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A novel, compact disk-like centrifugal microfluidics system for cell lysis and sample homogenization

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Abstract

In this paper, we present the design and characterization of a novel platform for mechanical cell lysis of even the most difficult to lyse cell types on a micro or nanoscale (maximum 70 μL total volume). The system incorporates a machined plastic circular disk assembly, magnetic field actuated microfluidics, centrifugal cells and tissue homogenizer and centrifugation system. The mechanism of tissue disruption of this novel cell homogenization apparatus derives from the relative motion of ferromagnetic metal disks and grinding matrices in a liquid medium within individual chambers of the disk in the presence of an oscillating magnetic field. The oscillation of the ferromagnetic disks or blades produces mechanical impaction and shear forces capable of disrupting cells within the chamber both by direct action of the blade and by the motion of the surrounding lysis matrix, and by motion induced vortexing of buffer fluid. Glass beads or other grinding media are integrated into each lysis chamber within the disk to enhance the transfer of energy from the oscillating metal blade to the cells. The system also achieves the centrifugal elimination of solids from each liquid sample and allows the elution of clarified supernatants via siphoning into a collection chamber fabricated into the plastic disk assembly. This article describes system design, implementation and validation of proof of concept on two samples—*Escherichia coli* and *Saccharomyces cerevisiae* representing model systems for cells that are easy and difficult to lyse, respectively.

Keywords: Mechanical lysis/homogenization; Molecular biology sample preparation; Cell disruption; Cell lysis; Microfluidics; CD; Disk

1. Introduction

Tissue homogenization and cell lysis are the first steps in virtually all molecular biology and molecular diagnostic techniques. The purpose of cell lysis is to disrupt the cells and release genomic and proteomic material to enable downstream processing. Cell lysis and subsequent nucleic acid or protein purification represent a considerable obstacle commonly encountered in molecular biology sample preparation protocols. Although easily attainable with certain cells and microorganisms, many cells of interest pose significant challenges due to increased cell wall structural integrity. Molecular biology sample preparation kits and equipment are part of a rapidly growing multi-billion dollar market [1]. It has been estimated that up to 15% of researchers' time is consumed with the simple and redundant task of sample preparation [2]. Cell lysis is routinely accomplished by one or more of several means, including osmotic lysis [3] (i.e. rapid dilution), freeze–thaw [4] (e.g. rapid freeze–thaw cycles in special buffers), enzymatic lysis [5,6] (i.e. application of enzymes such as lysozyme to digest cell walls), chemical lysis [7] (e.g. utilization of detergents to dissolve cell membranes), viral lysis [8,9] (e.g. exposure of cells to virus or bacteriophage, wherein the virii are employed to compromise the cell wall), plasma lysis [10] (e.g. a recently discovered technique in which electrical discharge is used to disrupt the cell membrane), thermal lysis [11] (e.g. application of an autoclave method) and various forms of mechanical lysis (i.e. utilization of mechanical forces such as shearing and impaction to mechanically destroy cell walls and

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membranes). Various aspects of these classical cell lysis technologies are well documented [16], however, a brief treatment is in order here.

While enzymatic and chemical lysis at first glance is a very elegant method [12], the largest drawback is introduction of the enzyme and/or chemical lysis reagents to facilitate downstream sample manipulation. This method is further complicated by the time required for efficient lysis and generally low yields obtained with difficult-to-lyse tissues. Often two techniques such as mechanical and enzymatic lysis are combined to improve the efficacy of cell disruption [15]. Among these methods, mechanical lysis is the most practical for downstream processing and is the most effective in hard-to-lyse cells and tissues such as yeast [13] and Gram-positive bacteria [14].

Optimal mechanical tissue homogenization and cell lysis is a compromise between two important parameters—efficiency of cell lysis, which is quantified by the amount of DNA or RNA extracted, and quality of the resulting macromolecules, referring to preservation of macromolecular structural integrity. In terms of mechanical lysis these two requirements are often mutually exclusive—the amount of energy required to achieve quantitative lysis may result in macromolecular shearing of the DNA, temperature enhanced enzymatic and thermal degradation of RNA and conformational denaturation of protein. Each of these is the undesirable result of mechanical work applied to the cell system. Thus, mechanical lysis is often punctuated into multiple steps to minimize macromolecular damage. Nevertheless, in order to extract intact genomic DNA, RNA and/or protein with well-preserved tertiary or ternary structures from cells that are difficult to lyse, one must compromise lysis efficiency for product quality.

Classical mechanical homogenization and lysis can be performed in a variety of ways including mechanical homogenization [17], French-press [18], ultrasonication [19] and bead-beating [20]. Mechanical homogenization is often accomplished with simple rotor-stator shear force homogenizers such as Fred Osius' Waring blender utilizing moving, cutting and impinging blades to shear and disrupt cellular tissue to generate lysates and homogenates [35]. Mechanical homogenizers range in design from such kitchen blenders to disk-like disintegrator systems with disposable blades and suction to maximize mixing efficiency [17]. Most mechanical homogenizers are designed with rotors and stators, where shearing occurs between these two components. In such cases most of the lysis is achieved by shear force alone. Ultrasonic lysis [19] is accomplished by applying ultrasound directly to the sample. During the sonication process, ultrasound waves rupture the cells by repeated rapid compression and expansion, and by the action of sonolytically generated free radicals. The increased thermal effects of sonification, including bubble generation in the surrounding medium, enhance lysis efficiency, but are detrimental to thermally labile macromolecules. Most ultrasound homogenizers are hand-held units, with the semi-disposable energy-transfer tips. The French press accomplishes cell lysis through the utilization of shear force and pressure differences. Cells are forced through the small orifice under extremely high pressure into an environment of low pressure. Cells are lysed during the turbulent and rapid change in pressure. Rapid decompression or "cell bomb" instruments work by rapidly applying and releasing extreme pressure on cells in a contained environment. Normally, inert gases such as Argon are employed to generate these high pressures. This method seldom works well with tissues and cells that are difficult to lyse.

Finally, the most efficient and elegant method of mechanical cell lysis is bead-beating [21,22]. Bead-beating is accomplished by combining a liquid or solid sample with milling beads in a closed container and exposing the entire mixture of grinding media, buffer and tissue to intense mixing by rapid and abrupt motion of the container. Lysis occurs predominantly as a result of two types of collisions between milling beads and cells, puncture by direct collision and friction-based shearing. If a component of angular motion is introduced into a bead-beating system, then additional lysis results from impaction and friction between cells and the lysing matrix as a result of the Coriolis effect [28]. The lysing matrix directly contributes to lysis again during centrifugation, as observed previously in a centrifugal CD-based homogenizer [28].

The instrumentation of bead-beating technology includes vortex-like shakers [22], linear motion shakers, such as mixer-mills [23] and orbital shakers, such as the FastPrep® family of machines, which are currently considered the optimal method for homogenization of difficult to lyse cells, as well as for sample preparation from complex matrices such as soil [24,25]. The FastPrep® system is able to lyse most tough samples in 40 s or less [26] and is superior to other mechanical lysis systems [27]. Another interesting new device for sample lysis is the disk-based homogenizer [28] wherein lysis results from a combination of both impaction and shear force, and Coriolis force induced collisions between cells and grinding media.

Sample scale is an extremely important factor in many laboratories where samples are miniscule and irreplaceable. However, most commercial lysis systems require sample volumes of at least 1 mL. Thus, there is a considerable need for a microfluidics sample preparation system capable of manipulating samples in the microliter range [29]. A microfluidics cell lysis system has been reported that utilizes local hydrogen peroxide generated radicals [30], however, we are currently unaware of a commercially available microfluidic lysis system. In this article, we describe the continued development of a novel, plastic disk-based system for small scale (up to 70 μL) sample homogenization and cell lysis utilizing bead-beating and mechanical stator-rotor lysis in a semi-disposable system. This concept is compatible with the CD-based centrifugal microfluidics bioanalytical instrumentation, as recently reviewed in Ref. [31]. The system herein described, implements magnetic field actuated microfluidics in a plastic disk assembly the size of a standard compact disk (CD). Individual chambers within the CD assembly harbor flat ferromagnetic metal disks and user-defined bead-beating matrices. The selection of lysis matrix material depends on the intended application and spans of wide variety of grinding materials, such as glass beads, silica and garnet. Tissue homogenization and cell lysis are achieved via the relative motion of these metal disks and bead matrices in an oscillating magnetic field. The mechanical impaction and shear forces