

# Cancer cells (MCF-7, Colo-357, and LNCaP) viability on amorphous hydrogenated carbon nitride film deposited by dielectric barrier discharge plasma

Abhijit Majumdar,<sup>1,a)</sup> Ramesh Ummanni,<sup>2</sup> Karsten Schröder,<sup>3</sup> Reinhard Walther,<sup>2</sup> and Rainer Hippler<sup>1</sup>

<sup>1</sup>*Institute for Physics, Ernst-Moritz-Arndt-University Greifswald, Felix-Hausdorff-Str. 6, 17489 Greifswald, Germany*

<sup>2</sup>*Institute for Medical Biochemistry and Molecular Biology, Klinikum, Ernst-Moritz-Arndt-University Greifswald, 17486 Greifswald, Germany*

<sup>3</sup>*Leibniz Institute for Plasma Science and Technology, Felix-Hausdorff-Str.2, 17489 Greifswald, Germany*

(Received 16 January 2009; accepted 5 July 2009; published online 11 August 2009)

Atmospheric pressure dielectric barrier discharge plasma in CH<sub>4</sub>/N<sub>2</sub> (1:1) gas mixture has been employed to deposit amorphous hydrogenated carbon nitride (*a*H-CN<sub>x</sub>) film. *In vitro* studies with three different cancer cell lines were carried out on the coated surfaces. Preliminary biocompatibility and effect of CH<sub>4</sub>/N<sub>2</sub> films have been investigated by measuring cell proliferation. Three different cancer cell (MCF-7, Colo-357, and LNCaP) suspensions have been exposed on the surface of *a*H-CN<sub>x</sub> film to investigate the effect of deposited films on viability of cells. Results from the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) proliferation assays indicated that the deposited *a*H-CN<sub>x</sub> film is cytotoxic to cancer cell lines. Time course cell viability assay indicated maximum cell death at 24 h after seeding the cells. This effect is dependant on physicochemical and mechanical properties of the deposited films. The deposited film has been characterized by x-ray photoelectron spectroscopy and Fourier transform infrared spectroscopy. The results confirm the presence of C-N, C≡N, C-H<sub>x</sub>, C-O, N-O, overlapping NH, and OH bonds in the film. © 2009 American Institute of Physics.

[DOI: [10.1063/1.3190558](https://doi.org/10.1063/1.3190558)]

## I. INTRODUCTION

Application of atmospheric pressure (AP) plasma in life sciences is a recent advancement. However, there are several limitations to use plasma in biomedical applications. Over the past 5 yr, enormous developments have been reported in biomedical plasma research and technology.<sup>1-4</sup> Now a day the biocompatibility of diamondlike coating (DLC) or carbon nitride film and the plasma-tissue interaction is one of the emerging issues in plasma biomedical application.<sup>1</sup> In this respect it would be a novel approach to study cytotoxic effect of different plasma deposited surfaces (hydrophobic, cytotoxic, etc.) on mammalian cancer cells. However, the plasma generated for biological applications should be compatible and suitable for the biological environment. AP plasmas have been developed for many applications such as surface modification of polymers,<sup>5-7</sup> air purification,<sup>8-10</sup> and sterilization.<sup>11-17</sup> CN<sub>x</sub> films have been prepared by magnetron sputtering,<sup>18</sup> electron cyclotron resonance plasma-assisted vapor deposition,<sup>19,20</sup> ion beam deposition,<sup>21</sup> plasma enhanced chemical vapor deposition (CVD),<sup>22,23</sup> dielectric barrier discharge plasma,<sup>24,25</sup> and microwave plasma CVD.<sup>20,26</sup> It is generally accepted that CN<sub>x</sub> and DLCs do not induce any cytotoxicity or inflammation on usual adherent cells<sup>27</sup> including human embryonic kidney (HEK) cells.<sup>28</sup>

Only one contradictory result has been reported regarding cytocompatibility [with respect to HEK and pheochromocytoma (PC) 12 cell lines] of *a*H-CN<sub>x</sub> film deposited by dielectric barrier discharge plasma.<sup>24</sup> Therefore it is very important that the behavior of special carbon-based coatings should be discussed based on the detailed information of the coating such as atomic bond structure, composition, and/or electronic structure.<sup>27</sup>

It happens in most of the cases that the applied plasma could develop a thin layer on the subjected tissue/cell lines. Thus before applying the CH<sub>4</sub>/N<sub>2</sub>, dielectric barrier discharge (DBD) plasma directly on the tissues or cancer cell lines, it would be important to check the impact of the thin layer on the targeted cell lines. In our previous work,<sup>24</sup> on cytotoxic effect, it has been observed that the different mammalian cell lines are not able to survive after a certain time span on the *a*H-CN<sub>x</sub> film deposited by CH<sub>4</sub>/N<sub>2</sub> DBD plasma. To proceed further in this respect we have tried to work out the same treatment/experiment on several cancer cell lines.

In the current study we have investigated the effect of the DBD assisted plasma deposited film (CH<sub>4</sub>/N<sub>2</sub>=1:1) on mammalian cancer cell lines to understand cytotoxicity of these films. The chemical properties/composition of the deposited film has been analyzed by x-ray photoelectron spectroscopy (XPS) and Fourier transform infrared (FTIR) spectroscopy. Here we report how CH<sub>4</sub>/N<sub>2</sub> plasma-deposited films influence proliferation rate of cancer cells in MTS

<sup>a)</sup>Author to whom correspondence should be addressed. Electronic mails: majumdar@physik.uni-greifswald.de and abhijit\_majumdar2005@yahoo.com. FAX: +493834864701.

(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) assays.

## II. MATERIALS AND METHODS

### A. Film preparation

Three films were deposited with  $\text{CH}_4:\text{N}_2$  gas mixture ratios at 3:1, 1:3, and 1:1, at a constant pressure 400 mbar with constant frequency 5.5 kHz. The time course cell viability assays has been studied on one of the film ( $\text{CH}_4/\text{N}_2 = 1:1$ ) among the three deposited films ( $\text{CH}_4/\text{N}_2 = 3:1, 1:1,$  and  $1:3$ ). The experimental setup of the dielectric barrier discharge has been explained in detail elsewhere.<sup>29-31</sup> Both the Ag electrodes are covered by dielectrics: the upper (powered) electrode is covered with aluminum oxide ( $\epsilon \sim 10$ ); and the lower (grounded) electrode with a glass plate ( $\epsilon \sim 3.8$ ). Glass electrode is used as dummy substrate. Electrodes are separated by 0.15 cm gap. The upper electrode is connected to a home-built high voltage power supply, while the lower electrode is grounded. The chamber is pumped by a membrane pump down to a base pressure of about 1 mbar. Pressure inside the plasma chamber was controlled by two gas flow controllers for methane and nitrogen and by an adjustable needle valve between the chamber and the membrane pump. The high voltage power supply consists of a frequency generator delivering a sinusoidal output that is fed into an audio amplifier. The amplifier can be operated at up to 500 W. Experiments were performed at 8.8 kV (peak-to-peak) and at 5.5 kHz. The dissipated power during plasma under these conditions was of 4.5 W and plasma surface power density is  $0.18 \text{ W/cm}^2$  and  $0.12 \text{ W/cm}^3$ .

### B. Chemical surface analysis

XPS measurements of the  $a\text{H}-\text{CN}_x$  films were performed on a multi-technique 100 mm hemispherical electron analyzer (CLAM2: VG Microtech), using Mg  $K\alpha$  radiation (photon energy 1253.6 eV) as the excitation source and the binding energy (BE) of Au (Au  $4f_{7/2}$ : 84.00 eV) as the reference. The XPS spectra were collected in a constant analyzer energy mode, at a chamber pressure of  $10^{-8}$  mbar and pass energy of 23.5 eV at 0.125 eV/step.

FTIR transmission spectra were obtained by means of FTIR spectrometer Bruker (Vector 22). The plain sample was placed in a vacuum chamber built inside the spectrometer in order to minimize the IR signal of water vapor,  $\text{CO}_2$  content, and noise. The measuring signal passed the optical way with an aperture diameter of 3 mm with spectral resolution  $4 \text{ cm}^{-1}$ . For optimal signal-to-noise ratio 50 scans were averaged per sample spectrum and apodized by applying of the Norton Beer apodization function for Fourier transformation. Interferograms were zero-filled using a zero-filling factor of 2. The background spectrum was independently measured on a pure silicon substrate.

### C. Cell culture

The prostate (LNCaP), breast (MCF-7), and pancreatic (Colo-357) carcinoma cell lines were purchased from DSMZ and maintained in RPMI1640 medium (Invitrogen, Ger-

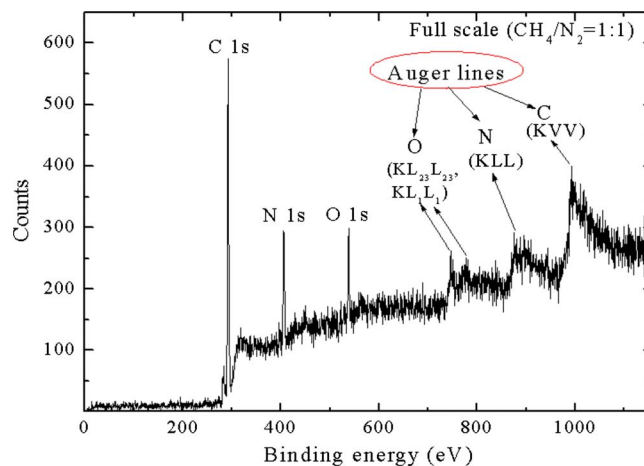


FIG. 1. (Color online) Full scale XPS spectrum of the deposited  $a\text{H}-\text{CN}_x$  film by  $\text{CH}_4/\text{N}_2 = 1:1$  DBD plasma ( $f = 5.5 \text{ kHz}$  and  $P = 400 \text{ mbar}$ ).

many) supplemented with 10% fetal calf serum (FCS), 100 units/ml penicillin and streptomycin, glucose, sodium pyruvate, sodium bicarbonate, and HEPES. Cells were grown in an incubator at  $37^\circ\text{C}$  with constant supply of 5%  $\text{CO}_2$  and split after reaching 85%–90% confluence. Cells were regularly tested for mycoplasma contamination using the MycoAlert™ mycoplasma detection kit (Cambrex).

The effect of  $\text{CH}_4:\text{N}_2$  films on proliferation rate of the carcinoma cell lines LNCaP was measured by the MTS assay. This assay uses the novel tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS] and the electron coupling reagent, phenazine methosulfate (PMS). MTS is chemically reduced by cells into formazan, which is soluble in tissue culture medium with maximum absorbance at 492 nm. In brief,  $6 \times 10^4$  cells in 300  $\mu\text{l}$  medium were seeded in each well on deposited films using rings and allowed to grow at  $37^\circ\text{C}/5\% \text{ CO}_2$ . Then MTS stock solution was added to the cells at final  $1 \times$  concentration and allowed to incubate for additional 2 h at  $37^\circ\text{C}/5\% \text{ CO}_2$ . 2 h after incubation, formazan production in culture medium was measured at 490 nm in an EILISA plate reader. Cell proliferation values are the mean of three independent experiments carried out with triplicate samples each. For calculation of significance, a  $t$ -test was performed using GRAPH PAD PRISM version 3.0.

## III. RESULTS AND DISCUSSION

### A. Chemical properties

#### 1. XPS

Full scale XPS spectra of the deposited films are shown in Fig. 1, with BE from 100 to 1160 eV for the film  $\text{CH}_4:\text{N}_2 = 1:1$ , supporting the presence of C 1s, N 1s, and O 1s bands in the deposited polymer film. Photoelectron and Auger electron are shown in one full scale XPS spectrum. After fitting the photoelectron peak (which is also the XPS peak area) area by Gaussian deconvolution method,<sup>25</sup> it helps to determine the percent amount of elements present in the film. The observed chemical shift have been calibrated by peak fitting program.<sup>25</sup> With the increase in nitrogen concen-

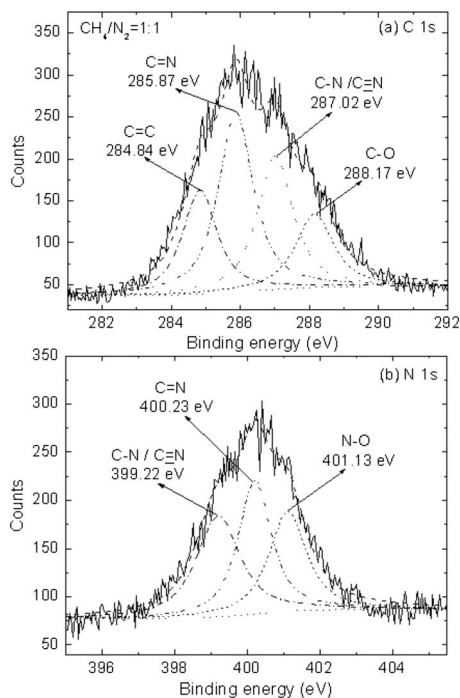


FIG. 2. Typical XPS spectrum of C 1s and N 1s of  $aH-CN_x$  film deposited by  $CH_4/N_2=1:1$  gas mixture DBD plasma.

tration, the C 1s peak broadens and also becomes more asymmetric in nature which indicate that the nitrogen is involved in chemical bonds with carbon in three possible chemical states: C–N, C=N, C≡N, and C–O bonds.<sup>25</sup> The best Gaussian fits to the XPS lines resulted in to four different peaks for the C 1s line and three peaks for the N 1s line. From Fig. 2(a), the C 1s spectrum ( $CH_4:N_2 \sim 1:1$ ) exhibits peaks at 284.84, 285.87, 287.02, and 288.12 eV, which are attributed to C=C, C=N, C–N or C≡N and C–O bonds, respectively.<sup>21,23–25,31</sup> Figure 2(b) shows, the N 1s spectrum ( $CH_4:N_2 \sim 1:1$ ) peaks at 399.22, 400.23, and 401.13 eV, which are attributed to C–N or C≡N, C=N, and N–O bonds, respectively.<sup>24,25</sup> In some cases, a peak appears at 286.4 eV and is attributed to the nitrile group (C≡N).<sup>21,31</sup> The energy of the  $sp^2$  C–N peak falls within 285.5–285.9 eV and that of the  $sp^3$  C–N peak is assigned in the range of 287.0–287.8 eV. Due to these wide spectral ranges a clear understanding of the differences between C–N and C≡N bonds<sup>21,31</sup> in the analysis of C 1s XPS spectra is quite difficult.

## 2. FTIR

FTIR absorption measures the vibrational, stretching, and symmetric/antisymmetric bands configuration (range of 4000–400  $cm^{-1}$ ) present in the deposited polymer film. The  $aH-CN_x$  films have been analyzed by FTIR technique. Typical IR transmission spectra are shown in Fig. 3, within the range from 4000 to 400  $cm^{-1}$ . Spectroscopic properties of the polymerized films deposited at different mixture concentrations of the reactive gas  $CH_4:N_2$  are presented in Fig. 3.<sup>21</sup> The band between 3395.83  $cm^{-1}$  is attributed to stretching vibrations of NH and OH functionally groups.<sup>25</sup> However, the separation of the overlapped bands is not possible

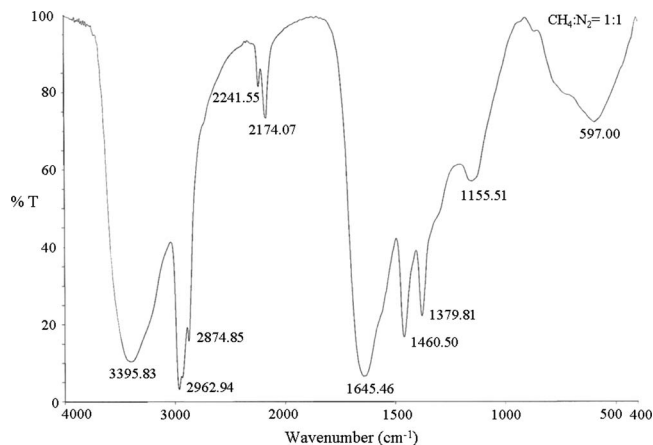


FIG. 3. FTIR absorption spectra of  $aH-CN_x$  film deposited by  $CH_4/N_2=1:1$  mixture DBD plasma.

due to intermolecular interactions as H-bridges, which are very intensive in this region and cause the broadening of the bands. The second interval (3000–2810  $cm^{-1}$ ) is the characteristic for  $CH_2$  and  $CH_3$  groups.<sup>25</sup> The intensity of the bands is low (absorption up to 1%) and is related with the concentration of methane in the gas discharge. The broad absorption single peak at 2174  $cm^{-1}$  is attributed to C≡N triple bond stretching vibration (so called nitrile group). The broad band at 1645.46  $cm^{-1}$  is attributed to C=C and C=N stretching mode.<sup>26</sup> The absorption band observed in the interval 1350–1480  $cm^{-1}$  corresponds to the C–N single bond stretch.<sup>26</sup> The absorption peak at 1155.51  $cm^{-1}$  is referred as C–O stretching mode.<sup>29</sup> The broad absorption from zone 820 to 450  $cm^{-1}$  corresponds to band.<sup>29</sup> From Fig. 3, we see that the bands corresponding to the intervals to 1350–1480, 1645–1665, and 2260–2290  $cm^{-1}$  indicate that the carbon and nitrogen atoms in the  $CN_x$  film are linked as C–N, C=N and C≡N bonds, respectively.

## B. Biological response

Figure 4 shows the rate of proliferation of cancer cells after an incubation period of 24 h on the  $aH-CN_x$ . MTS assay results showed a significantly decreased proliferation rate of all three carcinoma cell lines. The cells seeded on to glass surface without deposition represent respective controls

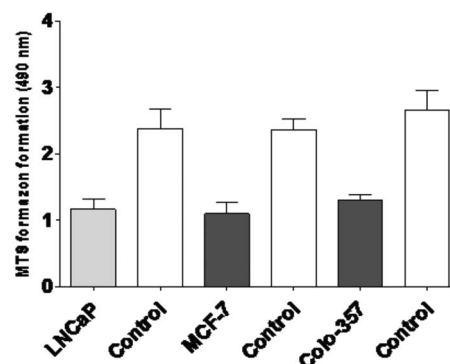


FIG. 4. Rate of proliferation of cancer cells after an incubation period of 24 h on the  $aH-CN_x$  ( $CH_4/N_2=1:1$ ) gray filled bars represent cell lines where white bars represent their respective controls.

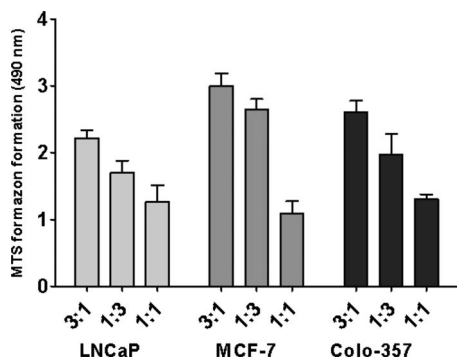


FIG. 5. The cell proliferation rate of cancer cells grown on  $aH-CN_x$  films deposited with different  $CH_4/N_2$  ratios ( $CH_4/N_2=3:1$ ,  $1:3$  and  $1:1$ ).

and the formazon formation is set at 100%. The proliferation rate of the cells on coated glass was 60% lower than their corresponding controls. The cell adhesion and proliferation at the biomaterial surface is determined by physical, chemical and mechanical characteristics. It has been shown that cells are highly sensitive to surface morphology and this interaction affects several cellular functions (cell shape and predominant type, migration, adhesion and tissue organization).<sup>32</sup> Therefore we deposited  $CH_4/N_2$  films at different ratios and tested them for their cytotoxic effects. The thickness of the coating depends upon the ratio of gases and current used in the DBD assisted plasma deposition. Figure 5 shows the cell proliferation rate of cancer cells grown on films deposited with different  $CH_4/N_2$  ratios. The results indicate that the films deposited with 1:1 ratio using  $CH_4/N_2$  are more efficient to induce cell death in cancer cells. On the other hand we would like to point that the DBD assisted plasma deposition with 3:1 or 1:3 produced dark yellow color films. These films produce brown color in culture medium after seeding the cells and it has nearly the same absorption maxima as in current assay used to measure cell proliferation. Our intention is to examine this aspect in a more controlled manner in the near future. Figure 6 shows the result of the proliferation assay carried out after 12 h, 1, 2, and 3 days on deposited films or sterile glass seeded with cells. The initial number of cells (50 000) is represented by the control and is set as 100%. The plot shows that the time dependant cytotoxic effect on cells. Only after 24 h the proliferation rate of cancer cells is significantly less than then controls.

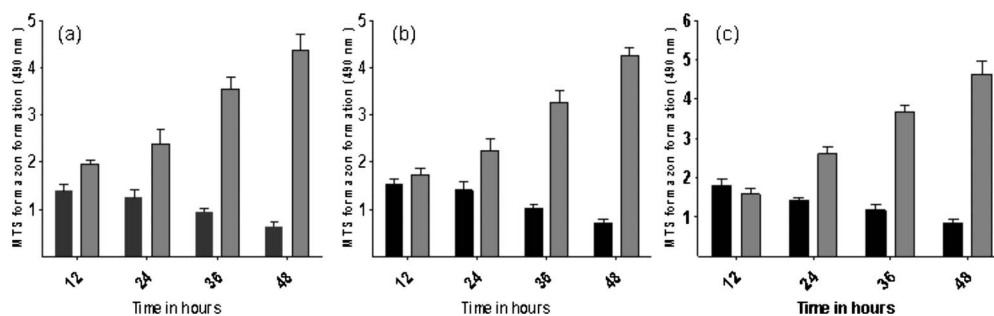


FIG. 6. The result of the proliferation assay carried out 12, 24, 36, and 48 h after seeding the cells on deposited  $aH-CN_x$  film (selected film with ratio,  $CH_4/N_2=1:1$ ) or sterile glass slide as control.

In cell culture proliferating cells needs cell to cell contacts. Usually when cells are directly treated with plasma, the growing cells will detach from each other<sup>24,33</sup> and.<sup>34</sup> Further the detached cells will enter into cell death.<sup>24,33-35</sup> This disruption of cell to cell contacts is dependant on the cultivation conditions. Therefore we have checked for time dependant effect of plasma deposited films on cell proliferation. In order to investigate the cytotoxicity of the  $aH-CN_x$  films we studied the changes in the rate of proliferation of prostate, pancreatic and breast cancer cells seeded on to the plasma coated glass slides by MTS assay. The assay measures dehydrogenase enzyme activity found in metabolically active cells. This method gave us direct information about the cytotoxicity of the deposited material. Results also indicate that the cytotoxic effect of the films to pancreatic cancer cells [Fig. 6(c)] is minimal compared to prostate (a) and breast (b) cancer cells. However, values are significant when compared with Student's  $t$ -test at  $p < 0.05$ . Both the spectroscopic (XPS and FTIR) results exhibit the common feature to the presence of nitrile ( $-C\equiv N$ ) and NH group in the deposited  $aH-CN_x$  film. The presence of nitrile and NH group could exhibit the toxicity toward cell lines. The deposited  $aH-CN_x$  film is adhesive and not soluble in water and has slightly acidic pH observed over pH indicator strips. In the contact angle measurement (which is not discussed here) we observed that the adhesive property is changing with time (approximately after 15 min). Also, the interesting thing is that the film is not soluble in those precursors. Still there are some arguments regarding this behavior.  $aH-CN_x$  films seem to have a tendency to impair the protein-based cell adhesion mechanism and to stop the cell growth process. Strong homo- and hetero-nuclear dipolar coupling properties of  $aH-CN_x$  film may break the peptide configuration and induce apoptotic signals.<sup>24</sup> At present stage, it is difficult to explain clearly from the biological, physical, and chemical point of view the actual mechanism of the cells death and growth on such an amorphous- $H-CN_x$  coated substrate. Such surfaces could be very interesting for the suppression of unwanted cell growth with help of biological implants. To support this assumption, a further study of those films in cell culture is necessary.

#### IV. CONCLUSION

Cytotoxic effect of the amorphous hydrogenated carbon nitride ( $aH-CN_x$ ) film was studied *in vitro* with three different adherent cancer cell lines (MCF-7, Colo-357, and LN-

CaP). Cytotoxic effect was deduced from the fact that the treated cells do not initially die but stop growth and die en masse after 24 h by the following treatment, where the untreated cells continue to grow and proliferate. Chemical properties of the films confirmed the presence of C–N, C $\equiv$ N, C–H $_x$ , C–O, N–O, overlapping NH and OH bands in the film. Deeper understanding of film–cell interaction and of the specific biochemistry is needed to answer how and why the plasma assisted deposited films promotes cytotoxic behavior toward mammalian cells. Further studies on these films (biological applications) might be worth pursuing for the development of novel biomedical implantation materials to control cellular processes.

## ACKNOWLEDGMENTS

Part of this work was supported by the Deutsche Forschungsgemeinschaft (DFG) through Sonderforschungsbereich SFB/TR24 *Fundamental of Complex Plasmas* and by the Federal Ministry of Education and Research (BMBF) through Verbundprojekt “Campus PlasmaMed.” Authors are also thankful to Professor Winfried Hinrichs, Dr. Rajesh Kumar Singh, and Dr. Gottfried J. Palm, Institute for Biochemistry, Greifswald, Germany, for helpful discussions.

- <sup>1</sup>G. Fridman, A. D. Brooks, M. Balasubramanian, A. Fridman, A. Gutsol, V. N. Vasilets, H. Ayan, and G. Friedman, *Plasma Processes Polym.* **4**, 370 (2007).
- <sup>2</sup>E. Stoffels, A. J. Flikweert, W. W. Stoffels, and G. M. W. Kroesen, *Plasma Sources Sci. Technol.* **11**, 383 (2002).
- <sup>3</sup>M. Laroussi, *Plasma Processes Polym.* **2**, 391 (2005).
- <sup>4</sup>R. Brandenburg, J. Ehlbeck, M. Stieber, T. von Woedtke, J. Zeymer, O. Schluter, and K. D. Weltmann, *Contrib. Plasma Phys.* **47**, 72 (2007).
- <sup>5</sup>M. Moisan, J. Barbeau, S. Moreau, J. Pelletier, M. Tabrizian, and L 'H. Yahia, *Int. J. Pharm.* **226** 1 (2001).
- <sup>6</sup>R. Dorai and M. J. Kushner, *J. Phys. D* **36**, 666 (2003).
- <sup>7</sup>M. Strobel, M. C. Branch, M. Ulsh, R. S. Kapaun, S. Kirk, and C. S. Lyons, *J. Adhes. Sci. Technol.* **10**, 515 (1996).
- <sup>8</sup>G. Tepper, R. Kessick, and D. Pestov, *J. Appl. Phys.* **102**, 113305 (2007).
- <sup>9</sup>H. H. Kim, G. Prieto, K. Takashima, S. Katsura, and A. Mizuno, *J. Electro.* **55**, 25 (2002).

- <sup>10</sup>R. Hackam and H. Akiyama, *IEEE Electr. Insul. Mag. (USA)* **17**, 8 (2001).
- <sup>11</sup>S. Tajima and K. Komvopoulos, *J. Appl. Phys.* **101**, 014307 (2007).
- <sup>12</sup>B. J. Park, D. H. Lee, J.-C. Park, I.-S. Lee, K.-Y. Lee, S. O. Hyun, M.-S. Chun, and K.-H. Chung, *Phys. Plasmas* **10**, 4539 (2003).
- <sup>13</sup>K. Kelly-Wintenberg, A. Hodge, T. C. Montie, L. Deleanu, D. Sherman, J. R. Roth, P. Tsai, and L. Wadsworth, *J. Vac. Sci. Technol. A* **17**, 1539 (1999).
- <sup>14</sup>N. S. Panikov, S. Paduraru, and R. Crowe, *IEEE Trans. Plasma Sci.* **30**, 1424 (2002).
- <sup>15</sup>M. Laroussi, G. S. Saylor, and B. B. Glascock, *IEEE Trans. Plasma Sci.* **27**, 34 (1999).
- <sup>16</sup>J. H. Choi, I. Han, H. K. Baik, M. H. Lee, D.-W. Han, J.-C. Park, I.-S. Lee, K. M. Song, and Y. S. Lim, *J. Electro.* **64**, 17 (2006).
- <sup>17</sup>X. T. Deng, J. J. Shi, and M. G. Kong, *J. Appl. Phys.* **101**, 074701 (2007).
- <sup>18</sup>J. R. Shi, *J. Appl. Phys.* **99**, 033505 (2006).
- <sup>19</sup>C. Godet, N. M. J. Conway, J. E. Boure, K. Bouamra, A. Grosman, and C. Ortega, *J. Appl. Phys.* **91**, 4154 (2002).
- <sup>20</sup>*Low Temperature Plasmas: Fundamentals, Technologies, and Techniques*, edited by R. Hippler, H. Kersten, M. Schmidt, and K. H. Schoenbach (Wiley-VCH, Berlin, 2008), Vols. 1 and 2.
- <sup>21</sup>S. Aisenberg and R. Chabot, *J. Appl. Phys.* **42**, 2953 (1971).
- <sup>22</sup>D. Liu, J. Zhou, and E. R. Fisher, *J. Appl. Phys.* **101**, 023304 (2007).
- <sup>23</sup>A. Grill, *J. Appl. Phys.* **93**, 1785 (2003).
- <sup>24</sup>A. Majumdar, K. Schröder, and R. Hippler, *J. Appl. Phys.* **104**, 074702 (2008).
- <sup>25</sup>A. Majumdar, J. Schäfer, P. Mishra, D. Ghose, J. Meichsner, and R. Hippler, *Surf. Coat. Technol.* **201**, 6437 (2007).
- <sup>26</sup>H. Windischmann, G. F. Epps, Y. Cong, and R. W. Collins, *J. Appl. Phys.* **69**, 2231 (1991).
- <sup>27</sup>R. K. Roy and K. R. Lee, *J. Biomed. Mater. Res., Part B: Appl. Biomater.* **83B**, 72 (2007).
- <sup>28</sup>M. Allen, F. Law, and N. Rushton, *Clin. Mater.* **17**, 1 (1994).
- <sup>29</sup>A. Majumdar and R. Hippler, *Rev. Sci. Instrum.* **78**, 075103 (2007).
- <sup>30</sup>A. Majumdar, J. F. Behnke, R. Hippler, K. Matyash, and R. Schneider, *J. Phys. Chem. A* **109**, 9371 (2005).
- <sup>31</sup>A. Majumdar, G. Das, N. Patel, P. Mishra, D. Ghose, and R. Hippler, *J. Electrochem. Soc.* **155**, D22 (2008).
- <sup>32</sup>A. Wennenberg, T. Albrektsson, and B. Andersson, *Int. J. Oral Maxillofac. Implants* **8**, 622 (1993).
- <sup>33</sup>G. Fridman, G. Friedman, A. Gutsol, A. B. Shekhter, V. N. Vasilets, and A. Fridman, *Plasma Processes Polym.* **5**, 503 (2008).
- <sup>34</sup>G. Fridman, A. Shereshevsky, M. M. Jost, A. D. Brooks, A. Fridman, A. Gutsol, V. Vasilets, and G. Friedman, *Plasma Chem. Plasma Process.* **27**, 163 (2007).
- <sup>35</sup>G. Fridman, M. Peddinghaus, M. Balasubramanian, H. Ayan, A. Fridman, A. Gutsol, and A. Brooks, *Plasma Chem. Plasma Process.* **26**, 425 (2006).