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Magnetic Recovery Method Of Magnetically Responsive High-Aspect Ratio Photoresist Microstructures

Tech ID: 21454 / UC Case 2010-528-0

PATENT STATUS

Country	Type	Number	Dated	Case
United States Of America	Issued Patent	9,340,417	05/17/2016	2010-528

BRIEF DESCRIPTION

The recent identification of rare cell populations within tissues that are associated with specific biological behaviors, for example, progenitor cells, has illuminated a limitation of current technologies to study such adherent cells directly from primary tissues. The micropallet array is a recently developed technology designed to address this limitation by virtue of its capacity to isolate and recover single adherent cells on individual micropallets.

The capacity to apply this technology to primary tissues and cells with restricted growth characteristics, particularly adhesion requirements, is critically dependent on the capacity to generate functional extracellular matrix (ECM) coatings. The discontinuous nature of the micropallet array surface provides specific constraints on the processes for generating the desired ECM coatings that are necessary to achieve the full functional capacity of the micropallet array. We have developed strategies, reported herein, to generate functional coatings with various ECM protein components: fibronectin, EHS tumor basement membrane extract, collagen, and laminin-5; confirmed by evaluation for rapid cellular adherence of four dissimilar cell types: fibroblast, breast epithelial, pancreatic epithelial, and myeloma.

These findings are important for the dissemination and expanded use of micropallet arrays and similar microtechnologies requiring the integrated use of ECM protein coatings to promote cellular adherence.

(GunnN.M., MS; Bachman M., Li G.P., Nelson E.L.Fabrication and biological evaluation of uniform extracellular matrix coatings on discontinuous photolithography generated micropallet arrays. J Biomed Mater Res A. 2010 Nov;95(2):401-12.)

FULL DESCRIPTION

A distinct advantage of the micropallet array is to be able to identify and recover viable single adherent cells from a heterogeneous mixture of cells. The unique geometry and characteristics of the micropallet array impose constraints on the use of ECM coatings. Such coatings are likely to be required for application of this technology to primary cells or cell lines with restricted growth conditions. Thus, specific issues surrounding the application of ECM components must be addressed, specifically the uniformity and stability of adhered ECM coatings, restriction of the ECM to the top micropallet surface and cell-type-specific affinities for the various ECM components. Early on we identified that although single cells could be placed onto the micropallet array, we frequently saw cells bridging from one micropallet to another. We postulated that this was potentially due to bridging of the ECM coating. To test the hypothesis and to evaluate the various parameters involved in ECM coating of the micropallet array, we also established imaging methods for the ECM coatings.

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OTHER INFORMATION

CATEGORIZED AS

- » **Biotechnology**
 - » Other
- » **Imaging**
 - » Other
- » **Medical**
 - » Devices
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Our findings support our hypothesis that the success of various ECM protein coatings for use with micropallet arrays to capture single cells depends on three properties: the adherence of the ECM coating to the micropallet top surfaces, the prevention of ECM protein networks that bridge neighboring micropallets, and the cells’ affinity for adherence to the ECM coating. We discovered that certain ECM coatings are problematic when applied in traditional manner to micropallet arrays because of poor adherence to the micropallets and/or formation of fibrillar bridging structures connecting multiple micropallets. This in turn, required the development of novel application strategies to overcome these issues and effectively coat micropallet arrays with each of four different ECM protein coatings: human FN, BME, collagen, and laminin-5.

We investigated the ability of each coating type to capture cells representing stromal, epithelial, and hematopoietic/inflammatory cell types on individual micropallets and were able to capture all cell types with one or more of the coatings. Cell adherence to each ECM coating was not affected by the micropallet-patterned surface in comparison with adherence experiments performed on solid 1002F photoresist surfaces. Finally, the capacity for the relative enrichment of a given cell population dependent on the strategic selection of ECM coatings reinforces the necessity to be able to effectively coat the micropallet array surface with various ECM components and provide the broadest range of applications for these types of technologies.

Micropallet arrays are a new technology with many versatile applications in the fields of adherent cellular analysis and separation, including single cell selection, colony selection, population characterization, and population purification. The successful application of this technology to the study of adherent cells is critically dependent on the ability to capture and isolate cells to single micropallets. The work presented in this article will enable and guide the use of micropallet arrays for a multitude of future uses in the isolation and study of discrete adherent cell populations. In addition to micropallet arrays, these findings have particular relevance for other systems that use ECM coatings and microscopically nonuniform surfaces. Such devices are increasingly common as advances in microtechnology are tailored for biological applications.

SUGGESTED USES

The work presented in this article will enable and guide the use of micropallet arrays for a multitude of future uses in the isolation and study of discrete adherent cell populations. In addition to micropallet arrays, these findings have particular relevance for other systems that use ECM coatings and microscopically nonuniform surfaces. Such devices are increasingly common as advances in microtechnology are tailored for biological applications.

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