Colloids and Surfaces B: Biointerfaces 134 (2015) 363–369

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

A laser-based technology for fabricating a soda-lime glass based microfluidic device for circulating tumour cell capture

Daniel Nieto[a,*,1], Ramiro Couceiro[b,*,1], Maria Aymerich[a], Rafael Lopez-Lopez[b], Miguel Abal[b], María Teresa Flores-Arias[a]

[a] Microoptics and GRIN Optics Group, Applied Physics Department, Faculty of Physics, University of Santiago de Compostela, Santiago de Compostela E15782, Spain
[b] Translational Medical Oncology, Health Research Institute of Santiago (IDIS), Fundacion Ramon Dominguez, SERGAS, 15706 Santiago de Compostela, Spain

ARTICLE INFO

Article history:
Received 20 January 2015
Received in revised form 2 July 2015
Accepted 3 July 2015
Available online 11 July 2015

Keywords:
Laser ablation
Glass materials
Microfluidics devices
Circulating tumour cells

ABSTRACT

We developed a laser-based technique for fabricating microfluidic microchips on soda-lime glass substrates. The proposed methodology combines a laser direct writing, as a manufacturing tool for the fabrication of the microfluidics structures, followed by a post-thermal treatment with a CO_2 laser. This treatment will allow reshaping and improving the morphological (roughness) and optical qualities (transparency) of the generated microfluidics structures. The use of lasers commonly implemented for material processing makes this technique highly competitive when compared with other glass microstructuring approaches. The manufactured chips were tested with tumour cells (Hec 1A) after being functionalized with an epithelial cell adhesion molecule (EpCAM) antibody coating. Cells were successfully arrested on the pillars after being flown through the device giving our technology a translational application in the field of cancer research.

1. Introduction

Microfluidics is a quickly developing engineering discipline targeting transportation and handling of small liquid volumes. An increasing number of applications derived from this field such as biomedical diagnostic, microfuel cells and cooling in microelectronics are currently being explored [1–4]. However, the materials used for microfluidic designs should gather optical quality properties in order to allow high resolution imaging; such as fluorescence microscopy, the analysis of parameters such as laminar flow, mass transport driven by diffusion and constant removal of waste products derived from manufacturing processes [5–7]. Glass materials present advantageous optical characteristics, nonetheless, other features such as surface stability, solvent compatibility, and well-known fabrication techniques make glass an interesting choice for microfluidic developments [8,9]. Glass also overcomes many limitations found in polymers by presenting a higher mechanical durability, reusability and low auto-fluorescence. However, high costs involved in glass processing and the material itself might limit a wider use as disposable devices.

An extensive range of manufacturing methods are available up to date for the fabrication of microfluidic devices, selecting the best method is mainly determined by parameters such as size and shape of the required features as well as the composition of the materials to be treated. Embossing, injection moulding, and similar thermoforming techniques, while providing excellent results, are ineffective for glass-based materials [10]. Alternatively, lithographic techniques require advanced facilities and numerous processing steps. A number of researchers have proved the feasibility of microchannel fabrication in glass using electron beam lithography, photolithography, along with wet and dry etching [11–14]. These methods provide high-quality microfluidic systems, still, several trade-offs such as sophisticated equipment located in clean rooms or the production of toxic waste, underlines significant limitations for fabricating low-cost reliable glass based microchannels. Due to its non-contact nature, laser micromachining, offers several advantages for manufacturing microfluidic channels, including the capability for producing complex shapes with minimal mechanical and thermal deformation. Fabrication of microfluidics devices on glass by laser ablation techniques has also been investigated and reported using CO_2, UV and ultra-short pulse lasers [15–17]. Microfluidic devices for detection of circulating

* Corresponding authors.
E-mail addresses: daniel.nieto@usc.es (D. Nieto), Ramiro.Couceiro.Otero@sergas.es (R. Couceiro).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.colsurfb.2015.07.007
0927-7765/

tumour cells have emerged as a promising minimally invasive diagnostic tool. Isolation of circulating tumour cells has become a central topic in cancer research where engineering and medical science converge with the common goal of capturing rare cell types in liquid biopsies as a starting point for early diagnose and the development of point of care and single cell analysis systems. Several designs have been tried in PDMS and glass substrates leading to panoply of features and arrangements able to trap cells flown through the device. In order to enhance the specificity and sensibility of such systems, a surface functionalization based on EpCAM antibodies seems to be the first choice for developing a micropillar coating, such antibodies are commonly employed for CTC isolation [18].

Although micropillars were some of the first features developed [19] as a toolbox for studying cell behaviour such as cell spreading, motility and mechanobiology, these are also interesting features to be further developed as a potential substrate easy to coat while exhibiting exceptional optical conditions for microscopy applications. Therefore, micropillar functionalization with a steady topographical control [20] could be a promising platform for a point of care device or lab-on-chip technology.

In this present work, a fast, simple and reliable process is reported for the fabrication of microfluidics devices using two lasers: a Nd:YVO_4 nanosecond IR laser for creating the desired structures and a CO_2 laser for reducing the damage created during the laser ablation (in terms of surface roughness) by the Nd:YVO_4 and improving the optical quality of the generated microstructures. The applicability of the manufactured chips was tested by functionalizing the pillars with an EpCAM antibody coating able to trap tumour cells (Hec 1A). Section 2 introduces materials and methods. In Section 3 we describe the micropillar fabrication procedure. Section 4 presents the 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 microfluidic microchannels 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, as seen on Fig. 1,

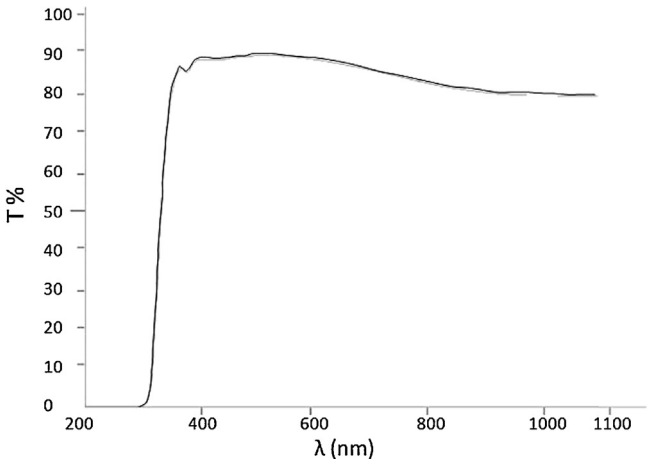

Fig. 1. Transmission spectrum of the soda-lime glass.

obtained with a Perkin Elmer Lambda 25 spectrometer with a spectral range between 200 and 1100 nm.

2.2. 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 ns and tuneable repetition rate. An Easy Mark CO_2 laser system with a wavelength of 10.6 μm, maximum power of 124 W (tuneable by changing the duty cycle), pulse durations around 20 μs and tuneable repetition rate was used for the thermal treatment.

2.3. 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. Channels were visually inspected with a Nikon MM-400 microscope; topographic and surface roughness assessment was performed with a SENSOFAR 2300 Plμ confocal microscope. For all the biological observations such as cell culture and trapping, a Zeiss Microscope (Zeiss Axio Vert A.1) and Zen software was used.

2.4. Micropillar functionalization

To chemically modify the device a variation of the protocol stated by Stott et al. [18] was followed; briefly, the microfluidic channels were treated with 4% (v/v) solution of 3-mercaptopropyl trimethoxysilane (Sigma–Aldrich, St. Louis, MO) in absolute ethanol for 1 h at room temperature, next step is an incubation in 0.01 μmol/mL N-y-maleimidobutyryloxysuccinimide ester (GMBS), an amine-to-sulfhydryl crosslinker with a short spacer arm of 7.3 Å (Pierce Biotechnology, Rockford, IL) in absolute ethanol for 30 min at room temperature.

Immediately after, the chip was treated with 10 μg/mL of NeutrAvidin (Pierce Biotechnology, Rockford, IL) solution in PBS for 45 min. The resulting functionalized device was soaked in avidin and kept overnight at 4 °C. Next day a biotinylated goat antihuman EpCAM (R and D Systems, Minneapolis, MN) solution (15 μg/mL) in PBS supplemented with 1% (w/v) bovine serum albumin (BSA) (Sigma–Aldrich, St. Louis, MO) and 0.10% (w/v) sodium azide were added to the chips. Non-bonded molecules were rinsed out of the chip after each step with an ethanol wash. For the coating a biotinylated goat IgG (R&D Systems, Minneapolis, MN) was used. Chips are finally cleared with 3% BSA with 0.05% Tween20 (Sigma–Aldrich, St. Louis, MO) in ultrapure water.

2.5. Cell line preparation

An endometrial cancer cell line, HEC-1A (ATCC, Manassas, VA), was cultured at 37 °C in 5% CO_2 in Mcoys-5A growth medium containing 1.5 mM L-glutamine supplemented with 10% FBS and 1% penicillin/streptomycin with media changes every 2–3 days. Prior to the spiking of cells into the media, all cells were pre-labelled with a fluorescent cellular dye (DiO, Invitrogen, Carlsbad, CA) following the manufacturers recommended protocol. Experiments were performed using HEC-1A cells suspended in serum-free medium.

3. Fabrication procedure

The laser direct-write technique for fabricating microfluidics micropost system is based on the ablation of a soda-lime glass substrate with a beam laser with a circular Gaussian profile followed