



## Self-folding nano- and micropatterned hydrogel tissue engineering scaffolds by single step photolithographic process <sup>☆</sup>

Iskandar Vasiev <sup>\*</sup>, Andrew I.M. Greer, Ali Z. Khokhar, John Stormonth-Darling, K. Elizabeth Tanner, Nikolaj Gadegaard <sup>\*</sup>

Biomedical Engineering Division, School of Engineering, University of Glasgow, Glasgow G12 8LT, UK

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### ABSTRACT

Current progress in tissue engineering is focused on the creation of environments in which cultures of relevant cells can adhere, grow and form functional tissue. We propose a method for controlled chemical and topographical cues through surface patterning of self-folding hydrogel films. This provides a conversion of 2D patterning techniques into a viable method of manufacturing a 3D scaffold. While similar bilayers have previously been demonstrated, here we present a faster and high throughput process for fabricating self-folding hydrogel devices incorporating controllable surface nanotopographies by serial hot embossing of sacrificial layers and photolithography.

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### 1. Introduction

Current progress in tissue engineering is focused on the creation of environments in which the relevant cells can adhere, grow and form functional tissue [1]. This proliferation can be controlled by chemical [2] or topographical [3] cues through surface patterning and functionalization. While there has been significant progress in 2D surface-cell interaction, most fall short of presenting the cells with the cues which exist in the native 3D cellular environment in a device combining surface patterning and self-folding methods [4–6]. While many 3D cellular tissue scaffold constructs have been demonstrated in the past [7], these do not offer much control over the geometry, and would not serve in making a purpose designed surface topography and structure. There is therefore a need to extend the applications of defined 2D micro- and nanopatterned methods to the third dimension. Self-folding is one technique of extending the existing and readily available 2D patterning techniques into a viable method of manufacturing more complex 3D cellular environments, and to create reconfigurable structures which can fold or unfold in response to specific environmental cues. An array of self-folding 3D structures [6], employing stimuli

responsive materials for cell capture [8] and drug delivery have been made in the past. These have often incorporated a differential bilayer structure [9] with varying environmental sensitivity [10]; from heat-shrink type hinges [11], shape memory polymers [12] to hydrogel films [13]. However these devices lack combined surface patterning on a material which will remain permeable to oxygen and nutrients. The challenge lies in the creation of permeable and patterned 2D templates composed of polymers and hydrogels to provide a basis for self-folding structures constructed using a wider range of biocompatible and biodegradable materials. One possible solution is optical patterning using photolithography [14,15] or soft lithographic methods [16] such as UV-NIL [13,16–18]. Some of these methods have previously been utilized to fabricate hingeless polymeric structures that roll up or fold spontaneously [14,15,17]. Hydrogels are of particular interest in this application because of their high water content, permittivity and mechanical properties which resemble those of nonosseous living tissues. Previous methods of manufacturing thin hydrogel scaffolds have used a two stage photolithographic process or manufacture by two-photon stereo-lithography. These processes are time consuming and require several stages of mask alignment or expensive equipment, as the micro-patterning of biocompatible hydrogel films is generally recognized as a complex task [9]. The key features for a self-folding tissue engineering scaffold are then: pattern-ability for cell contact guidance, nutrient permeability to avoid tissue necrosis, and ease and speed of manufacture.

We propose a novel method for producing environmentally triggered, self-folding, non-fouling and permeable hydrogel scaffolds, combined with nano- and micropatterned on both surfaces in a one-step photolithographic system. The manufacturing

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<sup>\*</sup> Corresponding authors. Address: Biomedical Engineering Division, School of Engineering, Rankine Building, University of Glasgow, Glasgow G12 8LT, UK. Tel.: +44 (0) 141 330 0441; fax: +44 (0) 141 330 4343 (I. Vasiev), tel.: +44 (0) 141 330 5243; fax: +44 (0) 141 330 4907 (N. Gadegaard).

E-mail addresses: [ivasiev.1@research.gla.ac.uk](mailto:ivasiev.1@research.gla.ac.uk) (I. Vasiev), [nikolaj.gadegaard@glasgow.ac.uk](mailto:nikolaj.gadegaard@glasgow.ac.uk) (N. Gadegaard).

process uses to the authors' knowledge a new method of sacrificial layer embossing and simultaneous double sided patterning and surface activation. The method allows for high throughput manufacture of "smart" hydrogel scaffolds with the added advantages of employing well developed 2D micro and nanopatterning techniques. In this approach, the previously demonstrated use of poly(acrylic acid) (PAA) as a uniform thin film sacrificial layer [19] is taken a step further with sacrificial layer embossing using an ordered micro- and nanopatterned stamp. This pre-patterning of the sacrificial film allows for the simultaneous patterning and activation of the hydrogel film applied on top of it, either by UV-NIL or UV photolithography. During the subsequent photolithographic step pendant groups of PAA are cross-linked to the poly(ethyleneglycol)dimethacrylate (PEGDMA) hydrogel matrix, forming an environmentally sensitive bilayer, where the grafted side of the thin film swells on exposure to a neutral pH 7 and collapses again in a more acidic pH 4 allowing for repeated spontaneous folding and unfolding with changes in the surrounding aqueous environment. Inter-sheet spacing is affected by exposure dose and ionic concentration of the surrounding media, or fixed in its folded state by additional UV exposure to further crosslink the hydrogel scaffold while it is in its folded state, preserving the roll like structure on further exposure to aqueous media. The finished device is a bifacially nanopatterned self-folding hydrogel scaffold, achieved by fast throughput methods.

## 2. Materials and methods

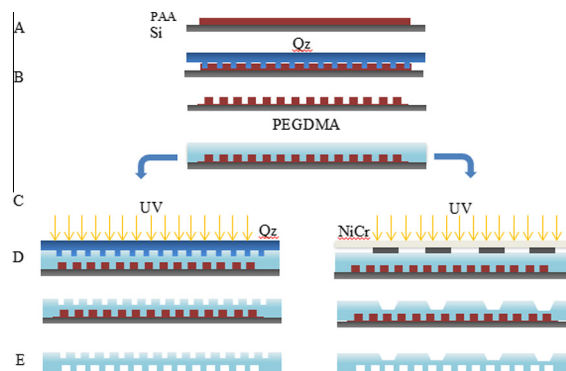
### 2.1. Materials

PEGDMA (550 Mn) and Phenylbis(2,4,6-trimethylbenzoyl)phosphine oxide (IR 819) were obtained from Sigma Aldrich, three different molecular weights of PAA (50,000  $M_w$  25% in  $H_2O$ , 100,000  $M_w$  25% in  $H_2O$  and 1800  $M_w$  67% in  $H_2O$ ) were obtained from Polysciences, the low molecular weight PAA was diluted to 25% aqueous concentration by the addition of RO Water, while the others were used as received. Sylgard 184 Polydimethylsiloxane (PDMS) and curing agent were obtained from Farnell Electronics and used as received according to manufacturer guidelines.

The micro- and nanopatterned hydrogel device were created through a series of stages to create the foundation for a high throughput one-step lithographic method. For each pattern, a mastering process was required in which quartz (Qz), silicon (Si) or PDMS stamps were prepared, to be used in subsequent hot embossing and photolithography steps of the sacrificial PAA base layer to facilitate lift-off and functionalization of the PEGDMA hydrogel layer.

### 2.2. Quartz stamp

The nanopatterned stamp utilised for both the pre-patterning of the sacrificial film and subsequent top-side patterning of the hydrogel film (as shown in Fig. 1) was fabricated from a 25 × 25 mm and 1 mm thick, quartz sample. Electron beam lithography, metal lift-off and reactive ion etching (RIE) were used to create the stamp. Electron sensitive poly(methylmethacrylate) (PMMA) 2010 4% was spun at 5000 rpm and baked at 180 °C for 15 min before repeating the process with a second layer of 2041 2.5% PMMA. The purpose of the bi-layer was to provide an undercut in the resist in order to aid a future stage of processing, metal lift-off. Prior to defining the desired nanopattern, with an electron beam lithography tool, a discharge surface layer is required as the quartz/PMMA stack has insufficient electrical conductivity and a build-up of surface charge from the electron beam exposure is known to occur on non-conductive substrates [20]. Such a charge build-up would cause uncontrollable pattern deformity [21]. In



**Fig. 1.** Fabrication process for manufacture of nano/nanopatterned and nano/micropatterned PEGDMA hydrogel films by PAA sacrificial layer embossing. (A) PAA is spun onto Si wafer. (B) PAA film is embossed with master stamp. (C) Hydrogel is applied to PAA surface. (D) Master stamp or mask is applied and assembly is exposed to UV and developed in IPA. (E) Wafer placed in RO water allowing for dissolution of PAA layer and subsequent lift-off of hydrogel patterned film.

this instance 10 nm of Al was evaporated onto the sample to act as the discharge layer.

The nanopattern design for this stamp was an ordered array of 250 nm diameter circles with a pitch of 500 nm covering a square area of 5 × 5 mm. This design was defined in the resist using a Vistec Gaussian Vector Beam 6 (100 kV) electron beam lithography tool.

Following exposure, the Al discharge layer was removed with tetramethylammonium hydroxide (TMAH) and the PMMA developed in MIBK:IPA solution. The sample was then desiccated in oxygen plasma. NiCr has been shown to be an effective hard mask for RIE quartz nanofeatures [22]. Therefore 50 nm of NiCr was evaporated onto the PMMA coated quartz face so as to create the actual nanofeature etch mask. Thereafter the PMMA resist was 'lifted-off' in acetone and a further oxygen plasma descum was performed. The penultimate step in the quartz stamp fabrication was the transfer of the defined hard mask features into the quartz. In order to achieve this, an Oxford Instruments Plasmalab 80 plus RIE machine was used with a mixture of  $CHF_3$  and Ar gases. The quartz was etched to a depth of ~250 nm producing robust 1:1 aspect ratio nanopillars. Finally any remaining NiCr on the top of the pillars was removed by giving the sample an agitated emersion in a solution of ceric ammonium nitrate and nitric acid (chrome etch).

### 2.3. Si stamp

The Silicon stamp was first fabricated for thermal NIL. A clean 15 × 15 mm Si substrate with a thickness of 500 μm was spin coated with HSQ in which the pattern was written. The design was further transferred to the Si substrate by inductively coupled plasma (ICP) dry-etching [23] The gas used for dry-etching was  $SF_6/C_4F_8$ . The dry-etching depth was found to be 260 nm as measured under SEM.

### 2.4. Release coating

Prior to use in UV-NIL or manufacture of PDMS stamps the Si and Qz masters were washed in acetone, methanol and isopropanol for 5 min each before being cleaned in an oxygen asher for 2 min. Silane deposition was carried out from a 0.0001% v/v solution of silane in heptane for 20 min. After the treatment was completed the master stamps were rinsed in heptane, acetone, and isopropanol.

### 2.5. PDMS stamps

Sylgard 184 PDMS was mixed at a ratio of 10:1 to the curing agent, and poured onto the master pattern in a salinized glass dish.

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