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# Electrospinning of Diphenylalanine Nanotubes**

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Electrospinning of polymers has recently become one of the most popular methods to produce polymer fibers from solutions and melts of polymers.[1,2] It is usually impossible to use monomers because only polymer solutions or melts are sufficiently viscous and provide the required degree of molecular entanglement.[3] Notable exceptions are found in proteins,[4,5] but for small molecules only in phospholipids.[6] Pure peptides, the logical extension towards smaller entities, have not yet been used in electrospinning. Here, we present electrospinning from concentrated diphenylalanine solutions in a low-boiling-point solvent, resulting in tubes that are chemically identical to self-assembled tubes but show different morphologies, especially, extreme lengths. Electrospinning of tubes offers more possibilities for manipulation, for example, bridging electrodes in parallel orientation; a possible patterning strategy for electrospun material.

In peptides with aromatic residues, for example, di-L-phenylalanine (Phe-Phe), $\pi$–$\pi$ interactions play a vital role in stabilizing the structures of the bulk crystal and of fibers.[7] Phe-Phe fibers, produced by self-assembly in solution, are tubular, and, either via confinement or by their chemical groups, act as templates to construct functional nanowires and nanotubes.[8] In contrast to the assembly process in solution chemistry, electrospinning requires a subtle balance between the solvent, the solution concentration and thus viscosity and surface tension, the voltage applied to the spinneret, and the flow rate that determines how fast a droplet builds up. Owing to the low solubility of peptides, achieving concentrated solutions is often impossible. In addition, the solvent should possess a high vapor pressure to ensure complete evaporation before the fiber hits the collector. After various tests, we chose 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, boiling point 59 °C), which is highly polar and miscible with water.[9]

We employed a homebuilt electrospinning setup with a vertically placed polarized syringe needle. After formation of a droplet, the voltage at the spinneret (a needle) was slowly increased, until a Taylor cone deformation became visible. A further voltage increase resulted in spinning only above a concentration of ca. 6 wt %, as evidenced by appearance of an easily visible, several millimeters long jet. Its end apparently sprayed very fine material, which we collected about 10 cm below the jet. The material consisted of fibers with large curvature radii (tens of micrometers) and quite uniform diameter. A mixture of fibers and beaded fibers was noticed up to at least 13.2% because of low entanglement of the assembled peptide molecules, which does not allow complete stretching of droplets during spinning. At higher concentrations, beadless fibers formed (Fig. 1A and B) owing to complete stretching of droplets, and the majority attained lengths well above 10 μm. The diameter was observed to increase slightly with increasing concentration (Fig. 1C). This small effect could be due to the increase in viscosity that results in greater resistance of the solution to be stretched by the electrostatic charges on the jet. The diameter of the fibers was shown to decrease very slightly with increasing applied electrostatic voltage (Fig. 1D); indeed, the resulting increase in charge density in the jet should lead to greater stretching of the jet owing to greater Coulombic forces on the jet surface. However, the system allowed only relatively small changes in concentrations and voltages.

With optimized parameters, pure fibers of practically infinite length were obtained (Fig. 2A; see also Supporting Information). The observation of fibers means that molecular chains interact very strongly – otherwise a continuous jet stream cannot be maintained, and microdroplet formation sets in. Because the first (rather large) jet emanates directly from the tip of the Taylor cone, and since this jet is very prone to break up into droplets, we assume that the Phe-Phe molecules assemble already at this point. The driving forces are the evaporation of HFIP and the high surface charge of the jet, which forces the molecules into ordered structures at least close to the surface. A clear indication for self-assembly is the observation of needlelike fibers (Fig. 1), much shorter than the curved structures (Fig. 2A), resembling self-assembled tubes (Fig. 2B), which we obtained from diluting the HFIP solution with water. Raman spectra of electrospun and self-assembled fibers show identical vibrational resonances (Fig. 2C and D; due to the higher background, the spectrum for the self-assembled fibers shows fewer details, but even the relative peak intensities compare very well with the spectrum of spun fibers). Moreover, the spun fibers, too, resemble tubes, as

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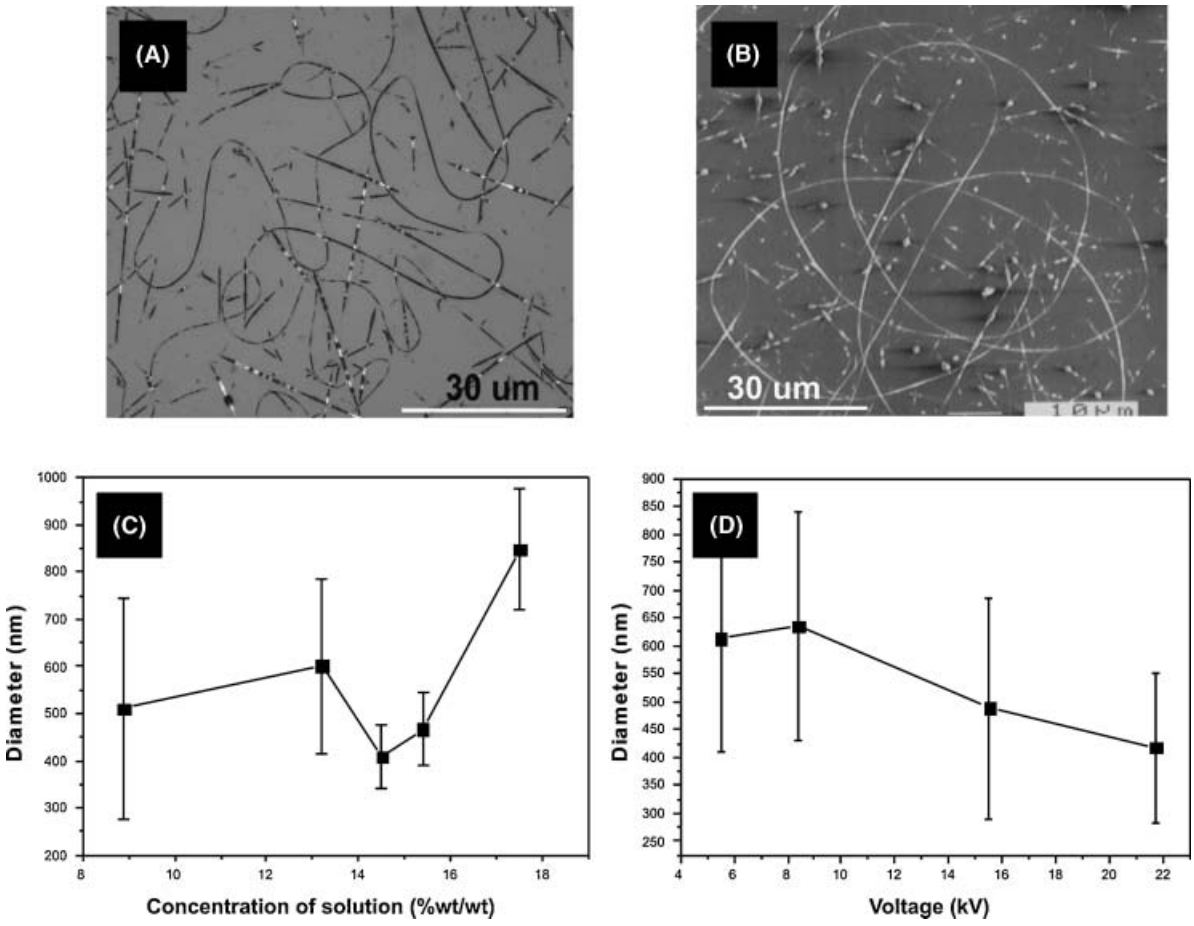


**Figure 1.** A) Optical microscopy image of electrospun Phe-Phe fibers on glass substrates. 13.2% Phe-Phe in HFIP, 0.6 mL $h^{-1}$ feed rate, 8.4 kV, $h = 9.8$ cm (distance spinneret – ground plate). B) Scanning electron microscopy image of long fibers and short needle-like structures (typical for self-assembly). C) Fiber diameter vs. concentration. D) Diameter vs. spinning voltage (collecting plate grounded).

shown by electron microscopy (Fig. 2E and F). The smallest diameters are only several tens of nanometers.

We found that the fibers can be aligned simply by employing conductive substrates with sub-millimeter gaps (Fig. 3).[10,11] The underlying mechanism is not yet elucidated; certainly the high charge density on the fiber during spinning is decisive (note that after deposition, the fibers lose the charges to the grounded substrate). Clearly, this very direct and fast way from monomers to microscale assemblies could be employed for device fabrication.[10,11] For example, casting metal wires in the tubes by electroless deposition in aqueous solution would offer a simple bottom-up way to connect two electrodes or other objects with a conductive nanoscale path.[9,12] Moreover, alignment of nanoscale tubes can open up new fabrication methods for nanofluidic devices, especially because the walls of the tubes are easily modified by choosing related peptides.

To this end, we concentrated first on the wetting behavior. Upon placing a micrometer-sized droplet of paraffin oil more than 5 mm from the observed area, a thin oil film moved along the fibers. Water droplets exhibited no flow, but complete dewetting. This very hydrophobic nature suggests that the surface might be completely composed of phenyl moieties, which is compatible with Görbitz' model,[7,13] when one removes the outermost molecular layer of the densely packed Phe-Phe entities.

For a detailed chemical and morphological characterization, we produced self-assembled Phe-Phe fibers for comparison.[7,8,12,13] In contrast to electrospun fibers, they are straight, variable in diameters and discontinuous in length (Fig. 2B and F). Atomic force microscopy (AFM) tests suggest that the spun fibers are damaged already by nanonewton forces, while self-assembled fibers are much stronger.[14,15] For both spun and self-assembled fibers, optical microscopy images show bright sections that extend up to tens of micrometers, often associated with fiber crossings. Upon increasing the temperature, the bright areas disappear; they are also modified by paraffin oil, suggesting that solely structural defects are responsible. We confirmed this by comparing optical with electron microscopy images of identical sample areas (Fig. 4A and B). Obviously, the number of defects (roughness) is higher for the bright sections, which results in light scattering and thus produces the bright appearance. We further analyzed the fibers

  

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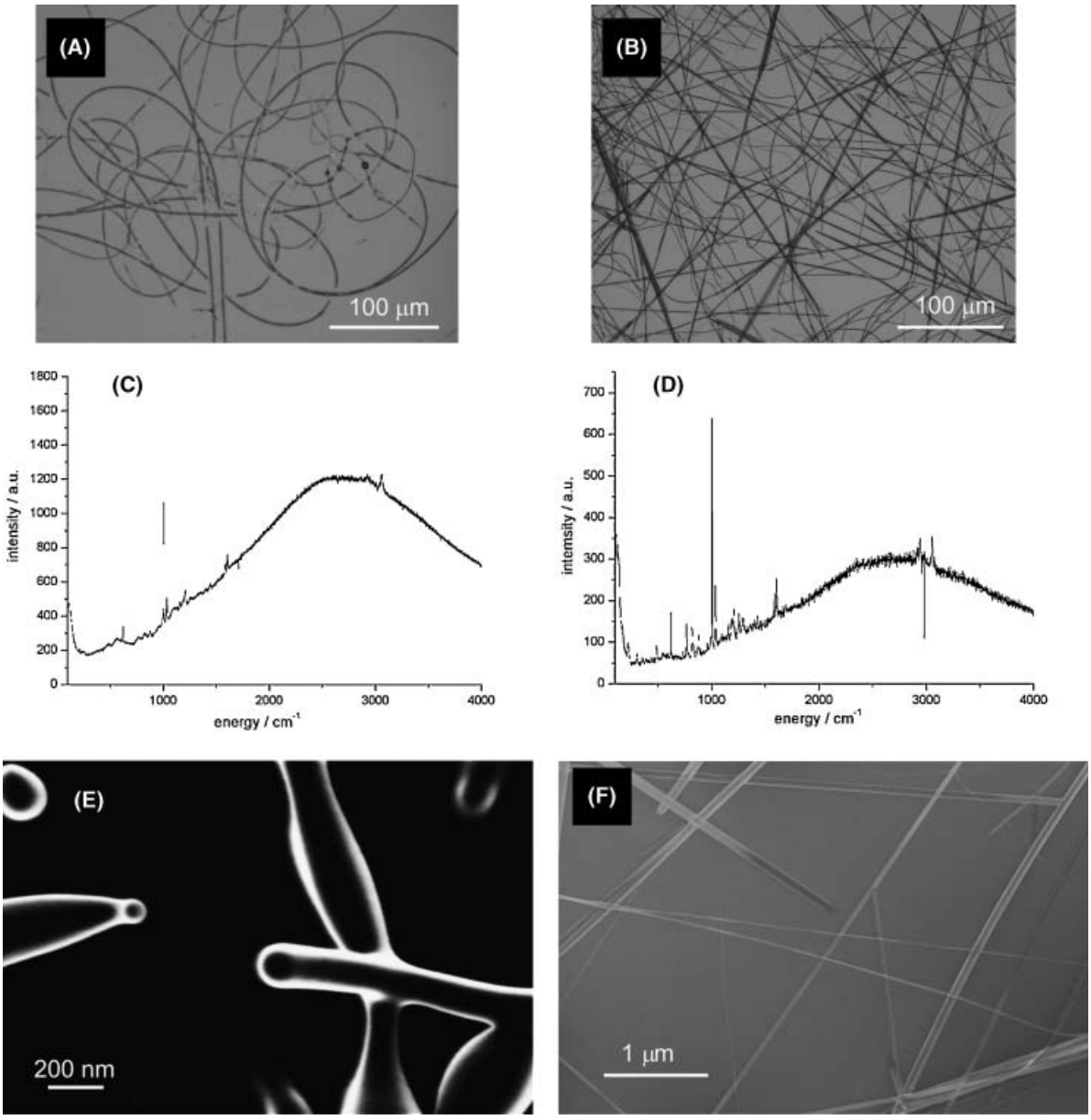


**Figure 2.** A) Electrospun Phe-Phe fibers of ''infinite'' length. 17.5% Phe-Phe, 2 mL $h^{-1}$, 13.2 kV, $h = 13.8$ cm. B) Optical microscopy image of self-assembled fibers at 0.08% (2.9 mM) concentration in HFIP/water. C) Raman spectrum of electrospun fibers. D) Raman spectrum of self-assembled fibers. E) Scanning electron microscopy (SEM) image of open ends of the tubular electrospun fibers. F) SEM image of self-assembled fibers showing a tubular structure.

with infrared spectroscopy (Supporting Information) and Raman spectroscopy (Fig. 4C). The observed vibrational bands compare well with data for bulk L-phenylalanine L-phenylalaninium perchlorate. (Supporting Information)[16] The only significant differences are the presence of $COO^-$ in the fibers (protonated in the perchlorate), and the absence of the N–H stretches, presumably owing to extensive hydrogen bonding, as suggested by Görbitz' structure model.[7] We did not find any of the easily detectable vibrations from $CF_3$, an indicator for complete evaporation of HFIP. As obvious from Figure 4C, all Raman bands appear with the same relative intensities on the various parts of the samples, so the bright areas are chemically identical. In fact, Phe-Phe tubes were recently shown to be thermally and thus chemically surprisingly stable.[17]

From our experiments we conclude that, in the case of small (bio) molecules, a highly concentrated solution is required in order to achieve entanglement or at least strong interactions between the forming assemblies, and to obtain fibers from electrospinning. Solution viscosity, solution concentration, and voltage play vital roles in the formation of fibers to control diameter and morphology. Specifically, diphenylalanine

  

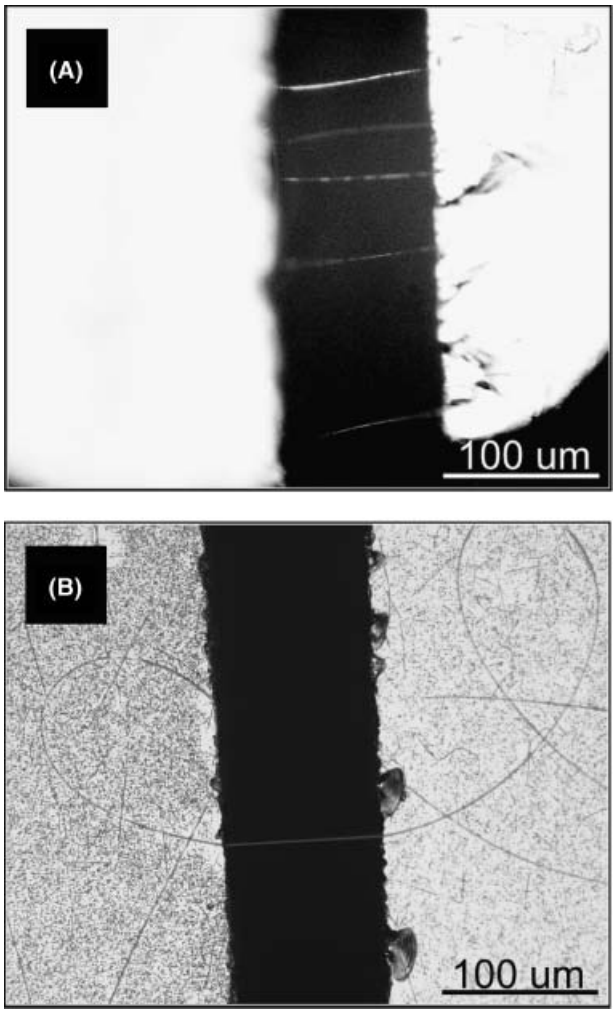


**Figure 3.** Microscopy images of Phe-Phe fibers bridging a gap between two silicon wafers (bright regions, height ca. 0.5 mm), fixed on a glass plate. 184.6 mg Phe-Phe in 620 μL HFIP, 2 mL $h^{-1}$, −13.3 kV, ground plate: +20.2 kV, $h = 15$ cm. B shows that the fibers arrange in the usual curves on top the electrodes.

(Phe-Phe) can be electrospun at high concentrations when dissolved in hexafluoroisopropyl alcohol, under conditions that do *not* produce a polymer (self-assembly of the molecules) in *absence* of the electrical potential. As for self-assembly of Phe-Phe, both micrometer- and nanometer-sized fibers are produced. They have a unique crystal structure which predicts that the surface of the fibers is completely hydrophobic (phenyl), or exposes additionally some amide groups. Our experimental results suggest complete hydrophobicity, and thus pure phenyl termination.

It should be interesting to investigate electrospinning procedures also for other peptides – we were able to spin a tripeptide with only one aromatic residue,[18] but also for other molecules that exhibit tendencies towards self-assembly. The straightforward and simple production and alignment of long tubular fibers opens up a wide range of possible applications.

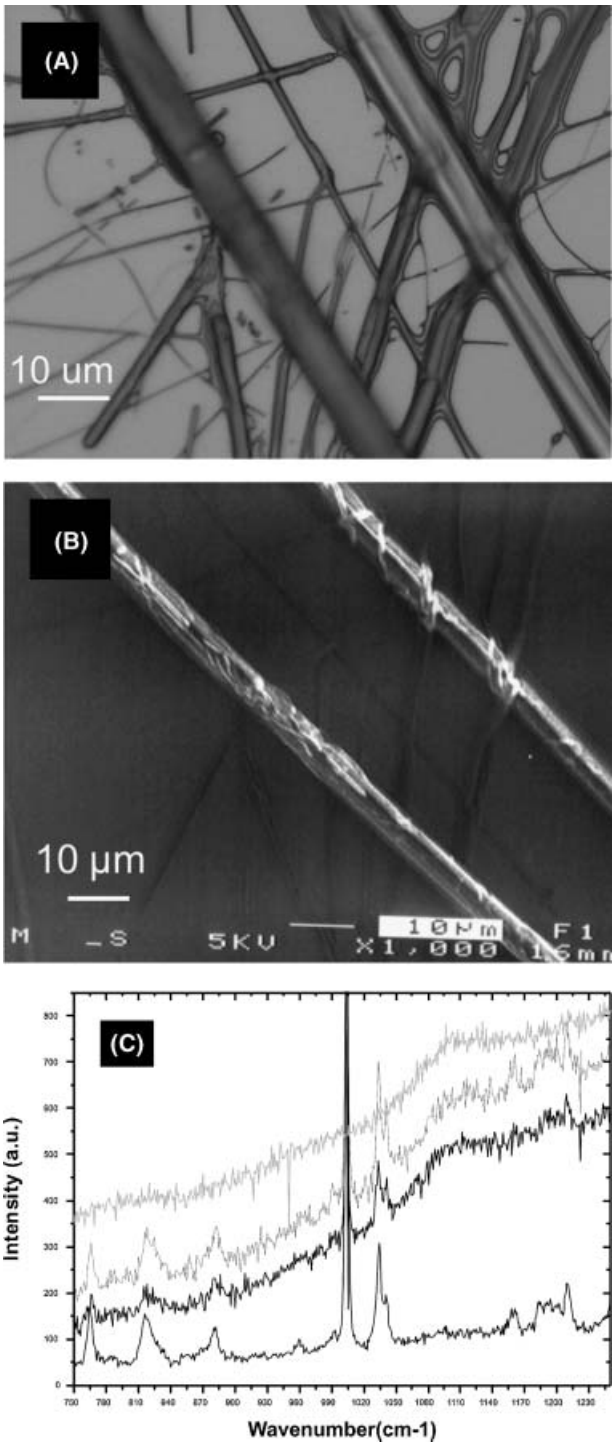


**Figure 4.** A) Self-assembled Phe-Phe on silicon wafer, optical microscopy. B) Scanning electron microscopy image of the same sample (slightly turned). C) Raman analysis of dark and bright portions of the sample; light grey trace on top: background (glass), bottom: bright sample sections, middle: dark sample sections, top: mixed (bright and dark parts).

  

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## *Experimental*

We used Applied Kilovolt modules in homebuilt power supplies to polarize the stainless steel syringe needle (inner diameters varying from 1.2 mm to 0.3 mm, length 53 mm) to ±30 kV, and to apply a negative voltage (up to 30 kV) to the collector, an aluminum plate covered by a glass plate. On the plate we placed glass substrates, or oxidized silicon (111) wafers. The needle was placed at various distances h above the collector, at the tip of a ~200 mm long polymer tube that guided the high voltage wire. The needle was connected with a thin fluoropolymer tube to a 1 mL syringe in a syringe pump (Harvard 11 plus). The experimental setup was well grounded and surrounded by transparent windows. All experiments were performed in air at ambient temperature.

We dissolved L-Phe-L-Phe (some batches donated from EMC microcollections in Tuebingen, others purchased from Sigma–Aldrich) in HFIP by ultrasonication at 60 °C for ca. 30 min. For self-assembly, we typically dissolved 10 mg L-Phe-L-Phe in 90 μl HFIP and ultrasonicated for 30 min at 25 °C. 11 mL water were added during sonication (final concentration 0.08% or 2.9 mM), resulting in formation of peptide fibers within a few minutes.

For Raman spectromicroscopy, we used a single monochromator, a He-Ne laser beam of spot size of 5–10μm and an objective with 100× magnification in a Jobin Yvon Labram system. The SEM instruments were field emission SEMs of types Hitachi S-4800 and Zeiss Gemini Ultra 55, operated at 1 to 5 keV, and JEOL JSM 6400 operated at 5 keV.

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