Nanomed. J. 7(3): 194-198, Summer 2020

RESEARCH PAPER

Emerging aluminium nitride nanoparticles: chemical synthesis and exploration of their biocompatibility and anticancer activity against cervical cancer cells

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ABSTRACT

Objective(s): Aluminium nitride (AlN) could be used in implantable biomedical sensor devices, for which cytotoxicity analysis is of utmost importance.

Materials and Methods: AlN nanoparticles were synthesized using a simple and effective solvothermal method. The X-ray diffraction results revealed the cubic phase of AlN, and the field emission scanning electron microscopy analysis demonstrated the structural morphology of the synthesized materials. In addition, the cytotoxicity of the AlN nanoparticles was assessed against healthy (HEK-293, HUVEC, and MCF10A) and cancerous cell line (HeLa). The intensity of the reactive oxygen species was also measured to determine the induced oxidative stress in the treated cells.

Results: The cytotoxicity analysis indicated that the AlN nanoparticles were nontoxic against the cancerous and normal cell lines. No significant changes were observed between the low doses of the AlN nanoparticles in the treated and control cells. However, morphological changes were detected by a phase contrast microscope, while insignificant changes were observed similar to the control cells.

Conclusion: The findings of this study could lay the groundwork for the development of AlN nanoparticles for further biomedical applications.

Keywords: *Aluminium Nitride, Biocompatibility, Cytotoxicity, Nanoparticles*

How to cite this article

Kaur M, Singh K, Singh P, Kaur A, Meena R, Pratap Singh G, Barabadi H, Saravanan M, Kumar A. Emerging aluminium nitride nanoparticles: chemical synthesis and exploration of their biocompatibility and anticancer activity against cervical cancer cells. Nanomed J. 2020; 7(3): 194-198. DOI: 10.22038/nmj.2020.07.0003

INTRODUCTION

Ceramics have attracted the attention of researchers for their biological applications owing to their exceptional electronic, thermal, and optical properties, as well as their biocompatibility [1, 2]. Boron nitride and gallium nitride have been investigated as the potential candidates for biomedical use [3-5].

Aluminium nitride (AlN) is a wide-bandgap semiconductor and nitride-based material. The wide applications of AlN are attributable to its high thermal conductivity with proper electrical insulation and high surface acoustic wave velocity. Furthermore, AlN possesses numerous other beneficial properties, such as hardness (up to 20 GPa), wide bandgap (~6.2 eV), high surface acoustic wave (SAW) velocity (depending on the crystal orientation) between 5760 and 10,500 m/s, electrical resistance (10^9-10^{11} Ωm), high resistance to oxidation (>600 °C), high thermal conductivity

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Note. This manuscript was submitted on February 10, 2020; approved on April 15, 2020

(up to 320 W/mK), and low thermal expansion coefficient ($4.2x10^{-6}$-$5.3x10^{-6}$/K), which depends on crystal orientation [6-9]. AlN also has great resistance to various materials.

AlN possesses unique properties that are considered efficient for biosensors and microelectromechanical devices. Implantable biomedical devices (e.g., smart sensors) have the potential to revolutionize the medical field. These devices should be able to communicate with an outside system via a wireless interface. AlN is considered to be a viable option for wireless and blue-tooth communications owing to its high SAW velocity [10, 11].

AlN could be used for several biomedical applications, such as SAW sensors and implantable biomedical and microelectromechanical devices. However, the development of such materials is bound to one requirement, which is biocompatibility. The materials showing great chemical inertness, stability, and high compatibility with biological samples are considered optimal in this regard to be utilized in biomedical devices. The main benefit of the lower cytotoxicity is that the device could function properly without disturbing the functioning of the biological media.

In the present study, AlN nanoparticles were synthesized using a simple and effective solvothermal method, and the structural study was performed using X-ray diffraction (XRD). The biocompatibility of the synthesized AlN nanoparticles was evaluated against normal human HEK 293, HUVEC, and MCF 10A cell lines using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay, and the anticancer potential of the nanoparticles was also assessed against cervical cancer cells. Additionally, the intensity of reactive oxygen species (ROS) was measured in order to determine the induced oxidative stress in the cell culture and cellular biocompatibility of the AlN nanoparticles.

MATERIALS AND METHODS

Synthesis of the aln nanoparticles

In a typical experiment, a solvothermal method was used for the synthesis of the AlN nanoparticles. To this end, a specifically designed stainless steel autoclave was employed. In brief, two grams of Aluminium oxide powder (Al_2O_3; 99.99% purity; Sigma-Aldrich, USA) and 25 milliliters of 1 M aqueous ammonia solution (NH_3) as a nitrogen source were applied as precursors. These materials were placed inside an autoclave, preserved at the temperature of 500°C for 18 hours, and cooled to room temperature naturally. Al_2O_3 was converted into AlN, and the solid product (AIN) was collected and used for further characterization.

Cytotoxicity assessment of the aln nanoparticles

The MTT assay was used for the screening of cell viability. The cytotoxicity of the AlN nanoparticles on the HeLa (cervical cancer), human embryonic kidney (HEK-293), human endothelial (HUVEC) and breast cell lines (MCF10A) was assessed. The cell lines were preserved in the Iscove's modified Dulbecco's medium. The medium was supplemented with penicillin (100 U/ml), GlutaMAX (2 mM), 10% fetal calf serum (FCS), and streptomycin (100 µg/ml), incubated at the temperature of 37°C in an atmosphere with 95% air and 5% CO_2, and maintained at 90% relative humidity. In brief, approximately 10^5 cells per well were incubated in 100 microliters of RPMI-1640 supplemented with 10% FCS, l-glutamine (2 mM), and various concentrations of the AlN nanoparticles (100, 200, 400, and 800 mg/l) for 24 hours. Four hours before termination, the supernatants were substituted with 10 microliters of the MTT solution (1 mg/ml) and 90 microliters of a fresh medium. After four hours of incubation at the temperature of 37°C, the medium was aspirated, and the formazan crystals were solubilized in 200 microliters of dimethyl sulfoxide. Optical density was obtained spectrophotometrically using Bio-Rad 840 at 570 nanometers. The relative cell viability (%) of the control cells (without AlN nanoparticles) was estimated based on the UV-Vis absorbance data obtained by the following formula [12, 13]:

$$Cell\ viability(\%) = \frac{A_{test}}{A_{control}} \times 100 \quad (1)$$

In the formula above, $[A]_{test}$ and $[A]_{control}$ show the absorbance of the test and control samples, respectively.

Following this method, the amount of cleaved MTT could be determined, which was proportional to the population of the viable cells. All the measurements were performed in triplicate.

Intracellular ros evaluation

The production of intracellular ROS was measured using DCFH-DA [14]. In brief, the DCFH-DA stock solution in methanol (10 mM) was

diluted in the culture medium to yield a working solution (100 µM). After exposure to the AlN nanoparticles, the cells were washed twice with phosphate buffered saline (PBS), incubated in one milliliter of the working solution of DCFH-DA at the temperature of 37°C for 30 minutes, lysed in an alkaline solution, and washed with PBS. In addition, hydrogen peroxide (H_2O_2) was used as the positive control to measure ROS. Fluorescence intensity was measured via excitation (485 nm) and emission filters (520 nm) using a fluorometer (model: RF-5301 PC Shimadzu spectrofluorometer, Nakagyo-ku, Kyoto, Japan).

Cellular morphological analysis

At this stage, the cells were treated using an AlN colloidal system (0.6 mg/ml) and incubated at the temperature of 37ºC in 5% CO_2 for 24 hours. In addition, 0.1 M PBS was used to wash the cells, and the cells were trypsinized by 0.05% trypsinase, fixed with methanol for 10 minutes, and dissolved in one milliliter of 0.1 M PBS. Imaging was performed using phase contrast microscopy at the magnification of 100X.

Results and Discussion

Structural and morphological analysis

In the current research, the XRD technique was used to investigate the structural properties of the synthesized AlN nanoparticles operated at 40 kV and 100 mA using the CuKα (λ=1.5406 Å) radiation. Fig 1-a shows the XRD pattern of the synthesized samples. As can be seen, the peaks were indexed with the cubic AlN (JCPDS card no. 00-046-1200). Moreover, the XRD results were used to calculate the mean crystallite size. The crystallite size of the AlN was calculated using the Debye Scherrer formula, as follows [15]:

$$D = \frac{K\lambda}{\beta Cos\theta} \qquad (2)$$

In the formula above, *D*, *k*, *λ*, *β*, and *ϑ* show the mean crystallite size, X-ray wavelength (1.54060 Å), Scherrer constant (0.94), full width at the half maximum (FWHM) in the radians, and diffraction angle, respectively. The calculated crystallite size of the synthesized material was ~59 nanometers. The lattice parameters of the synthesized samples were also calculated, and the correlation used to determine the lattice parameters for a cubic structure was as follows:

$$\frac{1}{d^2} = \frac{(h^2 + k^2 + l^2)}{a^2} \qquad (3)$$

In the equation above, *a* is the unit cell parameter, *hkl* is plane, and *d* represents the inner-planar distance. The calculated value of *a* was 4.053 Å, which was used to generate the FCC structure of AlN (Fig 1-b) using the VESTA software [16]. The structure demonstrated the least dense plane (111) of AlN.

Furthermore, the XRD pattern was used to calculate the texture coefficient, which provided the data on the preferred growth orientation of the material. For this calculation, the values of the standard intensities corresponding to the observed diffraction planes were used, and texture coefficient was calculated using the following equation [17, 18]:

$$TC(h_i k_i l_i) = \frac{I(h_i k_i l_i)}{I_0(h_i k_i l_i)} \left[\frac{1}{n}\sum_{i=1}^{n} \frac{I(h_i k_i l_i)}{I_0(h_i k_i l_i)}\right]^{-1} \qquad (4)$$

In the equation above, *TC (hkl)* is the texture coefficient of the plane specified by miller indices, *I (hkl)* and I_0 *(hkl)* represent the specimen and standard intensities, respectively, which correspond to a given diffraction peak, and the *n* value shows the number of various peaks. The texture coefficient analysis revealed that the synthesized material had higher growth (111), along with the texture coefficient value (2.22) (Table 1).

Table 1. Calculated Texture Coefficient of AlN Nanoparticles

Sample ID	Plane (hkl)	Texture Coefficient
Aluminium Nitride (AlN)	111	2.22
	200	0.316
	220	0.312
	311	1.05
	222	0.48

Fig 1-c depicts the FE-SEM image of the synthesized samples.

The AlN nanoparticles were formed with spherical morphology containing some agglomerations.

The mean particle size was calculated using XRD and confirmed by FE-SEM.

Cytotoxicity and rOS evaluation

MTT assay is a simple, rapid, relatively inexpensive, and widely used procedure for the screening of cell viability.

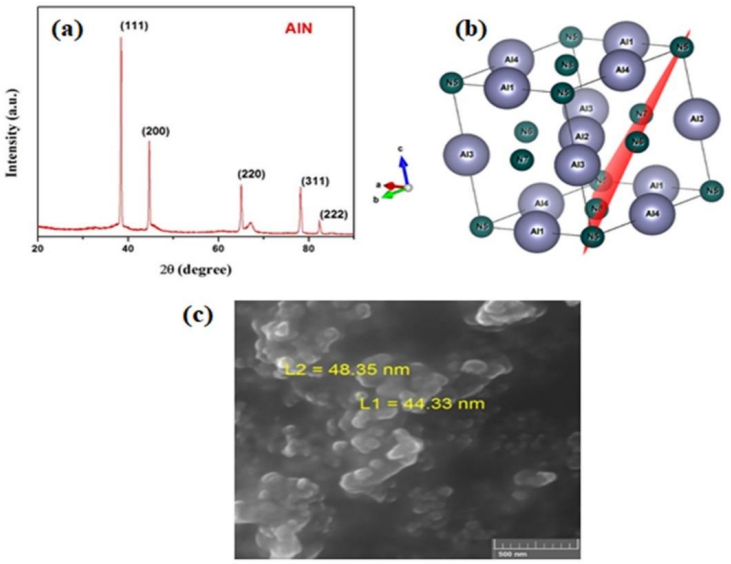

Fig 1. a) XRD Pattern of Synthesized Sample, b) Cubic Structure of AlN Nanoparticles Generated Using VESTA Software, c) FE-SEM Image of AlN Nanoparticles

In the present study, the viability of AlN on the normal and cancerous cell line decreased as a function of dose, while the changes were not considered significant compared to the controls (Fig 2).

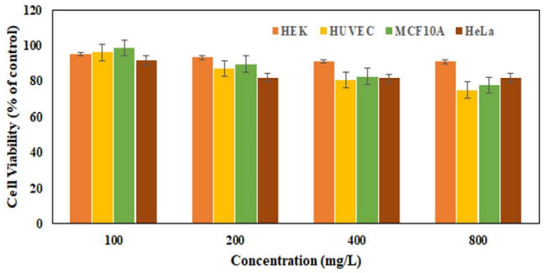

Fig 2. Comparative Cell Viability of AlN Nanoparticles on HEK-293, HUVEC, MCF10A, and HeLa Cell Lines

Furthermore, the findings indicated that the AlN nanoparticles were nontoxic to both the cancerous and normal cell lines and could be used safely for biomedical applications.

DCF fluorescence intensity is an indicator of oxidative stress in cells, which increased after 24 hours of exposure to the AlN nanoparticles at all the examined concentrations in the current research.

The oxidative stress level of the cells treated with the AlN nanoparticles (800 mg/l) was higher compared to the control cells (Fig 3).

The positive control showed oxidative stress, which was expected due to the use of hydrogen peroxide, while no significant changes were observed between the control cells and cells treated with lower doses of the AlN nanoparticles.

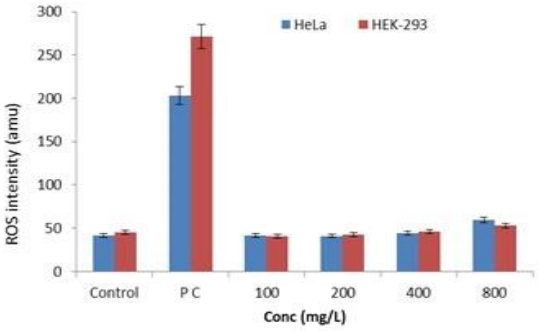

Fig 3. Comparative ROS Intensity of AlN Nanoparticles on HeLa and HEK-293 Cell Lines

Based on the mentioned findings, it could be concluded that the nanoparticles did not generate free radicals in significant amounts, which may not induce oxidative stress. Similarly, the obtained results regarding cell viability indicated that the AlN nanoparticles exerted no cytotoxic effects, rendering them a viable option for further biomedical applications.

Cellular morphological analysis

In the present study, morphological changes were observed using a phase contrast microscope, which were not considered significant similar to the control cells. On the other hand, the cells had an intact nuclear membrane and low density of the nuclear chromatin, and no cell death and apoptotic

features were observed in the nanostructured HeLa cells treated with AlN nanoparticles.

CONCLUSION

In this study, AlN nanoparticles were synthesized using a solvothermal method, precursor Aluminium powder, and ammonia solution at the temperature of 500ºC. The XRD analysis confirmed the formation of cubic-phase AlN nanoparticles, and the FE-SEM assessment demonstrated the spherical morphology of the synthesized samples. Moreover, cytotoxicity studies were used to analyze whether the nanoparticles could be used for biomedical applications. According to the cell viability results, the synthesized AlN nanoparticles were nontoxic to the healthy (HEK-293, HUVEC, and MCF10A) and cancerous cell lines (HeLa), causing no changes in cell morphology. These findings emphasized that AlN nanoparticles could be developed as a promising nontoxic material for further biomedical applications.

ACKNOWLEDGEMENTS

Hereby, we extend our gratitude to the Department of Science and Technology in India for the financial support of this research project under the Science and Engineering Research Board (SERB) project number EMR/2016/002815. This study was also supported by UPE-II, UGC in New Delhi, India.

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