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# A Novel Biophysical Technique for Treatment of Leukopenia in Human Blood Using Diamagnetic Composite Nanoparticles

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**Abstract** Exposure to radiation, suffering from severe diseases for long periods of time, regular intensive chemotherapy, and mineral deficiency may result in leukopenia, a depleted condition of white blood cells in the human body, depending on their levels of intensity. This elevates the body's susceptibility to infection. To date, the primary techniques in practice for the short-term treatment of leukopenia are cytokine therapy and steroid administration, each of which causes serious long-term health effects such as hormonal imbalance. This paper aims at the presentation and experimentation of a new technique for the treatment of leukopenia with minimal side effects by devising and using silver composite nanoparticles. This is the first paper to present a safe bio-physical technique using composite nanoparticles for the immediately effective short-term treatment of infection under leukopenia. The increase in efficiency of a blood sample in fighting against pathogenic bacteria cells on application of this technique in vitro is observed through plotted graphical representations of experimental results.

***Keywords:*** *white blood cells, leukopenia, infection, short-term treatment, composite nanoparticles*

## 1. Introduction

Radiation deposits energy as it traverses through materials or living tissue. As a consequence of this property, materials get deteriorated and cells destroyed or damaged depending upon the level of exposure. Certain kinds of cells are easily affected by radiation. Leukocytes are the cells in the body, responsible for fighting against pathogenic cells entering the body. They have a short life-span and are cells of constantly regenerating type. Such types of cells are stated to be very sensitive and are highly prone to damage by radiation [1].

People suffering from severe diseases such as HIV/AIDS, typhoid, malaria and some types of cancer have reduced leukocyte counts in their blood [2]. Such a condition is caused due the fact that a number of them get killed in the process of fighting against the disease causing germs. Chemotherapy basically attacks rapidly dividing cells including cells in the bone marrow that affects leukocytes [3]. Thus people undergoing regular chemotherapy get prone to leukopenia. Deficiency in certain minerals such as zinc and copper is also believed to result in low white blood cell counts [4].

The depletion of leukocytes leads to leukopenia in blood and consequently leads to a condition in which the body becomes highly prone to infection. The kind of leukocytes whose function is to engulf the foreign bodies, especially bacteria and fungi which enter the body are called neutrophils [5]. Neutrophils constitute about 60% of total leukocytes and thus practically, the depletion of leukocytes implies a decrease in the neutrophil count. Thus it can be approximated that the terms leukopenia and neutropenia imply the same health condition. Bone marrow transplantation is the only effective technique being used over the years for the long term treatment of leukopenia. Donors are necessary for bone marrow transplantation and donation of bone marrow being a biological safety-critical task, donors are difficult to get. The techniques available for short term treatment are primarily cytokine therapy [6] and steroid administration. Recombinant G-CSF (granulocyte-colony stimulating factor) is the cytokine therapy used in the treatment of neutropenia. The Recombinant Human G-CSF hormone can be kept in storage just for a few months from the time of preparation under extremely low temperatures after which it gets expired. Thus this technique cannot be used for the treatment of patients in remote locations.

Further, the use of steroids is associated with far-reaching side effects [7] which include hormonal imbalance, psychiatric disorders and increased tendencies to develop cancers and tumors. As such, the safety levels associated with the short-term treatment methods of leucopenia which are in application today can be observed to be considerably low.

The paper presents a safe bio-physical technique using composite nanoparticles for the immediately effective short-term treatment of infection under leukopenia using composite nanoparticles. The treatment helps to prevent

infection from spreading in any part of the victim's body as it works by increasing the number of neutrophils fighting against pathogens in a particular pathogen-affected region by using injected composite nanoparticles.

## 2. Materials Used

This section describes the materials used for the preparation of the composite nanoparticles along with their properties. The composite nanoparticles are basically a silver nanoparticle coated with a layer of dextran sulfate. Silver nanoparticles are an interesting proposition due to its strong diamagnetism and small size in the order of nanometers. Small size is a very important feature for the composite nanoparticles to undergo proper phagocytosis. Besides this, the particles have considerable biocompatibility [8]. Their presence in blood leads to a very moderate inflammation of acceptable levels. Dextran sulfate is a compound which provides nutrition to bacteria, especially to those of the Lactobacillus species. So these bacteria form a layer on the layer of dextran sulfate. Hence, using Dextran sulfate and Lactobacillus bacteria together is quite ideal for implementing this technique.

## 3. Proposed Method

The aim of the proposed methodology is to improve the rate and efficiency of neutrophils in fighting against foreign entities that enter into the body. According to the proposed methodology, firstly, a region of the victim's body which is being affected by pathogens is taken into consideration. Dextran sulfate-coated silver nanoparticles surrounded by weakened Lactobacillus bacteria (Lactobacillus bacteria are non-pathogenic) are injected into the blood in a region close to the infected area. Silver nanoparticles are strongly diamagnetic and can therefore be controlled by a magnetic field. Dextran sulfate coating attracts bacterial cells which further forms a layer around the nanoparticle as shown in Figure 1. The nanoparticle coated with Dextran and surrounded by weakened bacteria forms the required composite nanoparticles which can be magnetically controlled by repulsion on account of its diamagnetic property. The nanoparticles covered with dextran and weakened bacteria must be about submicrometer range and less moveable as compared to pure nanoparticles. After performing the injection, neutrophils get attracted towards the composite nanoparticles by the weakened bacterial layer through the phenomenon of chemotaxis and in a few minutes, the composite nanoparticles are phagocytosed [9] by them. The silver nanoparticles become a part of the neutrophils engulfed by it and thus the neutrophils can then be magnetically controlled as shown in Figure 2. A strong external magnetic field is applied in order to move the neutrophils move towards the infected region from the region of injection. Such a controlled magnetic field can be generated using Magnetic Resonance Equipment generally used in Magnetic Resonance Imaging (MRI).

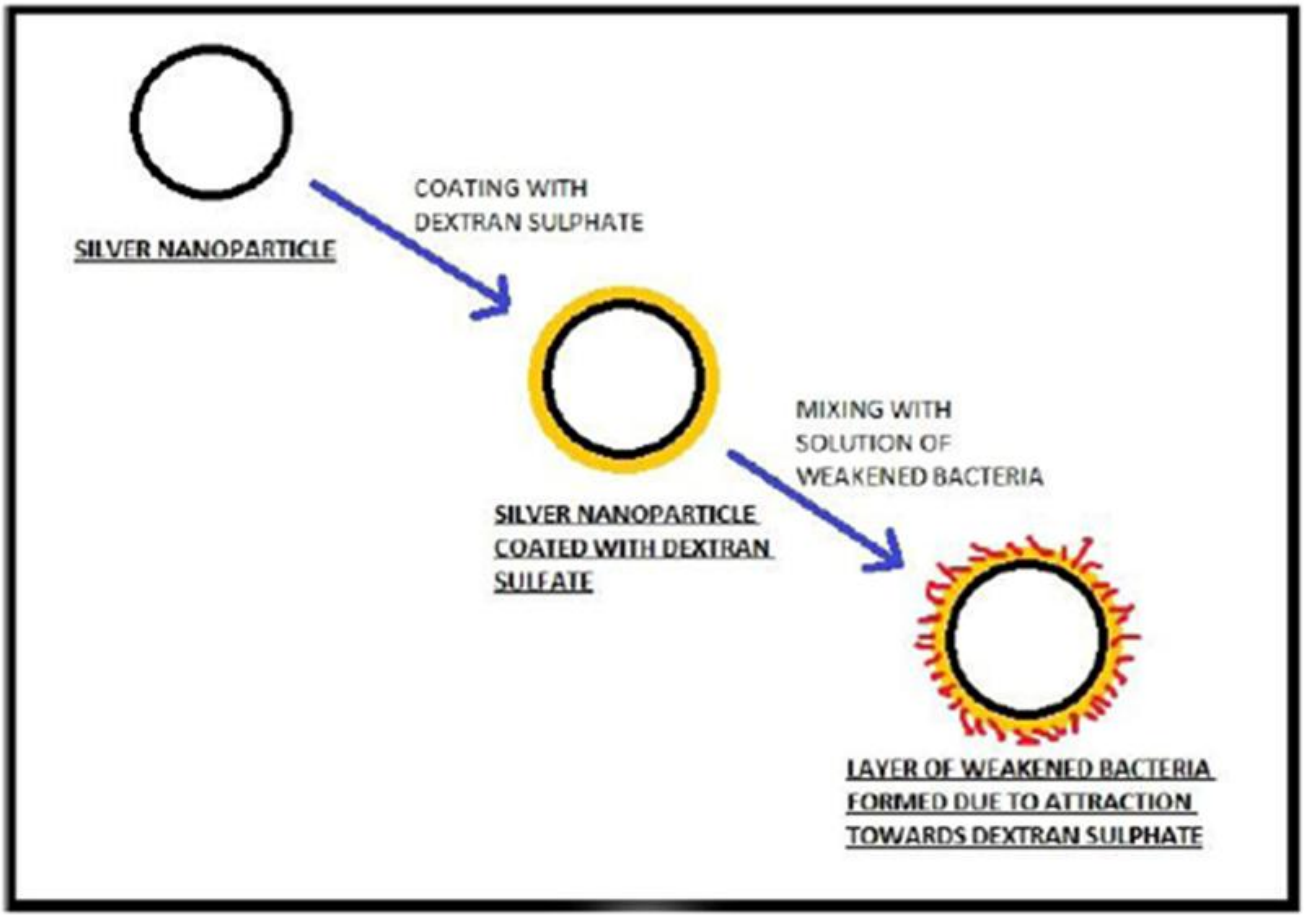


**Figure 1.** Preparation of the Composite nanoparticles

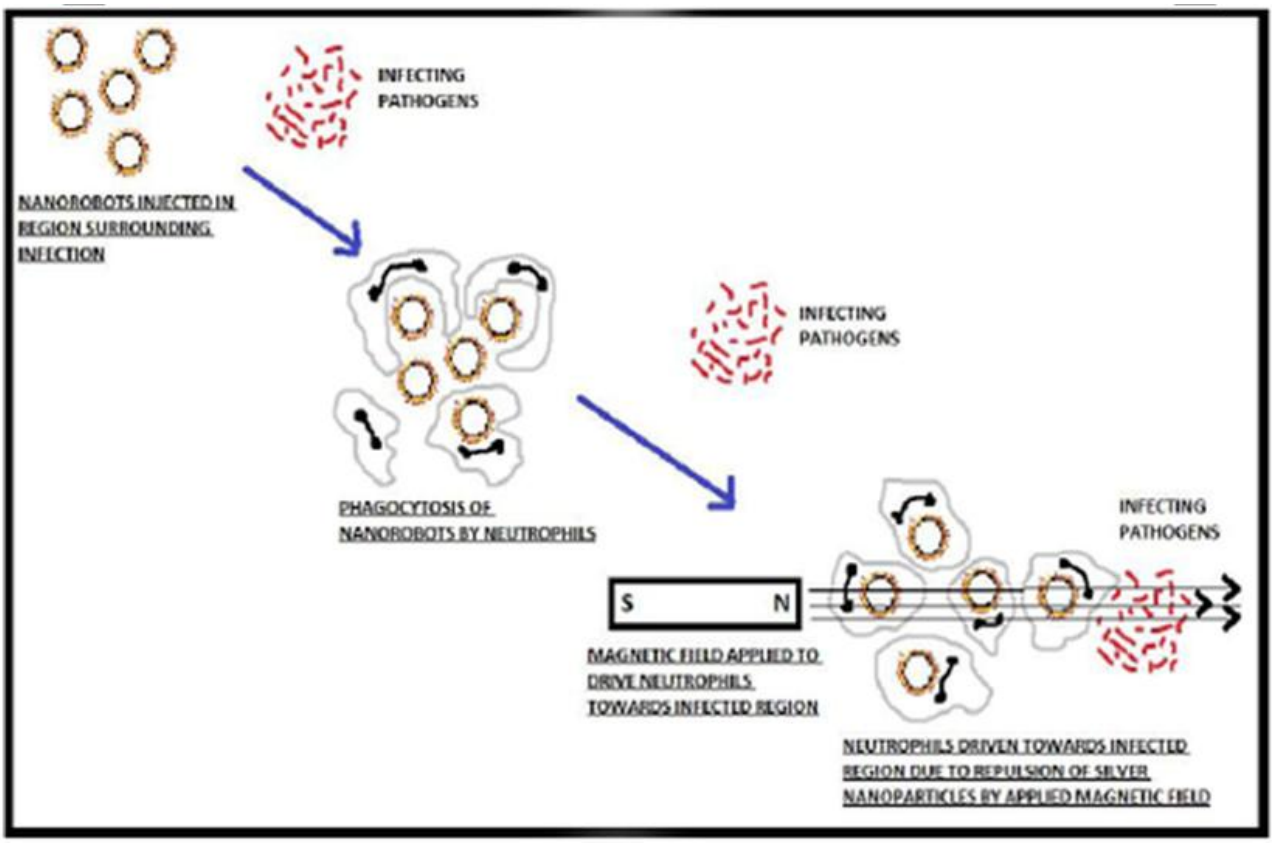


**Figure 2.** Illustration of the Proposed Methodology

This process increases the number of neutrophils fighting against the pathogens in the infected region thus decreasing the rate of spread of infection. Hence this technique can be implemented for immediately effective short-term infection treatment in cases of leukopenic patients whose neutrophil counts are abnormally low.

Since all substances possess a small magnitude diamagnetic property, and since diamagnetism is a weak property, the force exerted by the magnetic field on silver is just a little more than that on its surrounding substances. As a result, the nanoparticles can be reasonably projected to possess a negligible chance to break through the neutrophils under the applied magnetic field. Even if the composite nanoparticles break through the cell membranes of the leukocytes, phagocytosis executes again thus leading to their capture by leukocytes which is desired.

## 4. Experimentation

This section elaborates the experimentation for the analogical in vitro testing of the proposed methodology. The section explains in detail the formation of silver nanoparticles followed by the creation of composite nanoparticles which are tested invitro for observing the improvement in the efficiency of leukocytes in fighting against infection.

This paragraph describes in detail the preparation of silver nanoparticles. The initial step in this process is the aqueous-gaseous phase reaction of silver nitrate solution and ammonia gas [10]. A 50ml silver nitrate solution of concentration $10^{-2}$mol $L^{-1}$ is prepared using double distilled water and crystalline silver nitrate purchased from Merck Chemicals (India). This solution is collected in a 500ml round bottom flask. This flask is subjected to magnetic stirring by placing it on a magnetic stirrer. A 50ml aqueous solution of ammonia of concentration 10mol $L^{-1}$ is prepared from ammonia solution purchased from Alpha Chemika (India) and is collected in a 100ml round bottom flask. The 2 flasks are interconnected by a glass delivery tube. A 40W lamp is placed at a distance of 1 meter from the apparatus in order to maintain the temperature around ~39$^{o}$C by heating. The vivid experimental setup can be referred to in [10]. Ammonia volatilizes from its aqueous solution and travels to the other flask through the delivery tube. As a result, ammonia reacts with silver nitrate to form nanoparticles of silver.

The experimental procedure consists of 5 steps. The word heating in the following steps refers to keeping the 40W lamp in switched on condition. For the first 11 hours the apparatus is kept under stirring and heating. The stirring and heating are halted for the next 13 hours. The third step is to maintain the apparatus in the same condition as in the first step for a period of 10 hours. Step 4 is a repetition of step 2, i.e., maintaining the apparatus without stirring or heating for 13 hours. Finally, the conditions of step 1 are setup and maintained for 7 hours to complete the experiment. The reaction is completed in 54 hours as per the above instructions cited in the referred procedure [10]. The solution of silver nanoparticles thus obtained is taken up into several sample vials for centrifugation [11]. The centrifuged solutions are decanted and then the vials are exposed to sunlight to obtain nanoparticles in powder form. All the powder is collected in a single sample vial.

Once the nanoparticles have been generated, our aim is to prepare composite nanoparticles. The composite nanoparticles consists of a nanoparticle as the core particle

which is covered by a solution of Dextran sulfate with bacteria attached to this layer as discussed previously in the Materials Section. Once the composite nanoparticles are placed in blood, the neutrophils are attracted towards them. They finally engulf the composite nanoparticles. All foreign bodies which enter into the body attract neutrophils but the attraction due to bacteria is much high as compared to other foreign bodies as the attraction in the latter case is defined by weaker chemotaxis chemical reaction when compared to the former case. This is the reason for coating the silver nanoparticles with a layer of weakened bacteria. But for the sake of simplifying the experimental implementation for analogous testing of the design, the lesser effective choice of using silver nanoparticles exclusively is undertaken. In fact, silver particles have been experimentally proved to attract neutrophils as substantiated by an inflammation observed in an *in-vivo* implementation [8]. So we use silver nanoparticles directly for the further steps.

The next step is to apply magnetic field to a blood sample containing nanoparticles in order to drive the neutrophils in the desired direction. A magnetic field of 2kHz is applied to nanoparticles taking into consideration any health hazard from magnetic fields. The "in vivo" may not move in the desirable direction by applied magnetic field, because of the strong blood flow in arteries which can be adjusted by varying the strength of magnetic field. The experimentation is performed on four clean, dry, dust-free glass slides. A glass slide is taken up and using a glass cutter, one of its corners is cut off, leaving a cut edge width of 15mm. This slide serves as a spreader [12]. A volunteer's middle finger is swabbed with laboratory alcohol and pricked with a sterile needle and blood is dropped on two slides - SLIDE-1 and SLIDE-2. The blood sample on SLIDE-1 is taken a reference sample for the experiment. In the case of SLIDE-2, the drop is spread lightly using the spreader such that it occupies an approximate area of $2cm^2$ on the slide. Using silver nanoparticles, an aqueous solution of concentration $0.1mol\ L^{-1}$ is prepared and $0.2cm^3$ of this solution is injected uniformly into the blood sample on SLIDE-2 using a sterilized syringe. This slide is left unoperated for a time period of 65 minutes to allow neutrophils to get attracted towards the nanoparticles and phagocytose them. After phagocytosis, the diamagnetic silver nanoparticles become a part of the neutrophils which have engulfed them, so the neutrophils move away from any magnetic pole placed in their vicinity.

An electromagnet which provides a magnetic flux density of 8 Tesla is made using a soft iron core and an insulated copper wire wounded around the core and connected across a 200V D.C. power supply. This electromagnet is placed at a distance of 8 cm from one of the short edge of the slide. It is used to drive nanoparticles from the section of the blood sample located near the magnet to the section away from it. The motive of doing this is to increase the number of neutrophils in the farther section of the blood sample with respect to the blood sample. The farther half-section can be considered as an analogical equivalent to an infected region of a patient's body where the concentration of neutrophils is to be increased. The half-section which is nearer to the magnet can be considered as the analogical equivalent of the region from which neutrophils are magnetically directed towards the region of infection. The next step of the experimentation is the calculation of the neutrophil counts in the blood samples undertaken.

The following is the procedure for calculating the Differential Leukocyte Count (DLC) as per the standardized procedure for a blood sample. In the next step, blood from the half-section away from the magnet is shifted onto a third slide (SLIDE-3) with the help of the clean spreader from its current location on SLIDE-2. Thus we now have blood taken directly from the volunteer's finger on SLIDE-1, blood from the nearer half-section on SLIDE-2 and blood from the farther half-section on SLIDE-3. The spreader is placed at an angle of $45^o$ to the third slide on the sample and once the blood spreads on the edge of the spreader, it is pulled towards the end of the slide in a single stroke so as to form a thin blood smear. The smear is dried and stained for 2 minutes with Leishman's stain purchased from Alpha Chemika (India). Phosphate buffered saline of double the volume of stain is added to the smear and this setup is left untouched for 5 minutes. Phosphate buffered saline (PBS) of pH 7.2 has been purchased from Alpha Chemika (India). In the further step, the smear is drained and washed in double distilled water. The smear is allowed to dry after which it is observed microscopically at a magnification of 100 X using a microscope - Olympus CX21. All kinds of leukocytes are counted manually by identifying each type's characteristic shape of nucleus. The percentages of each kind of blood cells are observed and tabulated in Table 1.

This procedure is repeated for SLIDE-1 and SLIDE-2 as well and the observations are taken at the same magnification in each case. The Differential Leukocyte Count results are computed for each of the slides.

## 5. Results

The results obtained enable us to investigate the capabilities of our methodology applied in vitro. The superiority of the technique is validated by computing the neutrophils count in three different situations. The count of different cells for the first case (SLIDE-1) is shown in Table 1. The count for the first case acts as a reference value of neutrophils for the experiment. The counts for SLIDE-2 and SLIDE-3 are also shown in Table 1.

**Table 1. Results of Differential Leukocyte Count (DLC)**

| type of leukocyte | percentage in SLIDE-1 | percentage in SLIDE-2 | percentage in SLIDE-3 |
|---|---|---|---|
| Neutrophils | (+-) 51% | (+-) 44% | (+-) 65% |
| Lymphocytes | (+-) 36% | (+-) 43% | (+-) 32% |
| Monocytes | (+-) 7% | (+-) 8% | (+-) 4% |
| Eosinophils | (+-) 3% | (+-) 3% | (+-) 1% |
| Basophils | (+-) 1% | (+-) 1% | (+-) 0% |
| Bands | (+-) 0% | (+-) 0% | (+-) 0% |

It is observed that the percentage of neutrophils in the blood sample on SLIDE-3 is remarkably high when

compared to SLIDE-1 and SLIDE-2. This has happened because of the magnetic field applied on the complete blood sample initially present on SLIDE-2 before being split. As theoretically presumed before, the diamagnetic silver nanoparticles get repelled by the magnetic field and in this process, being a part of the neutrophils which had phagocytosed them, take the neutrophils along with them. As a result, there has been an abrupt increase in the neutrophil concentration in the half section of the blood sample which was located away from the electromagnetic pole on SLIDE-2 initially. This half-section of blood which was later transferred to SLIDE-3 can be analogously compared to a pathogen affected region to which neutrophils are driven from surrounding. Thus the effective working of this technique is clearly evident from the obtained results.

# 6. Conclusion

The concentration of neutrophils in a particular infected region of blood can be increased by gaining magnetic control over neutrophils by making them phagocytose injected diamagnetic silver particles and thus driving them from surrounding regions to the infected region. Therefore this technique can be employed for the immediately effective short-term treatment of infection under leukopenic conditions with the only prerequisites being silver nanoparticle powder and a strong magnetic field setup. Besides, the safety of this technique is justified from the fact that the silver nanoparticles injected are phagocytosed by neutrophils and are later released out from the body along with dead neutrophils and bacteria in the form of pus generated during inflammation thus causing minimal harm to the body. Hence, on account of the feasibility, efficiency, and safety of this technique, it could replace the existing techniques employed for the short-term treatment of infection under leukopenia especially in the emergency cases of patients suffering from leukopenia as a consequence of sudden exposure to intense radiation.

# References


[1] Richard wagner, Norma meyerriecks, Carroll z. Berman, "In Vitro Effects of X-radiation on White Blood Cells and Blood Platelets" *Blood*, Volume 12, No. 8, 733-745, 1957.

[2] Xu B, Liu L, Huang X, Ma H, Zhang Y, et al, "Metagenomic Analysis of Fever, Thrombocytopenia and Leukopenia Syndrome (FTLS) in Henan Province, China: Discovery of a New Bunyavirus", *Plospehogens Journal*, 2011.

[3] S Neudorf, A Nourani, P Kempert, V Shen, L Sender and I Kirov, "Chemotherapy and donor leukocyte infusions for relapsed juvenile myelomonocytic leukemia (JMML)", *Bone Marrow Transplantation* Volume 33, No. 10, 1069, 2004.

[4] Saskia C.A. de Jager, Ilze Bot, Adriaan O. Kraaijeveld, Suzanne J.A. Korporaal, Martine Bot, Peter J. van Santbrink, et all, "Leukocyte-Specific CCL3 Deficiency Inhibits Atherosclerotic Lesion Development by Affecting Neutrophil Accumulation", *Arterioscler Thromb Vasc Biol.* Volume 33, No. 3, e75-83. 2013.

[5] Véronique Witko-Sarsat, Philippe Rieu, Béatrice Descamps-Latscha, Philippe Lesavre and Lise Halbwachs-Mecarelli, Neutrophils: Molecules, Functions and Pathophysiological Aspects, Lab Invest 2000, *Nature*, Volume 80, No. 5, Pg. 617-653, February 2000.

[6] David A.Halfler, Cytokines and interventional immunology, Nature Reviews Immununology, *Nature*, Volume 7, Pg. 423, June 2007.

[7] Jay R. Hoffman, Nicholas A. Ratamess, Medical Issues Associated With Anabolic Steroid Use: Are They Exaggerated, *Journal of Sports Science and Medicine*, Volume 5, Pg. 182-193, June 2006.

[8] M. Böswald, K. Mende, W. Bernschneider, S. Bonakdar, H. Ruder, H. Kissler, E. Sieber and J. P. Guggenbichler, "Biocompatibility Testing of a New Silver-Impregnated Catheter *In-vivo*," *Infection*, Volume 27, Pg. S38-S42, 1999.

[9] Warren L. Lee, Rene E. Harrison, Sergio Grinstein, Phagocytosis by neutrophils, *Microbes and Infection*, Volume 5, Pg. 1299-1306, 2003.

[10] Chunhua Liu, Xiupei Yang, Hongyan Yuan, Zaide Zhou and Dan Xiao, Preparation of Silver Nanoparticle and Its Application to the Determination of ct-DNA, *Sensors*, Volume 7, No. 5, Pg. 708-718, May 2007.

[11] Luminita S. Munteanu, A. Dinu, Fractionation Of Granulocytes From Whole Human Blood By Centrifugation. Practical Hints, *Romanian Journal of Biophysics*, Volume 14, Nos. 1-4, Pg. 53–58, 2004.

[12] Pia Hartmann, Ralph Becker, Caspar Franzen, Elisabeth Schell-Frederick, Jens Romer , Michaela Jacobs, Gerd Fatkenheuer, and Georg Plum, Phagocytosis and killing of *Mycobacterium avium* complex by human neutrophils, *Journal of Leukocyte Biology*, Volume 68, Pg. 297-404, March 2001.