



# Study on Cell Viability, Skin Penetration, and Edema Inhibition Using Collagen-based Silver Nanoparticles

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## ABSTRACT

The goal of this study was to examine the behaviour of silver nanoparticles stabilised with collagen (AgNPcol) and to assess the skin's ability to absorb the substance and how it responded to carrageenan-induced paw edoema. Utilizing solutions of the reducing agent sodium borohydride (NaBH<sub>4</sub>), silver nitrate (AgNO<sub>3</sub>), and collagen, AgNPcol was created. The characterization process used dynamic. With 30% of all proteins and making up 6% of the human body's weight, collagen is thought to be the most plentiful protein in the animal kingdom. In order to comprehend the stability of the nanoparticle system, the impacts seen in biological systems and the emergence of new chemical medicinal products, studies that look at the interaction between silver nanoparticles and proteins have been highlighted in the literature. AFM, X-ray diffraction, and dynamic light scattering (DLS) were used for characterization. In order to examine the cellular viability of human melanoma cancer (MV3) and murine fibroblast (L929) cells, flow cytometry was used. Using a Franz diffusion cell, the skin permeation investigation was carried out, and AgNPcol's effectiveness in preventing mouse paw edoema was assessed. AgNPcol has a hydrodynamic diameter of 140.7 7.8 nm and a zeta potential of 20.1 0.7 mV, respectively. AgNPcol failed to cause necrosis, late apoptosis, or early apoptosis in L929 cells, although it was more hazardous to cancer cells (MV3) than to healthy cells (L929). AgNPcol promoted skin penetration and avoided paw edoema while showing elevated toxicological effects in cancer MV3 cells.

**Keywords:** Nanoparticle; Silver Collagen; Nanotoxicology; MV3 Cancer Cell

## INTRODUCTION

The fibrous protein collagen can be found in the skin, tendons, bones, and teeth. 30% of the overall body is made up of cartilage, blood arteries, and intestines. Proteins make up 6% of the weight of the human body. Hence, it is regarded as the animal kingdom's most plentiful protein [1]. It serves a variety of purposes in the body, including supporting numerous organs and tissues for energy storage. A rising number of people are interested in using collagen because of its many different qualities [2]. Low allergenicity, low antigenicity, and excellent biocompatibility characterise collagen. It is compatible with natural and synthetic polymers, biodegradable, non-toxic, hemostatic, bioabsorbable, and synergistic with bioactive components. Additionally, it has a strong affinity for water and excellent tensile strength [3]. Up to the 1980s, collagen's value as a biomaterial confined to the manufacture of surgical sutures. At the moment, its applications range from supporting the vascular prosthesis to coating big diameters positioning of developing nerve cells. The expansion of the use of collagen- Due to its abundant occurrence in nature and the diverse variety of ways it can be shaped Due to the intricacy and diversity of the

structure, the inclusion of non-helical domains, the structure of collagen's assembly, and its function, up to 26 different varieties of collagen were discovered in the 1990s [4]. Types I, II, III, V, and XI of collagen are those that form fibrils. Types IX, XII, XIV, XVI, XIX, and XX of collagen are those that are associated with fibrils (types IV, VI, VII, VIII, and X). The fibril-forming collagen is the most prevalent, making up 90% of all collagen in living things and having the ability to form structures with highly structured fibres. The most researched and prevalent type of collagen is type I. It is present in tendons, skin, and 90 percent of the organic bone mass [5].

## METHODS AND MATERIALS

### Synthesis of collagen-based silver nanoparticles

The main element that gives the structures around it stiffness is type I collagen. All of these characteristics imply that type I collagen is a strong contender in investigations using different materials. Studies on the various collagen types are often highly relevant since the variations in their structures and characteristics may be useful in

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therapeutic applications such medication delivery systems, growth factor and cell formation anchoring systems, and tissue repair.

Beyond the realms of biotechnology and health, researchers are interested in using proteins and silver nanoparticles (AgNPs) in their studies. In the literature, information on AgNPs' antibacterial activity, biocompatibility, and adsorption has been presented. The scientific community places a high priority on research involving medical devices for drug delivery and the interactions between AgNPs and proteins in order to change or stop cellular activity with implications for the general public. Understanding the stability of nanoparticle systems, the effects of discovery in biological systems, and the development of new pharmaceutical products used collagen type I, to stabilise the AgNPs starting from a silver nitrate solution can all benefit from research on the interaction of AgNPs with proteins [6]. The researchers carried out a study to evaluate the biocompatibility and antibacterial effects of collagen-stabilized AgNPs. In this investigation, flow cytometry, activity against inflammation, and biological testing with AgNPcol were done. Through the use of flow cytometry, in vitro permeation front analysis, and carrageenan-induced paw edoema, the current work sought to examine the behaviour of stabilised AgNPs with collagen.

### Structural Characterization of AgNPcol

On dried samples, powder X-ray diffraction (XRD) was used to ascertain the phase, crystallinity %, and crystallite size of the silver contained in the nanoparticles. The experiment was conducted utilising a diffractometer, CuK monochromatic radiation, step-scanning mode, range of 8–60°, exposure length of 5 s/step, and step-scanning resolution of 0.02°. Using the MAUD software, more research was done using the Rietveld refinement in an effort to fit the complete powder pattern received from the experiment to the starting model of the phases, which was found by exploring the preceding phase [7]. Model Building was used to geometrically optimise the buildings once they had been developed using HyperChem. Light scattering that is dynamic (DLS). The zeta potential measurements were carried out using an acid solution of AgNPcol on a Zetasizer Nano-ZS.

### Morphological Characterization of AgNPcol

AFM analysis was carried out utilising TT-AFM equipment in vibrating (tapping) mode from the AFM Workshop in the United States. After depositing 10 l of AgNPcol onto a clean mica substrate and allowing the sample to dry at room temperature, 6 m area scans were performed. Utilizing ACT-20 cantilevers with a resonance frequency of around 319 kHz, representative images were created after drying. With the help of Gwyddion software 2.40, images were examined [8].

## RESULTS AND DISCUSSION

AgNPcol's crystallinity profile was determined by XRD, and after being normalised based on the amorphous area, which belonged to the collagen phase, it was analysed using the Rietveld method. Silver and silver chlorides were present in the other phases discovered by ICSD (Inorganic Crystal Structure Database) searches (ICSD 44387 and ICSD 56538, respectively) [9]. The R indices calculated after each refinement cycle, which include the adjustment of the profiles by the least-square of the parameters for lattice cell, peak broadening, and background [10], were used to determine the method's convergence. a diffractogram will be produced once the With a smooth residual curve and low R-values, refinement

demonstrates the data's convergence:  $R_{exp} = 0.96508$ ,  $R_{wp} = 1.45883$ , and goodness of fit  $2 = 1.51161$ . The Rietveld technique determined that the silver phase's crystal size was 138.78832 nm. Calculating the ratio of the crystalline peak area to the entire area of the diffractogram, the percent crystallinity was determined to be 12.67667% crystallite phase [11]. The sample had the AgCl phase in addition to the pure silver phase. As the combination of the nanoparticles doesn't include chloride. The presence of chloride happens just in collagen cleaning and in view of this was the arrangement of AgCl. In any case, there were no periods of silver oxide, appearing that the metal nanoparticle was better balanced out. At an acidic pH, the amino acids along the sort I collagen particle are positively charged, giving the whole collagen particle a positive charge and making gathering arrangement around metallic particles possible. Consequently, the outcomes shown the arrangement of metallic groups encompassed by collagen. These groups collected themselves to lead to the nanoparticles, which were obvious on the off chance that the crystallinity information was broke down at around 12%. Molecule size and still up in the air by DLS and results showed a molecule size of  $140.7 \pm 7.8$  nm and a zeta capability of  $+ 20.1 \pm 0.7$ . The not entirely settled by DLS is in understanding with the crystallite size of the not entirely settled by the Rietveld strategy, regardless of the distinction in the estimation states [12]. Through this information, we can reason that the AgNPcol profile doesn't change with movements of strong media in fluid, and keeps up with its firm construction. The morphology of AgNPcol should be visible in which we can observe circular nanoparticles [13].

## CONCLUSION

Following exposure to nanoparticles, reactive oxygen species (ROS) production may lead to harmful consequences in cells. Based on our earlier findings in cytotoxicology and genotoxicology, as well as information from the literature about the toxic processes of AgNPs, it has been established that the main mechanism caused by nanoparticles is the creation of ROS. Our earlier research demonstrates that AgNPs coated with PVA (polyvinyl alcohol) caused ROS formation, which resulted in DNA damage in HepG2 human cancer (hepatocarcinoma) cells and primary normal human peripheral blood mononuclear cells (PBMC). This method may cause cancer cells to undergo significant extension of apoptosis or necrosis. The literature has described the electrostatic interactions between positively charged nanoparticles and the intended cancer cells. The majority of the time, cancer cells has a lot of anionic.

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