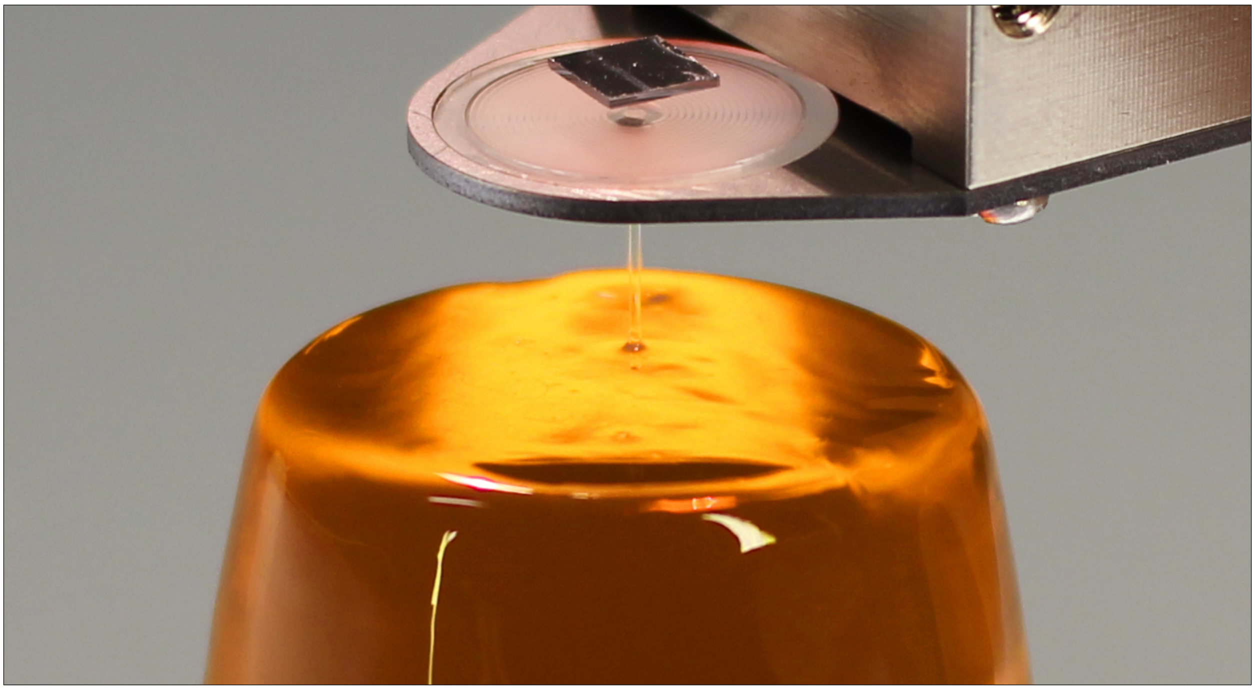
Application information

Viscoelasticity of hydrogels



Description of the problem / Objectives of the investigation

In-vitro tests with two-dimensional cell cultures have only limited significance because complex cell interactions can only take place in three-dimensional tissue. For this reason, there is ever-growing scientific interest in three-dimensionally structurable biomaterials.

These biomaterials are used, for example, as scaffolds for cell growth in tumour research, material research or as implants for tissue regeneration. The diversity of possible fields of application means that different requirements are placed upon the material. For this reason, a wide range of specific material properties have to be generated and precisely quantified.

One of the deficits in the field of research is that the current standard measurements (DIN 53513) inadequately describe the complex mechanical behaviour of many of the materials used in life science, because these materials exhibit frequency-dependent viscoelastic behaviour. In particular, the physiological frequency range of possible stresses (0.1 to 3 Hz) is not taken into consideration.

Fig.1: Dipl.-Ing. Holger Rothe, Institut für Bioprozess- und Analysenmesstechnik e.V. Heilbad Heiligenstadt

Objectives of the study

The objectives of the study are, on the one hand, to show that the viscoelastic parameters of a lactide-caprolactone copolymer can be specifically controlled by varying the degree of cross-linkage. These changes should primarily manifest themselves in the physiologically relevant frequency range. An evaluation routine should also be developed, that enables a continuous relaxation time spectrum to be derived from relaxation measurements made on a polymer material, from which viscoelastic parameters can be derived for any frequency.

Experimental set-up / Approach to a solution

Experiment:

The experiment consisted of a dynamic force measurement (indentation & relaxation) and its evaluation.
In this type of indentation measurement, the test piece is compressed vertically by a force up to a maximum of 0.5 N or by 20% of the height of the piece. The force was measured at a constant z-position for 300 seconds, during which time the relaxation behaviour of the piece was investigated. The sampling rate was 100 per second.
The raw data of the modulus/time curves were fitted with the aid of evolutive algorithms, and the relaxation time spectrum was derived from the data gained in this way.

Experimental object:

The experimental object was a material platform made of photopolymerisable copolymers. Their field of application lies in rapid prototyping processes, such as two-photon polymerisation. In the latter case, scaffolds are constructed from photosensitive precursors for use in 3D cell cultures. The precursors used in this case consisted of lactide and caprolactone mixtures (LCM), which had been methacrylated for the purpose of photopolymerisation. Both components are biodegradable and biocompatible, and are recognized by the FDA as medical products. Varying the proportions of the two base materials created a highly variable system in respect of moistening, rate of biological decomposition, and mechanical and biological functionality.

As well as varying the proportions of lactide and caprolactone, the mechanics of the polymers could also be effectively controlled by incorporating monofunctional precursors, thereby reducing the degree of polymerisation. Increasing the proportion of monofunctional precursors ought to reduce the stiffness of the polymer.

Measuring system:

A BASALT-N2 INDENTER made by TETRA, with a 1N force sensor, was used as the measuring system. A circular plate with a diameter of 6 mm was attached to it in order to apply a force to the entire surface of the cylindrical test piece, which had dimensions of d = 5.5 mm, l = 6 mm.

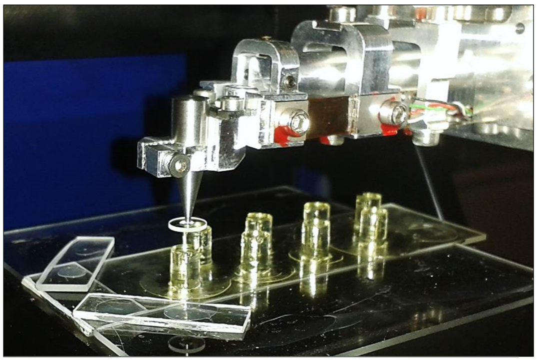


Fig. 2: Experimental set-up: BASALT-N2 INDENT and test pieces

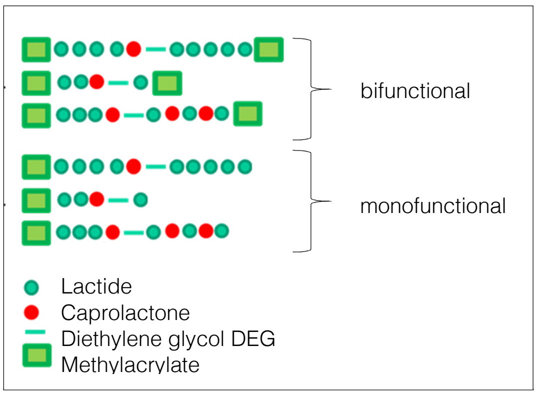


Fig. 3: Structure of the polymer platform (Experimental object)

Results

The results of the evaluation of the relaxation measurements produced frequency-dependent storage moduli and loss moduli for various polymer mixtures lying in the frequency range from 10^{-6}/s to 10^{6}/s. It could be shown that the selected material platform had a relaxation range lying within the physiologically relevant range. The storage modulus showed a significant dispersion step between 0.1 Hz and 10 Hz. At low frequencies, the storage module corresponded to the relaxed modulus of elasticity. Whereas at high frequencies, the polymer did not have an opportunity to relax, and the storage modulus corresponded to the unrelaxed modulus of elasticity. In this case, the loss modulus was equal to zero.

As the proportion of monofunctional components increased, the stiffness of the material could be significantly reduced. (Fig. 5-A) At the same time, the relative proportion of the loss modulus increased. As the proportion of monofunctional precursors increased, the range of relaxation itself shifted only slightly towards lower frequencies, but remained overall within the physiologically relevant range. (Fig. 5-B). This always led to higher values for the modulus of elasticity determined according to DIN in all the material modifications tested (Fig. 5-C), which is the reason why the measurement and evaluation strategy chosen here is to be preferred.

Conclusion

The investigations confirmed the initially formulated thesis that the incorporation of monofunctional LCMs could specifically adjust the viscoelastic behaviour over several orders of magnitude. Increasing the proportion of monofunctional LCMs increased the viscous proportion up until structural integrity was lost (100% monofunctional = no cross-linking agent). The recording of the measured values and the adjustable force range of the BASALT-N2 INDENT are ideally suited for the systematic investigation of the viscoelastic behaviour of biomaterials and hydrogels. It could thus be shown that the mechanical properties of photo-structurable polymers could be specifically varied by the addition of monofunctional precursors. These findings, in conjunction with rapid prototyping methods, such as two-photon polymerisation, enable structurally and mechanically optimised designer scaffolds to be created

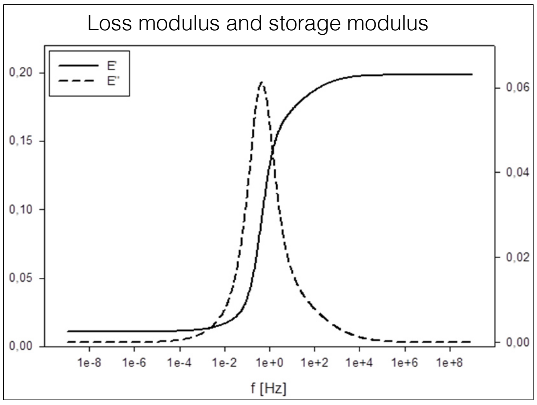


Fig. 4: Diagram of loss and storage modul

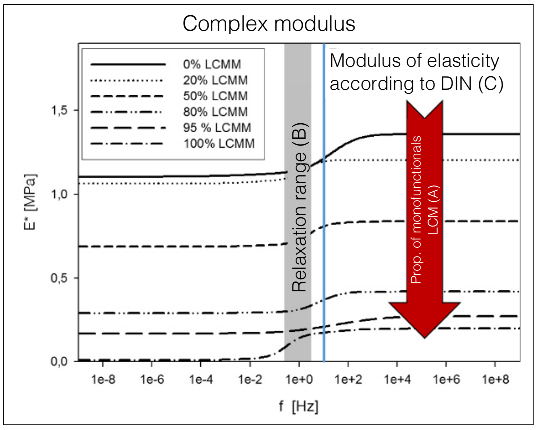


Fig. 5: Results of the experiment

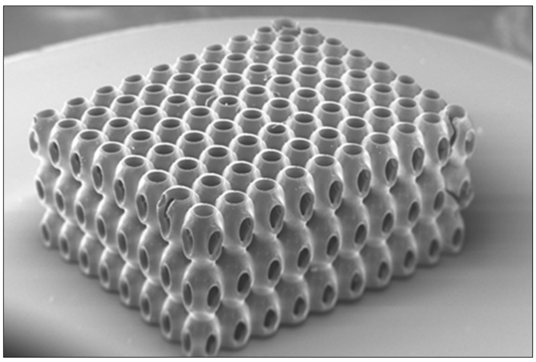


Fig. 6: Scaffold with the polymer platform that was investi-gated. It was structured by two-photon

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