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Research Article

Effect of Sterilized Ag Nano Particles using γ-irradiation on human blood (in Vitro) Biocompatibility

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Abstract

In this study we tested gamma irradiation method of sterilization which traditionally used in medical device industry on silver nano particles and monitored the effect on biocompatibility and other particles, here we investigate the effect of three gamma radiation equivalent dose (5, 10, 15) Sv on the biocompatibility of nano silver with particle size distribution (20 – 40) nm on human blood Exposing nano silver colloid samples to gamma irradiation caused increase in platelet aggregation within (in vitro) method compared to theor treated counterparts. We also observe positive tendency of sterilized silver nano particles to cause platelet aggregation, sensitive in vitro indication of thrombogencity, also positive effect on other blood parameters such as HCT.

Keywords: *Gamma irradiation, human blood Exposing, HCT*

Introduction

Silver nanoparticles as arch product from the field of nanotechnology has gained interest because of distinctive properties, such as good conductivity, chemical – physical stability, catalytic, antibacterial activity, antifungal and anti-viral [Gleiter, H, 2000]. Silver based medical products ranging from ointments and bandages for wounds healing to coated stents have been prove to be effective in retrading and preventing bacterial infections [Abdulrahman K., Dayah N. Raouf,2011]. Nanoparticles present higher surface to volume ratio in relevant for catalytic reactivity and other properties such as antimicrobial activity in nanoparticles. Nano enabled drag delivery has already been successful in delivering drugs to specific cells and also multi- targeting which is essential in the case of sever diseases [Roe D. Karandikar B, *et al*, 2008]· silver nanoparticles can be synthesized and stabilized by peptides protins, DNA and methods exist thus displaying different characteristics of nanoparticles [Ahn S. J. lee SJ, *et al*, 2009] . For nanparticles; like conventional chemical compounds; there are three main routes of exposure ; inhalation, skin absorption and ingestion. At the cellular level, nanoparticles can found in various compartments, and even in the cell nuclens, which contains all the gentic information [De bapriya Bandyopadhyay, *et al*, 2012]. Silver has been shown inviter to be more cytotoxic than gold, especially where the concentration of silver ion exceeds 5 mg/ml, but silver toxicity in highly debated and it is shown as low concentration as 16 -20 mg as sufficient for its antibacterial activity without any toxicity [Jiwen zheng, Jeffrey D. *et al*, 2011]. The smallest capillaries in the body are 5 – 6 mm in diameter. The size of particles being distributed into blood stream must be significantly smaller than 5mm without forming aggregates, to ensure that the particles do not an embolism [Steven K. N. *et al*, 2009]. In particular, silver and gold are the most commonly used nano particles for diagnostics and drug delivery.

The unique chemical properties of colloidal silver make it a promising targeted delivery approach for drugs regen specific cells. Due to small size in combination with varying properties ; it has been suggested that these small particles might show nano-toxicity. In virto studies have indeed demonstrated that high concentration of silver nano particles will have damaging effects cells that can lead to cell death. It remains to determine if silver nano particles will be safe in patients in the long run. In mean time, silver nano particles remain a hot item and these are incorporated in a number of products from device coating to cosmetics [Li T. Park HG, Choi sh, 2010]. when the human body sustains a wound ; blood platelets stick together to help clot the blood and heal the injury but blood clotting is not all ways trigged by an open wound but by a disorder in the bloody that can block entries. This type of platelet aggregation is serious and can life threading. For many patients, physicians prescribe anti- coagulant, but exact dosing can be a challenge. Too much of an anti- coagulant can cause blood bleeding either internally or through skin wound. Too little anti- coagulant and the patient

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arteries may close and cause a heart attack or stroke. To find safe solution to the problem, researchers test effectiveness of nano silver particles as an anti-coagulant. Researchers show nano silver particles has effective controlled on dumping of platelets irrespective of diseases that caused it [A. L. EL – Batal, *et al*, 2013]. The number of adhered platelets was strongly reduced on the coatings containing silver and lore heparin [Nakan T., *et al*, 2006]. This study investigated changes and while blood cells and platelets changes after injection of nano silver particles. The hematology analysis used under normal and treated blood samples was achieved. Erythrocyte parameters and thrombocyte parameters and leukocyte parameters for human blood donors were achieved. This study has examined the physiological effects of nano silver particles on the change of blood properties given the importance and novelty of studies on nano – biotechnology. This study used the first generation of nano silver as a colloidal which was spherical shape with particle size distribution (20- 40) nm. Nano silver particles exposing to sterilization process using standard Gamma irradiation dose on procedure with (5 -15) Sv/ hr for particle integrity [Kiruba Daniel SCG, *et al*, 2014]

Materials and Methods

Nano silver colloidal (20 – 40) nm particles size distribution were prepared by pulsed laser ablation technique using (Q – switched Nd : YAG, λ= 1064, 10 ns pulse duration and E = 600 mJ). The nano particles solution applied to UV– vis spectroscopy measurement (type : shomedzo 20) and atomic force microscopy (type : Angstrom advanced–type AA 3000) for nano silver characterization. For human blood compatibility test; platelets aggragration study, healthy volunteer blood specimens were collected in BD vactainer tubes containing sodium citrated as an anticoagulant; specimens from at least three donors were pooled. The blood specimens were treated with silver nano particles (volume ratio 0.25 ml). Single platelet count and other blood parameters were determined using high quality CBC system (type M-d3 – dermatology analyzer) were conducted using nanosilver solutions exposed to three gamma irradiation doses (5,10,15)Sv as sterirlization procedure for Ag colloidal for blood *in virto* application process.

Result and Discussion

Silver nano particles, after sterilization treatments using gamma irradiation processes were analyzed using UV- vis spectrometer and the spectra were recorded the absorption peak intensity of nanosilver particles indicated to the potential to beam light around (400 – 420)nm related to volume – surface ratio as in fig (1).

Colorant intensity related to prepration method and concentration of ionic particles silver in suspension medium for particle size distribution, fig (2) show spherical shapes of Ag nanoparticles before sterilization process.

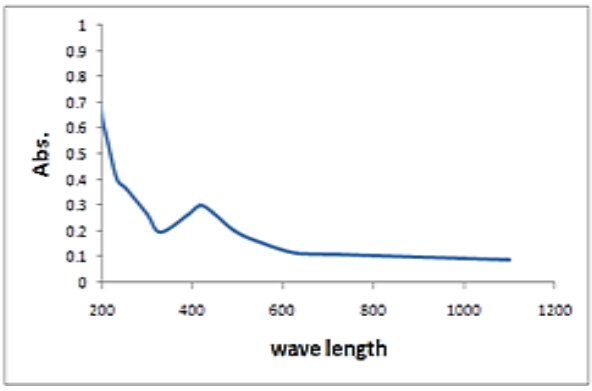


Figure 1: UV.vis spectra of NAg suspension

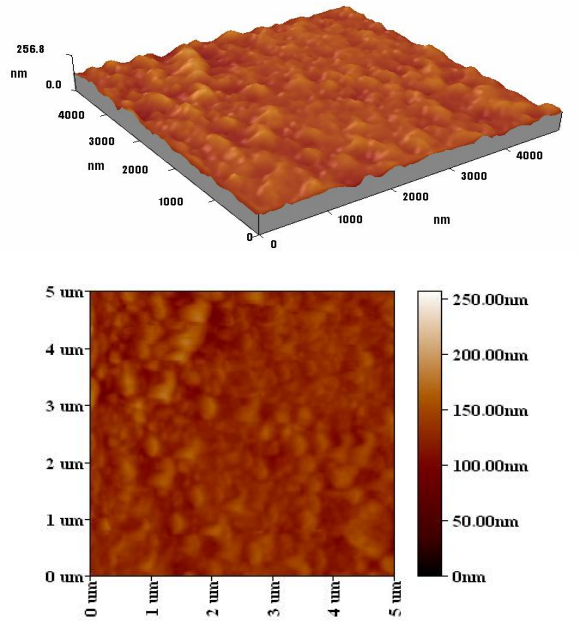


Figure 2: AFM image of Ag Ns solution as thin film

Fig. (2) shows the surface morphology of Ag Ns film with spherical and homogeneity of size distribution below 100 nm.

Table 1

Sample gamma Exposure Sv	Platelet (PLA) Cont. x 10^3		HCT		WBC X 10^3		RBC X 10^6	
N1 (5 Sv)	224	199	44.4	39	5.8	5.0	4.62	4.06
N2 (10 Sv)	280	23	48.1	42	7.9	7.0	5.4	4.7
N3 (15 Sv)	249	36	47.9	44.9	6.1	5.5	5.34	4.72

Gamma irradiation could cause a little aggregations of silver nano particles as shown by some studies. Using irradiated Ag solutions with one of the three dose levels added to the blood specimens and hematology analysis applied. We do observe a relationship between

the γ radiation exposed to silver nano particles and blood properties. The irradiated nano silver effects on the platelet count or reduction factor and HCT, WBC and RBC, as shown in table (1,2).

The reduction in platelet count demonstrate that nano silver particles effectively controlled clumping of platelets to normal human blood. With raising γ dose to (10, 15) Sv ; we observe acceleration in platelet aggregation; that also caused reduction in HTC, WBC and RBC values. Nano silver has added value of providing antibacterial action, and it does not collect in the body, because it is systematically eliminated by the liver and kidneys. The same effect showed to HCT, WBC and RBC parameters which is conduct benfits to high viscosity blood patients.

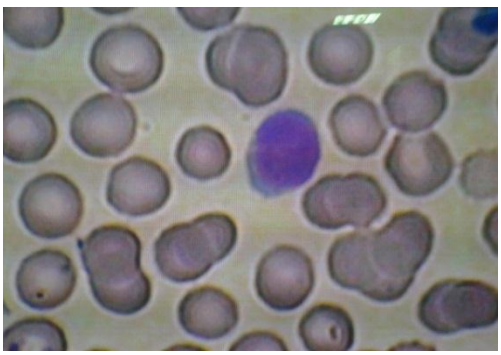
The timing parameter and silver nano particles concentration is too important factors. Table (2), show the count platelet of three blood samples test after (1 min., 30 min., 1 hr).

Table 2

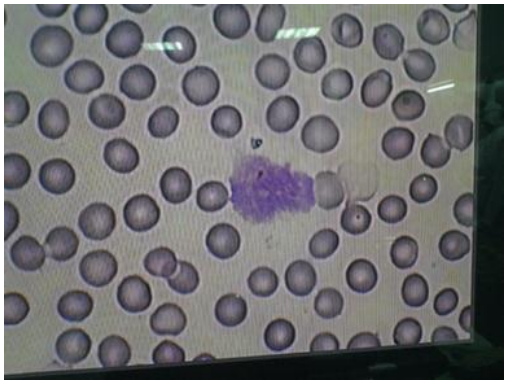
Sample	Time	PLA x10³ (Before adding AgNs)	PLA x10³ (After adding AgNs)
N1 (5 sv)	1 min.	224	199
	30 min.	201	204
	60 min.	208	187
N1 (10 sv)	1 min.	264	46
	30 min.	280	397
	60 min.	260	108
N3 (15 sv)	1 min.	249	36
	30 min.	238	57
	60 min.	249	49

The variation of platelet count to samples treated with Ag Ns compared with control sample detected after (1 min., 15 min. and 1 hour) show decrease in platelet count due to the platelet aggregation and could be used to calculated and repeated 3 times.

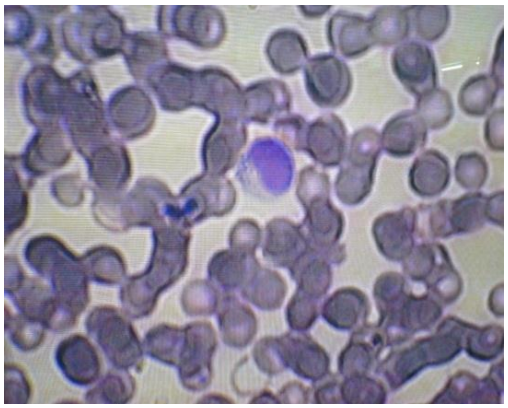
The γ dose (5 sv) show small effect of PLA after period of time, while other doses (10 -15 Sv) show high effect on PLA count and the effect continued even after 1 hr. The effect of Ag Ns on blood properties could be useful for wounds or for surface injuries as coagulant material since it is still effect after period of time (60 min).



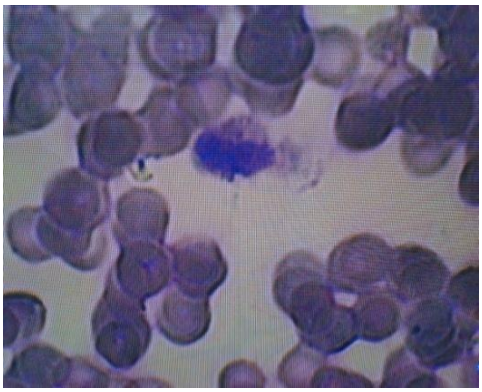
(1)



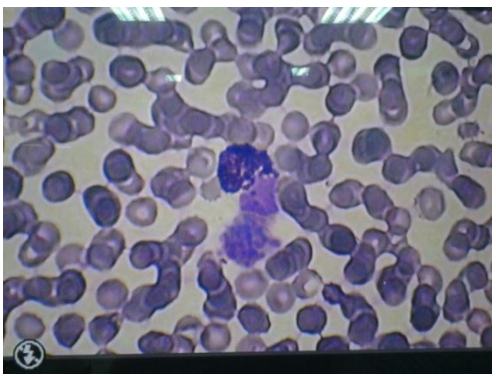
(2)



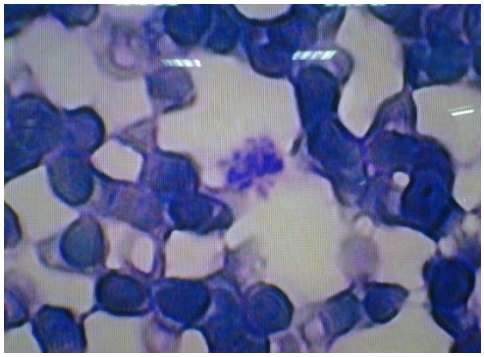
(3)



(4)



(5)



(6)

Figure 3: Effect of Colloidal Silver (AgNs) on PLT, WRC and RBC in vitro application

In this study we test the effect of sterilized silver nano particles on the blood (in vitro) application and observed for gamma irradiated AgNs particles, compared to their untreated samples cause increase in platelet aggregation as shown if fig.(3) where (1,2,3,4,5,6) show the agglomeration between red and white cell and platelet blood during period of time (1-15)min according to gamma irradiation dose.

Conclusions

While using AgNs irradiated with 10 Sv is almost samiler to sample irradiated with Sv, where PLA return to normal condition after 60 min or more. Blood sample N3 seems take long time to return to normal or first condition where PLA($z49 \times 10^2$). From this study, we observe effect of AgNs treated with (5, 10, 15) Sv gamma doses.

In vitro application, seems that AgNs has effect on the PLA count and other blood parameters (HCT, WBC, RBC). The reduction of platelet count related to the aggregation of plates caused by silver nano particles. This study open more fields of researches with different kind of blood doners (patients). From the results, the gamma dose (5-10)Sv seem controlled dose since the effect of AgNs could be accomplished after period of time (1- 60) min.

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