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Impedance-based detection of *Schistosoma mansoni* larvae viability for drug screening

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Abstract

Human schistosomiasis is a neglected tropical disease caused by trematodes, affecting almost 250 million people worldwide. For the past 30 years, treatment has relied on the large-scale administration of praziquantel. However, concerns regarding the appearance of drug-resistance parasites require efforts in identifying novel classes of suitable drugs against schistosomiasis. The current drug screening system is manual, slow and subjective. We present here a microfluidic platform capable of detecting changes in viability of *Schistosoma mansoni* larvae (Newly Transformed Schistosomula, NTS). This platform could serve as a pre-screening tool for the identification of drug candidates. It is composed of a pair of coplanar electrodes integrated in a microfluidic channel for the detection and quantification of NTS motility. Comparison of viability detection by using our platform with the standard visual evaluation shows that our method is able to reliably detect viable and non-viable NTS at high sensitivity, also in case of low-motility parasites, while enabling a 10 fold decrease in sample consumption.

Keywords

schistosomiasis; *Schistosoma mansoni*; impedance analysis; drug screening

I Introduction

Over 250 million people worldwide are affected by human schistosomiasis, a neglected tropical disease caused by helminths (parasitic worms) of the genus *Schistosoma* [1]. Current treatment in endemic regions has been relying for over 30 years on the periodic administration of praziquantel to reduce morbidity, however reduced sensitivity to the drug has already been reported. Hence there are concerns whether drug-resistant parasites would emerge [2]. Therefore, identification of new drug compounds to target the disease is of paramount importance.

The current gold standard for measuring drug efficacy *in vitro* is based on the phenotypical evaluation of the parasites when exposed to different drug candidates using manual microscopy (Fig. 1A). This procedure is extremely labor-intensive, requires a large number of parasites and trained operators for the evaluation, and might be affected by subjectivity

due to the operator evaluating the drug efficacy [3]. Different methods have already been proposed to try to overcome these limitations, however automated screening of the abundant larval-stage parasite (Newly Transformed Schistosomula, NTS) for drug assessment requires expensive instrumentation and extremely large numbers of larvae, which limits the throughput of the analysis, or falls below the sensitivity of conventional approaches [4,5].

Here, we present a novel microfluidic platform for the assessment of the viability of NTS of the species *Schistosoma mansoni* by using an impedance-based readout of NTS motility (Fig. 1B). NTS viability after exposure to the drug to be studied is detected by measuring the current fluctuations caused by NTS upon moving in a compartment between a pair of coplanar electrodes, patterned at the bottom of a microfluidic channel (Fig. 1C). The combination of a microfluidic structure for restraining the NTS to a compartment between the electrodes with an electrical-readout scheme enables a massive sample reduction, as only ~5 NTS and 20 μL of solution are required per measurement, as compared to 50-100 NTS in 200 μL solution for the standard evaluation. Furthermore, an electrical readout of the viability paves the way to devising parallelized and automatic platforms for the pre-screening process, which will increase the throughput of the analysis and eliminate subjectivity associated with a visual phenotype evaluation.

II Impedance-Based Motility Detection

The platform is composed of a microfluidic chip, made of polydimethylsiloxane (PDMS), bonded to a glass substrate with patterned platinum electrodes (Fig. 2A). The microfluidic structure comprises two large open reservoirs for medium storage, a smaller inlet for sample loading, two pillar structures and a narrow channel across the detection electrodes. Fig. 2A shows a schematic of the platform and the relevant dimensions. The dark red areas denote the large (~3 mm diameter) medium reservoirs and the smaller sample inlet (0.75 mm diameter); the pillar structures, in blue, retain the NTS in the detection area and prevent them from flowing into the reservoirs; the narrower channel is used to confine the parasites to the volume over the electrodes and to limit the overall electrolyte volume in order to increase the obtained signals and measurement sensitivity. The 150-nm-thick platinum electrodes are deposited on a glass substrate using a Ti/W adhesion layer and patterned via a lift-off process. The electrodes spans across the whole detection chamber of width 375 μm , and feature equal dimension and spacing of 150 μm (Figure 2A). The chip is contacted via a custom-made PCB presenting a set of multiplexers to route the connections from the impedance spectroscopy to the user-selected pair of electrodes. The electrode fabrication procedure and the principal elements of the control electronics have been reported in details in [6] for a similar platform.

Figure 2B shows the electrical equivalent circuit of the microfluidic platform and the experimental setup. An AC voltage signal is generated by a lock-in amplifier (HF2-LI, Zurich Instrument, Zurich, Switzerland) and fed to the microfluidic chip via leads, featuring a resistance R_{trace} . At the interfaces between the electrode and the electrolyte, a capacitive double layer, C_{dl} , is formed. The presence and movement of the worms in the channel is represented by a variable resistance and capacitance between the electrodes, R_{ch} and C_{ch} . The current flowing through the system is fed into a trans-impedance amplifier (HF2TA,

Zurich Instrument, Zurich, Switzerland) with 1 k Ω feedback resistor for current-to-voltage conversion, and the resulting voltage is finally sampled by the lock-in amplifier and subsequently analyzed for detecting current fluctuations caused by impedance variations in the channel.

The amplitude of the voltage-converted current flowing through the chip was acquired by applying a sinusoidal carrier signal of 333 kHz with 300 mV amplitude, and the current was sampled with a sampling frequency of 225 Hz and a cut-off frequency of 10 Hz of the lock-in low-pass filter. The data were then processed and analyzed in Matlab (MATLAB 2016b, The Mathworks Inc.). To separate signal fluctuations, caused by the movement of the NTS, from fluctuations of the baseline, caused by solution evaporation or sample flow, we applied a 0.2 Hz high-pass filter to the acquired signals. As different samples may contain different electrolyte concentrations, hence exhibit different conductivity, the high-pass-filtered signal was then normalized by the average amplitude of the raw signal. To calculate and assign a motility value as a function of the signal fluctuations, we calculated the power of the high-pass-filtered and normalized signal in a 1-5 Hz bandwidth. This approach minimizes the effect of readout noise, present at higher frequencies, while preserving the signal power related to the contraction and expansion of the larvae between the electrodes. This data analysis workflow enables a signal-to-noise ratio of over 30 between the detected signal for viable parasites and signal fluctuations caused by noise sources. Finally, the calculated power of the measured fluctuations was normalized by the number of loaded larvae to compare measurements with different number of NTS in the measurement compartment.

III Experimental procedure

The microfluidic chip was prepared by plasma bonding the PDMS structure on the platinum-patterned glass substrate, after alignment of the pillar structures with the on-chip electrodes. The microfluidic channel and reservoirs were subsequently treated with a hydrophilic coating (Biolipidure 206, NOF America Corporation, White Plains, NY, USA) before use. After rinsing the microfluidic channel with ethanol, the chip was left for 24 hours at room temperature to dry.

Fig. 3 shows the loading and operation of the platform. First, 15 μ L of NTS suspension (obtained as described elsewhere [4]) are loaded into the chip using the sample inlet (I). Then, 5 μ L of culture medium (Medium 199, 5% FCS, 1% Penicillin/ Streptomycin) are added to the chip reservoir at the left to generate a hydrostatic pressure to drive the NTS into the measurement channel and to move the larvae in the region of the detection electrodes for signal acquisition (II). Once all the NTS in the channel are dragged by the flow into the detection area, the voltage-converted current flowing through the system is recorded for one minute for detecting current fluctuations caused by the motion of the larvae (III). The hydrostatic pressure generated by the height difference of the medium in the reservoirs is sufficient to maintain the NTS in the region of the electrodes during the data acquisition so that no external pumping system is required. Lastly, the chip is emptied by drawing the sample solution from the sample inlet using vacuum suction to ensure that no solution is left in the chip (IV). The chip is now ready for a new measurement. The hydrophilic coating of the channel walls enables a highly reproducible loading of the sample solutions, minimizes

the trapping of air bubbles in the chip during sample injection and provides consistent flow conditions for subsequent experiments.

IV Results

Fig. 4 shows the behavior of the estimated motility parameter for untreated NTS as a function of the number of larvae in the electrode regions. Individual measurements show large variations in calculated power as a consequence of the differences in the behavior of individual NTS across different measurements. However, as soon as there are three or more NTS in the measurement compartment, the average power value falls within a narrow interval, which evidences that multiple measurements provide a robust estimation of motility even when dealing with such low numbers of larvae. Measurements with only one or two NTS between the electrodes should be discarded, as the variability between measurements is too dependent on the individual behavior of the NTS, which bears a large risk of incorrect estimation of the larvae motility parameter.

To validate that the microfluidic platform can be used to detect status differences between NTS samples and can be used for identifying drug candidates via the pre-screening phase with parasite larvae, we measured the viability of NTS after 24 hours of exposure to different concentrations of DMSO and compared our results with those of standard visual evaluation by an operator. Briefly, in a 96 well plate, DMSO was serially diluted in culture medium and 50 μ l of 50 NTS were added, for final assay DMSO concentrations of 0, 1, 2 and 5% v/v. The NTS were assessed 24 hours after DMSO treatment by visually scoring them microscopically using a viability scale from 0 to 3 (0 meaning dead and 3 meaning normal motility and healthy morphology, as previously described [7]). Thereafter, for every DMSO concentration between 3 to 12 NTS were loaded into the device and measured from the sample inlet port. (Fig. 5). These measurements were intended to mimic a viability screening of the larvae after incubation with a test compound. Comparison of our viability evaluation via impedance-based measurement with the standard visual evaluation shows a very good agreement between the two methods. By using our platform, we were able to measure the differences in viability between untreated larvae and DMSO-exposed NTS and to detect differences in motility between NTS in 1% DMSO, where they were still viable though less motile, and in higher concentrations of the DMSO (from 2% on), where the larvae could not survive. The larger variation measured with our platform between untreated larvae and larvae in 1% DMSO when compared to the standard evaluation can be explained by the longer exposure of the NTS to the drug (the standard evaluation was performed about 1 hour before the impedance-based readout) or could also be a consequence of the higher sensitivity of the electrical readout to motility changes in comparison to phenotypical optical evaluations, a phenomenon that has already been observed with adult *S. mansoni* [5].

V Conclusions

We have presented a microfluidic platform to measure the viability of *S. mansoni* in the larval stage using an impedance-based readout scheme. The platform was validated by measuring the viability status of live vs. killed NTS, as well as upon their exposure to different concentrations of DMSO. The impedance-based estimations showed good

correlation with the standard visual evaluation, performed by a trained operator. An all-electrical readout of the NTS viability provides an objective estimation of the larvae status and eliminates uncertainties related to the subjectivity in visual scoring by an operator. Furthermore, an electrical read-out scheme enables the parallelization of the analysis to simultaneously measure multiple drug conditions at potentially high throughput and paves the way to automatization. Automatization and higher throughput will significantly reduce the costs of the discovery of drugs against schistosomiasis.

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Fig. 1.

Comparison between the standard evaluation of motility of NTS through visual inspection in well plates (a) and our microfluidic platform relying on impedance changes induced by larvae motility (b). Live NTS induce fluctuations in the voltage-converted recorded signal, whereas a stable and flat signal is recorded for dead worms (c). The images in the insets show three screenshots of the worms in the chip during the measurements. The black regions at the top and bottom are the platinum electrodes while the pillars to contain the larvae in the sensing area are clearly visible.

Fig. 2.

a) Schematics of the microfluidic platform with relevant dimensions. The red areas are the solution-accessible regions, the darker red regions indicate open inlets. The platinum electrodes are displayed in grey and the PDMS structure in blue; b) electrical-equivalent circuit of the platform and measurement setup. The main components include a lock-in amplifier, in dark green, for generating the stimulation AC signal, the chip in grey, while the light red regions indicate the regions of liquid phase presence. Current-to-voltage conversion is obtained via a transimpedance amplifier (TIA), in light green, and then sampled by the lock-in amplifier. Control of the experimental parameters and signal analysis is performed by means of a PC.

Fig. 3.

Experimental procedure for loading and measurements of *S. mansoni* NTS in the microfluidic platform. No active pumping system is needed to move the sample into the detection region, which simplifies the experimental setup and the operation of the platform.

Fig. 4.

Signal power in a 1-5 Hz bandwidth as a function of the number of NTS between the electrodes. The signal power obtained upon measuring three or more viable NTS falls within a narrow interval. The error bars show the deviations from the mean power value obtained for each number of larvae in the chip. The average values were calculated from two or more measurements, as shown in the plot.

Fig. 5.

Measurements of motility of *S. mansoni* NTS after 24 hours exposure to different concentrations of DMSO. The impedance-based measurement results show good agreement with the results of operator-based visual evaluation of the larvae viability. The average value was calculated from 4 measurements for the motility evaluation by impedance and 3 measurements for the visual evaluation.