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Research review paper

Cell detachment: Post-isolation challenges

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ABSTRACT

Rare cells already have become established indicators for disease diagnosis, to help track prognosis, and in developing personalized therapy. Numerous techniques have been developed to effectively and specifically detect and sort rare cells and cell isolation techniques have gained much attention among researchers in the last few decades. Recent developments in nanotechnologies and microfluidics have been used with great promise towards these goals. The research emphasis has also shifted from simple detection with microfluidic devices to comprehensive isolation, collection and subsequent analysis with integrated and automated systems. The first challenge in post-isolation analysis is cell detachment from substrates, while keeping cells viable and unperturbed. In this review, various methods used for cell detachments are discussed. For effective cell sorting, the detachment is identified as critical criteria for selecting substrates and methods.

Contents

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1. Introduction

Cells with less than 1000 count in a milliliter of sample are defined as rare cells (Dharmasiri et al., 2010). These include circulating tumor cells (CTCs), circulating endothelial cells, hematopoietic stem cells, HIV infected CD4 + T cells and many others. Rare cells have garnered significant interest from the scientific and clinical communities as these may provide invaluable information for disease diagnostics, prognostics and therapeutics (Baum et al., 1992; De Coppi et al., 2007; Hansford et al., 2007; Iverson et al., 1981; Nagrath et al., 2007; Peterlin and Trono, 2003; Simpson, 1993; Singh et al., 2003; Toma et al., 2001; N. Uchida et al., 2000). To efficiently isolate rare cells in a viable state, a number of approaches have been reported, including devices that rely on mechanical forces, dielectrophoresis, optical interactions, magnetic sorting, flow cytometry, and microfluidic channels. Previous reviews have discussed many of these rare cell isolation approaches (Dharmasiri et al., 2010; Yu et al., 2011). Recently, nanostructured substrates have been promisingly employed in rare cell isolation; these not only provide better isolation sensitivity and specificity but also can keep captured rare cells viable on the surface, while requiring very little effort in sample preparation (Chen et al., 2011; Kim et al., 2010, 2012; Lee et al., 2012; Park et al., 2012; Sekine et al., 2011; Wan et al., 2012b; Wang et al., 2009, 2011; N. Zhang et al., 2012). Few devices have been tested with real patients' body fluids for rare cell isolation, and encouraging results have been observed with ligand functionalized nanostructured substrates (Wang et al., 2011; N. Zhang et al., 2012).

There is great opportunity in not just rare cell enumeration and isolation but also to be able to do further analysis like whole cell culture and molecular profiling. These may guide personalized therapy and track cell genotype during treatment. For this, the captured rare cells should remain viable and detached for subsequent analysis (den Toonder, 2011). For example, CTCs hold the key in understanding the biology of metastasis and heterogeneity among tumor cells (Yu et al., 2011). If perturbations occur during the isolation process, stem cells may not remain suitable for cell-based therapy anymore (Chen et al., 2010). Similarly, viable HIV infected CD4 + cells would better reveal the dynamics of T cell population if these stay in the native state (Hazenberg et al., 2000). Currently, most analysis on captured cells is done by directly lysing cells on the device surface for downstream enzymatic reactions to measure DNA, mRNA, miRNA, ncRNA, and proteins. With high definition cell assays, such as CellSearch™ system, cell counting and downstream characterization requires fixing of cells that renders subsequent whole cell culture and analysis impossible (Marrinucci et al., 2012). However, the value of captured rare cells is much more than that; for example, FACS analysis can simultaneously analyse approximately 18 different proteins, which can provide better and more comprehensive understanding of protein expression at the single cell level (Bar-Even et al., 2006; Newman et al., 2006; Sachs et al., 2005). Thus, detachment and collection of captured rare cells from substrates without perturbing the microenvironment is a pressing issue.

In order to detach cells from substrates, two major adhesive forces need to be overcome. These are (i) interactions between the receptors and capture ligand, and/or (ii) focal adhesion. Cells themselves can detach from surfaces during their migration by complex signal regulation and participation of multiple proteins, however cells cannot be harvested by this active detachment. On the contrary, by means of external forces, cells can be effectively lifted off in a passive manner. Theoretical models predict a logarithmic dependence of mechanical detachment on adhesive forces (Bell, 1978; Dembo et al., 1988), and data have been obtained to experimentally test these models (Kuo and Lauffenburger, 1993; Wang and Lin, 2007). It is now of utmost importance to find reasonable ways to overcome the adhesive forces while keeping cells viable and unperturbed.

Traditionally, enzymatic treatment for cell detachment is most common. This process digests extracellular matrix (ECM) proteins and cells are released. This treatment is invasive as other cell surface proteins (ion channels, receptors, cell-to-cell junction proteins, etc.) are also digested (Keizer et al., 1988). As an alternative, a temperature induced cell detachment method, based on the fact that ECM generally adheres to a hydrophobic surface rather than highly hydrophilic surface, was also developed (McAuslan and Johnson, 1987). Although the need for less invasive cell harvesting methods is not discounted by the community, only a few works like electricity-induced, pH change-induced, and light-induced methods have approached this issue (Hong et al., 2013). Only a few almost non-invasive cell detachment methods such as those using aptamers have been shown for cell detachment. It is thus necessary to summarize these available cell detachment technologies and have a comparison among them. This review introduces cell detachment strategies and their respective mechanisms, limitations, practical applications, and future directions. We define a few criteria for selecting ideal substrates for rare cell capture and detachment.

2. Cell detachment strategies

The cell detachment strategies span a wide range, including cell biology, molecular biology, biomaterial science, tissue engineering, and electrical engineering. Techniques developed from different backgrounds emphasize different purposes and practical applications. For example, in tissue engineering complete cell sheet lift-off is preferred; contrarily, laser mediated technique can precisely detach a targeted single cell while aptamer functionalized substrates show promise in the capability of surface regeneration. Therefore, a simple comparison of cell detachment efficiency and viability of lifted-off cells among these techniques may downplay the real foci of the respective works. We introduce various cell detachment approaches and discuss their respective advantages and limitations. This can help define various benchmarks for selecting or developing suitable materials and/or methods for reaching ideal cell detachment in future works. Cell detachment conditions, releasing times, detachment rates and cell viability rates for typical approaches are summarized in Table 1.

2.1. Active cell detachment

In cell biology, adhesion of cells to ECM is a key step in the regulation of cell morphology, migration, proliferation and differentiation (Ridley et al., 2003). Integrin heterodimers on cell membrane act as the predominant receptors to recognize ECM ligands, to form focal adhesions, and to mediate cell adhesion to ECM (Mitra et al., 2005). On the contrary, during migration, numerous proteins such as microtubules, Kinesin-1, phosphatases, focal adhesion kinase, Calpain-2, ZF21, etc. are involved in the disassembly of focal adhesion complexes. Therefore, cells can be released from ECM by reducing regional adhesive forces (Nagano et al., 2012). However, in rare cell capture and release, it might affect the functionalities of the cells while detaching the captured cells from substrate by regulation of biomolecules in cytoplasm, since the attachment mechanisms and regulation pathways are not fully known. Hence, passive detachment of cells could be more efficient and provide more manipulative power.

2.2. Flowing fluid mediated detachment

The shear stress generated by a controlled fluid, a measure of tangential force per unit area, is widely applied to detach the attached cells. The cells detach from the microfluidic device surfaces when the hydrodynamic force can overcome the cell adhesive force (Fig. 1). The effects of flow rate, flow acceleration, and flow type in cell detachment have been studied before (Abu-Reesh and Kargi, 1989; Cheung et al., 2009; Lu et al., 2004). Technically, applying flowing fluid to attached cells is not difficult; a well-sealed microfluidic flow-through device and a flow-rate controllable syringe pump can satisfy the basic configurations of cell detachment. However, the major issue is on choosing the suitable shear stress to attached cells. Various factors, such as antibody