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A microscale mechanical stimulator for generating identical in-plane surface strains toward live cells on multiple loading sites

Qian Wang, Xu Zhang, Yi Zhao*

Laboratory for Biomedical Microsystems, Department of Biomedical Engineering, The Ohio State University, 1080 Carmack Road, Columbus, OH 43210, United States

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ABSTRACT

To investigate complicated mechanobiological events at the cellular level, microdevices that are capable of delivering controlled and identical mechanical signals to multiple loading sites are of imperative need. Although current devices in this field can generate identical loads under static conditions, parallel delivery of dynamic loads with identical loading parameters often requires the use of a multi-channel pump or multiple pumps to avoid the differential patterns of load magnitudes caused by the compliant fluidic channels. This however increases the complexity of the devices and somewhat compromises the miniaturization nature. In this study, we design and fabricate a bi-layered microfluidic device driven by a single external pump that can simultaneously deliver identical strain profiles to all the loading membranes (each with 500 μm in diameter). The loading performances under both static and cyclic loading conditions were experimentally examined. The influences of the total membrane number and the loading frequency were also examined. By minimizing the number of external pumping units for parallel operation, this device allows further miniaturization of on-chip mechanical stimulators for various studies in the field of cellular mechanobiology.

1. Introduction

Miniaturization has been the trend of lab automation because the greatly reduced operation time and consumption of reagents, and the ease of operation have greatly expedited analytical research and development [1,2]. The miniaturized devices allow the integration of more than one functional units into one device for parallel and multiplexed operation [3–6]. Such devices are of significance for drug discovery [7–10], toxicology tests [11–13], and screening the most conducive stimuli to induce desired cellular responses [14,15]. In these devices, a fundamental need is the on-demand application of controlled signals to multiple subjects to obtain statistically meaningful measurements while retaining the miniaturization nature of the devices. For example, in the studies of electromechanical coupling of cardiac cells, electrical voltages of the same magnitude can be delivered to stimulate multiple cells in culture *via* the use of micropatterned electrodes [16]. For optogenetic investigations, optical stimulation can be applied through micropixellated light-emitting diodes [17]. For mechanical stimulation, flexible membranes are often used, where mechanical strains are delivered toward the cells cultured thereon by applying a differential pressure across the membranes [18,19], stretching the membranes laterally [20], or pushing the membranes against an extruded platen [21,22].

Nonetheless, the application of identical mechanical signals toward multiple membranes often needs a multi-channel pumping unit [18], which somewhat increases system complexity and compromises the beauty of miniaturization. In addition, the use of multiple pumps or a multi-channel pump inevitably increases the number of microfluidic inlets/outlets, thus imposing a risk of leaking, a most critical challenge in microfluidics. Although parallel loading of multiple membranes using a single pump has been used [23,24], the sizes of the membranes and the fluidic channels must be sufficiently large to reduce the fluidic damping effect. As the characteristic dimensions of the devices scale down, a differential pattern of loading parameters at different sites is expected, especially under dynamic loading conditions. This can be illustrated by Fig. 1a, where a number of thin circular membranes with the same diameter are arranged in series along a close-ended channel and connected to one external pump. Such a fluidic system can be modeled using equivalent electrical circuit analogy (Fig. 1b), where the distensible membranes can be represented by electrical capacitors; the microfluidic channels by electrical resistors; and the applied pressure by voltage input. Analysis showed that under static loading, all the membranes exhibit the same profiles of surface strains, as represented by the same maximal deflections at the membrane

* Corresponding author. Tel.: +1 6142477424.
E-mail address: zhao.178@osu.edu (Y. Zhao).

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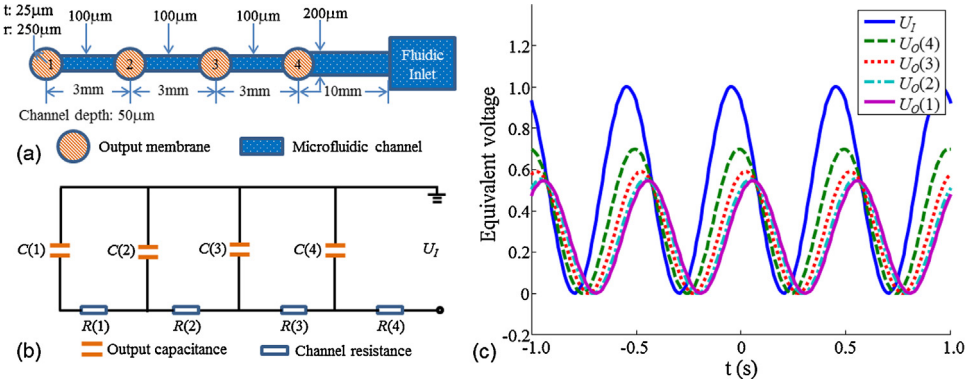

Fig. 1. A single-layered micromechanical stimulator with four membranes: (a) the two-dimensional layout of the microfluidic network; (b) the corresponding equivalent electrical circuit; and (c) the profile of the output voltages $U_O(i)$ at the loading frequency of 2.0 Hz. $R(i)$ denotes the hydraulic resistance of each segment of the microfluidic channel. $C(i)$ denotes the hydraulic capacitance of each membrane. U_I demotes the input voltage with the dimensionless amplitude of 1.0.

centers. Under dynamic loading conditions, nonetheless, the strain profiles vary. In particular, a membrane that is more distal to the fluidic inlet exhibits a smaller maximal deflection and a larger phase shift, and *vice versa* (Fig. 1c). Moreover, the inter-membrane strain disparity increases with the loading frequency. Therefore, although many groups reported parallel mechanical loading using a single pump, there was no experimental proof or convincing justification showing whether the loading outputs were identical under dynamic loading conditions, in spite of that several groups reported that delivery of different loading outputs to multiple membranes was feasible [19,21,22].

In this paper, we report an approach for delivery of identical strain profiles to multiple flexible membranes along a microfluidic channel under both static and dynamic conditions; and develop a bi-layered microfluidic device consisting of four output loading membranes for experimental validation. The strain profiles under various loading conditions were experimentally measured, and compared to a conventional single-layered micromechanical loading device. The influences of the loading frequency and the total membrane number were also examined.

2. Materials and methods

2.1. Microfluidic design

The delivery of identical strain profiles are implemented through a bi-layered microfluidic network (Fig. 2a). The lower layer consists of a close-ended microfluidic channel, which is connected to the fluidic inlet. The upper layer consists of four microfluidic channels that are in parallel; each connects to a circular output membrane. The lower and upper microfluidic channels interface at four intermediate membranes with the same size. Upon loading, the intermediate membranes are deformed by the increased hydrostatic pressure in the lower layer, which raises the hydrostatic pressure in the upper layer and in turn deforms the output membranes. The equivalent electrical circuit of such a bi-layered microfluidic network is shown in Fig. 2b and below:

$$\begin{bmatrix} (R_l(1)+Z(1)+Z(2)) & -Z(2) & & \\ -Z(2) & (R_l(2)+Z(2)+Z(3)) & -Z(3) & \\ & -Z(3) & (R_l(3)+Z(3)+Z(4)) & -Z(4) \\ & & -Z(4) & (R_l(4)+Z(4)) \end{bmatrix} \begin{bmatrix} I(1) \\ I(2) \\ I(3) \\ I(4) \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ U_I \end{bmatrix} \quad (1)$$

where $R_u(i)$ and $R_l(i)$ denote the hydraulic resistances of the microfluidic channels in the upper and the lower layers, respectively; $C_O(i)$ denotes the hydraulic capacitance of the output membranes; $C_x(i)$ denotes the hydraulic capacitance of the intermediate membranes; $Z(i) = 1/j\omega C_O(i) + R_u(i) + 1/j\omega C_x(i)$ denotes the electrical impedance in each branch; $I(i)$ denotes the current in each loop; and U_I denotes the input voltage bias, which is analogous to the difference between the applied pressure at the fluidic inlet and the atmospheric pressure. According to previous literatures, the channel resistance R and membrane capacitance C can be expressed as [25–27]:

$$R = \frac{12\mu L}{1 - 0.63\,(h/w)} \times \frac{1}{h^3 w} \quad (2)$$

$$C = \frac{\pi r^6 (1-\nu^2)}{16Et^3} \quad (3)$$

where L is the length of a microfluidic channel; h and w are the lengths of the rectangular cross-section ($h < w$); r and t are the radius and the thickness of the circular membrane; E and ν are the Young's modulus and the Poisson's ratio of the membrane material; $\mu = 10^{-3}$ Pa s for water. From Eqs. ((1)–(3)), the voltages at the four output membranes can be determined.

It is worth noting that water was selected as the actuation medium in this study, different from many previous reports where pneumatic actuation was often used. This is due to the observation that the membrane material, polydimethylsiloxane (PDMS), is gas permeable. When subject to a differential pressure, the membrane deflection changes with the holding time. Given the low loading frequency for most physiologic conditions, the strain drifting is inevitable. In addition, compressibility of the air needs be considered especially at high deflections. Moreover, water is used to ensure the device is leaking free, while the air leaking that may lead to measurement errors cannot be easily observed and was often ignored.

In order to generate identical strain profiles at the output membranes, the maximal deflections at the membrane centers should be identical. The pressure outputs at the four loading membranes should also be identical due to their proportionality with the maximal center deflections [28]. The ratio of hydraulic resistances of