UC Berkeley

UC Berkeley Previously Published Works

Title

Laser-made 3D Auxetic Metamaterial Scaffolds for Tissue Engineering Applications

Permalink

<https://escholarship.org/uc/item/9414h3d0>

Journal

Macromolecular Materials and Engineering, 305(7)

ISSN

1438-7492

Authors

Flamourakis, George
Spanos, Ioannis
Vangelatos, Zacharias
et al.

Publication Date

2020-07-01

DOI

10.1002/mame.202000238

Peer reviewed

Laser-made 3D Auxetic Metamaterial Scaffolds for Tissue Engineering Applications

George Flamourakis, Ioannis Spanos, Zacharias Vangelatos, Phane Manganas, Lina Papadimitriou, Costas Grigoropoulos, Anthi Ranella,* and Maria Farsari*

Exploiting the unique properties of three-dimensional (3D) auxetic scaffolds in tissue engineering and regenerative medicine applications provides new impetus to these fields. Herein, the results on the fabrication and characterization of 3D auxetic scaffolds for tissue engineering applications are presented. The scaffolds are based on the well-known re-entrant hexagonal geometry (bowtie) and they are fabricated by multiphoton lithography using the organic–inorganic photopolymer SZ2080. In situ scanning electron microscopy–microindentations and nanoindentation experiments are employed to characterize the photocurable resin SZ2080 and the scaffolds fabricated with it. Despite SZ2080 being a stiff material with a positive Poisson's ratio, the scaffolds exhibit a negative Poisson's ratio and high elasticity due to their architecture. Next, mouse fibroblasts are used to seed the scaffolds, showing that they can readily penetrate them and proliferate in them, adapting the scaffold shape to suit the cells' requirements. Moreover, the scaffold architecture provides the cells with a predilection to specific directions, an imperative parameter for regenerative medicine in many cell-based applications. This research paves the way for the utility of 3D auxetic metamaterials as the next-generation adaptable scaffolds for tissue engineering.

1. Introduction

Mechanical metamaterials are a class of artificial materials with exceptional mechanical properties.^[1] These are not a result of the chemical composition of the bulk material, but a consequence of the tailored architecture, employing novel design principles and combining the concept of hierarchical structures with material size effects at the micro and nanoscale. Mechanical metamaterials have properties that surmount the behavior of bulk materials, such as high strength and low-density,^[2,3] negative thermal expansion,^[4] and negative Poisson's ratio.^[5–7]

More specifically, materials with negative Poisson's ratio, known as auxetics,^[8] have extraordinary mechanical properties such as high energy absorption and fracture resistance.^[9–12] They are widely found in nature, as a large number of crystalline materials are auxetic.^[13] Biological tissues are also known to display auxetic characteristics; auxetic properties have been

reported in cat^[14] and salamander skin,^[15] arteries,^[16] tendons,^[17] and cancellous bone.^[18] Auxetic porous materials have been predicted to facilitate mass transport^[19] and, therefore, there is potential for optimal delivery of nutrients and therapeutic agents, and removal of metabolic wastes. For these reasons, auxetic architectures have been identified as candidates for the development of the next-generation, porous scaffolds for tissue engineering applications, based on the unique combination of suitable materials and structure design.^[20]

Until recently, the application of auxetics in scaffold fabrication was limited to the usage of two-dimensional (2D) structures or auxetic foams.^[21] However, the porosity, geometry, and other mechanical properties of such materials cannot be fine-tuned, as the creation of the foam is chemically based.^[19] Recent progress in additive manufacturing technologies has enabled the realization and investigation of complex mechanical metamaterials, with submicron features, using a wide variety of constituent materials.^[22] The most suitable additive manufacturing technique for mechanical metamaterial fabrication has been shown to be multiphoton lithography (MPL).^[23] It is a laser-based technique which allows the fabrication of three-dimensional (3D) structures with a resolution of as little as a few tens of nanometers. It is based on the phenomenon of multiphoton

G. Flamourakis, I. Spanos, Dr. P. Manganas, Dr. L. Papadimitriou, Dr. A. Ranella, Dr. M. Farsari
Institute of Electronic Structure and Laser
Foundation for Research and Technology-Hellas (FORTH/IESL)
Heraklion, Crete GR-70013, Greece
E-mail: ranthi@iesl.forth.gr; mfarsari@iesl.forth.gr

G. Flamourakis
Department of Materials Science and Technology
University of Crete
Heraklion, Crete GR-70013, Greece

Z. Vangelatos, Prof. C. Grigoropoulos
Department of Mechanical Engineering
University of California
Berkeley, California 94720, USA

Z. Vangelatos, Prof. C. Grigoropoulos
Laser Thermal Laboratory
Department of Mechanical Engineering
University of California
Berkeley, California 94720, USA

The ORCID identification number(s) for the author(s) of this article can be found under <https://doi.org/10.1002/mame.202000238>.

DOI: 10.1002/mame.202000238

absorption: when the beam of an ultrafast laser is focused inside the volume of a transparent, photosensitive material, the high intensity of the beam can induce the absorption of two or more photons within the focal volume, causing the material to cross-link locally. Moving the beam in a 3D pattern allows the writing of high-resolution 3D structures. Like any other lithographic technique, to obtain the structure, one needs to “develop” the sample, by immersing it into an appropriate solvent.

In this work, we demonstrate for the first time the application of MPL-made auxetic scaffolds for cell growth in 3D culture. As an auxetic we have chosen the re-entrant hexagonal structure, also known as the bowtie, a well-known and well-characterized architecture. For the structure fabrication, we employ an organic–inorganic hybrid material, SZ2080, known to enhance cell proliferation.^[24,25] We characterize its mechanical properties and we show that, despite the material itself being stiff, the fabricated scaffolds are flexible and exhibit auxetic behavior. Finally, we use two different types of the scaffolds in fibroblasts cell cultures aiming to study the response of cells thereto and vice versa. These two types of auxetic scaffolds differ in the size of their pores. The first of these exhibits smaller pore size in order to be used as a cell-substrate, without the possibility of cell penetration, while the second one has a larger pore size to study the penetration potential of fibroblasts.

2. Experimental Section

2.1. Material Synthesis and Sample Preparation

The material employed for the scaffold fabrication was the photosensitive organic–inorganic hybrid SZ2080.^[26] Samples were prepared by drop casting the liquid resin onto conventional glass coverslips and prebaking them at 70 °C for 1 h to form a hard gel. To promote structure adhesion, the coverslips were previously coated with the organic monomer compound 3-(trimethoxysilyl) propyl methacrylate (MAPTMS). After polymerization, the whole sample was immersed in a developing solution consisting of 1:1 4-methyl-2-pentanone/isopropanol for 15 min, washed with isopropanol for another 15min, and then allowed to air dry.

2.2. 3D Scaffold Fabrication Using MPL

The experimental setup used in this work is shown in **Figure 1**. It consists of two scanning systems, which have been described in detail.^[27] Both systems use the same irradiation source: a Ti:Sapphire pulsed fs laser operating at 800 nm (Femtolasers Fusion, pulse duration < 20 fs in the sample, repetition rate 80 MHz).

Figure 1. Multiphoton lithography setup (made using 3ds MAX 2020). All the scaffolds were 3D printed using the “nano” setup which is able to make very accurate and detailed structures up to few nanometers. A 3D computer-aided design (CAD) of the design is inserted into the computer and the movement of the stages, attenuator, and shutter are orchestrated by 3Dpoli software.

The first setup (referred to as nano setup) employs piezo-electric stages for the x–y–z movement (Physik Instrumente M-110.1DG). In this case, a second, oil-immersed, focusing microscope objective lens is also used (100×, NA = 1.4, Zeiss, Plan Apochromat). All the auxetic scaffolds were made using this setup, with a laser fluence of 106 mJ cm⁻² and 80 μm s⁻¹ scanning speed.

The second setup uses a galvanometric mirror system (Scan-labs Hurryscan II) to scan the laser beam inside the material on the xy-plane, while a linear translation stage moves the sample in the z-direction (Physik Instrumente M-500). The galvanometer was adapted to house a microscope objective lens (40×, NA = 0.95, Zeiss, Plan Apochromat). This setup was used to fabricate the structures for the mechanical characterization of the material

2.3. Nanodynamic Mechanical Measurement and Analysis (Nano-DMA)

The nanoscale mechanical measurements were carried out using Bruker's TI 950 TriboIndenter to conduct nano-DMA experiments, a dynamic testing technique equipped with Continuous Measurement of X (CMX) control algorithms that provides a continuous measurement of mechanical properties as a function of indentation depth, where X can be hardness *H*, storage modulus *E'*, loss modulus *E''*, complex modulus *E**, and the mechanical damping tan δ. The technique can be applied from ultrasoft hydrogels to hard coatings, with a greatly improved signal to noise ratio.^[28,29] Dynamic testing can be performed in a range of frequencies between 0.1 and 300 Hz. A quasi-static force, up to 10 mN, is applied to the indentation probe while superimposing a small oscillatory force of 5 mN maximum. A lock-in amplifier measures phase and amplitudes changes in the resulting force–displacement signal. To obtain the viscoelastic properties the material is modeled as two Kelvin–Voigt systems in parallel, with one end fixed and applying the indenters force to the other. This model requires four viscoelastic parameters to calculate the moduli of the material, providing highly accurate results compared to the simple Maxwell model or even the Standard Linear Solid.^[30]

2.4. Cell Culturing and Characterization

For this study, the mouse fibroblast cell line NIH-3T3 was used, which was maintained in a 5% CO₂ incubator at 37 °C in standard Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. In order to sterilize the scaffolds, the cover slips were immersed for 30 min in 70% ethanol and then left to dry inside a UV chamber for 1 h. Finally, they were placed in a 24-well plate and seeded with cells.

To prepare samples for scanning electron microscopy (SEM) imaging, 3 × 10⁴ cells/well were seeded and cultivated for 4 days. After the 4d timepoint, the cells were fixed using the cell fixation/preparation for SEM analysis protocol. Briefly, the culture medium was removed and the cover slips were washed twice with sodium cacodylate buffer 0.1 M (SCB). Then,

4% w/v glutaraldehyde (GDA) in SCB was added followed by two washes with SCB. Finally, the specimen was dehydrated in graded ethanol solutions (from 30% to 100%), underwent a critical point drying process (Baltec CPD 030) and 10 nm gold-sputter coat (Baltec CPD 030).

To prepare samples for confocal microscopy imaging, cells were cultured in the same way as for the SEM imaging. Once the cells reached the desired timepoint, they were washed three times with phosphate-buffered saline (PBS) and fixed with 4% w/v paraformaldehyde (PFA) in PBS for 10 min, followed by permeabilization with Triton X-100 0.5% v/v in PBS for 5 min. Then, the samples were blocked by incubating with 2% w/v bovine serum albumin (BSA) in PBS for 30 min. After washing three times with PBS, phalloidin-680 (1:1000 dilution) was used to dye the actin cytoskeleton in 0.5% w/v BSA/0.1% v/v Triton X-100 in PBS for 45 min. Finally, the specimens were placed upside down on an objective glass slide with slow fade mounting medium containing DAPI and kept at 4 °C until observation (Leica SP8 inverted confocal microscope).

3. Results and Discussion

3.1. Mechanical Properties of SZ2080

Materials used in MPL are viscoelastic, exhibiting both elastic and viscous behavior when deformed. Elasticity is a result of bond distortion when the material is stressed.^[31] Elastic deformation occurs almost instantly and the material returns to its original length when the stress is removed. Viscosity is attributed to larger scale rearrangements of molecules inside the material. These molecular allocations depend on the duration of the stress and are not necessarily reversible. In order to consider both responses, dynamic tests were performed on structures fabricated with MPL, in which sinusoidal stress is applied on them and the resulting strain is recorded. Stress and strain will both oscillate with the same frequency, but with the former displaying a phase delay, the magnitude of which depends on how viscous the material is. It is convenient to handle stress as a complex quantity whose real and imaginary parts are, respectively, in and out of phase with the strain. The ratio of in-phase stress to strain is used to define *storage modulus E'*, which represents the elastic portion of the material's response, while the ratio of out-of-phase stress to strain is the *loss modulus E''*, which represents the contribution of viscosity to the material's response. Identifying storage and loss modulus greatly of the bulk material is crucial to accurately model and simulate the mechanical response of architected scaffolds.

Two geometries were considered for the characterization of the material's mechanical properties: rectangular boxes (40 × 50 × 40 μm) and upright cylinders (radius of 4 μm and height of 80 μm) (Figure 2A,B). Arrays of such structures were fabricated with a variable laser fluence ranging from 90 to 150 mJ cm⁻² at a constant scanning speed of 10 μm s⁻¹.

For each geometry, eight arrays were prepared to consider for statistical errors. The boxes were measured using the nano-DMA to extract the *storage* and *loss modulus*, as well as the *hardness* of the material as a function of laser fluence during

Figure 2. A,B) Scanning electron microscopy (SEM) images of the boxes and the cylinders used for the calculation of the mechanical properties, C) Hardness, D) loss modulus, E) storage modulus as a function of laser fluence. There is an abrupt jump at the vicinity of $\approx 110 \text{ mJ cm}^{-2}$ in all three graphs attributed to the polymerization threshold and the writing strategy followed.

fabrication. The nano-DMA oscillation frequency ω was 220 Hz for all measurements. The upright cylinders were used in situ SEM—microindentation experiments to measure the material's Poisson's ratio. For the nano-DMA, a 50 μm diameter spherical probe was employed, while for the microindentation experiments, a 10 μm flat punch was utilized.

The storage modulus E' , loss modulus E'' and hardness H were graphed separately as a function of laser fluence. In the vicinity of 110 mJ cm^{-2} there appears to be an abrupt jump in all three graphs for their respective y-axes. Especially loss modulus and hardness graphs appear to transition from one constant value to another during this sudden increase. This step-function behavior can be attributed to the polymerization threshold and the polymer writing strategy which was followed: the motors use a line-by-line hatching strategy to write each structure, where the distance between each line is ≈ 200 nm. So, between two parallel lines, the distance between the center of the focus of the laser beam is ≈ 200 nm. At the same time, within the line, as the 80 MHz laser is scanned at a speed of 10 $\mu\text{m s}^{-1}$, the distance between consecutive laser pulses is in the order of 1 pm. The test samples therefore consist of very densely cross-linked plates, while the material holding together these plates is less dense with its cross-linking density being dependent on the photon flux received. Above a certain laser fluence, which in this case at approximately 110 mJ cm^{-2} , the cross-linking density reaches a saturation point, after which it can no longer increase.

SZ2080's Poisson's ratio was calculated from its original definition by measuring the cylinders' dimensions before and after deformation and was found to be independent of laser fluence with a constant value of 0.490 ± 0.002 .

3.2. Auxetic Scaffold: The Re-Entrant Hexagonal Structure (Bowtie)

The bowtie is probably the most thoroughly investigated auxetic geometry. It has been widely studied in 2D, to make meshes for stents, as well as for studying auxetics is sports safety equipment. In 3D, it was first built in the microscale by the Wegener group.^[32]

In this study, we fabricated scaffolds with two different unit cells: 8.6 μm (Figures 3–5) and 40 μm (Figure 6). Each size corresponds to the length of the top line of the bowtie unit cell as well as the height of it. The 8.6 μm unit cell size (small pore-size scaffold) was used for mechanical tests and 2D cell cultures, whereas the 40 μm unit cell (large pore-size scaffold) for 3D cell cultures. All scaffolds were fabricated using the nano setup.

The structures used to investigate the mechanical properties of the bowtie scaffold are shown in Figure 3, where the computer-aided design (CAD) model of the structure is compared to the SEM images. There is close matching between the experimental structure and the CAD model, since the unit cells are consistent and well-defined. Its auxetic behavior was investigated using the Hysitron PI 88 SEM PicoIndenter, enabling in situ mechanical testing and observation of the tested samples. Multiple videos of the mechanical tests were recorded to show the deformation of the scaffolds. Figure 4 shows that under compression, the scaffold shrinks homogeneously, without cracking until 60% deformation, thus demonstrating the auxetic behavior. A representative force displacement curve is presented in Figure 4G. While there is no fracture until 60% load, the curve has an undulated profile,

Figure 3. Scanning electron microscopy (SEM) images of the bowtie auxetic scaffold with X axis direction small pore size (8.6 μm) in comparison with the computer-aided design (CAD) model (designed in 3DS max2020). A) tilted view of the scaffold, B) top view, C) zoomed photo of the one edge of the scaffold. The red color was added in photoshop to make the bowtie unit cell clearer, and D) tilted view of the whole scaffold.

Figure 4. Microindentation scanning electron microscopy (SEM) pictures of the bowtie design. The white dashed box represents the initial uncompressed form of the scaffold. A) Uncompressed scaffold, B) at 40% load, some deformations are starting to be visible, C) 60% load, and D) at 100% load, the first layer of the scaffold is completely compressed and touches the lower one without cracking. E) 3D computer-aided design (CAD) design of the scaffold using ANSYS. F) Deformation of the scaffold while applying axial force on the top. The colors represent the total strain each node undergoes (red: maximum, blue: minimum). G) Diagram of the load in correlation to the indentation depth.

which is associated with the reconfiguration of the structure due to internal compression, altering the contact between the structure and the indentation tip. This reconfiguration occurs through the whole loading cycle, leading to instability of the structure due to buckling at 26% deformation (e.g. the slope of the curve becomes negative), causing large deformations and fracture at 60% (Figure 4C). These results reveal that the structure can sustain large deformations, shrinking during compression, and reconfiguration during loading, elucidating its utility as a bioengineering scaffold. The imaging of the tested samples also confirms the simulation results of 3D model created using the 2020 R1 academic version of ANSYS software, where a virtual axial force was applied on top of the scaffold, compressing it in all directions. The CAD simulation was made using ANSYS Design Modeler and the scaffold had 1350 nodes and 720 elements. The material used for the simulation had a Young's modulus of 200GP and Poisson's Ratio of 0.3.

3.3. Cultures of NIH-3T3 Cells on the Scaffolds

The bowtie structure with the small pore size was initially used as a cell substrate. This pore size is too small to allow cell penetration; however, it is useful in order to investigate the ability of fibroblasts to adhere and proliferate on the scaffold and to adapt and deform it. All the cells were able to create filopodia, adhere on the surface as well as the sides of the scaffold (Figure 5) and pull the whole structure. We observed major deformations of many unit cells of the scaffold, as well as buckling around and

on the scaffold. This was a very promising first indication that a hard and stiff material like SZ2080 can become soft and elastic only by tuning its geometry. Furthermore, no cell damage was observed.

In order to allow cell penetration and proliferation inside the scaffold, we built scaffolds with a large pore size of 40 μm, comparable to the size of a single cell (mouse fibroblasts have a diameter of ≈40–50 μm). Each scaffold consisted of two layers. To make the scaffold mechanically stronger, each line was 3D printed three times (scanning speed 100 μm s⁻¹ with a laser fluence of 131 mJ cm⁻²) with each line separated by 0.5 μm. After 4 days of cells in culture, SEM pictures showed that they were able to enter into the pores of the scaffold and successfully proliferate (Figure 6). The structure seemed to be completely occupied by cells and deformed, especially on the one side which is completely flat (Figure 6B). Compared to cells growing on the glass, cells that grow inside the scaffold seem to have proliferated more and be more directional due to the fact that the unit cell itself is designed in X-axis. It is clear that the 3D culture with auxetic properties is more efficient than the 2D in terms of both proliferation and directionality. This design is ideal for cell types that require environments with well-characterized mechanical properties and directionality.

3.4. Confocal Observation

We used the large pore size scaffolds and the same culture conditions as previously described to observe the cell-loaded

Figure 5. Culture of NIH-3T3 on small pore size auxetic scaffolds (I through III). A) General view of the three scaffolds where major deformations can be observed due to the mechanical forces applied by the fibroblasts. Due to the plasticity of the material, the scaffold is able to bend and deform without breaking, thus mimicking the mechanical properties of the extracellular matrix while providing the cells with an X axis directionality. B) Magnified image of the second scaffold. C) Further magnification of the filopodia of a single cell (yellow pseudocolor).

scaffolds under the confocal microscope and take multiple z-stacks. In **Figure 7**, the confocal images are displayed with z-stacks overlaying one another in two different channels. The blue channel represents the DAPI dyed nuclei, whereas the red channel represents the actin filaments of the cytoplasm. As we observed, the scaffold was fluorescent in both channels, with their overlay creating a pink color. Moreover, the fluorescence intensity was really high, making it hard to clearly observe the actin filaments of the cells inside the scaffolds. Nevertheless, with some small image adjustments, we were able to spot the cells at the bottom of the scaffold (layer of unit cells attached on the glass), as well as some others inside higher unit cells, thus showing that the cells are actually capable to exploit their 3D microenvironment.

4. Conclusion

Auxetic porous materials are predicted to facilitate mass transport. As a result, they have the potential for optimal delivery of nutrients and therapeutic agents, as well as removal of metabolic waste and have been identified as candidates for the development of adaptable scaffolds for tissue engineering applications. We have shown for the first time that 3D auxetic structures constructed through MPL can be used as scaffolds for cell cultures. We have characterized the mechanical properties of the fabrication material, SZ2080 using nano-DMA and the mechanical response of the 3D auxetic scaffolds using in situ SEM microindentation experiments. We have shown that, even though SZ2080 is fundamentally a stiff material with a

Figure 6. Culture of NIH-3T3 on large pore size auxetic scaffold. A) General tilted views of the auxetic scaffold with the large pore size. It is obvious that the cells were able to penetrate inside the pores of the structure making it the first 3D culture of an auxetic scaffold. The structure consisted of two layers and was able to withstand the forces. B) Top view of the scaffold with green colored cells (green pseudocolor). C) Zoomed picture of several unit cells on the surface of the scaffold. The bowtie geometry is able to bend inwards because of the mechanical forces applied by the cells (Yellow pseudocolor).

Figure 7. Confocal images of the large pore size scaffold with the fibroblasts. A) Brightfield image of the scaffold using a 20× objective lens, in which the cells are not visible. B) The same image with an overlay of the red and blue fluorescence channels. C) Image taken with a 40× objective lens showing the corner of the scaffold where a higher cell density was observed. The focal point in all images is on the bottom part of the scaffold.

positive Poisson's ratio, the bowtie structures fabricated with it exhibit auxetic behavior and high elasticity due to their architected design. In addition, we have shown that mouse fibroblasts can readily penetrate and proliferate in these scaffolds, adapting the scaffold shape to suit their requirements increasing their proliferation and acquiring desirable directionality. Our research promulgates for the use of 3D auxetic metamaterials as the next-generation of adaptable scaffolds for tissue engineering.

Acknowledgements

This research was partially sponsored by the Stavros Niarchos Foundation within the framework of the project ARCHERS ("Advancing Young Researchers' Human Capital in Cutting Edge Technologies in the Preservation of Cultural Heritage and the Tackling of Societal Challenges"). The authors also acknowledge the support of this work by the projects "Advanced Research Activities in Biomedical and Agro alimentary Technologies" (MIS 5002469) which is implemented under the "Action for the Strategic Development on the Research and Technological Sector," funded by the Operational Program "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014–2020), HELLAS-CH (MIS 5002735) implemented under "Action for Strengthening Research and Innovation Infrastructures," funded by the Operational Program "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014–2020) both co-financed by Greece and the European Union (European Regional Development Fund), and FEMTOSURF, the European Union's Horizon 2020 research and innovation program under grant agreement No. 825512. The authors thank Professor Peter Hosemann, Department of Nuclear Engineering, University of California, Berkeley, and Professor Andrew Minor, Department of Materials Science and Engineering, University of California, Berkeley for the use of the microindentation apparatuses. The SEM, and microindentation experiments were carried out at the California Institute of Quantitative Bioscience (QB3) at UC Berkeley.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

additive manufacturing, auxetic materials, mechanical metamaterials, multiphoton lithography, scaffolds for tissue engineering

Received: April 7, 2020

Revised: May 14, 2020

Published online:

- [1] M. Kadic, T. Bueckmann, R. Schittny, M. Wegener, *Rep. Prog. Phys.* **2013**, 76, 126501.
- [2] X. Zheng, H. Lee, T. H. Weisgraber, M. Shusteff, J. DeOtte, E. B. Duoss, J. D. Kuntz, M. M. Biener, Q. Ge, J. A. Jackson, S. O. Kucheyev, N. X. Fang, C. M. Spadaccini, *Science* **2014**, 344, 1373.
- [3] L. R. Meza, S. Das, J. R. Greer, *Science* **2014**, 345, 1322.
- [4] R. Lakes, K. W. Wojciechowski, *Phys. Status Solidi B* **2008**, 245, 545.
- [5] G. W. Milton, *J. Mech. Phys. Solids* **1992**, 40, 1105.
- [6] H. M. A. Kolken, A. A. Zadpoor, *RSC Adv.* **2017**, 7, 5111.
- [7] K. E. Evans, A. Alderson, *Adv. Mater.* **2000**, 12, 617.
- [8] K. E. Evans, M. A. Nkansah, I. J. Hutchinson, S. C. Rogers, *Nature* **1991**, 353, 124.
- [9] K. E. Evans, K. L. Alderson, *Eng. Sci. Educ. J.* **2000**, 9, 148.
- [10] C. Yang, H. D. Vora, Y. Chang, *Smart Mater. Struct.* **2018**, 27, 025012.
- [11] A. Bezazi, W. Boukharouba, F. Scarpa, *Phys. Status Solidi B* **2009**, 246, 2102.
- [12] H. Wan, H. Ohtaki, S. Kotosaka, G. Hu, *Eur. J. Mech.* **2004**, 23, 95.
- [13] R. H. Baughman, J. M. Shacklette, A. A. Zakhidov, S. Stafstrom, *Nature* **1998**, 392, 362.
- [14] D. R. Veronda, R. A. Westmann, *J. Biomech.* **1970**, 3, 111.
- [15] L. M. Frolich, M. Labarbera, W. P. Stevens, *J. Zool.* **1994**, 232, 231.
- [16] J. W. Lee, P. Soman, J. H. Park, S. Chen, D. W. Cho, *PLoS One* **2016**, 11, e0155681.
- [17] R. Gatt, M. V. Wood, A. Gatt, F. Zarb, C. Formosa, K. M. Azzopardi, A. Casha, T. P. Agius, P. Schembri-Wismayer, L. Attard, N. Chockalingam, J. N. Grima, *Acta Biomater.* **2015**, 24, 201.
- [18] J. L. Williams, J. L. Lewis, *J. Biomech. Eng.* **1982**, 104, 50.
- [19] A. Alderson, J. Rasburn, K. E. Evans, *Phys. Status Solidi B* **2007**, 244, 817.

- [20] J. J. Warner, A. R. Gillies, H. H. Hwang, H. Zhang, R. L. Lieber, S. Chen, *J. Mech. Behav. Biomed. Mater.* **2017**, 76, 145.
- [21] P. Soman, J. W. Lee, A. Phadke, S. Varghese, S. Chen, *Acta Biomater.* **2012**, 8, 2587.
- [22] S. Hengsbach, A. D. Lantada, *Smart Mater. Struct.* **2014**, 23, 085033.
- [23] T. Frenzel, M. Kadic, M. Wegener, *Science* **2017**, 358, 1072.
- [24] M. Chatzinikolaidou, S. Rekstyte, P. Danilevicius, C. Pontikoglou, H. Papadaki, M. Farsari, M. Vamvakaki, *Mater. Sci. Eng., C* **2015**, 48, 301.
- [25] S. Psycharakis, A. Tosca, V. Melissinaki, A. Giakoumaki, A. Ranella, *Biomed. Mater.* **2011**, 6, 045008.
- [26] A. Ovsianikov, J. Viertl, B. Chichkov, M. Oubaha, B. MacCraith, I. Sakellari, A. Giakoumaki, D. Gray, M. Vamvakaki, M. Farsari, C. Fotakis, *ACS Nano* **2008**, 2, 2257.
- [27] A. N. Giakoumaki, G. Kenanakis, A. Klini, M. Androulidaki, Z. Viskadourakis, M. Farsari, A. Selimis, *Sci. Rep.* **2017**, 7, 2100.
- [28] K. Liu, M. Ostadhassan, B. Bubach, R. Dietrich, V. Rasouli, *J. Mater. Sci.* **2018**, 53, 4417.
- [29] M. E. Dokukin, I. Sokolov, *Macromolecules* **2012**, 45, 4277.
- [30] W. N. Findley, J. S. Lai, K. Onaran, *Creep and Relaxation of Nonlinear Viscoelastic Materials, with An Introduction to Linear Viscoelasticity*, North-Holland Pub. Co., Amsterdam **1976**.
- [31] A. Skarmoutsou, G. Lolas, C. A. Charitidis, M. Chatzinikolaidou, M. Vamvakaki, M. Farsari, *J. Mech. Behav. Biomed. Mater.* **2013**, 25, 48.
- [32] T. Bückmann, N. Stenger, M. Kadic, J. Kaschke, A. Frölich, T. Kennerknecht, C. Eberl, M. Thiel, M. Wegener, *Adv. Mater.* **2012**, 24, 2710.