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Automated tissue dissociation for rapid extraction of viable cells

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Abstract

Viable cells from healthy tissues are a rich resource in high demand for many next-generation therapeutics and regenerative medicine applications. Cell extraction from the dense connective matrix of most tissues is a labor-intensive task and high variability makes cGMP compliance difficult. To reduce costs and ensure greater reproducibility, automated tissue dissociators compatible with robotic liquid handling systems are required. Here we demonstrate the utility of our automated tissue dissociator that is compatible with standard microtiter well plates for high-throughput processing. We show that viable cell yields match or exceed manual methods while reducing processing time by at least 85%.

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1. Introduction

Cells are a fundamental research tool with diverse downstream applications. The vast majority of biomedical research has been accomplished through use of cell culture in which transformed or cancerous cell lines are propagated in the lab indefinitely. These cancerous cells, while inexpensive, robust, and able to provide numerous key insights, ultimately fail to accurately recapitulate how healthy normal cells respond to their environments [1]. Although cell lines are a key tool for high-throughput drug screening by pharmaceutical companies [2], they are entirely unsuitable for regenerative medicine applications or for use as therapeutics in patients [3]. For these reasons, there is a substantial drive in biomedical research to extract primary cells directly from healthy tissues—of either animal or human origin.

Separating large quantities of viable cells from highly connected tissue matrices is a complex and labor-intensive task [4]. Tissues are composite materials comprised of diverse cell types suspended in an extracellular matrix—which itself varies drastically depending on tissue type. For most tissues (blood being a notable exception), the extracellular matrix is a highly adhesive material that cements fragile cells together to function as a cohesive whole [5]. Tissue dissociation is therefore a careful balance between disrupting the tissue while not lysing the cells.

Researchers have largely turned to a combination of enzymatic and mechanical methods to extract viable cells [4, 6]. Enzymatic methods are effective but have several significant drawbacks [7, 8]. Because each tissue is comprised of different extracellular matrices, enzyme digestion must be optimized for each application. Enzymes must also be carefully sourced as they are subject to lot-to-lot variability and may potentially introduce viral and prion contaminants. These latter points make compliance with cGMP protocols difficult. Mechanical separation methods center around cutting and grinding with scalpels, frosted glass, mortar and pestles, and rotor-stator systems [9, 10]. They have the significant advantage of reproducibility and minimal optimization but are more labor intensive and more difficult to automate for high-throughput manufacturing. A semi-automated method based on a rotor-stator platform has increased tissue dissociation throughput and is eminently suitable for the research setting [11]. However, for industrial-scale processing of tissues as the sample input for stem cell factories, alternate features are required. We desired

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a tissue dissociation system that was robust enough to effectively homogenize the tissue while maintaining cell viability, was compatible with commercially-available microtiter plates, yielded an output that was readily accessible by fluid handling robotics, could be used with multiple tissue types, would process multiple organs in parallel for high-throughput platforms, and would ultimately reduce the need for enzymatic digestion.

We have developed an automated multiplexed tissue dissociator that uses a pestle array to grind tissues against the side walls of standard microtiter plates [12, 13]. The operator can easily vary rotation speed, process time, and pestle shape to allow for rapid optimization of the procedure. Compared to the current state-of-the-art in automated tissue homogenization, our platform has significantly more throughput than the leading instrumentation (GentleMACS, Miltenyi Biotec) and is compatible with standard microtiter plates. Additionally, the novel integrated mechanical design reduces the complexity and cost of controlling the radial orbit.

To demonstrate the utility of our instrument, we monitored cell viability after dissociation of murine spleen, uterus, lymph nodes, lung, and liver. The uterus was included to represent a particularly tough tissue and lymph nodes as an example of an extremely small tissue sample. We additionally demonstrate homogenization of human umbilical cord as a rich source of mesenchymal stem cells—ideal for a number of downstream research and therapeutic applications.

2. Materials and Methods

2.1 Tissue sourcing and handling

Murine spleens, uteri, lymph nodes, lungs, and liver used in testing were generously provided by our industry partner. All animals were maintained and cared for according to protocols approved by their Institutional Animal Care and Use Committee. Organs were taken from four female mice and placed in individual microcentrifuge tubes in RPMI media and placed on ice for shipping immediately after reclamation. Samples were processed within 36 h of harvesting to ensure maximum cell viability. Human umbilical cords were purchased from Bioreclamation IVT and were shipped at 4 °C in sterile saline solution and processed within 48 h from birth.

2.2 Manual processing of tissues

All murine tissues were placed in sterile petri dishes, bathed in 250 µL sterile PEB buffer (1X phosphate buffered saline, pH 7.2; 0.5% bovine serum albumin; 2 mM ethylenediaminetetraacetic acid), and were randomly assigned to either manual or automated groups. Whole tissue was then placed between two frosted glass slides (VWR) and carefully ground to homogeneity. Average manual processing time for spleens, lymph nodes, lungs, and liver was 5 min per sample. Uterine tissue typically required 8–10 min per sample. Tissues were then incubated for 5 min at 37 °C in an enzymatic digestion kit (Mouse Lung Dissociation Kit, Miltenyi Biotec). Next, tissues were filtered through 70-µm filters to remove any large remaining debris to achieve single cell suspensions (MACS SmartStrainer, Miltenyi Biotec). Cells were washed with 10 mL PEB buffer, spun down at 650 x *g* for 5 min at 4 °C, and resuspended in 300 µL PEB in preparation for cell viability staining. Human umbilical cord tissue was received in sterile saline solution and was immediately washed in 70% ethanol. Cord tissue was cut into 1-cm segments and temporarily stored in media (Dulbecco's Modified Eagle Medium, ThermoFisher). Segments for manual processing (1 cm) were minced using fine scissors for 15 min. Samples were then incubated in 10 mL PBS containing 2 U collagenase and 1 U hyaluronidase (SigmaAldrich) for 30 min at 37 °C.

2.3 Automated processing of tissue

Murine tissues were placed in wells of a 24-well microtiter plate (VWR) and 250 µL sterile PEB buffer was added. Tissues were ground at 800 RPM for either 30 s or 60 s, as indicated. Tissues were incubated with digestion buffer, filtered, and resuspended under identical conditions to manual processing. Human umbilical cord (250 mg) was placed in a 24-well microtiter plate (VWR) and 250 µL sterile PBS buffer was added. Tissues were ground at 650 RPM for 2 min and incubated in digestion buffer as before.

2.4 Cell viability determination

Cells were mixed with trypan blue exclusion buffer and counted on a hemacytometer according to standard protocols. Total numbers of viable cells were compared between manual and automated methods.

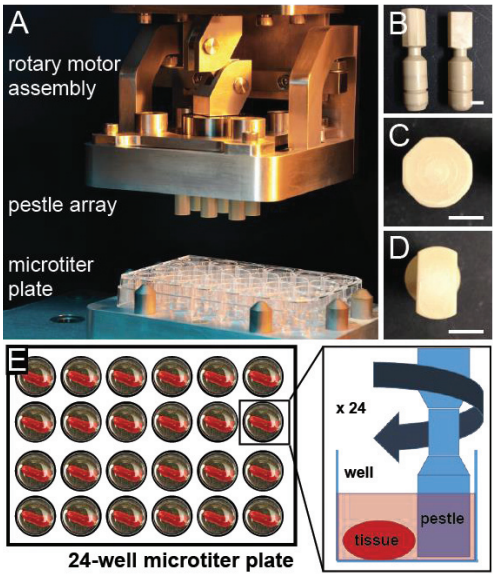

Figure 1. Automated tissue dissociator utilizes a pestle array orbiting the well of a standard microtiter plate to dissociate tissue (A). Pestles snap in and out of the array for easy optimization and tool switching (B). The octagonal pestle design was used for murine organs (C) while human umbilical cord used the pestle design shown in (D). 24 samples can be processed simultaneously (E). Scale bars= 0.5 cm