Array of Biodegradable Microelements for Isolation and Implantation of Living, Adherent Cells

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Statement of Purpose: Implantation of viable cells is used to create xenograft tumor models, to study stem cell differentiation, and potentially for tissue repair.[1] The use of adherent cells for this purpose requires a series of steps starting with cell detachment from a culture dish, sorting of cells to obtain the desired phenotype, cell re-attachment on biodegradable microcarriers for final implantation into an animal. A new approach is described for isolation and direct implantation of adherent cells using a recently developed microarray platform for culture and selection of adherent cells.[2,3]

Methods: Biodegradable microelements (termed microrafts) were fabricated in a high-density array format on a polydimethylsiloxane (PDMS) microwell template. The array elements served as both culture surfaces and microcarriers for living, adherent cells. Poly(lactic-co-glycolic acid) (PLGA) materials were screened for manufacturing the microrafts. Degradation rates of the microcarriers in vitro and in vivo were investigated. A mixed population of wild-type and GFP-expressing H1299 cells were plated on the array and imaged by brightfield and epifluorescence microscopy (Fig. 1a-i and ii). Cells of interest (e.g. GFP cells) were identified and collected by dislodging individual microrafts from the array with a microneedle device (Fig. 1a-iii and iv). The isolated microrafts with their attached cells could then be transferred directly into mice for implantation. AsPC-1 cells stably expressing luciferase (AsPC-1-Luc) were used for implantation. Bio-luminescence in vivo imaging was used to follow the growth of implanted cells into a tumor in the recipient animals (Fig. 1b). Mice were euthanized and tumors harvested after 3 months. Photomicrograph of H&E stained sections confirmed the establishment of tumors in the mice (Fig. 1c).

Results: A simple drain coating process was used to micromold a high density array of PLGA microrafts. Three types of PLGA material were found to be suitable, showing bulk erosion starting 2-12 days after immersion in a buffer. H1299 cells plated on the PLGA microrafts array (Fig. 1a-i and ii) attached and grew. The array was optically clear permitting brightfield imaging to assess cell morphology. Microrafts and the attached cells were isolated from the array using a simple needle release device (Fig. 1a-iii and iv). Using the microraft carriers, the AsPC-1-Luc cells were efficiently transferred by subcutaneous implantation in a nude mouse to generate a tumor model system (Fig. 1b and c). Degradation of the implanted microrafts was confirmed over a 3-month period. The results demonstrated that the microraft array served as an effective means for selective cell or colony isolation and enabled efficient implantation of sorted cells.

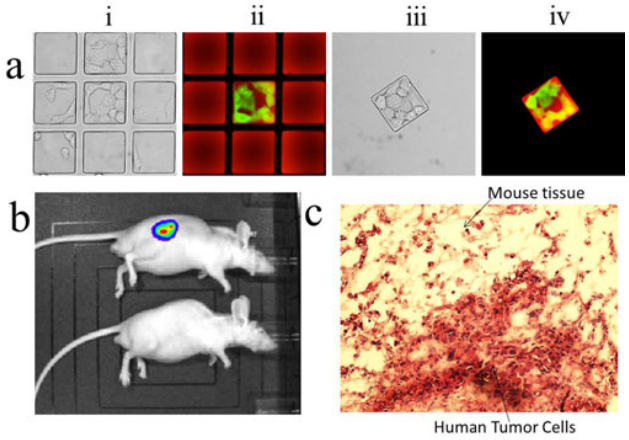

Figure 1. Array of microrafts composed of biodegradable PLGA for sorting and implantation of living, adherent cells. (a) Isolation of H1299-GFP cells on microrafts formed from PLGA conjugated with TF3 dye. (i-ii) Brightfield (i) and fluorescence (ii) images of a region of an array containing mixed fluorescent and non-fluorescent colonies of cells after 72-h culture. (iii-iv) Images of a fluorescent colony released and collected in a separate dish. The size of microrafts was 100 μm. (b) In vivo imaging of the growth of a xenograft tumor in a mouse (top) after implantation of 500 microrafts with AsPC-1-Luc cells over 53 days. A mouse lacking implanted cells was used as a control (bottom). (c) Photomicrographs of an H&E stained tissue section. The tumor was harvested from a mouse at 3 months after implantation. Tumor cells are seen surrounded by normal subcutaneous tissue (100×).

Conclusions: We have demonstrated a new strategy for the isolation and delivery of living, adherent cells for transplantation using an array composed of biodegradable PLGA microrafts. Cells of a desired phenotype can be directly imaged and identified on the array based on a variety of selection criteria including fluorescence signature, morphology, and growth rate among others. The strategy is most advantageous when a highly purified sample of cells is needed for direct implantation into animal models.

References:

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