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Short communication

A prototype microfluidic chip using fluorescent yeast for detection of toxic compounds

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ABSTRACT

A microfluidic chip has been developed to enable the screening of chemicals for environmental toxicity. The microfluidic approach offers several advantages over macro-scale systems for toxicity screening, including low cost and flexibility in design. This design flexibility means the chips can be produced with multiple channels or chambers which can be used to screen for different toxic compounds, or the same toxicant at different concentrations. *Saccharomyces cerevisiae* containing fluorescent markers are ideal candidates for the microfluidic screening system as fluorescence is emitted without the need of additional reagents. Microfluidic chips containing eight multi-parallel channels have been developed to retain yeast within the chip and allow exposure of them to toxic compounds. The recombinant yeast used was GreenScreen™ which expresses green fluorescent proteins when is exposed to genotoxins. After exposure of the yeast to target compounds, the fluorescence emission was detected using an inverted microscope. Qualitative and quantitative comparisons of the fluorescent emission were performed. Results indicated that fluorescent intensity per area significantly increases upon exposure to methyl-methanesulfonate, a well known genotoxic compound.

The microfluidic approach reported here is an excellent tool for cell-based screening and detection of different toxicities. The device has the potential for use by industrial manufacturers to detect and reduce the production and discharge of toxic compounds, as well as to characterise already polluted environments.

1. Introduction

The requirement for toxicity testing of chemical substances is of growing concern as most manufactured chemical products are formulations or mixtures of substances and the toxicity of each formulation could change depending on particle sizes, volatility, etc. The new EU REACH (Registration, Evaluation, Authorisation and registration of CHemicals) regulations will mean much more testing is required, and unless rapid, meaningful screening tests can be developed, animal testing will inevitably be used. Both, *in vivo* and *in vitro* bioassays are currently used to study toxic effects in whole organisms or at cell level, respectively.

For toxicity testing, miniaturized systems have many advantages including small sample and reagent volumes and a biomimetic microenvironment within microfluidic systems ideal for microorganism maintenance. Accordingly, the microfludic environment with its inherent high surface area-to-volume ratio, provides a tool that creates a more *in vivo*-like cellular microenvironment *in vitro* than current methodology offers. The ability to control the spatial distribution within a microfluidic device readily allows for the isolation of single cells or small groups of cells and their interactions with other stimuli can be monitored (Inoue et al., 2001). Wet-etching channel geometries in glass and sealing with elastomeric siloxane polymers such as poly-dimethylsiloxane (PDMS) to generate a PDMS–glass microfluidic chip offers an ideal method for the prototyping of microfluidic chips as they have low fabrication costs and good chemical compatibility with most biological fluids. In addition, the integration of optical and/or electrochemical detectors onto the microfluidic system forms a complete device or "chip" with overall dimensions of a few centimetres (Watts and Haswell, 2005).

A number of elegant microfluidic cell based handling applications have been described for drug development, tissue engineering, molecular diagnostics and biosensors (Cho et al., 2003; MacDonald et al., 2003; Rhee et al., 2004; Zeringue et al., 2004). Microfluidic systems have also been used to analyse single cells, including bacterial, fungal, yeast and mammalian cells

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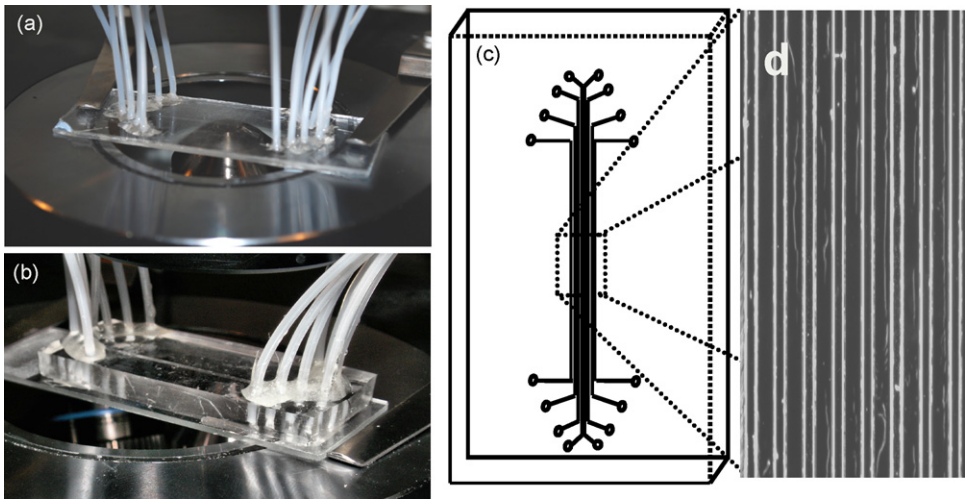

Fig. 1. Microchips with 8 multi-parallel channels. (a) Glass–glass device and (b) PDMS–glass chip on an inverted microscope. (c) Scheme showing the design of the chips. (d) Microphotography of the channels (40× magnifications).

(Tourovskaia et al., 2004; Werdich et al., 2004; Shackman et al., 2004).

Despite the advantages of using yeast (i.e. a robust eukaryotic cell line) and their widespread use in biotechnology, few microsystems have been developed using yeast. Incorporating cells within a microfluidic device would allow fast high throughput screening to test different metabolic responses to toxicants/drugs on a cellular level (e.g. human cell lines) as well as an organismal level (e.g. yeasts and bacteria).

Toxicity screening using yeast is widely used for different target compounds, such as genotoxic chemicals (Cahill et al., 2004) endocrine disrupting chemicals (EDCs, Michellini et al., 2005) or oxidative stress factors (de Souza and Geibel, 1999). Recombinant budding yeast (*Saccharomyces cerevisiae*) containing fluorescent markers such as green or red fluorescent protein (GFP or RFP) are ideal candidates for microscreening, because they fluoresce without the addition of substrates. GreenScreen™ yeast cells have been genetically modified to express the GFP whenever the cells repair damaged DNA. This yeast is being used to simultaneously detect genotoxicity and cytotoxicity (Cahill et al., 2004). Under genotoxic conditions, the fluorescence emission increases, whilst cytotoxicity is determined by a reduction in cell proliferation as compared to an untreated control. GreenScreen™ has been employed for screening both industrial products and for environmental samples (Gompel et al., 2005; Knight et al., 2004).

In this paper we describe a simple microfluidic based toxicity screening test, using glass and polydimethylsiloxane (PDMS)–glass microchips with viable recombinant yeast and fluorescence quantification. The device was designed to have a low cost and to be used by non-experts in small- to medium-sized enterprises to screen chemicals that had not been previously evaluated for their toxicity in aquatic environment.

2. Materials and Methods

2.1. Microchips

Glass (Fig. 1a) and PDMS–glass microchips (Fig. 1b) incorporating eight 20 mm long parallel channels (40 μm wide and 80 μm deep) were fabricated by wet-etching the glass using a technique similar to a previously published method (McCreedy, 2001). Briefly, the design was drawn using autoCAD software and transferred, by a commercial process (J.D. Phototools, Oldham, UK) to a film photomask. Crown white glass (B270) plates coated with chrome and photoresist (Telic Co., CA, USA) were contacted with a photomask design and exposed to UV radiation. The plates were then treated with photoresist developer followed by chrome etch solution (Rohm-Haas Ltd., UK). The exposed glass channels were etched at a rate of 4 μm per minute in a 1% hydrofluoric acid/5% ammonium fluoride solution at 65 °C. After a thorough cleaning process, the etched plates were thermally bonded (595 °C for 3 h) to top-plates with drilled access holes. The fabrication of the PDMS–glass microchips used the same methodology as described above for the glass base plates. The upper layer of PDMS (5 mm) was produced by polymerisation and was then hardened for 2 h at 90 °C before being plasma bonded to the 2 mm glass base plate (Fig. 1b).

TFZL tubes (i.d. 1/16 in., Upchurch Scientific) were used to connect the microfluidic channels to pumps and the recombinant yeast was pumped inside the chambers using a 250/500 μl syringes (SGA) connected to the system with a two-way valve. The specific assay culture media for keeping the cells alive was then pumped into the microfluidic device at a very low flow rate of 0.1 $\mu l\,min^{-1}$ using two pumps (KDS-200CE, kdScientific®).

2.2. Recombinant Yeast and Reagents

The GreenScreen™ yeast strains, specialist resuscitation and assay media were supplied by Gentronix Ltd. (Manchester, UK). A DNA repair-competent strain of the brewer's yeast *S. cerevisiae* was employed as the host strain for a reporter of DNA repair activity (the "test" strain). The reporter consisted of a fusion of the DNA damage-inducible promoter from an endogenous DNA repair gene, RAD54, with a gene encoding a yeast enhanced green fluorescent protein (yEGFP). The yeast cells are genetically modified to express a yeast enhanced GFP under the control of a copy of the promoter from the native yeast gene RAD54. RAD54 is known to be specifically up regulated by the cells in response to DNA damage. Thus, on exposure to a genotoxic agent the cells become increasingly fluorescent as GFP accumulates. A second "control" strain was used to correct for cellular or test article auto-fluorescence. The control strain contained a disabled reporter plasmid, and thus was unable to express GFP despite being identical to the test strain in every other way. Methyl methanesulfonate (MMS #M4016) as a genotoxic standard and dimethyl sulfoxide (DMSO #D8418) as diluent were purchased from Sigma–Aldrich.