MICROCARRIER ENABLED MICROFLUIDIC ASSAYS OF ANCHORAGE DEPENDANT CELLS

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Abstract:
We present the use of microcarriers as a method to greatly expand the cell types available for microchip based cell assays that rely on fluidic transport of cell suspensions through functional zones. Cells attached to the microcarriers remains viable and responsive to cell stimulation even after flow at high linear velocities through microfluidic systems.

Keywords: Microcarrier, Cell signaling, Protein pathways

Introduction:
Many microchip based cell assays rely on fluidic transport of cell suspensions through functional zones, such as cell sorting areas [1] or cell stimulation and lysis zones [2]. Most mammalian cells are adherent and anchorage dependant cells that can only be put in suspension by treatment with a proteolytic agent such as trypsin. However, this treatment and the loss of adhesion severely perturb the biological machinery of the cells causing interference in many assays. Instead we show that by attaching the cells to microcarriers cells can be suspended while retaining their normal anchorage dependent functionality. We further demonstrate this technique in a microfluidic device for cell signaling applications (i.e., studies of protein expression). Large microcarrier particles (100μm-1000μm), not suitable for transport in microfluidic systems, have preciously been utilized in cell culture systems, both conventional and microscale [3], because of the increased surface to volume ratio that can be achieved with microcarrier culture. The use of smaller carriers in microfluidic systems is, however, not trivial because below a certain (cell dependant) size geometric effects can induce apoptosis in the cells [4].

Experimental
To minimize geometrical effects while keeping the beads sufficiently small for compatibility with microfluidic devices, we have used medium sized beads (30μm-50μm) for cell attachment. The beads were coated with collagen in order to enhance cell adhesion. HeLa cells were incubated with the beads in a 25mL spinner flask under gentle stirring for 24 hours before experiments. To test the applicability of the microcarriers for cell based assays a microfluidic device was fabricated using soft lithography in a PDMS layer that was bonded to 50mm x 75mm glass slide. The main channel is 3 m long, 300μm wide and 200μm deep with 3 inlets for the microcarrier suspension, cell stimulus, and gas (Figure 1). The latter can be used to create segmented

gas liquid flow for enhanced mixing [2]. The channel length ensures long residence times in the system even at relatively high linear velocities, which represents a worst case scenario with respect to the influence of shear in the fluidic system on cell viability and function.

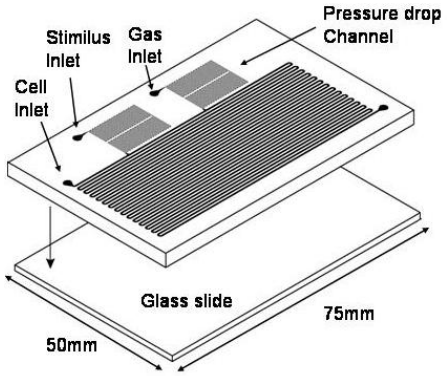

Figure 1. Schematic of the microfluidic system, with inlets for microcarrier introduction, cell stimulus and a gas inlet that can be used to form segmented gas-liquid flow. The main channel is 300µm wide, 200µm deep and approximately 300cm long.

Results

After 24 hours of incubation of HeLa cells with the beads in a 10:1 ratio, almost all cells adhered to the microcarriers and normal cell behavior such as cell division could be observed (Figure 2).

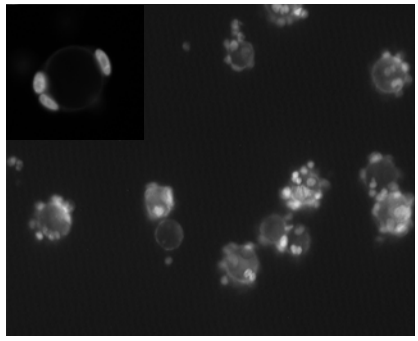

Figure 2. HeLa cells attached to collagen coated microcarrier beads. The histones in the nuclei have been transfected with green fluorescence protein (GFP) while the mitochondria in the cells have been dyed with a red fluoresce dye. Inserted in the upper left corner of the figure is a close up of what appears to be HeLa cells that have undergone cell division as indicated by the two closely separated nuclei.

Even after the microcarriers with the cells were flowed through the microfludic system at an average linear velocity of ~14mm/s (50µL/min) the cells remained viable and no difference could be observed in cell attachment compared to the populations in the spinner flask (Figure 3A). As an application in cell pathway activation, we have found that cells on the microcarriers continue to respond strongly to EGF and TNF stimulation, with a clear increase in the activated ERK signal of the cell stress sensitive MAP kinase pathway (Figure 3B). This shows that microcarrier techniques are applicable for cell signaling assays.

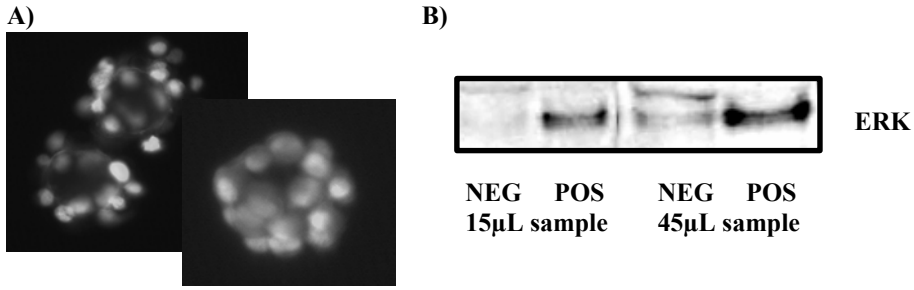

Figure 3. A) To the left is a micrograph close up of microcarriers after flow through the microfluidic system at 50µL/min. The cells appear to be attached to the same extend as those on the microcarriers directly from the spinner flask (Figure 2). To the right is a micrograph of regular (non-GFP) HeLa cells stained with a green viability dye after flow through the microfluidic system. All cells are viable after flow through the device.
B) Western blot of P-ERK from cell lysates of HeLa cells on microcarriers. Lanes marked "POS" are from cells treated with EGF/TNF while lanes marked with "NEG" are from untreated cells. There are clear signals (scaling with sample size) in the positive samples. The negative control samples only show a weak signal.

Acknowledgments: This research was funded by National Institutes of Health (GM-68762)

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