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Protocols

Laser microfabrication of a microheater chip for cell culture outside a cell incubator

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ABSTRACT

Microfluidic chips have demonstrated their significant application potentials in microbiological processing and chemical reactions, with the goal of developing monolithic and compact chip-sized multifunctional systems. Heat generation and thermal control are critical in some of the biochemical processes. The paper presents a laser direct-write technique for rapid prototyping and manufacturing of microheater chips and its applicability for lab-on-a-chip cell culture outside a cell incubator. The aim of the microheater is to take the role of conventional incubators for cell culture for facilitating microscopic observation and/or other online monitoring activities during cell culture and provides portability of cell culture operation. Microheaters (5 mm × 5 mm) have been successfully fabricated on soda-lime glass substrates covered with aluminium layer of thickness 120 nm. Experimental results show that the microheaters exhibit good performance in temperature rise and decay characteristics, with localized heating at targeted spatial domains. These microheaters were suitable for a maximum long-term operation temperature of 120 °C and validated for operation at 37 °C for 48 h. Results demonstrated that the microheaters are suitable for the culture of immortalised cell lines. The growth and viability of SW480 colon adenocarcinoma cells cultured the developed microheater chip were comparable to the results obtained in a conventional cell incubator.

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1. Introduction

Microfluidic chips have demonstrated their significant application potentials in microbiological processing and chemical reactions [1], with the goal of developing monolithic and compact chip-sized multifunctional systems. The wide application of microfluidic chips in chemical, biomedical and biological research or industrial fields calls for multifunctional chips with more complex systems and high integration. Heat generation and thermal control are critical in some of the biochemical processes, particularly in the development of *in vitro* cell culture, where high thermal stability is a necessity in order to effectively mimic *in vivo* conditions. Cell cultures are an invaluable laboratory method for the investigation of cell behavior and proliferation under various conditions and stimuli, whether it be chemical, electrical or thermal. They are central to the study of cell biochemistry and physiology, including stem cells [2] and chondrocytes [3] and the investigation of drug-induced chemical reactions in cells [4]. Conventional cell culture systems typically involve the usage of a closed incubator to simulate the appropriate thermal conditions for cell proliferation. However, these systems can be expensive, non-compact and are rarely compatible with real-time microscopic imaging; this can result in periodic removal and replacement of the cell culture medium from its desired environment. The ability to provide a hospitable environment for cell growth by maintaining stringent control over the parameters of the culture conditions while simultaneously conducting real-time monitoring of the cell viability provides significant advantages for the study of cells. In recent years, progress has been made on the integration of microelectrodes such as microheaters with microfluidic devices towards the development of compact, multi-functional platforms for cell proliferation and analysis. Microfluidic platforms

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exploiting incorporated microheater have been designed for the study of the metastatic potential of lung cells [5]. Additionally, micro cell culture chambers have been developed using indium tin oxide (ITO) thin films – which are conductive and transparent to facilitate microscopic observation – to provide thermal stimulation through resistive heating for chondrocyte perfusion cell cultures [6]. Other applications include the integration of microheaters with micropumps and temperature sensors for the generation of a perfusion-based cell culture device [7]. Integrated microheater devices have also been extensively utilised in other biological reaction such as the implementation of polymerase chain reaction (PCR) procedures [8], commonly used for quantitative analysis of nucleic acids. Here again, high control over the thermal parameters are essential for the viability of the process; DNA amplification is performed using a temperature cycling concept and as such high control of the thermal parameters is essential [9]. As with the cell culture, procedures requiring PCR benefit from real-time monitoring not available when the technique was initially developed [10] but has since become commonplace [11]. Integrated microfluidic chips to carry out real-time PCR which incorporate microheaters for thermal cycling have since been developed [12,13]

Fabrication of aluminium microheaters has been carried out using both lithographic techniques [26] and UV nanosecond laser direct write [27]. Direct-write laser machining is a non-contact process utilised in the addition, subtraction or modification of a surface by means of laser-material interactions. In this paper, we propose the use of an Infrared laser for selectively eliminating an aluminium thin film from the surface of a soda-lime glass. The ablation mechanism of glass is strongly related with the wavelength absorption, the soda-lime glass present high absorption at UV (355 nm) and poor absorption at (1064 nm) IR wavelength. By using IR wavelength we are able avoid any damage at surface of glass while eliminating the aluminium thin film, which turns into high surface quality and optical transparence of the glass which makes possible optical inspection of biological experiments. Such a method can be used for the removal of matter in a defined pattern where the resulting patterns are highly dependent on laser parameters such as the focused spot size and the corresponding scan patterns. Dimensions as low as a few microns can be achieved through direct-write structuring using the nanosecond pulse laser. Furthermore, laser processing allows the removal of material without the need for masks or other predetermined patterns associated with the lithographic techniques commonly used for the fabrication of such microelectrode devices. For this reason, selective laser ablation is a powerful, reconfigurable and versatile technique, useful for rapid prototyping. Selective nanosecond laser processing can be used for the ablation of bulk material such as the fabrication of microchannels [14] or micropillars [15] in glass for microfluidic applications or the removal of thin films from a substrate [16,17] as is the case for the fabrication of the microheater device described in this paper. Glass substrates are often used for thin film heaters due to their high electrical resistivity and low thermal conductivity. The microheater is designed so as to have small resistance; this results in a higher current and hence a greater and more efficient generation of heat. Materials reported in the fabrication of microheaters include aluminium [18,19], silicon [20], tungsten [21], Au/Ti [22] Ag/Pd [23] and ITO [24,25]. This paper outlines a rapid laser direct-write technique for the fabrication of aluminium thin film microheaters using a Q-Switched Nd:YVO4 laser operating at 1064 nm for the thermal activation of SW480 colon adenocarcinoma cells. Section 2 introduces materials and methods. In Section 3 we describe the microheater fabrication procedure and results and Section 5 is devoted to discussion and conclusions.

2. Materials and methods

2.1. Materials

The glass used as a substrate for fabricating the thermal microheater was a commercial soda-lime glass, provided by a local supplier. The composition of this glass (O 50.25%, Na 9.08%, Mg 2.19%, Al 0.54%, Si 33.08%, Ca 4.87%) was determined by using a scanning electron microscope (SEM) Zeiss FESEM-ULTRA Plus issued with EDX analysis. In addition, the soda-lime glass was also characterized by its transmission spectrum obtained using a Perkin Elmer Lambda 25 spectrometer with a spectral range between 200 and 1100 nm.

2.2. Thin metal layer deposition

An aluminium layer of thickness 161 Å was deposited over soda-lime glass using a physical vapor deposition (PVD) machine (Balzers BAE 250 coating system). The aluminium used for coating the glass has a purity of 99.98%. The PVD parameters used for each thickness aluminium layer were: pressure chamber: 2 × 10–5 mbar, coating speed: 7 Å/s. Before PVD deposition, a six-step cleaning process was used for cleaning glass substrates. The samples were first brush scrubbed in an aqueous and soap bath. They were then ultrasonically pulsed in a second deionized water and soap bath heated to 30 °C and for 30 min. Then we changed the water and repeated the process. The fourth and fifth baths contained isopropyl alcohol, heated to 30 °C for 30 min. Finally, the samples were dried using air pressure.

2.3. Microheater fabrication tools

In order to perform the microstructuring of the glass, a Rofin Nd:YVO_4 laser was used. This is a Q-Switch pulsed solid-state laser, operating at 1064 nm wavelength, with an average power of 20 W, pulse durations of 20 nanoseconds (ns) and tuneable repetition rate (Single pulse to 200 kHz). An aluminium layer of thickness 161 Å was deposited over soda-lime glass using a physical vapor deposition (PVD) machine (Balzers BAE 250 coating system).

2.4. Characterization tools

A PerkinElmer Lamb25 spectrometer was used for measuring the transmission spectrum. The sample composition was determined by a Scanning electron microscope FESEM ULTRA Plus. Aluminium tracks were visually inspected with a Nikon MM-400 microscope; topographic and surface roughness assessment was performed with a SENSOFAR 2300 Plμ confocal microscope. The thermal characterization of the microheater were performed using a FLIR A325sc Infrared Camera. For all the biological observations such as cell culture and trapping, a Zeiss Microscope (Zeiss Axio Vert A.1) and Zen software was used.

2.5. Cell culture

The human colon adenocarcinoma cell line SW480 was obtained from ATCC (CCL-228^{TM}). Cells were grown in DUBELCCOís modified Eagle's medium (DMEM) (Sigma-Aldrich, Saint Louis,MO) supplemented with 10% foetal bovine serum (Gibco, Gaithersburg, MD), 100 U/mL penicillin and 100 μg/mL streptomycin (Sigma-Aldrich), at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Cells were passed before reaching 80% confluence, 2–3 times a week, and the culture medium replaced every second day.

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