**Supplementary Materials**

# Human mesenchymal stem cell (hMSC) differentiation towards cardiac cells using a new microbioanalytical method.

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## 1. Fabrication of the microdispenser

A poly(methyl methacrylate) (PMMA) chip with the microchannels (the main part of the working module of the microdispenser) were made by micromilling using a CNC micromilling machine (Mini-Mill/3 from Minitech Machinery). The micromilling was carried out in several steps. In the first step, the microchannels were made using a flat-end milling cutter (2-flute milling cutter from Kyocera) with a diameter of 200 μm. Then, inlet holes for the microchannels

were made using a milling cutter with a diameter of 500 μm. In the last step, pockets and mounting holes were made using a 1mm diameter milling cutter and the final shape of the chip was cut out. After the micromilling, the plates were cleaned under a stream of water until all shavings were rinsed out from the microchannels and then washed in detergent, deionized water and isopropanol. After drying, the plates were bonded by solvent-assisted thermal bonding. The top plate was placed in chloroform vapor preheated at 77 °C for 4 s. Then, the plates were combined and placed between the press plates of the press. Bonding step was carried out at 96°C under a pressure of 30 kg cm-2 for 15 min. The developed PMMA chip was integrated with a microvalve, which consist of a gasket, valve body, stepper motor, a spring, and valve housing. The gasket made of Viton® rubber was chosen due to its properties, i.e. any shape can be milled in this material at the rotation of the spindle motor at 30 000 rpm, and microchannels can be fabricated on the surface of this material. A microchannel with a square cross-section and dimensions: 200 μm wide and 200 μm deep and 3.5 mm long was made on the gasket surface. The gasket was glued to a valve body made of an aluminum alloy. The next element of the microvalve is a housing which was made in a polyetheretherketone (PEEK) block of 40 mm x 40 mm and a thickness of 30 mm. In this housing, also by the micromilling technique, slots housing a compression spring as well as a valve body with a glued gasket were fabricated.

The next element of the microdispsenser is the micropump, which consist of a rotor assembly made of aluminum. Three PEEK rollers are placed in the rotor. Silicone tubing is wrapped around the rotor, the inlet of which is connected to the end of the outlet channel from the microvalve, while the other end is connected to the port connecting the microdispenser to the microsystem for cell culture. This tubing is pressed by the rollers to the compression block made in PEEK and therefore peristaltic flow is forced. The motors have been placed in the PMMA block, in which pockets for both types of motors and wells for the vials with reagents were made by micromilling. Flat panels made of black PMMA have been glued to the exterior

walls of the block. The housing provides protection against adverse conditions on five sides. From the top, PMMA chip is placed. In addition, any small leaks are eliminated by a flat Viton rubber gasket placed between the housing and the PMMA chip.

## 2. Calibration of a microdispenser

Before the usage of the microdispenser, it is necessary to calibrate the rotary microvalve and set the initial position of the microvalve. For this purpose, the buttons with blue arrows and home are used. Each time the appropriate button is pressed, the text command assigned to this button is sent to the microcontroller. This starts the appropriate function performed by the microcontroller. The pressing blue arrow button causes the stepper motor shaft to rotate by 3.6° in the appropriate direction. In turn, the pressing the buttons marked with numbers 1 - 7 causes the microvalve to be set in the right position (the vials with different reagents). At the lower part of the application screen there are six buttons controlling the speed of the DC motor directly and the flow generated by the micropump indirectly (1 ÷ 45 μl min-1). In addition, the application is equipped with two buttons (blue tool, green arrow) sending commands that start more complex functions in the microcontroller. The button with blue tool causes automatic filling with the reagents of all microchannels in the microdispenser. Such a procedure guarantees the removal of air residues from microchannels. The button with green arrow causes automatic introduction of the reagent from one vial, every 24 h for 10 min (a flow rate of 1 μl min-1). For this button, any procedure/function of reagent introduction can be developed.