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Enabling actuation and sensing in organs-on-chip using electroactive polymers

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***Abstract*— Organ-on-chip (OoC) devices are in rising demand for high-throughput and low-cost development and toxicological screening of chemicals and pharmaceuticals, as they accurately mimic *in vitro* physiological conditions as in the human body. In particular, OoCs are urgently needed for screening cardiovascular drug toxicity. Physiological relevance of cardiovascular cell cultures requires moving substrates. To date cell culture substrates have been commonly actuated by pneumatic systems, which are bulky, expensive and non-user-friendly, and may thus limit the adoption of OoCs by researchers and industry. In this paper we propose the first actuating *and* sensing smart material-based OoC device and demonstrate its functionality by culturing human vascular smooth muscle cells (vSMC). Our device utilizes a single ionic polymer metal composite (IPMC)-based transducer to provide both actuation and sensing. The soft IPMC substrate allows to intermittently apply cyclic loading to tissues and to sense their spontaneous contractions. We integrated the transducer within a compact, easy-to-operate, economically affordable and scalable OoC prototype, which achieves an actuation range of 0.2 mm and 0.72 V/mm sensing resolution. The 0.1 % strain induced by actuation on cells accurately corresponds to *in vivo* strains for vSMCs. We successfully grew vSMCs on the IPMC substrate, and actuated them for 150 min at 1 Hz. Fluorescent staining showed no evidence of adverse effects. These results are a major step towards versatile OoCs for a wide variety of biological modelling of human tissues.**

I. Introduction

Drug development is presently a decade-long and expensive process confronted by a decreasing number of yearly accepted drugs in spite of rising total development costs [1]. One of the reasons of this problematic situation is that available models for drug screening and testing, including standard cell culture methods and animal models, only partially recapitulate human biology [2]. In particular, screening cardiovascular drug toxicity is currently a priority, since it represents the most prevalent reason for drug candidate withdrawal [1]–[3]. Organs-on-chip (OoCs) offer a promising solution to this conundrum. OoCs are microfluidic devices used to culture fundamental units of human organs *in vitro* under physiologically relevant conditions [3]. OoCs make use of soft biocompatible substrates, fluid flow, periodic mechanical loading and other dynamic stimuli to help the cultured cell tissues experience an *in vivo*-like microenvironment [4].

The majority of available OoCs, both commercial and in development, use external pneumatic systems to drive tissue perfusion and substrate actuation [5]. Such solution allows to simplify the design of the OoCs but requires pneumatic control systems. The latter are bulky, expensive and ultimately non-user-friendly, and may thus prevent broader acceptance of OoCs by biology researchers and industrial companies [2]. For this reason, recent research has been directed towards novel approaches to reduce the reliance of OoCs on pneumatics for substrate functionality.

Lind *et al.* reported a pneumatics-free strain sensor for muscular thin film motion detection based on an elastic cantilever [6]. The device, compatible with well-plates for high-throughput screening assays, included strain gauges that could sense the contraction of cardiac tissue; however, the device lacked tissue actuation capability. Poulin *et al.* proposed to use dielectric elastomer actuators (DEAs) for streching human cells [7]. They could achieve high strain at high speed despite the inherent drawbacks of using DEAs, which include high actuation voltage (*i.e.*, hundreds of V, possibly cytotoxic) and short device viability. Ionic polymers are an attractive alternative to dielectric elastomers for integrated transduction in microfluidics due to their intrinsic compliance, miniaturizability and low driving voltages. Jager *et al.* first proposed polypyrrole-based actuators in lab-on-chip applications [8]. Their device was intended to manipulate only single human cells, and it is therefore not suitable for tissues including thousands of living cells as typical in OoCs.

Among ionic polymers, ionic polymer metal composites (IPMCs) are a particularly attractive class of smart materials. First reported in 1992 by Oguro *et al.* [9] and later extended by Shahinpoor *et al.* [10], in their basic form IPMCs are composed of an ionic polymer membrane encapsulated by a pair of conducting electrodes. IPMCs hold high potential as substrates for sensors and actuators in a wide variety of soft robotics applications [11]. Though IPMCs may currently be relatively immature for some of such applications [12], their capabilities are readily suitable for OoCs. Using IPMCs as active substrates for cell cultures can afford several advantages, since IPMCs have been reported as biocompatible [13], [14] and can work in wet conditions at low enough voltages to avoid hydrolysis and cytotoxic effects [15]. Hitsumoto *et al.* first reported a cell tissue stretching device based on IPMC [16], which however did not fit standard cell

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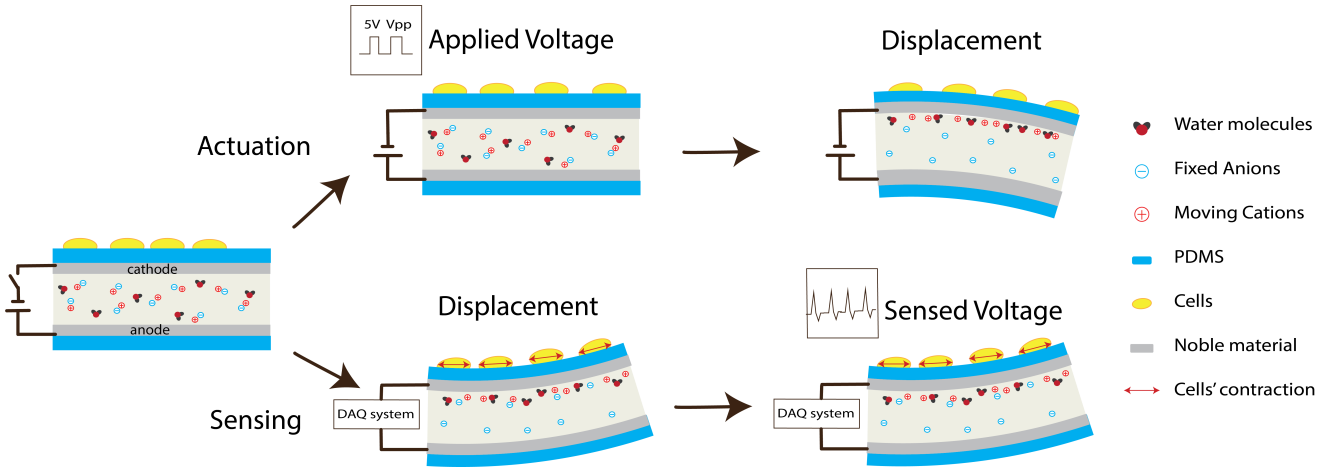

Fig. 1. Sketch of the cross-section of the IPMC-based OoC working as actuator and sensor. In the actuation mode (top), the voltage applied between the electrodes induces a displacement of the polymer. In the sensing mode (bottom), cells contraction deforms the IPMC substrate, triggering cation migration and causing a charge imbalance, measureable as a voltage difference at the electrodes.

culture systems, and had no sensing capabilities. In fact, the possibility of using IPMCs as active substrates for electro-mechanical stimulation *and* sensing of living cell cultures has not been explored to date.

In this paper we present the first OoC device that can both actuate human cell tissues *and* measure their spontaneous contraction by means of the same single electroactive polymer-based substrate. We designed and fabricated a multi-well-compatible and electronically controlled OoC device incorporating an IPMC as the cell culture substrate which can be programmed via a user-friendly interface through a single USB connection. We experimentally characterised actuation and sensing performance of the system both in deionized water and saline buffer solution, and we successfully validated the viability of the concept by culturing cells under dynamic loading conditions.

II. Materials and Methods

The working principle of the proposed IPMC-based OoC device is depicted in Fig. 1. In *actuation mode*, upon application of an input voltage difference across the electrode pair, the loosely coupled cations within the ionic polymer membrane migrate towards the cathode, causing the cantilever-shaped IPMC to bend towards the anode. The bending thus achieved imparts mechanical loading to the cells cultivated on the surface of the IPMC. In *sensing mode*, the intrinsic contraction of the cells induce a deformation of the IPMC and consequent displacement of the cations, leading to a voltage drop across the electrodes that can be measured as a readout signal.

The proposed device is composed of two distinct parts (Fig. 2). The first includes a standard 12-well plate in which the IPMC substrate is fixed by means of a tailored 3D-printed insertion system (Section II-A). The second is the driving circuitry, consisting of an electronic control board which drives the IPMC substrate and is run through a Matlab®-based graphical user interface (Section II-B).

A. IPMC based substrate

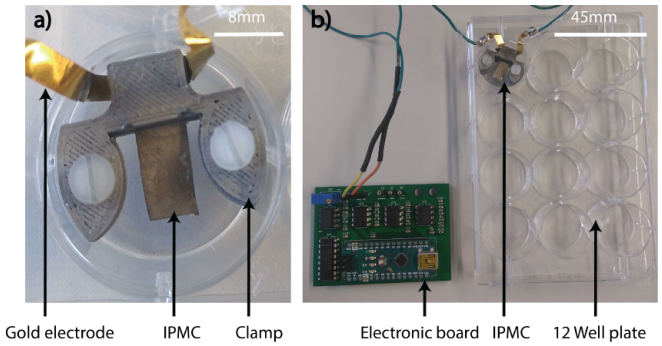

Fig. 2. The IPCM-based OoC device. a) The clamping system together with the gold electrodes and the PDMS-coated IPMC in a 20 mm-diameter well. b) The full OoC system, including the control electronics, the 12-well plate and the clamped IPMC cantilever.

The IPMC substrate was manufactured by electroless deposition of platinum on a commercial Nafion-117 membrane (thickness 180 μm, Alfa Aesar) using iterative immersion-reduction steps, as described in [10]. Fig. 3 shows the superficial 100 nm-thick Pt film and Pt nanoparticles after diffusion inside the polymer matrix. Controlling the depth of Pt penetration is cornerstone to obtain a mechanically robust IPMC. We tuned the manufacturing process to obtain sufficiently deep penetration of the Pt electrodes within the polymer to improve adhesion and avoid delamination. The IPMC sample was then ion-exchanged into Na^+ form by immersing it overnight in 2.4M NaOH solution. Afterwards the IPMC substrate was laser-cut into a rectangular cantilever shape (10x3 mm^2) using a micro laser etching machine (Optec) and dip-coated with a thin layer of polydimethylsiloxane (PDMS) to shield cells from actuation voltage and promote cell adhesion. The IPMC was finally clamped between a pair of gold electrodes in a standard 12-well plate using a custom 3D-printed clamp (Fig. 2).

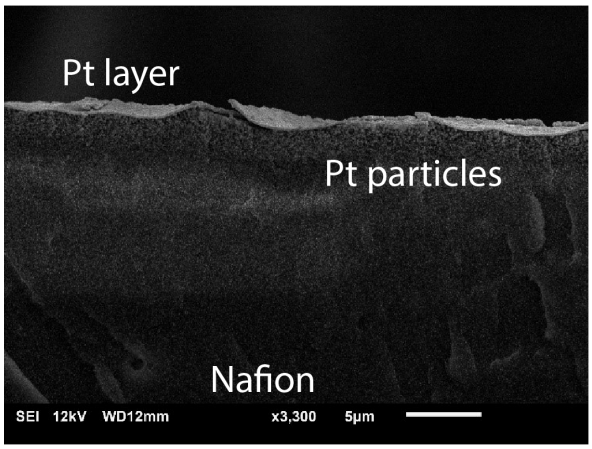

Fig. 3. Scanning electron microscope picture of an IPMC cross-section, evidencing the thin Pt film on the surface and the penetration of Pt nanoparticles inside the polymer backbone.

B. Control electronics for actuation and sensing

The electronic board was designed 1) to provide precise control over cantilever shape, displacement and actuation frequency in actuation mode, and 2) to extract an accurate and noise-free readout signal in sensing mode, corresponding to the cantilever deformation induced by tissue contraction (Fig. 4). Actuation and sensing modes can not be operated simultaneously, and time division was adopted to allocate an optimal time interval to each mode. We enabled all these functions via a customised printed circuit board (PCB) centered around a small, inexpensive and easy-to-use microcontroller (Arduino nano) (Fig. 5).

The actuation electronics included a digital-to-analog converter (DAC) used in series to the digital output of the microcontroller to actuate the IPMC substrate. Since proper actuation of the IPMC requires a current of around 100 mA, a standard amplifier was added after the DAC (Fig. 4). Both amplifier and DAC were powered by the microcontroller. A 2.5 V reference was used to deliver a full square wave with 5 Vpp amplitude.

A second electronic circuit was designed on the same PCB to support sensing capabilities using the same IPMC substrate. The sensing circuit included a band-pass filter to remove parasitic and environmental noise, and a differential amplifier together with a standard amplifier and active low-pass filters to read, amplify and filter the sensing signal from the IPMC substrate. The gain of the last amplification stage could be tuned up to 5000 using a trimmer.

Finally, switching between actuation and sensing modes of the system was enabled via a multiplexer controlled through the microcontroller (Fig. 5).

C. System characterization

Actuation and sensing were performed using the aforementioned electronic board (Fig. 5). Characterization of the actuation mode was performed by tracking the voltage-induced displacement of the IPMC cantilever using a laser triangulation sensor (ILD1750-2, Micro-Epsilon) connected

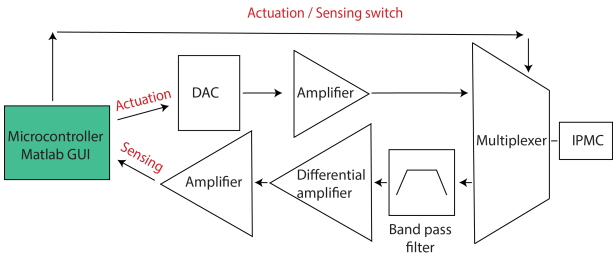

Fig. 4. Schematic of the electronic control circuit. Time-divided sensing and actuation modes are enabled on the same substrate by a multiplexer controlled through the microcontroller.

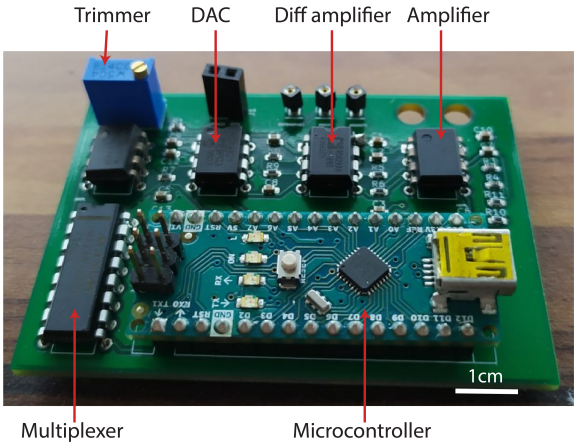

Fig. 5. Close-up view of the printed circuit board for the electronic control of the IPMC-based OoC, including the single USB external connection, the microcontroller, the multiplexer, the DAC, the trimmer and the amplifiers.

to a data acquisition board (National Instruments) run through LabView®. To characterise the sensing mode, a displacement was manually induced in the IPMC and quantified using the laser triangulation sensor while the corresponding electric output signal was recorded through the control board. The sensitivity was calculated as the average of the ratio between displacement and readout voltage.

The setup to perform the frequency response analysis is sketched in Fig. 6 and shown in Fig. 7. The measurements were performed by applying sine waves of frequency in the physiological range of 0.1 Hz to 20 Hz and recording the corresponding displacement of the cantilever tip. The spectral response was obtained by analysing magnitude of displacement and phase difference between the applied and recorded signal. The experiments were performed in different solutions by alternating deionized water and phosphate buffered saline (PBS) solution. PBS contains the same amount of cations as standard culture media, and is commonly used to wash cell cultures during culture medium change.

Finally, the Young's modulus of the fabricated IPMC substrate was characterized by means of tensile tests (Dynamic Mechanical Analysis Q800 TA).

The strain applied to the cells adhering to the top surface of the cantilever by the cantilever deformation was calculated as follows. Using traditional elastic beam theory, the lateral displacement of the beam in the vertical direction $(+y)$

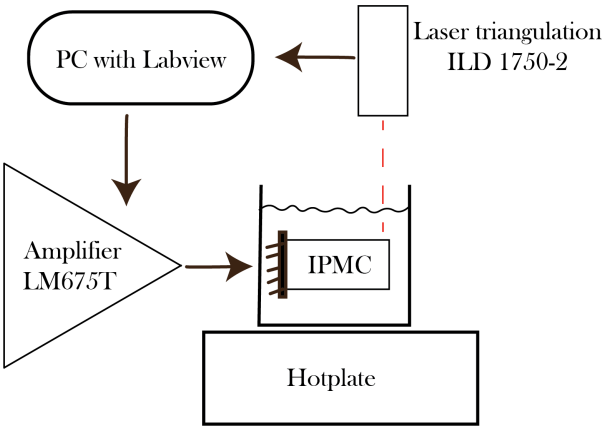

Fig. 6. The experimental setup used to perform the frequency analysis.

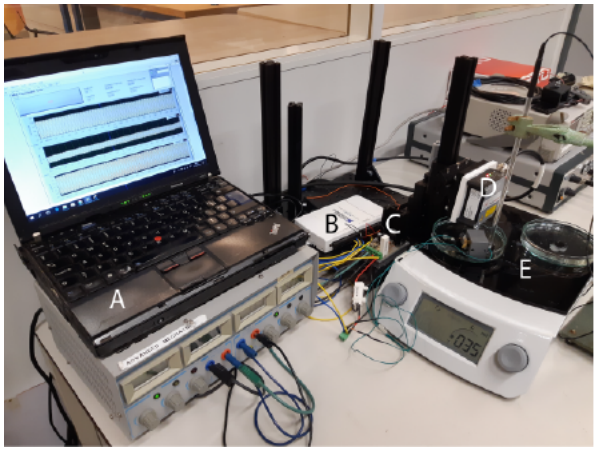

Fig. 7. The frequency analysis setup, including a computer (A) and data acquisition unit (B), an amplifier (C), the laser triangulation system (D) and an hotplate for the control of the temperature of the solution (E).

is $v(x)$. In the elastic deformation regime the curvature is related to the bending moment by:

$$\frac{d^2v(x)}{dx^2} = \frac{M(x)}{EI} = -\frac{P(L-x)}{EI} \tag{1}$$

with E the Young's modulus, $M(x)$ the bending moment, L the length and I the second moment of area of the beam. By integrating Eq. (1) twice and assuming no displacement ($x = 0$) and no rotation ($x' = 0$) at the base of the cantilever, the following equation for the displacement field is obtained:

$$v(x) = -\frac{Px^2}{6EI}(3L - x) \tag{2}$$

P being the (point) force applied to the cantilever. Accordingly, the lateral displacement δ (assumed downward) at the tip of the cantilever ($x = L$) is:

$$\delta = \frac{PL^3}{3EI} \tag{3}$$

Using the following definition of strain ϵ:

$$\varepsilon = \frac{\sigma}{E} = -\frac{M(x)t}{EI} \tag{4}$$

t being the thickness of the cantilever. For $x = L$ and $t = \frac{h}{2}$, the strain can be correlated to the tip displacement:

$$\varepsilon = \frac{PLh}{2EI} = \delta\frac{3}{2}\frac{h}{L^2} \tag{5}$$

D. Validation

The main purpose of the present study was to show biocompatibility and non-cytoxicity of the IPMC substrate during actuation of living cells cultured on its top surface. Vascular smooth muscle cells (vSMCs) were obtained by differentiation from induced pluripotent stem cells (iPSCs). High-density vSMCs were seeded on the substrate pre-coated with fibronectin (Sigma Aldrich) and left in an incubator overnight at 37 °C, 5% CO_2, in Gibco® IMDM and Gibco® F12 culture media (1:1) to allow cell growth on the PDMS substrate. The IPMC was then connected to the printed circuit board to perform actuation. The cells were actuated for 150 minutes with a square wave input signal with a frequency of 1 Hz and amplitude of 3 Vpp. After the experiments, the cells were stained using Calcein Am fluorescent dye (Sigma Aldrich) which only stains living cells.

III. Results and Discussion

A. Actuation capabilities and mechanical characterization

The measured Young's modulus of the IPMC was 650 MPa, which aligns with the values reported in literature [17].

The characterization of the actuation mode showed a 0.04 mm/V actuation capability (Fig. 8). In the sensing mode, the measured sensitivity was 0.72 V/mm after amplification (Fig. 9), corresponding to 0.28 mV/mm before amplification. The sensitivity was measured with a trimmed gain of 2500 which showed no parasitic noise. The sensivity of our device is therefore lower than the one (10mV/mm) reported for voltage-based active sensing methods [17]. We additionally noticed that the actuation characteristics did not change over an actuation period protracted for several hours.

In the experiments we measured cantilever tip displacements of the order of 0.2 mm. Given the dimensions of the moving cantilever ($L = 8$ mm excluding the clamped fraction, and $t = 200$ μm including the PDMS layers), we estimated via Eq. (5) an induced strain of 0.094%. This strain value corresponds to the circumferential strain value experienced *in vivo* by cells in the jugular vein, as previously reported in rats [18]. It should therefore be pointed out that the actuation capabilities outlined by this device are already sufficient to mimic the strain experienced by cells in vasculature, even if IPMC actuation capabilities, in particular maximum strain, can achieve higher values (of the order of 15 %) according to literature [17]. When used at their full potential, IPMC-based actuators could potentially meet all mechanical and sensing requirements no matter the OoC biological model studied.

The frequency analysis showed a difference in actuation magnitude between PBS and DI water (Fig. 10). In particular, the magnitude of the response was higher in DI water than in PBS at high frequencies (1 to 20 Hz). We hypothesize that the saturation of cations within the IPMC immersed

in PBS with high cation concentration leads to a smaller concentration gradient in the vicinity of the Pt electrodes, and thus to lesser stress upon voltage application. Conversely, at lower frequencies (0.1 to 03 Hz) the magnitude was higher for actuation in saline solution.

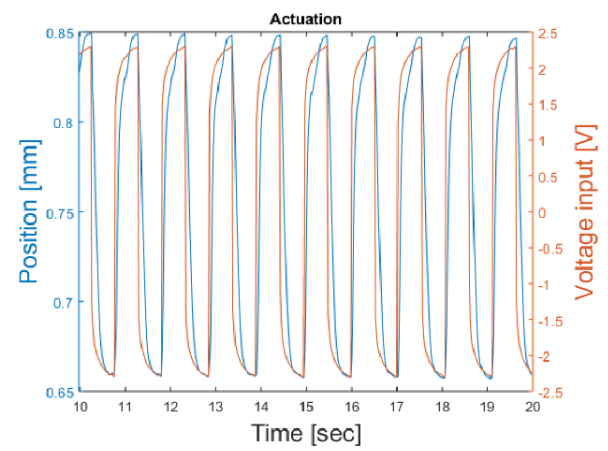

Fig. 8. Characterization of actuation mode. A square wave signal of 1 Hz frequency and 5 Vpp amplitude was used to drive the IPMC motion. The capacitive behavior of the IPMC is manifested by the low-pass filtered output electric signal recorded at the electrodes of the cantilever.

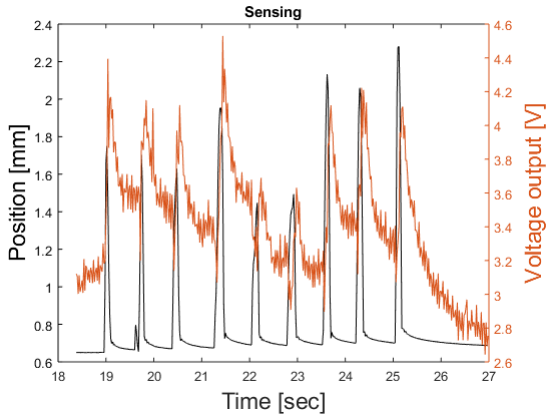

Fig. 9. Characterisation of the actuation mode. The impulsive and manually induced displacement was recorded using the electronic control board and monitored using the laser trangulation system.

We also noticed that, under the cell culture conditions we used for the long-term actuation (see Section II-D), the high concentration of sodium ions inside the culture medium were conducive to good actuation capabilities and prolonged viability of the IPMC. The IPMC requires mobile cations in the polymer backbone, and we hypothesize that the high concentration of sodium cations in the medium reduced the loss of the cations from the IPMC upon actuation. Accordingly, and while many authors do outline cations loss over time as a critical reliability issue for IPMCs [19], our device may better withstand this failure modality and extend its lifetime thanks to the cations present in common cell culture media.

Performing further experiments under realistic conditions to quantify the actual strain experienced by the thin cell layer during actuation and to assess the possibility of detecting cell contraction via the substrate is reserved for future work.

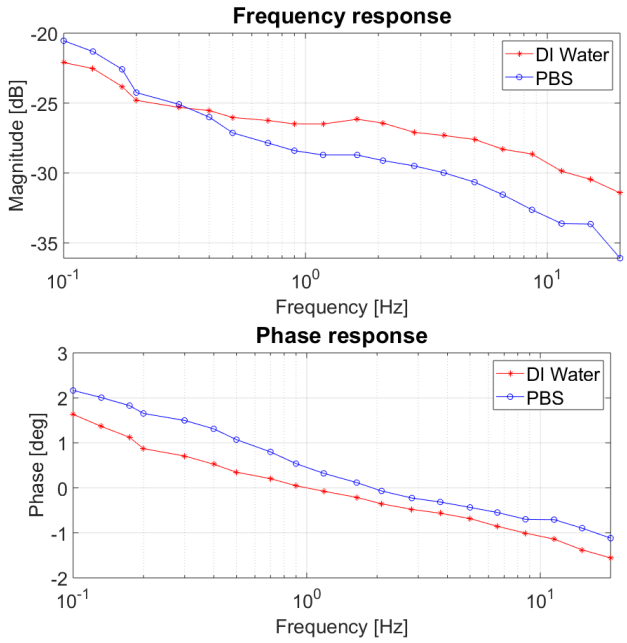

Fig. 10. Bode plot for frequency and phase response of the IPMC cantilever sequencially actuated in PBS and DI water at 37° C.

B. Cell culture

We successfully actuated the vSMC tissue cultivated on the IPMC substrate clamped to a 12-well plate (Fig. 2) inside a standard incubator for 150 minutes and with no detectable hydrolysis. The subsequent Calcein AM staining proved that after actuation the smooth muscle cells were still alive with no adverse effects (Fig. 11). Moreover, the vSMCs showed an elongated shape, proving that they did not delaminate from the substrate despite the prolonged mechanical loading and that they were not affected by the actuation voltage.

IV. Conclusion

We have presented a novel, simple, inexpensive and easy-to-use OoC device that could for the first time perform both actuation and sensing using the same, smart material-based substrate. Our ionic polymer-based OoC allows to stretch human cell tissues thanks to the unique properties of the IPMC under electronic control of a microntroller. Periodic actuation was successfully performed for more than two hours to induce mechanical load to a vascular smooth muscle cells culture (about 0.1% strain and no hydrolysis issues). The range of applied strain corresponds to the one experienced by cells *in vivo*, proving the relevance of using smart materials to mimic human microenvironment for OoC applications. No adverse affects nor delimination of the human tissue were detected after actuation. Sensing was chracterized thanks to an externally-induced displacement

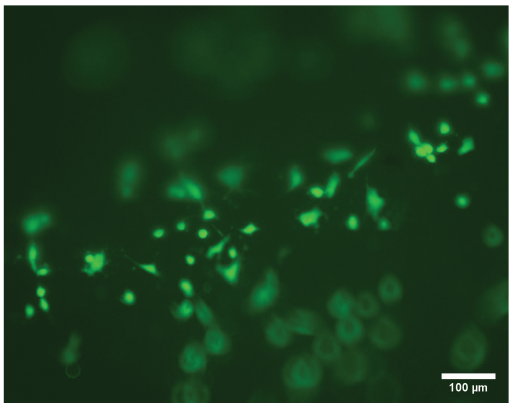

Fig. 11. vSMCs on the substrate after 150 min actuation with 1Hz, 3 Vpp input signal. The picture shows living and elongated cells, proving that vSMCs did not delaminate despite prolonged mechanical loading and that they were not affected by the actuation voltage.

of the IPMC cantilever, showing good performance (0.72 V/mm). Future improvements will address the recording of the intrinsic contraction of the living tissue together with full cytotoxicity testing after prolonged actuation.

The proposed device could be easily tailored to many other uses and cell types, such as cardiomyocytes for heart-on-chip applications. The present IPMC-based device anticipates a potentially significant impact of smart materials in the development of OoCs for drug screening.

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