Biocompatibility testing of aluminium and zirconium based hybrid organic-inorganic materials

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Introduction

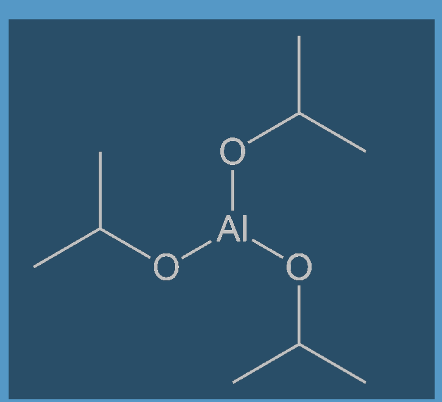
Tissue engineering is a field based on the idea that the majority of human tissues and organs can be replaced by autologous artificial tissues, composed of cells and scaffolds [1]. There are many scaffold fabrication techniques, but one of the most promising ones is laser multiphoton polymerisation [2]. A wide range of materials can be structured via this technique, but hybrid organic-inorganic materials are among the most widely investigated due to their high structuring quality and ease of workflow [3]. Here, we present an aluminium-based hybrid organic-inorganic material that is relatively simple to prepare, tune and structure in 3D. We investigate its biocompatibility by comparing it to a hybrid organic-inorganic material based on zirconium and a commercially available OrmoComp (Micro Resist Technology GmbH).

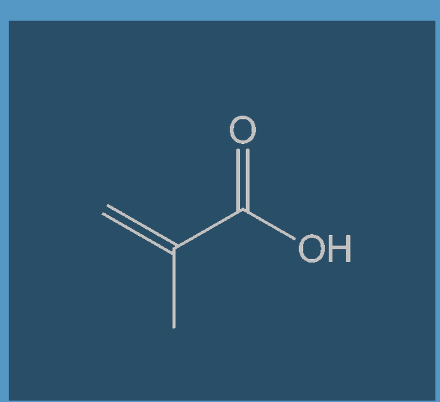
Materials

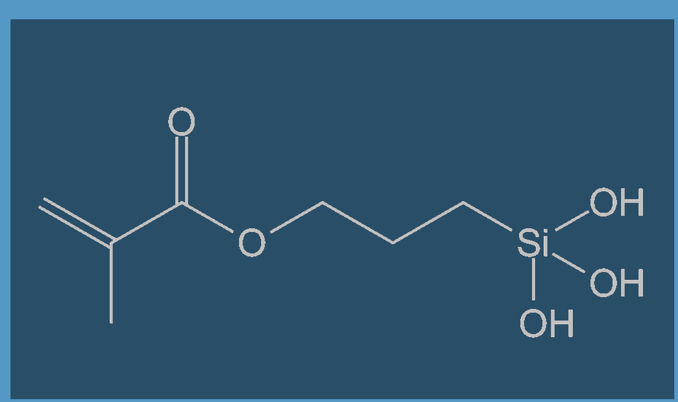
The aluminium-based hybrid organic-inorganic (HPA) material was prepared by dissolving aluminium isopropoxide (AIP) in toluene and subsequently adding methacrylic acid (MAA) and 3-methacryloxypropyltrihydroxysilane (MAPTHS) and a photoinitiator (PI, 4,4′-bis(diethylamino)benzophenone). Three different compositions were made - 1:1:9, 1:1:4 and 1:1:2, where the numbers denote molar ratios between the components - AIP:MAA:MAPTHS, 1 % PI was added by weight.

The zirconium-based hybrid organic-inorganic (HPC) material was prepared in an analogous fashion - zirconium isopropoxide (ZPO) was mixed with MAA and then with MAPTHS at a 1:1:4 ZPO:MAA:MAPTHS molar ratio, 1 % PI was added by weight.

For laser fabrication, small droplets of the material were drop-cast on cover glass slides and left in a fume hood to evaporate. For biocompatibility testing, the materials were spin-coated on circular glass slides slides and polymerised under UV light.

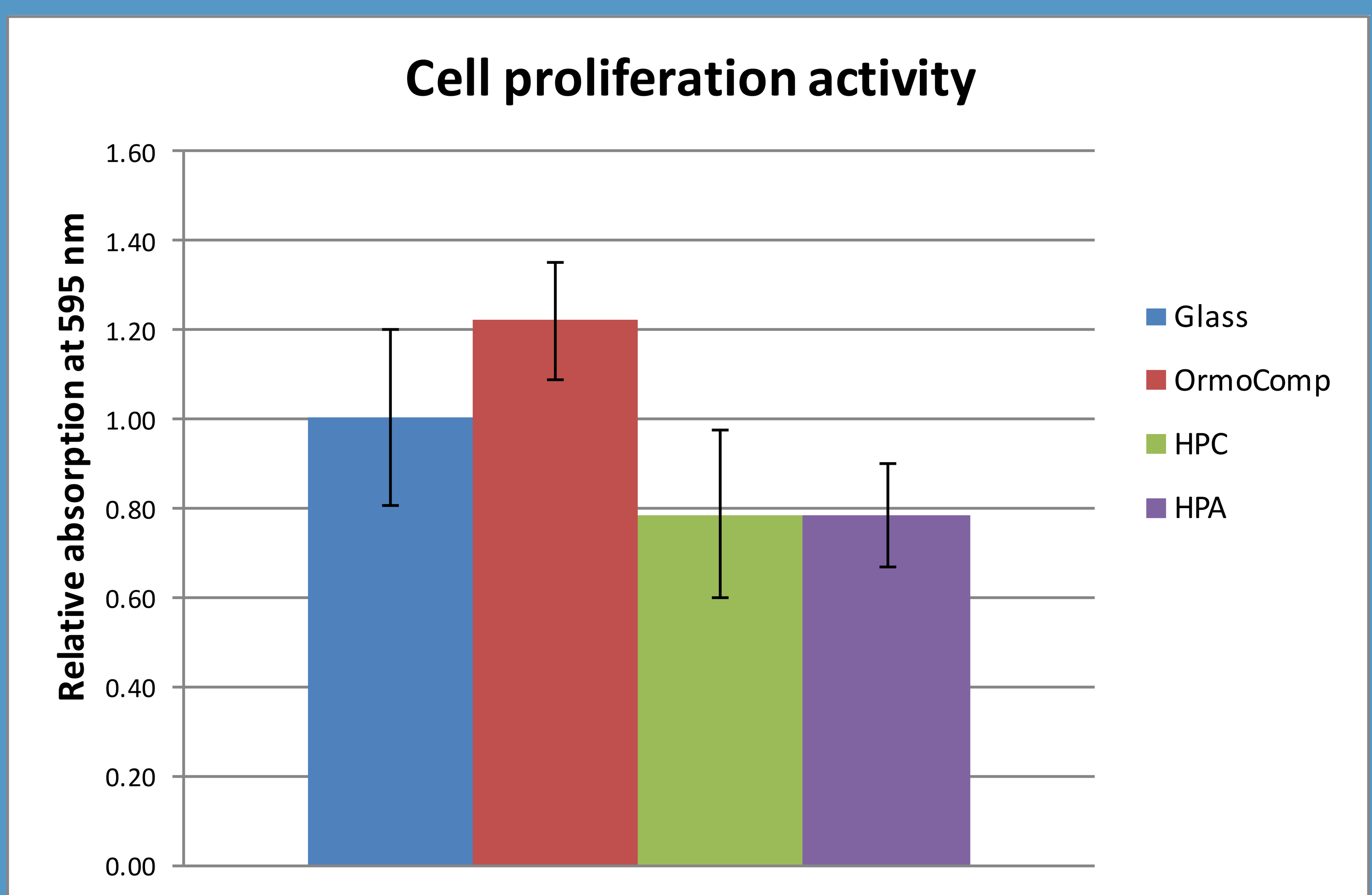






Biocompatibility

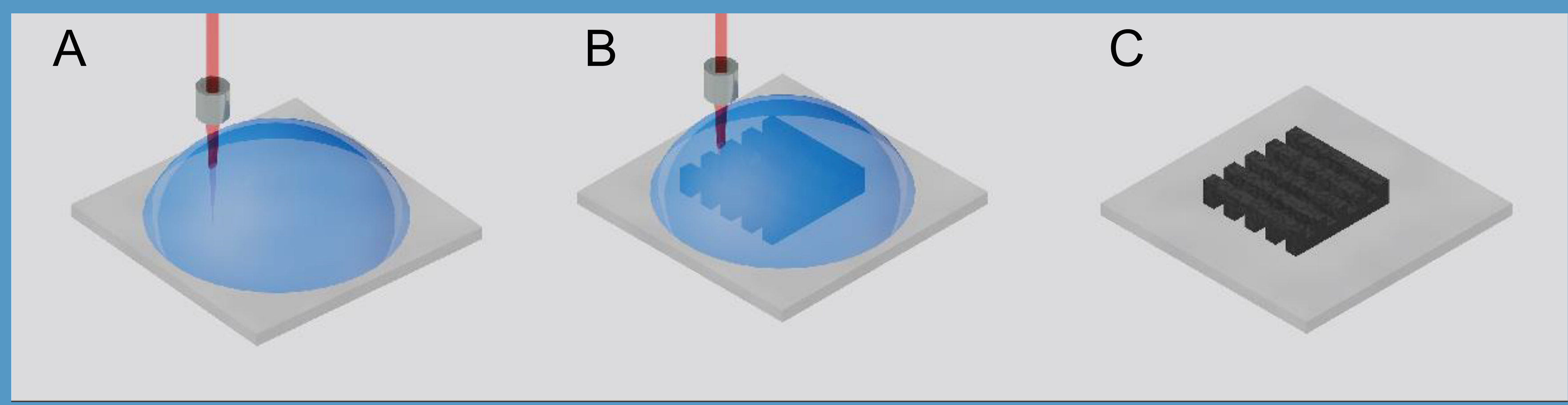
NIH 3T3 mouse embryonic fibroblasts were seeded at a density of 2 x 10^4 cells/well on spin-coated and UV-cured polymer films in 24 well tissue culture plates. After 48 hours in a CO_2 incubator (5 %) at 37 °C, cell proliferation activity was measured by exchanging the medium with MTT (0,5 mg/mL in PBS) and incubating for 1 hour. The formazan crystals were then dissolved in DMSO and measured spectrophotometrically at 595 nm wavelength.



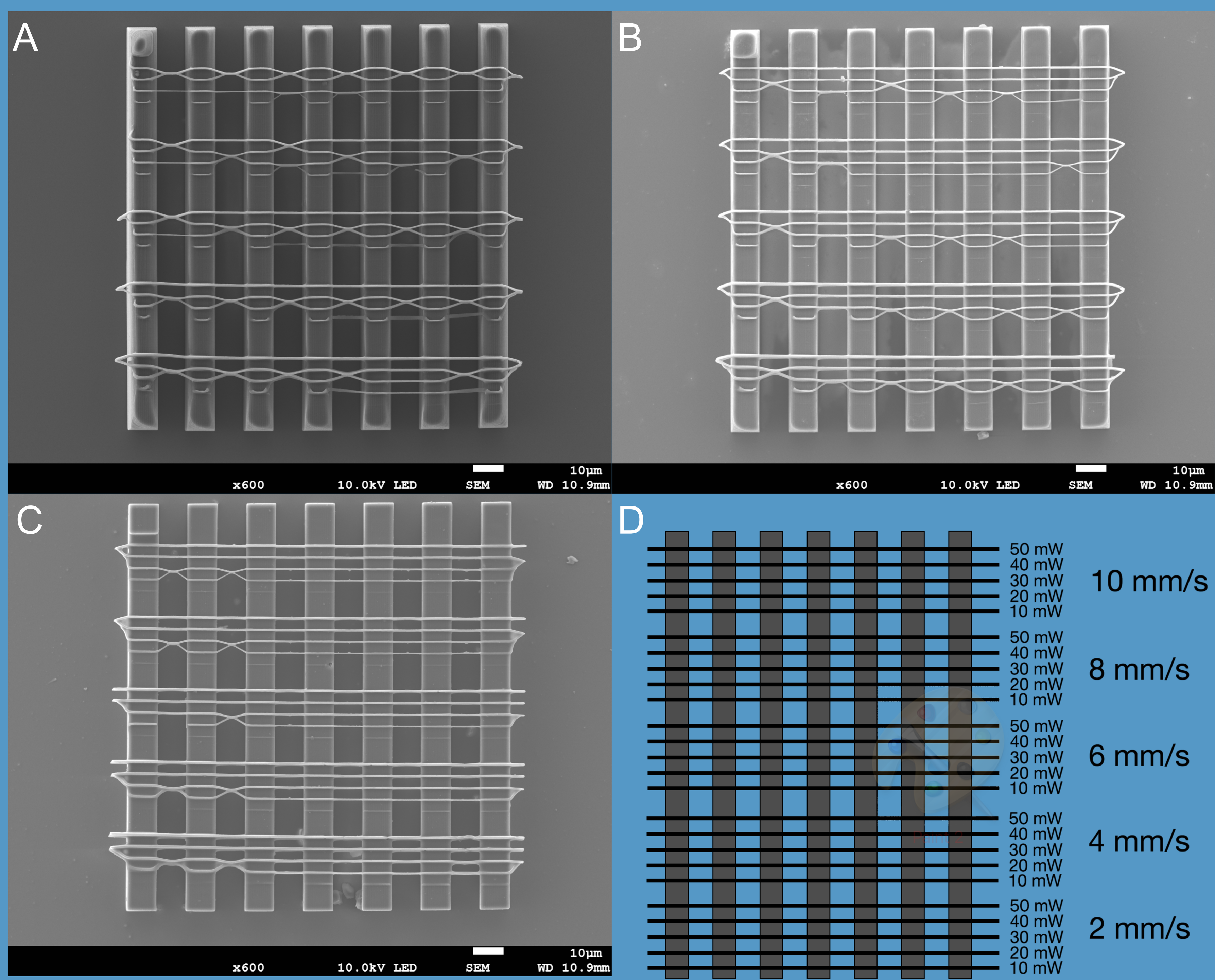
Results are expressed as relative absorption, using glass as a reference point. The chart shows averages from 5 measurements +/- standard deviation.

Laser Fabrication

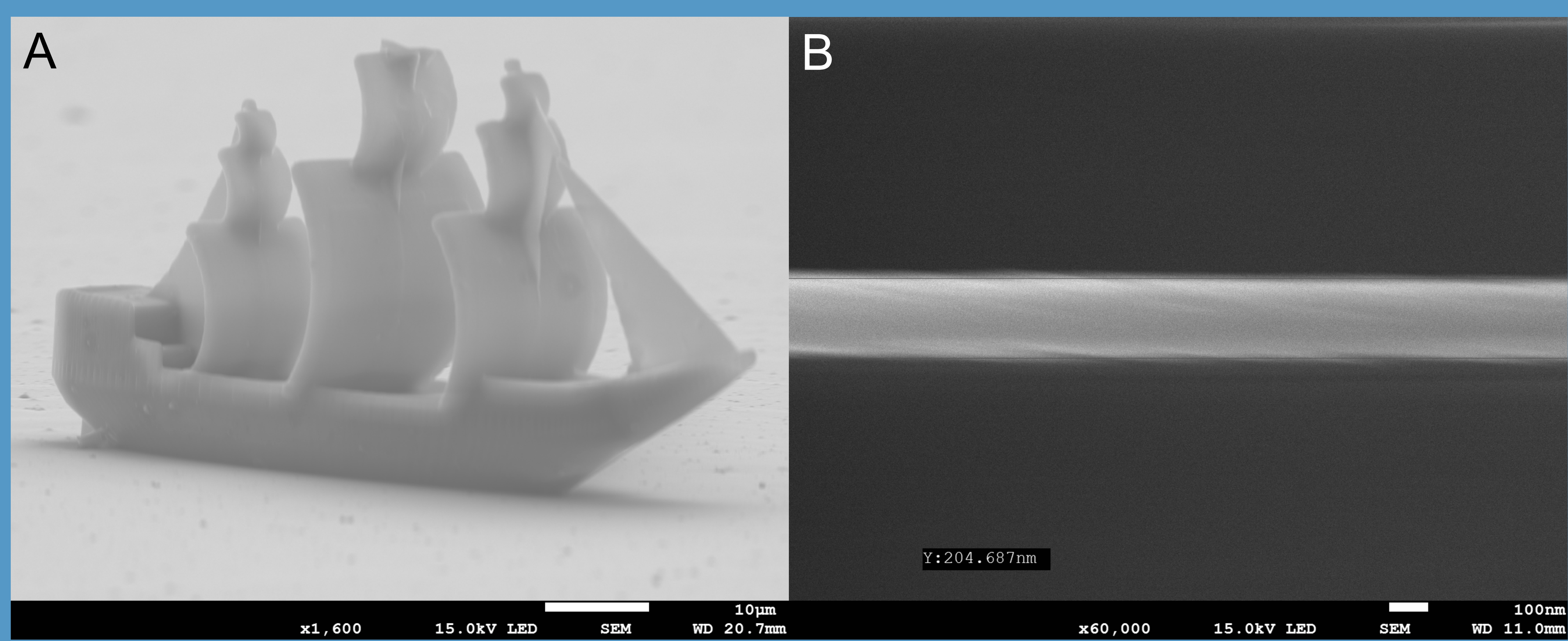
The structures were made using a commercially available Photonic Professional GT system (Nanoscribe GmbH). Generally, the fabrication process comprises 3 steps: A - an ultra-short pulse laser beam is focused in the material; B - the beam is guided in 2D by galvanometric mirrors, while a stepper motor moves the sample in the Z axis according to CAD sketches; C - the structure, when complete, is immersed in a solvent to wash out unexposed parts of the material.



The Nanoscribe system was used to fabricate suspended lines between bulky support structures to assess the resolution and fabrication windows of the material.



HPA material with 1:1:9 (A), 1:1:5 (B), 1:1:2 (C) AIP:MAA:MATMS molar ratios. D shows the arrangement of lines, where each corresponds to a different laser power and scanning speed.



A - 3D structure with fine features and high aspect ratio. B - suspended lines with down to 200 nm can be fabricated. Both structures were fabricated using 40 % (20 mW) laser power and 10 mm/s scanning speed out of 1:1:2 material using a 63x immersion lens.

References

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Acknowledgements

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