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结合微针及AFM的单细胞精准激励与力学特性同步测量*

冯雅琦[1,2,3] 于 鹏[1,2,3] 施佳林[1,2,3] 李 密[1,2,3]**
([1] 中国科学院沈阳自动化研究所，机器人学国家重点实验室，沈阳 110016；
[2] 中国科学院机器人与智能制造创新研究院，沈阳 110169；[3] 中国科学院大学，北京 100049）

摘要 目的 细胞力学特性与细胞生理病理变化过程及机体健康状态密切相关，研究细胞力学特性对于揭示生命活动内在机制具有重要科学意义。原子力显微镜（AFM）的出现为单细胞研究提供了新的技术手段，它不仅可以在溶液环境下对单个活细胞的形貌结构进行高分辨率成像，还能够对细胞力学特性进行定量测量。基于AFM的单细胞力学特性研究在过去数十年中取得了巨大的成功，为细胞生理病理行为带来了大量新的认识，已成为生命科学领域的重要研究方法。然而，由于AFM探针自身难以进行药物递送，目前在超微量药物刺激下的AFM细胞力学特性实时探测方面仍然面临巨大挑战。本文通过将微针与AFM结合，发展了可对单细胞进行精准药物激励及力学特性同步测量的方法。**方法** 基于三维操纵仪、微量注射泵、医用注射器、聚四氟管和玻璃微针在荧光倒置显微镜上搭建了基于微针的单细胞显微注射系统，并利用拉针仪对毛细玻璃管拉制得到玻璃微针。选取NIH 3T3（小鼠胚胎成纤维细胞）、HEK 293（人胚胎肾细胞）和MCF-7（人乳腺癌细胞）3种细胞进行实验。在光学显微镜导引下利用微针将染色剂/药物分子注射到单个细胞，随后控制AFM探针移动到被注射的细胞表面获取力曲线。利用Hertz-Sneddon模型对力曲线进行分析得到细胞杨氏模量。**结果** 首先分析了微针针尖孔径尺寸对细胞注射的影响，针尖尺寸较大（针尖外径大于1 μm）时容易对细胞造成明显机械损伤。随后在光学显微镜导引下利用微针将蓝色墨水/PI染液注射到单个细胞并对目标细胞进行连续光学成像，结果显示墨水/PI染液被成功注射至目标细胞。最后将微针和AFM结合对超微量化疗药物（阿糖胞苷）刺激下单个细胞杨氏模量变化进行了测量，结果显示化疗药物刺激后会导致细胞力学特性改变。**结论** 结合微针和AFM可对单个细胞施加精准化学刺激并对化学刺激后的细胞力学特性进行同步测量，为超微量药物作用下的单细胞力学特性分析提供了新的思路。

关键词 原子力显微镜，微针注射，细胞，力学特性，杨氏模量
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细胞力学特性及其动态变化在细胞生理病理活动过程中起着重要的调控和指示作用。近年来人们越来越深刻地认识到细胞不仅是生化系统（如基因变化、蛋白质结构、信号转导等），同时也是机械系统（如生成并传递机械力以驱动细胞运动和变形等）[1]。机械力几乎在所有尺度（如分子、细胞、组织、器官）的生命活动过程中均起着重要作用[2]，如SNARE蛋白产生的熵力驱动囊泡融合[3]，细胞在进行有丝分裂时会产生向外的推力以便在三维微环境中进行增殖[4]，而在组织生长过程中张力驱动成纤维细胞与肌成纤维细胞之间的可逆转变[5]。对于细胞来说，生物体内的细胞存在于由细胞外基质、邻近细胞等构成的三维微环境中[6]。一方面，微环境的力学特性（如纳米形貌[7]、刚度[8]、黏弹特性[9]等）对于细胞生理活动及行为具有重要影响，如研究表明在不同刚度基底上生长的干细胞分化为不同类型的细胞[10]等。此外，微环境力学特性还可对细胞黏附、细胞迁

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** 通讯联系人。
Tel：024-23970203，E-mail：limi@sia.cn
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移、细胞铺展、细胞增殖等进行调控[11]。另一方面，细胞在其生理病理活动过程中也会改变微环境的力学特性进而调控自身生理活动[12]，如在癌症、纤维化、心血管等疾病过程中通常伴随着细胞外基质的硬化[13]。细胞-微环境之间的相互作用不仅影响细胞生化特性，还通常伴随着细胞力学特性的相应变化。在癌症发生发展过程中，癌细胞的刚度明显小于正常细胞的刚度[14]，且癌细胞比正常细胞更容易变形[15]，而在疟原虫感染红细胞后红细胞的变形能力显著减弱[16]，显示细胞力学特性已成为指示疾病发生发展的重要指标[17-18]。因此，开展细胞力学特性研究对于揭示生命活动奥秘及相关疾病的诊疗具有重要的科学意义。

原子力显微镜（atomic force microscope，AFM）[19]的发明为单细胞力学特性研究提供了新的技术手段。AFM利用压电陶瓷驱动一根末端集成有极细针尖的微悬臂梁对样本表面进行光栅扫描，同时通过一束照射到悬臂梁背面的激光检测悬臂梁信号（如偏转量、振幅、频率等）变化来感知扫描过程中针尖表面原子与样本表面原子之间的相互作用以获取反映样本表面形貌起伏的三维图像。AFM的独特优势是可以直接在溶液环境下对活体状态的无需预先处理（如固定、染色、标记等）的生物样本进行高精度（纳米级空间分辨率、毫秒级时间分辨率和皮牛级力灵敏度[20]）观测。AFM不仅可对样本表面形貌结构进行成像，还能在力谱模式下通过在垂直方向对样本表面进行压痕测试来分析样本力学特性[21-25]。过去的数十年中，研究人员利用AFM对细胞力学特性开展了广泛的研究，如细胞弹性特性测量[26]、基底效应消除[27]、细胞黏弹特性测量[28]、细胞流变特性测量[29]、癌细胞力学特性探测[30]、临床病例样本探测[31]、软基底表面细胞力学特性探测[32]、外泌体对细胞力学特性的影响[33]、细胞膜张力[34]等，极大地促进了力学生物学[35]的发展。然而需要指出的是，由于常规AFM探针自身难以进行药物递送，目前利用AFM对化学刺激（如化疗药物[36]、蛋白抑制剂[37]、细胞松弛素[38]等）下的细胞力学特性进行测量时通常需要预先将化学分子添加到细胞培养皿中以对培养皿中的全体细胞进行刺激，这种测量方式的不足之处是难以实现超微量药物精准刺激单个细胞情况下的细胞力学特性动态变化实时监测。虽然研究人员通过在AFM探针中构建中空管道，实现了基于AFM探针的药物递送[39]，但这种方法需要特殊制备的中空AFM探针，且会不可避免地影响AFM探针测量精度。

针对上述问题，本文通过将微针显微注射技术与AFM相结合，实现了单细胞精准激励及力学特性同步测量。通过搭建微针注射系统，实现了对单个细胞的荧光染液递送，并建立了超微量药物精准激励下的单细胞力学特性同步检测方法。在此基础上，揭示了化疗药物刺激前后单个细胞力学特性的实时变化。研究结果为细胞-药物之间相互作用的精准定量动态分析提供了新的方法和思路。

1　材料与方法

1.1　细胞培养与样本制作

本文选用NIH 3T3（小鼠胚胎成纤维细胞）、HEK 293（人胚胎肾细胞）和MCF-7（人乳腺癌细胞）开展实验，细胞购自中国科学院细胞库（上海）。细胞在含有10%胎牛血清（以色列Biological Industries公司）和1%青霉素-链霉素（美国Hyclone公司）的DMEM（高糖）培养基（美国Hyclon公司）中培养（37℃，5% CO_2）。NIH 3T3、HEK293和MCF-7为贴壁生长型细胞，可以直接贴附在基底表面并在基底表面延展生长，因此将细胞接种于培养皿中进行培养。当细胞铺满80%培养皿基底时将细胞传代到新的培养皿，随后将培养皿置于细胞培养箱中，培养2~3 d后直接将培养皿置于结合微针和AFM的单细胞分析实验平台（图1a）进行实验（微针注射、AFM探测、荧光观察等）。

1.2　原子力显微镜（AFM）

本文采用的AFM型号为Bioscope Catalyst AFM（美国Bruker公司）（图1b）。AFM样品台安装在荧光倒置显微镜（Ti，日本Nikon公司）上，使得可以在光学/荧光显微镜导引下移动AFM探针到目标细胞进行探测（图1g）。将含有细胞的培养皿置于AFM样品台后，即可控制AFM探头浸入培养皿液面并对目标细胞的力学特性进行探测。本文细胞力学特性测量实验使用的AFM探针型号为MLCT（美国Bruker公司），探针具体参数如下：探针的材料为氮化硅，悬臂梁弹性系数为0.01 N/m，悬臂梁长度为310 μm，悬臂梁宽度为20 μm，针尖曲率半径为20 nm，针尖高度为2.5~8 μm。

1.3　细胞显微注射系统与微针制作

细胞显微注射系统由三维操纵仪（MP-225，

美国Sutter仪器公司）、微量注射泵（2000，美国Harvard仪器公司）、医用注射器（1 ml容量）、聚四氟管（PTFE tube）、玻璃微针等组成（图1c）。利用P-2000型拉针仪（美国Sutter仪器公司）对毛细玻璃针管进行拉制得到微针（图1e，f）。具体来说，设置拉针仪的拉制参数（温度、速度、拉力等），拉针仪将自动按照设置的参数得到所需尺寸的微针。需要指出的是，刚拉制出的微针尖端为封闭状态（图2a），因此需要将针尖折断以得到用于细胞注射的微针。在光学显微镜的导引下，控制微针针尖与坚硬基底接触摩擦，即可实现针尖部位的折断。本文制作的微针（折断后）管口直径（外径）范围为0.7~1.2 μm。需要指出的是，本文采用针尖摩擦折断方法制作的针尖参数（形状、尺寸）难以控制。为了得到针尖形状和尺寸可控的微针，可以采用商用的锻针仪（microforge）对微针进行熔融折断[40]。将制作的微针固定在三维操纵仪上，并通过聚四氟管将微针与安装在微量注射泵的医用注射器相连。利用与AFM集成的荧光倒置显微镜（图1b）对微针注射操作进行视觉导引。将用于实验注射的试剂溶液装载于玻璃针内后，在光学显微镜成像导引下利用三维操纵仪可控制玻璃针沿水平和/或垂直方向移动，从而将针尖移动至目标细胞上（图1d，h），随后通过微量注射泵推动注射器施加正压以实现单细胞注射。

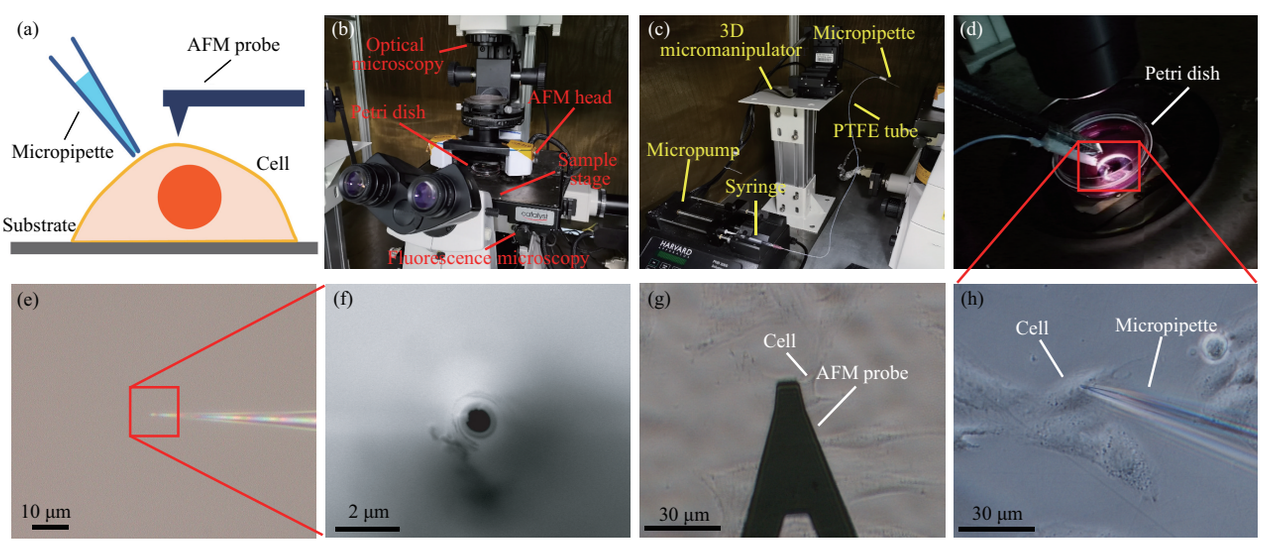

Fig. 1　Single-cell analysis experimental platform based on micropipette and AFM

(a) Schematic of combining micropipette and AFM for single-cell drug delivery and simultaneous cell mechanics measurement. (b) Actual photograph of the AFM system. (c) Actual photograph of the micropipette system. (d) Single-cell injection by micropipette under the guidance of optical microscopy. (e, f) Optical bright field image (e) and scanning electron microscope (SEM) image (f) of a micropipette. (g) Moving AFM probe to the targeted cell under the guidance of optical microscopy. (h) Optical image showing single-cell injection by micropipette.

1.4　微针细胞注射

本文首先选用蓝色墨水（上海晨光文具公司）和碘化丙啶（propidium iodide，PI）（上海翊圣生物科技有限公司）荧光染液作为试剂进行细胞注射实验，以验证微针内溶液是否成功注入细胞。在此基础上，通过微针将临床使用的化疗药物阿糖胞苷溶液（中国人民解放军总医院第五医学中心提供）注射至单个细胞开展精准激励下的细胞力学特性同步检测研究。具体来说，在将微针安装在三维操纵仪前，首先用注射器将待注射溶液装载于微针内部。需要指出的是，由于微针针尖尺寸较小，气泡的存在将严重影响针管内试剂的流出，因此装载溶液后的微针需针尖朝下悬空放置，并轻弹管壁，帮助针尖处的气泡上浮至微针尾部的液面。将排净气泡后的微针通过聚四氟管与注射器连接，并将微针固定在三维操纵仪上。注射器和聚四氟管内预先装载有PBS溶液，因此整个注射系统形成密封的纯液体环境。利用注射泵推动注射器推杆形成一定的正压，以控制微针内溶液的流出。控制三维操纵仪移动微针，同时调节显微镜聚焦平面，使微针针尖与显微镜聚焦平面重合，从而在光学显微镜成像视野中观察到微针。控制微针与显微镜聚焦平面向基

底移动，直至在视野中同时观察到微针与细胞。随后控制针尖移动至目标细胞附近，并以45°方向刺入细胞。当针尖进入细胞后，针管内溶液将在正压作用下从针尖流向细胞内部，从而将整个细胞染色或进行药物刺激。

1.5　AFM细胞力学特性测量

利用AFM对微针注射前后同一细胞进行力学特性测量的过程如下。在进行微针注射前，将细胞培养皿置于AFM样品台，在光学显微镜的导引下，控制AFM探针在基底空白区域获取力曲线以校正悬臂梁的偏转灵敏度，并利用AFM热噪声模块对悬臂梁的弹性系数进行校正。随后控制AFM探针在力谱模式下在细胞表面中央区域不同位置点进行垂直方向的逼近-回退运动，以得到力曲线[41]。在测量化疗药物注射前后单细胞力学特性动态变化的实验中，在每个细胞的中央区域不同点获取15条力曲线。为了使测量结果具有可比性，力曲线获取的所有参数均保持一致（探针逼近速度为10 μm/s，力曲线范围为5 μm）。AFM细胞力学特性测量完毕后，在光学显微镜导引下控制微针对目标细胞进行药物注射。注射完成后，再次控制AFM探针在目标细胞（被注射药物）表面获取力曲线以测量药物刺激后的细胞力学特性。

1.6　细胞杨氏模量计算

利用Hertz-Sneddon模型（锥形针尖）对获取的力曲线进行分析以得到细胞杨氏模量[41]：

$$F = \frac{2E\delta^2\tan\theta}{\pi(1-\upsilon^2)} \quad (1)$$

（1）式中υ为细胞的泊松比（细胞通常被考虑为不可压缩材料，因此υ=0.5[42-43]），F为探针加载力，δ为压痕深度，E为细胞杨氏模量，θ为锥形针尖半开角。探针加载力F可以根据胡克定律从悬臂梁偏转量x得到：

$$F = kx \quad (2)$$

（2）式中k为悬臂梁弹性系数。从获取的力曲线上可以得到悬臂梁偏转量x和压电陶瓷驱动器在垂直方向的距离变化d。根据逼近曲线上的接触点，将逼近曲线转为压痕曲线（压痕深度等于压电陶瓷驱动器垂直方向的变化量d与悬臂梁偏转量x的差值），随后利用Hertz-Sneddon模型对压痕曲线进行拟合即得到细胞杨氏模量。拟合过程利用Matlab（美国Mathworks公司）编写的程序完成。

1.7　扫描电镜（SEM）成像

利用SEM（德国Zeiss公司）更精确地观察微针针尖形貌并确定针尖孔径尺寸。具体来说，用导电胶分别将折断后与未折断的微针固定在样品台上，并用离子溅射仪对样品进行镀金处理。随后将制备好的微针样品置于SEM样品室中进行SEM扫描成像。

2　结果与讨论

首先建立了基于玻璃微针的单细胞注射方法并进行了实验验证。利用拉针仪对玻璃毛细管进行拉制后，微针针尖为封闭状态（图2a）。将微针置于光学显微镜下进行折断以使针尖由封闭状态变为开口状态（图2b，c），以确保针管内试剂溶液的顺利流出（图2d）。此外，微针针尖的尺寸直接影响注射效果。实验发现，如果针尖外径大于1 μm，则注射过程会直接对细胞造成机械损伤，导致细胞形态发生明显改变（图2e，f），而在利用针尖尺寸较小的微针对细胞进行注射时，细胞形态保持完整（图2g，h）。为了验证微针内部溶液是否被注射至细胞，首先选取蓝色墨水作为被注射试剂。实验结果清晰地显示了细胞在被注射蓝色墨水后呈现蓝色（图3），表明了玻璃针管内部的蓝色墨水溶液成功被注射至细胞。进一步采用PI染液作为试剂，利用微针将PI染液注射至细胞，并在注射过程中动态记录PI荧光图像（图4）。图中清晰地显示目标细胞（图4a黄色箭头指示）的细胞核荧光显著增强的动态过程（图4b~h），证明了PI染液成功被注射至细胞。

随后结合微针注射和AFM建立了单细胞精准化学激励下的细胞力学特性同步测量流程（图5）。首先在光学显微镜的导引下，控制微针对目标细胞进行PI染液注射（图5a）。可以看到注射后的目标细胞呈现明亮的荧光（图5c），表明PI溶液被递送至细胞内部。随后即可在PI荧光的导引下，控制AFM移动到目标（发光）细胞（图5d）并在细胞表面进行压痕实验以获取力曲线（图5e）。通过对力曲线进行分析即可得到细胞杨氏模量（图5f），可以看到理论拟合曲线与实验压痕曲线一致。近年来，研究人员广泛研究了基于微针的单细胞操作及测量技术，包括发展用于胚胎注射的自动化微注射系统[44]，对贴壁细胞进行机器人化注射以研究细胞间相互作用[45]，抽取细胞内部物质（如细胞器[46]、DNA[47]等），以及基于微针吸吮的细胞力学特性测量等[48]。2019年，Daza等[49]进一步比较了基于AFM的细胞局部力学特性测量和基于微

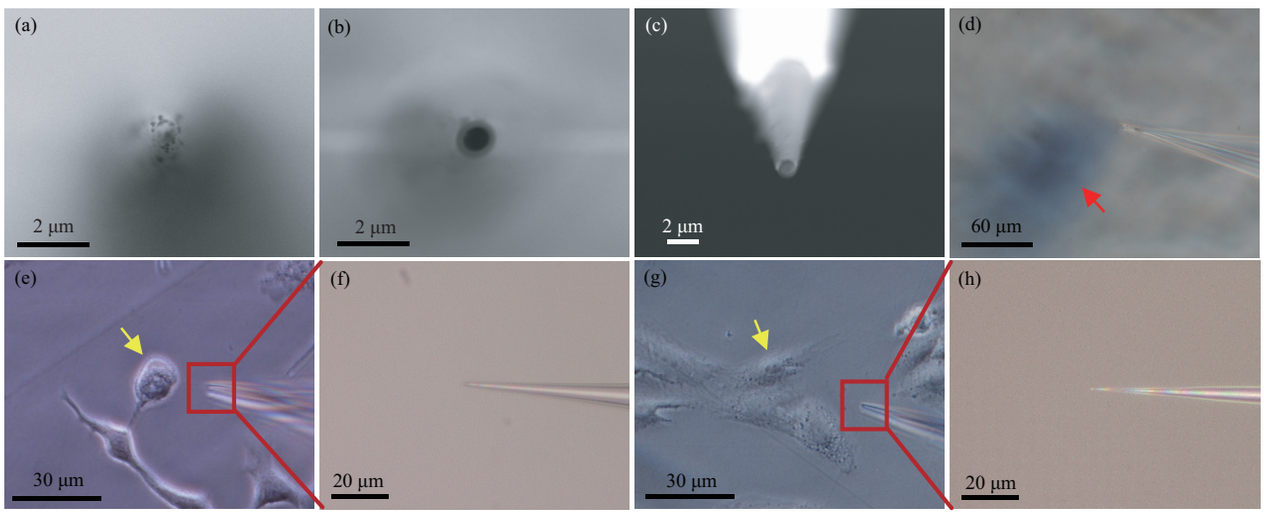

Fig. 2　Effects of the size of micropipette tip on intracellular injection

(a) SEM image of micropipette with closed tip. (b, c) SEM images of micropipette after breaking the closed tip. (d) Optical image showing that the solution inside the micropipette is flowing out under positive pressure (denoted by the red arrow). (e, f) Intracellular injection by micropipette with large tip size. (e) Optical image of the targeted HEK 293 cell (denoted by the yellow arrow) after injection. (f) Optical image of the micropipette. (g, h) Intracellular injection by micropipette with small tip size. (g) Optical image of the targeted NIH 3T3 cell (denoted by the yellow arrow) after injection. (h) Optical image of the micropipette.

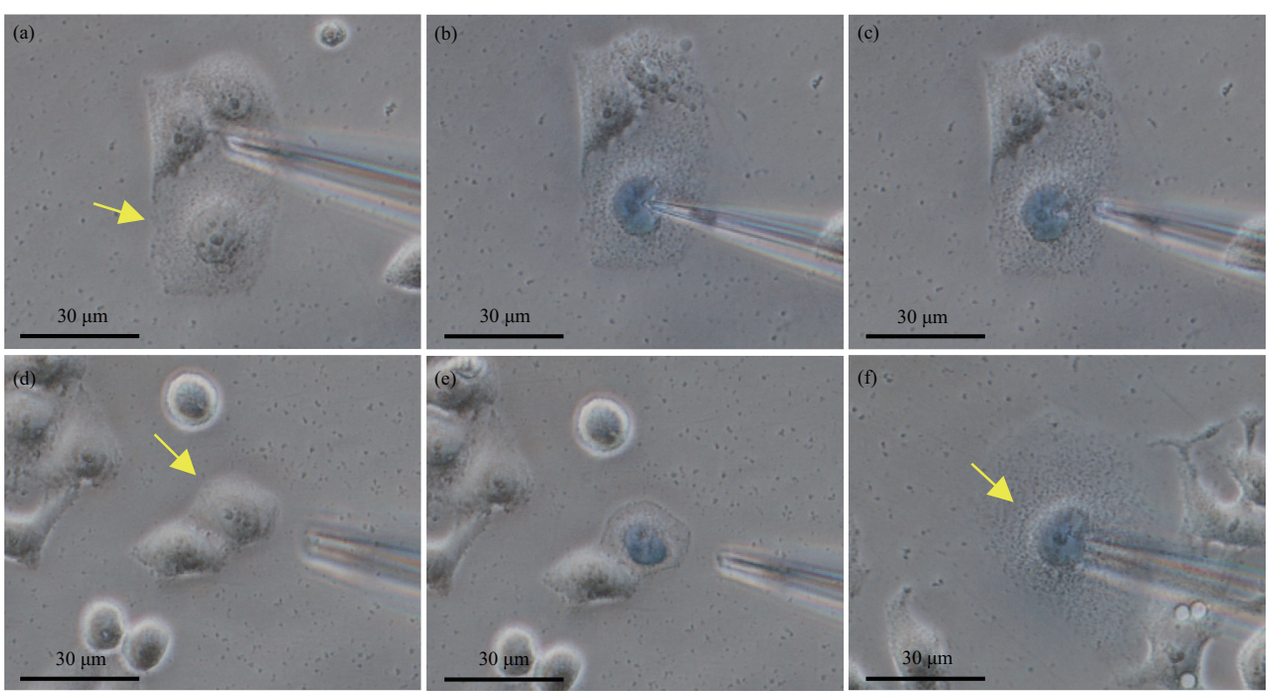

Fig. 3　Injecting blue ink into single cells by micropipette

(a−c) Optical images showing the dynamic processes of injecting blue ink into the targeted MCF-7 cell (denoted by the yellow arrow) by using micropipette. (a) Before injection, (b) during injection, (c) after injection. (d−f) Optical images of injecting blue ink into other MCF-7 cells (denoted by the yellow arrows).

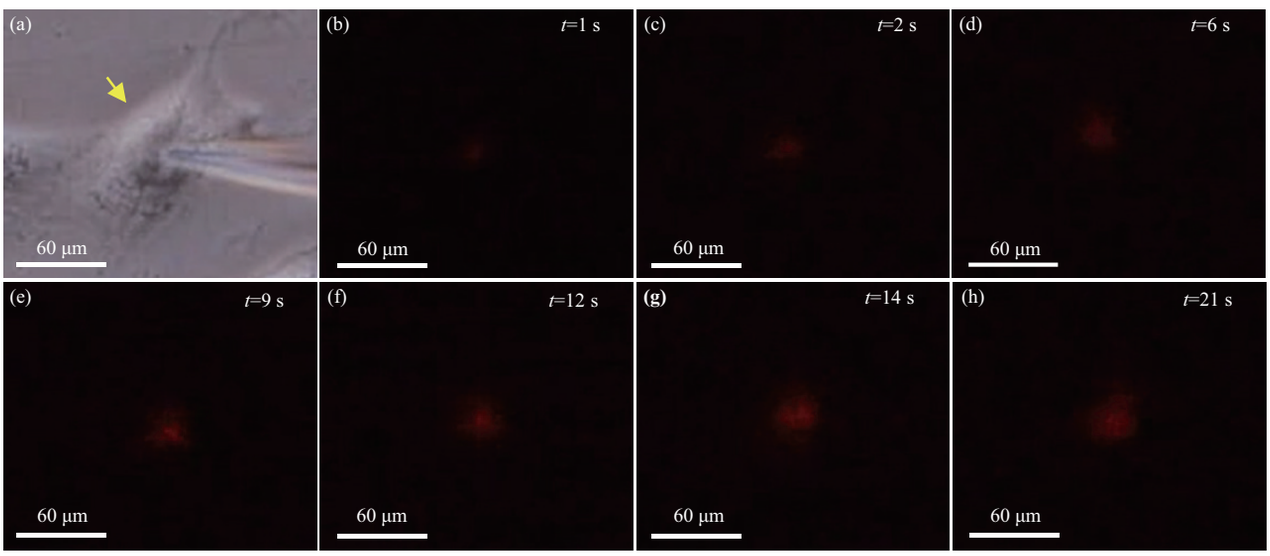

Fig. 4　The dynamic processes of injecting PI staining solution into single cells by micropipette

(a) Optical bright field image of the targeted NIH 3T3 cell (denoted by the yellow arrow) before injection. (b-h) Successive PI fluorescent images acquired during the injection.

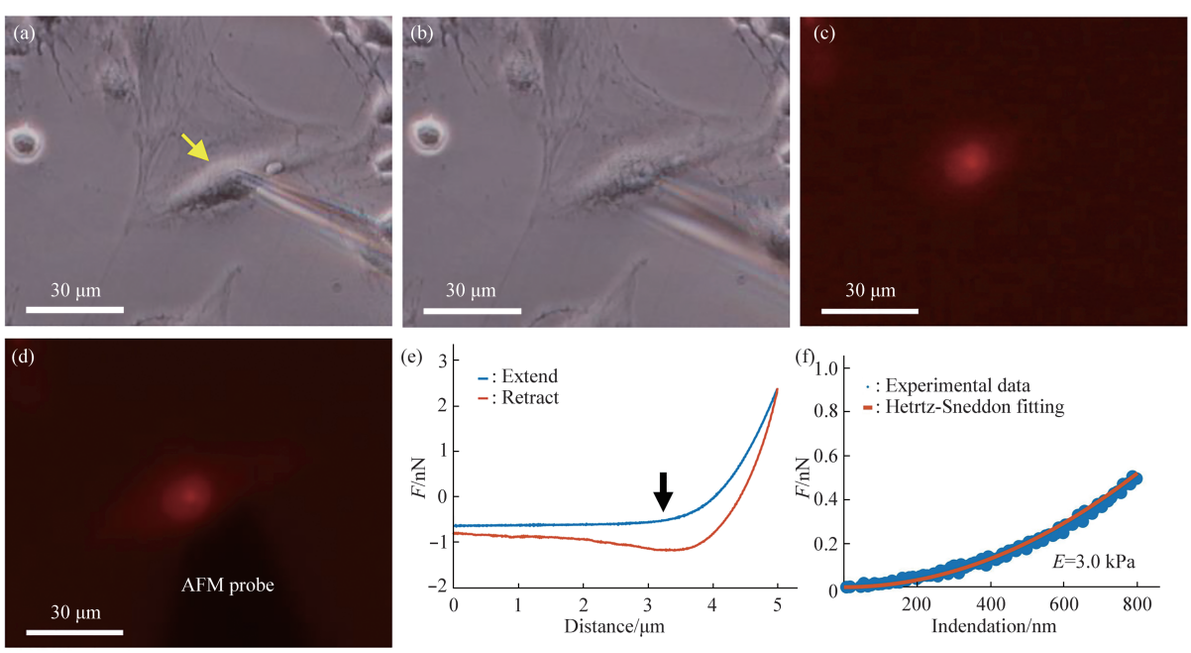

Fig. 5　Single-cell PI staining solution delivery and simultaneous cell mechanics measurement based on the combination of micropipette and AFM

PI staining solution was delivered to the targeted NIH 3T3 cell, after which AFM probe was moved to the same cell to detect the mechanics of the cell under the guidance of PI fluorescence. (a) Optical bright field image of the targeted cell (denoted by the yellow arrow). (b, c) Optical bright field image (b) and the corresponding PI fluorescent image (c) of the cell after injection. (d) Moving AFM probe to the targeted cell under the guidance of PI fluorescence. (e) A typical force curve obtained on the targeted cell with PI fluorescence. The approach curve was converted into indentation curve according to the contact point (denoted by the black arrow) in the approach curve. (f) Cellular Young's modulus was extracted by fitting the indentation curve with Hertz-Sneddon theoretical model.

针的细胞整体细胞力学特性之间的差异。需要指出的是，在结合微针单细胞化学刺激精准递送及AFM细胞力学特性同步测量方面的研究还不多见。本文的实验结果不仅显示了基于微针的单细胞精准化学刺激的可行性（图2~4），同时表明了结合微针和AFM可对培养皿中单个目标细胞进行有效化学刺激并对刺激后的细胞力学特性进行同步测量（图5），为细胞力学特性研究提供了新的可能。

基于所建立的方法，本文研究了化疗药物刺激单个细胞过程中的细胞力学特性动态变化。选取临床肿瘤治疗中广泛使用的阿糖胞苷[50]作为化学药物试剂对细胞进行刺激，并探测细胞在刺激前后力学特性变化。阿糖胞苷经由核苷转运蛋白内化至细胞内部后转化为三磷酸阿糖胞苷，随后三磷酸阿糖胞苷在细胞核内与DNA嵌合从而抑制DNA的复制和翻译[51]。首先利用AFM在目标MCF-7细胞（此时未被注射阿糖胞苷）表面获取力曲线以测量细胞杨氏模量（图6a），随后控制装载有阿糖胞苷溶液的微针对目标细胞进行注射操作以将阿糖胞苷递送到细胞内部（图6b）。最后再次控制AFM探针在

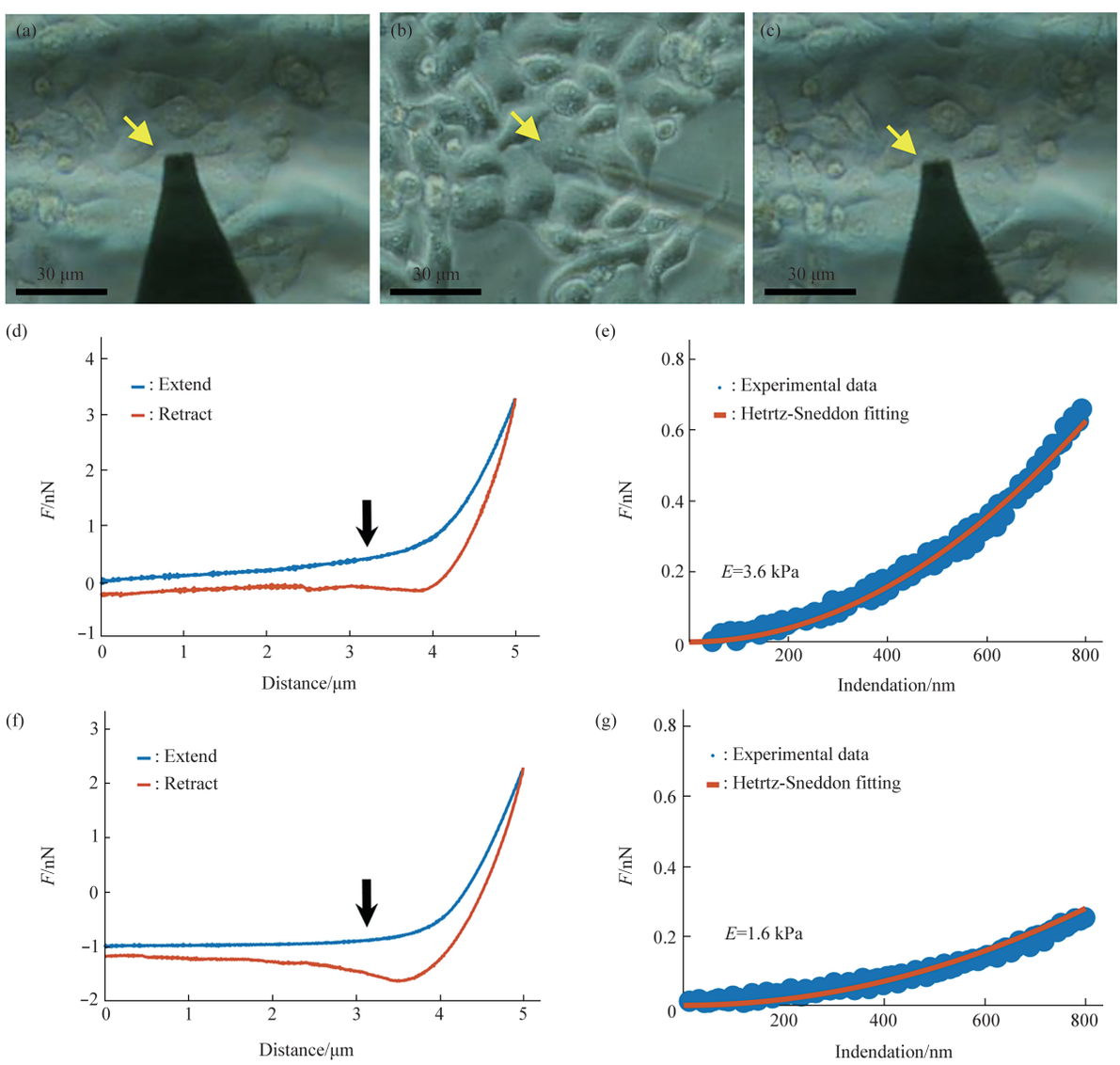

Fig. 6　Single-cell chemotherapeutic drug（cytarabine）delivery and simultaneous cell mechanics measurement by combining micropipette and AFM

(a) Optical bright field image of controlling AFM probe to obtain force curves on the targeted MCF-7 cell (denoted by the yellow arrow) before cytarabine injection. (b) Optical image showing the cytarabine injection of the targeted cell. (c) AFM probe was moved to the targeted cell to obtain force curves again after the cytarabine injection. (d, e) A typical force curve obtained before cytarabine injection (d) and Young's modulus extraction (e). (f, g) A typical force curve obtained after cytarabine injection (f) and Young's modulus extraction (g).

目标细胞表面获取力曲线以监测阿糖胞苷刺激后细胞力学特性变化（图6c）。图6d，e为阿糖胞苷刺激前在细胞表面获取的典型力曲线及应用Hertz-Sneddon理论模型对力曲线进行拟合以得到细胞杨氏模量的结果。图6f，g为阿糖胞苷刺激后在同一细胞表面获取的典型力曲线及拟合结果。可以看到拟合曲线与压痕曲线吻合，表明了Hertz-Sneddon理论模型的有效性。分别对10个MCF-7细胞进行阿糖胞苷注射并对阿糖胞苷刺激前后细胞力学特性进行测量（图7）。图中各个细胞的药物注射前后杨氏模量值及测量时间间隔（从药物注射到注射后杨氏模量测量之间的时间间隔）如表1所示。可以看到总体上阿糖胞苷被微针递送至细胞内部后，细胞杨氏模量减小，显示了阿糖胞苷作用过程中细胞力学特性的动态变化。微针注射已经被广泛应用于单细胞转染[52]，单细胞人工细胞器递送[53]，以及构建基因编辑的小鼠胚胎模型[54]等。本文的实验结果证明了结合微针注射和AFM可以实现单细胞精准化学激励及细胞力学特性同步测量，对于单细胞生理活动行为探测具有积极意义。需要指出的是微针注射本身有可能造成机械损伤，为了消除微针注射对细胞造成的损伤，可以采取微针与细胞接触后依靠药物分子的扩散作用来进行单细胞刺激[55]，但该方法的不足之处是药物自由扩散耗时，从而影响递送效率。

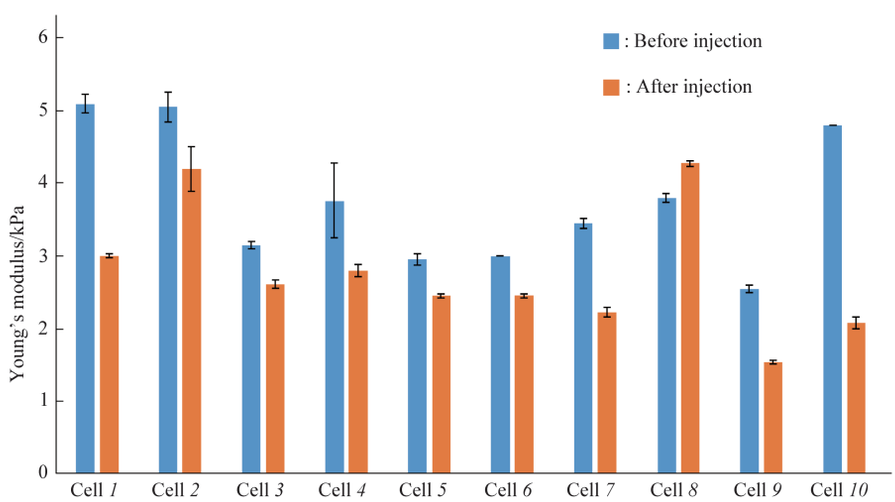

Fig. 7　Statistical histograms showing the Young's modulus changes of individual MCF−7 cells after the injection of cytarabine

With the use of micropipette and AFM, cytarabine solution was injected into individual MCF-7 cells which were grown in dish for precise drug stimulation, and the Young's modulus changes of the cells before and after injection were measured. Ten MCF-7 cells were measured.

Table 1　Young's modulus changes of MCF−7 cells after the injection of cytarabine

Cell number	Cellular Young's modulus before injection/kPa	Cellular Young's modulus after injection/kPa	Time interval between injection and Young's modulus measurement/min
1	5.10±0.12	3.00±0.03	56
2	5.05±0.20	4.20±0.31	52
3	3.15±0.05	2.61±0.05	47
4	3.77±0.52	2.80±0.08	43
5	2.95±0.07	2.45±0.03	38
6	3.00±0.00	2.45±0.03	33
7	3.45±0.06	2.23±0.06	29
8	3.80±0.07	4.28±0.03	24
9	2.55±0.05	1.54±0.02	20
10	4.80±0.00	2.08±0.08	9

结合微针注射和AFM的单细胞精准激励以及力学特性同步测量将有助于研究细胞-药物之间相互作用的内在机理。现有细胞-药物之间相互作用的研究主要采用集群平均实验[56-58]，即将药物分子加入到整个培养皿中，对培养皿中的所有细胞进行刺激，并在培养一定时间后对细胞进行分析（如统计细胞存活率等），以评价药物对细胞的作用效果。这种研究方式的不足之处是，研究结果仅仅反映了所有细胞对药物响应的平均行为，掩盖了单个细胞或少数细胞的独特响应行为[59]。研究表明，细胞之间的异质性在肿瘤治疗及耐药性等方面起着关键性的作用[60]。因此研究单个细胞的行为特性对于深入理解细胞生理行为及药物作用机制等具有广泛的基础意义。特别是细胞力学特性在细胞生理病理变化过程中起着重要的指示作用[61]。本文的实验结果表明，采用微针直接对培养皿中单个目标细胞进行超微量精准化学刺激（图2~4），并利用AFM对化学刺激下的细胞力学特性进行实时动态检测（图5~7），可以直观地研究单个细胞在可控药物作用下的响应行为（如细胞力学特性动态变化），在单细胞尺度为药物作用机制提供新的认识。需要指出的是，在利用微针将药物溶液递送至单个细胞时，通常情况下，药物溶液的无色透明特征导致难以对注射进细胞的药物含量进行准确测定。针对这个问题，可以在药物溶液中加入荧光试剂[62]，根据荧光范围对注射的药物含量进行标定。

3 结　论

总结起来，本文基于微针和AFM实现了对单个细胞的超微量精准激励及细胞力学特性同步测量，为单细胞力学特性研究提供了新的方法和技术，对于实时动态高精度观测细胞-药物之间相互作用具有积极的意义。

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Combining Micropipette and Atomic Force Microscopy for Single-cell Drug Delivery and Simultaneous Cell Mechanics Measurement*

FENG Ya-Qi[1,2,3], YU Peng[1,2,3], SHI Jia-Lin[1,2,3], LI Mi[1,2,3]**

([1])*State Key Laboratory of Robotics, Shenyang Institute of Automation, Chinese Academy of Sciences, Shenyang* 110016, China;

[2])*Institutes for Robotics and Intelligent Manufacturing, Chinese Academy of Sciences, Shenyang* 110169, China;

[3])*University of Chinese Academy of Sciences, Beijing* 100049, China)

Abstract **Objective** Cell mechanics plays an important role in cellular physiological and pathological processes and is closely related to the health states of living organisms. Investigating cell mechanics significantly benefits revealing the underlying mechanisms guiding life activities. The advent of atomic force microscope (AFM) provides a novel instrument for single-cell assay. AFM is able to not only visualize the morphology of singe living cells under aqueous conditions with high resolution, but also quantitatively measure the mechanical properties of cells. Utilizing AFM to investigate the mechanics of individual cells has achieved great success in the past decades, which provides numerous new insights into cellular physiological and pathological processes and has become an important tool in the field of life sciences. However, due to the fact that AFM probe itself is unable to perform drug delivery, so far it is still challenging for the simultaneous measurements of cell mechanics by AFM in response to the stimulation of ultra-trace drug. Here, by combining micropipette and AFM, a method allowing single-cell precise drug delivery and simultaneous measurements of cell mechanics is presented. **Methods** The micropipette-based single-cell microinjection system was built on an inverted fluorescent microscope by using a 3D manipulator, a micropump, a syringe, a PTFE tube and a micropipette. The micropipette was obtained from the glass capillary by using the micropipette puller. NIH 3T3 cells (mouse embryonic fibroblast), HEK 293 cells (human embryonic kidney cell) and MCF-7 cells (human breast cancer cell) were used for the experiments. Under the guidance of optical microscopy, staining reagents or drug molecules were delivered to individual cells, and then AFM probe was moved to the targeted cells to obtain force curves. Cellular Young's modulus was calculated from the force curves by applying Hertz-Sneddon model. **Results** The effects of the pore size of micropipette tip on cell injection were analyzed firstly, and the results showed that larger pore size tip (the outer diameter of the tip was larger than 1 μm) could cause obvious mechanical damage to the cell. Then blue ink or PI staining solution was injected to single cells by micropipette under the guidance of optical microscopy, and the recorded optical/fluorescent images after injection clearly showed that the targeted cells were successfully injected. Finally, micropipette was integrated with AFM to measure the Young's modulus changes of single cells after the treatment of chemotherapeutic drug (cytarabine), and the results showed that stimulation of cytarabine could cause the changes of cellular mechanical properties. **Conclusion** Combining micropipette and AFM enables applying precise chemical stimulation to a single cell while simultaneously measuring cellular mechanical properties after chemical stimulation, providing a novel idea for single-cell mechanical analysis in response to ultra-trace drugs.

Key words atomic force microscope, micropipette injection, cell, mechanical properties, Young's modulus

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** Corresponding author.

Tel：86-24-23970203，E-mail：limi@sia.cn

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