Scaffold-like Micro-well Structures Fabricated by Two-photon-absorption Photopolymerization

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Abstract

We demonstrate a novel two-photon-absorption (TPA) microfabrication technique with potential applications in tissue engineering and other biomedical applications. Complex 3-dimension (3D) microstructures can be constructed using this technique with the advantages of being maskless, single step, real 3D structure and high resolution. By use of this technique, we fabricated scaffold-like micro-well structures with sub-micron resolution and high aspect ratio by both raster scanning and pinpoint scanning methods. The linewidth and height of the resulted micro-wells are controlled by an ultraviolet (UV) pre-exposure step. The finest linewidth is 0.28 μm and the highest aspect ratio reaches 6.7.

***Keywords*:** Tissue engineering, High aspect ratio, Two photon absorption, Photopolymerization, Sub diffraction limit

Introduction

Microstructures fabricated on a substrate surface have been shown to influence the behavior of cells growing on the substrate [1-5]. Surface microstructures affect the cell functions such as attachment, spreading, growth, migration and proliferation. Except in the field of cell growth, 3-dimension (3D) microfabrication techniques also find potential applications in tissue engineering such as scaffold fabrication. Since tissues are well organized and highly oriented *in vivo*, the structure randomness in many existing scaffolds proves to be a significant limitation for tissue growth. Due to this reason, one of the current research interests in tissue engineering is to develop complex 3D scaffolds with precise spatial control of cell growth *in vivo* and *in vitro*. One of the approaches uses Micro-Electro-Mechanical-Systems (MEMS) technology to fabricate complex 3D scaffolds or array bioreactors, which provide engineered growth control of tissue [6-9]. Its inherent features easily satisfy the requirements of scaffold design in the issues of accuracy, regularity and reproducibility.

Usually the scaffolds are porous, degradable structures made of either natural materials (collagen, fibrin) or synthetic polymers (polyglycolide, polylactide, polylactide coglycolide). Injection molding [10], 3D-printing technique [11] and microsyringe [12] methods have been used to fabricate such structures with a resolution ranging from 300 μm to 10 μm. To achieve better resolution, optical lithography, ion beam etching and laser ablation, etc., can be employed. We demonstrate a novel two-photon-absorption (TPA) photopolymerization technique to create well-defined and reproducible 3D microstructures at the sub-micron level. Compared with other methods of microfabrication, this method has the advantages of being maskless, single step, real 3D structure and high resolution.

Optical stereolithography (one of the traditional one-photon photopolymerization techniques) has long been used in biomedicine [13]. For example, out of the sectioned-medical imaging data (CT-Scanner or MRI) one can build complex anatomical parts for surgical planning, surgical templates, design of implants and models for pedagogy. These parts are fabricated layer by layer in a vat of photosensitive liquid resin that hardens on ultraviolet (UV) light exposure. Models with feature sizes of tens of microns are routinely produced with this method. Recently, TPA photopolymerization [14] of UV-curable polymers has been extensively studied and employed to fabricate complex 3D structures with sub-micron resolution in the fields of micromachines [15-19], photonic devices [20-22] and 3D optical data storage [23-25]. Using this unique method one can fabricate sub-diffraction-limit 3D objects and even functional devices such as micro oscillators [15], micro gearwheels [16], light-driven rotors [17], micro-tweezers [18], micro-chains [19] and photonic crystals [20-22], etc. To achieve TPA photopolymerization in the liquid photopolymerizable resin, intense near-infrared (near-IR) femtosecond (fs) lasers are used as light sources. Usually high

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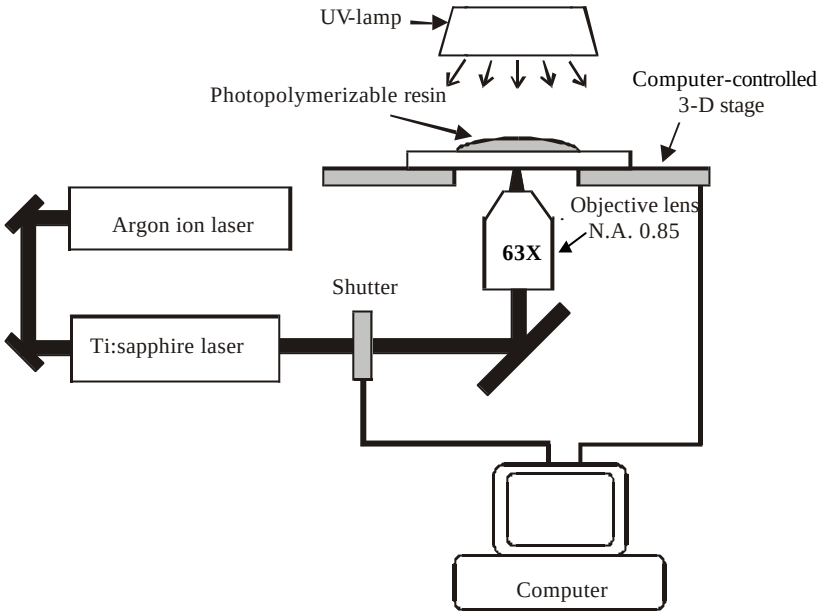

Figure 1. Schematic drawing of the experimental setup. A 4W UV-lamp is held at 3 inches above the samples to precure the liquid resin.

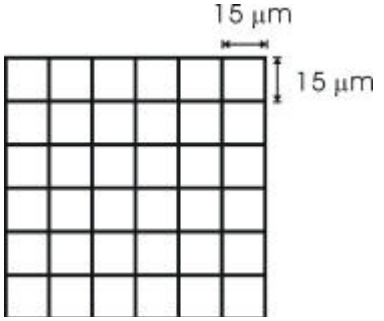

Figure 2. The micro-well pattern used for single-line scanning TPA photopolymerization.

numerical aperture (N.A.) microscope objective lenses are employed to focus the fs laser beam tightly into the resin to reach a very high photon density near the focal spot. In general, these UV-curable resins consist of photoinitiators, monomers and oligomers. When the UV light irradiates on the resin, photoinitiators absorb UV photons to generate radicals for the subsequent polymerization process, but the process can alternatively be initiated by near-IR light via two- or multi-photon absorption under high-intensity illumination. In nonlinear optical TPA process, molecules exposed to high-intensity light simultaneously absorb two photons when the combined energy of two photons matches the transition energy between the ground state and the excited state, and the rate of TPA is proportional to the square of the incident light intensity. The quadratic dependence of TPA rate on light intensity confines the process to the vicinity of the focal point. This principle has been applied to fluorescence imaging [26], microfabrication [14-22] and photodynamic therapy [27], etc. For single-photon-absorption (SPA) process, polymerization can occur outside the focal region along the light path and consequently lower spatial resolution in both lateral and axial directions is resulted. In contrast, the photopolymerized volume in TPA is more confined within the focal region owing to the nonlinear dependence on the light intensity. In fact, the volume of solidified resin (voxel) can be made smaller than the focal spot by properly controlling the laser power and exposure. In addition, its ease of 3D manipulation is unachievable by conventional lithography techniques. Based on these advantages, one can fabricate complex stereo microstructures with sub-diffraction-limit resolution by scanning the focal spot in a predetermined path through the liquid resin.

In this paper, we demonstrate the fabrication of a high-aspect-ratio micro-well structure in high-speed, single-layer scans using TPA photopolymerization with an extra step of SPA pre-exposure. The resulted linewidth is as thin as 0.70 μm and the aspect ratio is 6.7. Our preliminary experimental results suggest that TPA photopolymerization technique can be used to fabricate microstructures, such as 3D scaffolds and surface patterns, in tissue engineering and other biomedical applications.

Materials and Methods

The microfabrication apparatus is shown in Figure 1. We put a drop of commercially available UV-photopolymerizable resin on top of a cover slip. The resin (3D system, SL-5510) is a mixture of photoinitiators and monomers in liquid state. It is transparent for near-IR light and solidifies when irradiated by UV light. A mode-locked Ti:sapphire laser operating at 780 nm wavelength generates near-IR light pulses whose pulse width and repetition rate are 45 fs and 86 MHz, respectively. The fs laser pulses are tightly focused onto the liquid resin by a 63x objective lens with a moderate N.A. of 0.85. At the focus,

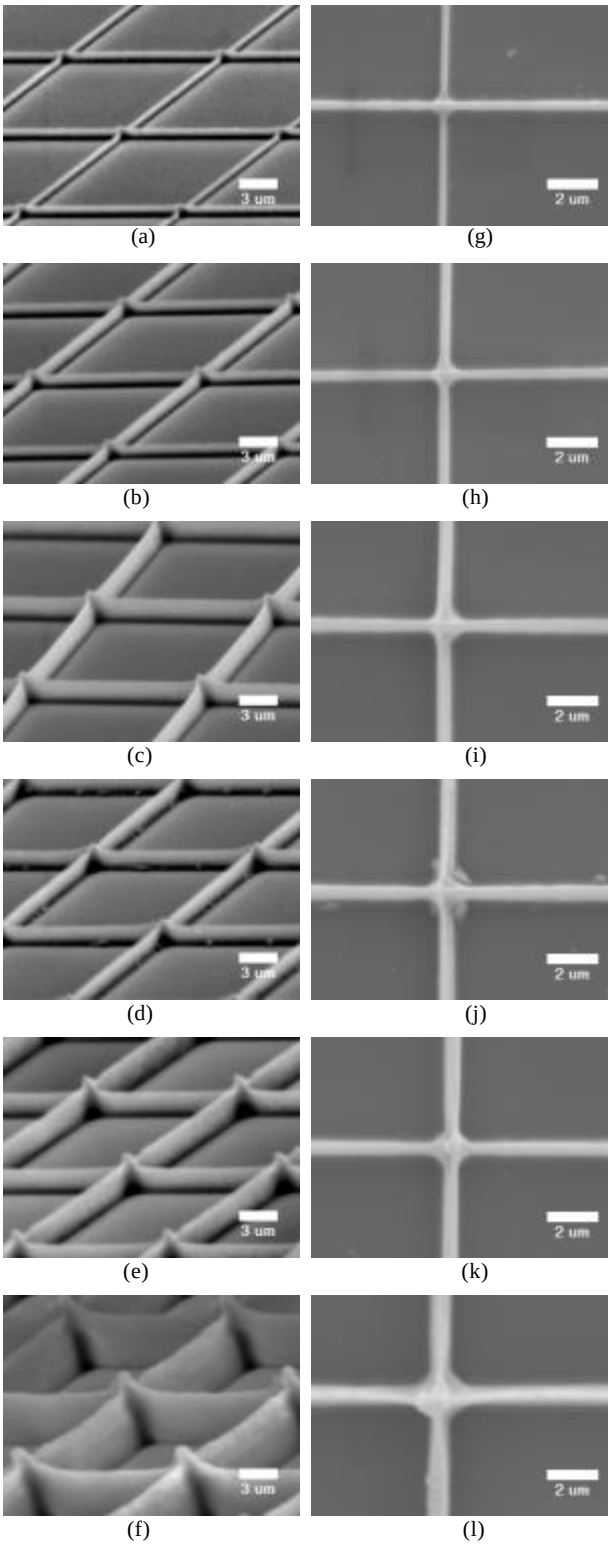

Figure 3. The SEM images of the micro-well structure made by TPA photopolymerization with single-line scanning method. Their pre-exposure time varies from 0 to 25 s in steps of 5 sec. (a) to (f) are taken form 75° tilt from normal direction and (g) to (l) are taken from the top of the samples.

the estimated lateral spot size (diameter) and the confocal parameter are 0.87 μm and 1.51 μm, respectively. The samples are scanned with a computer-controlled piezo-electric 3-D translation stage (Physik Instrumente, P527.3), which moves along the pre-programmed path. To control the exposure time, a computer-controlled mechanical shutter with a 10-ms rise time is used.

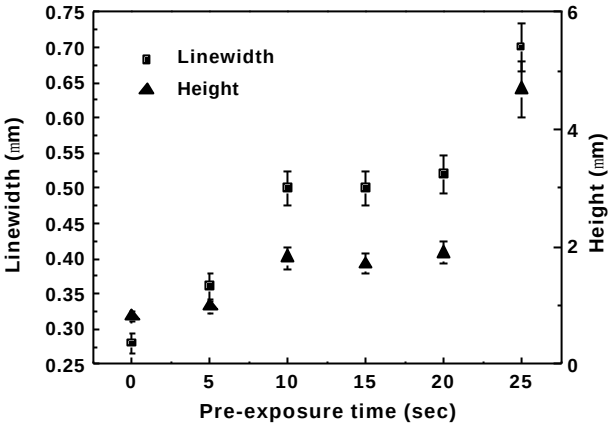

Figure 4. The measured linewidths and heights vs. UV pre-exposure time in samples made by raster scanning method.

In this study, we use both raster-scan and pinpoint-scan modes to direct-write square micro-well structures in the liquid photopolymer on top of glass substrate surface. After fabrication, the unsolidified resin is removed by acetone. Finally, the fabricated micro-well structures are examined and measured by an inverted optical microscope (Nikon, TS-200) and a high-resolution scanning electron microscope (SEM) (Hitachi, S-3500N).

Results and Discussions

Single-line scanning method

Figure 2 shows the layout of the micro-well structure, which is composed of 36 unit wells. Each unit-well is 15μm X 15μm in dimension. For all samples, the incident laser power before entering the cover slip is 8.7 mW and the scanning velocity is 15 μm/s. A complete scan takes less than 3 minutes. In addition to the regular TPA writing process, we introduce an extra *in-situ* pre-exposure step to improve the overall quality of our micro-wells. This pre-exposure process employs a 4W UV-lamp held at 3 inches above the samples to irradiate the liquid resin, the pre-exposure time varies from 0 to 25 sec in steps of 5 sec. Figure 3 shows the SEM images of the samples made by the single-line scanning process with different UV pre-exposure time. Figure 3(a) to (f) are taken from 75° tilt from normal direction and Figure 3(g) to (l) are taken from the top of the samples. The UV pre-exposure time for each image is listed in table 1. The measured linewidths and heights of the micro-wells are also shown in table 1 and illustrated in Figure 4. Note that all samples are made with the same TPA photopolymerization parameters. It is evident that the UV pre-exposure significantly influences the feature size of the microstructures, especially in the longitudinal direction. This phenomenon can be explained as follows: First, UV pre-exposure creates short-chain photopolymers in the unsolidified resin, which effectively reduce the near-IR dose

Table 1. The measured linewidths, heights and aspect ratios for micorwells with various SPA pre-exposure time.

Image (Figure 3)	SPA exposure time (sec)	Linewidth (μm)	Height (μm)	Aspect ratio (height/linewidth)
(a), (g)	0	0.28	0.82	2.92
(b), (h)	5	0.36	0.98	2.73
(c), (i)	10	0.50	1.81	3.62
(d), (j)	15	0.50	1.71	3.42
(e), (k)	20	0.52	1.89	3.64
(f), (l)	25	0.70	4.69	6.70

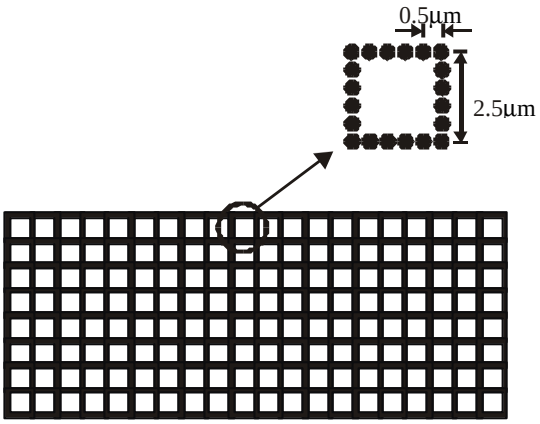

Figure 5. Layout of the micro-well structure for pinpoint scanning method.

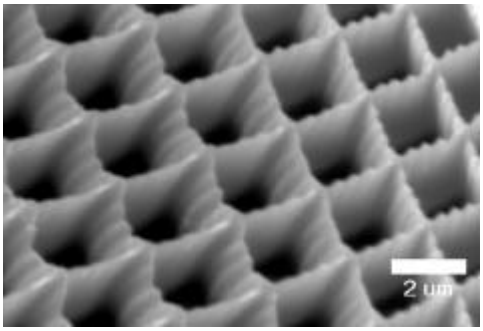

Figure 6. The SEM image of the micro-well structure made by pinpoint scanning method. Each point is exposed to IR laser pulse for 100 ms. (without UV pre-exposure)

required to reach solidification threshold. Given the same IR exposure, the feature size should increase proportionally with the pre-exposure time. Second, instead of high N.A. lenses commonly used to achieve high resolution in the microfabrication process, we employ a moderate N.A. objective lens to obtain a much longer depth of focus (DOF). Note that DOF increases as $(N.A.)^{-2}$, while the spot size is only proportional to $(N.A.)^{-1}$. This effect is easy seen in the heights of these wells. The finest linewidth is about 0.28μm, which is 32 % of the laser spot size, for the sample without SPA pre-exposure. When the sample undergoes *in-situ* SPA pre-exposure for about 25 sec, the thickness of the sidewall increases to 0.70 μm (or 80 % the diffraction limit), twice as thick as the previous sample, but its height is 5.8 times as high and the aspect ratio reaches 6.7. To the best of our knowledge, this is the first work that uses TPA photopolymerization to successfully fabricate micro structures with a sub-micrometer linewidth and an aspect ratio as high as 6.7 in *single-scan writing*.

Pinpoint scanning method

In this experiments, the micro-well layout is shown in Figure 5. Each well is 2.5 μm X 2.5 μm in dimension. The whole dimension is 50 X 20 μm with 160 wells. To fabricate this structure, we expose the resin point by point with the focused laser spot following the programmed path. No UV pre-exposure is applied. The incident laser power before entering the cover slip is 8.7 mW. The laser exposure time per point is 100 ms and the step between neighboring points is 0.5 μm. Figure 6 is the SEM image of the resulted structure, it is taken from 45° tilt from normal direction. The wall thickness is about 0.5 μm and the height is about 2 μm. The wavy profile of the well wall is due to the pinpoint scan nature, it can be smoothed by reducing the step distance between neighborhood points.

Conclusions

In summary, we have demonstrated the use of a novel TPA photopolymerization technique to fabricate high-aspect-ratio 3D micro-well structures. By using a moderate N.A. objective lens and a UV pre-exposure step, we have successfully fabricated micro-wells with sidewalls as thin as 0.70 μm and an aspect ratio approaching 7 in high-speed single-layer scans. We believe that this technique can be applied in general to tissue engineering and biotechnology to fabricate complex 3D micro-objects such as scaffolds and surface microstructures with sub-diffraction-limit resolution.

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以雙光子吸收光致聚合法製作類支架之微井結構

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摘 要

使用微製造技術製作之表面微結構或立體支架以為體內或體外的細胞及組織生長環境，已經是組織工程領域中重要之研究題目，其目的是希望藉由空間上的微結構引導及控制組織之成長，經由控制細胞與組織之成長，最終期能達到人工器官之目標。

我們展示了一個有潛力應用於組織工程及生醫領域之新穎的微製造技術—雙光子吸收光致聚合法，它所使用之基材為液態高分子材料。與其它微加工技術比較，使用此方法除可製作複雜的三維微結構外，同時它又具有免用光罩、製作步驟簡單、真正三維的立體結構及高解析度之優點。在本研究中我們使用此技術配合線掃描與點掃描之方式，製作出具次微米解析度及高深寬比之類似支架之微井結構，同時我們也提出一種可增加結構之深寬比的方法—紫外光預曝光法，藉此可控制微井結構之線寬及高度。我們所達到的最小線寬為 0.28 微米，最高之深寬比可達 6.7。

關鍵詞：組織工程、高深寬比、雙光子吸收、光致聚合、次繞射極限

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