Публикация научно-исследовательской работы

Лекция 4

Основные составляющие статьи

- название;
- введение;
- методы исследований;
- основные результаты и их обсуждение;
- заключение (выводы);
- список использованной литературы.

Название статьи

- максимально краткое изложение ее содержания.
- составляется, как правило, после написания основного текста, когда материалы, подготавливаемые к публикации, их суть и взаимосвязи сформулированы автором на системном уровне.

Название статьи

pharmaceutics

MDPI

Article

A Comparative Study of Cancer Cells Susceptibility to Silver Nanoparticles Produced by Electron Beam

Evgenii V. Plotnikov [1,2], Maria S. Tretayakova [1,3], Diana Garibo-Ruíz [4], Ana G. Rodríguez-Hernández [4], Alexey N. Pestryakov [1,*], Yanis Toledano-Magaña [5] and Nina Bogdanchikova [4,*]

1 Research School of Chemistry & Applied Biomedical Sciences, Tomsk Polytechnic University, Tomsk 634050, Russia
2 Mental Health Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk 634014, Russia
3 Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk 634009, Russia
4 Nanoscience and Nanotechnology Center (CNyN), Campus Ensenada, National Autonomous University of Mexico (UNAM), Mexico City 04510, Mexico
5 Escuela de Ciencias de la Salud, Universidad Autónoma de Baja California, Ensenada 22890, Mexico
* Correspondence: pestryakov@tpu.ru (A.N.P.); nina@ens.cnyn.unam.mx (N.B.); Tel.: +7-3822606117 (A.N.P.); +52-6461366446 (N.B.)

Abstract (Резюме)

Abstract: Introduction: Silver nanoparticles (AgNPs) have a wide range of bioactivity, which is highly dependent on particle size, shape, stabilizer, and production method. Here, we present the results of studies of AgNPs cytotoxic properties obtained by irradiation treatment of silver nitrate solution and various stabilizers by accelerating electron beam in a liquid medium. Methods: The results of studies of morphological characteristics of silver nanoparticles were obtained by transmission electron microscopy, UV-vis spectroscopy, and dynamic light scattering measurements. MTT test, alamar blue test, flow cytometry, and fluorescence microscopy were used to study the anti-cancer properties. As biological objects for standard tests, adhesive and suspension cell cultures of normal and tumor origin, including prostate cancer, ovarian cancer, breast cancer, colon cancer, neuroblastoma, and leukemia, were studied. Results: The results showed that the silver nanoparticles obtained by irradiation with polyvinylpyrrolidone and collagen hydrolysate are stable in solutions. Samples with different stabilizers were characterized by a wide average size distribution from 2 to 50 nm and low zeta potential from −7.3 to +12.4 mV. All AgNPs formulations showed a dose-dependent cytotoxic effect on tumor cells. It has been established that the particles obtained with the combination of polyvinylpyrrolidone/collagen hydrolysate have a relatively more pronounced cytotoxic effect in comparison to samples stabilized with only collagen or only polyvinylpyrrolidone. The minimum inhibitory concentrations for nanoparticles were less than 1 μg/mL for various types of tumor cells. It was found that neuroblastoma (SH-SY5Y) is the most susceptible, and ovarian cancer (SKOV-3) is the most resistant to the action of silver nanoparticles. The activity of the AgNPs formulation prepared with a mixture of PVP and PH studied in this work was higher that activity of other AgNPs formulations reported in the literature by about 50 times. Conclusions: The results indicate that the AgNPs formulations synthesized with an electron beam and stabilized with polyvinylpyrrolidone and protein hydrolysate deserve deep study for their further use in selective cancer treatment without harming healthy cells in the patient organism.

Keywords: electron beam; cytotoxicity; silver nanoparticles; tumor cells; anticancer agent

Введение

- информирует читателя о цели и задачах исследования;
- освещает используемые методы, их достоинства и недостатки;
- содержит обоснование актуальности решения рассматриваемой проблемы;
- представляет факты, подтверждающие новизну полученных результатов.

Актуальность темы

- характеризует степень ее важности в текущий момент времени и в рассматриваемой ситуации для решения данной проблемы (задачи, вопроса);
- показывает необходимость и своевременность проведенного научного исследования.

Введение

1. Introduction

Nanoparticles are widely used in various technology fields, but from a medical point of view, their potential is still far from being discovered. Currently, nanoparticles are used for the visualization of some molecular markers of diseases, diagnosis, malignant tumors treatment, and targeted delivery of drugs with controlled release and accumulation in tissues and organs. Nanoparticles are used as active components, for example, photosensitizers in photodynamic therapy of cancers or hyperthermic tumor destruction by heating nanoparticles [1]. However, the toxicity of nanoparticles for living organisms limits their medical use [2]. The biological properties of nanoparticles significantly depend on their size, shape, stabilizer type, and method of preparation [3,4]. In addition, particle nanosizing often leads to the appearance of new material properties or the enhancement of existing ones. However, this can also increase the potential hazard to human health [2]. The small sizes of nanoparticles (1–100 nm) allow them to penetrate through the epithelial and endothelial layers into the internal environment and body fluids, while migrating and being carried by the blood, penetrating even through dense histohematological barriers including the blood–brain barrier [5]. In this regard, the toxicity of nanoparticles is mainly realized through the following mechanisms: mechanical impact of nanoparticles and, in some cases, the formation of their aggregates with biological molecules; membrane integrity alteration and perforation; catalytic action of nanoparticles; enzymes damage and inhibition with cell metabolism disruption; deactivation of antioxidants and oxidative stress induced by nanoparticles; damage to cell cytoskeleton and internal organelles, primarily mitochondria; tissue inflammatory response and tissue damage due to immune response [6]. In many cases, toxicity is determined by the metal ions' action during the dissolution of nanoparticles [7].

Цель исследования

- должна быть связана с разработкой или применением на практике новых методик, классификаций, приборов, программ, алгоритмов, структур, технологий и т. п.;
- формулируется, как правило, начиная с глаголов типа: выяснить, выявить, сформировать, обосновать, проверить, определить, создать, построить, описать.

Задачи

- основные составляющие исследования, которые необходимо выполнить для достижения указанной цели.

Цель исследования

Therefore, the aim of the present work was to perform a systematic study of the hemolytic properties of Argovit^{TM} AgNPs in a wide interval of concentrations. For this, a variation of parameters important for biomedical applications, such as pH, erythrocytes source (healthy and diabetic donors), and compound type (AgNPs and $AgNO_3$), were performed. The results obtained in this work revealed an unusual and unexpected bell shape of the hemolysis curve. A meta-analysis permitted to reveal the reason why previously this bell-shaped curve of hemolysis induced by AgNPs was registered (but not discussed) only in one publication. The importance of the bell shape for hemolysis data extrapolations is discussed.

Основная часть статьи

- представляет само исследование, использованные методы, полученные результаты и практические рекомендации.

Экспериментальная часть

- Описание изучаемых материалов, методов их синтеза
- Методы исследования с описанием применяемой аппаратуры и условий ее работы
- Условия испытаний изучаемых материалов с формулами расчета параметров/показателей эффективности образцов

Экспериментальная часть

2. Materials and Methods

2.1. Synthesis of Silver Nanoparticles

Nanoparticles were obtained according to the methods described in patents [20,21]. Briefly, the method includes the following steps. First, a solution of collagen hydrolysate 18.8 wt.% (to obtain sample No. 1) or polyvinylpyrrolidone with a concentration of 18.8 wt.% (for samples No. 2 and No. 3). Then, silver nitrate solution necessary to reach 1.2% wt. of AgNPs (12 mg/mL of metallic silver) was prepared and stirred at room temperature until completely dissolved. The resulting silver salt solution was added to a vessel with the appropriate amount of stabilizer solution, intensively mixed, and exposed to an accelerated electron beam (voltage 30 kV) of high-energy (2–2.5 MeV) electrons with an absorbed dose of 15 kGy generated on a linear accelerator ILU-10 (Institute of Nuclear Physics, Novosibirsk, Russia). Electron beam treatment led to stable AgNPs formation. In general, the accelerated electrons have a relatively low damaging effect on organic polymers compared to gamma radiation [30]. For the comparative test of biological activity, all samples were diluted with distilled water. The samples for all tests were denominated as sample #1 (with collagen hydrolysate stabilizer), sample #2 (with polyvinylpyrrolidone stabilizer), and sample #3 (with a mixture of 70% of collagen hydrolysate and 30% of polyvinylpyrrolidone).

2.2. Characterization of the Silver Nanoparticles

2.2.1. UV-Vis Spectroscopy

The optical properties of silver nanoparticles were characterized by measuring their absorption spectrum at the wavelength range from 200 to 800 nm at room temperature (25 °C) by UV−vis spectroscopy (Cary 60 UV-Vis Spectrophotometer, Agilent Technologies, Santa Clara, CA, USA). The absorption spectra of all samples were recorded for dilute aqueous solutions of the corresponding samples. Distilled water was used as a reference sample.

2.2.2. Hydrodynamic Diameter and Zeta-Potential Analysis

AgNPs samples charge and hydrodynamic diameter distribution were determined by dynamic light scattering (Nano-ZS (Malvern Instruments Ltd., Malvern, UK)). The size distribution characteristics and Zeta-potential were measured in aqueous solutions at room temperature 25 °C with an equilibration time of 2 min. All samples were analyzed in triplicate.

Результаты и обсуждение

- Приводятся полученные результаты в виде таблиц, рисунков, графиков
- Приводится описание полученных результатов
- Обсуждение результатов с объяснением наблюдаемых эффектов, выявлением закономерностей, корреляций, предположительных механизмов изучаемых процессов

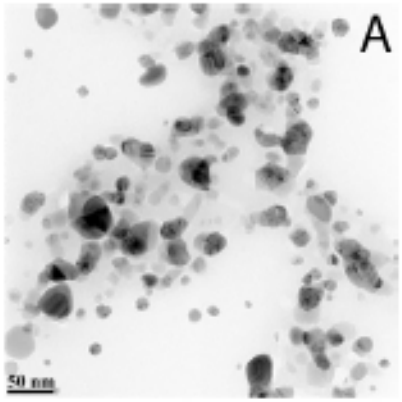
Результаты и обсуждение

3. Results

3.1. Characterization of the Silver Nanoparticles

3.1.1. Transmission Electron Microscopy (TEM)

TEM is the main method for objective assessment of the morphology and size of nanoparticles. Micrographs of AgNPs samples showed that all samples contain detectable particles, located both in an isolated and grouped order (Figure 1).



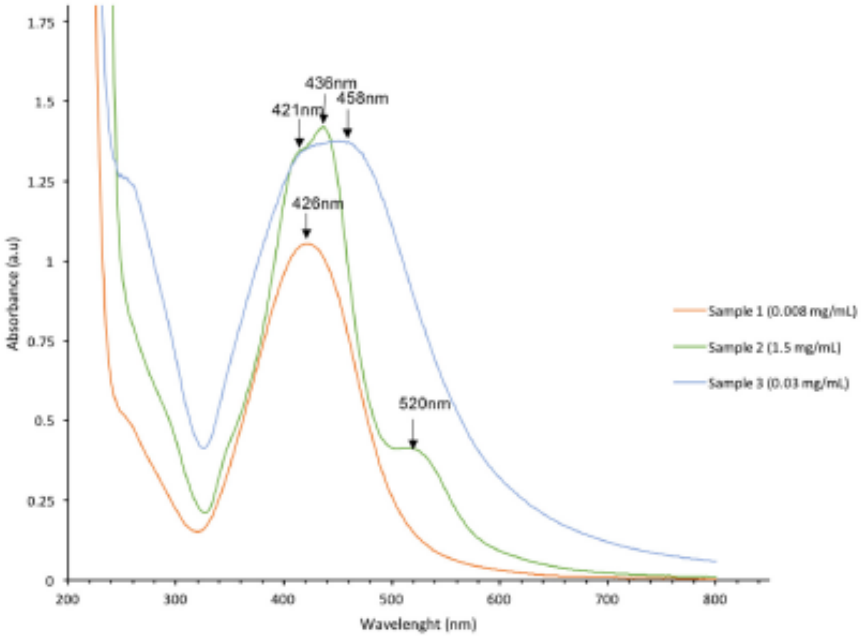


Figure 2. UV-visible spectra of AgNPs samples #1–3 with concentrations of: sample #1—0.008 mg/mL; sample #2—1.5 mg/mL; and sample #3—0.03 mg/mL.

Результаты и обсуждение

Table 1. IC50 of cancer cell lines for different AgNPs formulations.

Preparation Method	Particle Size, nm	Stabilizer	Ag/Stabilizer Concentrations Ratio	Hydrodynamic Diameter, nm	Zeta Potential, mV	Cell Type	IC50, μg/mL	Reference
Commercial product (Colorobbia S.p.A., Vinci, Italy), series PARNASOS NAMA		Solutions were prepared by dissolving AgNPs in culture medium	AgNP 1% in water	20		SH-SY5Y	30.73 ± 3.20	[41]
Bio-reduction of silver nitrate	18			30		SH-SY5Y	10	[42]
Silver nitrate reduction by accelerated electron beam		Combined stabilizer PVP/ protein hydrolysate	1.2/18.8 (wt.%)	142.6	+9.15	SH-SY5Y	0.15	This paper
Silver nitrate reduction by *B. funiculus* cultures supernatant.				20		MDA-MB-231	8.7	[43]
Commercial product (Argovit). Silver nitrate reduction by accelerated electron beam	35 ± 15	PVP	1.2/18.8 (wt.%)	70	−15	MDA-MB-231 MCF-7	2.62 ± 0.027 3.06 ± 0.014	[44]
Silver nitrate reduction by accelerated electron beam		Combined stabilizer PVP/ protein hydrolysate	1.2/18.8 (wt.%)	142.6	+9.15	MDA-MB-231	0.6	This paper
Commercial product (Huzheng Nano Technology Limited Company (Shanghai, China)) 5, 20 and 50 nm.	5.9 ±3.3, 23.8 ± 6.7 47.5 ± 22.1	PVP				MCF-7	0.51 ± 0.02 14.33 ± 5.61 47.64 ± 14.67	[45]
Silver nitrate reduction by *P. fulgens* extracts	10 to 15 nm	*Potentilla fulgens* extract		39.04	−18 mV	MCF-7	4.91	[46]
Silver nitrate reduction by accelerated electron beam		Combined stabilizer PVP/ protein hydrolysate	1.2/18.8 (wt.%)	142.6	+9.15	MCF-7	0.21	This paper
Silver nitrate reduction by flavonoid naringenin	6	naringenin (NAR)	NAR (50 μM) mixed with 2 mM $AgNO_3$	6 ± 1		HCT-116	5	[47]
Silver nitrate thermal reduction by NaBH4		Trisodium citrate		57.4 ± 3.8	−39.4	HCT-116	28.11	[48]
Silver nitrate reduction by accelerated electron beam		Combined stabilizer PVP/ protein hydrolysate	1.2/18.8 (wt.%)	142.6	+9.15	HCT-116	0.63	This paper

Результаты и обсуждение

4. Discussion

4.1. Bell-Shaped Hemolysis Profile

Surprisingly, the curves passing through a well-defined maximum were registered for hemolysis caused by AgNPs at pH 7.4 and 6.8 in both HDE and DDE (Figure 1, blue lines). The segment of the hemolysis curve where hemolysis increased, for AgNPs and $AgNO_3$, was the same, but at concentrations $\geq$1 mM, hemolysis of $AgNO_3$ practically remained constant (except for the case of pH 6.8 in HDE) (Figure 1). Consequently, at high concentrations, $AgNO_3$ was slightly more hemolytic than AgNPs.

For pH 5.6, the profile of the hemolysis curves for AgNPs and $AgNO_3$ drastically changed (Figure 1), which certainly indicates a change in the mechanism of hemolysis. For AgNPs, the bell shape changed to a step shape with a subsequent sharp increase, and for $AgNO_3$ it changed to a usual gradual increase. From Figure 1, it is apparent that pH 6.2 represents a transitional state. While for HDE at pH 6.2, the hemolysis curves were like the corresponding curves at pH 7.4 and 6.8, for DDE, they were more like the profiles corresponding to pH 5.6.

4.2. The Possible Reason for the Bell-Shaped Hemolysis Profile

The study of mechanisms of the processes occurring during hemolysis can explain the bell-shaped profiles, but this requires a series of future experiments. Nevertheless, the approaches presented below could help to guide these future experiments. Erythrocytes-based experimental systems, like many other biological models, are complex. In part, it is because of their chemical content. They include erythrocytes and dilution media, which maintains erythrocytes in an environment similar to human blood. In addition, they contain substances under test, in our case, the AgNPs and $AgNO_3$. Below are some clues to better comprehend the complexities of the erythrocytes-based hemolysis experimental system:

AgNPs complexity. Hemolysis experiments are carried out in an atmosphere containing oxygen, which can oxidize the metallic silver of AgNPs to form Ag^+ silver ions. In solutions containing AgNPs, there is always a balance between AgNPs and Ag^+ ions, which depends on the effectiveness of the protection of metallic silver by stabilizers, AgNPs size, ζ potential, etc.

Результаты и обсуждение

- Приводятся полученные результаты в виде таблиц, рисунков, графиков
- Приводится описание полученных результатов
- Обсуждение результатов с объяснением наблюдаемых эффектов, выявлением закономерностей, корреляций, предположительных механизмов изучаемых процессов

Заключение

- содержит краткую формулировку результатов работы;
- отражает интерпретацию результатов автором;
-содержит выводы, обобщения и рекомендации, вытекающие из проведенного исследования;
- характеризует практическую значимость полученных результатов.

Заключение (Выводы)

5. Conclusions

In this work, we present the cancer cell growth inhibition induced by three AgNPs formulations prepared by an accelerated electron beam of high-energy electrons which confers their unique biological properties. For the three studied AgNPs formulations, it was revealed that cancer cell inhibition by AgNPs was dose dependent, and the main mechanism of cell death was apoptosis. Moreover, the half-max inhibitory concentration for the seven studied cancer cell cultures varies by more than an order of magnitude, possibly due to different proliferation rates and tissue specificity of different tumor cell types. For all three studied AgNPs formulations, the sensitivity of seven cancer cell lines towards AgNPs decreases in the following order: SKOV-3 > PC-3 > HCT-116 > MDA-231 > Jurkat > MCF-7 > SH-SY5Y. The most sensitive to AgNPs were neuroblastic cells (SH-SY5Y), while the less sensitive ones were ovarian cancer cells (SKOV-3). The activity of the AgNPs formulation prepared with a mixture of PVP and PH, studied in this work, was about 50 times higher than the activity of other AgNPs formulations reported in the literature.

Список использованной литературы

– перечень цитированных в статье книг, журналов, статей.

Последовательность формирования списка может быть различной:

- *по алфавиту фамилий авторов или названий документов;*
- *по мере появления сносок;*
- *по значимости документов (нормативные акты, документальные источники, монографии, статьи, другая литература);*
- *по хронологии издания документов.*

Примеры оформления списка использованной литературы

References

1. Darvishi, V.; Navidbakhsh, M.; Amanpour, S. Heat and mass transfer in the hyperthermia cancer treatment by magnetic nanoparticles. *Heat Mass Transf.* **2022**, *58*, 1029–1039. [CrossRef]
2. Sukhanova, A.; Bozrova, S.; Sokolov, P.; Berestovoy, M.; Karaulov, A.; Nabiev, I. Dependence of Nanoparticle Toxicity on Their Physical and Chemical Properties. *Nanoscale Res. Lett.* **2018**, *13*, 44. [CrossRef] [PubMed]
3. Woźniak, A.; Malankowska, A.; Nowaczyk, G.; Grześkowiak, B.F.; Tuśnio, K.; Słomski, R.; Zaleska-Medynska, A.; Jurga, S. Size and shape-dependent cytotoxicity profile of gold nanoparticles for biomedical applications. *J. Mater. Sci. Mater. Med.* **2017**, *28*, 92. [CrossRef] [PubMed]
4. Zhang, Y.; Xu, D.; Li, W.; Yu, J.; Chen, Y. Effect of Size, Shape, and Surface Modification on Cytotoxicity of Gold Nanoparticles to Human HEp-2 and Canine MDCK Cells. *J. Nanomater.* **2012**, *2012*, 375496. [CrossRef]
5. Lombardo, S.M.; Schneider, M.; Türeli, A.E.; Günday Türeli, N. Key for crossing the BBB with nanoparticles: The rational design. *Beilstein J. Nanotechnol.* **2020**, *11*, 866–883. [CrossRef]
6. Ajdary, M.; Moosavi, M.A.; Rahmati, M.; Falahati, M.; Mahboubi, M.; Mandegary, A.; Jangjoo, S.; Mohammadinejad, R.; Varma, R.S. Health Concerns of Various Nanoparticles: A Review of Their in Vitro and in Vivo Toxicity. *Nanomaterials* **2018**, *8*, 634. [CrossRef]
7. Nguyen, K.C.; Rippstein, P.; Tayabali, A.F.; Willmore, W.G. Mitochondrial Toxicity of Cadmium Telluride Quantum Dot Nanoparticles in Mammalian Hepatocytes. *Toxicol. Sci.* **2015**, *146*, 31–42. [CrossRef]
8. Ochoa-Meza, A.R.; Álvarez-Sánchez, A.R.; Romo-Quiñonez, C.R.; Barraza, A.; Magallón-Barajas, F.J.; Chávez-Sánchez, A.; García-Ramos, J.C.; Toledano-Magaña, Y.; Bogdanchikova, N.; Pestryakov, A.; et al. Silver nanoparticles enhance survival of white spot syndrome virus infected Penaeus vannamei shrimps by activation of its immunological system. *Fish Shellfish Immunol.* **2019**, *84*, 1083–1089. [CrossRef]

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Conflicts of Interest: The authors declare no conflict of interest.

Домашнее задание № 2

1. Придумать название статьи
2. Написать Резюме (Абстракт)
3. Описать Экспериментальную часть
4. Написать Выводы