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24 Abstract

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Rapid prototyping of multi-scale biomedical microdevices by combining additive manufacturing technologies

Stefan Hengsbach · Andrés Díaz Lantada

Abstract The possibility of designing and manufacturing biomedical microdevices with multiple length-scale geometries can help to promote special interactions both with their environment and with surrounding biological systems. These interactions aim to enhance biocompatibility and overall performance by using biomimetic approaches. In this paper, we present a design and manufacturing procedure for obtaining multi-scale biomedical microsystems based on the combination of two additive manufacturing processes: a conventional laser writer to manufacture the overall device structure, and a direct-laser writer based on two-photon polymerization to yield finer details. The process excels for its versatility, accuracy and manufacturing speed and allows for the manufacture of microsystems and implants with overall sizes up to several millimeters and with details down to sub-micrometric structures. As an application example we have focused on manufacturing a biomedical microsystem to analyze the impact of microtextured surfaces on cell motility. This process yielded a relevant increase in precision and manufacturing speed when compared with more conventional rapid prototyping procedures.

Keywords Fractals · Surface topography · Material texture · Materials design · Computer-aided design · Additive manufacturing · Direct laser writing

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1 Introduction

Biomedical devices that include geometries and functions on multiple length scales and at different locations are able to interact with their environment and surrounding living systems in a more controlled and accurate way. Multi-scale biomedical devices help to promote biomimetic approaches, as living organisms also exhibit forms and functions at different scales (Place et al. 2009), thus helping to improve aspects such as biocompatibility and overall performance. Therefore, progressive research into design and manufacturing strategies that promote hierarchical materials and structures and their integration into complex appliances is helping to improve both the diagnostic and therapeutic results of several biodevices. In biomedical sciences, fields such as prosthetics (Ponche et al. 2010; Anselme et al. 2010), health-monitoring and diagnosis (Reljin & Reljin 2002), tissue engineering (Hosseinkhani et al. 2010; Hosseinkhani et al. 2007) and even biofabrication (Borchers et al. 2012) are already starting to take advantage of multi-scale approaches, the applications of which are continuously evolving.

Directly related to the concept of multi-scale geometries, material surface topography has an extraordinary influence on several relevant properties linked to final material (and device) performance. These properties include friction coefficient (Archard 1974), wear resistance (Bushan et al. 1995), self-cleaning ability (Barthlott & Neinhuis 1997), biocompatibility (Buxboim & Discher 2010), optical response (Berginski et al. 2007), touch perception, overall aesthetic aspect and even flavor (Briones et al. 2006), to cite just a few. Thus, topography also plays a determinant role in material selection in engineering design, especially in the field of micro and nanosystem development for biomedical engineering, where the effects of topography on the incorporation of advanced properties are even more remarkable.

Normally, material surface topography is a consequence of a material's natural state. It can also be the result of machining processes, chemical attacks or post-processes used to manufacture a device or product. Several strategies for modifying material topographies and surface properties (towards hierarchical materials, structures and multi-scale devices) have taken advantage of conventional surface micromachining (Madou 2002), laser ablation (Chandra et al. 2010), micromolding (Martin & Aksay 2005), biomimetic templating (Pulsifier & Lakhtakia 2011), physical and chemical vapor deposition processes (Kwasny 2009), sol–gel procedures (Jedlicka et al. 2007) and molecular self-assembly (Rahmawan et al. 2013). All these processes require enormous hands-on expertise and the final result depends on several control parameters whose interdependencies are normally complex to understand, characterize, model and master (Gad-el-Hak 2003). As can be seen from the previously cited documents, top-down and bottom-up approaches for controlling surface properties co-exist and in many cases complement each other (Naik et al. 2009). The former are more focused on mass-production (as they are derived from the microelectronic industry), while the latter provide remarkable geometric versatility.

Combinations of top-down and bottom-up approaches are frequent and have usually focused on manufacturing the larger micrometric features by means of top-down processes (micromachining, etching, etc.). The smaller nanometric details, such as for the rapid prototyping of patterned functional nanostructures (Fan et al. 2000), are made using bottom-up techniques (like CVD, PVD, sol–gel, self-assembly, ink-jet printing). Normally these combinations are not aimed at obtaining 3D features at different scales, but at incorporating some surface patterns, 2D ½ geometries or some sort of physical-chemical functionality, such as enhancing biocompatibility and implementing special actuating-sensing functions.

Currently, advances in computer-aided design and in high-precision additive manufacturing technologies based on layer-by-layer deposition or construction are opening new horizons for controlling surface topography. They are being used from the design stage and can be applied in a manner that is very direct, rapid and simple. This is enabling the prototyping of multi-scale designs and hierarchical structures. Even though conventional computer-aided design packages are only capable of handling Euclidean geometries and mainly rely on simple operations (sketch based operations, extrusions, pads, holes, circular grooves, etc.) for obtaining "soft" solids and surfaces, recent approaches relying on the use of matrix-based programming have already proved to be useful for designing rough surfaces and textured objects adequately described by fractal geometries (Mandelbrot 1982a; Falconer 2003a). In parallel, the continued progress in additive manufacturing technologies (also called "solid free-form fabrication" due to the complex geometries attainable), especially during the last decade, has increased the range of materials capable of being additively processed and greatly promoted their precision, even down to nanometric features. This has implications in the development of advanced materials and metamaterials, many of which benefit from multi-scale approaches (Bückmann et al. 2012; Röhrig et al. 2012).

Ultra-high precision additive manufacturing technologies, however, mainly direct-laser writing based on two-photon polymerization, despite being capable of yielding nanometric details, are very slow and the attainable devices are normally smaller than 1 mm^3. Such tiny devices are normally aimed at very specific studies (i.e. single-cell mechanical-biological experiments). Obtaining successful implants, as well as easy-to-handle microsystems, is still challenging since most biodevices and medical appliances, either for diagnostic or for therapeutic tasks, are at least several mm^3. On the other hand, industrial rapid prototyping (i.e. laser stereolithography, digital-light processing and selective laser sintering), in spite of being fast and capable of yielding larger devices, is limited to manufacturing precisions typically in the 50–250 μm range. It is thus still unable to produce biomedical microdevices with ad hoc features for interacting at the molecular or even cellular level.

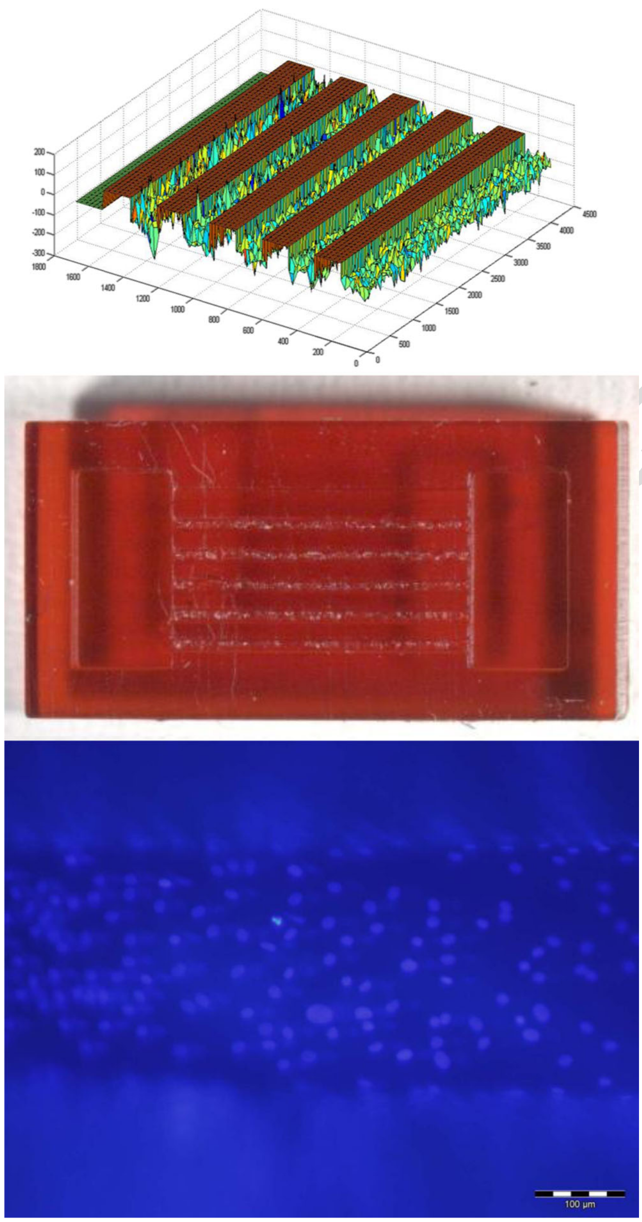
In this paper, we present a design and manufacturing procedure for obtaining multi-scale biomedical microsystems that is based on the combination of two additive manufacturing processes: a conventional laser writer to manufacture the overall device structure, and a direct-laser writer based on two-photon polymerization to yield the smallest details. The process stands out for its versatility, accuracy and manufacturing speed and allows for the manufacture of microsystems and implants with overall sizes up to several millimeters and with details down to sub-micrometric structures. The following section explains the methods and materials used. We then present our main results, propose some future directions and detail our concluding remarks.

2 Materials and methods

2.1 Design process

As application example we have selected a biomedical microsystem aimed at addressing the influence of microtextures on cell motility. The system includes two microchambers connected by several microchannels to guide cell movement, each with a different texture at its bottom. The cell motility experiment should begin adding cells to one of the chambers and growth factors to the other one, so as to promote cell movement from one chamber to another.

The design presented here is inspired by existing devices (Díaz 2013), though it has been adapted to scales better suited to interacting at a cellular level. Previous designs and prototypes included 300-μm wide and 3-mm long channels and were manufactured using conventional digital light processing. Figure 1 shows the matrix-based design (see description below) of microtextured channels, with the aforementioned preliminary rapid prototype obtained by digital light processing, and cell culture results that exhibit adequate attachment of cells within a textured channel. One of the main limitations of this preliminary device is that the microchannels are too wide for adequate assessment of cell motility, since several cells can enter the channel at once. In addition, the microtextures



Q1 **Fig 1** Matrix-based design of microtextured channels. Rapid prototype obtained by digital-light processing and results from cell culture, showing adequate attachment of cells within a textured channel. Adapted from: A. Díaz Lantada, Handbook on advanced design and manufacturing technologies for biomedical devices, Springer, 2013

attainable by conventional rapid prototyping have a typical height of 50–250 μm, what is not perceived by single cells as a real texture.

For more adequate interactions at a cellular level, 30-μm wide channels and 1–5 μm high textures, similar to the dimensions of pseudopods and cytoplasmatic deformations, would be advisable. At the same time, the overall device size cannot be importantly reduced if it is to remain manipulable. Fulfilling both requirements suggests a multi-scale approach, as we will attempt to explain further on. This approach uses one technology and related material to manufacture the overall structure, and another technology and related material for the smallest details.

The design process, then, also includes combinations of different processes. First, the overall structure, which mainly comprises the different walls of the two circular microchambers and the six microchannels, is designed using conventional 3D computer-aided design methods. The CAD files can be converted into .stl (standard tessellation language) format, currently the most common file type used in 3D additive manufacturing. Different technologies including as digital light processing, conventional laser stereolithography, selective laser sintering or melting and fused deposition modeling allow .stl file as information input. The specific method chosen would depend on the desired material and precision (in our case we used a Heidelberg Instruments DWL66fs laserwriter). There is also the possibility of converting the 3D design into a black-white mask for 2D½ manufacture of the overall structure using lithographic approaches typical to the electronic industry.

Subsequently, to incorporate the desired high-precision microtextures (capable of interacting at a cellular level), additional design operations rely on the generation of simple geometries via matrix-based approaches. In such matrix-based designs the geometries are stored in the form of [X, Y, Z (x, y)] matrices, where X and Y are column vectors with the x and y components of the working grid, and Z (x, y) is a column vector whose components are the height values for each (x, y) couple (spherical and cylindrical coordinates can be used for the cases of spherical and cylindrical meshes). Then, fractal features can be introduced to incorporate controlled random textures to the initially regular meshes (z_0), as previously detailed (Díaz Lantada et al. 2010). In this paper we use fractional Brownian surface models (Mandelbrot 1982b; Falconer 2003b) to incorporate the desired height fluctuations by means of the following equation:

$$z(x,y) = z_0 + m\cdot\sum_{k=1}^{\infty} C_k\cdot\lambda^{-\alpha k}\cdot\sin\left(\lambda^k\left[x\cdot\cos(B_k) + y\cdot\sin(B_k) + A_k\right]\right)$$ Q2

The models use several random functions (A_k, B_k, C_k) and control constants (λ, α, m), and an initial height function "z_0" can also be introduced. It is interesting to note that in fractional

Brownian models (Mandelbrot 1982b; Falconer 2003b), the fractal dimension can be related to the exponent α, where D= 3 - α, with $0<\alpha<1$. Therefore, higher values of "alfa" lead to more "planar" surfaces or textures and lower values of "alfa" lead to more "three-dimensional" or spiky surfaces or textures, as shown in Figs. 1a and 2b. Adequately assessing the most beneficial values of "alfa" for different applications is still a matter of research; for instance, our team has addressed its impact on cell culture (Díaz Lantada et al. 2011). By truncating the aforementioned sum of infinite terms, basic fractal geometries can be obtained in matrix form and further converted into recognizable CAD formats, typically .stl (standard tessellation language) .igs (initial graphics exchange specification) or .dxf (drawing exchange format). In our case the surface generation has been programmed using Matlab (The Mathworks Inc.). The use of additional "mesh to solid" converters leads to the final solid files, which can be used as normal CAD parts for further design, simulation, modeling and computer-aided manufacturing tasks. The process can be adapted to the surfaces of any computer-aided designed implant and multi-scale designs are possible, normally using conventional Euclidean surfaces for micrometric – milimetric features. The fractal term would usually be added for the 100 nm – 10 μm range, in order to promote interactions at cellular level.

One problem associated with incorporating micrometric textures and microstructures to computer-aided designs involves the final file size. For instance, a micrometric grid of 300 ×300 points with a clearance between points of 1 μm leads to a .stl file of around 7 MB and to a .dxf file of around 30 MB. For a useful part measuring several mm^3, the incorporation of a micrometric texture can result in file sizes of several hundred MB or even a few GB, which is currently very difficult to manage with computer-aided design resources.

The fact is that the "universal" .stl, .igs, .dxf and other formats are not optimal, especially for fractal-based designs, which can be described and programmed with just one line of code. For instance a binary .stl file, similar to those we have used, has typically an 80 character header (generally ignored, but which should not begin with the word "solid" because that will lead most software to assume that it is an ASCII .stl file).

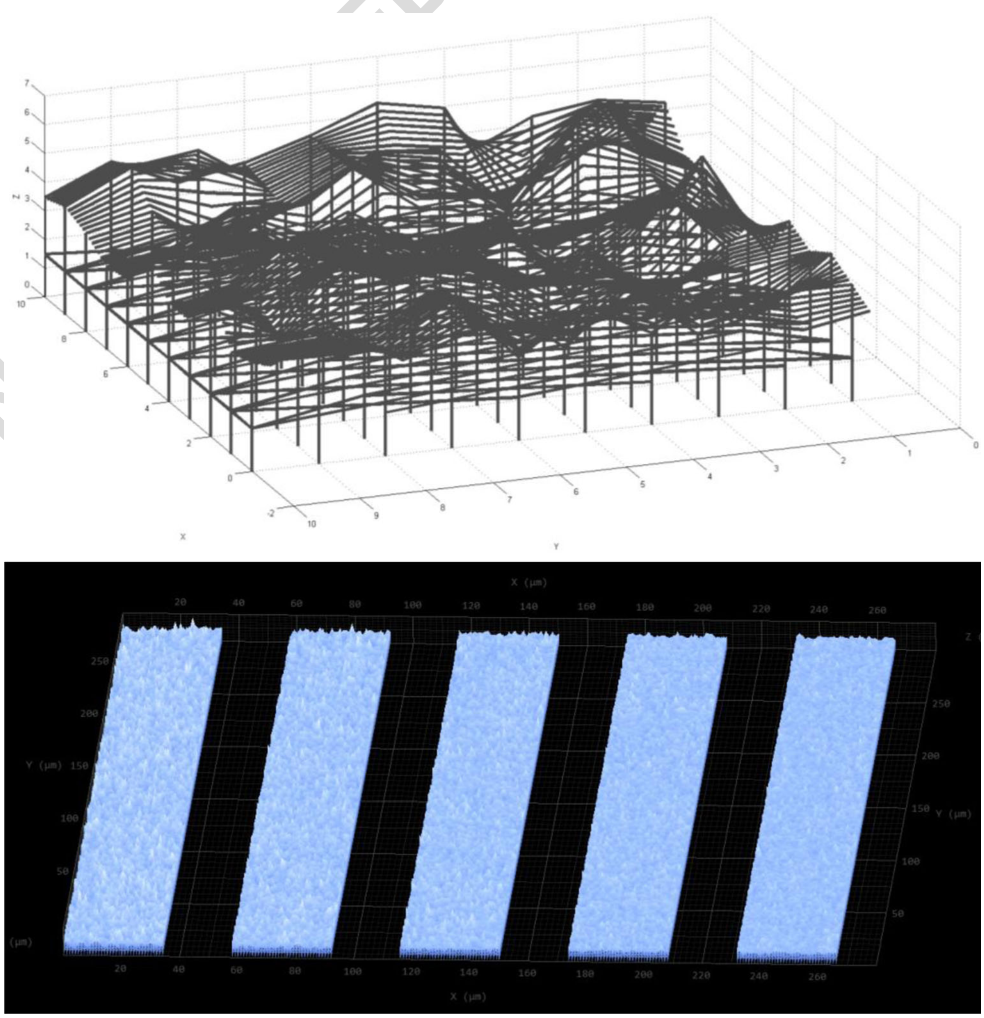


Fig 2 **a** Microtextures as lines supported by pillars, as determined by the manufacturing technology. **b** Overview of the different microtextures designed for the channels in the microsystem

Following the header, a 4 byte unsigned integer indicates the number of triangular facets in the file. After that integer, each triangle is described by twelve 32-bit-floating point numbers: three for the normal vector and then three for the Cartesian coordinates of each vertex. In consequence, a vertex common to four triangles of the surface is repeated four times in the .stl structure and such description is not optimal. The conventional CAD geometrical description of these designs unnecessarily increases file size. The shift to an algorithmic, rather than descriptive, geometry is a key factor to promote material properties and structure by design and to the further application of these knowledge-based materials to product development (Lipson 2012).

Even though CAD resources can be utilized to almost directly convert the surfaces generated into solid .stl files, any subsequent slicing of the geometry (a typical operation of the software used to control layer-by-layer manufacturing machines) leads to very slow and expensive manufacturing processes. In our case, a microtextured surface created on 30 ×300 μm^2 channels in which points on the grid are separated by 1 μm, once converted into a solid and sliced, leads to a manufacturing time of more than 50 h using direct laser writing.

In addition, the resist and direct laser writing process used in this study require a distance between parallel written (polymerized) lines of 250 nm, meaning the initial matrix-based design (Fig. 1a) has to be adapted to the manufacturing process. Using a square grid (for each channel) of 30 ×300 μm^2, in which the grid points are separated by 1 μm, the fractal surfaces are generated again and stored in matrix form. Each matrix is completed, as shown schematically in Fig. 2a, by incorporating additional column vectors that store interpolated paths, separated by 250 nm, between the original vectors separated by 1 μm. Vertical parallel lines, also separated by 250 nm, are generated under each fractal path so as to provide a supporting structure for surface construction.

The design shown in Fig. 2b can be manufactured in just a couple of hours. This is an increase in production speed of more than one order of magnitude when compared with the initial solid model. Material and laser power consumption are also reduced by a similar rate. The time and material saved can be used to manufacture several prototypes so as to methodically compare the effects of different control parameters, such as fractal dimension, laser power used, pre-polymer employed or post-processing operations. These can include the use of critical point dryers or additional post-curing so as to precisely adjust the prototypes to the final production stage. Additional details regarding the manufacturing process are included below.

2.2 Manufacturing process

Materials: For the initial stage in which the overall structure of the microdevices is manufactured, we used SU-8 spin coated on a silicon wafer. SU-8 (MicroChem Corp.) is a commonly used epoxy-based negative photoresist. It is highly functional, optically transparent and photo imageable to near UV (365 nm) radiation. Cured films or microstructures are very resistant to solvents, acids and bases and have excellent thermal and mechanical stability. They are also important for the promotion of medical applications and studies in the field of tissue repair and engineering (White R. SU-8 Photoresist processing: Standard operating procedure. (Online), January, 19 2012).

For the detailed microtextures within the different channels, a resist with a much lower voxel size than that of the SU-8 is needed. In our case, the resist is also linked to the two-photon polymerization process used. In this study we used the IP-Dip resist (NanoScribe GmbH and related data sheets for additional information), a specially designed photoresist that guarantees ideal focusing and has the highest resolution of any NanoScribe IP-Photoresist (with feature sizes down to 150 nm and minimized shrinkage). This is because its refractive index is matched to the focusing optic (Bückmann et al. 2012).

Process: The multi-scale manufacturing process followed is schematically described in Fig. 3 and consists mainly of the following stages. First, a silicon wafer is spin coated with SU-8 and the overall structure of the microsystem is obtained after photopolymerization (using a Heidelberg Instruments DWL66fs laserwriter) and further development. Subsequently, the channels are filled with the IP-Dip photoresist and the microtextures are obtained using the Photonic Professional System from NanoScribe GmbH, the first commercial direct laser writing system based on two-photon polymerization. NanoScribe GmbH (www.nanoscribe.de) was founded in 2007 by scientists in the field of photonics as a spin-off company of the Karlsruhe Institute of Technology (www.kit.edu). The company specializes in the innovative technique of 3D laser lithography and produces compact and easy-to-operate table-top laser lithography systems (Photonic Professional). Final super critical drying and development lead to the desired multi-scaled microsystem.

The direct laser writing process is noted for its accuracy and versatility, since several resists and even polymer-ceramic mixtures can be manufactured. This process can also be used additively without the need for supporting structures, which allows for the manufacture of especially complex parts with inner details. In short, when focused onto the volume of a photosensitive material, the laser pulses initiate two-photon polymerization via two-photon absorption and subsequent polymerization, normally perceived as a change of resist viscosity. Polymerization only occurs at the focal point, where the intensity of the absorbed light is highest, thus enhancing the accuracy. After illumination of the desired structures inside the resist volume and final development (washing out of the non-illuminated regions) the polymerized material remains in the written 3D form (Ostendorf & Chichkov 2006; Hermatsweiler 2013).

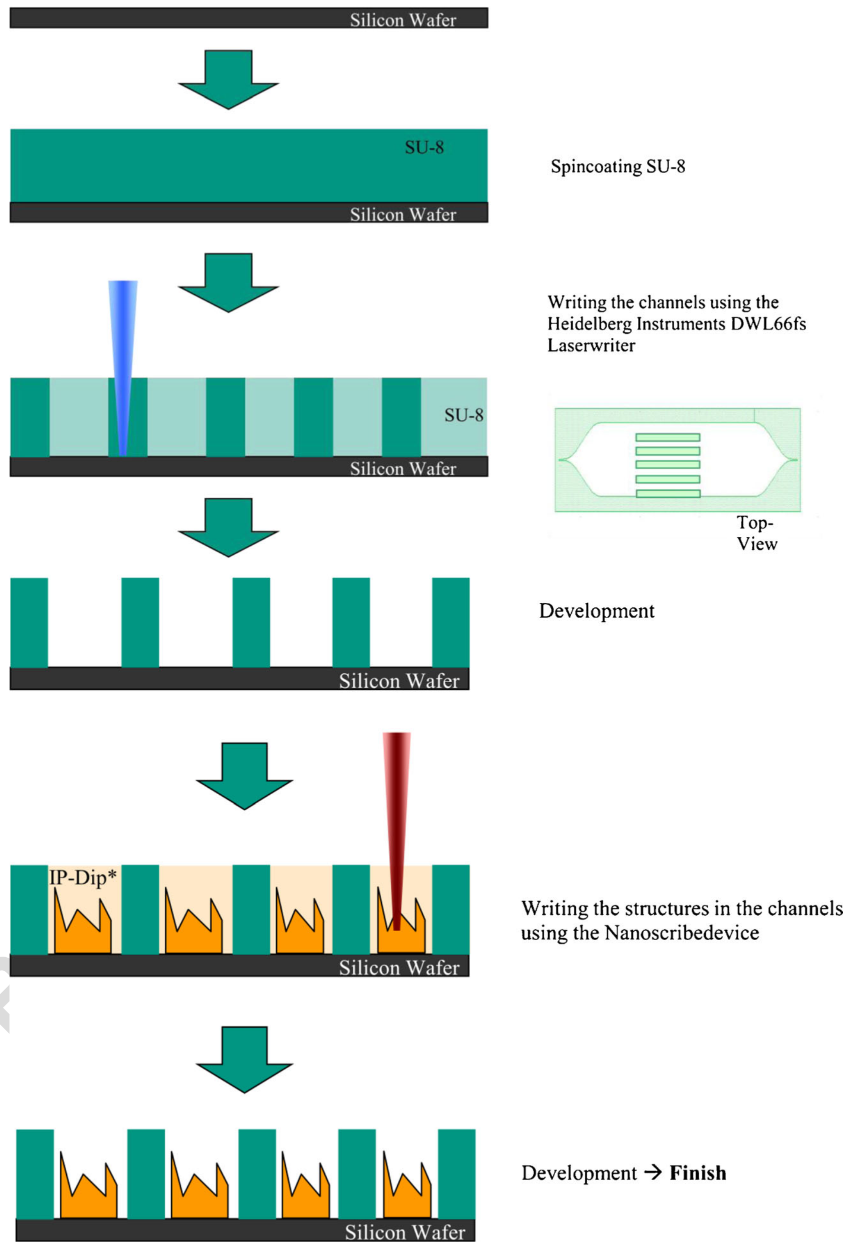


Fig. 3 Schematic depiction of the multi-scale rapid prototyping process

It is important to note that the NanoScribe direct laser writing technology writes the structures differently than conventional additive or "layer by layer" manufacturing technologies. In other additive technologies, such as normal laser stereolithography, selective laser sintering or melting or ink-jet printing, the manufacturing process starts from a 3D computer-aided design file, which is sliced into layers with the help of ad hoc software. Then, the manufacturing is accomplished layer by layer, by photopolimerization or deposition of material along the boundaries of each layer and subsequent filling of layers with parallel lines of material. In the NanoScribe process, the structures are not written layer-by-layer, but by following three-dimensional paths connected from the beginning to the end of the writing process. This means that additional programming is usually needed to convert the original CAD files into writable structures, as already schematized in Fig. 2. In addition, it is important to establish an adequate writing strategy in order to avoid writing through already polymerized resist. This can lead to unwanted optical effects because the polymerized resist has a different refractive index when compared to the unexposed resist.

As mentioned earlier, Matlab (The Mathworks Inc.) is used to create the structures and also to create the information exchange files that can be used directly in Nanoscribe Photonic Professional. The advantage over using more conventional additive-manufacturing slicing software is that the structure can be calculated and optimized based on the writing strategy and taking into account energy and time saving issues. Time can be saved by wiring lines in the correct order. Another advantage is that additional control variables can be used and parameter variation can be easily promoted by writing ad hoc programs. Parameter variation (i.e. distance between lines, structure scales, etc.) is especially useful for systematic research and matrix-based designs are helpful for providing this versatility and freedom of design. Finally, complex mathematical variables can be used to create complex structures, in keeping with recent tendencies intended to minimize .stl file size by resorting to algorithmic approaches (Lipson 2012).

The choice of laser power depends on the material being processed and has a direct influence on the attainable voxel (here defined as the minimal building block in additive manufacture approaches) size. Lower powers lead to smaller voxel sizes, although to start the polymerization at one point, a minimum threshold has to be overcome. This threshold is the minimum laser power that promotes enough energy density at the focal point to start polymerization. Below that power, the possibility of two photons being absorbed at the focus point is too low. If the density at the focal point is too high, inner explosions in the resist occur. In our case, for the fractal structures a minimal possible laser power of 5.5 mW was chosen to create a very detailed surface. At optimal conditions a line width of 150 nm at an aspect ratio of 3.5 can be reached.

One of the major problems in lithography involves shrinking, which affects the accuracy. There are two types of shrink, one linked to the material being processed and one linked to the structure geometry. The former depends on the contractility of the material being processed, and the latter is related to possible structure contractions and collapse during the manufacture and subsequent development. There are also possible adhesion effects.

Another limiting factor for some applications is the difficulty indirectly processing metals through direct laser writing. However, it is important to note that organic photoresists, like SU-8 (MicroChem Corp.) or the IP-Photoresists (NanoScribe GmbH), hybrid materials, such as the Ormocere® organic–inorganic hybrid polymer family (Fraunhofer-Gesellschaft e. V.), and the amorphous semiconductor As_2S_3 are capable of two-photon polymerization, which provides a wide range of possibilities. In addition, through CVD/PVD coating processes, or just by electroplating, final metallization is possible and casting processes can also be used for additional versatility. Moreover, advanced research groups, as well as companies, are focusing on the continuous development of novel materials, including photoelastomers, photopolymers and polymer-ceramic composites. These materials, even when used for medical applications, can be structured by means of direct laser writing (Ostendorf & Chichkov 2006).

3 Results

Figure 4 shows the final multiscale biomedical microsystem for assessing the effect of surface texture on cell motility. Its outer structure (circular chambers and channel walls) was obtained using the Heidelberg Laser Writer, and the textured channels were created using the NanoScribe system. Figure 5 shows several details from the different micro-textured channels obtained via direct-laser writing and helps to highlight the influence of control parameter “alfa” on surface topography. This parameter is linked to roughness and fractal dimension. In short, higher values of “alfa” lead to more planar surfaces and lower values of “alfa” lead to more spiky surfaces. In our case we used a different value of “alfa” for each channel so as to control the textures of the different channels from the design (Fig. 2b) stage. Figure 5 shows the different values of “alfa” used: 0.1; 0.3; 0.5; 0.7 & 0.9, with related fractal dimensions of 2.9; 2.7; 2.5; 2.3 & 2.1. An additional planar (with fractal dimension equal 2) was also included for use as a control channel in forthcoming *in vitro* trials.

The detailed images included in Figs. 5 and 6a help to show the accuracy of the micro-texturing process. The similarity between the initial design and the final prototype validates the proposed approach for controlling surface topography in microsystems. It is interesting to note that the typical “steps” that can be seen in several additive manufactured devices when using more conventional technologies, cannot be appreciated here. This is because the NanoScribe process does not work using a sliced CAD file, but by writing lines in three-dimensional space (in a similar way as schematically depicted in Fig. 2a). Consequently, the process is additive but not “layer by layer”: instead of appreciating the different slices and steps, several lines can be perceived upon the different surfaces, according to the different paths followed by the laser. In any case, for the purpose of the microsystem, these lines do not affect the functionality as much as the layered and stepped geometries usually obtained by other high-precision rapid prototyping technologies, including digital-light processing and micro-stereolithography.

The detailed image in Fig. 6b shows the fractal surface and supporting pillars obtained by two-photon polymerization of the previously rapid manufactured microsystem structure of channels and chambers, which shows the benefits of combining processes and materials towards multi-scale microsystems. Some shrinking during the critical drying process (around 4 %) is present and has led to some de-attachment between the microtextured surfaces and the channel walls. This shrinking can be reduced to values of around 1-2 % by incorporating some additional outer pillars connected to the surface. These pillars act as support structures and absorb stress, as previous research has shown (Norman et al. 2013).

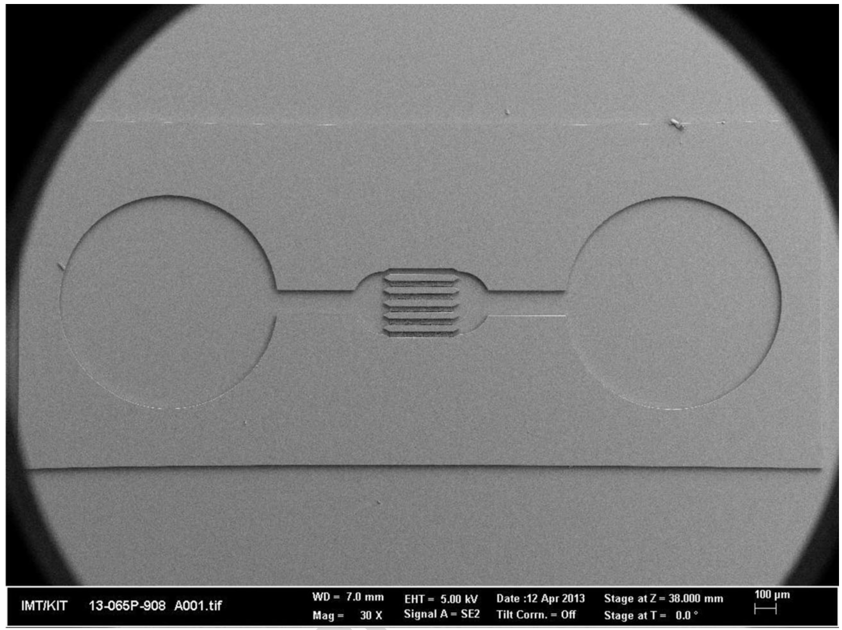


Fig. 4 Overview of the multiscale biomedical microsystem, with the outer structure obtained using the Heidelberg Laser Writer and with the textured channels obtained using the NanoScribe system

Besides, the detailed view helps to verify that the microtextured surfaces are adequately supported by the structure of pillars, which do not penetrate through the surface due to adequate photopolymerization. Lower laser powers lead to lower degrees of polymerization and to the collapse of fractal surfaces, as happened in some of our preliminary manufacturing tests. On the other hand, increased laser power can promote multi-photon, instead of two-photon, absorption. This results in lower accuracy and in an uncontrolled response of the resist during polymerization, normally leading to significant defects. The process must thus be adequately adjusted so as to reach the adequate polymerization level.

Some design improvements, such as the incorporation of a progressive ramp at the beginning of each channel to help the cells crawl on the microtextured surfaces supported by pillars and enter the different channels, as well as the inclusion of some additional micro-gripping structures at the edge of the microsystem to simplify its handling, can enhance the final functionality. Regarding manufacturing, improvements in the final critical drying process can also help to reduce residual stresses, hence minimizing shrinkage of the IP-Dip photoresist and preventing de-attachments. In spite of these possible improvements, it is important to note that the writing speed for the direct laser writing part of the process can be increased by more than one order of magnitude by using a surface design supported by pillars, when compared with a solid design. The quantity of resist used and the laser power consumed are similarly reduced, hence resulting in a remarkably low-cost and sustainable solution.

The surfaces and prototypes obtained can be used as final parts, they can be have additional coatings or functionalities, i.e. for micromolding (Norman et al. 2013), and they can be

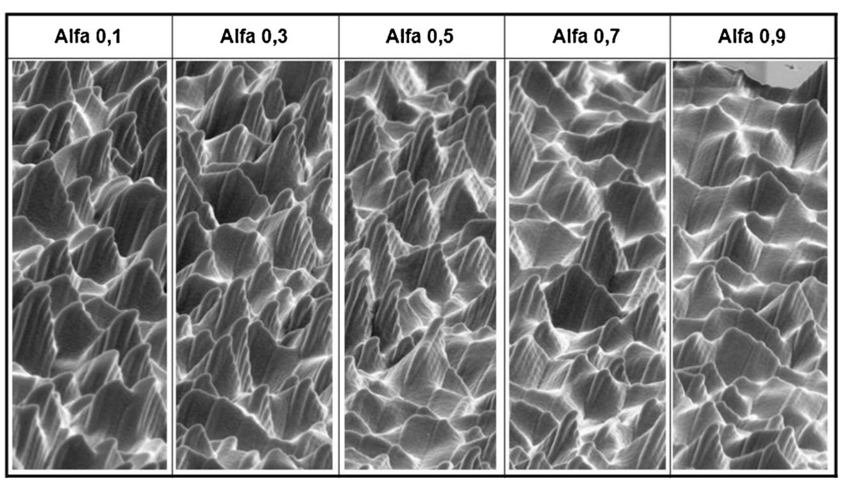


Fig. 5 Close-up of the different micro-textured channels obtained via direct-laser writing. Influence of control parameter "alfa" on surface topography, related to roughness and fractal dimension

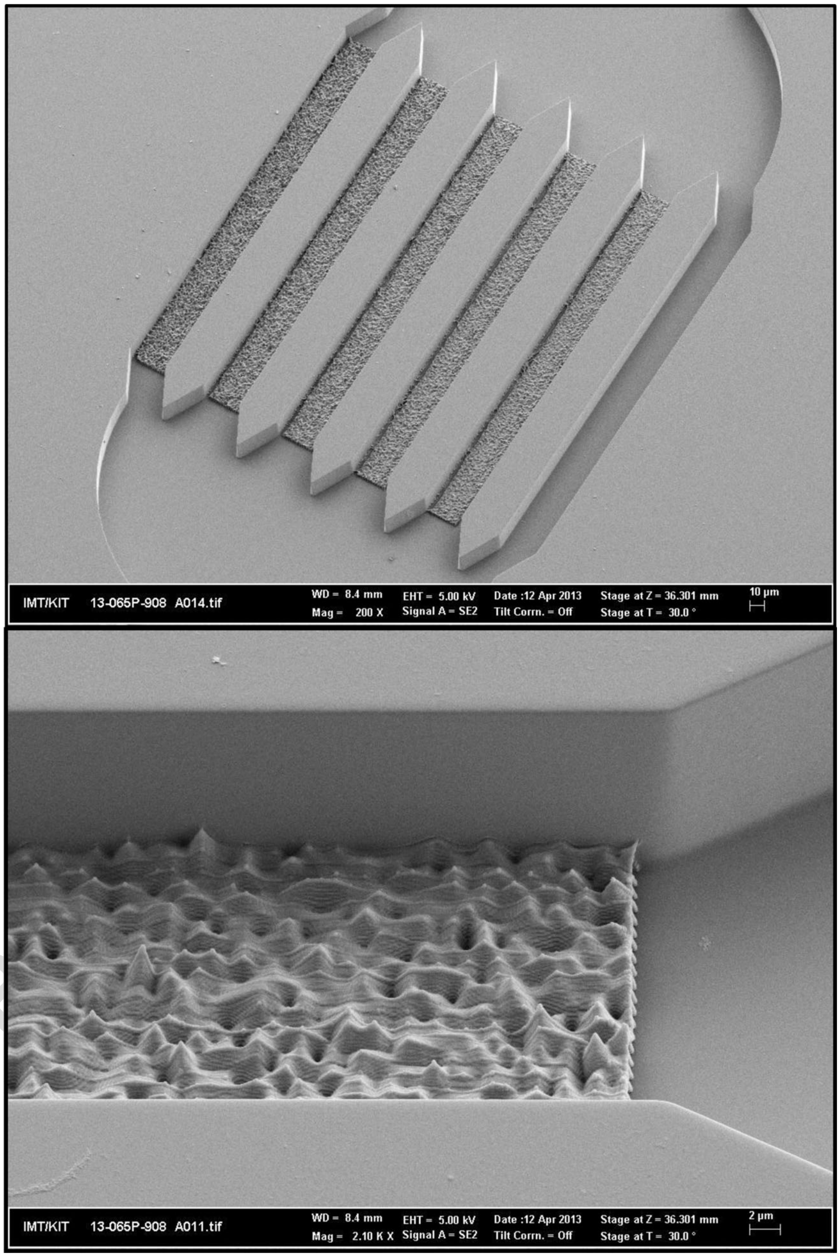


Fig. 6 Close-up of the different micro-textured channels (upper image) Close-up of the fractal surface and supporting pillars obtained by two-photon polymerization of the previously rapid manufactured microsystem structure of channels and chambers (lower image)

used as green parts for obtaining replicas in other materials, depending on the application. For instance, following metallic chemical- or physical-vapor deposition to enhance surface conductivity, the surfaces can be electroplated with nickel and further used as inserts for injection molding of thermoplastics or of ceramic powders with bonding agents before final sintering. PDMS molds can be also directly obtained by casting upon the surfaces and used as rapid molds for casting several polymers. Interesting functionalizations for further integration with electronics (Simon et al. 2013) may also open new horizons. These combinations of prototyping and mass-production processes will help to increase the range of applications of these micro-textured surfaces, providing a wider palette of materials whose surface topography can be precisely controlled from the design stage.

Future trials will focus on assessing the possibilities of the designed and manufactured microsystems by culturing real cells on them. The material is adequate for cell culture and the manufacturing precision allows for real interaction at the cellular level, as previous ground-breaking research has shown (Klein et al. 2010). However, we still need to improve some capabilities and resources from our labs involving micromanipulation facilities, cell culture related equipment and the cells themselves, in preparation for these trials. In any case the device has the potential to address cell motility and the influence of surface topography on the cells, with roughness in the range of 1–5 μm, which is much more adequate than the 200–350 μm from the original proof-of-concept from Fig. 1 (Díaz 2013). The channel width of 30 μm is aimed at preventing several cells from crawling in parallel and at promoting single-cell tracking, which could not be obtained with our previous device (Díaz 2013). The capabilities of these microsystems can be complemented by the use of other fractal features that affect cell dynamics, behavior and differentiation into relevant tissues (Díaz Lantada et al. 2013).

Finally we would like to emphasize the level of accuracy achieved and the quality of the microsystem obtained, even when considering the aforementioned minor defects inherently related to the multi-scale process utilized. The channels obtained have a length of 300 μm and a width of 30 μm, which will prevent several cells from entering a channel at once and allow for single cell tracking. It will also enhance the motility monitoring process in future *in vitro* trials. In addition, the fractal microtextures obtained are in the initially desired range of 1–5 μm, thus having the same order of magnitude as cytoskeleton deformations and allowing for a more adequate interaction at a cellular level. Future trials will allow us to assess the actual impact of fractal dimension on cell motility. In an effort to promote the use of biomimetic approaches or as a complement to recent biomimetic proposals in the field of cancer cell migration (Huang et al. 2013), similar approaches could potentially be used to control the textures of several microsystems and implants.

4 Conclusions

We have presented an enhanced design and manufacturing process for obtaining multi-scale biomedical microdevices that is based on the combination of two additive manufacturing processes: a conventional laser writer to manufacture the overall device structure; and a direct-laser writer based on two-photon polymerization to yield the smallest details. The process excels for its versatility, accuracy and manufacturing speed and allows for the manufacture of microsystems and implants with overall sizes up to several millimeters and with details down to sub-micrometric structures. As an application example we have focused on manufacturing a biomedical microsystem to analyze the impact of microtextured surfaces on cell motility. This process yielded a relevant increase in precision and manufacturing speed when compared with more conventional rapid prototyping procedures.

Regarding future studies, we consider it important to focus on exploring in depth the possible applications of design-controlled multi-scale biomedical microdevices, especially in areas such as cell mechanobiology and multi-scale integration across organic and inorganic interfaces for several types of implantable (either active or passive) medical devices. In addition, we believe it relevant to address further combinations of micro-nanomanufacturing technologies. This includes the possibility of complementing the procedures detailed herein with other mass-replication technologies, including micro-injection molding and hot-embossing.

We foresee relevant implications of the processes described in areas such as: tribology, due to the potential promotion of adhesion using fractal textures; microfluidics, due to the possibility of controlling the hydrophobicity and hydrophilicity of surfaces by acting on their topography; optics, due to the option of changing surface reflection properties and overall aesthetics; and biomedical engineering, for the promotion of biomimetic designs. Currently we are working to improve the versatility of the design process by allowing for the introduction of controlled texture gradients and different kinds of texture variations within the surfaces of interest.

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