Exp. Theo. NANOTECHNOLOGY 9 (2025) 395-404 https://doi.org/10.56053/9.S.395

Effect of Ag:Au nanoparticles prepared by plasma jet on breast cancer (MDA) cells line

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Received 11/2/2025, *Received in revised form* 29/3/2025, *Accepted* 20/4/2025, *Published* 15/5/2025

This work involved the synthesis of nanoparticles utilizing the plasma jet technology and the evaluation of their impact on a breast cancer cell line (MDA). The nanoparticle is created by coating silver with gold in various ratios (1:9, 2:8, and 3:7) and then compared to silver alone. Then the examination by UV-visible, X-Ray Diffraction, and Field Emission Scanning Electron Microscopy is done to show the effect of on both cancer cell lines and normal cell lines. After 48 hours, the cancer cell inhibition rate reached a maximum of 60% when the concentration is 100% for a ratio of 3:7. In contrast, the toxicity rate to normal cells is highest at 15.3% under the same conditions. The nanoparticle preparation method is simple, rapid, cost-effective, and efficient in suppressing breast cancer cells without harming normal cells.

Keywords: Ag:Au; NPs; Plasma jet; XRD.

1. INTRODUCTION

Nanotechnology is the study and application of materials with sizes as small as 100 nanometers (nm).They are utilized in several disciplines such as material science, agriculture, the food industry, cosmetics, medicine, and diagnostics [1]. Aside from their material, nanoparticles come in a variety of sizes, shapes, and dimensions [2]. For the synthesis of NPs, a variety of techniques can be used, although they can be roughly divided into two categories: (1) the top-down approach and (2) the bottom-up approach [3]. These methods further subdivide into several groups according to the protocols that have been established, the operation, and the reaction condition.

Noble metal nanoparticles (NPs), including gold and silver, are the most desirable elements because of their unique visual, chemical, physical, and electrical characteristics. Because of the dispersion and absorption of photons, Au and Ag NPs both exhibit a plasmonic effect. Au NPs and Ag NPs are

insensitive to physical variables such as light, air, electrical qualities related to size, magnetic properties, excellent conductivity, and chemical stability [4]. Compared to monometallic nanoparticles (NPs), the synthesis of Silver (shell) and Au NPs as a core-shell system improves the catalytic process and finds applications in molecular sensing, photo thermal treatments, optical diagnostic sensors, and antimicrobials [5].

Plasma, or the fourth state of matter, is a quasi-neutral gas composed of charged and neutral particles that act together. Gaseous nebulae, star interiors, atmospheres, and interstellar hydrogen are examples of plasma.Plasma is made up of dissociated negative electrons and positive ions of atoms, and it makes up around 99% of all matter in the universe.Plasma is a gaseous mixture of electrons and negative or positive ions. It can be partially ionized, as in the case of fluorescent lamps, or totally ionized, as in the sun [6]. Produce silver nanoparticles (Ag NPs) and gold nanoparticles (Au NPs) using a plasma jet for application in cancer treatment. The examination of the nanoparticles using field emission scanning electron microscopy showed that the Ag NPs and Au NPs are evenly distributed and had a spherical shape. Additionally, their particle size distributions are found to be comparable to those determined from the x-ray diffraction analysis [7]. The aim of the research is to prepare Ag:Au NPs and studies their influence on Breast cancer (MDA) cells line.

2. MATERIALS AND METHODS

2.1 Synthesis Ag:Au nanoparticles by plasma jet

The plasma system which used in this work consist of the following, as shown in Figure 1:
a- Gas argon (Ar).
b- A gas flow meter that is connected to a hollow metal tube and has a calibrator of (1–5) L/min.
c- Hold stainless steel metal tubes that are 10 cm long and have various internal sizes of 1 mm.
They are made specifically for this purpose and connect to the power supply's cathode, which is outfitted with a high voltage that is both continuous and intermittent. The tubes are capable of processing voltage up to 25 kV and cutting at a frequency of 25 kHz. Their length of conductor measure is 7 cm, and their width is 5 mm. The strip ends feature a 1 x 1 flat end that is connected to the anode. They also include a metal tube holder for the glass beaker containing the solution used to prepare the samples for silver nanoparticles (Ag NPs) and gold nanoparticles (Au NPs).
d-A vertical metal tube attached by the catcher. Its higher end attaches to the gas regulator by a rubber tube, and the gas regulator is connected to the air compressor by another rubber tube. The beaker is mounted on a moving holder beneath the metal tube, and the solution brine is deposited in a little flask with a capacity of 25 mm. The volts' anode (anode) is equipped.

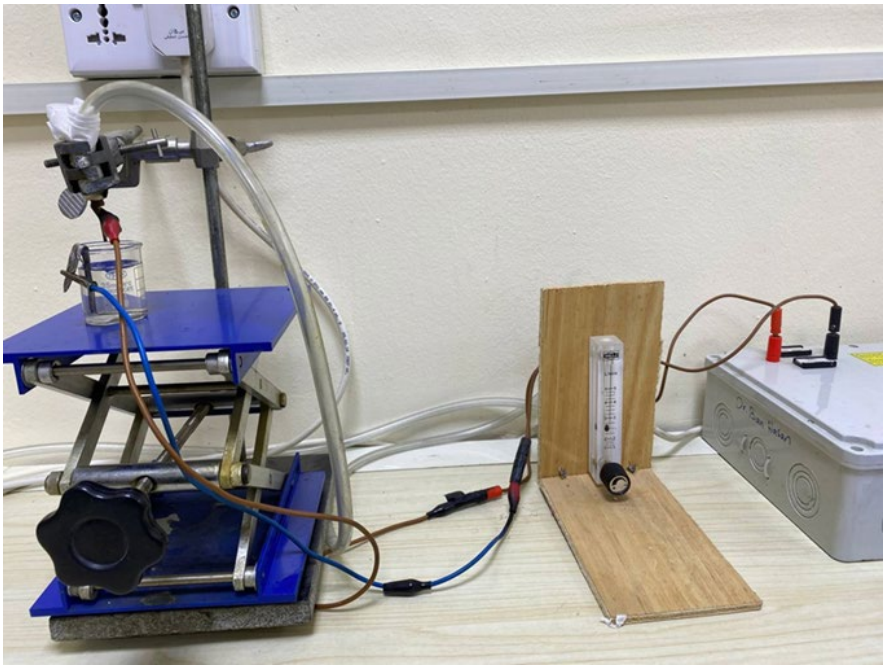

Figure 1 The system of plasma jet.

2.2 Preparation of chemical solution

Two types of salts are used, one to prepare Ag and the second to prepare Au:
1- Silver nitrate ($AgNo_3$) exhibiting a partial weight of 169.872 g/mol at a 1 mM concentration.
2- Gold salt ($AuCl_4.3H_2O$) hydrogen tetrachloroauratetrihydrate, exhibiting a partial weight of 393.83 g/mol at a 0.5 mM concentration and a purity of > 99.9%. The necessary weight can be computed using equation (1) [7].

$$concentration\ \left(\frac{mol}{liter}\right) = \frac{mass(g)}{Molecular\ weight\ \left(\frac{g}{mol}\right) * Volume\ (liter)} \quad (1)$$

2.3 Preparation of Ag NPs

It is taken 10 mL of silver salts (molecular weight 169.872 g/mol and concentration 1 mM) are exposed to the plasma jet system for 3 minutes; when a yellow color is observed, it is removed from the system , as shown in Figure. (2a).

2.4 Preparation of Ag:Au NPs

The catcher fixes a metal tube one millimeter in diameter vertically. Using the plasma jet technique, the length of the plasma between the two electrodes is 1 cm, the flow meter is 3 liters/minute, the voltage is 20 kV, and the gas utilized is argon. Then, a ratios mixture of Ag NPs and gold salts are taken. The ratios of Ag NPs to gold salt are (1:9, 2:8, and 3:7), respectively, and exposed them to the plasma system for 3 minutes until the solution color changed to violet, as shown in Figure (2b).

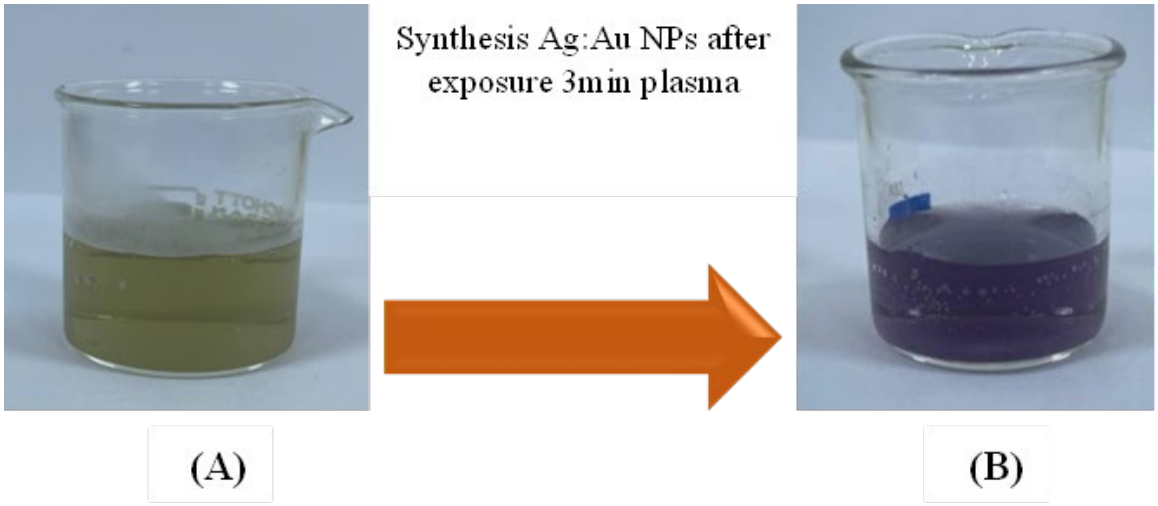

Figure 2 Synthesis Ag:Au NPs by plasma jet (A) Ag NPs (B) Ag:Au NPs.

2.5. Cytotoxicity assay

In vitro work is done at the Biotechnology Research Centre at Al-Nahrain University. In this experiment, cancer cell lines are employed. In terms of tissue culture, the research employed 96 (12×8) microtiterplates, each one of the cells utilized for seeding 10,000 cells. The cells are then allowed to incubate at 37 °C for 24 hours in order to create a monolayer, as demonstrated by the inverted microscope. Furthermore, a series of diluted Ag NPs and Au NPs are applied to the cells, while no treatment is applied to the control wells. The steps mentioned have been done in triplicate, after which they are exposed to reincubation at a degree of 37 °C. The growth medium is decanted after 48 hours of incubation. The steps have been done three times to check the authenticity, using a 50µl crystal violet assay, then incubated for 20 minutes. The cells are viewed under an inverted microscope, shot using a digital camera and recorded individually for the results. To examine the experiment outcomes, Graph Pad Prism v 8 is applied [7].

3. RESULTS AND DISCUSSION

The reaction process is changing the solutions color, which is related to the production of metal NPs that are prepared using the Ar jet technique as well as the metal salts prepared solutions. The first color about the creation of NPs during mixing in metal salt solutions is provided by the color shift. When a metal's particle diameter approaches a nanometer, such as gold or silver, surface plasmon resonance (SPR) occurs, giving metal particles their non-metal color. Thus, the UV-visible absorption spectra connected to colloidal Ag:Au NPs as different ratio between Ag:Au NPs (1:9,2:8 and 3:7) , and compare result (Ag NPs) only and at visible light wavelengths (526,519,517)nm ,respectively ,the spectral analysis equipment has been employed to prove the creation of NPs. The ultraviolet-visible spectrum for Ag NPs at 3 minutes exposure of plasma jet different ratios, the sharp peak at roughly 408 nm. The absorption spectrum increased as the ratio of silver to Ag:Au NPs increased, a result of an increase in the number of active sites on the particle's surface, which leads to an increase in surface interactions that contribute to light absorption , are displayed in Figure 3.

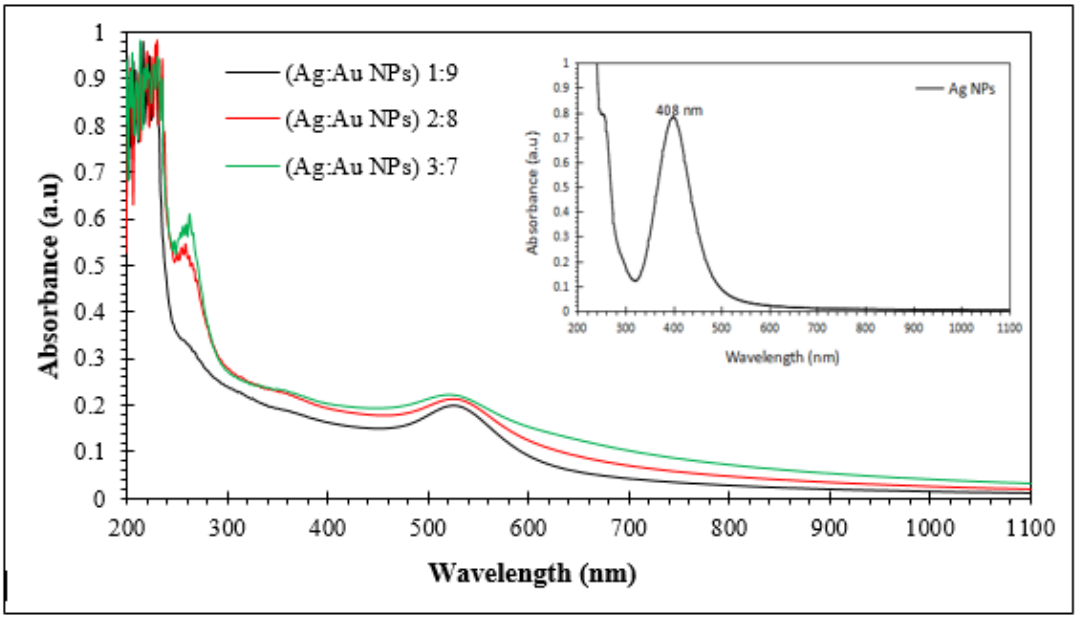

Figure 3 UV-visible absorption spectrum of NPs prepared using plasma jet at a different ratios functions: Ag NPs and Ag:Au NPs.

3.1. X-Ray diffraction (XRD)

The dried Ag NPs by a hot plate at 30°C that are synthesized using the Ar jet technique had changed XRD patterns that displayed the reflection of Bragg, the structures of the FCC of the Ag NPs are indicative. Figure.4 (A) shows the XRD Ag NPs spectrum. After matching with the standardized X-ray models of the Ag (JCPDS No.04-0783), the peaks at angles of (29.44, 38.12, and 44.32) corresponds to (210), (111), (200) planes of Ag, and the XRD results for Ag:Au NPs are calculated by the ratios which the peaks at angles (38.2, 44.44, 64.56 and 77.64) corresponds to (111) ,(200) ,(220) and (311) planes ,respectively. The aforementioned pattern has demonstrated that peaks' diffraction patterns cannot be replicated in other kinds of material, proving the prepared samples purity and lack of additional impurities [8].

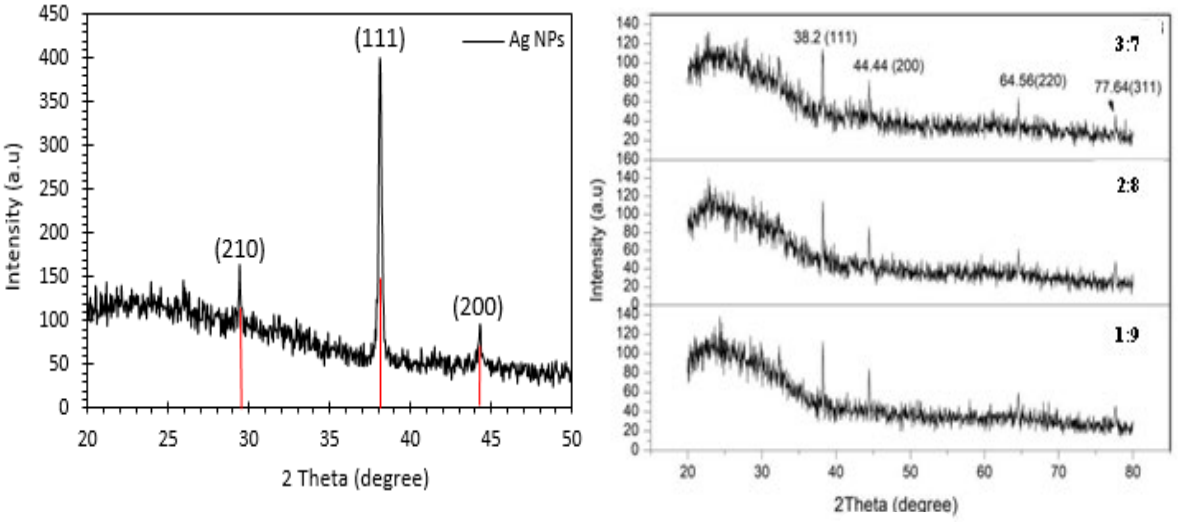

Figure 4 X-ray patterns of NPs prepared using the plasma jet as different ratios functions: (A) Ag NPs (B) Ag:Au NPs.

3.2. FESEM

The morphological characteristics of synthesized Ag NPs and Ag:Au NPs using a plasma jet are illustrated in FE-SEM pictures (see Figure (5)). The well-dispersed Ag NPs image in Figure.5 (a) shows that the NPs have a spherical form and range in size from (32 to 50) nm. The images revealed highly agglomerated spherical Ag:Au NPs in the sample, according to FESEM analysis. It's possible that chemical reactions or the conditions before the experiments caused the spherical particles to clump together into agglomerated structures. This can happen because of forces between the particles, like superposition and surface cross-linking. Agglomerated nanoparticles showed an important advantage in preparation, as they could affect the physical and chemical properties of the particles. It may lead to an increase in particle interface area, which can lead to improved surface interactions and performance.

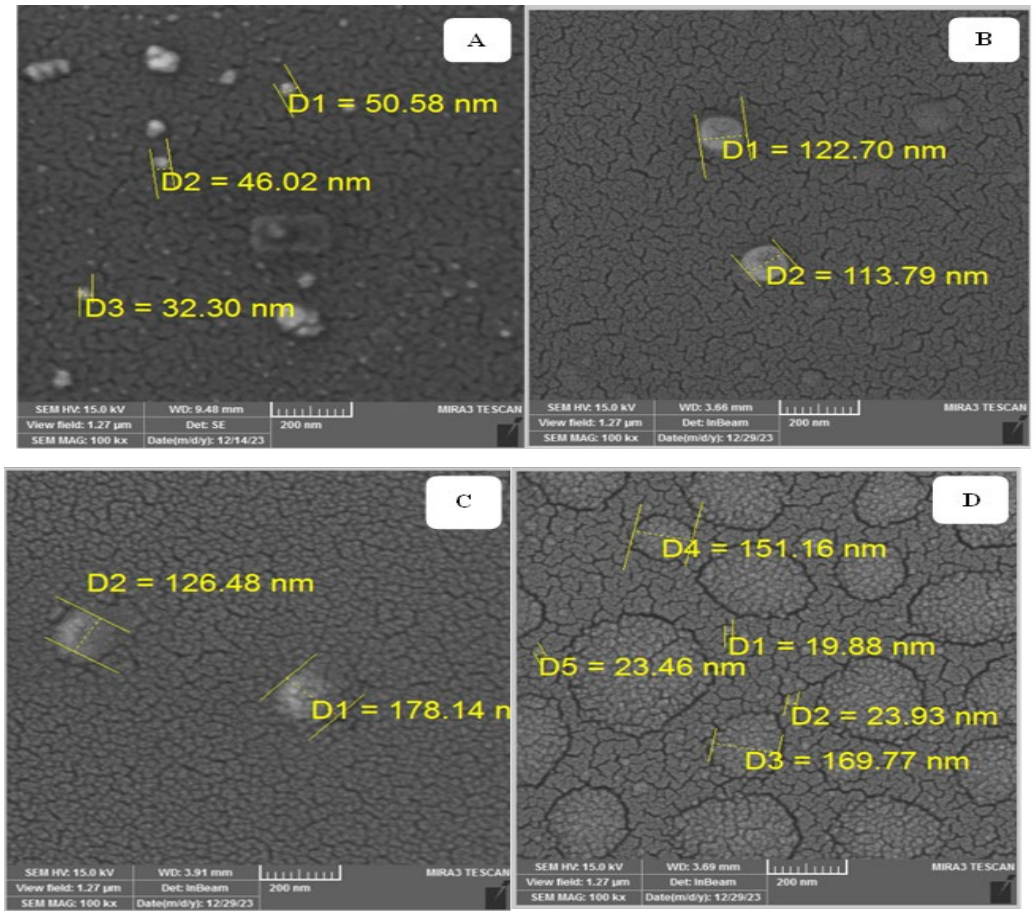

Figure 5 FESEM pictures of the colloidal nanoparticles: (A) Ag NPS (B) Ag:Au NPs 1:9 (C) Ag:Au NPs 2:8 (D) Ag:Au NPs 3:7.

3.3. Cytotoxicity assay

The toxicity tests are assessed in vitro against the normal embryonic cell line (REF) and the breast cancer cell line (MDA) under a variety of dilutions of the Ag:Au NPs in different ratios after 24 and 48 hours of exposure. The highest rate of destruction for the breast cancer cell line (MDA) is 54% after 24 hours when the concentration of Ag:Au NPs is 100% for 1:9, and the greatest rate is 60% after 48 hours for 3:7, as shown in Figures (6) and (7), respectively . For the normal cell line (REF), the greatest toxicity is 12% after 24 hours when the concentration of Ag:Au NPs is 100% for 3:7, and the greatest toxicity is 15.3% after 48 hours when the concentration is 100% for 3:7, as shown in Figures (8) and (9), respectively.

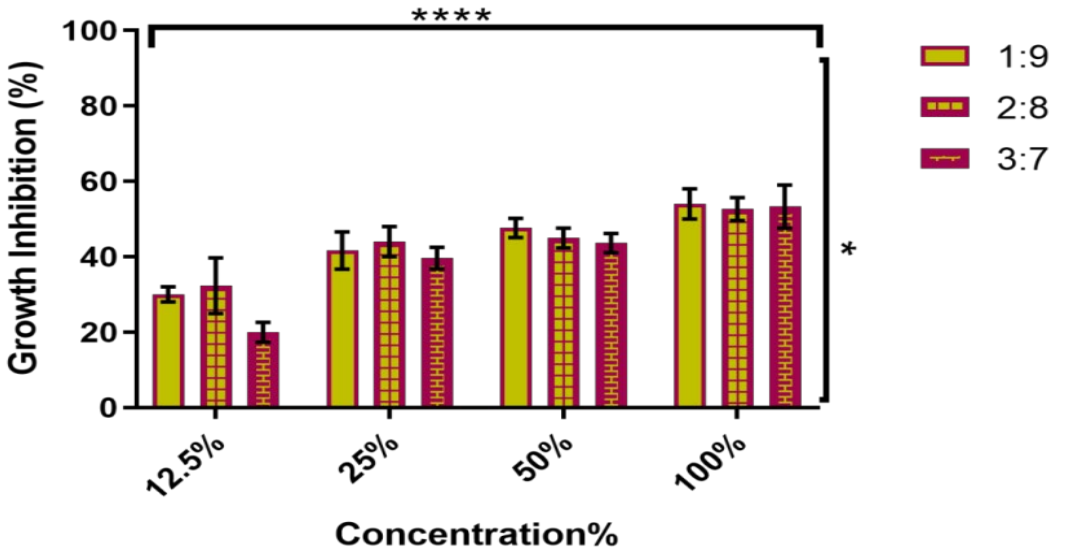

Figure 6 Growth inhibition of the breast cancer cells line (MDA) after 24h as different ratios of Ag:Au NP.

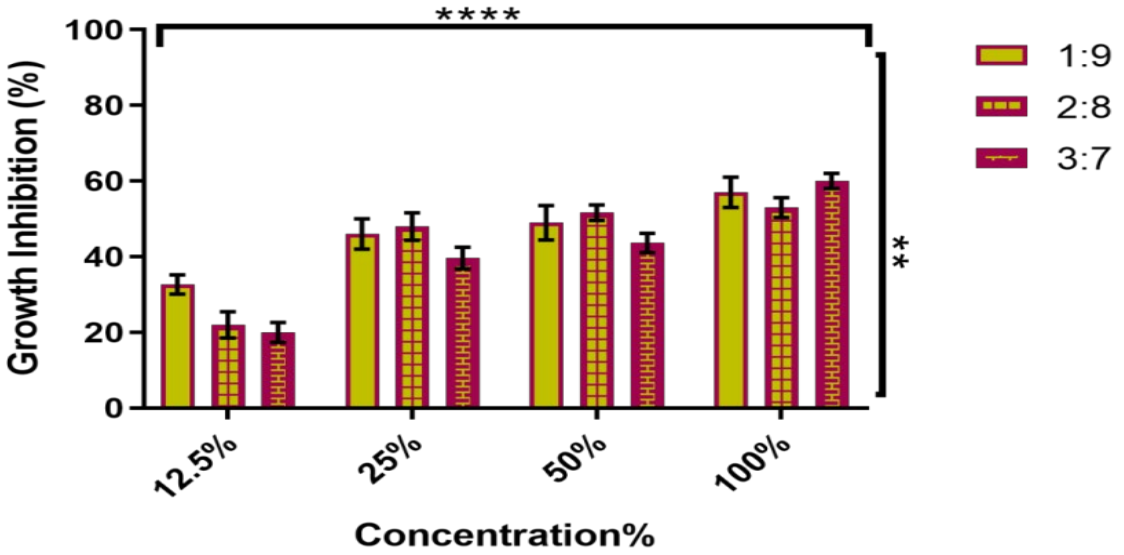

Figure 7 Growth inhibition of the breast cancer cells line (MDA) after 48h at different ratios of Ag:Au NPs.

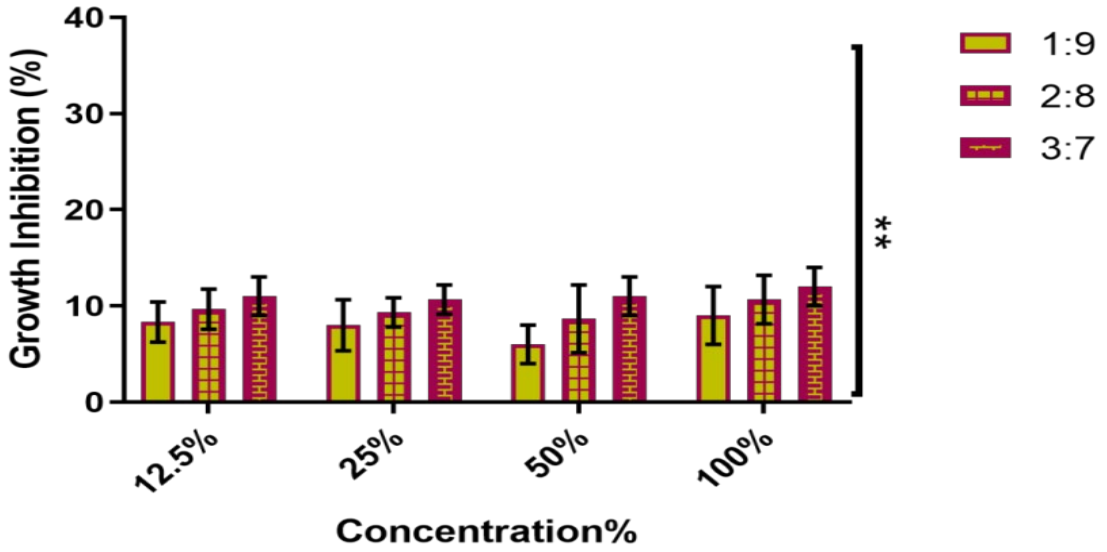

Figure 8 Growth inhibition of the normal cells line (REF) after 24h as different ratios of Ag:Au NPs.

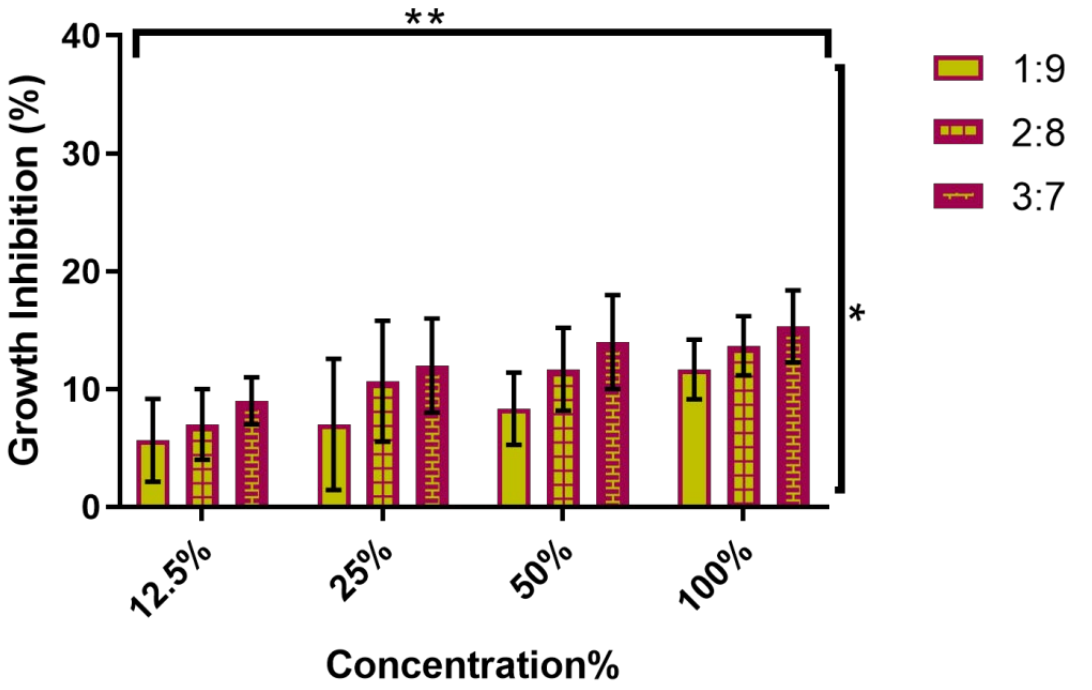

Figure 9 Growth inhibition of the normal cells line (REF) after 48h at different ratios of Ag:Au NPs.

4. CONCLUSIONS

In conclusion, employing a plasma jet system for material production was a straightforward, environmentally safe, and expeditious procedure. Silver exhibits cytotoxicity towards cancer cells and, upon the creation of the base shell, a distinct material with altered properties is generated. The properties of this material were enhanced until a material that is both easy to make and inexpensive was achieved. Simultaneously, cancer cells were eradicated with reduced toxicity, without causing harm to healthy cells, and hold great potential in the fields of medication delivery and cancer treatment. The FE-SEM images reveal that the nanoparticles possess a spherical morphology and have a size distribution ranging from 32 to 50 nm. This characteristic promotes their efficacy in targeting cancer cells, since they closely resemble natural cells. Ag and Au nanoparticles were found to exhibit reduced toxicity in normal cells.

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