

曲线型微纳拓扑结构的制备及细胞行为调控

郭敏^{1,2}, 刘享洋^{1,2}, 董贤子¹, 刘洁¹, 金峰¹, 郑美玲^{1*}¹中国科学院理化技术研究所仿生材料与界面科学重点实验室, 北京 100190;²中国科学院大学未来技术学院, 北京 101407

摘要 生物材料的表面拓扑结构能够显著影响细胞的黏附、增殖、迁移和分化等行为。为有效模拟体内细胞微环境,利用飞秒激光无掩模光学投影光刻技术制备了一系列曲线型拓扑结构。结果表明:细胞在沟槽、折线和三种不同曲率的波浪形拓扑结构上严格按照拓扑结构形貌进行生长、迁移。当波浪形结构曲率过大时,细胞改变原有的迁移方向,产生沿弯曲方向的迁移行为。共聚焦荧光显微图像显示:细胞在折线结构和波浪线结构的拐角区域发生骨架重排,相较于线区域细胞圆度增加。据此提出了细胞在曲线型拓扑结构上的迁移机制。该研究揭示了细胞对曲线型拓扑结构的响应机制,将为体外植入材料的设计提供科学依据。

关键词 医用光学; 飞秒激光; 无掩模光学投影光刻; 曲线型拓扑结构; 细胞迁移; 细胞骨架

中图分类号 O436

文献标志码 A

DOI: 10.3788/CJL230591

1 引言

细胞微环境作为细胞生命活动的场所,由细胞外基质(ECM)、同型或异型细胞及生长因子等生物因子组成^[1-3]。细胞能够感知微环境中的物理化学信号并将其转导为生物信号,从而改变形态和功能^[4-6]。近年来,研究人员通过在体外模拟细胞微环境对细胞的黏附、增殖、迁移和分化等行为进行了大量的研究。如,通过在特定形貌的基底上涂覆细胞源基质,可以增强细胞骨架收缩并诱导骨髓间充质干细胞的成骨分化^[7]。将精氨酸-甘氨酸-天冬氨酸(RGD)肽序列接枝到聚二甲基硅氧烷(PDMS)表面,可促进人成纤维细胞的黏附、增殖和胶原分泌^[8]。除化学组分外,ECM的物理性质也是影响细胞行为的重要因素。Engler等^[9]在体外构筑了不同硬度的水凝胶基底,研究了间充质干细胞的分化行为,发现在与大脑基质硬度相似的基底上,细胞倾向于向神经细胞分化,而在与骨骼硬度相似的基底上细胞倾向于向成骨细胞分化。

随着微纳加工技术的发展,ECM表面拓扑结构与细胞的相互作用引起了越来越多研究者的兴趣^[10-12]。沟槽结构作为最典型的拓扑结构已经被证明可以使多种细胞产生接触导向行为,且细胞的迁移速度与平面基底相比得到了明显的提升^[13-17]。细胞在柱状结构表面通常具有较小的黏附强度和铺展面

积^[18-20]。然而,规则形状的沟槽结构和凸起结构并不能充分反映细胞与微环境几何拓扑结构的相互作用。体内正常组织的ECM常表现为弯曲或波浪形拓扑结构^[21-24]。因此,在体外构筑多样化拓扑结构有助于进一步理解细胞对ECM表面形貌的响应机制。

传统紫外曝光^[25]、电子束光刻^[26]、聚焦离子束光刻^[27-28]、纳米压印^[29]和静电纺丝^[30]等微纳加工技术已被广泛用于微纳拓扑结构的制备,但仍然难以满足大面积、高精度和高灵活性的微纳结构的制备需求。飞秒激光直写虽然可以灵活地实现任意结构的加工,但在制备大面积拓扑结构时效率较低^[31-35]。飞秒激光无掩模光学投影光刻(MOPL)技术不需要实体掩模版、加工工艺简单且成本低^[36-41],可同时进行多像素点加工,在制备大面积结构时具备高效性^[42-43]。在前期工作中,利用水凝胶材料和MOPL技术制备了不同形状的基底结构,发现结构形貌对细胞行为起到了主要的调控作用^[41]。因此,MOPL技术有望实现图案化拓扑结构的快速制备。

本文利用MOPL技术制备了一系列曲线型微纳拓扑结构,并将其应用于细胞行为调控。通过活细胞工作站对细胞在拓扑结构上的动态迁移行为进行延时成像观察。进一步地,利用荧光染色对拓扑结构上的细胞形态进行可视化观察,并对细胞圆度、细胞周长和细胞铺展面积进行了量化分析,提出了细胞在曲线型拓扑结构上的迁移机制。并且,我们制备了圆环形和

收稿日期: 2023-03-06; 修回日期: 2023-03-22; 录用日期: 2023-03-28; 网络首发日期: 2023-04-07

基金项目: 国家自然科学基金(61975213, 61475164)、中国科学院国际伙伴计划(GJHZ2021130)、中奥国际合作项目(GJHZ1720)、国家重点研发计划(2016YFA0200501)

通信作者: *zhengmeiling@mail.ipc.ac.cn

六角形图案化拓扑结构,细胞能够严格按照图案化结构形貌进行定向迁移,从而实现细胞图案化。细胞共培养实验验证了所制备的拓扑结构基底具备良好的生物相容性。该研究揭示了曲线型微纳拓扑结构对细胞迁移和细胞形态的影响规律,加深了拓扑结构与细胞相互作用机制的理解,为细胞图案化以及体外植入材料的设计提供了科学依据。

2 实验部分

2.1 实验试剂及仪器

本实验中所用的试剂包括:正性光刻胶 AZ P4620;显影液 AZ 400K;1,2-丙二醇单甲醚醋酸酯(PGMEA);多聚赖氨酸(PDL);4',6-二脒基-2-苯基吡啶(DAPI)染色液;RPMI培养基;青霉素-链霉素;胎牛血清;人肾透明细胞腺癌细胞(786-O细胞);罗丹明标记的鬼笔环肽;Mito-Tracker Green 荧光探针;碘化丙啶(PI);NaCl、KCl、 KH_2PO_4 和 Na_2HPO_4 ;无水乙醇;超纯水。实验中所有试剂均直接使用,未作纯化处理。

本实验中所用仪器包括:台式匀胶机;飞秒激光器;无掩模光学投影光刻系统(自主搭建);等离子体表面处理仪;离子溅射仪;扫描电子显微镜(SEM);原子力显微镜(AFM);激光扫描共聚焦显微镜(LSCM);接触角仪。

2.2 拓扑结构的制备

首先将稀释的正性光刻胶 AZ P4620(质量比为 $M_{\text{AZ P4620}}:M_{\text{PGMEA}}=1:1$)以 4000 r/min 的速度旋涂在干净的盖玻片上,并在 95 °C 的加热板上烘 1 min。然后,将样品固定在 MOPL 的三维移动台上进行程序化曝光。曝光完毕后将样品置于稀释的 AZ 400K 显影液(体积比为 $V_{\text{AZ400K}}:V_{\text{水}}=1:4$)中。由于是正胶,曝光部分在显影液中产生酸分解,未曝光部分被保留,从而形成所设计的图案化拓扑结构。

2.3 拓扑结构的形貌表征

首先将所制备的拓扑结构用去离子水冲洗干净并晾干。然后,在拓扑结构表面喷镀一层金膜,利用 SEM 进行形貌观察。SEM 图像拍摄过程中采用的加速电压为 10 keV。采用 AFM 表征拓扑结构的三维形貌,将获得的 AFM 图像在 NanoScope Analysis 1.9 软件中处理,可得到拓扑结构的三维形貌图像、截面轮廓图以及拓扑结构的高度分布信息。

2.4 细胞培养

本研究选取人肾透明细胞腺癌细胞(786-O)进行实验研究。786-O 细胞培养在含有 10%(体积分数)胎牛血清和 1%(体积分数)青霉素-链霉素的 RPMI 培养基中,放置在 37 °C 和含有 5%(体积分数)CO₂的培养箱中。待细胞覆盖率达到 80% 时,对细胞进行传代。接种细胞前,将拓扑结构在 O₂ 等离子体中处理 7 min,并在 PDL 中浸泡过夜。

2.5 细胞延时成像

待细胞接种在拓扑结构上 5 h 后,将培养皿从培养箱中转移至活细胞工作站(37 °C, CO₂ 体积分数为 5%),利用激光扫描共聚焦显微镜对细胞进行时间延时成像,每 10 min 拍摄一次并持续 20 h。

2.6 荧光染色和成像

细胞在拓扑结构上生长 24 h 后,对细胞进行固定、染色和观察。首先去除培养基,用 PBS 润洗 3 次,每次 3 min。然后在室温下加入 4% 多聚甲醛固定细胞 15 min,之后用磷酸盐缓冲溶液(PBS)润洗 3 次。之后加入曲拉通 TritonX-100 渗透 5 min,用 PBS 润洗 3 次。最后分别加入鬼笔环肽和 DAPI 染色液在黑暗中孵育细胞 30 min 和 10 min,并用 PBS 润洗 3 次即可观察。利用 LSCM 对细胞进行荧光成像,细胞核的荧光采用 405 nm 连续激光激发,并在 410~460 nm 的波段内记录共聚焦荧光图像。肌动蛋白的荧光采用 561 nm 连续激光激发,在 570~620 nm 的波段内记录共聚焦荧光图像。

细胞活死染色步骤:细胞培养在拓扑结构上 24 h 后,分别加入 Mito-Tracker Green 和 PI 荧光探针孵育 20 min 和 10 min,然后用温热的培养基润洗 2 次,每次 3 min。利用 LSCM 对细胞进行成像,线粒体的荧光采用 488 nm 连续激光激发,并在 490~540 nm 波段内记录共聚焦荧光图像。细胞核的荧光采用 561 nm 连续激光激发,并在 570~620 nm 的波段内记录共聚焦荧光图像。

2.7 数据分析

微纳拓扑结构的尺寸采用 ImageJ 软件测量。细胞轨迹追踪采用 ImageJ 软件中的 Manual tracking 进行分析,每个样品至少有 10 个细胞被分析。细胞轮廓在 ImageJ 中手动描出,测量得出细胞铺展面积和细胞周长。通过对细胞进行椭圆拟合,得出细胞长轴与短轴的值。细胞圆度定义为短轴与长轴的比值,圆度接近 1 代表细胞趋于圆形,圆度接近 0 代表细胞拉长度大。每个样品至少有 30 个细胞被测量。

3 分析与讨论

3.1 拓扑结构的制备与表征

MOPL 技术是一种低成本、高通量且灵活的微纳拓扑结构制备方法。在加工的过程中,MOPL 中的数字微镜器件(DMD)能够根据载入的图形进行空间光场调制,从而形成相应图形的数字掩模。因此,可以通过图形设计实现所需要的微纳结构的制备。在本研究中,我们利用 MOPL 技术制备了一系列曲线型微纳拓扑结构,实现了细胞行为的定向调控(图 1)。

考虑到正常组织所处的细胞外基质表面为弯曲形或波浪状结构,为了更有效地模拟细胞微环境,我们分别制备了折线形微纳拓扑结构和不同曲率的波浪形微

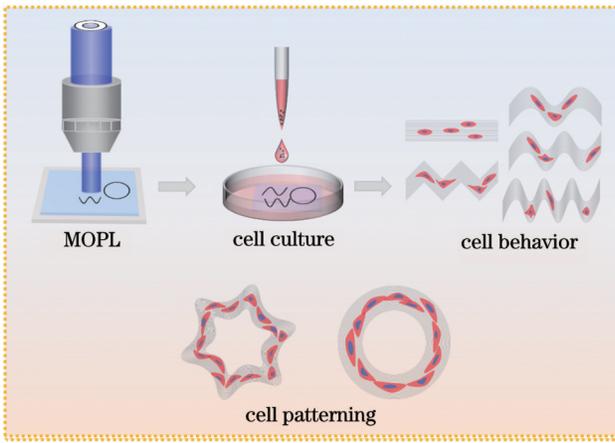


图 1 大面积微纳拓扑结构的制备及细胞行为调控示意图
Fig.1 Schematics of fabrication of large-area micro-nano topography structures and regulation of cell behavior

纳拓扑结构来研究细胞行为,以沟槽结构作为对照。如图 2 所示,所制备的沟槽结构的脊线宽度为 $1.3\ \mu\text{m}$,脊线间距为 $8\ \mu\text{m}$,该结构用 G 表示。折线结构的脊线宽度为 $1.3\ \mu\text{m}$,脊线间距为 $5.2\ \mu\text{m}$,该结构用 BL 表示。

波浪线 1 的脊线宽度为 $1.3\ \mu\text{m}$,弯角处间距为 $7.7\ \mu\text{m}$,弯角曲率为 0.067 ,该结构用 W1 表示。波浪线 2 的脊线宽度为 $1.3\ \mu\text{m}$,弯角处间距为 $7.7\ \mu\text{m}$,弯角曲率为 0.086 ,该结构用 W2 表示。波浪线 3 的脊线宽度为 $1.3\ \mu\text{m}$,弯角处间距为 $7.7\ \mu\text{m}$,弯角曲率为 0.14 ,该结构用 W3 表示。图 2 中局部放大的 SEM 图像表明了所制备的微纳结构形貌完整、均一。

我们利用原子力显微镜对所制备的微纳拓扑结构的三维形貌进行了表征。如图 3(a)~(f) 所示,三维形貌图下方为对应结构的截面轮廓,可以看出所制备的微纳结构形貌具备良好的保真度。通过对截面轮廓图进行测量,可以得出 G、BL、W1、W2 和 W3 结构的脊线高度分别为 804 、 865 、 853 、 856 和 $817\ \text{nm}$ [图 3(g)]。各个结构的脊线高度值近似一致,这可以排除高度差异引起的细胞行为变化。此外,此高度值远大于细胞对凸起结构的感应阈值^[15],能够对细胞行为进行有效诱导。由于细胞在中度亲水的环境中更容易存活和生长^[44],我们对所制备的拓扑结构进行了 O_2 等离子体处理。为了促进细胞的黏附,利用 PDL 对结构进行包

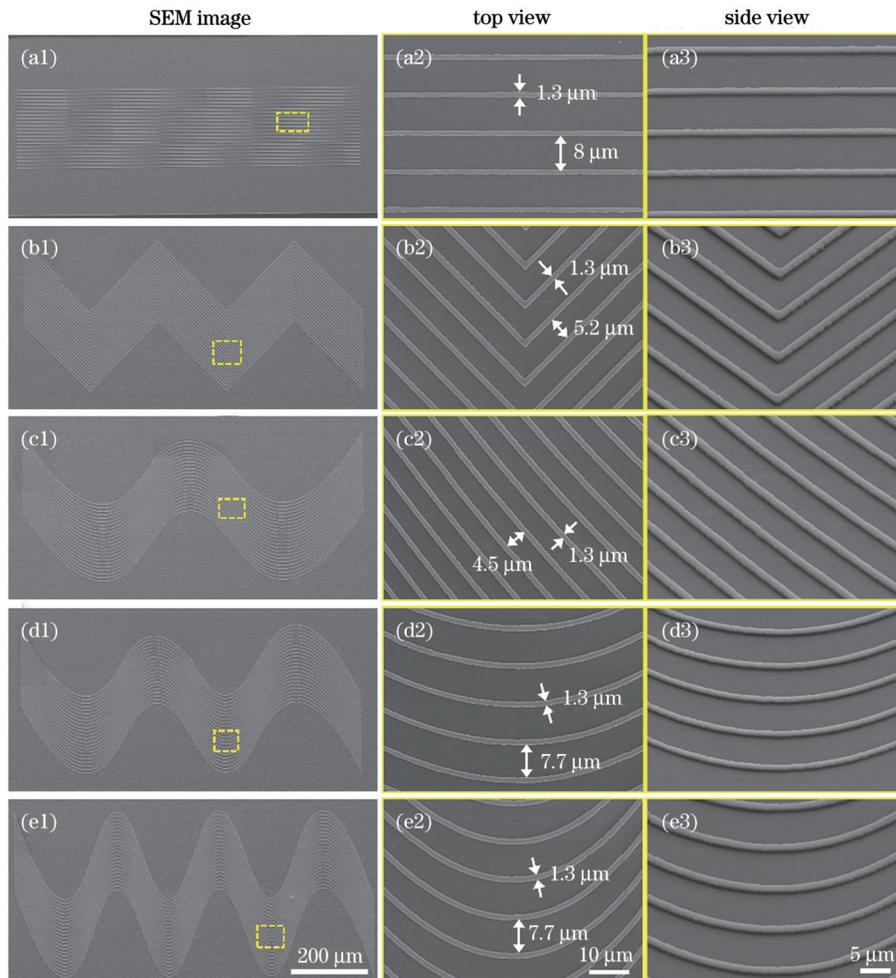


图 2 所制备的微纳拓扑结构。(a1)~(a3)沟槽结构;(b1)~(b3)折线结构;(c1)~(c3)波浪线 1 结构;(d1)~(d3)波浪线 2 结构;(e1)~(e3)波浪线 3 结构

Fig. 2 Prepared micro-nano topography structures. (a1)–(a3) Groove structure; (b1)–(b3) broken line structure; (c1)–(c3) wave 1 structure; (d1)–(d3) wave 2 structure; (e1)–(e3) wave 3 structure

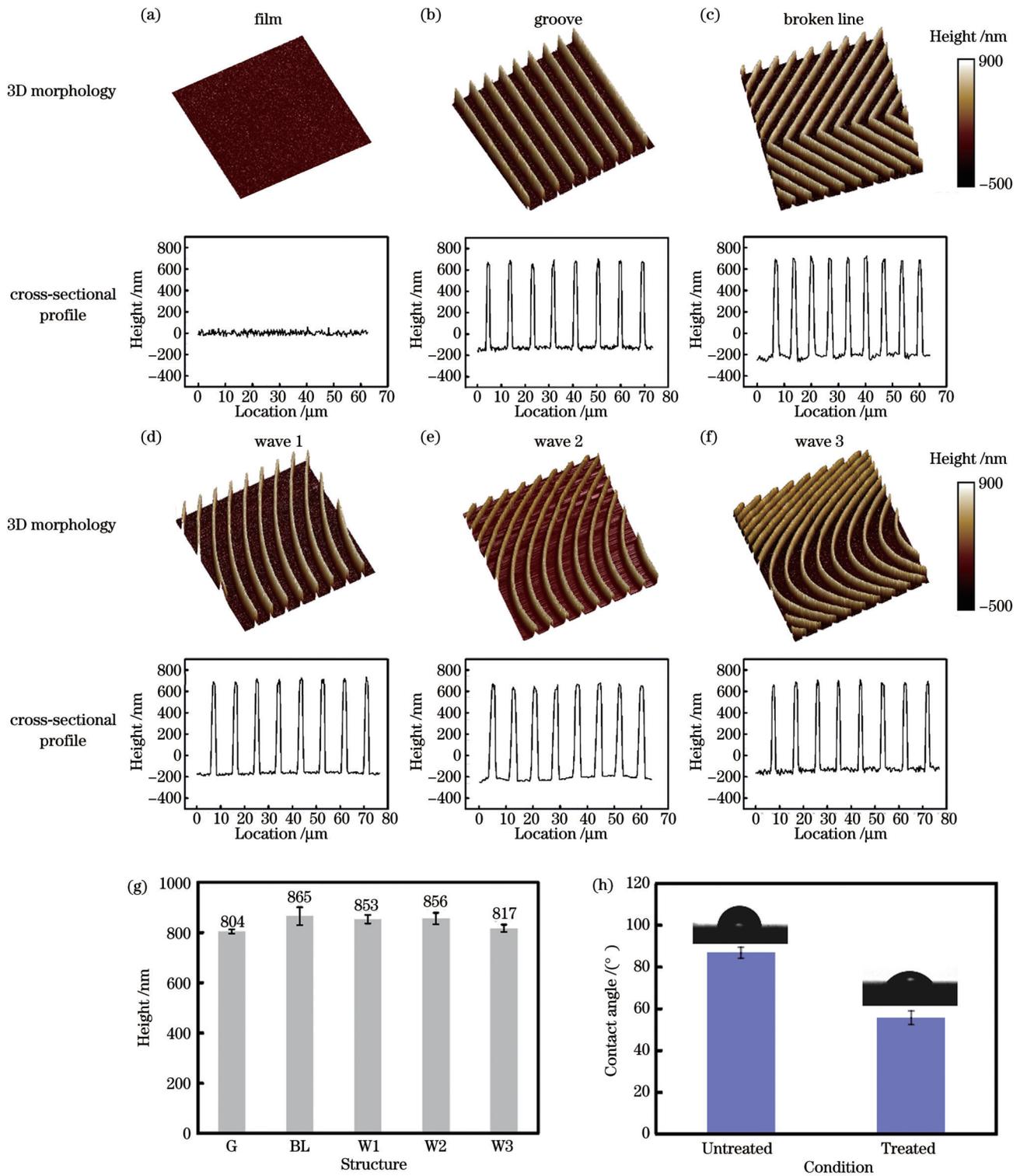


图3 拓扑结构的AFM图像和接触角。(a)平面、(b)沟槽、(c)折线、(d)波浪线1、(e)波浪线2和(f)波浪线3结构的三维形貌图,下方为对应结构的截面轮廓图;(g)拓扑结构的高度统计;(h)未处理的和处理的平面基底的接触角

Fig. 3 AFM images and contact angles of topography structures. 3D morphology of (a) film, (b) groove, (c) broken line, (d) wave 1, (e) wave 2, and (f) wave 3 structures with corresponding cross-sectional profile images shown below; (g) height statistic of topography structure; (h) contact angles of untreated and treated film substrates

覆。如图3(h)所示,经过处理和包覆的基底接触角从86.7°降为55.6°,表明基底表面亲水性增加。以上微纳拓扑结构高度和表面化学性质的一致性保证了在后续的实验中可以单一地比较拓扑几何结构对细胞行为的影响。

3.2 拓扑结构对细胞迁移行为的影响

为了研究拓扑结构对细胞迁移行为的影响,我们将786-O细胞培养在一系列所制备的微纳拓扑结构上,待细胞在结构上粘附5h后,将细胞转移至活细胞工作站中进行延时成像观察。图4为细胞在微纳拓扑

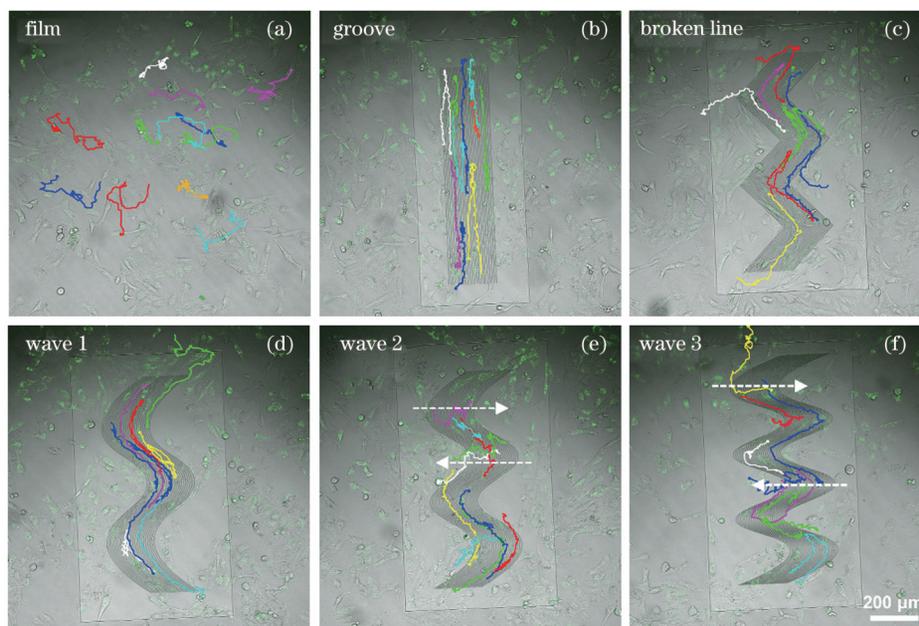


图4 细胞在不同结构上培养 20 h 的迁移轨迹,箭头指示细胞迁移方向。(a)平面;(b)沟槽;(c)折线;(d)波浪线 1;(e)波浪线 2;(f)波浪线 3
Fig. 4 Migration trajectories of cells cultured on different structures for 20 h with directions of cell migration shown by arrows.
(a) Film; (b) groove; (c) broken line; (d) wave 1; (e) wave 2; (f) wave 3

结构基底上培养 20 h 的迁移轨迹。细胞在平面基底上以随机游走的方式迁移。在沟槽结构上,细胞表现出与以往研究一致的接触导向行为,细胞沿着脊线的方向定向迁移。在折线结构上,细胞迁移轨迹严格按照折线路径。折线结构区域外的细胞一旦接触到折线结构,细胞便调整形态按照折线路径迁移。这表明细胞能够感知拓扑结构的角度偏折,灵活地调整迁移方向。在三种波浪线结构上,细胞能够灵活地适应拓扑结构角度的变化,形成对应拓扑结构的曲线迁移轨迹。值得注意的是,在波浪线 2 和波浪线 3 结构上,细胞出现沿着弯曲方向的迁移行为(虚线箭头所示)。这可能是波浪线 2 和波浪线 3 结构弯角处曲率过大引起的弯曲诱导效应。细胞沿拓扑结构形成了相应模式的迁移路径,这表明拓扑结构的形貌对细胞运动方向起着决定性作用。

3.3 拓扑结构对细胞形态的影响与细胞行为变化机制

细胞在迁移的过程中,首先伸出细窄的丝状伪足感知外部环境,然后在迁移的方向上形成宽大的板状伪足与基底形成稳定黏附,之后细胞后方变窄并逐渐与基底脱离^[45-47]。为了理解细胞在所制备的微纳拓扑结构上的迁移机制,我们利用荧光染色对拓扑结构上的细胞形态进行了可视化处理并进行了量化分析。

图 5(a)展示了细胞在一系列所制备的微纳拓扑结构基底上的共聚焦荧光图像,右侧两栏分别为拓扑结构上线区域和拐角区域的细胞荧光图像。细胞核和肌动蛋白分别被 DAPI 和鬼笔环肽荧光探针标记。在沟槽结构上,可以清楚地看到细胞沿脊线形成了定向

排列的肌动蛋白应力纤维,细胞核亦表现出沿脊线方向的拉长,细胞形态整体为拉长状。对于折线结构,细胞在线区域上形成沿脊线排列的肌动蛋白应力纤维,细胞沿脊线的方向拉长。在拐角区域上,细胞的肌动蛋白应力纤维呈直角状排布,细胞外缘沿着折线结构呈直角形态。在波浪线结构中,细胞在线区域上沿脊线方向呈拉长的形态。在 W1 结构上的拐角区域中,大部分应力纤维沿着基底脊线方向排布,少数应力纤维与基底脊线形成一定方向的夹角排布。细胞整体呈现沿脊线的拉长状。在 W2 和 W3 结构的拐角区域中,细胞形态明显不同于线区域。细胞沿着弯角方向形成了排列的肌动蛋白应力纤维,细胞形态呈多角状。

图 5(b)、(c)分别为各个拓扑结构基底上的细胞周长和细胞铺展面积统计。各个基底上的细胞周长无明显差异,但拓扑结构上的细胞铺展面积小于平面基底,表明结构化基底限制了细胞的铺展,改变了细胞的形态。为了进一步说明同一结构上线区域和拐角区域细胞形态的变化,我们对各个基底上细胞的圆度进行了分析。从图 5(d)可以得出:各个基底中线区域上的细胞圆度值近似相等,接近沟槽结构上的细胞圆度;各个基底中拐角区域上的细胞圆度值远远大于线区域,接近平面基底上的细胞圆度。此外,对于三种曲线结构,随着波浪线曲率的增加,拐角区域的细胞圆度值增加。

通过分析细胞在一系列拓扑结构基底上的行为,我们提出了细胞在曲线型拓扑结构上的迁移机制。如图 5(e)所示,细胞在迁移的过程中,首先沿着脊线拉长并沿着脊线方向迁移。当细胞遇到拐角结构时,细

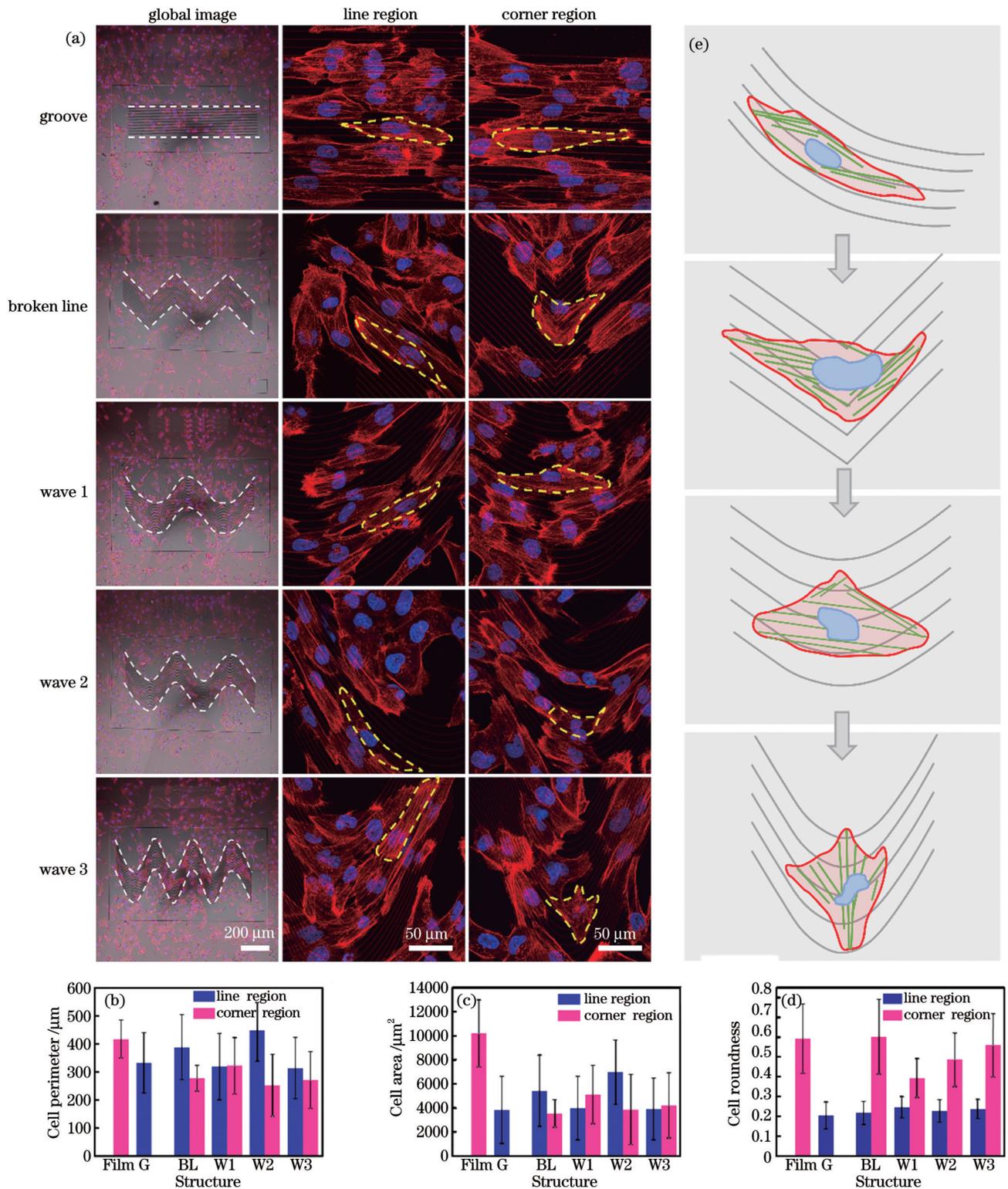


图 5 拓扑结构上细胞的形态分析。(a)细胞在不同拓扑结构上的共聚焦荧光图像;(b)细胞周长统计;(c)细胞铺展面积统计;(d)细胞圆度统计;(e)细胞在不同拓扑结构上的迁移机制示意图

Fig. 5 Analysis of cell morphology on topography structures. (a) Confocal fluorescence images of cells on different topography structures; (b) statistic of cell perimeter; (c) statistic of cell spreading area; (d) statistic of cell roundness; (e) schematics of migration mechanism of cells on different topography structures

胞改变形态适应拓扑结构变化。在折线结构拐角区域,肌动蛋白应力纤维排列成直角,细胞呈直角状形态。在三种不同曲率的波浪线结构上,细胞在拐角区域的形态不同。对于低曲率的波浪线结构,细胞分别

形成沿脊线方向的应力纤维和与脊线成一定夹角的应力纤维,相比于线区域,细胞形态拉长度减小。在高曲率的波浪线结构上,细胞形成沿弯曲方向的肌动蛋白应力纤维,细胞形态表现为多角状。这表明细胞能够

感知拓扑结构形貌,将拓扑结构的物理信号由细胞骨架传导至细胞核。细胞核内在控制细胞骨架进行重排,从而改变细胞形态和迁移方向。当波浪线拓扑结构拐角处曲率过大时,细胞不能适应脊线弯度,细胞形态发生变化,导致迁移方向发生改变。曲线弯度诱导细胞形成了新的迁移方向。

3.4 细胞图案化调控

上述讨论表明细胞能够适应基底拓扑结构的弯曲

形成相应方向的定向迁移。进一步地,我们制备了圆环和六角形图案化结构来诱导细胞的定向迁移。图 6(a1)~(b3)为所制备的圆环结构和六角形结构的 SEM 图像和局部的放大图像,其中脊线宽度为 $1.3\ \mu\text{m}$ 。图 6(c)、(d)分别为圆环结构和六角形结构的 AFM 图像,脊线高度分别为 $817\ \text{nm}$ 和 $824\ \text{nm}$ 。图 6 表明所制备的图案化结构和设计图形一致且形貌完好、均一。

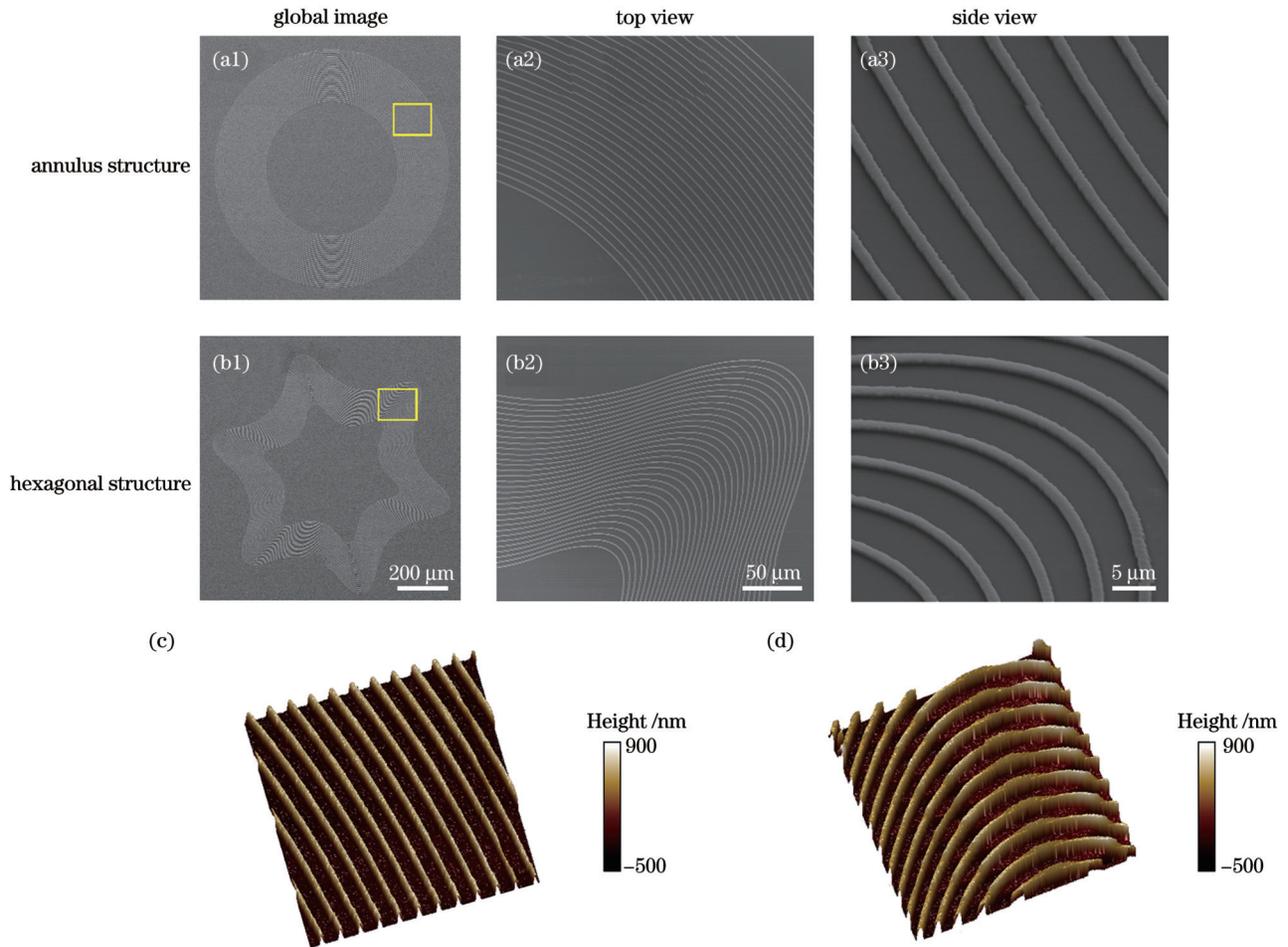


图 6 圆环形结构和六角形结构的形貌表征。(a1)~(a3)圆环形结构的 SEM 图像;(b1)~(b3)六角形结构的 SEM 图像;(c)圆环形结构的 AFM 图像;(d)六角形结构的 AFM 图像

Fig. 6 Morphological characterization of annulus and hexagonal structures. (a1)~(a3) SEM images of annulus structures; (b1)~(b3) SEM images of hexagonal structures; (c) AFM image of annulus structure; (d) AFM image of hexagonal structure

在图案化拓扑结构上培养细胞 20 h 后,对其进行固定和染色观察。图 7 为圆环形、六角形和平面结构上细胞的共聚焦荧光图像及局部放大图像。在圆环结构上,可以看出细胞沿着圆环结构形成圆形排布。局部放大图像表明细胞在圆环结构上形成了沿圆环脊线分布的应力纤维,细胞拉长度大,细胞沿着圆环排列。由于圆环形结构为各向同性结构,细胞在圆环各处形态一致。在六角形结构上,细胞沿着所处拓扑结构排布成六角形形状。图 7(b2)、(b3)分别为六角形结构中线性区域和拐角区域的局部放大图像,可以看出线性区域上细胞拉长并沿着脊线排布。在拐角区域上,细胞

适应拓扑结构弯度形成半弯形态。在平面基底上,细胞随机分布,细胞形态无明显取向。

3.5 拓扑结构的生物相容性

为了验证所制备的微纳拓扑结构的生物相容性,我们将细胞分别接种在拓扑结构和光刻胶薄膜上进行共培养。24 h 后,分别使用 Mito-Tracker Green 和 PI 荧光探针对活细胞的线粒体和死细胞的细胞核进行染色。如图 8 所示,Mito-Tracker Green 通道显示细胞生长良好,PI 通道几乎没有死细胞。这表明我们所使用的结构基底具备良好的生物相容性,有望应用于组织工程领域。

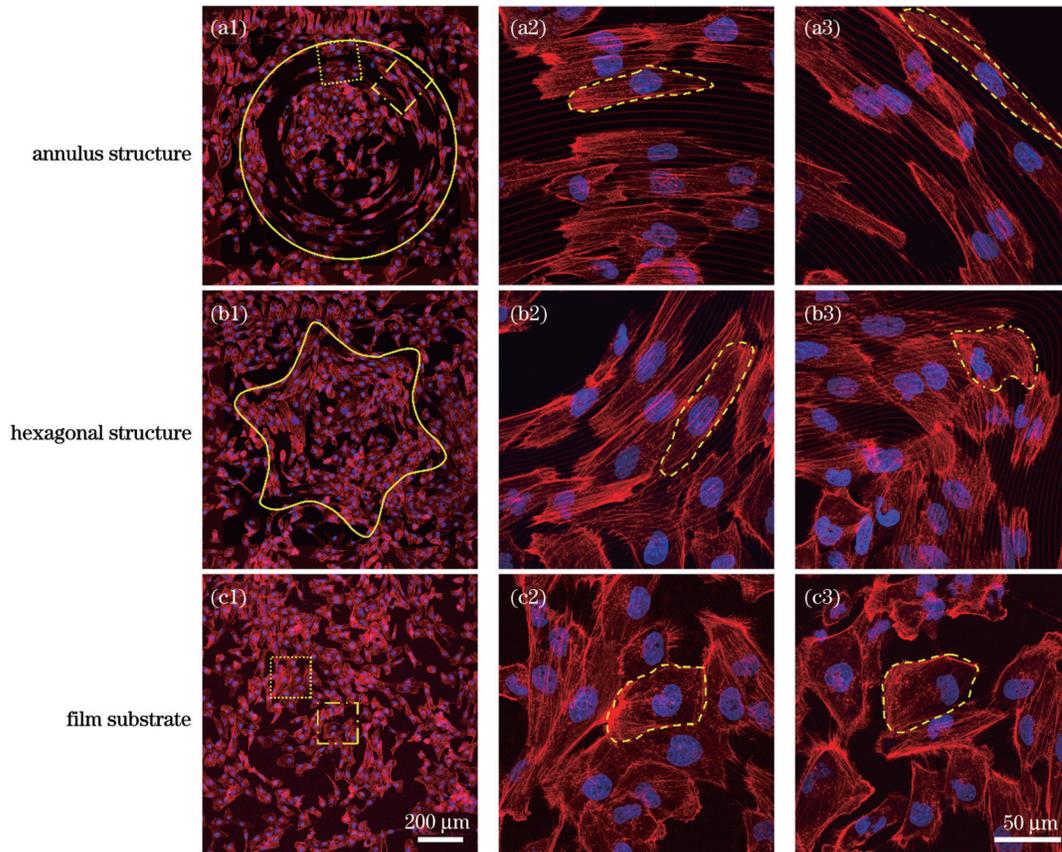


图 7 细胞在图案化拓扑结构上的共聚焦荧光图像,虚线表示细胞轮廓。(a1)圆环形结构上细胞的荧光图像;(a2)图 7(a1)点框处放大的荧光图像;(a3)图 7(a1)点线框处放大的荧光图像;(b1)六角形结构上细胞的荧光图像;(b2)局部放大的线区域的荧光图像;(b3)局部放大的拐角区域的荧光图像;(c1)平面基底上细胞的荧光图像;(c2)图 7(c1)中点框处放大的荧光图像;(c3)图 7(c1)中点线框处放大的荧光图像

Fig. 7 Confocal fluorescence images of cells on patterned topography structures with cell outline shown by dashed line. (a1) Fluorescence image of cells on annulus structure; (a2) magnified fluorescence image at dot box in Fig. 7(a1); (a3) magnified fluorescence image at dotted wireframe in Fig. 7(a1); (b1) fluorescence image of cells on hexagonal structure; (b2) local magnification of fluorescence image in line region; (b3) local magnification of fluorescence image in corner region; (c1) fluorescence image of cell on film substrate; (c2) magnified fluorescence image at dot box in Fig. 7(c1); (c3) magnified fluorescence image at dotted wireframe in Fig. 7(c1)

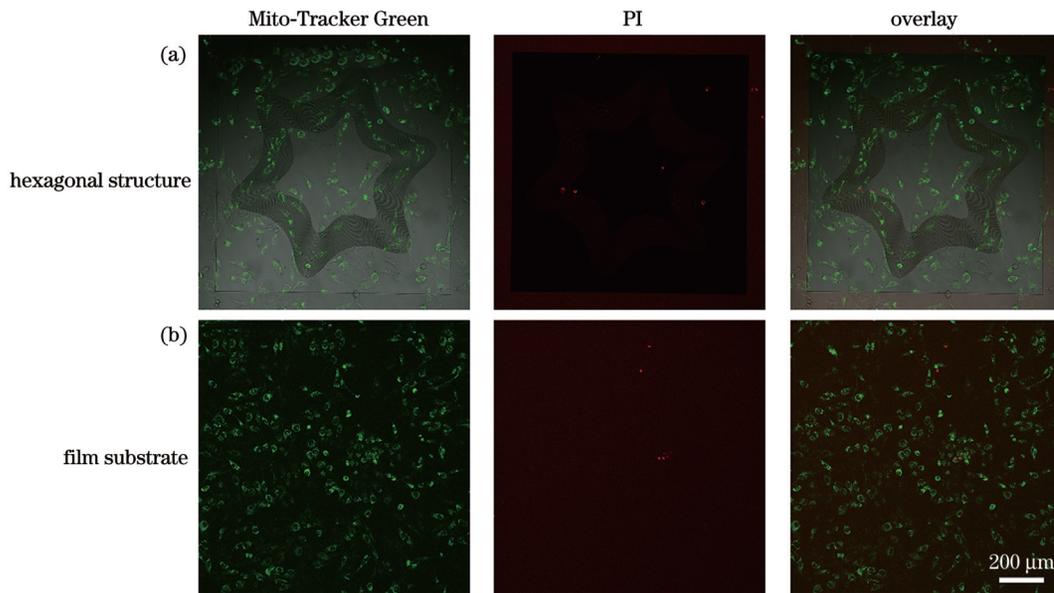


图 8 拓扑结构上的细胞活性
Fig. 8 Cell viability on topography structures

4 结 论

利用飞秒激光无掩模投影光刻技术制备了系列曲线型微纳拓扑结构,并详细研究了细胞的迁移行为和细胞形态变化。结果表明:细胞能够按照一系列直线、折线、曲线拓扑结构进行生长、迁移。当波浪线结构曲率过高时,细胞改变原有的迁移方向,出现沿弯角方向的迁移行为。这是由于在波浪线结构的拐角区域,细胞骨架发生重排,因此细胞形态发生变化,进而细胞的迁移方向发生改变。因此,拓扑结构的形貌在细胞的迁移过程中发挥着重要作用。进一步地,制备了圆环形和六角形图案化拓扑结构,细胞能够严格按照图案化结构形貌进行定向迁移,从而实现细胞图案化。所制备的拓扑结构与细胞的共培养实验证实其具备良好的生物相容性。该研究表明,拓扑结构可以明显调控细胞的形态、生长和迁移行为。研究结果进一步加深了对拓扑结构调控细胞行为机制的理解。

参 考 文 献

- [1] Lee D B, Kim D W, Cho J Y. Role of growth factors in hematopoietic stem cell niche[J]. *Cell Biology and Toxicology*, 2020, 36(2): 131-144.
- [2] Zhang H L, Zheng X W, Ahmed W, et al. Design and applications of cell-selective surfaces and interfaces[J]. *Biomacromolecules*, 2018, 19(6): 1746-1763.
- [3] Charras G, Sahai E. Physical influences of the extracellular environment on cell migration[J]. *Nature Reviews Molecular Cell Biology*, 2014, 15(12): 813-824.
- [4] Li J, Di Russo J, Hua X M, et al. Surface immobilized E-cadherin mimetic peptide regulates the adhesion and clustering of epithelial cells[J]. *Advanced Healthcare Materials*, 2019, 8(8): 1801384.
- [5] Damiani L A, Tsimbouri M P, Hernandez V L, et al. Materials-driven fibronectin assembly on nanoscale topography enhances mesenchymal stem cell adhesion, protecting cells from bacterial virulence factors and preventing biofilm formation[J]. *Biomaterials*, 2022, 280: 121263.
- [6] Han L, Yin Q D, Yang L L, et al. Biointerface topography regulates phenotypic switching and cell apoptosis in vascular smooth muscle cells[J]. *Biochemical and Biophysical Research Communications*, 2020, 526(3): 841-847.
- [7] Yang L L, Ge L, van Rijn P. Synergistic effect of cell-derived extracellular matrices and topography on osteogenesis of mesenchymal stem cells[J]. *ACS Applied Materials & Interfaces*, 2020, 12(23): 25591-25603.
- [8] Li B, Chen J X, Wang J H C. RGD peptide-conjugated poly(dimethylsiloxane) promotes adhesion, proliferation, and collagen secretion of human fibroblasts[J]. *Journal of Biomedical Materials Research Part A*, 2006, 79(4): 989-998.
- [9] Engler A J, Sen S, Sweeney H L, et al. Matrix elasticity directs stem cell lineage specification[J]. *Cell*, 2006, 126(4): 677-689.
- [10] Wang S, Hashemi S, Stratton S, et al. The effect of physical cues of biomaterial scaffolds on stem cell behavior[J]. *Advanced Healthcare Materials*, 2021, 10(3): 2001244.
- [11] Liu X L, Wang S T. Three-dimensional nano-biointerface as a new platform for guiding cell fate[J]. *Chemical Society Reviews*, 2014, 43(8): 2385-2401.
- [12] Niari S A, Rahbarghazi R, Geranmayeh M H, et al. Biomaterials patterning regulates neural stem cells fate and behavior: the interface of biology and material science[J]. *Journal of Biomedical Materials Research Part A*, 2022, 110(3): 725-737.
- [13] Thiruvikraman G, JagieHo A, Lai V K, et al. Cell contact guidance via sensing anisotropy of network mechanical resistance[J]. *Proceedings of the National Academy of Sciences of the United States of America*, 2021, 118(29): e2024942118.
- [14] Kim J, Bae W G, Kim Y J, et al. Directional matrix nanotopography with varied sizes for engineering wound healing[J]. *Advanced Healthcare Materials*, 2017, 6(19): 1700297.
- [15] Gui N, Xu W, Myers D E, et al. The effect of ordered and partially ordered surface topography on bone cell responses: a review[J]. *Biomaterials Science*, 2018, 6(2): 250-264.
- [16] Li G C, Li S J, Zhang L L, et al. Construction of biofunctionalized anisotropic hydrogel micropatterns and their effect on schwann cell behavior in peripheral nerve regeneration[J]. *ACS Applied Materials & Interfaces*, 2019, 11(41): 37397-37410.
- [17] Tamiello C, Buskermolen A B C, Baaijens F P T, et al. Heading in the right direction: understanding cellular orientation responses to complex biophysical environments[J]. *Cellular and Molecular Bioengineering*, 2016, 9(1): 12-37.
- [18] Matschegewski C, Staehlke S, Loeffler R, et al. Cell architecture-cell function dependencies on titanium arrays with regular geometry [J]. *Biomaterials*, 2010, 31(22): 5729-5740.
- [19] Staehlke S, Koertge A, Nebe B. Intracellular calcium dynamics dependent on defined microtopographical features of titanium[J]. *Biomaterials*, 2015, 46: 48-57.
- [20] Matschegewski C, Staehlke S, Birkholz H, et al. Automatic actin filament quantification of osteoblasts and their morphometric analysis on microtextured silicon-titanium arrays[J]. *Materials*, 2012, 5(7): 1176-1195.
- [21] Fischer R S, Sun X Y, Baird M A, et al. Contractility, focal adhesion orientation, and stress fiber orientation drive cancer cell polarity and migration along wavy ECM substrates[J]. *Proceedings of the National Academy of Sciences of the United States of America*, 2021, 118(22): 2021135118.
- [22] Tilbury K B, Campbell K R, Eliceiri K W, et al. Stromal alterations in ovarian cancers via wavelength dependent Second Harmonic Generation microscopy and optical scattering[J]. *BMC Cancer*, 2017, 17(1): 102.
- [23] Provenzano P P, Eliceiri K W, Campbell J M, et al. Collagen reorganization at the tumor-stromal interface facilitates local invasion[J]. *BMC Medicine*, 2006, 4(1): 38.
- [24] Conklin M W, Gangnon R E, Sprague B L, et al. Collagen alignment as a predictor of recurrence after ductal carcinoma *in situ* [J]. *Cancer Epidemiology, Biomarkers & Prevention*, 2018, 27(2): 138-145.
- [25] Chuang Y J, Tseng F G, Lin W K. Reduction of diffraction effect of UV exposure on SU-8 negative thick photoresist by air gap elimination[J]. *Microsystem Technologies*, 2002, 8(4): 308-313.
- [26] Park J H, Steingart D A, Kodambaka S, et al. Electrochemical electron beam lithography: write, read, and erase metallic nanocrystals on demand[J]. *Science Advances*, 2017, 3(7): e1700234.
- [27] Córdoba R, Ibarra A, Maily D, et al. Vertical growth of superconducting crystalline hollow nanowires by He⁺ focused ion beam induced deposition[J]. *Nano Letters*, 2018, 18(2): 1379-1386.
- [28] McGehee W R, Michels T, Aksyuk V, et al. Two-dimensional imaging and modification of nanophotonic resonator modes using a focused ion beam[J]. *Optica*, 2017, 4(11): 1444-1450.
- [29] Malloy M, Litt L C. Technology review and assessment of nanoimprint lithography for semiconductor and patterned media manufacturing[J]. *Journal of Micro/Nanolithography, MEMS, and MOEMS*, 2011, 10(3): 032001.
- [30] Bhardwaj N, Kundu S C. Electrospinning: a fascinating fiber fabrication technique[J]. *Biotechnology Advances*, 2010, 28(3): 325-347.
- [31] Jin F, Liu J, Zhao Y Y, et al. $\lambda/30$ inorganic features achieved by multi-photon 3D lithography[J]. *Nature Communications*, 2022, 13

- (1): 1-10.
- [32] Wang J Y, Jin F, Dong X Z, et al. Flytrap inspired pH-driven 3D hydrogel actuator by femtosecond laser microfabrication[J]. *Advanced Materials Technologies*, 2022, 7(8): 2200276.
- [33] Gao W, Chao H, Zheng Y C, et al. Ionic carbazole-based water-soluble two-photon photoinitiator and the fabrication of biocompatible 3D hydrogel scaffold[J]. *ACS Applied Materials & Interfaces*, 2021, 13(24): 27796-27805.
- [34] 高文, 郑美玲, 金峰, 等. 飞秒激光快速制备大面积二维微纳结构[J]. *激光与光电子学进展*, 2020, 57(11): 111421.
Gao W, Zheng M L, Jin F, et al. Fast fabrication of large-area two-dimensional micro/nanostructure by femtosecond laser[J]. *Laser & Optoelectronics Progress*, 2020, 57(11): 111421.
- [35] 陈林森, 乔文, 叶燕, 等. 面向柔性光电子器件的微纳光制造关键技术与应用[J]. *光学学报*, 2021, 41(8): 0823018.
Chen L S, Qiao W, Ye Y, et al. Critical technologies of micro-nano-manufacturing and its applications for flexible optoelectronic devices[J]. *Acta Optica Sinica*, 2021, 41(8): 0823018.
- [36] Wang T W, Dong X Z, Jin F, et al. Consistent pattern printing of the gap structure in femtosecond laser DMD projection lithography [J]. *Optics Express*, 2022, 30(20): 36791-36801.
- [37] 匡珺洁, 罗宁宇, 张静雅, 等. 基于空间光调制器的并行微纳光刻技术研究进展[J]. *激光与光电子学进展*, 2022, 59(11): 1100009.
Kuang J J, Luo N N, Zhang J Y, et al. Review on the progress of parallel micro/nano lithography based on spatial light modulator[J]. *Laser & Optoelectronics Progress*, 2022, 59(11): 1100009.
- [38] 周子逸, 董贤子, 郑美玲. 数字微镜无掩模光刻技术进展及应用[J]. *激光与光电子学进展*, 2022, 59(9): 0922030.
Zhou Z Y, Dong X Z, Zheng M L. Evolution and application of digital micromirror device based maskless photolithography[J]. *Laser & Optoelectronics Progress*, 2022, 59(9): 0922030.
- [39] Liu Y H, Zhao Y Y, Jin F, et al. $\lambda/12$ super resolution achieved in maskless optical projection nanolithography for efficient cross-scale patterning[J]. *Nano Letters*, 2021, 21(9): 3915-3921.
- [40] 王荣荣, 张维彩, 金峰, 等. 双光子聚合制备聚苯胺微结构[J]. *中国激光*, 2021, 48(2): 0202006.
Wang R R, Zhang W C, Jin F, et al. Fabrication of polyaniline microstructure via two-photon polymerization[J]. *Chinese Journal of Lasers*, 2021, 48(2): 0202006.
- [41] 张维彩, 郑美玲, 董贤子, 等. 高精度水凝胶微图案的快速制备及其对细胞行为的诱导[J]. *光电工程*, 2022, 49(2): 0210336.
Zhang W C, Zheng M L, Dong X Z, et al. Rapid preparation of high-precision hydrogel micropatterns and its induction of cell behavior[J]. *Opto-Electronic Engineering*, 2022, 49(2): 0210336.
- [42] Kang M S, Han C, Jeon H. Submicrometer-scale pattern generation via maskless digital photolithography[J]. *Optica*, 2020, 7(12): 1788-1795.
- [43] Jung Y, Lee H, Park T J, et al. Programmable gradational micropatterning of functional materials using maskless lithography controlling absorption[J]. *Scientific Reports*, 2015, 5(1): 1-7.
- [44] Shi W X, Xu T L, Xu L P, et al. Cell micropatterns based on silicone-oil-modified slippery surfaces[J]. *Nanoscale*, 2016, 8(44): 18612-18615.
- [45] Ridley A J, Schwartz M A, Burridge K, et al. Cell migration: integrating signals from front to back[J]. *Science*, 2003, 302(5651): 1704-1709.
- [46] Pollard T D, Borisy G G. Cellular motility driven by assembly and disassembly of actin filaments[J]. *Cell*, 2003, 112(4): 453-465.
- [47] Ladoux B, Nicolas A. Physically based principles of cell adhesion mechanosensitivity in tissues[J]. *Reports on Progress in Physics*, 2012, 75(11): 116601.

Fabrication of Curved Micro-Nano Topography Structures and Regulation of Cell Behavior

Guo Min^{1,2}, Liu Xiangyang^{1,2}, Dong Xianzi¹, Liu Jie¹, Jin Feng¹, Zheng Meiling^{1*}

¹Key Laboratory of Bionic Materials and Interface Science, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing 100190, China;

²School of Future Technology, University of Chinese Academy of Sciences, Beijing 101407, China

Abstract

Objective The fields of tissue engineering and regenerative medicine have seen significant advancements, prompting extensive research into interactions between biomaterials and cells. Factors, such as surface chemical composition, stiffness, and topography, can influence cell adhesion, proliferation, migration, and differentiation. Advanced micro-nanofabrication technologies have made it possible to study cell-responsive topographical structures. Many cell types exhibit contact guidance behavior on groove structures, which in turn affects cell function. However, *in vivo*, normal tissues often exhibit curved and wavy extracellular matrix (ECM) structures, which differ significantly from previously fabricated regular topographies. Hence, in this study, maskless optical projection lithography (MOPL) was utilized to fabricate broken lines and wavy structures with varying curvatures. Groove structures were also created as controls. We examined cell migration behavior and morphological changes on the fabricated structures, proposing a cell migration mechanism specific to curved structures. This research aims to enhance our understanding of cell-surface topography interactions and provide valuable insights for designing implant materials.

Methods A series of micro-nano topography structures were successfully created using maskless optical projection lithography. The surface morphologies of these structures were characterized via scanning electron microscopy (SEM) and atomic force microscopy (AFM). To promote cell adhesion, the fabricated structures were treated with O₂ plasma and coated with poly-D-lysine (PDL). Cells were then cultured on the topography structures, and their migration behavior was monitored using a live cell station. After cell culture on the topography structures for 24 h, the cells were fixed and labeled with DAPI and phalloidin fluorescent probes for nuclei and actin, respectively. Quantitative analysis of cell roundness, cell perimeter, and cell spreading area was conducted to determine changes in cell morphology. The fabricated topography structures were found to induce cell patterning, and their biocompatibility was

confirmed via cell co-culture experiments.

Results and Discussions We successfully fabricated grooves, broken lines, and wavy structures using the MOPL technique. SEM images revealed the integrity and uniformity of the topography structures, while AFM images displayed 3D morphology of these structures. According to the AFM cross-sectional profiles, ridge heights within individual topography structures are consistent. Moreover, we subjected the surfaces of these structures to the same chemical treatment, thus isolating the impact of structural topography on cell behavior. Cell migration tracking experiments demonstrated that cells form straight, broken, and curved migration paths in accordance with the underlying topography. This finding suggests that topography structures significantly influence cell migration. Interestingly, cells on wavy structures with high curvature alter their original migration direction from along the ridge to along the curve. High curvature wavy structures prompt cell migration in the curved direction. Confocal fluorescence imaging revealed cell morphology on various topography structures. We observed that cells elongate and migrate along ridges in line regions, whereas they display reduced elongation and adopt curved morphologies in corner regions. In the corner regions of broken-line structures, cells exhibit right-angled morphologies. Actin stress fibers in cells align with the curved direction in high-curvature wavy structure corners whereas these fibers align with ridge direction in wavy structure line regions. We propose that this alignment represents an intrinsic mechanism driving cell migration in curved directions. Furthermore, we cultured cells on annular and hexagonal structures, resulting in cell patterns influenced by the induction effect of curved structures on cell migration. Live/dead fluorescence staining revealed that cells grown with the Mito-Tracker Green channel thrive, with almost no dead cells detected in PI channels. This finding suggests that the fabricated topography structures exhibit excellent biocompatibility.

Conclusions We fabricated custom-designed topography structures using the MOPL technique, which presents a flexible and efficient method for creating micro/nanostructures. The cells exhibit migration trajectories that strictly adhere to the topography of grooves, broken lines, and wavy structures. In line regions, cells adopt an elongated morphology, while they assume a polygonal shape and increased roundness in corner regions of the topography structures. We posit that the high curvature of wavy structures generates an induction effect on cells, causing cytoskeletal rearrangement and cell migration along the curved direction. This observation suggests that topography plays a role in directing cell migration. By designing patterned structures based on cells' responsiveness to wavy structures, cell patterning can be realized. This study demonstrates that topography can significantly alter cell morphology and migration, enhancing our understanding of cell response mechanisms to topography. Additionally, our findings serve as a reference for designing implant materials.

Key words medical optics; femtosecond laser; maskless optical projection lithography; curve topography structures; cell migration; cytoskeleton