基于AFM的食虫植物天然水凝胶黏液纳米结构原位成像与分析

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摘要 天然高分子水凝胶以其良好的生物相容性以及生物可降解性在生物医学领域得到了广泛应用, 然而由于缺乏合适的观测手段, 目前对于纳米尺度下天然状态水凝胶的精细结构及其特性仍然不完全清楚. 原子力显微镜(atomic force microscopy, AFM)的出现为生物材料研究提供了新的强大技术手段, 但目前利用AFM对天然高分子水凝胶纳米结构进行原位成像的研究还较为缺乏. 本文利用AFM对4种不同类型食虫植物(茅膏菜、捕虫堇、瓶子草、捕蝇草)分泌的天然水凝胶黏液的结构进行了高分辨率原位成像与分析. 分别将茅膏菜黏液和捕虫堇黏液平铺至云母表面, 在空气中进行的AFM成像结果清晰地显示, 茅膏菜黏液和捕虫堇黏液中均含有大量纳米纤维结构, 且纳米纤维自组装行为具有多样性. 分别将瓶子草黏液和捕蝇草黏液平铺至载玻片表面, 在去离子水溶液中进行AFM成像结果揭示了瓶子草黏液和捕蝇草黏液中均含有大量纳米颗粒, 对纳米颗粒进行的统计分析显示, 捕蝇草黏液中的纳米颗粒尺寸显著大于瓶子草黏液中纳米颗粒的尺寸. 研究结果加深了人们对食虫植物分泌的天然水凝胶黏液的认识, 为天然生物材料精细纳米结构研究提供了新的方法和视角.

关键词 原子力显微镜, 食虫植物, 天然水凝胶, 纳米纤维, 纳米颗粒

天然高分子水凝胶对于细胞行为及功能研究具有重要意义. 水凝胶是一类由亲水多聚物链交联形成的具有三维网络结构的聚合物材料^[1]. 水凝胶按照多聚物来源可分为天然水凝胶、合成水凝胶以及天然-合成混合水凝胶^[2]. 天然水凝胶主要包括胶原蛋白、

纤维蛋白、海藻酸盐等, 合成水凝胶主要包括聚丙烯酰胺、聚乙二醇等, 混合水凝胶主要包括透明质酸和多肽^[3]. 近年来, 合成水凝胶以其更好的吸水能力、更长的使用寿命、结构特性可控性好以及化学原料多样化等优点在生物医学领域得到了越来越多的应

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用^[4,5], 如细胞-细胞外基质相互作用^[6]、结肠伤口修复^[7]、软骨生物打印^[8]、干细胞免疫调节^[9]等。尽管如此, 天然水凝胶仍然有其重要的意义。首先天然水凝胶具有良好的生物相容性和生物可降解性。许多天然水凝胶成分本身来源于细胞外基质, 可以更好地调节细胞行为与功能(如分裂、黏附、分化、迁移)^[10]。其次天然水凝胶自身拥有大量的天然功能基团, 这些基团一方面使得天然水凝胶可以黏附细胞, 另一方面提供了更多可选的化学耦合方式, 而合成水凝胶则往往需要额外进行化学处理与修饰以使其能黏附细胞^[11]。事实上, 天然水凝胶在药物递送^[12]、湿表面黏附^[13]、细胞增殖^[14]、生物墨水^[15]、组织工程支架^[16]、细胞封装再生医学^[17]等细胞相关研究中展现出了独特的性能, 对于理解细胞生理活动行为内在机理具有重要作用。

原子力显微镜(atomic force microscopy, AFM)的出现为研究天然状态下的水凝胶超微结构提供了新的技术手段。水凝胶的微观结构及特性与水凝胶的功能及应用有着密切的联系^[18], 如水凝胶网格结构与水凝胶所包裹药物的扩散及释放有关^[12], 水凝胶硬度影响干细胞分化^[19,20], 水凝胶局部蛋白沉积以及结构重塑对于干细胞命运决定具有重要调控作用^[21], 水凝胶黏弹性影响细胞表面蛋白表达^[22]等。目前对水凝胶微观结构进行成像表征的方法主要是扫描电子显微镜(scanning electron microscopy, SEM)^[23,24]、透射电子显微镜(transmission electron microscopy, TEM)^[25,26]和荧光染色^[27,28]。电子显微镜技术要求样本冷冻干燥, 难以对天然状态下的水凝胶进行观察, 而荧光技术则受限于较低空间分辨率, 难以对水凝胶的精细结构进行成像, 这些问题导致目前对于天然状态下水凝胶的超微结构及其在水凝胶形成过程中调控作用的认知还很不足。AFM可以直接对未经处理的水凝胶样本以纳米级的空间分辨率进行成像, 能够揭示纳米尺度下天然状态水凝胶的精细结构^[29]并对水凝胶纳米结构的力学特性进行测量^[30], 为水凝胶研究提供了新的方法, 是对传统水凝胶表征方法的有力补充, 对于认识水凝胶的微观分子机理具有重要的基础意义。

本文基于AFM高分辨原位成像技术对4种不同类型食虫植物分泌的天然水凝胶黏液的纳米结构进行了研究。食虫植物在其叶子表面分泌的黏液在食虫植物的捕食机制中起重要作用^[31]。尽管人们对食虫植物分

泌的水凝胶黏液进行了大量研究, 如测量黏液黏弹性^[32], 对黏液进行微流变特性分析^[33], 测量黏液黏附特性^[34]等, 但这些研究^[32-34]均集中在宏观尺度, 且由于缺乏合适的观测手段, 目前直接对食虫植物分泌的天然状态水凝胶黏液纳米结构及其特性进行的研究还鲜有报道。本文利用AFM对天然状态下食虫植物分泌的水凝胶黏液进行了原位成像, 结果显著揭示了食虫植物分泌的水凝胶黏液中的精细纳米结构, 为人们理解食虫植物分泌的天然水凝胶提供了新的认识, 对于天然水凝胶研究具有重要的意义。

1 材料与方法

1.1 植物培养

本文选取的4种不同类型食虫植物分别为茅膏菜(*Drosera capensis*)、捕虫堇(*Pinguicula cyclosecta*)、瓶子草(*Sarracenia rubra*)和捕蝇草(*Dionaea muscipula*)。4种盆栽食虫植物均购自山东贵翔光电有限公司(潍坊)。根据供应商提供的食虫植物培养手册对购买的食虫植物进行培养。将购买的盆栽食虫植物利用去离子水进行培养。茅膏菜、瓶子草、捕蝇草为喜光植物, 置于阳台接收太阳光照培养, 而捕虫堇则置于室内阴凉有太阳散射光环境培养。环境温度为20~25℃, 环境湿度为50%~70%。

1.2 样本制作

当培养的食虫植物叶子表面分泌水凝胶黏液后, 采集水凝胶黏液样本进行实验。从食虫植物叶子的光学图像(图1)可以清晰地观察到食虫植物分泌的水凝胶黏液。具体来说, 茅膏菜(图1A)和捕虫堇(图1B)的叶子表面分泌水凝胶黏液(图1A和B II中箭头指示的透亮露珠), 瓶子草在瓶口边缘及内壁分泌黏液(图1C II中箭头指示), 捕蝇草则在夹子内表面分泌大量黏液(图1D II中箭头指示)。为了制备食虫植物分泌的水凝胶黏液样本, 将干净的云母/载玻片轻轻接触食虫植物分泌的水凝胶黏液并涂片, 在载玻片表面不同区域形成水凝胶黏液薄层。随后将覆盖有水凝胶黏液薄层的载玻片样本置于AFM样品台(图2)进行AFM成像实验以观察天然状态下的水凝胶黏液微观结构。对于每种食虫植物, 制备多个黏液涂片载玻片样本以进行重复实验。

图 1 4种食虫植物分泌的水凝胶黏液。A: 茅膏菜; B: 捕虫堇; C: 瓶子草; D: 捕蝇草。(I)盆栽食虫植物整体图像。(II)食虫植物单个叶子(I中方框指示区域)及叶子表面分泌的水凝胶黏液(箭头指示)(网络版彩图)

Figure 1 Adhesive mucilage secreted by four types of carnivorous plants. A: *Drosera capensis*; B: *Pinguicula cyclosecta*; C: *Sarracenia rubra*; D: *Dionaea muscipula*. (I) Overall images of the potted carnivorous plants. (II) Images of the individual leaves (denoted by the squares in I) of carnivorous plants and mucilage (typically denoted by arrows) on leaves (color online)

1.3 AFM成像

利用美国Bruker公司的Dimension Icon AFM(图2)分别在空气中和去离子水溶液(Milli-Q, 德国)中对制备的天然状态水凝胶黏液样本进行成像以观察水凝胶黏液中的纳米结构。在AFM自带的光学显微镜引导下(图2A), 控制AFM探针对样品表面不同区域进行扫描成像。空气中成像时使用的探针型号为Bruker公司的ScanAsyst-Air(图2B), 探针材料为氮化硅, 悬臂梁(悬臂梁背面有铝反射涂层)弹性系数为0.4 N/m, 悬臂梁长度和宽度分别为115 μm 和25 μm , 针尖曲率半径为2 nm, 针尖高度为2.5~8 μm ; 溶液中成像时使用的探针型号为Bruker公司的ScanAsyst-Fluid(图2C), 悬臂梁(悬臂梁背面有金反射涂层)弹性系数为0.7 N/m, 悬臂梁长度和宽度分别为70 μm 和10 μm , 针尖曲率半径为20 nm, 针尖高度为2.5~8 μm 。空气中成像时直接将载玻片样本置于AFM样品台进行AFM扫描成像。溶液中成像时将载玻片样本置于含去离子水的培养皿中, 随后将培养皿置于AFM样品台进行AFM扫描成像。

为了观测天然状态水凝胶中的纳米结构, 采用的AFM成像模式为峰值力轻敲(PeakForce tapping, PFT)模式^[35]。PFT成像原理如图2D和E所示。为了得到水凝

胶黏液精细结构, 每幅图像的扫描线设置为512条, 每条扫描线的采样点数为512。空气和溶液中进行PFT成像时, 探针驱动频率均为2 kHz。首先控制探针在基底表面获取力曲线校正探针悬臂梁的偏转灵敏度, 紧接着利用AFM的热噪声模块对探针的弹性系数进行精确校正, 随后即可对水凝胶结构进行扫描成像。与传统AFM接触模式成像和轻敲模式成像相比, PFT模式成像具有独特的优势^[36]。接触模式的缺点是扫描过程中施加的侧向力容易导致样本结构的改变进而影响成像分辨率, 特别是对于与基底贴附不牢固的样本而言, 接触模式成像容易造成样本位置的改变从而导致难以成像。轻敲模式成像消除了侧向力的影响, 但轻敲模式下的探针驱动频率接近悬臂梁的共振频率, 而PFT模式下探针驱动频率显著小于悬臂梁共振频率, 因此与传统轻敲模式相比, PFT模式下探针与样本之间的相互作用力更小^[37], 这使得PFT更适合探测脆弱的生物样本。因此, 本研究采用PFT成像模式原位观察天然状态水凝胶黏液的精细结构。

2 结果与讨论

空气中AFM成像结果清晰地揭示了天然状态下

图 2 AFM高分辨原位成像实验平台. A: Dimension Icon AFM实物图; B和C: 光学显微镜引导下控制AFM探针对样品进行探测. B: 空气中成像探针; C: 溶液中成像探针; D和E: PFT成像原理. PFT成像模式下, 振动探针在 xy 水平面对样本进行扫描时, 探针在垂直(z)方向对每个采样点进行逼近(approach)-回退(retract)探测(D). (D)中每个小方格对应样本表面一个采样点. 记录逼近-回退过程中悬臂梁偏转量以及压电陶瓷驱动器垂直方向的位置变化即可得到力曲线(E). 探针逼近过程中悬臂梁最大偏转量对应的力即为峰值力(绿色箭头指示). 通过控制压电陶瓷驱动器在垂直方向进行运动以维持峰值力不变即可得到样本表面形貌图像(网络版彩图)

Figure 2 *In situ* high-resolution AFM imaging platform. A: Actual photograph of the Dimension Icon AFM used here; B and C: AFM probe is moved to specimens under the guidance of optical microscopy. B: AFM probe for imaging in air; C: AFM probe for imaging in water; D and E: principle of PFT imaging mode. In the PFT mode, the vibrating probe raster scans the specimen in the horizontal plane (xy), and the probe performs approach-retract cycles in the vertical direction (z) at each sampling points on specimens. Each small square in (D) corresponds to a sampling point on the specimen. During the approach-retract process, the deflections of the cantilever and the vertical displacements of the piezoelectric driver are recorded, yielding the force curves (E). The peak force is the force exerted by the cantilever with the maximum deflection during the approach process (denoted by the green arrow). The piezoelectric driver moves vertically to maintain the peak force constant, which yields the topography image of the specimen (color online)

茅膏菜/捕虫堇水凝胶黏液的纳米结构. 将茅膏菜/捕虫堇水凝胶黏液平铺至新剥开的云母表面后, 可以直接对黏液进行AFM成像. 对茅膏菜水凝胶黏液进行的AFM成像结果显著显示, 茅膏菜黏液中含有纳米纤维和纳米颗粒(图3A~D). 不仅可以观察到吸附于纳米纤维表面的纳米颗粒(图3B, 蓝色箭头指示), 还可以观察到以分散形式单独存在的纳米颗粒(图3D, 蓝色箭头指示). 对纳米纤维进行的成像结果显示了纳米纤维具有螺旋状结构特征(图3C, 黄色箭头指示). 从纳米纤维的纵向截面曲线上可以观察到大量明显的突起峰(图3E和F, 黑色箭头指示), 表明了纳米纤维结构在纵向的异质性. 图3G为两个纳米颗粒的截面曲线, 图中显示这两个纳米颗粒高度约为4 nm. 与茅膏菜水凝胶黏液相似, 捕虫堇水凝胶黏液中也含有大量纳米纤维结构(图4). 从AFM图像上可以看到捕虫堇水凝胶黏液中的纳米纤维结构呈现出不同的行为特征, 包括纳米纤维交

织形成网络结构(图4A和C), 纳米纤维平行聚集成束(图4B, 蓝色箭头指示), 以及离散分布的较短纳米纤维(图4D). 此外, 在纳米纤维束表面可以观察到较短的纳米纤维(图4B, 黄色箭头指示). 纳米纤维的纵向截面曲线上同样存在很多突起峰(图4E和G, 黑色箭头指示), 表明纳米纤维结构的异质性. 单根纳米纤维的横向截面曲线(图4F)显示纳米纤维高度范围为5~15 nm. 图3和图4的AFM成像结果显示, 茅膏菜/捕虫堇叶子表面分泌的水凝胶黏液中含有大量纳米纤维结构. 这些纳米纤维结构可能与茅膏菜/捕虫堇的捕食功能有关. 茅膏菜和捕虫堇具有相似的捕食机制, 均为通过叶子表面分泌的黏性水凝胶黏液粘住昆虫使其难以逃脱, 进而分泌消化酶将昆虫消化以吸取养分^[38-40]. 此外, 研究发现, 纳米纤维结构的引入可以显著提升水凝胶的黏性^[41-43]. 因此可推测本文观察到的纳米纤维结构(图3和图4)对于维持茅膏菜/捕虫堇水凝胶黏液的黏性以

图3 茅膏菜黏液的AFM成像与分析。A~D: 在空气中获取的云母表面茅膏菜黏液AFM高度图, 显示茅膏菜黏液中含有纳米纤维和纳米颗粒。A: 大范围成像; B: 小范围成像, 蓝色箭头指示吸附在纳米纤维上的纳米颗粒; C: 螺旋状纳米纤维结构(黄色箭头指示); D: 分散存在的纳米颗粒(蓝色箭头指示); E~G: 从AFM图像中获取的纳米结构(纳米纤维, 纳米颗粒)截面曲线, E~G分别对应B~D中的#1, #2和#3

Figure 3 AFM imaging and analysis of mucilage secreted by the leaves of *Drosera capensis*. A~D: AFM height images of the mucilage-coated mica recorded in air showing the nanofibers and nanoparticles. A: Large-size scan; B: small-size scan, the blue arrows denote the nanoparticles attached on nanofibers; C: nanofibers with helical structures (denoted by yellow arrows); D: disperse nanoparticles (denoted by blue arrows); E~G: section profile curves of nanostructures (nanofiber, nanoparticle) taken along the AFM images, E~G corresponds to the lines labeled with #1, #2, and #3 in B~D, respectively

捕获昆虫具有重要意义。需要指出的是, 有关植物分泌黏液中含有纳米纤维结构的研究已有大量报道^[44]。许多植物的种子在发生水合作用时会在种子表面形成黏液^[45], 植物种子黏液作为一种新兴亲水胶体近年来在食品工业研究中得到了大量关注^[46]。植物种子黏液主要由纤维素、半纤维素和果胶等多糖构成^[45]。对植物种子黏液进行的电子显微镜成像显示其包含大量纳米纤维结构, 如水芹(*Lepidium sativum*)种子黏液^[47]、罗勒(*Ocimum basilicum*)种子黏液^[48]、鼠尾草(*Salvia hispanica*)种子黏液^[49]、车前草(*Plantago major*)种子黏液^[50]等。本文结果(图3和图4)清晰地表明, 茅膏菜/捕虫堇叶子表面分泌的黏液同样含有大量纳米纤维结构, 为植物分泌的水凝胶黏液以及天然纳米材料研究提供了新的思路。

水溶液环境下的AFM成像结果揭示了瓶子草/捕蝇草水凝胶黏液中含有大量纳米颗粒。在实验中发现, 瓶子草/捕蝇草黏液涂片标本难以干燥, 导致在空气中难以对瓶子草/捕蝇草黏液进行高质量AFM成像。将瓶

子草/捕蝇草黏液涂片标本置于Milli-Q超纯水溶液中后, 可以在溶液环境下对黏液进行AFM成像。溶液环境下的成像结果清晰地显示出瓶子草黏液中含有大量纳米颗粒结构(图5)。大范围成像结果可以明显观察到聚集分布的纳米颗粒簇(图5A, 黄色箭头指示), 而小范围成像则可以清晰地观察到纳米颗粒结构(图5B)。此外, 还可以观察到分散分布于基底表面的纳米颗粒(图5C)。从截面曲线上可以测量纳米颗粒的高度(图5D和E)。统计结果显示, 瓶子草黏液纳米颗粒高度约为 (11.15 ± 4.9) nm。与瓶子草类似, AFM溶液中成像结果显示, 捕蝇草黏液中也含有大量纳米颗粒(图6)。不仅可以观察到聚集的纳米颗粒簇(图6A和B), 还可以观察到分散的单个纳米颗粒(图6C)。统计结果显示, 捕蝇草黏液纳米颗粒高度约为 (16.66 ± 6.5) nm。本实验表明, 捕蝇草黏液中的纳米颗粒尺寸显著大于瓶子草黏液中纳米颗粒的尺寸, 反映了瓶子草纳米颗粒与捕蝇草纳米颗粒之间的差异性。比较图3~图6, 可以看到茅膏菜/捕虫堇黏液中含有大量纳米纤维结构(图3和图4), 而瓶子

图 4 捕虫堇黏液的AFM成像与分析. A~D: 在空气中获取的云母表面捕虫堇黏液AFM高度图. 可以观察到捕虫堇黏液中含有大量纳米纤维且纳米纤维呈现不同的行为. A: 纳米纤维构成网络结构; B: 纳米纤维聚集束, 蓝色箭头指示纳米纤维束, 黄色箭头指示纤维束上分布的较短纳米纤维; C: 单根纳米纤维; D: 离散分布的较短纳米纤维; E~G: 截面曲线, E~G分别对应C和D中的#1, #2和#3

Figure 4 AFM imaging and analysis of mucilage secreted by the leaves of *Pinguicula cyclosecta*. A~D: AFM height images of the mucilage-coated mica recorded in air showing the nanofibers with different behaviors. A: Network structures constructed by nanofibers; B: nanofibers aggregate together to form bundled nanofibers, the blue arrows denote the bundled nanofibers and the yellow arrows denote the nanofibers with short length on the bundled nanofibers; C: individual nanofibers; D: discrete nanofibers with short length; E~G: section profile curves, E~G corresponds to the lines labeled with #1, #2, and #3 in C and D, respectively

草和捕蝇草黏液中则含有大量纳米颗粒(图5和图6), 这可能与食虫植物黏液的不同功能有关. 瓶子草和捕蝇草利用其分泌的香甜蜜汁黏液吸引昆虫, 昆虫在进食蜜汁黏液移动时掉进瓶子草的陷阱底部或被捕蝇草叶子夹住^[51-53]. 瓶子草和捕蝇草分泌的黏液主要用于给昆虫提供花蜜以吸引昆虫, 而茅膏菜/捕虫堇分泌的黏液主要用于捕获昆虫^[38-40], 因此食虫植物黏液中不同纳米结构(纳米纤维、纳米颗粒)构成反映了黏液在食虫植物捕食机制中所起作用的多样性. 植物分泌的蜜汁是一种糖-水溶液, 其中的糖类分子主要由蔗糖、果糖、葡萄糖等组成^[54]. 研究人员利用SEM和TEM对不同植物类型的蜜腺表面结构以及蜜汁黏液进行了大量成像观察, 如疣果匙荠(*Bunias orientalis*)^[55]、麦卢卡(*Leptospermum scoparium*)^[56]、花格贝母(*Fritillaria meleagris*)^[57]等. 但需要指出的是, 这些成像结果^[55-57]的分辨率较低(微米级), 难以观察蜜汁黏液中的精细

结构. 本文的结果(图5和图6)清晰地揭示了瓶子草/捕蝇草分泌的蜜汁黏液中含有的纳米颗粒结构, 加深了人们对植物分泌的天然蜜汁黏液的认识.

本文实验结果证明了AFM对天然状态下未经处理的水凝胶纳米结构进行原位高分辨率成像的能力, 为人们理解植物分泌的天然水凝胶黏液提供了新的认识. 大自然亿万年进化形成的天然生物材料及其精巧结构为仿生材料研究与设计提供了丰富的资源宝库, 阐明天然生物材料的精细结构形成分子机理对于发展面向各类生物医学应用的下一代超分子生物材料具有重要的基础意义^[58-60]. 然而由于缺乏合适的观测手段, 目前对水凝胶生物材料结构形貌的成像主要依赖于需要对样本进行大量预处理(如干燥、镀金)的电子显微镜成像技术^[61,62], 这种成像方式得到的结果无法完全反映天然状态下的水凝胶真实结构. 本文的研究结果表明, AFM不仅可以在空气中直接对无需处理的天然

图 5 瓶子草黏液的AFM成像与分析. A~C: 在去离子水溶液中获取的载玻片表面瓶子草黏液AFM高度图, 显示黏液中含有纳米颗粒. A: 大范围成像; B: 小范围成像结果; C: 分散的单个纳米颗粒; D和E: 截面曲线, D和E分别对应B和C中的#1和#2; F: 瓶子草黏液纳米颗粒高度统计柱状图(网络版彩图)

Figure 5 AFM imaging and analysis of mucilage secreted by the leaves of *Sarracenia rubra*. A~C: AFM height images of the mucilage-coated glass slides recorded in deionized water showing the nanoparticles. A: Large-size scan; B: small-size scan; C: individual dispersed nanoparticles; D and E: section profile curves, D and E corresponds to the lines labeled with #1 and #2 in B and C, respectively; F: statistical histogram of the height of the nanoparticles (color online)

图 6 捕蝇草黏液的AFM成像与分析. A~C: 在去离子水溶液中获取的载玻片表面捕蝇草黏液AFM高度图. A: 大范围成像; B: 小范围成像; C: 分散的单个纳米颗粒; D和E: 截面曲线, D和E分别对应A和C中的#1和#2; F: 捕蝇草黏液纳米颗粒高度统计柱状图(网络版彩图)

Figure 6 AFM imaging and analysis of mucilage secreted by the leaves of *Dionaea muscipula*. A~C: AFM height images of the mucilage-coated glass slides recorded in deionized water. A: Large-size scan; B: small-size scan; C: individual dispersed nanoparticles; D and E: section profile curves, D and E corresponds to the lines labeled with #1 and #2 in A and C, respectively; F: statistical histogram of the height of the nanoparticles (color online)

状态水凝胶纳米结构进行成像(图3和图4), 还可以在溶液环境下对水凝胶纳米结构进行高质量成像(图5和图6). 本文以食虫植物分泌的水凝胶黏液为实验对象

揭示了食虫植物水凝胶中的多种纳米结构(纳米纤维、纳米颗粒), 为研究水凝胶材料的真实精细结构及其行为特性提供了新的方法和思路. 本文建立的方法

(植物培养、样本制作、AFM成像)可直接用于其他类似天然生物材料研究, 对于发展新型仿生或生物启发生物材料具有积极的意义。

3 结论

天然水凝胶以其独有的特点在生物医学领域得到了广泛应用, 但由于观测手段的限制, 目前对于天然状态下水凝胶精细结构的认识还很缺乏。本文利用AFM

对4种不同类型食虫植物(茅膏菜、捕虫堇、瓶子草、捕蝇草)分泌的天然水凝胶黏液进行了成像, 分别在空气中和溶液中显著揭示了天然状态下的食虫植物水凝胶黏液中的纳米结构, 发现了不同类型食虫植物之间在水凝胶黏液纳米结构方面的差异, 并对水凝胶黏液纳米结构与水凝胶黏液实际功能进行了比较分析, 展示了AFM应用于水凝胶生物材料原位高分辨率成像观测的巨大潜力, 对于研究生物材料的纳米结构具有广泛的基础意义。

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***In situ* imaging and analysis of the nanostructures in natural hydrogels from carnivorous plants by atomic force microscopy**

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Natural hydrogels have been widely applied in the field of biomedicine for their biocompatible and biodegradable characteristics. However, due to the lack of adequate observation tools, so far the detailed organizations and structures of native natural hydrogels at the nanoscale are still not fully understood. The advent of atomic force microscopy (AFM) provides a novel powerful tool for characterizing biomaterials, but studies about utilizing AFM to investigate the nanostructures of native natural hydrogels are still scarce. In this paper, AFM *in situ* high-resolution imaging was used to reveal the nanoscopic fine structures of the natural hydrogels secreted by four types of carnivorous plants (*Drosera capensis*, *Pinguicula cyclosecta*, *Sarracenia rubra*, and *Dionaea muscipula*). Mucilage secreted by *Drosera capensis* or *Pinguicula cyclosecta* was coated on the surface of mica, and the subsequent AFM imaging of the mucilage-coated mica in air clearly revealed that both the mucilage secreted by *Drosera capensis* and *Pinguicula cyclosecta* contained a large number of nanofibers which had diverse self-assembly behaviors. Nectar secreted by *Sarracenia rubra* or *Dionaea muscipula* was coated on the surface of glass slides, and AFM images of the nectar-coated glass slide recorded in deionized water showed that both the nectar secreted by *Sarracenia rubra* and *Dionaea muscipula* considerably contained nanoparticles. Statistical results showed that the size of nanoparticles in the nectar of *Dionaea muscipula* was significantly larger than that of nanoparticles in the nectar of *Sarracenia rubra*. The study improves our understanding of the natural hydrogels secreted by carnivorous plants and provides a novel approach for characterizing the nanostructures of natural biomaterials in their native states, offering new possibilities for the studies of supramolecular biomaterials.

atomic force microscopy, carnivorous plant, natural hydrogel, nanofiber, nanoparticle

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