



Ordering of diphenylalanine micro/nanotubes prepared in PDMS microchannels with and without electric field

Giovana Bonano Carlos^a, Carla Carolina Silva Bandeira^a, André Mourão Batista^{a,b}, Benjamin Osebi Elegbe^a, Eduardo C. Lima^a, Breno Marques^a, Herculano Martinho^a,*

^a Center for Natural and Human Sciences, Federal University of ABC (UFABC), Av. dos Estados, 5001, Santo André, 09210-580, SP, Brazil

^b Nuclear and Energy Research Institute, Av. Prof. Almeida Prado, 2242, São Paulo, 05508-000, SP, Brazil

ARTICLE INFO

Keywords:

Diphenylalanine
Nanotubes
Unidirectionality
PDMS
Microchannel

ABSTRACT

Diphenylalanine is a dipeptide known for its capacity to self-assemble into various nanostructures, including nanotubes and nanowires, particularly in aqueous environments. These nanostructures possess distinctive properties that render them suitable for a variety of applications, such as biosensors and optical devices. Here we report a method of growing unidirectional diphenylalanine micro/nanotubes inside a microchannel device. Characteristic and potential diagnostic applications are discussed. Micro/nanotubes were synthesized within polydimethylsiloxane (PDMS) polymeric microchannels, both with and without the application of an electric field. Morphological analyses revealed the potential for alignment of the microstructures within the microchannels. The findings indicate that micro/nanotubes can be effectively generated in PDMS microchannels under both experimental conditions, highlighting notable morphological differences and a pronounced degree of unidirectionality of the microstructures. In spite of being reported on literature, electric field application is not guarantee of unidirectional growing requiring special care due to field inhomogeneity.

1. Introduction

L,L-diphenylalanine (FF) (Fig. 1a) is a dipeptide, a molecule composed of two amino acids, recognized for its ability to self-assemble. This property allows FF to spontaneously organize into various nanostructures, including nanotubes, nanowires, nanorods, and nanospheres, without requiring external assistance [1].

In an aqueous environment, FF can form stable nanotubes and microtubes (FFMNTs) when exposed to solvents. Furthermore, intermolecular interactions such as hydrogen bonds and interaction between aromatic side chains (π -stacking) are essential for understanding the self-aggregation phenomenon and are a relevant aggregative motif for FF conjugates with, e.g., dimers of guanine-cytosine [2]. Research on diphenylalanine nanostructures is also significant due to the multiple interesting properties that this material exhibits, including rigidity [3], optical properties evolving photoluminescence in both UV [4] and visible regions [5], temperature-dependent effects and thermal stability [6], and nonlinear optical properties [7]. The ability of nanotubes to act as waveguides is also noteworthy [8,9]. In addition, diphenylalanine nanostructures exhibit piezoelectricity [10], and other electronic properties [11], making them suitable for various applications.

Recent research has demonstrated that a device made with copper electrodes, polypropylene (PP) tape, and a polydimethylsiloxane

(PDMS, 1b) substrate was utilized for the growth of various FF nanostructures, including nanospheres, nanosheets, and nanorods, through the condensation method. The purpose of this device was to enable real-time monitoring of vital signs by leveraging the piezoelectric properties of FF nanostructures [12].

The application of self-assembled materials in biosensors, whether used in diagnostic medicine, medical therapies, or real-time monitoring via biophotonics, poses several challenges that have been examined in recent years. Several studies have been conducted to produce FFMNTs that are aligned in one direction to achieve this objective. One example of such research involves the use of surface-patterned substrates created through a UV/ozone exposure treatment, which allows for the generation of masks with varying separations. This process resulted in the growth of FFMNTs on these silicon substrates, leading to the oriented development of nanostructures [13–15]. Through a methodology that involves unidirectional evaporation-initiated growth, it is possible to obtain nanoforests formed by FFMNTs aligned vertically to the substrate, in addition to being possible to grow peptide MNTs aligned horizontally with the presence of an external magnetic field [16]. Furthermore, it was also possible to develop a methodology involving meniscus-driven self-assembly, which consists of a vertically immersed

* Corresponding author.

E-mail address: herculano.martinho@ufabc.edu.br (H. Martinho).

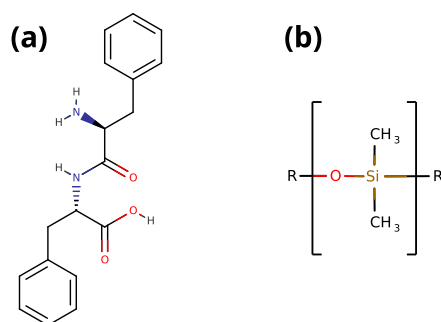


Fig. 1. Molecular structure of diphenylalanine (FF) dipeptide (a) and polydimethylsiloxane (PDMS) synthetic polymer (b).

substrate in an FF solution and its subsequent removal in a controlled amount of time [17,18]. With the presence of an external electric field, it was also possible to develop techniques for growing FFMNTs aligned vertically to the substrate and parallel to the applied electric field, factors that involve the electronic properties of FF previously discussed [19].

However, to the best of our knowledge, there is a lack in reports considering devices and substrates that allow the unidirectional growth of FFMNTs in closed microchannel substrates with methodologies that involve the presence of organic solvent acetic acid instead of using aggressive solvents such as HFP and that do not involve the use of high temperatures. A similar process could improve the development of low-cost technologies and an easy methodology for the construction of devices with FFMNTs aligned in closed microchannels for the most diverse applications.

The present investigation focuses on a novel methodology that evolves the synthesis and characterization of FFMNTs within PDMS microchannels subjected to an electric field for unidirectionality induction. The microstructures developed in the PDMS microchannels, along with various modifications, will be examined to explore the relationship between the optical and electronic properties of the material, its morphology, and chemical composition of the FF. This research aims to contribute to enhancing control over microstructure properties in order to optimize FFMNT applicability.

2. Materials and methods

2.1. Microchannel device

Extran MA 02 Neutral from Merck (Germany) and isopropyl alcohol from Dinâmica (Brazil) were used to clean the substrates. To prepare the PDMS samples, the Avipol two-component kit was used: Silicone 8000/3.5 M and Catalyst 73 (Pholiplac). The 3D printed matrices were composed of a polymer, in addition to the use of metal rods of diameters of 0.5 mm, 0.6 mm and 0.7 mm. These diameters are typical ones in micropipettes or similar tools used to manipulate biofluids, and for this reason were chosen here.

Initially, 3D printed matrices were created using an Anycubic Photon S 3D printer. These matrices were then used to prepare PDMS substrates, as shown in Fig. 2 (a), left side. For each sample, metal rods of different sizes (approximately 0.5, 0.6, and 0.7 mm) were inserted through the holes in the walls of the matrices to obtain the desired shape.

The Pholipac Kit mixture was prepared, consisting of Silicone 8000/3.5 M and Catalyst 73 with a ratio of 5 : 1, respectively. The mixture was homogenized and poured into the printed matrices with the presence of metal rods passing through the holes. Thus, the PDMS was left to dry at a temperature of 20°C and controlled humidity along 24 h. PDMS substrates were obtained with microchannels with diameters

$d = 0.5, 0.6$, and 0.7 mm (Fig. 2a, right side). The diameters relative to the metal rods are approximately larger, since the elastomeric behavior of the PDMS led to a decrease in this diameter after the removal of the metal rods. PDMS substrates were cleaned sequentially along 15 min in diluted Extran solution, isopropyl alcohol, and deionized water. To prepare samples under electric field, a voltage to $V = 1$ kV was applied to parallel plates perpendicular to the microchannel (Fig. 2b). The voltage remained stable during all experiments.

2.2. FF solution

The physical and morphological properties of FFMNTs are affected by the concentration and composition of solvents in their self-assembly process. Furthermore, the evaporation rate is also crucial [20]. Understanding these factors is crucial for understanding the mechanisms of peptide self-assembly in specific environments and for analyzing their physical characteristics. For example, the diameter of the microtubes may vary depending on the assembly methodology used, or the ordered growth of the nanostructures might follow a unidirectional pattern. Furthermore, the choice of solvent has a significant impact on the kinetics of nanostructure formation [21]. Although researchers have explored the use of various solvents to produce FFMNTs with different morphologies and properties, few studies have investigated the potential of acetic acid as an alternative solvent. Interestingly, FF has a higher solubility in acetic acid (approximately 430 gL^{-1}) compared to solubility in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) of 240 gL^{-1} , which makes it a promising option for producing FFMNTs [22]. In work published elsewhere [21], we investigated FF formation at three different concentrations, in addition to kinetic monitoring for FF formation with acetic acid, using time-resolved Raman spectroscopy. These results showed important information about the aggregation process that follows the auto-catalytic model.

Solutions were prepared using L-Phenylalanyl-L-phenylalanine from Alfa Aesar Thermofisher (Massachusetts, USA) and glacial acetic acid (HAc) from Merck (Darmstadt, Germany). To prepare the FFMNTs samples in PDMS microchannels, a stock solution of FF in acetic acid at a concentration of 150 gL^{-1} of FF was initially prepared. This solution was then homogenized in an ultrasonic bath along 15 min. After that, an aliquot of $1 \mu\text{L}$ of the stock solution was inserted into a container with $60 \mu\text{L}$ of ultrapure water at a final concentration of 2.5 gL^{-1} of FF. The final solution obtained was then immediately inserted into the PDMS substrate microchannel with the aid of a micropipette in order to fill the entire length of the microchannel (Fig. 2c). In order to avoid short-circuiting the system and to allow the presence of an electric field in the sample region, the microchannel was not completely filled with the final solution for samples that were prepared in the setup with a uniform electric field. After the solution was pipetted into the microchannel, samples were immediately inserted into the electric field.

2.3. Optical microscopy

A first morphological inspection of the FF samples contained in microchannels for all parameters was performed using bright field optical microscopy using Axio Scope A1 microscope from Carl Zeiss. For this purpose, a polarizing filter was used to allow a more detailed observation of the FF microstructures in relation to the substrate and the surrounding environment due to birefringence of FF [23].

For the analysis of the optical microscopy images, the *ImageJ* software using *OrientationJ* plugin was used to obtain the degree of orientation of the FFMNTs in the microchannels for each sample analyzed, in addition to being able to estimate its coherence. This analysis is based on structure tensor which computes the orientation and isotropy properties in a local window [24]. The gradient of structure tensor was computed using cubic spline in a window of 20 pixels. The histogram of orientation degree was analyzed and fitted to Gaussian distribution. The coherence, C was estimated comparing the width of Gaussian distribution, σ , to central value of histogram, θ_c : $C = \frac{1}{1 + \sigma/\theta_c^2}$.

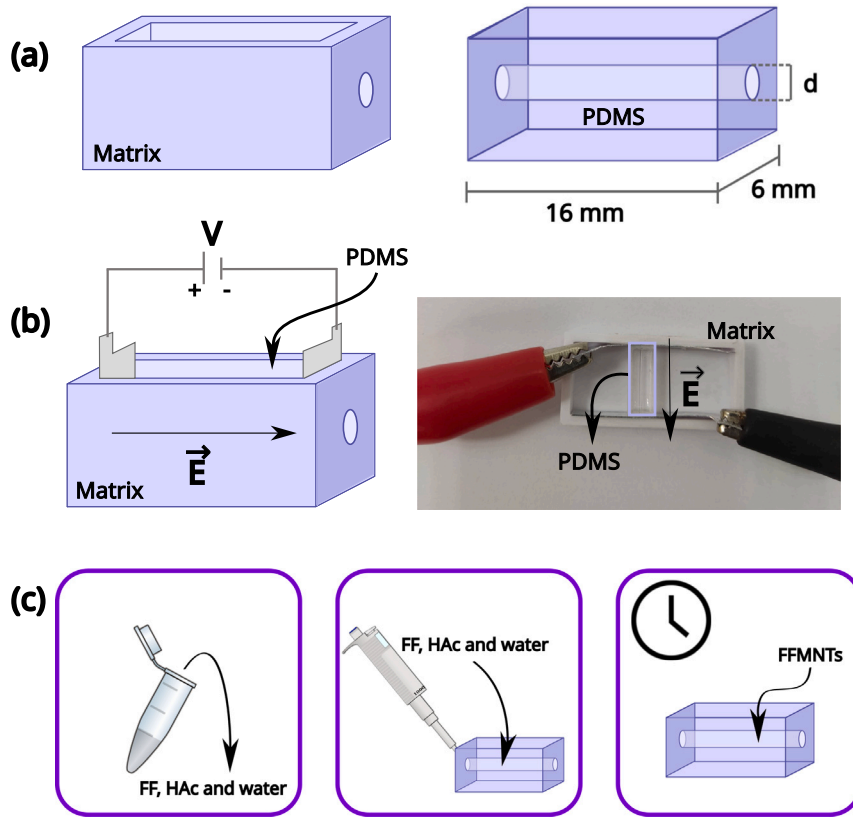


Fig. 2. (a) PDMS matrix prepared by 3D printing (left) and PDMS substrates with diameters $d = 0.5, 0.6$, and 0.7 mm (right). (b) PDMS substrate setup representation (left) including electrodes to apply voltage V which generates an electric field $\vec{E}$ along microchannel as shown in photo (right) (c). Schematization of the FFMNTs preparation methodology on the PDMS substrate.

2.4. Scanning electron microscopy

To perform a detailed morphological analysis of the samples, the JSM-6010LA Compact Scanning Electron Microscope from JEOL was employed. To ensure reliable results, the samples were coated with a layer of gold. Samples analysis was performed longitudinally to the microchannel.

2.5. Absorption spectroscopy

The UV-Visible absorption spectroscopy analysis were performed using the UV-visible spectrophotometer (mono-beam) equipment UV-M51 from Bel Engineering.

2.6. Fluorescence spectroscopy

Photoluminescence analyses were conducted using the Cary Eclipse Fluorescence Spectrophotometer (Varian) equipment. To analyze FFMNTs samples, the sample holder accessory for solid samples was utilized. The equipment was used to conduct photoluminescence analysis in individual spectra of FFMNTs grown on plane substrate on a similar way as done for wave-guiding experiments. To ensure accurate results, the PDMS substrate was also analyzed without the presence of FFMNTs samples inside the microchannel to account for any absorption bands of the substrate material that could interfere with the results.

2.7. Wave-guiding experiments

One microtube of FF about 1 mm long was placed on top of a photonic integrated circuit to use an already mounted setup from other experiments. The microtube was illuminated with a tunable

diode laser (TOPTICA/DL PRO) centered at 795 nm. The coupling is done with lensed fibers. The image is captured by a CCD camera (Thorlabs/CS165MU).

2.8. Raman spectroscopy

For spectral acquisition, the Horiba Jobin-Yvon T64000 triple Raman spectrometer was used. The excitation laser used was the Verdi G5 Laser (Coherent Inc.) operating at 532 nm. Measurements were performed using a 100 $\times$ objective and 1 mW power on the sample at room temperature.

2.9. Simulation of electric field for a parallel plate capacitor

In order to discuss the FF growing process under influence of electric field, we consider a simulation of electric dipole dynamics inside a real capacitor using the real set of parameters. FF subunits present a noticeable electric dipole being the dipolar interaction the driving force of micro/nanotubes self-assembling [25]. The electric field in the system was computed using numerical solutions of Laplace's equation for a parallel plate capacitor geometry, where one plate is at $z = 0$ (grounded) and the other at $z = L$ (charged to a voltage difference V_0):

$$\nabla \cdot (\epsilon \nabla \Phi) = 0 \quad (1)$$

where Φ is the electrostatic potential, ϵ is the permittivity (with a constant value of $\epsilon = 3.0$ for the PDMS material), and the equation is solved for a discretized 3D grid. The boundary conditions were defined as:

$$\begin{cases} \Phi(x, y, z = 0) = 0 \\ \Phi(x, y, z = L) = V_0 \end{cases} \quad (2)$$

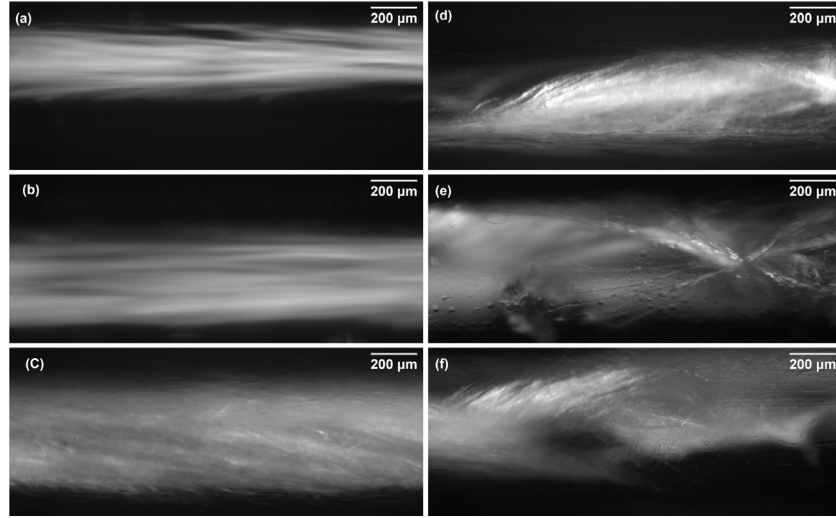


Fig. 3. Bright-field optical microscopy images of FFMNTs on PDMS microchannel in the absence (a–c) and under presence (d–f) of the electric field for microchannel with diameters $d = 0.5$ (a, d), 0.6 (b, e), and 0.7 (c, f) mm.

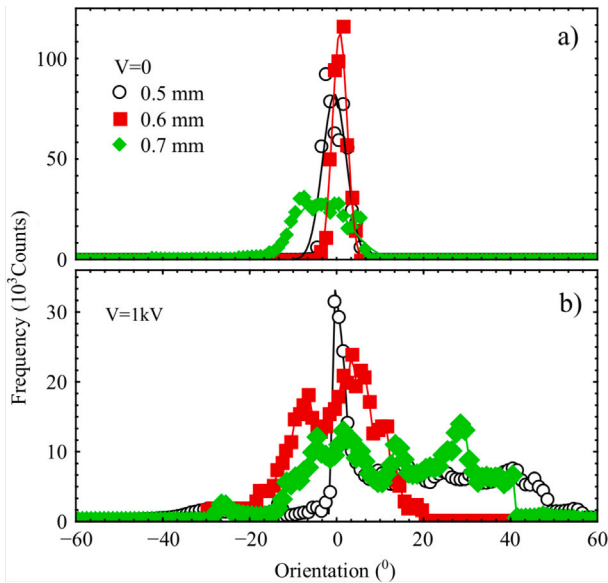


Fig. 4. Orientation distribution of microtubes in PMDS channels grew at zero voltage (a) and at $V = 1$ kV (b) for microchannel diameters of 0.5 (open circles), 0.6 (red solid square), and 0.7 (green solid diamond) mm. Solid lines in (a) and (b) are data fitting to Gaussian model and guide to the eyes curves, respectively.

Using the *Jacobi iteration method* (Python code implementation from [26]), the potential field $\Phi(x, y, z)$ was solved on a grid of dimensions $60 \times 60 \times 200$ points in the x , y , and z directions, respectively. The solution converged when the maximum difference between successive iterations fell below $\times 10^{-5}$.

The electric field components $\mathbf{E} = (E_x, E_y, E_z)$ were computed using the finite difference method:

$$\begin{cases} E_x = -\frac{\Phi(x+dx, y, z) - \Phi(x-dx, y, z)}{2dx} \\ E_y = -\frac{\Phi(x, y+dy, z) - \Phi(x, y-dy, z)}{2dy} \\ E_z = -\frac{\Phi(x, y, z+dz) - \Phi(x, y, z-dz)}{2dz} \end{cases} \quad (3)$$

Field lines were visualized by solving for the trajectory of test particles within the electric field. The direction of movement at each point was determined by the local electric field vector, which is used

to compute the velocity of the particles as they move along the field:

$$\mathbf{v} = \frac{\mathbf{E}}{|\mathbf{E}|} \cdot v_{\max} \quad (4)$$

where $v_{\max}$ is a scaling factor for velocity. These field lines were drawn by integrating the equation of motion for each particle using a *Runge–Kutta method* to provide smooth paths. The resulting plot shows the streamlines of the electric field within the region of interest, highlighting the spatial configuration of the electric field between the plates. This was achieved using *matplotlib's streamplot* function [27].

To simulate the motion of dipoles, we employed a numerical integration of the trajectory equation, where each dipole moves under the influence of the local electric field. The dipole motion was modeled using the following equation of motion:

$$\mathbf{r}(t + dt) = \mathbf{r}(t) + \frac{\mathbf{E}(\mathbf{r})}{|\mathbf{E}|} \cdot v_{\max} \cdot dt \quad (5)$$

where $\mathbf{r}(t)$ is the position of the dipole at time t , and $v_{\max}$ is the maximum drift velocity of the dipole. This equation was discretized using a first-order Euler method, with time steps of $dt = 0.005$ mm. The initial positions of the dipoles were randomly generated on the top surface of the cylinder, with each dipole constrained to lie within the circular region defined by the cylinder radius $R = 3.0$ mm. The initial orientations of the dipoles were chosen randomly from a unit sphere. The resulting dipole trajectories were computed over a total of 6000 time steps. The trajectories were constrained to stay within the cylindrical boundary by projecting the positions back onto the surface of the cylinder whenever they moved beyond its boundaries. The final output includes a 3D plot showing the electric field lines in the region between the plates and the trajectories of the dipoles. The animation visualizes the dynamical motion of the 100 dipoles, with each dipole's movement along its calculated trajectory, constrained within the cylinder.

3. Results and discussion

Inspection using optical microscopy were carried out on samples of FFMNTs grown in microchannels with (Figs. 3a–c) and without (Figs. 3d–f) the presence of an electric field. It is possible to observe bunches of microtubes around the entire microchannel region, regardless of its diameter. Due to birefringent nature of the FFMNTs, they appear bright under polarized light in opposition to a possible amorphous phase. Furthermore, we observed that all micro/nanotubes appeared oriented longitudinally to the PDMS microchannel at zero

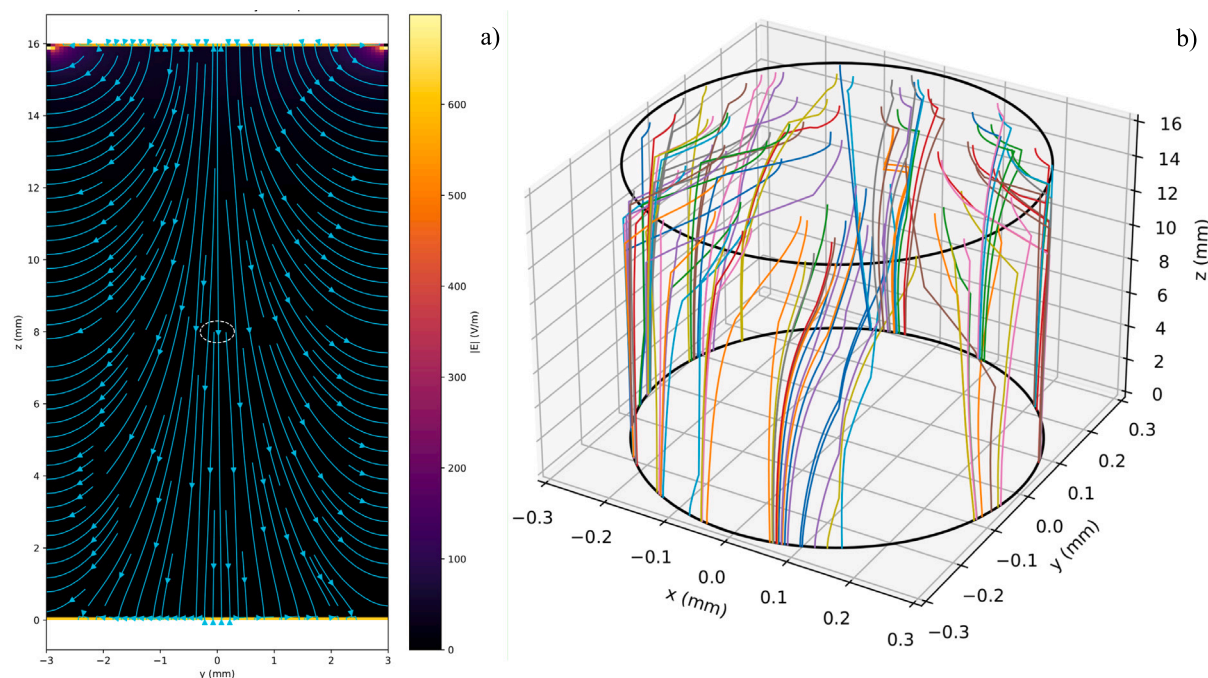


Fig. 5. (a) Electric field lines in the y – z plane for voltage of 1 kV applied across capacitor. Circled region at center represents the PDMS channel (b) Trajectories for 100 dipoles placed randomly over top ($z = 16$ mm) of capacitor.

field, which can be justified due to their heterogeneous nucleation from the surface of the PDMS substrate walls. Under electric field presence microtubes are still present but a helical orientation along axis.

The orientation analysis of the structures in the microchannels furnished quantitative estimates about degree of orientation. The obtained results are summarized in Fig. 4. For zero field (Fig. 4a) it clear that for microchannel with 0.5 and 0.6 mm almost all microtubes are oriented along microchannel direction. The average orientation angle were $\theta_c = 0.5 \pm 3^\circ$ and $0.7 \pm 3^\circ$ for $d = 0.5$ and 0.6 mm, respectively. Coherence were $C = 90\%$ and 80% for 0.5 and 0.6 mm cases. For microchannel with $d = 0.7$ mm it was observed a larger dispersion in orientation degree. In this case $\theta_c = 4 \pm 13^\circ$ and $C = 70\%$. From this finding it is clear that the diameter of microchannel is a critical parameter for homogeneity of degree of alignment of formed microtubes. On the other hand presence of electric field contributed to increase the dispersion of orientation of microtubes (Fig. 4b). Histograms presented at least 3 preferential orientation directions at well defined angles. Our simulations (Fig. 5) of electric dipole dynamics indicated that dipoles trajectories are strongly dependent of initial positions of dipoles on top since there are an appreciable field inhomogeneity along PDMS channel.

The relative differences when FFMNTs are grown in microchannels with the presence of an electric field, as can be seen in Figs. 3d, 3e and 3(f). Unlike the samples grown without the presence of an electric field, these show growth beyond the longitudinal direction of the microchannel, and it is possible to state that the presence of the electric field resulted in multi-unidirectional growth. This fact was corroborated by the data obtained for the degree of orientation in relation to the dominant direction.

SEM images obtained from samples of FFMNTs grown in PDMS microchannels without the presence of an electric field show that the grown FFMNTs seem to have detached from the walls of the microchannel, as shown in Fig. 6a. It is important to note the detailed analysis provided by Figs. 6b and 6c. The figures clearly illustrates the unidirectional alignment of FFMNTs, forming a substantial mass of microtubes. These microtubes are aligned towards the microchannel, indicating a distinct alignment compared to FFMNTs grown on flat substrates under similar conditions. The images from samples grown under electric field (Fig. 6d) been a detachment of the mass of FFMNTs

from the walls of the microchannel during the preparation of the sample for analysis. Despite some similarities in the unidirectionality of the FFMNTs towards the microchannel, there are clear differences between the samples. Sample of FFMNTs grown with the presence of an electric field exhibits multi-directionality compared to the others, showing a somewhat helical growth as observed in Fig. 6(d). These observations indicated that growing sees to be influenced by the presence of an electric field, indicating the distinct properties of FFMNTs when they are subjected to an electric field. This alignment as discussed in the literature previously [19]. More SEM images are available in the Supplementary Material in Figure S1.

There are a sort of reports respect growing of FFMNTs in microchannels in a unidirectionally aligned manner using various methodologies. For instance, one investigation involves the growth of vertically aligned FFMNTs on a substrate via the vapor deposition method [28]. However, the cycle-FF microstructure obtained in this investigation differs from the linear FF ones in other methodologies not involving phase change at high temperatures, presenting distinct properties. Another methodology, the meniscus-driven process [29], also produces satisfactory results regarding the alignment of FFMNTs but does not involve the use of microchannels. On the other hand, a investigation uses microchannels formed by PDMS to obtain FFMNTs [30]. This investigation results in FFMNTs grown in a dendritic manner within the microchannel, a unique outcome, but it does not exhibit unidirectional growth and presents a microchannel open to the environment. Thus, while various studies demonstrate results related to the unidirectionality of these nanostructures and their growth in microchannels, there are a lack of reported methodologies involving the growth of these nanostructures in a closed microchannel with unidirectionality.

Thus, it is possible to state that, in the case of samples that present superior unidirectionality and alignment, the FFMNTs sample grown in PDMS microchannels without the presence of the electric field presented more satisfactory results. However, both results obtained demonstrate that it is possible to obtain microstructures through the novel methodology developed, which differs from previously studied methodologies, given that it involves the use of closed microchannels, unidirectionality with sufficiently long FFMNTs, and unidirectional growth along the longitudinal axis of the microchannel simultaneously.

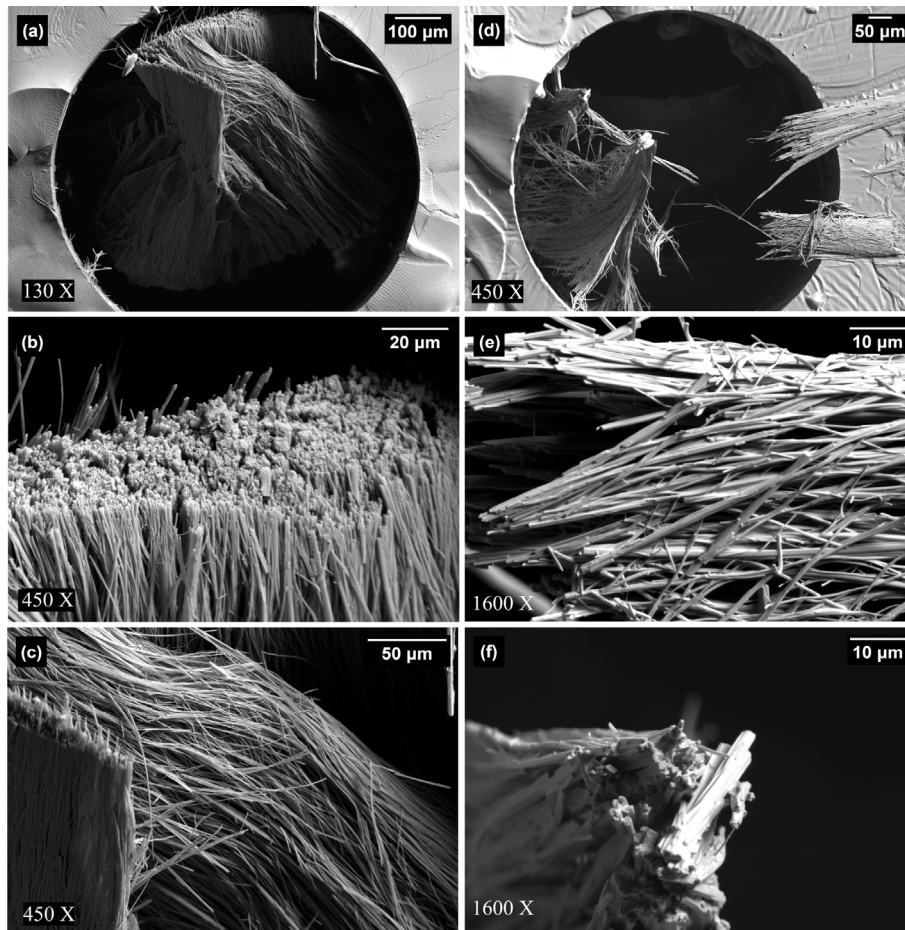


Fig. 6. SEM images of FFMNTs on PDMS microchannel with diameters $d = 0.5$ (a, d), 0.6 (b, e), and 0.7 (c, f) mm in the absence (a, b, c) and in the presence (d, e, f) of electric field. (a) Details of the substrate microchannel as a whole and (b, c) unidirectionality grown FFMNTs inside the microchannel without presence of electric field. (d) Details of the microchannel and FFMNTs (e) inside and (f) outside de microchannel in the presence of electric field with details.

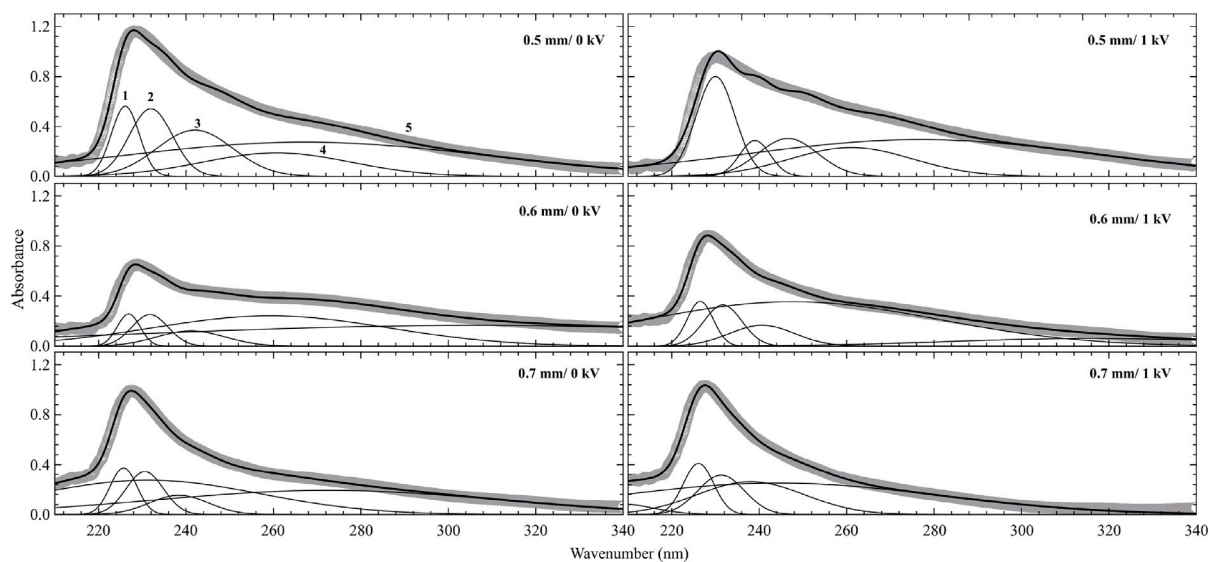


Fig. 7. Absorption spectra of FFMNTs samples in PDMS microchannels with varying diameters and with or without the presence of an electric field.

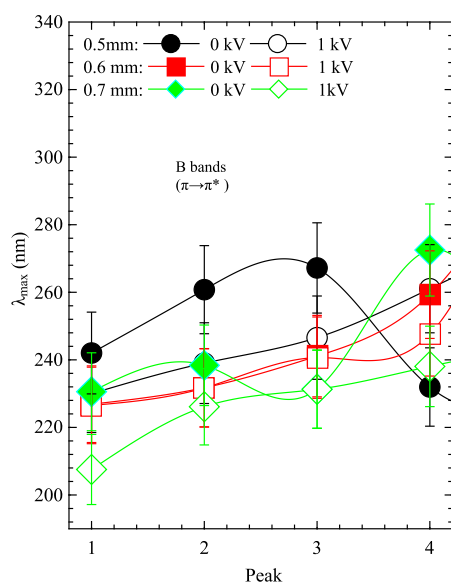


Fig. 8. Center position of de-convoluted spectra of UV-Vis of FF shown in Fig. 7.

This suggests that the methodology used has potential for further exploration and could lead to significant advancements in the field of nanotechnology.

UV-Vis absorption spectroscopy analyses of FFMNTs in PDMS were performed and corresponding results shown in Fig. 7. The samples were assessed for light absorption in the wavelength range of 200 to 700 nm. Spectra were composed by convolution of 5 peaks whose maxima are shown on Fig. 8. The broad band at higher wavenumber (240–320 nm) is a residual band from PDMS [31]. Remaining bands are related to $\pi \rightarrow \pi^*$ transition in phenyl rings (Bbands). Usually they are strong, sharp and used to monitor aromatic character or self-assembly. The red-shift observed in lower diameters zero-field case is probably related to $\pi - \pi$ stacking and excitonic coupling. The multiple B bands observed is probably related to vibronic coupling or Davydov splitting (exciton interactions in ordered arrays). Shaper bands (peaks 1–2) are related to ordered FF while the broad. The broad bands resolved in spectra as peak 3–4 are related to some degree of disorder, solvent interactions, and lack of $\pi - \pi$ stacking. The amount of disordered phase was estimated computing area of broad peaks compared overall area. The proportion of disordered phase was 18% for samples with $d = 0.5$ mm being $\sim 8\%$ for others samples. We interpret this fact as related to reduced water evaporation rate in the smaller diameter microchannel which slows the conversion of FF to FFMNTs. However it is important mention that ordered phase predominates in all samples.

According to studies [32], diphenylalanine nanostructures absorb light in the middle-UV range, between 200 and 300 nm. Consequently, it can be concluded that FFMNTs grown in PDMS microchannels exhibit optical properties similar to those of FFMNTs grown on flat substrates or in solution. While the FFMNTs on microchannel substrate showed values within the 200 nm to 300 nm range, there was no noticeable variation in the location of the absorption peak for samples with different microchannel diameters and when an electric field was present, however, it was possible to observe a considerable variation in the intensity of the absorption peak of the samples. This factor can be explained by analyses in regions with higher optical densities, justified by the heterogeneity of material inside the microchannels.

From analyses performed with a fluorimeter on FFMNT samples on flat substrates, it was possible to obtain the spectrum shown in Fig. 2 in Supplementary Material. The sample was excited at a wavelength of 255 nm with excitation and emission slit of 2.5 nm. The results

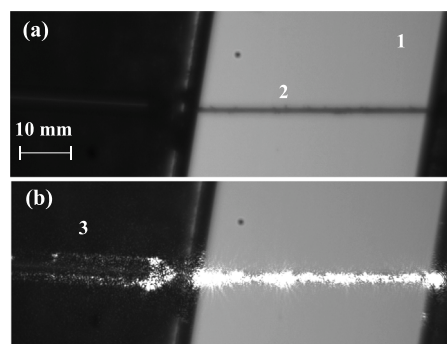


Fig. 9. (a) Silicon substrate (1) showing an isolated FFMNT (2) near to a lasing optical fiber tip (3). (b) Laser passing through the FFMNT which is acting as a waveguide.

demonstrate the existence of a band around 300 nm of emission in the UV region, which strongly corroborates data already consolidated in the literature [4].

The wave-guiding experiment is relevant to reveal the ordering nature of FFMNT. Fig. 9(a) shows a single microtube of FF over a photonic chip. The acquired image on Fig. 9(b) shows a bright, linear intensity profile consistent with laser light coupled into the FFMNT. This type of experiment is often conducted to study light-matter interactions in self-assembled peptide nanostructures for potential photonic or biosensing applications. There light coupling and propagation through FFMNTs where light enters the peptide structure and continues propagating along its length. This visible transmission of light through the microtube indicates successful optical coupling at the interface between the laser and the FF structure. This behavior confirms our grew FFMNT acts as optical waveguides. Their crystalline molecular arrangement and high aspect ratio allow confinement of light within the structure through internal reflection mechanisms [33]. The even brightness along the axis of the microtube suggests that the structure has high optical transparency at the wavelength of the laser, indicating low scattering and absorption losses. This is essential for waveguide efficiency, particularly in biosensing and integrated photonic circuits. The internal molecular packing of FFMNTs which is typically hexagonal or orthorhombic supports such propagation by minimizing structural defects that lead to scattering [34]. The image reveals periodic intensity variations where there are bright spots or bands along the tube. This suggest the presence of interference patterns or resonant optical modes. This behavior is characteristic of Whispering-Gallery Modes (WGMs) or Fabry-Pérot resonances, both of which occur when light reflects repeatedly within a confined structure. In cylindrical structures like FFMNTs, WGMs arise from total internal reflection of light at the curved inner wall. These resonances can store light temporarily, enhancing light-matter interactions [35]. These periodic patterns observed in the image imply the FF microtube can act as a biocompatible optical resonator, capable of detecting minute environmental changes (e.g., refractive index shifts caused by molecule binding), making it valuable for label-free biosensing. In addition to regular intensity variations, there may be localized scattering centers visible as bright or diffuse spots in the image. These arise from structural irregularities, molecular defects, or surface roughness at the FF-silicon interface. While scattering typically denotes energy loss, in optical systems it can also reveal mode coupling or localized photonic states [33].

Fig. 10 shows the zero to one normalized Raman spectra for FFMNT samples under PDMS microchannel. The laser was focused on the longitudinal axis of the microchannel, directly targeting the FFMNTs. This set of data is important the confirm crystallinity of samples and probe heterogeneity inside microchannels. Additionally, the existence of FFMNTs can be confirmed through their distinctive fingerprint spectra.

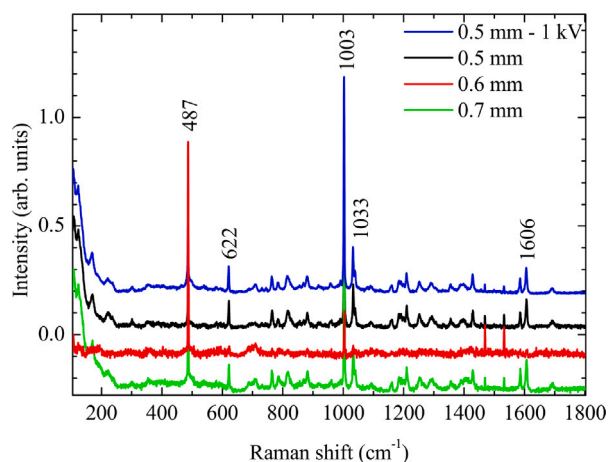


Fig. 10. Normalized Raman spectra of FFMNT samples on PDMS microchannels with diameters of 0.5 (black), 0.6 (red), and 0.7 (green) mm. Addition spectrum for sample on $d = 0.5$ mm microchannel under electric field (blue) is also shown. Spectra were vertically translated for comparison. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Spectra revealed the presence of distinct characteristic bands of FFMNT at [21,36]: (i) 486 cm^{-1} , related to the twisting of the phenylalanine rings of NTs; (ii) 621 cm^{-1} , which corresponds to the twisting of the C–C bond of NTs; (iii) 1002 cm^{-1} , related to the breathing of phenylalanine in NTs; (iv) 1032 cm^{-1} out-of-plane bending of the C–H bond of phenylalanine and the C–H bond in the bending plane of phenylalanine; (v) 1606 cm^{-1} band, which corresponds to Amide I and breathing of phenylalanine in NTs. Thus the majority of the vibrational bands observed in the FFMNTs samples grown on a PDMS microchannel substrate are correctly assigned to FFMNT bands previously reported. However, notable differences exist between the FFMNTs samples on PDMS substrate in terms of the presence and intensity of these bands. Specifically, for the FFMNTs samples on PDMS substrate with microchannels of 0.5 mm and 0.7 mm without the presence of an electric field, as well as the 0.5 mm sample with the presence of an electric field, it can be observed that all the previously mentioned bands and other characteristic bands are maintained, albeit with varying intensities. In contrast, for the FFMNTs sample in a PDMS microchannel with a diameter of 0.6 mm, the vibrational mode bands at 486 cm^{-1} and 1002 cm^{-1} appear with significantly lower intensities, and some characteristic bands are not apparent. We observed that the amount of microtubes for this sample is smaller since the intensity of UV–Vis spectra is significantly reduced in this case (Fig. 7) which explain the observed features for $d = 0.6$ mm sample.

4. Conclusions

As can be discussed, the investigation of self-assembled nanostructures is a field of importance in the development of new materials, nanomaterials and devices for various fields. FF is an example of a dipeptide with potential for application in diverse areas, a factor justified by the presence of properties attributed to both nanostructured and self-assembled materials, in addition to the unique characteristics of this dipeptide.

The development of new methodologies was crucial for growing FFMNTs in PDMS microchannels with and without the presence of an electric field. The influence of parameters such as the presence of an electric field and dimensions of microchannel were observed.

We showed that it is possible to grow FFMNTs with high degree of micro/nanotubes ordering along microchannels in the presence and in the absence of an electric field. For FFMNTs grown without electric

field, it was possible to confirm the ordering along microchannels in unidirectional form. On the other hand, we observed that electric field induced greater microtubes dispersion and helical distortion along the channel, showing that the presence of the electric field is essential for helical ordering of FFMNTs on microchannels. We showed that the helical arrangement observed is derived from field inhomogeneity inside the PDMS channel. This helical arrangement would be useful for applications requiring, e.g., large surface area of fluid permeation. On the other hand we argue that electric field geometry could be improved for unidirectional growth. Another promising approach would also employ electromagnetic lenses to produce well defined field lines geometries.

The combination of FF micro/nanostructures with PDMS microchips offers a vast field for innovative scientific research, exploiting the unique properties of both materials. Promising areas include the development of biosensors for disease diagnosis, tissue engineering and regenerative medicine, nanomaterial fabrication, and fundamental studies on peptide self-assembly and biomolecular interactions. The biocompatibility of PDMS and the self-assembly capacity of diphenylalanine are crucial features for these applications, enabling the creation of complex and multifunctional systems. The presence of a closed microchannel is essential for applications that require low or absence of contact with samples, avoiding contamination. Another important parameter is the optical path length (16 mm), that is around 80 times higher for the device developed on this work compared to a biofilm formed on a plane substrate (~ 200 microns), considering longitudinal analysis through the microchannel. Since Raman power signal is proportional to sample volume scattering our device is able to increase Raman signal itself by 80 factor. This process is relevant enabling *in situ* biosensing as, e.g., application as SERS substrates with advantage of minimum external manipulation of potential contaminant samples. However additional work need be devoted to develop and test this set of applications for PDMS microchannels developed here.

CRediT authorship contribution statement

Giovana Bonano Carlos: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Carla Carolina Silva Bandeira:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Data curation, Conceptualization. **André Mourão Batista:** Writing – review & editing, Visualization, Validation, Methodology, Formal analysis, Conceptualization. **Herculano Martinho:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank the Brazilian agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico and Institutos Nacionais de Ciência e Tecnologia INTERAS (311933/2021-1)/(406761/2022-1), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior on PROEX (88887.818251/2023-00) for the financial support. The authors also thank Multiuser Central Facilities at UFABC (CEM/UFABC) and their technicians for the experimental support.

Appendix A. Supplementary data

Supplementary material related to this article can be found online at <https://doi.org/10.1016/j.mseb.2026.119285>.

Data availability

Data will be made available on request.

References

- [1] Carl Henrik Görbitz, Nanotube formation by hydrophobic dipeptides, *Chem. Eur. J.* 7 (23) (2001).
- [2] Carlo Diaferia, Concetta Avitabile, Marilisa Leone, Enrico Gallo, Michele Saviano, Antonella Accardo, Alessandra Romanelli, Diphenylalanine motif drives self-assembling in hybrid PNA-peptide conjugates, *Chem. Eur. J.* 27 (57) (2021) 14307–14316.
- [3] Pavel Zelenovskiy, Igor Kornev, Semen Vasilev, Andrei Kholkin, On the origin of the great rigidity of self-assembled diphenylalanine nanotubes, *Phys. Chem. Chem. Phys.* 18 (43) (2016) 29681–29685.
- [4] Zhixing Gan, Hao Xu, Photoluminescence of diphenylalanine peptide nano/microstructures: From mechanisms to applications, *Macromol. Rapid Comm.* 38 (22) (2017).
- [5] T. Nikitin, S. Kopyl, V. Ya Shur, Y.V. Kopelevich, A.L. Kholkin, Low-temperature photoluminescence in self-assembled diphenylalanine microtubes, *Phys. Lett. A* 380 (18–19) (2016).
- [6] Lihi Adler-Abramovich, Meital Rechtes, Victoria L. Sedman, Stephanie Allen, Saul J.B. Tendler, Ehud Gazit, Thermal and chemical stability of diphenylalanine peptide nanotubes: implications for nanotechnological applications, *Langmuir* 22 (3) (2006).
- [7] Amir Handelman, Boris Apter, Nir Turko, Gil Rosenman, Linear and nonlinear optical waveguiding in bio-inspired peptide nanotubes, *Acta Biomater.* 30 (2016) 72–77.
- [8] Becky Bank-Srouer, Peter Becker, Leonid Krasovitsky, Alexander Gladkikh, Yuri Rosenberg, Zahava Barkay, Gil Rosenman, Physical vapor deposition of peptide nanostructures, *Polym. J.* 45 (5) (2013).
- [9] Xuehai Yan, Ying Su, Junbai Li, Johannes Früh, Helmuth Möhwald, Uniaxially oriented peptide crystals for active optical waveguiding, *Angew. Chem. Int. Ed.* 50 (47) (2011) 11186–11191.
- [10] Konstantin Romanyuk, Vladislav Slabov, Denis Alikin, Pavel Zelenovskiy, Maria Rosario P. Correia, Kirill Keller, Rute A.S. Ferreira, Semen Vasilev, Svetlana Kopyl, Andrei Kholkin, Piezoactive dense diphenylalanine thin films via solid-phase crystallization, *Appl. Mater. Today* 26 (2022).
- [11] Brahim Akdim, Ruth Pachter, Rajesh R. Naik, Self-assembled peptide nanotubes as electronic materials: An evaluation from first-principles calculations, *Appl. Phys. Lett.* 106 (18) (2015) 183707.
- [12] Ayyanu Ravikumar, Vishal Natraj, Adarsh Verma, Subramaniam Sivagnanam, Yuvaraj Sivalingam, Priyadip Das, Velappa Jayaraman Surya, WeiHua Han, Nan Liu, Wearable sensors for real-time physiological monitoring based on self-assembled diphenylalanine peptide nanostructures, *Surfaces Interfaces* 39 (2023) 102986.
- [13] Sawsan Almohammed, Agata Fularz, Fengyuan Zhang, Diana Alvarez-Ruiz, Frank Bello, David D. O'Regan, Brian J. Rodriguez, James H. Rice, Flexing piezoelectric diphenylalanine-plasmonic metal nanocomposites to increase SERS signal strength, *ACS Appl. Mater. Inter.* (2020).
- [14] Sawsan Almohammed, Stefano Fedele, Brian J. Rodriguez, James H. Rice, Aligned diphenylalanine nanotube-silver nanoparticle templates for high-sensitivity surface-enhanced Raman scattering, *J. Raman Spectrosc.* 48 (12) (2017) 1799–1807.
- [15] Sawsan Almohammed, Sarah O. Oladapo, Kate Ryan, Andrei L. Kholkin, James H. Rice, Brian J. Rodriguez, Wettability gradient-induced alignment of peptide nanotubes as templates for biosensing applications, *RSC Adv.* 6 (48) (2016) 41809–41815.
- [16] Meital Rechtes, Ehud Gazit, Controlled patterning of aligned self-assembled peptide nanotubes, *Nat. Nanotechnol.* 1 (3) (2006).
- [17] Hyojin Park, Yuseok Kim, Yerin Kim, Cheoljae Lee, Hyosik Park, Hyeonseo Joo, Ju Hun Lee, Ju-Hyuck Lee, Self-assembly of unidirectionally polarized piezoelectric peptide nanotubes using environmentally friendly solvents, *Appl. Surf. Sci.* 618 (2023) 156588.
- [18] Ju Hyuck Lee, Kwang Heo, Konstantin Schulz-Schönhagen, Ju Hun Lee, Malav S. Desai, Hyo Eon Jin, Seung Wuk Lee, Diphenylalanine peptide nanotube energy harvesters, *ACS Nano* 12 (8) (2018).
- [19] Jinlei Zhang, Xinglong Wu, Zhixing Gan, Xiaobin Zhu, Yamin Jin, Unidirectionally aligned diphenylalanine nanotube/microtube arrays with excellent supercapacitive performance, *Nano Res.* 7 (6) (2014).
- [20] Jiarui Xia, Bingbing Sun, Chenlei Wang, Nan Sun, Hongqian Cao, Yi Jia, Yang Yang, Junbai Li, Disassembly and reassembly of diphenylalanine crystals through evaporation of solvent, *J. Colloid Interf. Sci.* 599 (2021).
- [21] Carla Carolina Silva Bandeira, Leticia Marques Caviola Foiani, Giovana Bonano Carlos, Mariana Sayuri Ishikawa, Paula Maria Gabriela Leal Ferreira, Herculan da Silva Martinho, Solvent-dependent formation kinetics of L,L-diphenylalanine micro/nanotubes, *Phys. Chem. Chem. Phys.* 25 (6) (2023) 5107–5113.
- [22] Thomas O. Mason, Dimitri Y. Chirgadze, Aviad Levin, Lihi Adler-Abramovich, Ehud Gazit, Tuomas P.J. Knowles, Alexander K. Buell, Expanding the solvent chemical space for self-assembly of dipeptide nanostructures, *ACS Nano* 8 (2) (2014).
- [23] V.S. Bystrov, E. Paramonova, I. Bdkin, S. Kopyl, A. Heredia, R.C. Pullar, A.L. Kholkin, Bioferroelectricity: Diphenylalanine peptide nanotubes computational modeling and ferroelectric properties at the nanoscale, *Ferroelectrics* 440 (1) (2012) 3–24.
- [24] Zsuzsanna Püspöki, Martin Storath, Daniel Sage, Michael Unser, Transforms and operators for directional bioimage analysis: A survey, in: Winnok H. De Vos, Sebastian Munck, Jean-Pierre Timmermans (Eds.), *Focus on Bio-Image Informatics*, Springer International Publishing, 2016, pp. 69–93.
- [25] M.S. Ishikawa, C. Busch, M. Motzkus, H. Martinho, T. Buckup, Two-step kinetic model of the self-assembly mechanism for diphenylalanine micro/nanotube formation, *Phys. Chem. Chem. Phys.* 19 (47) (2017) 31647–31654.
- [26] Python Software Foundation, Python language reference, version 3.9, <https://www.python.org/>.
- [27] John D. Hunter, Matplotlib: A 2D graphics environment, *Comput. Sci. Eng.* 9 (03) (2007) 90–95.
- [28] Lihi Adler-Abramovich, Daniel Aronov, Peter Beker, Maya Yevnin, Shiri Stempler, Ludmila Buzhansky, Gil Rosenman, Ehud Gazit, Self-assembled arrays of peptide nanotubes by vapour deposition, *Nat. Nanotechnol.* 4 (12) (2009) 849–854.
- [29] Lipeng Zu, Huiyao Shi, Jia Yang, Chuang Zhang, Yuan Yuan Fu, Ning Xi, Lianqing Liu, Wenxue Wang, Unidirectional diphenylalanine nanotubes for dynamically guiding neurite outgrowth, *Biomed. Mater.* 18 (1) (2022) 015017.
- [30] Tae Hee Han, Jun Kyun Oh, Gyoung-Ja Lee, Su-Il Pyun, Sang Ouk Kim, Hierarchical assembly of diphenylalanine into dendritic nanoarchitectures, *Colloid Surf. B* 79 (2) (2010) 440–445.
- [31] Yanzi Gou, Hao Wang, Ke Jian, Yingde Wang, Jun Wang, Yongcai Song, Zhengfang Xie, Facile synthesis of melt-spinnable polyaluminocarbosilane using low-softening-point polycarbosilane for Si–C–Al–O fibers, *J. Mater. Sci.* 51 (2016) 8240–8249.
- [32] Jungki Ryu, Seong Yoon Lim, Chan Beum Park, Photoluminescent peptide nanotubes, *Adv. Mater.* 21 (16) (2009) 1577–1581.
- [33] Qi Li, Yi Jia, Luru Dai, Yang Yang, Junbai Li, Controlled rod nanostructured assembly of diphenylalanine and their optical waveguide properties, *ACS Nano* 9 (3) (2015) 2689–2695.
- [34] Xuehai Yan, Junbai Li, Helmuth Möhwald, Self-assembly of hexagonal peptide microtubes and their optical waveguiding, *Adv. Mater.* 23 (25) (2011).
- [35] Nadav Amdursky, Michel Molotskii, Daniel Aronov, Lihi Adler-Abramovich, Ehud Gazit, Gil Rosenman, Blue luminescence based on quantum confinement at peptide nanotubes, *Nano Lett.* 9 (9) (2009) 3111–3115.
- [36] P.M.G.L. Ferreira, M.S. Ishikawa, S. Kogikoski, W.A. Alves, H. Martinho, Relaxation dynamics of deeply supercooled confined water in L, L-diphenylalanine micro/nanotubes, *Phys. Chem. Chem. Phys.* 17 (48) (2015) 32126–32131.