilage nanostructures in pure water and showed significant differences in the mechanics between nanoparticles and nanofibers. This study provides a novel approach for investigating the ultra-microstructures and mechanics of mucus' functional interfaces in their native states *in situ*, which has fundamental impacts on research focused on biomaterials.

atomic force microscopy, mucus, functional interface, nanoparticle, nanofiber, mechanics

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