ijlpr 2020; doi 10.22376/ijpbs/lpr.2020.10.5.L127-137

International Journal of Life science and Pharma Research

Research Article

Biotechnology for prospective medical science

Fabrication of Silver Nanocomposite on Copper Metal- A Potential Strategy to Alleviate Pelvic Inflammatory Disease

B. Prabasheela[1]* and V.Sakithya[1]

[1]*Department of Biotechnology, Aarupadai Veedu Institute of Technology, Vinayaka Mission's Research Foundation Paiyanoor, Kancheepuram district, Tamil Nadu, India*

Abstract: Nano silver particle (NSPs), is one of the most attractive nano materials, and has been widely used in a range of biomedical applications, including diagnosis, treatment, drug delivery, medical device coating, and for personal health care. Silver nanoparticles can be synthesized by various methods but citrate reduced and stabilized particles gain an advantage over other methods because of its morphology, stable nature, cost effective and easy scale-up nature. In the present study, the silver nanoparticle was synthesized by reducing silver salt with sodium citrate that showed the characteristic peak at 410 nm by means of UV-Visible spectroscopy. Antibacterial activity was performed against *S.aureus and P.aeruginosa, and S.aureusand* was found to be more susceptible. Characterized colloidal silver nanoparticles were used for the coating process. Coating on copper plate was achieved by Dip method, and characterized using SEM, EDAX and FTIR. Resulting EDAX and SEM images showed the morphology of the coated surface with their elemental composition. FTIR interferogram showed the characteristic functional groups at 1290.09 cm^{-1} and 1483.68cm^{-1} responsible for the reduction of silver salt into silver nanoparticles. Antimicrobial activity was performed for the coated metal against the *Trichomonasvaginalis*, a sexual transmitted pathogen that attribute to pelvic inflammatory disease (PID).The inhibition diameter is of about 31mm, thereby considering the fabricated nano composite to be effective against the disease causing microbial species. Invitro cytotoxicity studies in the *Vero* cell line show 81% viability with no significant morphological alterations. Hence, the fabricated nano composite could be a future benchmark for implant functionalization.

Keywords: Silver nanoparticles, *Trichomonas vaginalis,* intrauterine device, copper, pelvic inflammatory disease

***Corresponding Author**

B. Prabasheela , Department of Biotechnology, Aarupadaiveedu Institute of Technology, Vinayaka Mission's Research Foundation Paiyanoor,Kancheepuram district, Tamil Nadu, India

Recieved On 05 June 2020
Revised On 16 November 2020
Accepted On 18 November 2020
Published On 03 December 2020

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation B. Prabasheela* and V.Sakithya , Fabrication of Silver Nano composite on Copper Metal- A Potential Strategy To Alleviate Pelvic Inflammatory Disease.(2020).Int. J. Life Sci. Pharma Res.10(5), L127-137 http://dx.doi.org/10.22376/ijpbs/lpr.2020.10.5.L127-137

1. INTRODUCTION

Advancement in Nanotechnology has opened new therapeutic horizon and especially Silver Nanoparticles has gained importance because of its extraordinary physicochemical properties and biological activity. The therapeutic action of Silver Nanoparticles against bacterial and viral infection is due to the interaction of microbial biomolecules and silver Nanoparticles[1]. It is widely accepted that NSPs (Nano silver particles) can anchor and penetrate into the bacterial cell wall, thereby producing structural changes in the cell membrane and increase cell permeability, leading to cell death[2]. The other mechanism includes free radicals formation and subsequent free radical-induced membrane damage according to Kim *et al* [3], NSPs can also act by releasing silver ions and interacting with the thiol groups of many vital enzymes and phosphorus-containing bases, thus inhibit certain cell functions, such as preventing cell division and DNA replication [4]. In addition, the antibacterial mechanism of NSPs can modulate signal transduction by changing the phosphotyrosine profile of bacterial peptides [5]. Nano particles has been widely used for diagnosis[6], treating sexually transmitted diseases, including syphilis and gonorrhea[7] drug delivery[8], medical device coating[9], wound dressings and contraceptive devices[10]. Coating of Silver Nanoparticles (AgNps) in different biomaterials to improve their antimicrobial characteristics is presently being studied extensively and has been vastly used in medicine. Intrauterine devices (IUD) are small devices implanted into the uterus for a certain period of time to prevent pregnancy by disrupting the uterine wall or slowly releasing hormones[11-13]. IUD is a safe, long acting and effective method of contraception.[14,15] Copper-containing intrauterine device (Cu-IUD) is the most widely accepted among many kind of IUDs since 1900s[16]. All IUDs, whether inert or medicated, profoundly alter the composition of uterine fluid and the morphology of the human endometrium. The action of contraception is mainly to release the cupric ions uninterruptedly from the device. This release of ions induces inflammatory reactions in the endometrium in response to the foreign elements; create an unfriendly environment that decreases the motility, viability of sperm and the endometrium to implant embryos [17-20]. However, Cu-IUD insertion causes side effects such as pelvic inflammatory disease, a poly microbial infection of the female reproductive tract, and is associated with pelvic pain, abnormal uterine bleeding, and tubal damage that can lead to ectopic pregnancies and infertility. These long-term implants are associated with the problem of bacterial infection by microorganisms besides aseptic loosening and lack of tissue integration during implantation[21]. Thus novel strategies that prevent implant colonization by microorganisms during perioperative period are strongly required. Anti-adhesive surfaces on intravascular and intra urethral devices show reduction of both bacterial cell and protein adhesion [22]. Nanotechnology was employed in the contraceptive field to reduce the side effects and increase contraceptive efficacy.. One way of reducing the side effects and cytotoxicity is to decrease the initial release of copper and recently, different methods were proposed to improve the performance of IUDs[23]. Hence, the present study was aimed to chemically synthesize silver nanoparticles and coat the synthesized particles on copper plates of 1mm thickness similar to that of Copper T and also to determine its dissolution property in simulated uterine medium. In addition, the study also evaluated the cytotoxicity of novel composite in the *Vero* cell line. This may lend credence to the feasibility and safety for applying it as contraceptive material.

2. MATERIALS AND METHODS

2.1. Synthesis of colloidal Silver nanoparticles by chemical reduction method

Silver Nanoparticles were prepared from Silver nitrate and trisodium citrate by using chemical reduction method[24]. In this preparation, 50 ml of different molar concentrations of $AgNO_3$ was heated to 70 °C. To this 5 mL of 1 % trisodium citrate was added drop wise. The solutions were mixed vigorously and heated until change of color was evident (pale yellow). Then it was removed and stirred until cooled to 37°C.

2.2 *Characterization of colloidal silver Nanoparticles using UV-Vis spectrophotometer*

Formation of Silver Nanoparticles was confirmed using UV–Vis spectroscopy Systronic UV 117. The absorbance spectrum of the colloidal sample should be in the range of 200–800 nm[25].

2.3 *Antimicrobial activity*

Antimicrobial activity was carried out with Muller Hinton Agar[26]

2.3.1. *Antibiotic sensitivity test*

Approximately 20ml of melted and cooled media was poured in sterilized petri plates, 24 hours growing culture of *Trichomonasvaginalis* (ATCC 50143), *Pseudomonas aeruginosa* (MTTC 1687) and *Streptococcus aureus* (MTTC 3160) were used. Hi-media discs containing Nanoparticle in solution were placed on the agar plate [27].The plates were then incubated at 37 °C for 24 hours. The inhibition diameter was then measured.

2.4 *Coating (silver nanoparticle to copper metal)*

2.4.1. *Dip coating*

A copper plate of dimension 1x1mm is dipped into the colloidal silver nanoparticle solution. To increase the thickness of the film, the dipping time was increased. Then the coated metal was dried and stored in the oxygen free zip lock cover until the time of characterization [28].

2.5 Characterization of the coated metal

2.5.1 *Surface characterization studies*

The nature of the film formed on the surface of the coated metal plate and control metal were analyzed by various surface analysis techniques like Scanning electron microscope (SEM), Energy dispersive X-ray analysis (EDAX) and Fourier transform infrared spectroscopy (FTIR)[29]

2.6. *Preparation of simulated uterine medium*

Table I: Composition of simulated uterine fluid g/L	
Components	**Weight (Gram)**
NaCL	4.97
KCL	0.224
$CaCl_2$	0.167
$NaHCO_3$	0.25
Glucose	0.5
$NaH_2O_4.2H_2O$	0.072

Chemical immersion tests were carried out in the simulated uterine solution. Table I indicates the composition of the simulated uterine solution [30]. The coated metal was immersed in the solution for 24 hrs to check its dissolution nature, Cu and Ag content were analyzed by EDX analysis.

2.7. *In Vitro Cytotoxicity assay*

Cytotoxicity assays were carried out in SashamBiologicals, Ashok Nagar, Chennai. MTT assay was used to evaluate the cytotoxicity of the composite [31].

2.7.1. *Cell line and culture studies*

VERO cell lines were obtained from King Institute, Guindy, Chennai. The cells were maintained in Minimal Essential Medium supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humid atmosphere of 50 µg/ml CO_2 at 37 °C.

2.7.2. *Reagents*

MEM was purchased from Hi Media Laboratories Fetal Bovine Serum (FBS) was purchased from Cistron laboratories Trypsin methyl thiazolyldiphenyl- tetrazolium bromide (MTT) and Dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of the other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

2.7.3. *In Vitro assay (MTT assay)*

Mosmann method of *in vitro* MTT assay was performed [32].1 × 10^5 well cells were plated in 6-well plates and incubated at 37^0C with 5% CO_2. As the cell reaches the confluence, the sample was added and incubated for 24hrs.After incubation,100µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) was added and incubated for 4 hours followed by 1ml of DMSO to all the wells .The absorbance was measured with UV-Spectrophotometer at 570nm using DMSO as the blank. The % cell viability was calculated using the following formula:

$$\text{Percentage cell viability} = A_{570} \text{ of treated cells} / A_{570} \text{ of control cells} \times 100$$

3. STATISTICAL ANALYSIS

The data were analysed by using MS Excel 2007 and expressed as mean ± SD as three replicates. One way analysis of variance (ANOVA) and Tukey test were performed by using "Stat plus 2009 professional" software to determine the significance and the means were considered as statistically significant if $p<0.05$

4. RESULTS

4.1 *Characterization using UV-Vis Spectrophotometer*

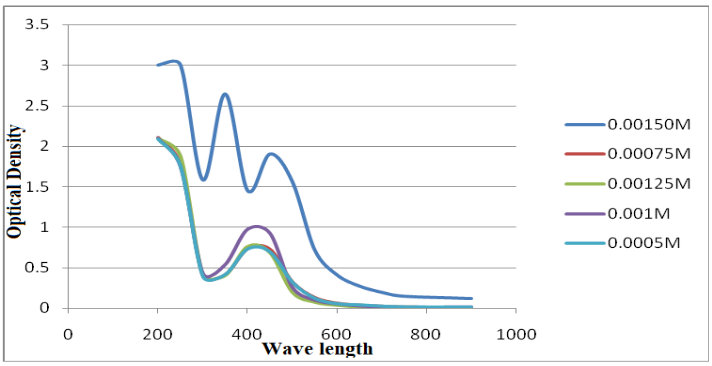
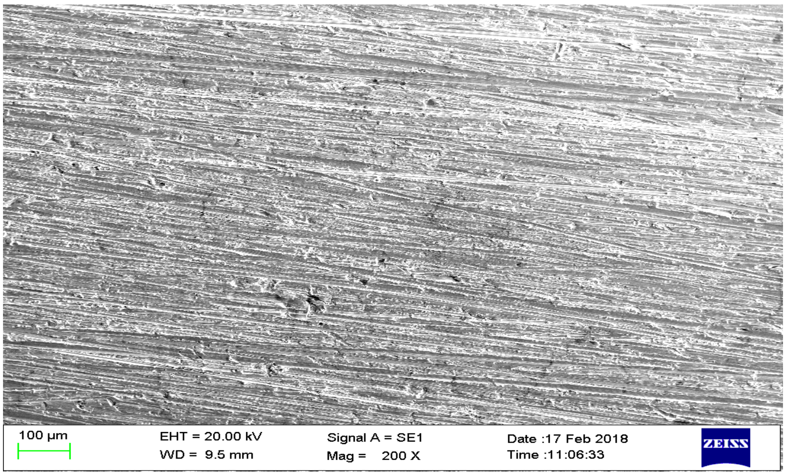


Fig 1: UV-Vis spectroscopy , Characteristic peak in the region of 380nm and 420nm is typical for nanoparticle synthesis.

4.2 *Characterization of coated metal*



The SEM analysis of the copper plate revealed the rough surface of the metal and the elementary analysis shows the purity of the metal plate

Fig.2: SEM image of uncoated copper plate

Table II: Elemental composition of uncoated copper plate

Element	Atomic (%)	Weight (%)
O K	1.35	5.09
Si K	0.84	1.81
Cu K	97.81	93.10

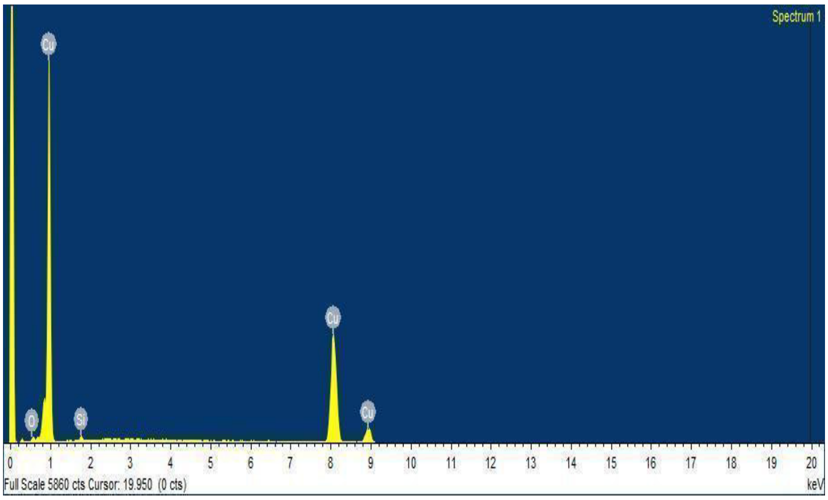


Fig.3: EDAX analysis of uncoated copper plate shows a strong signal for Copper atoms, as well as weaker signals for O and Si atoms.

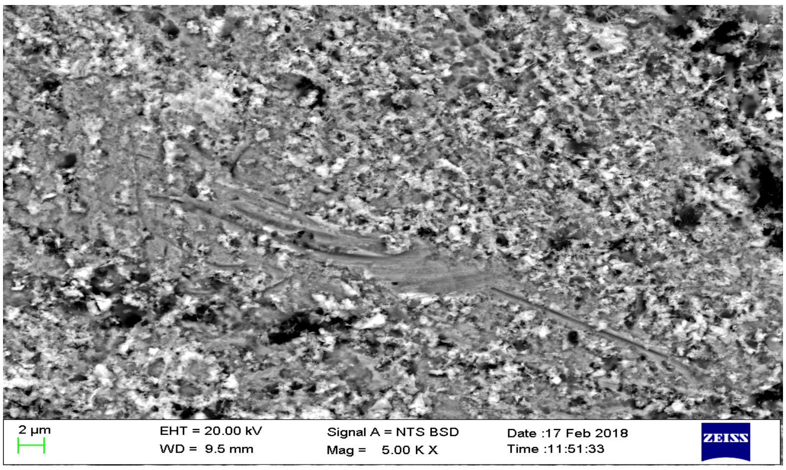


Fig.4: SEM image of dip coated copper plate shows the morphology and uniform coating of silver Nanoparticles over copper plate as aggregates of mixed phase.

Table III: Elemental composition of dip coated copper plate		
Element	**Atomic(%)**	**Weight(%)**
O K	8.23	32.85
Cu K	31.10	31.24
Ag K	60.67	35.91

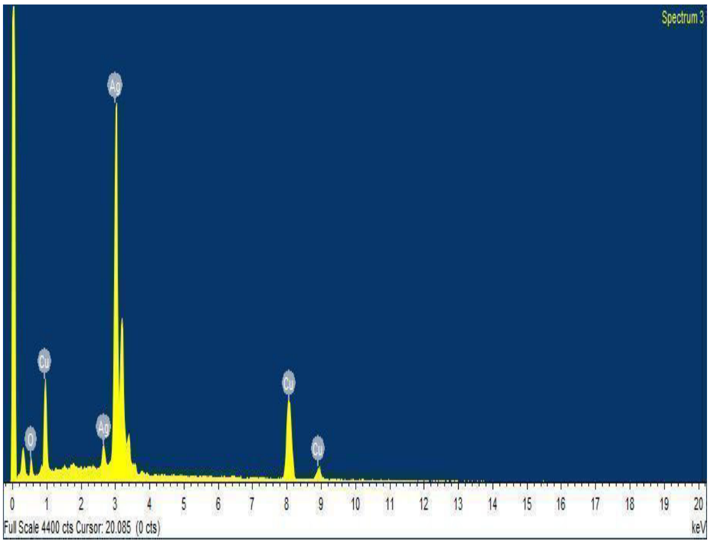


Fig.5: EDAX analysis of dip coated copper plate shows the elemental constituents and relative abundance of AgNPs.The EDX spectrum reveals the percentage of AgNPs coated over copper plate

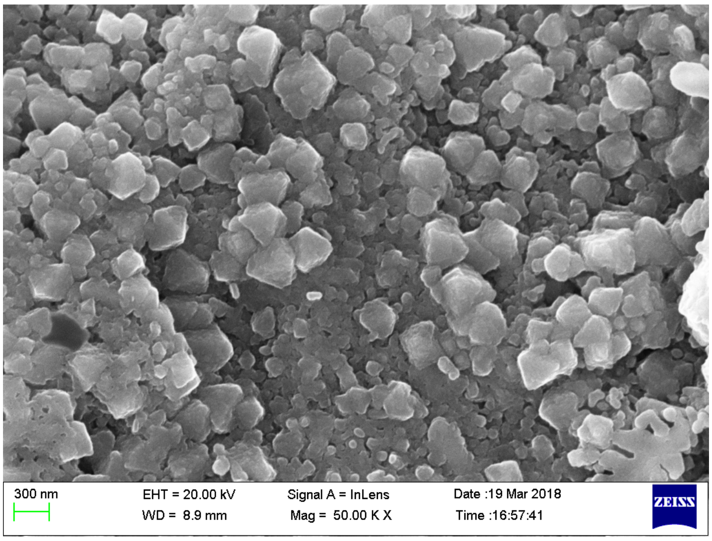


Fig.6: SEM image shows the morphology of the Silver Nanoparticles in Copper plate after dipping in simulated uterine medium. The elementary analysis reveals the percentage of silver Nanoparticles dissolute in the medium.

Table IV: Elemental composition of coated plate dipped in simulated uterine medium		
Element	**Atomic (%)**	**Weight (%)**
O K	13.82	44.49
Si K	0.94	1.72
Cu K	39.32	31.87
Ag K	45.93	21.93

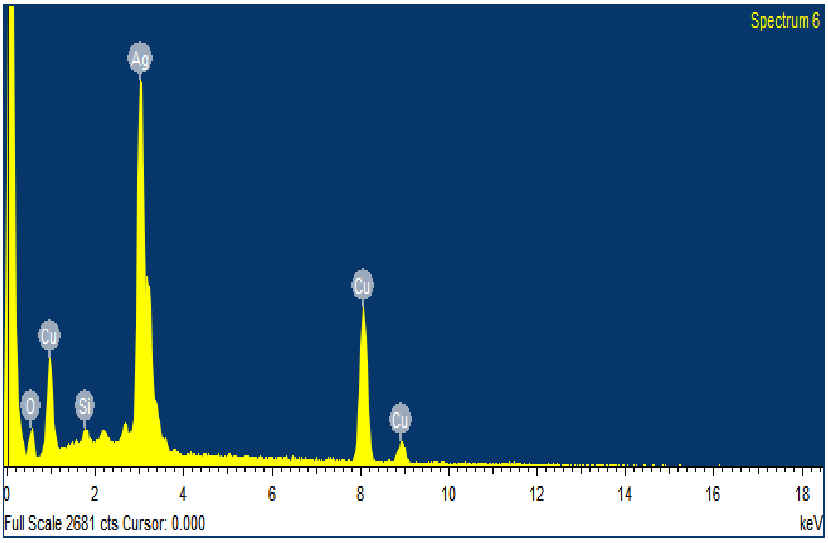


Fig.7.EDAX analysis of coated copper plate after overnight dipping in simulated uterine medium reveals that nearly 45percent of silver Nanoparticles were attached to the copper plate.

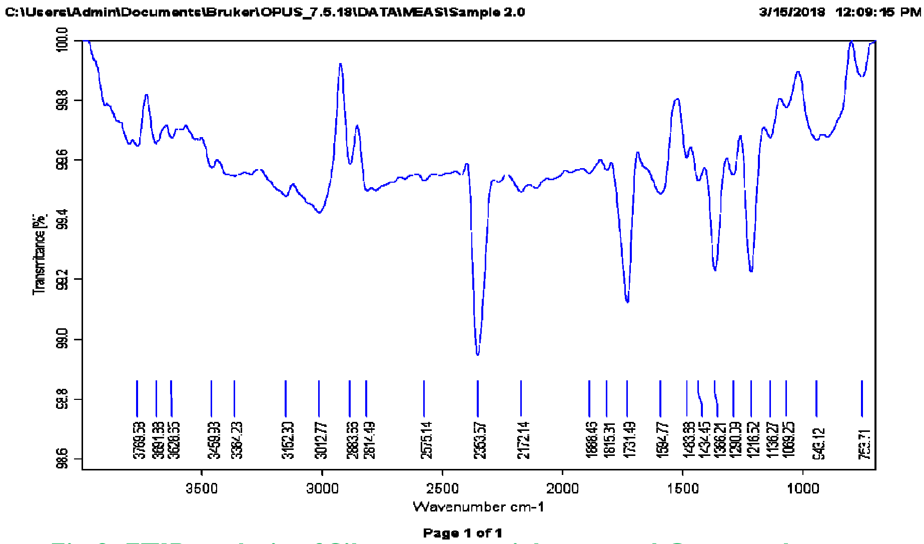


Fig.8. FTIR analysis of Silvernanoparticles coated Copper plate

reveals clear peaks throughout the whole range of observation. FTIR analysis expressed visible bands at 2883.66,2814.49,2353.57,1731.49,1463.68,1366.21,1216.52,943.12,753.71.

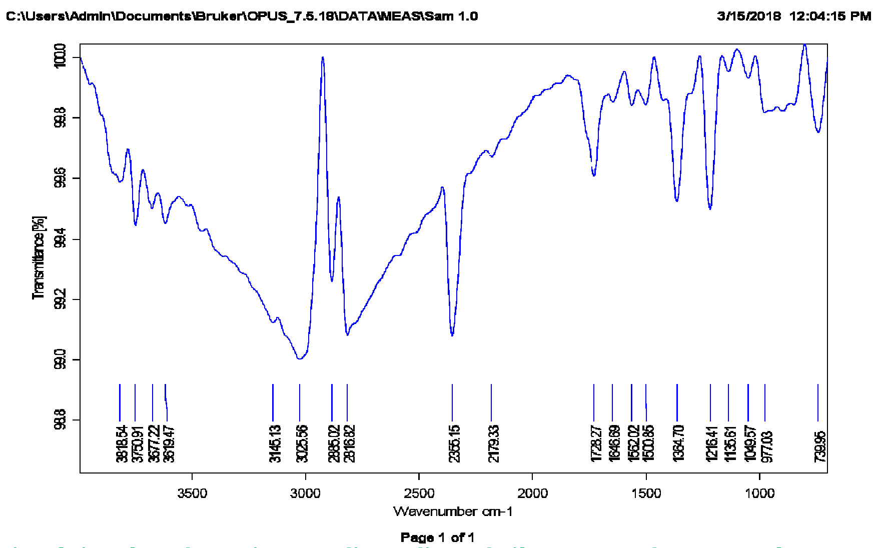


Fig.9. FTIR analysis of simulated uterine medium dipped silver coated copper plate reveals clear peak at 3750.91,3677.22,3619.47,3025.56,2885.02,2816.82,2355.15,1728.27,1364.70,1216.41,977.03,739.

4.3 Antibacterial activity

Fig.10. *S.aureus*

Fig.11.*P.aeruginosa*

The mean of three replicates of the diameter of inhibition zones (in millimeters) around each well with different concentration of AgNPs solution is represented in Table V. *In both the species the Zone of Inhibition was found to increase as the concentration increases* when compared to control whereas the inhibition rate of S.aureus when compared to P.aeruginosa . Hence its potency is dosage dependent.

Table V: Inhibition diameter for antibacterial activity

Organism	Control ± SD	20µl	30µl ± SD	40 µl ± SD	50 µl ± SD
S.aureus	24.5±1.55mm	0 mm	10.4±0.53 mm	12.45±0.98 mm	15.65±1.24mm
P.aeruginosa	25.6±1.89mm	0 mm	8.4±0.20 mm	10.45±0.45mm	14.55±0.50mm

Values are mean ± standard deviation of triplicates. The values were found to be significant ($p<0.05$) when compared to control.

4.4 *Antiparasitic activity*

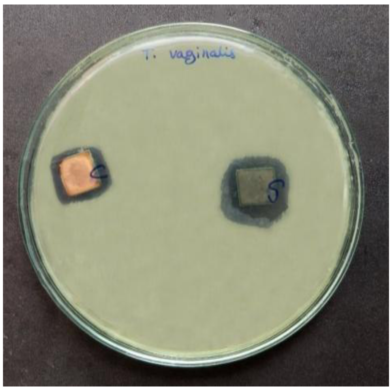


Fig.12. *Trichomonasvaginalis*

Growth inhibition zone of silvernano particles coated copper metal and copper metal on T.Vaginalis. The Zone of Inhibition was found to be higher in silver doped copper metal when compared to uncoated copper metal(Control).This indicate that silvernano particles has more antiparasitic activity when compared to compared to copper metal.

Table VI: Inhibition diameter for antiparasitic activity

Organism	Control ± SD	Sample ± SD
Trichomonasvaginalis	25.65±0.98 mm	31.54±2.35mm

Values are mean ± standard deviation of triplicates. The values were found to be significant ($p<0.05$) when compared to control.

4.5 In vitro cytotoxicity assay

4.5.1 Micrographs of normal and sample treated VERO cell line

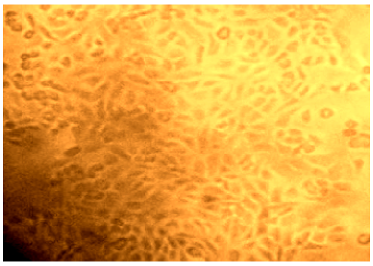


Fig.13.Normal *VERO* cell line

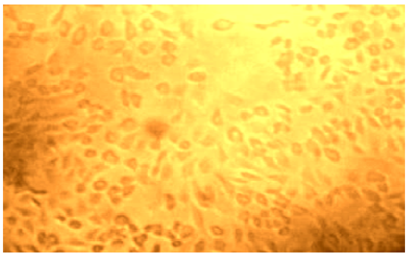


Fig.14.Sample treated cells after 24 hrs

Morphological alteration in Vero cells lines showed no massive cell death on exposure to silver nano composite.

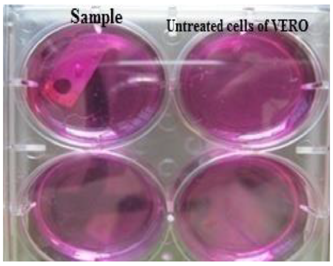


Fig 15 Dip coated copper metal sample in 6 well plate, purple colour change indicates that the reaction has taken place and the viability is proportional to the colour intensity.

Table VII: Cytotoxicity effect of silver nanocomposite on *VERO* Cell line

S.No	Samples	After 24 hrs	
		Optical density(O.D)	Percentage Cell Viability
1.	Sample	0.48	81.35
2.	Control	0.59	100

The viability of cells after exposed to silver Nanoparticles doped copper metal was evaluated by the amount of viable cells stained by MTT assay. The absorbance was read at 570nm by incubating for 24 hours. Percentage cell viability of Vero cell lines indicate that the synthesized silver Nanoparticles are most suitable to perform Cytotoxicity studies.

5. DISCUSSION

Bioengineered nano materials have witnessed a dramatic growth within the previous couple of years to satisfy the growing demand for novel technologies in both the scientific and industrial sectors [33-36].Medical implants though successful in treating disease and injury, it suffers from various complications like infections and inflammatory reactions instigated by bacteria colonizing and biofilm formation. This can be minimized either by Impregnating or coating with antibacterial agent which is one of the strategy and silver Nanoparticles has gained enormous attention [37-38]. Silver nanoparticle is one of the most vital and fascinating metallic nano materials in biomedical application. Plasmon resonance properties of the silver nanoparticles have become the focus of extensive research due to their wide range of applications in the field of medicine, agriculture, catalysis[39], colorimetric sensors, optical encoding, and metal-enhanced fluorescence [40]. SNPs (Silver Nanoparticles) are conventionally synthesized by using chemical reducing agents and stabilizers [41,42]. Different molar concentrations (0.00050mM, 0.00075mM, 0.001mM, 0.00125mM, and 0.00150mM) of aqueous silver nitrate solution and 1% tri sodium citrate solution was used in the present study to synthesis Silver Nanoparticles. Confirmation of synthesized silver Nanoparticles was made by the colour change from colorless to pale yellow. The most widely used technique for nanoparticle confirmation is based on the surface Plasmon absorption band and the synthesized nanoparticle showed the characteristic peak at 410nm at 0.001mM as in Fig1that indicates citrate is a reducing agent. Further investigations were carried out with 0.001mM concentration. The absorption band ranging between 350 nm to 450 nm is typical for the silver nanoparticles [43] .The Ag-NPs has found to be an effective biocide against a broad-spectrum bacteria both Gram-negative and Gram-positive bacteria[44].The antibacterial activity against *Pseudomonas aeruginosa* and *streptococcus aureus* were performed in Mueller Hinton Agar to determine effectiveness of different millimolar concentrations of the synthesized silver nanoparticle. *Streptococcus aureus* were found to be more susceptible than *Pseudomonas aeruginosa as in* Fig 10 and 11. High inhibition zones were observed in 50µl concentration as in Table 5 that might be due to the release of silver ions, from the SNPs–the reservoirs [45]. Fig.3, 4 and 5 showed typical SEM and EDAX results for copper specimen immersed in synthesized nanoparticle solution in comparison to that of uncoated metal represented in Fig.2 .The SEM image of silver nanoparticles was due to interactions of hydrogen bond and electrostatic interactions between the bioorganic capping molecules bound to the AgNPs. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent [46]. FTIR spectra was carried out to investigate the possible functional groups and chemical bonds of citrate-reduced and stabilized SNPs. Interferogram from the FTIR analysis as shown in Fig 8 revealed the presence of characteristic functional groups at 1290.09 cm-1(Nitro compounds), 1483.68cm^{-1}(Aromatics) are in agreement with the findings of [47] and these groups are responsible for the reduction of silver nitrate into silver nanoparticle. Table 2, 3 and 4 establishes the elemental composition of coated plate as their atomic and weight percentage. The oxygen presence indicates oxidation and agglomeration of particles by the formation of silver oxide, in a very minute quantity, whereas the rest of them are still stabilized and also provided with no other new elements on the surface of the composite. Chemical immersion tests were carried out in the SUF (simulated uterine fluid) solution. Prerequisite for *in vitro* measurements is creation of an environment similar to the human womb. As it is well known, human uterine fluid is a complicated mixture. Conventionally, a synthetic medium is prepared for *in vitro* measurements. The fluid consists of the compounds encountered predominantly in human uterine fluid and believed to play an important role in corrosion. This might be the reason for the shift in bands between Fig 8 and 9 that the components in the simulated uterine fluid could have reacted with the silver nano particle coated copper plate. Coated plate was immersed in the simulated uterine solution overnight to check its dissolution nature. Then the overnight immersed metal was dried and characterized using SEM (Fig 6), EDAX (Fig 7) and FTIR (Fig 9), it was observed that immersed metal shows no dissolution. Hence it confirms that the fabricated nano composite was found to be effective. Ag-NPs exhibit excellent antimicrobial activity against various species of fungi [48] such as strains of *Trichophytonmentagrophytes (T. mentagrophytes)* and *Candida albanicans*(*C. albanicans*) that showed 80% inhibitory concentration (IC80). So fabricated metals can destroy the membrane integrity and might cause morphogical changes in the protozoa species (*Trichomonasvaginalis*), one of the causative agents of pelvic inflammatory disease common among IUD users leading to cell death. Result of the antiprotozoa activity showed more inhibition (31mm) in nanocomposite sample than the copper control (25mm) as in the Figure 12 and Table 6 .This confirms that Ag-NPs can act against pelvic inflammatory disease and can overcome various microbial infections among IUD users. Although silver is toxic to mammals but at a low concentration it has proven to be non-toxic to human cells [49], hence it has numerous uses in *in-vitro* and *in-vivo* studies. In order to understand the cytotoxic effect of silver nano composite in non-cancerous VERO cell line, the present study demonstrates the cytotoxicity indices as a measure of percentage cell mortality by MTT assay at the end of 24hr incubation. Resulting MTT assay as in Table 7 showed 81% viability in the sample treated Vero cell line when compared to normal. [50] Studies in cell viability analysis on titanium implants modified with copper found 27ng/mm^2 has 80% viability in fibroblast cell lines. Morphological alteration in V*ero* cells lines upon exposure to Nano composite was observed under phase contrast microscope and showed no indication of massive cell death (Fig 13 and 14) [51] and thus evaluated the cytotoxicity level of TCu220C and Cu/LDPE (Copper/ low density polyethylene nano composite intrauterine device) in L929 fibroblasts . We found that burst release of cupric ions from TCu220C induced high level of cytotoxicity and also significant decrease in cell viability when compared to Cu/LDPE in terms of RGR (Relative growth rate) and CTG (Cytotoxicity grade) . The RGR, CTG of TCu220C and Cu/LDPE were found to be 11.4, 4 and 87.82, 1 respectively[32]. Hence, in our present study the activity demonstrated by the Fabricated Nano composite in the Vero cell line, *in vitro* provides is rationale and suitable for IUD users.

6. CONCLUSION

In conclusion, the most widely used reversible form of contraception worldwide is the intrauterine device (IUD) in the world. The IUD is also the most cost-effective reversible contraceptive method because of its long-acting properties and excellent efficacy. But the problem is development of Pelvic inflammatory disease and no protection against sexually transmitted diseases. Hence the present study focused on coating the silver nanoparticles over copper metal plate of similar thickness to that of Copper T. Characterization studies like SEM, EDAX, UV-Vis and FTIR confirmed the surface morphology, elemental composition and functional groups of coated metal. Fabricated metal were tested for its dissolution rate and activity against *Trichomonasvaginalis*, which is one of the causative for pelvic inflammatory disease common among IUD users in artificial uterine fluid and found to be (31mm zone of inhibition) to be effective. In vitro cytotoxic studies of the fabricated nanocomposite in Vero cell line exhibited 81% cell viability and hence it could be used as future benchmark for implant functionalization.

7. AUTHORS CONTRIBUTION STATEMENT

Dr.B.Prabasheela conceptualized and designed the work. Ms.V.Sakithya carried out the work under the mentorship of Dr.B.Prabasheela. All authors discussed the methodology and results and contributed to the final manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

9. REFERENCES

1. Lara HH, Garza-Treviño EN, Ixtepan-Turrent L, Singh DK. Silver nanoparticles are broad-spectrum bactericidal and virucidal compounds. Lara et al. J Nanobiotechnology. 2011;9:30.
doi: 10.1186/1477-3155-9-30, PMID 21812950.
2. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J Colloid Interface Sci. 2004;275(1):177-82.
doi: 10.1016/j.jcis.2004.02.012, PMID 15158396.
3. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK, Lee YS, Jeong DH, Cho MH. Antimicrobial effects of silver nano particles. Nano medicine. 2007;3(1):95-101.
doi: 10.1016/j.nano.2006.12.001, PMID 17379174.
4. Matsumura Y, Yoshikata K, Kunisaki S, Tsuchido T. Mode of bactericidal action of silver zeolite and its comparison with that of silver nitrate. Appl Environ Microbiol. 2003;69(7):4278-81.

doi: 10.1128/aem.69.7.4278-4281.2003, PMID 12839814.
5. Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D. Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nano technology. 2007;18(22). doi: 10.1088/0957-4484/18/22/225103, PMID 225103.
6. Uchihara T. Silver diagnosis in neuropathology: principles, practice and revised interpretation. ActaNeuropathol. 2007;113(5):483-99. doi: 10.1007/s00401-007-0200-2, PMID 17401570.
7. Gougeon ML, Lecoeur H, Dulioust A, Enouf MG, Crouvoiser M, Goujard C, Debord T, Montagnier L. Programmed cell death in peripheral lymphocytes from HIV-infected persons: increased susceptibility to apoptosis of CD4 and CD8 T cells correlates with lymphocyte activation and with disease progression. J Immunol. 1996;156(9):3509-20. PMID 8617980.
8. Skirtach AG, Muñoz Javier A, Kreft O, Köhler K, PieraAlberola A, Möhwald H, Parak WJ, Sukhorukov GB. Laser-induced release of encapsulated materials inside living cells. AngewChemInt Ed Engl. 2006;45(28):4612-7. doi: 10.1002/anie.200504599, PMID 16791887.
9. Galiano K, Pleifer C, Engelhardt K, Brössner G, Lackner P, Huck C, Lass-Flörl C, Obwegeser A. Silver segregation and bacterial growth of intraventricular catheters impregnated with silver nanoparticles in cerebrospinal fluid drainages. Neurol Res. 2008;30(3):285-7. doi: 10.1179/016164107X229902, PMID 17767809.
10. Chen X, Schluesener HJ. Nanosilver: a nanoproduct in medical application. ToxicolLett. 2008;176(1):1-12. doi: 10.1016/j.toxlet.2007.10.004, PMID 18022772.
11. Stubbs E, Schamp A. The evidence is in. Why are IUDs still out?: family physicians' perceptions of risk and indications. Can Fam Phys. 2008;54(4):560-6. PMID 18411385.
12. , Heinberg EM, McCoy TW, Pasic R. The perforated intrauterine device: endoscopic retrieval. JSLS. 2008;12(1):97-100. PMID 18402749.
13. The ESHRE Capri Workshop Group. Intrauterine devices and intrauterine systems. Hum Reprod Update. 2008;14(3):197-208. doi: 10.1093/humupd/dmn003, PMID 18400840.
14. ACOG practice bulletin. Clinical management guidelines for obstetrician gynecologists. Intrauterine Dev.ObstetGynecol .. 2005;105;59(January):223-32.
15. Sitruk-Ware R, Nath A, Mishell DR Jr. Contraception technology: past, present and future. Contraception. 2013;87(3):319-30. doi: 10.1016/j.contraception.2012.08.002, PMID 22995540.
16. Stanford JB, Mikolajczyk RT. Mechanisms of action of intrauterine devices: update and estimation of postfertilization effects. Am J Obstet Gynecol. 2002;187(6):1699-708. doi: 10.1067/mob.2002.128091, PMID 12501086.
17. Arancibia V, Peña C, Allen HE, Lagos G. Characterization of copper in uterine fluids of patients who use the copper T-380A intrauterine device. ClinChimActa. 2003;332(1-2):69-78. doi: 10.1016/s0009-8981(03)00124-4, PMID 12763282.
18. Beltran-Garcia MJ, Espinosa A, Herrera N, Perez-Zapata AJ, Beltran-Garcia C, Ogura T. Formation of copper oxychloride and reactive oxygen species as causes of uterine injury during copper oxidation of Cu-IUD. Contraception. 2000;61(2):99-103. doi: 10.1016/s0010-7824(00)00085-8, PMID 10802274.
19. Hagenfeldt K. Intrauterine contraception with the copper-T device. 4. Influence on protein and copper concentrations and enzyme activities in uterine washings. Contraception. 1972;6(3):219-30. doi: 10.1016/0010-7824(72)90062-5, PMID 4568282.
20. Roblero L, Guadarrama A, Lopez T, Zegers-Hochschild F. Effect of copper ion on the motility, viability, acrosome reaction and fertilizing capacity of human spermatozoa in vitro. ReprodFertil Dev. 1996;8(5):871-4. doi: 10.1071/rd9960871, PMID 8876046.
21. Horan TC, Gaynes RP, Martone WJ, Jarvis WR, Emori TG. CDC definitions of nosocomial surgical site infections, 1992: A modification of CDC definitions of surgical wound infections. Am J Infect Control. 1992;20(5):271-4. doi: 10.1016/s0196-6553(05)80201-9, PMID 1332552.
22. Pechey A, Elwood CN, Wignall GR, Dalsin JL, Lee BP, Vanjecek M, Welch I, Ko R, Razvi H, Cadieux PA. Anti-adhesive coating and clearance of device associated uropathogenicEscherichiacolicystitis. J Urol. 2009;182(4):1628-36. doi: 10.1016/j.juro.2009.06.008, PMID 19683735.
23. Xu XX, Nie FL, Wang YB, Zhang JX, Zheng W, Li L, Zheng YF. ActaBiomater. 2012;8:886−896.9.
24. Li WR, Xie XB, Shi QS, Zeng HY, Ou-Yang YS, Chen YB.Antibacterial activity and mechanism of silver nano particles on Escherichia coli.ApplMicrobiolBiotechnol. 2010;85(4):1115-22. doi: 10.1007/s00253-009-2159-5, PMID 19669753.
25. Govindaraju K, Tamilselvan S, Kiruthiga V, Singaravelu G. Biogenic silver nanoparticles by *Solanumtorvum* and their promising antimicrobial activity. J Biopesticides. 2010;3(1):394-9.
26. Anandalakshmi, Venugobaland V. Ramasamy. Characterization of silver nanoparticles by green synthesis method using *Pedaliummurex* leaf extract and their antibacterial activity.ApplNanosci. 2016;6(3):399-408. doi: 10.1007/s13204-015-0449-z.
27. MubarakAli D, Thajuddin N, Jeganathan K, Gunasekaran M. Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. Colloids and Surfaces B: Biointerfaces. 2011;85(2):360-5. doi: 10.1016/j.colsurfb.2011.03.009.
28. Innocenzi PC, Guglielmi M, Gobbin M, Colombo P. Coating of metals by the sol–gel dip-coating method. J Eur Ceram Soc. 1992;10(6):431-6. doi: 10.1016/0955-2219(92)90018-9.
29. yadav A, kaushik A, joshi A. Green synthesis of silver nanoparticles using Ocimum sanctum l. and Ocimumamericanum l. for their antibacterial potential. IJLPR. 2018;8(1):42-9.
30. Zhang C, Xu N, Yang B. The corrosion behaviour of copper in simulated uterine fluid.Corros Sci. 1996;38(4):635-41. doi: 10.1016/0010-938X(95)00154-C.

31. Chen ZL, Huang XB, Suo JP, Li J, Sun L. The contraceptive effect of a novel filtering-type nano-copper complex/polymer composites intra-vas device on male animals.Int J Androl. 2010;33(6):810-7. doi: 10.1111/j.1365-2605.2009.01028.x, PMID 20236368.
32. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1-2):55-63. doi: 10.1016/0022-1759(83)90303-4, PMID 6606682.
33. Ninan GB R, N, Muthoosamy K, Graphene MS. A versatile platform for nanotheranostics and tissue engineering.Prog Mater Sci. 2018;91:24-69.
34. Zhu H, Goswami N, Yao Q, Chen T, Liu Y, Xu Q, Chen D, Lu J, Xie J. Cyclodextrin–gold nanocluster decorated TiO2 enhances photocatalytic decomposition of organic pollutants. J Mater Chem A. 2018;6(3):1102-8. doi: 10.1039/C7TA09443D.
35. Hodges BC, Cates EL, Kim JH. Challenges and prospects of advanced oxidation water treatment processes using catalytic nanomaterials. Nat Nanotechnol. 2018;13(8):642-50. doi: 10.1038/s41565-018-0216-x, PMID 30082806.
36. George S, Xia T, Rallo R, Zhao Y, Ji Z, Lin S, Wang X, Zhang H, France B, Schoenfeld D, Damoiseaux R, Liu R, Lin S, Bradley KA, Cohen Y, Nel AE. Use of a high-throughput screening approach coupled with in vivo zebrafish embryo screening to develop hazard ranking for engineered nanomaterials. ACS Nano. 2011;5(3):1805-17. doi: 10.1021/nn102734s, PMID 21323332.
37. Yanez M, BlanchetteJ, Jabbarzadeh E. Modulation of inflammatory response to implanted biomaterials using natural compounds. Curr Pharm Des. 2017;23(41):6347-57. doi: 10.2174/1381612823666170510124348, PMID 28521709.
38. Pallotta A, Clarot I, Sobocinski J, Fattal E, Boudier A. Nanotechnologies for medical devices: potentialities and risks. ACS Appl Bio Mater. 2019;2(1):1-13. doi: 10.1021/acsabm.8b00612.
39. Hebeish A, Hashem M, El-Hady MM, Sharaf S. Development of CMC hydrogels loaded with silver nano-particles for medical applications. CarbohydrPolym. 2013;92(1):407-13. doi: 10.1016/j.carbpol.2012.08.094, PMID 23218313.
40. Shafer-Peltier KE, Haynes CL, Glucksberg MR, Van Duyne RP. Toward a glucose biosensor based on surface-enhanced Raman scattering. J Am Chem Soc. 2003;125(2):588-93. doi: 10.1021/ja028255v, PMID 12517176.
41. Kanmani P, Lim ST. Synthesis and structural characterization of silver nanoparticles using bacterial exopolysaccharide and its antimicrobial activity against food and multidrug resistant pathogens. Process Biochem. 2013a;48(7):1099-106. doi: 10.1016/j.procbio.2013.05.011.
42. Kanmani P, Lim ST. Synthesis and characterization of pullulan-mediated silver nanoparticles and its antimicrobial activities. CarbohydrPolym. 2013b;97(2):421-8. doi: 10.1016/j.carbpol.2013.04.048, PMID 23911466.
43. Aslan K, Lakowicz JR, Geddes CD. Rapid deposition of triangular silver nanoplates on planar surfaces: application to metal-enhanced fluorescence. J PhysChem B. 2005;109(13):6247-51. doi: 10.1021/jp044235z, PMID 16851692.
44. Marambio-Jones CM, Hoek EMV. A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. J Nanopart Res. 2010;12(5):1531-51. doi: 10.1007/s11051-010-9900-y.
45. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. J Biomed Mater Res. 2000;52(4):662-8. doi: 10.1002/1097-4636(20001215)52:4<662::aid-jbm10>3.0.co;2-3, PMID 11033548.
46. Priya AM, Selvan RK, Senthilkumar B, Satheeshkumar MK, Sanjeeviraja C. Synthesis and characterization of $CdWO_4$ nanocrystals. Ceram Int. 2011;37(7):2485-8. doi: 10.1016/j.ceramint.2011.03.040.
47. Janardhanan R, Karuppaiah M, Hebalkar N, Rao TN. Synthesis and surface chemistry of nano silver particles. Polyhedron. 2009;28(12):2522-30. doi: 10.1016/j.poly.2009.05.038.
48. Kim KJ, Sung WS, Moon SK, Choi JS, Kim JG, Lee DG.Antifungal effect of silver nanoparticles on dermatophytes.JMicrobiolBiotechnol. 2008;18(8):1482-4. PMID 18756112.
49. Gurunathan S, Kalishwaralal K, Vaidyanathan R, Venkataraman D, Pandian SR, Muniyandi J, Hariharan N, Eom SH. Biosynthesis, purification and characterization of silver nanoparticles using Escherichia coli. Colloids Surf B Biointerfaces. 2009;74(1):328-35. doi: 10.1016/j.colsurfb.2009.07.048, PMID 19716685.
50. Jung C, Mathes S, Bono E, Walker C, Kessler A, Straumann L, de Wild M, de Haller EB. Cell viability on titanium implant surfaces modified with antibacterial copper. Eur Cells Mater. 2015;29(2):24.
51. Hu LX, He J, Hou L, Wang H, Li J, Xie C, Duan Z, Sun LK, Wang X, Zhu C. Biological evaluation of the copper/low-density polyethylene nanocomposite intrauterine device. PLOS ONE. 2013;8(9):e74128. doi: 10.1371/journal.pone.0074128, PMID 24058521.