2010

Destruction of α -Synuclein Based Amyloid Fibrils by a Low Temperature Plasma Jet

Erdinc Karakas

Agatha Munyanyi

Lesley Greene

Mounir Laroussi
Old Dominion University, mlarouss@odu.edu

Original Publication Citation

Karakas, E., Munyanyi, A., Greene, L., & Laroussi, M. (2010). Destruction of α -synuclein based amyloid fibrils by a low temperature plasma jet. *Applied Physics Letters, 97*(143702), 1-3. doi: 10.1063/1.3499277

AIP | Applied Physics Letters

Destruction of α -synuclein based amyloid fibrils by a low temperature plasma jet

Erdinc Karakas, Agatha Munyanyi, Lesley Greene, and Mounir Laroussi

Citation: Applied Physics Letters **97**, 143702 (2010); doi: 10.1063/1.3499277
View online: http://dx.doi.org/10.1063/1.3499277
View Table of Contents: http://scitation.aip.org/content/aip/journal/apl/97/14?ver=pdfcov
Published by the AIP Publishing

Destruction of α-synuclein based amyloid fibrils by a low temperature plasma jet

Erdinc Karakas,[1] Agatha Munyanyi,[2] Lesley Greene,[2,a)] and Mounir Laroussi[1,a)]
[1]*Laser and Plasma Engineering Institute, Old Dominion University, Norfolk, Virginia 23529, USA*
[2]*Department of Chemistry and Biochemistry, Old Dominion University, Norfolk, Virginia 23529, USA*

(Received 20 August 2010; accepted 18 September 2010; published online 5 October 2010)

Amyloid fibrils are ordered beta-sheet aggregates that are associated with a number of neurodegenerative diseases such as Alzheimer and Parkinson. At present, there is no cure for these progressive and debilitating diseases. Here we report initial studies that indicate that low temperature atmospheric pressure plasma can break amyloid fibrils into smaller units *in vitro*. The plasma was generated by the "plasma pencil," a device capable of emitting a long, low temperature plasma plume/jet. This avenue of research may facilitate the development of a plasma-based medical treatment. © *2010 American Institute of Physics*. [doi:10.1063/1.3499277]

The biomedical application of low temperature plasmas is emerging as a field of great interest to physicists, engineers, chemists, and medical researchers. In the past decade various groups have shown that nonequilibrium plasmas can inactivate bacteria, help the proliferation of fibroblasts, coagulate blood, etc.[1] These important findings indicate that plasma can play an important role in various medical and therapeutic practices such as, in wound healing and the treatment of some types of cancer.[1,2]

Because they can generate plasmas unbound by electrodes and into ambient air, plasma jets, or plumes are well suited for biomedical applications.[3] The plasma jet/plume used in our study is generated by a device (the plasma pencil) capable of emitting a long cold plasma plume in ambient air.[4] The plasma pencil is driven by short (nanoseconds to microseconds in width) high voltage pulses and uses helium as a carrier gas. Other gas mixtures can also be used (such as helium/oxygen mixtures, argon/oxygen mixtures, air, etc.). The plasma plume which appears as a continuous plasma jet is in fact a train of small packets of plasma (generally known as "plasma bullets") traveling at supersonic velocities.[5,6] These plasma bullets are vehicles whereby chemically reactive species can be delivered to biological matter such as proteins or cells. In this letter, we show that the plasma bullets emitted by the plasma pencil can break amyloid fibrils into smaller units. Amyloid fibrils are otherwise very stable and extremely hard to destroy.

Amyloid fibrils are ordered beta-sheet aggregates that are associated with a number of neurodegenerative diseases such as Alzheimer and Parkinson.[7] Amyloid fibrils can also be found in other parts of the human body such as joint spaces in patients undergoing prolonged renal dialysis.[8] There are approximately twenty proteins that have been found to form fibrils in humans and are associated with disease states.[7] However, it has been postulated that all proteins can be induced to form this low energy state if subjected to the right conditions.[9] We are investigating potential methods involving the use of cold plasma that will lead to the destruction of amyloid fibrils, formed by the protein α-synuclein, which underlies Parkinson disease and the amyloid-β peptide which is associated with Alzheimer disease.[10] Parkinson disease is a neurodegenerative movement disorder. It results from the degeneration of dopaminergic neurons in the substantia nigra, a region of the brain that controls movement. Death of dopaminergic neurons by the formation of amyloid fibrils with the protein α-synuclein results in decreased production of dopamine. Lack of dopamine results in the following major symptoms of Parkinson disease: rigidity to muscles constantly contracted, resting tremor, postural instability, and slowness in initiating movement. Alzheimer disease is believed to be caused by the fibrillation of a peptide called amyloid-β after it is cleaved from the precursor protein on the cell surface in the brain. The residues composing this peptide are mainly 1–42 and spontaneously form amyloid fibrils. The fibrillation, which is believed to cause Alzheimer, begins in the temporal lobes of the brain and then spreads to other parts of the brain, thus killing cells and interfering with neural transmissions. At present, there is no cure for these progressive and debilitating diseases. We propose that this work may also be applicable to the destruction of prion proteins and have value in decontamination processes.

In our experiments, the human protein α-synuclein was selected as the initial model system. It is produced by expressing it in *Eschericia coli* from a DNA clone inserted into the bacteria. The soluble protein is then extracted from the bacterial cells and purified by ion-exchange and gel filtration chromatography. The α-synuclein protein (4–6 mg/ml) is

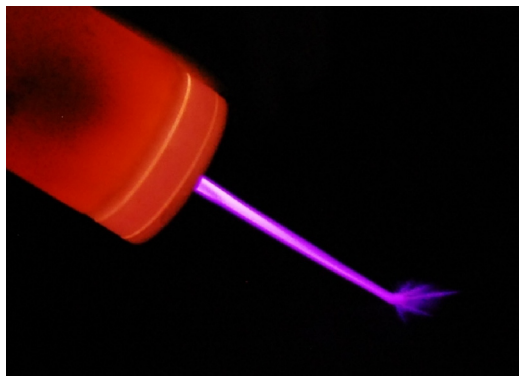

FIG. 1. (Color online) Photograph of the plasma pencil.

a)Authors to whom correspondence should be addressed. Electronic addresses: lgreene@odu.edu and mlarouss@odu.edu.

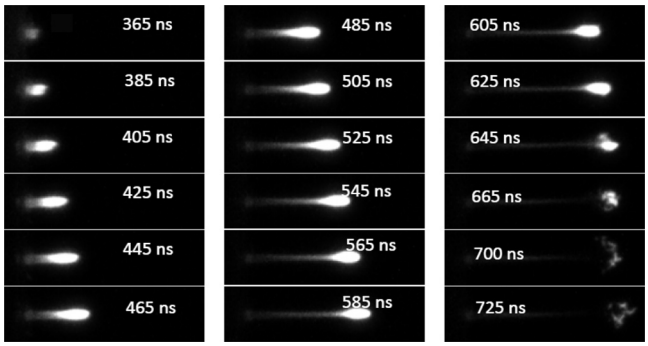

FIG. 2. High-speed camera images showing that the plasma plume, in fact, consists of a train of packets structures called plasma bullets, propagating in the surrounding air with supersonic velocities in order of 10^4–10^5 m/s without any external electric field. The exposure time of the camera is 20 ns.

dissolved in a buffer solution of 0.2 M NaCl in 20 mM Tris Base, *p*H 7.5 and incubated at 37 °C in an incubator shaking at 150–190 rpm. After 15 days mature fibrils analogous to those in patient's brain are formed in our test tubes.

The plasma pencil, which was described in details in prior publications,[3–5] was used to generate the plasma bullets that are applied directly on preprepared amyloid fibrils samples. The operating conditions of the plasma were the following: Voltage pulse magnitude was 7.5 kV, pulse width and frequency were 1 μs and 5 kHz, respectively, and feed gas was helium at a flow rate of 5 slpm. Figure 1 is a photograph of the plasma plume and Fig. 2 is a series of fast images taken with an intensified charge coupled device camera showing that the plume is in fact a train of plasma bullets traveling at high velocities. Figure 3 shows a spectrum of emission from the plume/bullets. It is dominated by emission lines from excited nitrogen, nitrogen molecular ions, and helium. Atomic oxygen and hydroxyl lines are also present. Figure 4 shows the spatial distributions of the important chemical species along the axial position. This information allows us to determine the optimum placement of the biological samples downstream of the plasma jet.

The amyloid fibrils in solution are placed into small tubes (0.2 ml) or glass slides and exposed to the plasma pencil for varying lengths of time (up to 10 min). The distance from the nozzle of the device to the samples is 2 cm.

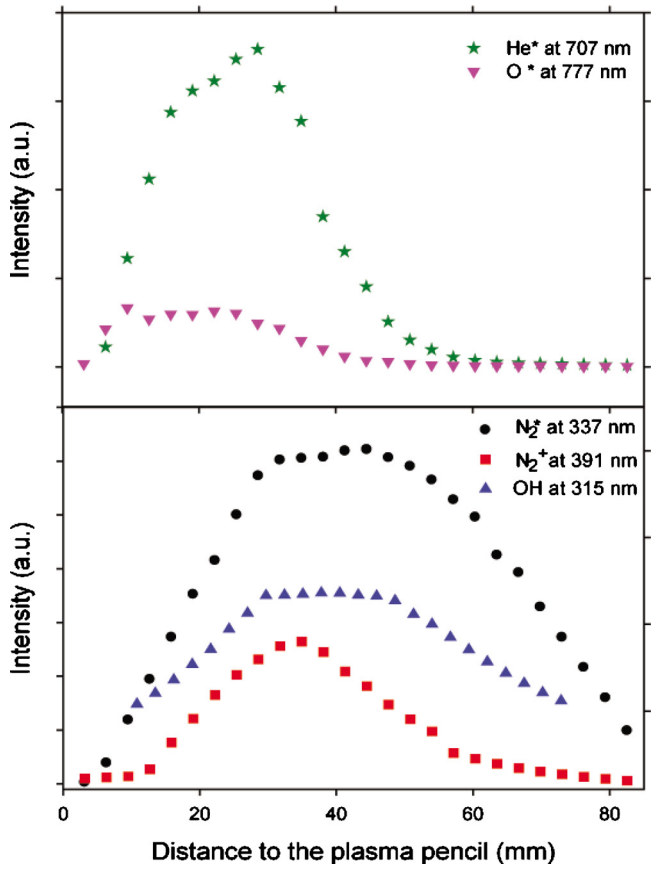

FIG. 4. (Color online) Spatial distribution of the emitting species along the plasma plume showing that N_2, N_2^+, OH, and O play important roles in the plasma chemistry in addition to He excited species. Lines selection of the emitting species are determined in terms of their order of magnitude within their spectral systems. Experimental conditions are as follows: HV amplitude: 7.5 kV; pulse repetition frequency: 5 kHz; helium gas flow rate: 5 L/min; pulse width: 500 ns.

After exposure the fibrils are immediately fixed onto 400 mesh formvar coated copper grids for analysis by electron transmission microscopy. Figure 5(a) shows mature intact α-synuclein fibrils while Fig. 5(b) shows the morphology of fibrils after 6 min exposure to the plasma. It clearly shows that a 6 min exposure induces severe damage to the fibril and

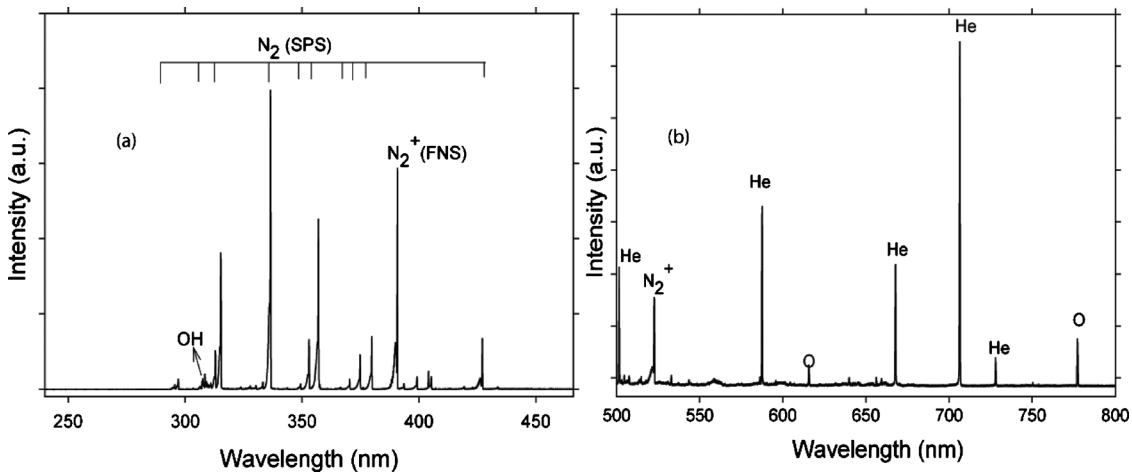

FIG. 3. (a) A typical ultraviolet (UV) emission spectrum showing emission of OH and N_2 second positive system (SPS), and N_2^+ First Negative System (FNS); (b) A typical visible-infrared (visible-IR) spectrum showing emission of N_2^+, He, and O [high voltage (HV) amplitude: 7.5 kV; pulse repetition frequency: 5 kHz; helium gas flow rate: 5 L/min; pulse width: 500 ns].

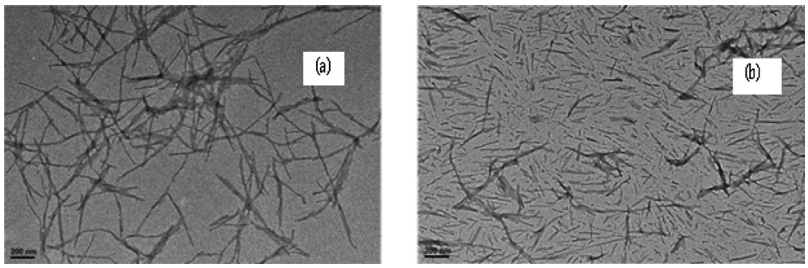

FIG. 5. (a) Transmission electron microscopic (TEM) image of mature intact α-synuclein fibrils; (b) TEM image of the fibrils after 6 min exposure to the plasma plume, showing clear evidence of extensive breakage.

causes extensive breakage. It is of note to mention that evidence of breakage starts showing up after only 2 min exposure to the plasma. Although preliminary, these are extremely important results as this methodology provides a facile mechanism whereby amyloid fibrils can be easily destroyed. This work is also very timely as quite recently, three other methods have also been shown to break fibrils. These are laser beam irradiation,[11,12] ultrasonication[13] and mechanical breakage by stirring at 1000 rpm.[14] However unlike these methods which rely on physical mechanisms, our method is based on the "dry" chemistry of the plasma. Nonequilibrium plasmas such the one generated by the plasma pencil are sources of reactive oxygen species, ROS (such as atomic oxygen and superoxides) and reactive nitrogen species, RNS (such as NO_x) which are known to chemically denature cellular lipids and proteins. So we expect that under plasma exposure the fibrils undergo chemical reactions that compromise their structural as well as chemical integrity.

The fact that amyloid fibrils are the cause behind such debilitating disease as Parkinson and probably also Alzheimer makes these results of even greater relevance. However, what remains to be tested is the cytotoxicity of plasma with regard to neurons/neuronal cells. Although studies on other type of eukaryotic cells have shown that low power doses of cold plasma do not cause them irreversible damage,[1,2] to date there are no known tests on neurons/neuronal cells. We also need to determine if the broken fibrils reassociate and are benign to brain cells as fragmented fibrils have very recently been proposed to have cytotoxic effect[14] and induce propagation.[11,12,14] It will also be very interesting and key to elucidate if the broken fibrils are taken up by microglial cells thus providing a mechanism for how the body may eliminate broken fibrils. The outcome of such tests is of utmost importance if plasma is to be used as a therapy against diseases caused by amyloid fibrils. If the fibrils are indeed chemically altered by the plasma (still to be determined experimentally) they may not be able to reassemble and their smaller units (broken by the plasma) may not be cytotoxic as those obtained by mechanical agitation, for example, These are in fact important issues that need to be carefully investigated.

Work partly supported by an AFOSR Grant No. FA9550-08-1-0487 (Laroussi).

[1]M. Laroussi, IEEE Trans. Plasma Sci. **37**, 714 (2009).
[2]M. G. Kong, G. Kroesen, G. Morfill, T. Nosenko, T. Shimizu, J. van Dijk, and J. L. Zimmermann, New J. Phys. **11**, 115012 (2009).
[3]M. Laroussi and T. Akan, Plasma Processes Polym. **4**, 777 (2007).
[4]M. Laroussi and X. Lu, Appl. Phys. Lett. **87**, 113902 (2005).
[5]N. Mericam-Bourdet, M. Laroussi, A. Begum, and E. Karakas, J. Phys. D: Appl. Phys. **42**, 055207 (2009).
[6]J. Shi, F. Zhong, J. Zhang, W. D. Liu, and M. G. Kong, Phys. Plasmas **15**, 013504 (2008).
[7]F. Chiti and C. M. Dobson, Annu. Rev. Biochem. **75**, 333 (2006).
[8]C. M. Eakin and A. D. Miranker, Biochim. Biophys. Acta **1753**, 99 (2005).
[9]F. Chiti, P. Webster, N. Taddei, A. Clark, M. Stefani, G. Ramponi, and C. M. Dobson, Proc. Natl. Acad. Sci. U.S.A. **96**, 3590 (1999).
[10]G. B. Irvine, O. M. El-Anof, G. M. Shankar, and D. M. Walsh, Mol. Med. **14**, 451 (2008).
[11]D. Ozawa, H. Yagi, T. Ban, A. Kameda, T. Kawakami, H. Naiki, and Y. Goto, J. Biol. Chem. **284**, 1009 (2009).
[12]H. Yagi, D. Ozawa, K. Sakurai, T. Kawakami, H. Kuyama, O. Nishimura, T. Shimanouchi, R. Kuboi, H. Naiki, and Y. Goto, J. Biol. Chem. **285**, 19660 (2010).
[13]E. Chatani, Y. H. Lee, H. Yagi, Y. Yoshimura, H. Naiki, and Y. Goto, Proc. Natl. Acad. Sci. U.S.A. **106**, 11119 (2009).
[14]W.-F. Xue, A. L. Hellewell, W. S. Gosal, S. W. Homans, E. W. Hewitt, and S. E. Radford, J. Biol. Chem. **284**, 34272 (2009).