

Exploring Cell-Material Interactions at Focal Adhesion Points

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Background

In tissue engineering, electrospun fibers are pivotal scaffolds, shaping cell interactions through their mechanical strength and surface characteristics. Focal adhesion sites, multi-protein complexes, regulating cell adhesion dynamics, form mechanical links between intracellular actin bundles and the substrate. Widely used in bioengineering, PMMA fibers play a crucial role as an integral component of bone implants, serving as an adhesive platform for various cell types (1). The aim of the research is to investigate the distribution and structure of proteins paxillin and vinculin in focal adhesion (FA) sites using advanced microscopy techniques, particularly AiryScan confocal super-resolution microscopy and the Density-Based Spatial Clustering of Applications with Noise (DBSCAN) cluster algorithm. Moreover, this research is performed on cells bind to polymer fibers commonly used in tissue scaffolds and compared to standard glass slides. Therefore, our results contribute to designing scaffold and understanding the interactions between cells and materials applied in/for tissue regeneration.

Methods

PMMA fibers were electrospun using the EC-DIG apparatus with climate control. Studies involved human osteoblast-like cell line MG-63 on PMMA electrospun fibers. Cells were cultured under standard conditions, fixed, and permeabilized after 3 days. Actin filaments were visualized with Alexa Fluor 488 Phalloidin, and nuclear DNA was stained with DAPI. Focal adhesion proteins (vinculin and paxillin) were labeled with immunofluorescence. Confocal microscopy, specifically Zeiss LSM 900 with the high-resolution concept Airyscan, captured multicolor 3D microscopy images of cells connecting to PMMA fibers. Image analysis was performed by using ImageJ.

Results

AiryScan microscopy offered detailed insight into cell adhesion onto PMMA fibers, confirming the binding process. Additionally, DBSCAN cluster analysis revealed substrate-specific correlations in osteoblast behavior, providing a quantitative understanding of cell-PMMA interactions. Effective adhesion correlated with well-organized, larger focal adhesions characterized by increased protein accumulation. Interestingly, a shift in paxillin and vinculin signals was observed during cell attachment to both glass and polymer fibers. Focal adhesions on polymer fibers appeared smaller and elliptical but exhibited higher protein density compared to glass (2). These attributes, influenced by paxillin and vinculin, likely signify cell adhesion strength. This innovative cluster analysis uncovers variations in adhesion clusters, offering valuable insights for scaffold design refinement, diverse substrate adhesion evaluation, and enhanced cellular interactions in biomedical applications.

Conclusion

We introduce a new method for analyzing protein distribution in cell adhesion to polymer fibers, crucial for tissue engineering scaffolds. Our study reveals significant differences in adhesion complex distribution compared to glass, with a focus on vinculin and paxillin. By quantitatively comparing osteoblast adhesion on glass and polymer scaffolds, we provide the first detailed analysis in a model incorporating polymer fibers, paving the way for understanding cell-material interactions in medical biomaterial research.

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