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Automating hESC differentiation with 3D printing and legacy liquid handling solutions

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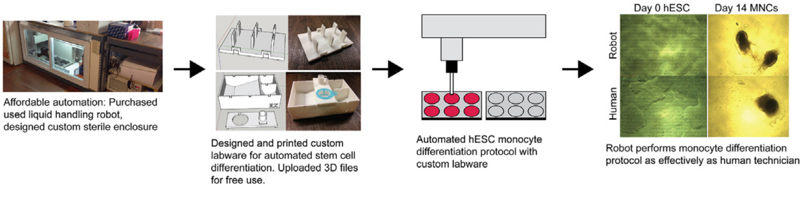
GRAPHICAL ABSTRACT



ABSTRACT

Historically, the routine use of laboratory automation solutions has been prohibitively expensive for many laboratories. As legacy hardware has begun to emerge on the secondary market, automation is becoming an increasingly affordable option to augment workflow in virtually any laboratory. To assess the utility of legacy liquid handling in stem cell differentiation, a used liquid handling robot was purchased at auction to automate a stem cell differentiation protocol that gives rise to CD14 + CD45+ mononuclear cells. To maintain sterility, the automated liquid handling robot was housed in a custom constructed HEPA filtered enclosure. A custom cell scraper and a disposable filter box were designed and 3D printed to permit the robot intricate cell culture actions required by the protocol. All files for the 3D printed labware are uploaded and are freely available.

- A used liquid handling robot was used to automate an hESC to monocyte differentiation protocol.
- The robot-performed protocol induced monocytes as effectively as human technicians.
- Custom 3D printed labware was made to permit certain cell culture actions and are uploaded for free access.

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Methods detail

Here, we assessed the utility of a legacy liquid handling robot at performing a stem cell differentiation protocol that requires intricate and accurate movements. An automated liquid handling robot was purchased used at auction ($100 from eBay.com) and was programmed to perform a stem cell differentiation protocol initially developed by Wilgenburg et al. that gives rise to mononuclear cells [1]. Briefly, the automated liquid handling robot is controlled by a computer and consists of a robotic arm that controls modular pipette attachment tools. Like standard pipettes, the automated liquid handling robot's pipetting tools are used to transfer liquid from one location to another. The robot also has a gripper tool that can manipulate culture dishes and other lab equipment on the work surface while housed in a custom sterile enclosure. We developed and used custom 3D printed tools to aid the robot in the differentiation protocol and provide the 3D files others to download.

Modifications to hESC differentiation protocol for use with the liquid handling robot

1. Minor modifications were made to the protocol by Wilgenburg et al. to optimize it for use on the automated liquid handling robot:
   a Wilgenburg et al. plated 20 embryoid bodies from a microplate to a 35 mm well of a 6-well as part of the protocol. However, the multichannel pipette of the automated liquid handling robot has 8 positions, so the robot is instead programmed to transfer 16 embryoid bodies to each well for convenience.
   b Wilgenburg et al. culture hESCs on mouse embryonic fibroblast feeder layers but for this method were maintained on stem cell culture plates with mTeSR-1 since this culture system is more amenable for automation and appears comparable to the co-culture method (data not shown).
2. Custom tools were made to accommodate the robots physical limitations.
   a Trituration combined with enzymatic detachment solution works for detaching loosely adherent cells such as HEK 293 cells, but hESCs strongly adhere to the vessel surface and require a cell scraper. The robot is not equipped with a cell scraper tool, however, its gripper arm can be programmed to readily pick up and manipulate customized pieces of lab equipment. We exploited this functionality and designed and 3D printed a custom cell scraper with 6 positions so that each scraper fit into one well of a 6-well plate for parallel processing (Fig. 1A).
   b We also 3D printed a custom disposable "filter box" that allowed the automated liquid handling robot to filter the cells as described in the protocol by Wilgenburg et al. (Fig. 1B). The filter box was designed to allow the robot's pipette to dispense cells through a filter and into a main reservoir (155 mL capacity), which could be accessed by the pipette tool to continue the protocol. To utilize as much of the robot's work surface as possible, the filter box was designed in thirds so that it also contained two small reservoirs for holding phosphate buffered saline (PBS) for washes and Accutase for enzymatic detachment solution (the two chambers each hold ~35 mL). The filter box also features a holster for a micro centrifuge tube, which was used to collect an aliquot of cells to determine cell concentration. The custom filter box that contained necessary components for the protocol saved significant work surface space. The equipment that would normally occupy 3–4 work surface modules was reduced to 1 module.
   c The cell-scraper and filter box were designed in Google SketchUp® 3D modeling software. We found it convenient to design custom tools that are based on the dimensions of a typical microplate in the x,y dimensions (~127.76 mm × 76.49 mm) The schematics were exported as an . stl file and printed on a MakerBot Replicator® 2 3D printer with white poly lactic acid (PLA)