Fabrication and modelling of fractal, biomimetic, micro and nano-topographical surfaces

Daniel J T Kyle, Antonios Oikonomou, Ernie Hill, Aravind Vijayaraghavan and Ardeshir Bayat

1 Plastic and Reconstructive Surgery Research, Manchester Institute of Biotechnology, The University of Manchester, Manchester, UK
2 School of Computer Science, Centre for Mesoscience and Nanotechnology, The University of Manchester, Manchester, UK
3 National Graphene Institute, The University of Manchester, Manchester, UK
4 School of Materials, The University of Manchester, Manchester, UK

E-mail: Ardeshir.Bayat@manchester.ac.uk

Keywords: biomimetics, fractal micro and nanotopography, implant biocompatibility, fabrication and modelling, 3D maskless grayscale photolithography, modified deep reactive ion etching, extracellular topographical cues

Supplementary material for this article is available online

Abstract

Natural surface topographies are often self-similar with hierarchical features at the micro and nanoscale, which may be mimicked to overcome modern tissue engineering and biomaterial design limitations. Specifically, a cell’s microenvironment within the human body contains highly optimised, fractal topographical cues, which directs precise cell behaviour. However, recreating biomimetic, fractal topographies in vitro is not a trivial process and a number of fabrication methods have been proposed but often fail to precisely control the spatial resolution of features at different lengths scales and hence, to provide true biomimetic properties. Here, we propose a method of accurately reproducing the self-similar, micro and nanoscale topography of a human biological tissue into a synthetic polymer through an innovative fabrication process. The biological tissue surface was characterised using atomic force microscopy (AFM) to obtain spatial data in X, Y and Z, which was converted into a grayscale ‘digital photomask’. As a result of maskless grayscale optical lithography followed by modified deep reactive ion etching and replica molding, we were able to accurately reproduce the fractal topography of acellular dermal matrix (ADM) into polydimethylsiloxane (PDMS). Characterisation using AFM at three different length scales revealed that the nano and micro-topographical features, in addition to the fractal dimension, of native ADM were reproduced in PDMS. In conclusion, it has been shown that the fractal topography of biological surfaces can be mimicked in synthetic materials using the novel fabrication process outlined, which may be applied to significantly enhance medical device biocompatibility and performance.

Abbreviations

ADM Acellular dermal matrix
AFM Atomic force microscopy
DRIE Deep reactive ion etching
PDMS Polydimethylsiloxane
SEM Scanning electron microscopy

1. Introduction

Bio-inspired, functionalized surfaces, containing hierarchical features at the micro and nano-topographical scale, may significantly enhance device performance [1–3]. As advanced fabrication techniques are expanding the boundaries of nanoscale surface design, we are increasingly seeking inspiration from nature to overcome and progress from modern manufacturing.
limitsations [4]. Many natural surfaces contain complex, hierarchical, topographical features, which have evolved over billions of years, to perform a specific function exceptionally efficiently [5]. Applying the concept of bio-mimicry has previously led to the design and fabrication of a diverse variety of advanced devices, such as super-adhesive materials, self-cleaning materials, miniature flying machines, soft-flexible robotics and biomimetic implant surfaces which promote implant biocompatibility [6–9]. The gecko foot, for example, is covered in millions of 0.2–0.5 μm diameter spatula-shaped projections, which interact with the underlying surface through van der Waals forces, allowing the Gecko to hang upside down on almost any surface due to a remarkable adhesive force of 10 N cm² [10, 11]. Attempts at mimicking the exceptional adhesive properties of the Gecko foot in polyimide have been attempted, through a fabrication method using E-beam lithography and dry etching with oxygen, with potential applications in robotics, sports clothing, healthcare and the military [12–14].

Moreover, the design of devices containing three-dimensional (3D), high spatial resolution features have considerable potential application in fields such as microelectronics, microelectronic mechanical systems (MEMS), microfluidics and tissue engineering [15–22]. However, in order to realise the vast potential of biomimetic topographies, a reliable fabrication process capable of reproducing complex, self-similar, micro and nanoscale features is required. There are currently a number of methods available for fabricating 3D micro and nano-topographical structures, including grayscale photolithography (mask [23, 24] and maskless [25, 26]), interference lithography [27], ion-beam lithography [28], two-photon polimerisation [29], E-beam lithography [30, 31], nano-imprint lithography [32] and soft lithography [33]; with each method possessing inherent advantages and disadvantages. In particular, grayscale photolithography processes are capable of high-throughput patterning of large areas containing high vertical and lateral spatial resolution, which makes it an attractive method for the fabrication of 3D, biomimetic, surface designs [26, 34].

In contrast to binary photolithography which produces planar structures, grayscale photolithography utilises a variable-dose exposure, which controls development depth and allows the fabrication of 3D structures [34]. During grayscale photolithography, a UV-sensitive photoresist is exposed to a controlled, variable dose of UV-light, which is dictated by the grayscale level contained in the pattern. The dose corresponds with penetration depth, volume of cross-linked photoresist and feature depth after development (for positive tone resists) [26]. Grayscale photolithography is typically performed using grey-tone masks (GTM’s) and is a high-throughput process primarily used to mass produce a final object of interest, despite disadvantages including substantial mask optimisation and limited flexibility [35].

In contrast, maskless grayscale photolithography, performed using a direct laser-writing system (laser lithography) and a computer generated grayscale ‘digital mask’ is a more flexible, cost and time effective fabrication process if the aim is to manufacture a template from which the mass production of the desired surface can be made [35].

Devices fabricated to date using maskless 3D grayscale photolithography include spherical micro-lens arrays [25], cantilevers, miniature bridges [26], pyramids and miniature buildings [36], however, to the best of our knowledge, maskless 3D grayscale lithography has not yet been used to produce hierarchical, 3D, micro and nano-topographical templates for high-throughput duplication of biomimetic topographies through replica molding.

Thus, in this work, an innovative grayscale fabrication technique was developed and optimised, which utilised three dimensional digital data, obtained by atomic force microscopy (AFM) of a biological surface as a grayscale ‘digital mask’. Maskless grayscale photolithography was followed by deep reactive ion etching (DRIE), to fabricate a hard template, and replica molding, to produce polydimethylsiloxane (PDMS) topographical surfaces which were replicas of the native biological surface; thereby creating a biomimetic synthetic surface (figure 1).

As a proof of concept, the hierarchical, micro and nano-topographical features of acellular dermal matrix (ADM) are replicated in PDMS. ADM is an allogenic, decellularized, extracellular matrix (ECM) protein construct containing precisely optimised topographical cues which promote wound healing and minimisation of the foreign body reaction when utilised in vivo [37, 38]. Replicating the topographical cues contained in ADM into PDMS may significantly enhance implant performance while reducing complications, as cells may be less likely to develop an acute foreign body reaction towards an implant surface it recognises “as self”. Biomimetic surface topography may significantly enhance silicone biocompatibility through modification of cell attachment, proliferation, differentiation and attenuated foreign body reaction [39–42].

Lastly, data gathered during quantitative characterisation of ADM was used to model an ADM surface in MATLAB. This further proof of concept project aimed to determine whether it is possible to create a computer generated model of the ADM surface, containing similar roughness values and fractal properties to the native ADM images obtained through AFM, but without needing to perform the AFM measurements. Collecting a sufficient number of high quality AFM images was a significant rate limiting step in the fabrication of the biomimetic surfaces, which could be ameliorated through the application of a modelled surface. The ability to accurately model biological surfaces may facilitate quick and efficient upsampling of the grayscale technology outlined in this paper to fabricate
biomimetic implant surface topographies at industrial scale.

2. Materials and methods

2.1. Fabrication of ADM PDMS surfaces

2.1.1. Characterising ADM using AFM

ADM was imaged using a Bruker Dimension Icon® AFM. Samples were imaged using ScanAsyst™ Air probes (silicon nitride, nominal $k = 0.4 \text{ N m}^{-1}$, tip radius $= 2 \text{ nm}$) and conducted in ScanAsyst™ mode. The method of preparing ADM for characterisation can be found in supplementary data. Peak Force Tapping™ (PFT) amplitude was 150–100 nm, and PFT frequency was 1 kHz. Scan rates varied between 0.5 and 1 Hz. Images were taken with 512 samples per line and using a Z-limit of 15 µm. A large, intact area of ADM was imaged through obtaining numerous $90 \times 90 \mu m^2$ AFM scans using offsets in the X and Y direction. Scans were performed in at least three different areas of the ADM sample and on three different patient samples. Further details on optimising the use of AFM for the collection of reliable biological topographical data can be found in supplementary data and Supplementary figures S1 and S2.

2.1.2. Creating a grayscale ‘digital mask’ of ADM for photolithography

Using the stitching feature within Mountain Maps® 7 imaging software (Digital Surf®, France) numerous, adjacent $90 \times 90 \mu m^2$ AFM images of ADM (figure 2(a)) were stitched together, to create a large area pattern of ADM. The montage was produced using X and Y offsets to correctly align images; thereby forming a large intact area of ADM without leaving stitch lines (figures 2(c) and (d)).

As the minimum resolution of the laser lithography system used was $0.5 \mu m$ in X and Y, images were re-scaled prior to exposure, through averaging the heights of surrounding pixels. For example, if exposing a single $90 \times 90 \mu m^2$ grayscale AFM image (figure 2(b)), the pattern is re-scaled to 180 pixels per
prior to exposure and in the laser lithography system, pixel size is set at 0.5 μm in X and Y.

A two-dimensional (2D) topographical AFM image of ADM, where colour within the image represents height data, can be converted to a grayscale ‘digital mask’, where each pixel is assigned a grayscale level which corresponds relatively to a feature height on the biological surface. To achieve this, the ADM montage was converted to an 8 bit grayscale image, consisting of 256 grayscale levels, using the open source scanning probe analysis software Gwyddion (http://gwyddion.net/) which could then be read by a laser lithography system (figures 2(b) and (c)).

2.1.3. Silicon wafer preparation

The following fabrication protocol was optimised for the particular equipment used and further details can be found in the patent application [43]. All processing was carried out in a class 100 clean room, to ensure the surfaces remained free from airborne contamination. A 2 × 2 cm² plain silicon wafer was sonicated for 5 min each in acetone, deionised water and isopropyl alcohol, dried with a stream of dry nitrogen gas and dehydrated on a hot plate set at 200 °C for 10 min. Resist adhesion was promoted using hexamethyldisilazane (HMDS) which was spun onto the wafer at 4000 RPM for 60 s.

2.1.4. Lowering photoresist contrast, improving exposure linearity and optimisation

Immediately following the application of HMDS, positive tone photoresist S1813® was spun onto the silicon wafer at 4000 RPM for 60 s using a photoresist spinner, producing a thickness of 1.3 μm.

Following exposure, the resist was developed in MF-319® developer solution for 30 s with gentle agitation followed by 30 s in deionised water to stop the development process.

Figure 2. Creating a grayscale digital mask of acellular dermal matrix (ADM) for maskless grayscale photolithography. First, a 90 × 90 μm² atomic force microscopy (AFM) scan of ADM was obtained (a), which was converted to an 8 bit (256 levels) grayscale image (b), and after stitching of numerous AFM images, formed an ADM grayscale montage for exposure (c). A three dimensional (3D) colour representation of the ADM montage is shown in (d). Scale bar = 20 μm. Reproduced from [64].
Table 1. Grayscale photolithography process parameters.

| Substrate | Thickness | Spin Speed | Soft-bake | Exposure | Develop | Conditions |
|-----------|-----------|------------|-----------|----------|---------|------------|
| Plain silicon | 1300 nm | 4000 RPM/60 s | 72°C for 1 min 30 s on hotplate | MicroTech Laserwriter, GaN solid state laser (57.7 mW/1% filter), h-line (405 nm), 40× objective, 0.65 NA, 0 J cm⁻² (black pixels) – 0.11 J cm⁻² (white pixels). | Gentle agitation in MICROPOSIT MF-319 Developer for 30 s followed by 30 s distilled H₂O. Dried with N₂ gas. | 21 ± 2°C and humidity: 55 ± 10%.

Note. RPM = revolutions per minute; nm = nanometres; GaN = Gallium nitride; J = Joule; NA = numerical aperture.

A grayscale wedge design was used to optimise exposure dose/development linearity of the grayscale photolithography process (supplementary figures S4 and S5).

2.1.5. Maskless grayscale 3D photolithography

Maskless grayscale photolithography was performed using a laser writer (Microtech Laserwriter LW405). The prepared grayscale bitmap image was loaded into the instrument and the pixel size was set at 0.5 μm in X and Y. A gallium nitride (GaN) solid state laser, operating at 405 nm wavelength (h-line), was used with a 40× objective having 0.65 NA (table 1). A laser power of 57.7 mW and a filter of 1% were applied. An effective exposure dose of 0 J cm⁻² was assigned to black pixels (pixel 0, no exposure) and a dose of 0.11 J cm⁻² was assigned to white pixels (pixel 256, maximum exposure), with corresponding doses within this dose range assigned to each pixel according to the grayscale pattern. The grayscale levels are created through different lengths of laser pulses of between 5 and 100 nanoseconds.

2.1.6. Pattern transfer into silicon using modified DRIE to create master template

An Oxford Plasmalab 100 ICP65 System (Oxford Instruments, UK) deep reactive ion etcher (DRIE), running a modified Bosch process recipe (discussed in results) was used to permanently transfer the exposed ADM pattern from the photoresist into the silicon wafer, which subsequently acted as a template to produce PDMS stamps through replica molding. Increased isotropic etching and lower etch selectivity were required for the accurate transfer of the ADM pattern from the photoresist into the silicon. Therefore, etch selectivity was optimised through the addition of an oxygen (O₂) only step to a recipe containing optimised pressures, gases, flow rates, RF and ICP powers, step times and repeats. The modified DRIE parameters can be found in table 2.

2.1.7. Replica molding to create biomimetic silicone surfaces from master template

The PDMS used to create ADM PDMS surfaces for characterisation was kindly donated by Mentor Corporation (Mentor Corporation, Texas, USA) and was the same medical grade mixed dimethyl dispersion used to manufacture the Company’s commercially available implant surfaces. The silicon master was vapour treated with a silanizing agent, Trichloro (1H, 1H, 2H, 2H-perfluorooctyl) silane (FDTS) for 10 min in a desicator under vacuum. This reduced surface free energy in order to ease the release of PDMS from the silicon master. The PDMS was spun onto the silicon wafer (100 RPM) and de-gassed in a desiccator for 1 h to remove any bubbles and to aid the transfer of features within the silicon template into the PDMS. To cure and crosslink the PDMS, it was baked for 18 h in an oven at 80°C. A 1.5 × 1.5 cm² PDMS stamp containing the fabricated surface topography of ADM was cut out using a scalpel and characterised.

2.2. Surface characterisation

The grayscale fabrication process was optimised using an exposed grayscale wedge (supplementary figures S4 and S5) and was characterised using a contact profilometer (Veeco Dektak stylus profiler). Native ADM and ADM PDMS samples were quantitatively characterised using AFM as described previously. All samples were qualitatively characterised using SEM after coating with 2 nm chromium (Cr) and 12 nm of gold (Au). Imaging was performed on a Carl Zeiss ULTRA PLUS SEM system using a working distance of 2.7 mm and EHT voltage level of 7–10 kV.

2.3. Modelled ADM surface generated in MATLAB

The significant rate limiting step in the fabrication of the biomimetic surface was obtaining AFM images of the ADM surface. Modelling an ADM surface which has the same topographical, fractal and roughness values as native ADM, would accelerate the fabrication process while also facilitating efficient upscaling and distribution of the technology to the manufacturing industry.

Therefore, as proof of concept, using the quantitative data gathered from the comprehensive characterisation and analysis of ADM, a model ADM surface was generated using MATLAB code derived from Kroese and Botev [44]. The surface was modelled, as a fractional Brownian process, based on the intrinsic embedding method proposed by Stein [45]. A fractal dimension of 2.3 was used to reflect that obtained for the reference ADM surface. Several surfaces were generated to test the method, but only the surface with statistical parameters closest to that obtained from the ADM data shown in this work is presented.
Table 2. Grayscale deep reactive ion etch (DRIE) recipe. Reproduced from [64].

| Step            | Step time (Seconds) | Pressure (mTorr) | RF power (Watt) | ICP power (Watt) | SF₆ (Sccm) | C₄F₈ (Sccm) | O₂ (Sccm) |
|-----------------|---------------------|------------------|-----------------|------------------|------------|-------------|-----------|
| Etch            | 3                   | 10               | 5               | 300              | 100        | 5           | 0         |
| O₂ Etch         | 3                   | 10               | 5               | 300              | 0          | 0           | 30        |
| Deposition      | 4                   | 10               | 5               | 300              | 5          | 100         | 0         |
| Repeats         | 80–100              |                  |                 |                  |            |             |           |

Note. SF₆ = Sulfur hexafluoride, C₄F₈ = Octafluorocyclobutane, SCCM = Standard cubic centimetres per minute.

3. Results

3.1. Exposure of ADM pattern into photoresist through maskless grayscale photolithography

A 90 × 90 μm² grayscale AFM image of ADM was exposed, etched and replica moulded to demonstrate the optimised grayscale fabrication process, in a proof of concept approach.

The grayscale ADM pattern to be exposed is illustrated (figure 3(a.i)); alongside a corresponding line profile of the ADM image (figure 3(a.ii)), as indicated by the white dashed line. The Sz value of the ADM pattern is 3.2 μm.

The exposed ADM pattern in photoresist is shown (figure 3(b.i)); alongside the corresponding line profile (figure 3(b.ii)). The grayscale ADM pattern was deliberately inverted in photoresist (figure 3(b)), and is returned to correct profile after replica molding of the template. The grayscale photolithography parameters were optimised so that features present in the native ADM image were scaled down when exposed into photoresist. It was necessary to scale-down the features of ADM in the photoresist, as the feature sizes present in ADM (~3–6 μm) were greater than the thickness of the photoresist (1.3 μm). Therefore, the Sz value of the exposed ADM pattern in photoresist is 0.35 μm.

3.2. DRIE step to transfer ADM pattern from photoresist into silicon

The etch selectivity during DRIE has to be tailored to the feature size present in the original image and to the feature sizes exposed into photoresist. In this example, an etch selectivity of 9.2:1 was required to scale-up ADM features exposed in photoresist to their original size in the native ADM pattern.

The etched ADM pattern in silicon after DRIE is shown (figure 3(c.i)); alongside the corresponding line profile (figure 3(c.ii)). The Sz value is 3.4 μm which indicates an etch selectivity of 9.6:1 was achieved using the optimised DRIE recipe. The ADM pattern transferred into silicon had a Sz value of 3.4 μm which is very similar to the native ADM Sz value of 3.2 μm, demonstrating that the features were appropriately scaled-up during DRIE (figures 3(c.i) and (c.ii)). SEM images (figures 4(a)–(d)) reveal that the nano-scale roughness created during DRIE (figures 4(c) and (d)) in silicon resembles the nano-scale roughness present within native ADM (figures 4(a) and (b)).

3.3. Replica molding to transfer ADM pattern from silicon into PDMS

The replica molding technique was able to reproduce the features of the master template in PDMS accurately (figures 3(d.i) and (d.ii), 4 and table 3). The Sz value of ADM PDMS was 3.4 μm, which is the same as the ADM pattern in silicon, indicating that there was no loss of features during the replica molding step. The ADM PDMS surface was also comparably to the native ADM surface, in both roughness and topography as seen in figures 4 and 5.

3.4. Characterisation of ADM PDMS surfaces and comparison to native ADM

ADM PDMS surfaces were characterised and compared with native ADM, to evaluate the accuracy of the grayscale fabrication technique to reproduce ADM features in PDMS and is summarised in table 3. Further analysis of native ADM and discussion of the fractal properties can be found in supplementary data.

The grayscale fabrication technique was able to reproduce the topography of ADM at micro and nano length scales, which can be seen in figure 5.

In comparisons at 90 × 90 μm², the Sa (arithmetic mean) values of ADM PDMS (figure 5(b)) were within 5 nm of native ADM (figure 5(a)), while the Sz (maximum peak to valley distance) value was within 655 nm. The excess Sku (kurtosis) and Ssk (skewness) were all ~0 in ADM PDMS surfaces, which is representative of native ADM, indicating that in addition to the reproduction of roughness, topographical features have also been replicated. The FD (fractal dimension) value of ADM PDMS at 90 × 90 μm² was 2.29, which is the same value as in native ADM and indicates that the fractal properties of the surface at this length scale have also reproduced.

At 10 × 10 μm², the topography of ADM was also accurately mimicked in PDMS and the Sa value of ADM PDMS (figure 5(d)) was within 27 nm of native ADM (figure 5(c)), while the Sz value was within 200 nm. Again, excess Sku and Ssk were approximately 0, as they are in native ADM. The FD value of ADM PDMS at this length scale was 2.27, in comparison to 2.28 in native ADM.
Lastly, at $1 \times 1 \mu m^2$, the Sa value of ADM PDMS (figure 5(f)) was within 1 nm of native ADM (figure 5(e)), while the Sz value was within 2 nm. The excess Sku and Ssk values were all close to 0, as they are in native ADM, and the FD value is 2.25 in ADM PDMS at this length scale, in comparison to 2.29 in native ADM.
3.5. Modelled ADM surface generated in MATLAB

Figure 6 (a.i) shows a model surface of ADM generated as a fractional Brownian process based on the intrinsic embedding method proposed by Stein [45], adjacent to the native ADM surface on which it was modelled (b.i). The modelled ADM surface (a.i and ii) possess similar fractional dimension (2.34), Sa Value (620 nm), Sku (2.3) and Ssk (−0.051) as the reference surface obtained from native ADM image and shown for comparison in figures 6 (b.i and ii).

This figure demonstrates that it’s possible to accurately model the ADM surface, which possesses similar roughness and topographical characteristics as the native ADM surface. Therefore, the modelled ADM grayscale image could replace the native AFM image of ADM as the grayscale pattern exposed into photoresist, thus removing the need to obtain AFM images of the surface. Utilising the modelled surface could enable a significantly faster fabrication process, in addition to efficient upscaling and distribution of the grayscale technology outlined in this paper to the manufacturing industry.

4. Discussion

Inspired by the concept of bio-mimicry, ADM topology was reproduced in silicone through an innovative 3D, grayscale fabrication technique. This carefully optimised and characterised process is capable of high throughput reproduction of 3D, fractal, nano and micro-scale biomimetic topographies in synthetic surfaces.

For the first time, X, Y and Z spatial data acquired through AFM was used to expose a biological surface topography into a positive-tone photoresist, using maskless grayscale photolithography; followed by pattern transfer into silicon through a modified DRIE recipe. A master template was created and replica molding was used to produce PDMS stamps containing 3D biomimetic topography (figure 7).

Technologies capable of 3D surface patterning in silicon, metals and polymers, have potential applications in micro-optics and diffractive optical elements [46–48], MEMS [23, 35, 36], plasmonics [49, 50], micro-fluids [51, 52], micro-sensors [53, 54] and pertinentiy, biomimetic, functionalised medical devices [55, 56]. The fabrication of 3D biomimetic surfaces may potentially enhance medical device performance and is currently being realised in implantable devices such as dental implants, orthopaedic implants, vascular stents, nerve conduits [57–63]. The fabricated surface outlined in this manuscript was biologically evaluated as a potential topography for silicone breast implant surfaces, designed to minimise fibrotic capsule formation [64]. The biological results are published elsewhere so as to not distract from the emphasis of this paper which was on the fabrication process but crucially, the fabricated, ADM inspired silicone surface was found to significantly attenuate the in vitro foreign body reaction of breast-derived fibroblasts, in comparison to commercially available
| Surface          | Scan size ($\mu$m$^2$) | Sa (nm) | Sq (nm) | Sz (nm) | Sv (nm) | Sp (nm) | Ssk | Sku | Fractal dimension (FD) |
|------------------|------------------------|---------|---------|---------|---------|---------|-----|-----|------------------------|
|                  |                        | Mean    | ±       | Mean    | ±       | Mean    | ±   | Mean ± | Mean ±                 |
| ADM Native       | 90 × 90                | 480.44  | 177.00  | 607.63  | 221.90  | 4020.68 | 1352.28 | −2099.8 | 827.42 | 2010.89 | 644.18 | 0.01 | 0.32 | 0.03 | 0.49 | 2.33 | 0.05 |
|                  | 10 × 10                | 69.30   | 29.18   | 87.64   | 34.25   | 572.06  | 177.76 | −268.2  | 98.70  | 303.69  | 92.60  | 0.02 | 0.23 | −0.05 | 0.43 | 2.28 | 0.05 |
|                  | 1 × 1                  | 5.84    | 2.33    | 7.39    | 2.94    | 46.89   | 15.60 | −23.85  | 7.65   | 23.00   | 9.18  | −0.04 | 0.14 | −0.10 | 0.73 | 2.29 | 0.02 |
| ADM PDMS         | 90 × 90                | 484.00  | 187.88  | 550.00  | 231.57  | 3366.00 | 1119.39 | −1692.0 | 648.16 | 1674.00 | 472.55 | −0.07 | 0.06 | −0.18 | 0.23 | 2.29 | 0.07 |
|                  | 10 × 10                | 95.62   | 27.27   | 122.78  | 35.97   | 762.00  | 315.77 | −436.80 | 213.10 | 325.00  | 105.93 | −0.15 | 0.31 | 0.22  | 0.55 | 2.27 | 0.03 |
|                  | 1 × 1                  | 5.93    | 0.04    | 7.44    | 0.01    | 48.40   | 0.28  | −23.40  | 0.84   | 25.00   | 1.13  | −0.01 | 0.28 | 0.14  | 0.09 | 2.25 | 0.03 |

**Note.** ± = SD, Sa = arithmetic mean; Sq = Root mean squared roughness; Sz = maximum peak to valley distance; Sv = maximum valley depth; Sp = maximum peak height; Ssk = skewness; Sku = kurtosis and FD = fractal dimension.
silicone implant surfaces [64]. The biomimetic surface holds potential as being able to reduce the acute inflammatory cellular response towards it, which requires further in vivo confirmation in the future.

Figure 5. A comparison of native acellular dermal matrix (ADM) topography and roughness, at 90 × 90 μm² (a), 10 × 10 μm² (c) and 1 × 1 μm² (e) with acellular dermal matrix polydimethylsiloxane (ADM PDMS) at 90 × 90 μm² (b), 10 × 10 μm² (d) and 1 × 1 μm² (f) to analyse the accuracy of the novel grayscale fabrication method of reproducing ADM topography in silicone. Two dimensional (2D) atomic force microscopy (AFM) images are shown in (i), alongside the corresponding three dimensional (3D) images shown in (ii). Section profiles of 2D images (i) (indicated by white dashed line) are shown in (iii). 3D areal analysis measurements are shown in (iv). The figure demonstrates the accuracy of the optimised grayscale fabrication process to reproduce the hierarchical, self-similar features within native ADM in silicone at each length scale analysed.

Maskless grayscale photolithography followed by DRIE and replica molding offers a number of advantages for the fabrication of biomimetic micro and nano textured surfaces. The resolution of features
produced by maskless photolithography is in theory only limited by the diffraction limit of the wave-length of light as the numerical apertures used when fabricating miniaturised devices is typically $> 0.5$ [65]. Therefore, in general, the smallest feature which can be reproduced is equivalent to, or slightly smaller than, the wavelength of light used [65]. Sub-micron features are possible with precise optimisation of the whole optical lithography process including sample preparation, soft-bake, photoresist contrast and development. Furthermore, the maskless system provides the flexibility to adjust and optimise the grayscale ‘digital mask’ design, with minimal cost and time, which is a significant advantage over the use of a GTM for fabricating templates. Lastly, maskless grayscale photolithography is a single exposure method which makes processing time quicker than multi-exposure systems or E-beam lithography, which is a high resolution, but slow and expensive patterning process [35].

However, grayscale fabrication processes require a low contrast photoresist which develops linearly with exposure dose, to allow more grey levels to be realised and the fabrication of varying relief structures (supplementary figures S3 and S4). To achieve this, the soft-bake temperature was reduced from 100 °C to 72 °C, and soft-bake time from 3 min to 1 min 30 s. Reducing the soft-bake time and temperature results in increased solvent retained in the photoresist. This increases the amount of dark erosion (areas of photoresist which are removed at low dose) during development; thereby lowering the contrast [66]. This allows 3D structures to be exposed in the photoresist as the feature height after development corresponds with exposure dose, which corresponds with grayscale level. (Further discussion on lowering photoresist contrast for the fabrication of 3D features is provided in supplementary data.)

Finding a balance between exposure dose and development time is also important to enable the accurate reproduction of micro and nano-topographical features into thin (1.3 μm) resists. The penetration depth of light is greater than resist thickness in thin resists, even at lowest exposure dose, and over-development results in the loss of resolution. Therefore, the development time was correlated with the optimised resist contrast, to achieve the fabrication of high resolution features at the correct scale. In the final optimised process, the development time was shortened to 30 s from 40 s, to account for the increased dark erosion as a result of the cooler/shorter soft-bake.

Features of ADM were scaled-down to accommodate them in resist during photolithography and were subsequently scaled-up to their original size during DRIE. In the demonstrated example, an etch selectivity of 9.2:1 was calculated (etch rate of silicon: etch rate of photoresist) to scale features back up to their original size. However, the Bosch process was designed to fabricate high-aspect ratio features, capable of etch selectivity’s greater than 75:1, which is significantly greater than what is required here [67]. Etch selectivity during DRIE has previously been reduced through the addition of an oxygen (O₂) only step which increases the photoresist etch rate [23, 24, 68]. O₂ plasma reacts with organic material in the photoresist forming CO, CO₂ and H₂O, which are readily removed from the surface [69]. Furthermore, these reactions are exothermic, which increases the temperature at the sample surface, further increasing reaction rate [23]. Therefore, it was decided to include a 3 s
The oxygen only step at a flow rate of 30 sccm, between etch and passivation steps, which reduced etch selectivity to 9.6:1. Furthermore, an unacceptable amount of 'scalloping' was observed with the first DRIE recipes, which was diminished through decreasing the silicon etch step time (SFt) from 6 to 3 s, which reduces the amount of silicon etched each step [70–72]. The oxygen only step may also have contributed to reduced scalloping [73, 74]. The etch rate was also reduced as a result of the shorter etch step time and therefore the number of repeats was increased from 30–40 to 80–100 to achieve the desired feature depth (Etch rate 0.25 μm min⁻¹). Further discussion on the application of DRIE to fabricate nanoscale topographies can be found in supplementary data. Replica molding is an established technique used to create replicas from a hard master template in a soft polymer based material and has been shown to replicate features down to 1.5 nm in PDMS [33, 75]. Using this technique ADM topography was reliably reproduced with characterisation in PDMS with characterisation demonstrating no loss of features above the lithography resolution limit.

Interestingly, characterisation of ADM revealed a fractal, self-similar surface containing micro and nano-scale hierarchical features. The nature of ADM self-similarity suggested there was potential to model the ADM surface, allowing features to be modified (either enhanced or reduced), depending on the requirements of the fabricated surface. Therefore, as proof of concept, the spatial data gathered from the comprehensive characterisation of ADM, was input into modelling software (MATLAB) and using MATLAB code derived from Kroese and Botev [44], a computer generated replica of ADM topography was produced, which could be used for grayscale fabrication. The ADM surface was accurately modelled in MATLAB and possessed the same fractal dimension (2.3) as the reference surface (figure 6). This potentially replaces the need to use grayscale AFM images of biological surfaces and facilitates faster fabrication speeds in addition to effortless distribution to industry and large scale manufacturing. Furthermore, the flexibility offered by a modelled surface enhances the ease at which this technology can be implemented and the number of potential applications.

The primary application of this technology is the enhancement of medical device performance, through reproducing biomimetic, fractal, ECM topographical features onto synthetic prosthesis surfaces, which may encourage implant integration while reducing foreign body reaction as has been demonstrated in previous studies [76, 77]. Medical implant biocompatibility is of great current interest and significance to the National Health Service, as an ageing population increases the demand for tissue replacements [78]. Thus, the design and manufacture of novel, micro and nano-topographical implant surfaces, which improve tissue integration, reduce complications and enhance outcomes, are of paramount importance in improving long term patient healthcare [79]. However, the techniques described in this paper can be used to fabricate biomimetic, nano and micro-scale topographical surfaces for any application.

5. Conclusions

For the first time, a biomimetic, 3D, nano and micro-textured silicone surface has been fabricated utilising the X, Y and Z spatial data of a biological surface (ADM), obtained through AFM. The rendered 2D topographical grayscale pattern was precisely exposed into an optimised photore sist using maskless grayscale photolithography. The pattern was successfully transferred into silicon using a modified version of the Bosch process and was used to create PDMS stamps through replica moulding. This comprehensively characterised and optimised process can fabricate hard
templates containing precisely controlled 3D, biomimetic features at a faster rate, with more flexibility and at lower cost, than conventional grayscale photolithography requiring a mask. Potential applications include significantly enhancing microelectronic and medical device performance.

Acknowledgments

We would like to thank all our clinical colleagues and collaborators who have contributed toward our ongoing research in this interesting and important subject area.

Author contributions

The manuscript was written through contributions of all authors. Daniel J T Kyle planned, optimised and performed all experiments, performed data analysis and wrote the manuscript. Antonios Oikonomou assisted the experimental design, performed optimisation experiments and provided critical technical and experimental guidance. Dr Aravind Vijayaraghavan provided technical and experimental guidance and reviewed manuscript. Dr Ernie Hill and Dr Ardeshir Bayat conceived the study, interpreted results, critically read and edited the manuscript. All co-authors revised the manuscript and have given approval to the final version of the manuscript.

Funding sources

This work was funded by Mentor Corporation, USA. Mentor Corporation did not take any part in the study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

AO and AV acknowledge funding from the Engineering and Physical Sciences Research Council (EPSRC) grant EP/K009451/1.

Conflict of interest

The authors declare no competing conflict of interest.

References

[1] Flemming R G, Murphy C J, Abrams G A, Goodman S L and Nealey P F 1999 Effects of synthetic micro- and nanostructured surfaces on cell behavior Biomaterials 20 573–88
[2] Anselme K and Bigerele M 2011 Role of materials surface topography on mammalian cell response Int. Mater. Rev. 56 243–66
[3] Stevens M M and George J H 2004 Use of nanotopography to study mechanotransduction in fibroblasts—methods and perspectives Eur. J. Cell Biol. 83 159–69
[4] Antonsen K et al 2012 Hierarchical three-dimensional microbattery electrodes combining bottom-up self-assembly and top-down micromachining ACS Nano 6 6422–32
[5] Waits C M, Morgan B, Kastantin M and Ghodssi R 2005 Microfabrication of 3D silicon MEMS structures using gray-scale lithography and deep reactive ion etching Sensors Actuators A 119 445–53
[6] Waits C M, Modeca A and Ghodssi R 2003 Investigation of gray-scale technology for large area 3D silicon MEMS structures J. Micromech. Microeng. 13 170
[7] Totsu K, Fukushima K, Tanaka S and Esashi M 2006 Fabrication of three-dimensional microstructure using maskless gray-scale lithography Sensors Actuators A 130–131 387–92
[8] Rammohan A, Dwivedi P K, Martinez-Duarte R, Katepalli H, Madow M J and Sharma A 2011 One-step maskless grayscale lithography for the fabrication of 3-dimensional structures in SU–8 Sensors Actuators B 153 125–34
[9] Campbell M, Sharp D N, Harrison M T, Denning R G and Turberfield A J 2000 Fabrication of photonic crystals for the visible spectrum by holographic lithography Nature 404 53–6
[10] Yong-Qi F, Kok Ann Bryan N and Shing O 2000 Diffractive optical elements with continuous relief fabricated by focused ion beam for monomode fiber coupling Opt. Express 7 141–7
Bioinspir. Biomim. 11 (2016) 046009

[29] Kawata S, Sun H-B, Tanaka T and Takada K 2001 Finer features for functional microdevices Nature 412 697–8

[30] Hu F and Soo-Young L 2003 Dose control for fabrication of grayscale structures using a single step electron-beam lithographic process J. Vac. Sci. Technol. B 21 2672–9

[31] Kim J, Joy D C and Lee S Y 2007 Controlling resist thickness and etch depth for fabrication of 3D structures in electron-beam grayscale lithography Microelectron. Eng. 84 2859–69

[32] Li M, Chen L and Chou S Y 2001 Direct three-dimensional patterning using nanoimprint lithography Appl. Phys. Lett. 78 3322–4

[33] Xia Y and Whitesides G M 1998 Soft lithography Annu. Rev. Mater. Sci. 28 153–84

[34] Mosher L, Waits C M, Morgan B and Ghodssi R 2009 Double-exposure grayscale photolithography J. Microelectromech. Syst. 18 308–15

[35] Lake J H, Cambron S D, Walsh K M and McNamara S 2011 Maskless grayscale lithography using a positive-tone photodefinable polyimide for MEMS applications J. Microelectromech. Syst. 20 1483–8

[36] McKenna C, Walsh K, Crain M and Lake J (ed) 2010 Maskless direct write grayscale lithography for MEMS applications 2010 18th Biennial University/Government/Industry-Micro/Nano Symp. (UGIM) (Piscataway, NJ: IEEE)

[37] Basu C B, Leong M and Hicks M J 2010 Acellular cadaveric dermis decreases the inflammatory response in capsule formation in reconstructive breast surgery Plast. Reconstr. Surg. 126 1842–7

[38] Reyzelman A et al 2009 Clinical effectiveness of an acellular dermal regenerative tissue matrix compared to standard wound management in healing diabetic foot ulcers: a prospective, randomised, multicentre study Int. Wound J. 6 196–208

[39] Chen S, Jones J A, Xu Y, Low H Y, Anderson J M and Leong K W 2010 Characterization of topographical effects on macrophage behavior in a foreign body response model Biomaterials 31 3479–91

[40] Dalley M J 2005 Topographically induced direct cell mechanotransduction Med. Eng. Phys. 27 730–42

[41] Yang J et al 2014 Nanotopographical induction of osteogenesis through adhesion, bone morphogenic protein cosignaling, and regulation of MicroRNAs ACS Nano 8 9941–53

[42] Teo B K K et al 2013 Nanotopography modulates mechanotransduction of stem cells and induces differentiation through focal adhesion kinase ACS Nano 7 4785–98

[43] Kyle D J T, Oikonomou A, Hill E W and Bayat A Inventors. Issuing Organisation: United Kingdom Intellectual Property Office 2014 Filing Date: 17.02.2014

[44] Kroese D P and Botez Z I 2013 Spatial process generation arXiv:13080399

[45] Stein M L 2002 Fast and exact simulation of fractional Brownian surfaces J. Comput. Graph. Stat. 11 587–99

[46] Morgan B, Waits C M, Krimanicki J and Ghodssii R 2004 Development of a deep silicon phase Fresnel lens using grayscale lithography and deep reactive ion etching J. Microelectromech. Syst. 13 113–20

[47] Yu W and Yuan X 2003 Fabrication of refractive microlens in hybrid SiO2/TiO2 sol–gel glass by electron beam lithography Opt. Express 11 899–903

[48] Zhong K, Gao Y, Li F, Zhang Z and Luo N 2014 Fabrication of PDMS microarrays by arraying digital maskless grayscale lithography and replica molding technique Optik 125 2413–6

[49] Yang J C, Gao H, Su H Y, Zhou W, Lee M H and Odom T W 2010 Enhanced optical transmission mediated by localized plasmons in anisotropic, three-dimensional nanohole arrays Nano Lett. 10 3173–8

[50] Zentgraf T, Liu Y, Mikkelsen M H, Valentine J and Zhang X 2011 Plasmonic lumenburg and electron lenses Nat. Nano 6 151–5

[51] Atencio J, Barnes S, Douglas J, Meacham M and Locascio L E 2007 Using pattern homogenization of binary grayscale masks to fabricate microfluidic structures with 3D topography Lab Chip 7 1567–73

[52] Huh D, Hamilton G A and Inger D E 2011 From 3D cell culture to organs-on-chips Trends Cell Biol. 21 745–54

[53] Higurashi E, Chino D, Sugia T and Sawada R 2009 Au–Au surface-activated bonding and its application to optical microsensors with 3D structure IEEE J. Sel. Top. Quantum Electron. 15 1500–5

[54] Varadan V and Varadan V 2000 Microsensors microelectromechanical systems (MEMS) and electronics for smart structures and systems Smart Mater. Struct. 9 953

[55] Soumya S, Sreerekha P R, Menon D, Naik S V and Chennazhi K P 2012 Generation of a biomimetic 3D microporous nano–fibrous scaffold on titanium surfaces for better osteointegration of orthopedic implants J. Mater. Chem. 22 1904–15

[56] Nikkhah M, Edalat F, Manoucheri S and Khademhosseini A 2012 Engineering microscale topographies to control the cell-substrate interface Biomaterials 33 5330–46

[57] Dahiya V, Shukla P and Gupta S 2014 Surface topography of dental implants: a review J. Dental Implants 46 66–71

[58] Sullivan M P, McHale K J, Parviz I and Mehta S 2014 Nanotechnology: current concepts in orthopaedic surgery and future directions Bone Joint J. 96 B 569–73

[59] Qi P, Maizt M F and Huang N 2013 Surface modification of cardiovascular materials and implants Surf. Coat. Technol. 233 80–90

[60] Perán M, García M A, López–Ruiz E, Jiménez G and Marchal J A 2013 How can nanotechnology help to repair the body? Advances in cardiac, skin, bone, cartilage and nerve tissue regeneration Materials 6 1335–59

[61] Liu R, Qin J, Zhao L, Zhang X and Xue X 2014 The Microstructures and materials of nerve conduits used in peripheral nerve regeneration J. Biomater. Tissue Eng. 4 65–83

[62] de Mel A, Jell G, Stevens M M and Seifalian A M 2008 Biofunctionalization of biomaterials for accelerated in situ endothelialization: a review Biomacromolecules 9 2969–79

[63] Trask R, Williams H and Bond I 2007 Self-healing polymer composites: mimicking nature to enhance performance Bioinsp. Biomim. 2 1–9

[64] Kyle DJ, Oikonomou A, Hill E and Bayat A 2015 Development and functional evaluation of biomimetic silicone surfaces with hierarchical micro–nano–topographical features demonstrates favourable in vitro foreign body response of breast-derived fibroblasts Biomaterials 52 88–102

[65] Ito T and Okazaki S 2000 Pushing the limits of lithography Nature 406 1027–31

[66] Gnilk M 2011 Grey scale lithography with ‘thin’ photoresists, MicroChemicals Data Sheet

[67] Westerman R, Martinez L, Pays-Volard D, Mackenzie K and Lazerand T (ed) 2014 Deep silicon etching: current capabilities and future directions SPIE MOEMS–MEMS (International Society for Optics and Photonics)

[68] Wang X, Chen Y, Wang L and Cui Z 2008 Fabrication of nanoimprint template in Si with high etch rate by non-switch DRIE process Microelectron. Eng. 85 1015–7

[69] Quirk M and Serda J 2001 Semiconductor Manufacturing Technology (Upper Saddle River, NJ: Prentice–Hall)

[70] Lai S, Johnson D, Westerman R J, Nolan J J, Purser D and Devre M (ed) 2003 Scallop minimizing in deep Si etching on Unaxis DSE tools

[71] Wang X, Zeng W and Eisenbraun E 2007 Sub-0.25 micron silicon via etching for 3D interconnects J. Micromech. Microeng. 17 155

[72] Chang C et al 2005 Etching submicrometer trenches by using the Bosch process and its application to the fabrication of antirefection structures J. Micromech. Microeng. 15 580

[73] Jensen J et al 2010 Black silicon method XI: oxygen pulses in SF6 plasma J. Micromech. Microeng. 20 075027

[74] Guo M, Chou X, Mu J, Liu B and Xiong J 2013 Fabrication of micro–trench structures with high aspect ratio based on DRIE
process for MEMS device applications Microsyst. Technol. 19 1097–103

[75] Gates B D, Xu Q, Stewart M, Ryan D, Willson C G and Whitesides G M 2005 New approaches to nanofabrication: molding, printing, and other techniques Chem Rev. 105 1171–96

[76] Harvey A G, Hill E W and Bayat A 2013 Designing implant surface topography for improved biocompatibility Expert Rev. Med. Devices 10 257–67

[77] Mwenifumbo S and Stevens M M 2007 ECM interactions with cells from the macro- to nanoscale Biomedical Nanostructures (New York: Wiley) pp 223–60

[78] Campoccia D, Montanaro L and Arciola C R 2013 A review of the clinical implications of anti-infective biomaterials and infection-resistant surfaces Biomaterials 34 8018–29

[79] Williams D F 2008 On the mechanisms of biocompatibility Biomaterials 29 2941–53