Tensile Testing of Individual Ultrathin Electrospun Poly(L-lactic acid) Fibers

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Received 14 July 2008; accepted 28 May 2009
DOI 10.1002/app.30959
Published online 17 August 2009 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The mechanical properties of ultrathin electrospun poly(L-lactic acid) (PLLA) fibers were studied by performing tensile tests on individual fibers. The tests were carried out on microelectronic mechanical systems (MEMSs) which were developed for characterizing the mechanical properties of thin polymer fibers. Force–displacement curves were obtained from video recordings of the experiments which were carried out in a scanning electron microscope. Each video was processed with an image processing routine to determine the elongation of the fiber and the elastic deformation of a component of the MEMS which yielded the force acting on the specimen. PLLA fibers with diameters ranging from 150 nm to 2 μm were tested. The elastic modulus and the ultimate tensile strength of the fibers increased significantly for fiber diameters below 1 μm. This indicates a higher degree of orientation of the polymer chains in ultrathin fibers. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 114: 3774–3779, 2009

Key words: ultrathin fiber; electrostatic spinning; poly(L-lactic acid); mechanical properties; elastic modulus; ultimate tensile strength; MEMS; single fiber testing

INTRODUCTION

The electrostatic spinning process attracts interest both from fundamental and applied perspectives because of its ability to generate polymer fibers with diameters in the submicrometer scale. The areas of potential applications of electrospun nonwovens range from filter applications, wound dressings, scaffolds for tissue engineering, fiber-reinforced composites, and membranes to nanofiber-based electronic and optical devices.[1] Important characteristics of ultrathin fibers are their high surface-to-volume ratio and high draw ratio. It is known from conventional spinning processes that a high draw ratio results in an alignment of the long axis of the polymer chains with the fiber axis which in turn results in superior mechanical properties of the fiber.[2] Therefore, the relationship between the draw ratio or the diameter of ultrathin fibers and their structure and mechanical properties attracted considerable interest ever since the research activities into the electrostatic spinning process gained new momentum in the early nineties of the last century.[3]

In the last decade a significant part of the research activities and patent applications involving electrospun fibers focused on biomedical applications.[4,5] Many biocompatible and bioresorbable polymers can be spun with the electrostatic spinning process.[6–8] The porous nonwoven which is typically obtained by the electrospinning process can be used as a two-dimensional scaffold for tissue engineering.[9–12] Initial research work focused on the characterization of electrospun nonwovens as scaffolds, e.g., the effects of the type of polymer and the structure of the nonwoven on the cell seeding and cell growth and differentiation.

A basic requirement of scaffolds is sufficient mechanical strength which allows a safe handling of the scaffold and provides an adequate stability of the seeded scaffold if external forces (e.g., in a bioreactor or *in vivo*) are applied. Furthermore, a mechanical stimulation of the cells is advantageous in order to stimulate cell growth and appropriate cell differentiation.[13–15] The scaffold plays an important role in the transmission of mechanical stimuli to the cells. Forces can either be directly applied to the scaffold, or the scaffold and the cells experience the drag force of the culture medium which passes through the scaffold in a flow bioreactor. The drag forces can change the structure of the in general rather flexible nonwovens and in turn alter the transport processes of nutrients and metabolic products. An insight into the mechanical behavior of

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electrospun nonwovens is therefore essential in order to optimize their performance. The mechanical properties of an electrospun single fiber are—amongst other characteristics such as the structure of the nonwoven and the adhesive forces between fibers—important parameters if one wishes to obtain a mechanical characterization of the nonwoven.

The mechanical properties of ultrathin fibers are closely linked to their structure and morphology. Because the handling and mechanical testing of an individual fiber with a submicrometer diameter is a formidable experimental task, early attempts to assess the mechanical properties of electrospun fibers focused on detecting an extended chain morphology and an alignment of the polymer chains and fiber axis[16,17] which would be an indication of superior mechanical properties.

In recent years, different tensile testing devices for ultrathin fibers have been developed.[18] Yu et al.[19,20] investigated the mechanical behavior of carbon nanotubes under stretching. Each end of the tube was attached to an atomic force microscope (AFM) tip. The connection was achieved by localized electron impact-induced dissociation. The force was detected with a "soft" cantilever and the stretching was carried out by a "stiff" cantilever. This procedure, however, would be problematic for polymeric fibers. Attaching a fiber to the cantilever using a focused electron beam would severely damage the fiber, and this would compromise the quality of the results of the tensile test. This method is therefore not suitable for polymer fibers. In a modified setup, Tan et al.[21] used a piezoelectric cantilever to detect the applied force directly. The fibers were collected parallel on a frame which was mounted on a light microscope stage. The fiber was connected to the AFM piezoelectric tip and the stretching was carried out by moving the microscope stage. Naraghi et al.[22] placed the electrospun fibers on a microelectronic mechanical system (MEMS) which was etched from a silicon chip. An epoxy adhesive was used to attach the fiber to a stationary grip and a leaf-spring grip. Again, an AFM cantilever without a tip was used to perform the stretching.

Another tool for testing thin fibers is a commercially available nanotensile testing system (e.g., NanoBionix System; MTS, Eden Prairie, MN).[23–27] After mounting the fibers in the testing system, a sensitive force transducer is used to determine the mechanical properties of the specimens. This setup was used till date for testing fibers with diameters larger than 100 nm.

In this article we describe how the elastic modulus, tensile strength, and elongation at break of electrospun fibers are measured using an MEMS. The micromechanical devices were designed by the Fraunhofer-IWM (Halle, Germany) and etched from a silicon chip. The authors used the micromechanical devices in order to study the influence of the diameter of electrospun poly(L-lactic acid) (PLLA) fibers on their mechanical performance.

The mechanical characteristics of bioresorbable fibers are important parameters for optimizing mechanical performance of electrospun scaffolds. The mechanical data of the fibers will be used in future research as input parameters for a numerical model which describes the mechanical behavior of electrospun nonwovens which is currently under development in our research group.

MATERIALS AND METHODS

Materials

PLLA with a molecular weight of $M_w = 125{,}000$ g/mol, purchased from Sigma-Aldrich, Fluka, was used to prepare 10 wt % PLLA-dichloromethane (DCM) solutions. The PLLA solution was prepared by gently stirring the polymer for 4 h at room temperature in DCM.

Methods

Electrostatic spinning of the fibers

The spinning solution was contained in a syringe with a needle with 0.33 mm inner diameter. The fluid flow was controlled by pressurized air (0.2 bar). The needle was connected to a high-voltage source (FUG Elektronik GmbH, Rosenheim, Germany). Between the needle and a grounded shield (distance: 7 cm), a voltage of 7 kV was applied. The spinning process was carried out in air at room temperature. To collect fibers for the mechanical testing, a grounded metallic frame (1×5 cm^2) was positioned between the charged needle and the counter electrode.

Micromechanical testers

The design of the micromechanical testing device was developed by the Fraunhofer-IWM. The MEMS were etched from a silicon chip through several etching processes by MEMSCAP (France).[28] A micromechanical tester essentially comprises two movable components, the pulling and the bending cantilever (see Fig. 1). The pulling cantilever consists of a pulling ring and a sample platform and is guided by two rails attached to the silicon chip in order to achieve a linear motion. The bending cantilever consists of a sample platform and a silicon bar connected at both ends to the silicon chip which is used as a flat spring. The fiber which is tested is attached to the two sample platforms such that the pulling and the bending cantilever are joined by the fiber

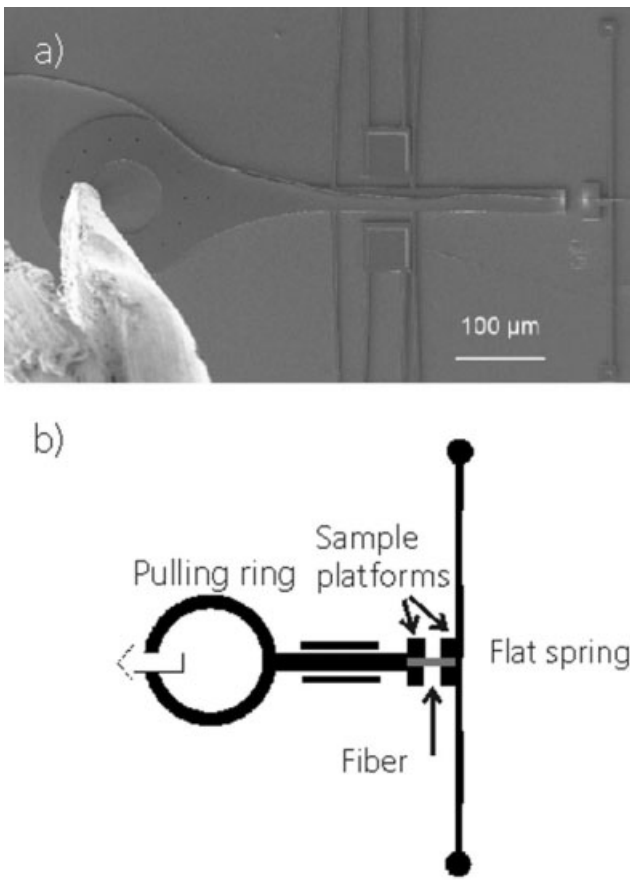

Figure 1 (a) SEM image of an MEMS; left: tip of the needle manipulator moving the pulling cantilever. (b) Sketch of a single fiber tensile tester; left: pulling cantilever; right: bending cantilever.

[see Fig. 2(a,b)]. When the pulling cantilever is moved, the flat spring exerts a force on the fiber. The force can be calculated from the displacement of the flat spring, assuming a linear-elastic behavior of the silicon bar. The strain of the fiber is the difference between the displacements of the two sample platforms.

Tensile tests on ultrathin fibers

The fibers which were spun onto a frame (see section "Electrostatic spinning of the fibers") were transferred to the micromechanical testers under a light microscope using a needle manipulator. A focused ion beam microscope (type: FEI611) was used to attach the fibers on the sample platforms (see Fig. 2). Projecting ends of the fibers were cut off using the focused ion beam. Subsequently, the silicon chip which carried the micromechanical testers was transferred into a scanning electron microscope (Zeiss crossbeam 1540EsB). The Zeiss crossbeam SEM can be operated at low currents in order to prevent the fibers from being damaged by the electron beam. A high-resolution SEM image was taken in order to determine the fiber diameter d and fiber length l_0. As SEM images of the fibers before and after the tensile tests were available, it was possible to determine the diameter of the fiber on the images prior to the test in the vicinity of the region where the fiber ruptured during the test. A needle manipulator (Kleindiek manipulator system) was inserted into the pulling ring and used to move the pulling cantilever with an average speed of 2.5 ± 0.5 µm/s in fiber direction in order to perform the tensile test. The test was recorded on a video (10 frames/s, reduction = 2, resolution = 512 × 384 pixels). An image processing routine was employed in order to extract the displacements of the edges of the two sample platforms which are used to calculate stress and strain.

Data processing

CImg, an open source C++ toolkit for image processing, was employed to analyze the frames of the videos. The user selects manually a segment of the fiber fixation on the micromechanical tester. This segment is used by the program as "marker" when tracking the movement of the fixation points. The procedure is carried out using the autocorrelation

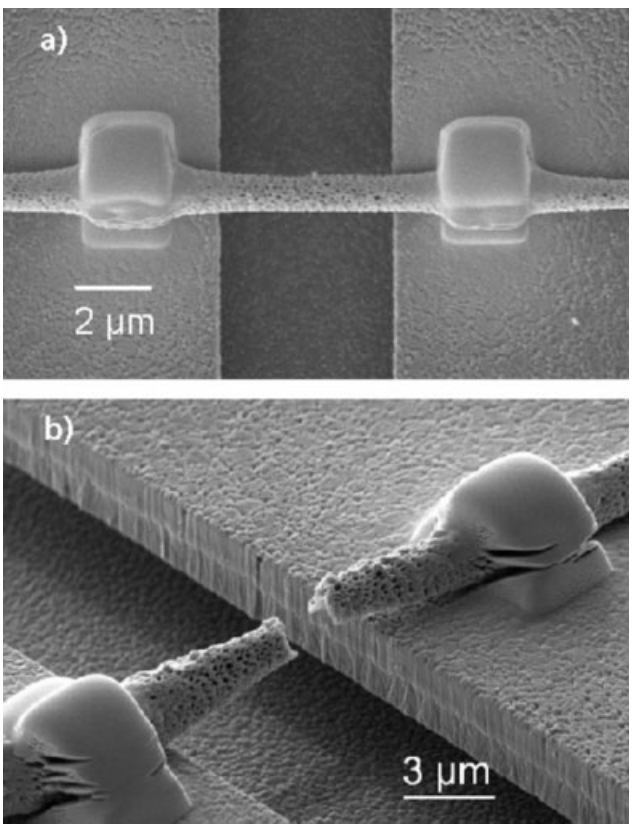

Figure 2 SEM images of a single fiber (a) before the tensile test and (b) after the tensile test.

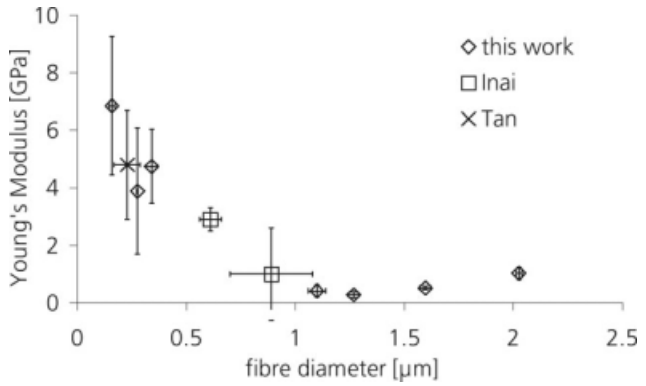

Figure 3 Young's modulus versus fiber diameter. Displayed are the results of this work and those of Inai et al.[24] and Tan and Lim.[27]

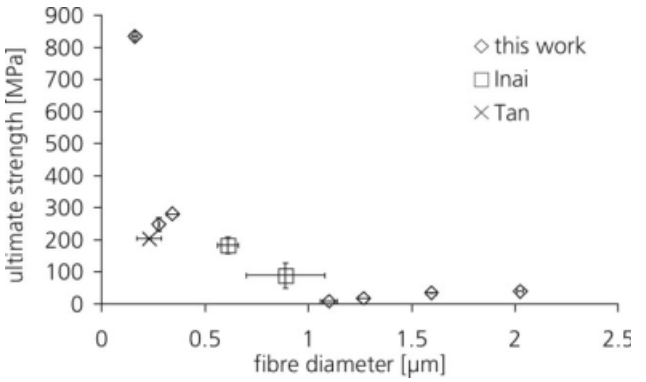

Figure 4 Ultimate tensile strength versus fiber diameter. Displayed are results of this work and of Inai et al.[24] and Tan and Lim[27] are displayed.

function and employing a next neighbor interpolation in order to reduce the noise within a frame. It is repeated for each video five times, where the displacement of the sample platforms is determined with different markers for each run of the procedure. The strain of the fiber $\varepsilon(t)$ is the difference between the position of the two markers $x_1(t)$ on the bending cantilever and $x_2(t)$ on the pulling cantilever divided by the initial length of the fiber l_0:

$$\varepsilon(t) = \frac{|x_1(t) - x_2(t)|}{l_0}$$

The force acting on the fiber is calculated by the bending of the flat spring:

$$F(t) = -D\,(x_1(t) - x_1(0)) = -D\,\Delta x_1(t)$$

$\Delta x_1(t)$ denotes the displacement of the bending cantilever at time t. The setup is equal to a three-point bending arrangement where both ends of the cantilever are fixed. The bending constant D is calculated by using the Young's modulus of silicon E_{Si} and the height h, width w, and length l of the silicon bar:

$$D = \frac{E_{Si}\,h\,w^3}{l}$$

RESULTS

The geometry of the collecting frame changes the electrostatic field during spinning, which causes an alignment of the fibers orthogonal to the longer frame side. This effect was observed by Li and Xia.[1] The electrostatic spinning process produced fibers with diameters ranging from 150 nm to 2 µm. Fiber damage at the fixation points was not observed; all fibers failed in the center, i.e., between these two points. Stress–strain diagrams were obtained from the processing of the videos of the tensile tests. These diagrams were used to determine the modulus, the ultimate tensile strength (UTS), and the elongation at rupture of the fibers.

Figures 3–5 show the results of the experiments on fibers with diameters ranging from 150 nm to 2 µm. In addition, the results obtained by Tan and Lim[27] and Inai et al.[24] are displayed in the diagrams. Both Young's modulus and UTS increase when the fiber diameter decreases below 1 µm. The fibers with a diameter greater than 1 µm show no significant difference in their mechanical behavior. No significant changes of the elongation at rupture of the fibers as a function of their diameter could be observed. The increase in the elastic modulus for the thinner fibers (which would result in a smaller elongation at rupture) is compensated with the higher UTS of thinner fibers. Necking of the polymer fibers was not observed. The results obtained in our study agree well with the results of Inai et al. and Tan et al. and with the results on melt-spun PLLA fibres.[29]

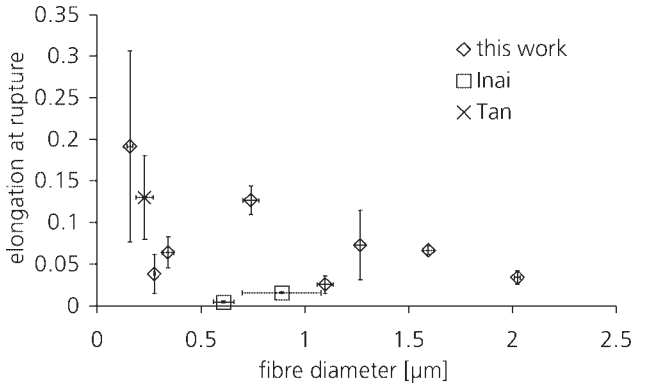

Figure 5 Elongation at rupture versus fiber diameter. Displayed are the results of this work and those of Inai et al.[24] and Tan and Lim[27] are displayed.

DISCUSSION

The experimental method presented in this article combines the advantages of the experimental approaches chosen by Inai et al.[24] and Tan et al.[21] while avoiding some of the disadvantages. The design of microtensile tester employed in our study facilitates gentle handling of the fibers prior to the experiments, similar to the method used by Inai et al. with the nanotensile testing system: Fibers are spun onto a frame and then placed carefully on the testing arrangement. In Inai's procedure, all except one ideally positioned fiber were discarded prior to the experiment. In the procedure described in this article, only suitably positioned fibers or fibers which required only minimal shifting were attached to the sample platforms. However, the testing system employed by Inai et al. is used till date to test only fibers with diameters larger than 100 nm, whereas the microtensile tester used in the current work can, in principle, be used to test fibers with diameters below 100 nm. An experimental setup employing an AFM cantilever can also be used to examine fibers with diameters below 100 nm. However, attaching an electrospun fiber to an AFM cantilever is a challenging task, most likely requiring an extensive manipulation of the fiber position.[18] This, in turn, can result in mechanical loads which potentially damage ultrathin fibers prior to the tests.

As the video observation of the tensile test is an integral part of the testing procedure described in this article, the dimensions, deformation, and rupture of the fiber are documented. Critical notches in the specimen or a potential failure of the sample fixation can be identified. As a result, the presented testing method is reliable and transparent.

SUMMARY AND OUTLOOK

In this study the mechanical properties of electrospun PLLA fibers with diameters in the micron and submicron range were measured employing an MEMS. An integral part of the measurement was a video documentation of the tensile test carried out in a SEM which was subsequently analyzed in order to obtain the stress–strain curves.

The experiments showed that the mechanical performance of the fibers increases for fiber diameters below 1 μm. The results agree well with the results of Inai et al. and Tan et al., who focused on testing fibers with a defined submicrometer diameter. Inai et al. showed that the molecular orientation of electrospun fibers can be improved by selecting suitable processing parameters and that the increased alignment of the polymer chains results in increased strength and modulus of the PLLA fibers. Considering the significant increase of the elastic modulus and UTS of the fibers below 500 nm observed in our experiments, it is fair to assume that the PLLA fibers with diameters of a few hundred nanometers will exhibit an even stronger orientation of the polymer chains and a higher crystallinity.

The MEMS developed by the Fraunhofer-IWM can be used to determine the mechanical behavior of single ultrathin fibers over a wide range of fiber diameters. The force can be detected with an accuracy of ±8 nN. One of the factors which currently limit the accuracy of the stress and strain measurements is the resolution of the video recording which is employed to analyze the tensile tests. Increasing the image resolution and the recording frequency and possibly decreasing the stiffness of the flat spring are ways to further improve the accuracy of the technique for experiments in the subnano-Newton force range.

The mechanical characterization of individual electrospun fibers is a useful tool to gain greater insight into the electrospinning process and the potential applications of ultrathin fibers. Future work will focus on a combination of microtensile tests on individual fibers and simulations for customizing nonwovens for different applications.

The support of the Fraunhofer-Gesellschaft is gratefully acknowledged.

References

1. Li, D.; Xia, Y. Adv Mater 2004, 16, 1151.
2. Hoogsteen, W.; Postema, A.; Pennings, A.; ten Brinke, G.; Zugenmaier, P. Macromolecules 1990, 23, 634.
3. Doshi, J.; Reneker, D. H. In Proceedings of the Industry Applications Society Annual Meeting, Conference Record of the 1993 IEEE, Vol. 3, p 1698.
4. Lannutti, J.; Reneker, D.; Ma, T.; Tomasko, D.; Farson, D. Mater Sci Eng C 2007, 27, 345.
5. Yoshimoto, H.; Shin, Y. M.; Terai, H.; Vacanti, J. P. Biomaterials 2003, 24, 2077.
6. Xinhua, Z.; Kwangsok, K.; Dufei, F.; Shaofeng, R.; Benjamin, S. H.; Benjamin, C. Polymer 2002, 43, 16.
7. Li, M.; Guo, Y.; Yen, A. W. Biomaterials 2006, 27, 2705.
8. Bhuvanesh, G.; Nilesh, R.; Hilborn, J. Prog Polym Sci 2007, 32, 455.
9. Kim, K.; Yu, M.; Zong, X.; Chiuc, E. A. Biomaterials 2003, 24, 4977.
10. Smith, L.; Ma, P. Colloids Surf B 2004, 39, 125.
11. Yang, F.; Murugan, R.; Wang, S. S. R. Biomaterials 2005, 26, 2603.
12. Sill, T. J.; von Recum, H. A. Biomaterials 29,2008,1989.
13. Yeung, T.; Georges, P. C.; Flanagan, L. A.; Marg, B.; Ortiz, M.; Funaki, M.; Zahir, N.; Ming, W.; Weaver, V.; Janmey, P. A. Cell Motil Cytoskeleton 2005, 60, 24.
14. Peyton, S. R.; Putnam, A. J. J Cell Physiol 2005, 204, 198.
15. Brown, X. Q.; Ookawa, K.; Wong, J. Y. Biomaterials 2005, 26, 3123.
16. Srinivasan, G.; Reneker, D. H. Polym Int 1995, 36, 195.
17. Jaeger, R.; Schönherr, H.; Vancso, G. J. Macromolecules 1996, 29, 7634.
18. Tan, E. P. S.; Lim, C. T. Compos Sci Technol 2006, 66, 1102.
19. Yu, M. F.; Dyer, M. J.; Skidmore, G. D.; Rohrs, H. W.; Lu, X. K. Nanotechnology 1999, 10, 244.

20. Yu, M. F.; Lourie, O.; Dyer, M. J.; Moloni, K.; Kelly, T. F.; Tuoff, R. S. Science 2000, 287, 637.
21. Tan, E.; Goh, C.; Sow, C.; Lim, C. Appl Phys Lett 2005, 86, 073115.
22. Naraghi, M.; Chasiotis, I.; Kahn, H.; Wen, Y.; Dzenis, Y. Rev Sci Instrum 2007, 78, 1.
23. Tan, E. P. S.; Ng, S. Y.; Lim, C. T. Biomaterials 2005, 26, 1453.
24. Inai, R.; Kotaki, M.; Ramakrishna, S. M. Nanotechnology 2005, 16, 208.
25. Wong, S.-C.; Baji, A.; Leng, S. Polymer 2008, 49, 4713.
26. Chen, F.; Peng, X.; Li, T.; Chen, S.; Wu, X.-F.; Reneker, D. H.; Hou, H. J Phys D: Appl Phys 2008, 41, 1.
27. Tan, E. P. S.; Lim, C. T. Nanotechnology 2006, 17, 2649.
28. MEMSCAP. PolyMUMPs Design Handbook; MEMSCAP, Research Triangle Park, North Carolina, 2005.
29. Fambri, L.; Pegoretti, A.; Fenner, R.; Incardona, S. D.; Migliaresi, C. Polymer 1997, 38, 79.