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Small molecule absorption by PDMS in the context of drug response bioassays

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ABSTRACT

The polymer polydimethylsiloxane (PDMS) is widely used to build microfluidic devices compatible with cell culture. Whilst convenient in manufacture, PDMS has the disadvantage that it can absorb small molecules such as drugs. In microfluidic devices like "Organs-on-Chip", designed to examine cell behavior and test the effects of drugs, this might impact drug bioavailability. Here we developed an assay to compare the absorption of a test set of four cardiac drugs by PDMS based on measuring the residual non-absorbed compound by High Pressure Liquid Chromatography (HPLC). We showed that absorption was variable and time dependent and not determined exclusively by hydrophobicity as claimed previously. We demonstrated that two commercially available lipophilic coatings and the presence of cells affected absorption. The use of lipophilic coatings may be useful in preventing small molecule absorption by PDMS.

1. Introduction

Differences in physiology can compromise the ability of animal models to predict drug responses in humans accurately. As a result, inappropriate drugs may enter clinical trials or useful drugs discarded unnecessarily early in development. One of the most common adverse drug effects is still cardiotoxicity despite use of ion channel ("hERG") assays to predict arrhythmic risk, illustrating the urgent need for improvement in human cardiac models [1]. In this context, the Food and Drug Association (FDA) recently initiated the Comprehensive In Vitro Proarrhythmia Assay (CIPA) (cipaproject.org), to assess cardiac drug safety using cardiomyocytes from human pluripotent stem cells. Among the models being examined are several incorporating cardiomyocytes into polymer-based devices or microtissues [2–4] rather than conventional tissue culture substrates like glass and plastic, which are orders of magnitude harder and less elastic than cells would normally encounter in tissue. Resulting stress can influence cell behavior profoundly and thus drug responses [5–7]. Soft polymer substrates like polydimethylsiloxane (PDMS) are preferred by bioengineers because they can be used to fabricate microfluidic channels, flexible membranes and provide topological cues to recapitulate blood vessels, mechanical tissue strain and induce cell orientation [8–10]. Furthermore, sensors that measure electrical activity, metabolic behavior, ion transport and force of contraction can be integrated as readouts [2,11]. PDMS-based Organ-on-Chip devices can also be used for live cell imaging and are compatible with immunoassays, disease phenotyping and drug testing [12,13]. Soft polymer-based microfluidic assays have long been used for multiple biological purposes [14] but PDMS most commonly because of its biocompatibility, transparency, ease of microfabrication, molding properties and tunable substrate

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mechanics [15]. Its elasticity has made it particularly useful in mimicking tissues that undergo cyclic stretch and strain in the body, such as the lungs or heart [2,8,11]. One of its drawbacks, however, is its absorption of compounds added to culture medium in bioassays, most particularly, hydrophobic small molecules like many drugs [16–18]. This reduces the available drug dose, shifts the dose response curve and thus limits the predictive value of assays.

There have been few direct comparisons of absorption of different compounds by PDMS with other (more inert) substrates and how cell culture affects absorption has not been investigated. Toepke et al. examined the absorption of hydrophobic small molecules qualitatively with a fluorescence assay but this was not quantitative [17]. Wang et al. did assess final compound concentration quantitatively over time and determined a threshold that distinguished compounds with negligible absorption from those where it was significant, based on the log P value [18], a measure of hydrophobicity. It was claimed that all compounds with a log P value greater than 2.67 would show high absorption by PDMS substrates. In addition, they tested whether glass and titanium dioxide coatings reduced compound binding. These coatings have been reported to be biocompatible [16], but the presence of cells on compound absorption or coating degradation has not been investigated. Parylene coating has also been reported [19], but it compromises important properties of PDMS, such as its elasticity and gas permeability.

Here, we determined small molecule absorption by PDMS-coated culture wells versus standard culture plastic for four drugs that affect the heart, our primary research area [20,21] for which there is interest in knowing their toxic threshold: verapamil, bepridil, Bay K 8644 and nifedipine. Toxic thresholds can only be determined accurately if the compound availability to cells is known. We therefore investigated whether cell density affected absorption and tested the ability of two commercial coatings (available on request) to reduce absorption, without affecting biocompatibility or cell plating [22]. Finally, we combined a commercial coating with cell culture to investigate the combined effect on the free drug concentration over time of incubation.

2. Experimental

2.1. Preparation of plates

Standard tissue culture grade polystyrene (TCPS) 96-well CELLSTAR® multiplates (Greiner Bio-One, Germany) were used. For PDMS absorption experiments, bottoms of wells were coated with 20 μL PDMS Sylgard 184 (Dow Corning, Auburn, MI), 1:10 wt ratio curing agent to elastomer. LipoCoat Cellbinder (LipoCoat, Enschede, The Netherlands) was used to coat oxygen plasma treated PDMS-coated 96-wells plates (PDMS Sylgard 184, Dow Corning, Auburn, MI).

2.2. Experimental procedure and sample retrieval

Verapamil, nifedipine (Sigma Aldrich, Saint Louis, MO), bepridil and Bay K 8644 (Tocris, United Kingdom) were diluted in dimethylsulfoxide (DMSO; Sigma Aldrich) to obtain 1 mM stock. This was diluted 1:1000 in Dulbecco's Phosphate Buffered Saline without calcium and magnesium (DPBS) or in Tyrode's buffer when cells were present, to a working concentration of 1 μM. All solutions were prepared in disposable glass vials. 250 μL of the working solution was pipetted into the TCPS or PDMS wells and incubated for 0.5, 1, 2 or 3 h before being gently mixed with a pipette and transferred to a glass vial which was sealed immediately. In addition, 250 μL of the working solution was pipetted into a glass vial for every sample group at the start of the experiment to provide baseline values (concentration at time zero). Time points were chosen to allow <10% change in absorption at the final time point for all compounds. Experiments were in duplicate. Cell culture experiments were at 37 °C; other experiments were at room temperature.

3. HPLC

Drug concentrations were determined by HPLC using a solvent delivery system from Gilson (Middleton, WI) equipped with a Gilson auto injector. A VI/VIZ detector (Applied Biosystems 759A; Thermo Fisher Scientific, Waltham, MA) was used, monitoring the UV absorption maximum of the compounds under investigation. A Discovery® column (125 × 4.6 mm; Supelco, Bellefonte, PA) was applied, with a stationary phase of C18, 5 μM particles. As mobile phase, MeCN was used at 40–65% (depending on the compound), 5 mM SDS and 0.1% TFA. Flow speed was 0.8 ml/min and the injection volume 100 μl. Recording and processing of data was performed using ADchom software (Leiden University, the Netherlands). In every experiment a calibration curve was included to validate the system and the linearity of the compound UV absorption response. Measurements were performed in duplicate or triplicate, as indicated. Averages from individual experiments are shown in the graphs. Since replicates showed little spread, n = 2 was standard for non-biological assays.

3.1. Cell culture

Human Embryonic Kidney 293T (HEK293T) cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2 mM L-glutamine, 10 mM non-essential amino acids (NEAA), 25 U/ml penicillin, 25 μg/ml streptomycin (Thermo Fisher Scientific), and 10% fetal calf serum (FCS) (Sigma Aldrich). Culture medium was refreshed every 3–4 days. For experiments, cells were dissociated from standard 6 well tissue culture plates (Greiner Bio-One, Germany) with 0.1% trypsin and plated in 96-well plates at three densities: 9×10^3, 37×10^3 and 117×10^3 cells per well (0.3 cm^2) corresponding to low, medium and high surface coverage, respectively.

3.2. Cell staining

Cells were briefly washed with PBS before fixation with 2% neutral paraformaldehyde for 30 min at room temperature. Next, cells were permeabilized for 8 min with 0.1% Triton X-100 (Sigma Aldrich) in PBS then preincubated with 4% FCS in PBS for 1 h to block non-specific binding. Cells were incubated with PBS plus Alexa Fluor 488 Phalloidin (1:40; Invitrogen) and 4% FCS for 30 min. Finally, cells were incubated with PBS plus DAPI Nucleic Acid Stain (1:1000; Invitrogen) before glass coverslips were mounted on top of the PDMS with ProlongGold (Thermo Fisher Scientific). Between every step, cells were washed with PBS and prior to Phalloidin with PBS plus 0.05% Tween 20 (Merck, Germany).

4. Results

HPLC was used to measure the free drug concentrations in PBS. Four compounds that affect cardiac behaviour and of interest to validate in cardiotoxicity models *in vitro* were incubated for 0.5, 1, 2 and 3 h on TCPS wells with PDMS as the base and compared to the absorption by standard TCPS wells after 3 h (Fig. 1). Absorption by TCPS, measured as loss of compound from solution, was negligible or low but detectable for Bay K 8644, verapamil and nifedipine and somewhat higher for bepridil. PDMS however absorbed compounds much more variably: verapamil and nifedipine were