Size dependent inhibition of sperm motility by copper particles as a path towards reversible male contraception

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Abstract

Effective inhibition of sperm motility using a spermicide can be a promising approach in developing non-invasive male contraceptive agents. Copper is known to have contraceptive properties and has been used clinically for decades as intrauterine contraceptive devices (IUDs) for contraception in females. Beyond that, the spermicidal use of copper has not been explored much further, even though its use could also subdue the harmful effects caused by the hormonal contraceptive agents on the environment. Herein, we study the size, concentration and time dependent *in vitro* inhibition of bovine spermatozoa by copper microparticles. The effectivity in inhibiting the sperm

motility is correlated to the amount of Cu^{2+} ions released by the particles during incubation. The copper particles cause direct suppression of sperm cell motility upon incubation and thereby show potential as sperm inhibiting, hormone free candidate for male contraception beyond condoms.

Introduction

Regulation of fertility and reproduction is one of the main targets in the human health sector, with egalitarian access to effective contraception means for males[1] and females moving further to the public attention. Even in the 21st century, the female-only contraception methods dominate the market: female sterilization, intrauterine devices, hormonal treatment through oral, injectable and implant administration.[2] Some of these methods are invasive and associated to health problems, for instance, hormonal birth control in women are reported to have mild to severe side effects.[3] In the male contraceptive sector there has not been much advancement besides barrier methods and vasectomy.[1,4] Despite growing demand on the market, only around 30% of couples use a form of male contraception. Lately, efforts in the development of hormonal contraception for men have increased, but many have been associated with side effects and social stigma, so they have yet to be approved. Hormonal approaches are divided into administration of pure androgens, combinations of testosterone with progestogens and combinations with gonadotropin-releasing hormone antagonists,[5] all showing potential as male contraceptives, but longevity and reversibility of action still have to be improved.[6]

Besides the medical drawbacks of hormonal contraception, the drastic effects on the environments have to be considered. The release of hormones into waste water and finally exposure to aquatic life has shown to alter aquatic ecology.[7–10] Both estrogens and progestins have been considered as a potent endocrine disrupters for both humans and aquatic lives even at a very low concentrations (as low as $\mathrm{ng\,L^{-1}}$).[10–12] At lower concentrations it causes ineffective reproduction or feminization of male fishes, and chronic exposure can even lead to extinc-

tion of a fish population.[9] Further, these hormones or their metabolites are very resistant to biodegradation and show high removal inefficiencies.[13] Thus, the focus of contraceptive research toward a more sustainable approach is a viable strategy to restrain the overuse of these hormonal means.

The efficacy of the contraceptive methods is also an important parameter to consider. These efficacy rates can be measured in terms of the pearl index which calculates unintended pregnancies in 100 women in a year of exposure. Male condoms have a pearl index of 2.5 - 5.9 which is higher compared to methods such as intrauterine devices (IUDs) for females (0.16 -1.26).[14] Therefore, prospective male contraceptives will only be impactful if they provide improved efficacy.

The development of a contraceptive that completely ceases the hyperactivation or motility of the sperm cells and avoids the crossing of "blood-testis" barrier has been envisioned as a promising approach in the advancement of future male contraception.[4,15] As a drug, they can be used as injectables,[16,17] where they stay within the seminal fluids and are ejaculated along with the sperms, or if they have an immediate response, they can be even administered just before the intercourse as component of gels or condoms.[4] Here, we demonstrate the suitability of copper as such motion inhibiting agent, bypassing the environmentally critical use of hormones.

The earliest reference of inhibition by cupric ions on mammalian spermatozoa was carried out by de Quatrefages in 1850.[18] Later, further effects of copper in reproductive processes have been investigated.[19,20]

On the one side, copper is a nutritional trace element required for hemoglobin formation,[21] maintaining health of embryos and also involved in the ovulation process.[22] On the other side, copper in excess concentration ($>62.5\,\mu M$ concentration of Cu^{2+} ions[23]) can cause infertility or inhibition of reproductive processes.[24] As an inhibitor, copper affects spermatozoa motility,[25] by suppressing metabolic processes such as glucose consumption and oxidative processes of sperm cells.[23,26] Besides, the Cu^{2+} ions released from Cu form copper chelation

complexes with mucoids which can curb spermatogenesis.[23,24] Additionally, when present in the genital tract, it can induce inflammatory responses which are toxic for spermatozoa and embryos.[27] The main spermicidal action of copper can be attributed to the metabolic and kinetic inhibition of spermatozoa, as found in several other heavy metals as well.

These spermicidal effects of copper in reproductive processes allowed the clinical development of Cu-IUDs in the early 1920s, with Ernst Gräfenberg as one of the pioneers whose work is believed to have led to the first copper compounds in these devices (introduced through a silver wire).[28] Following that, Zipper *et al.*, in 1969 demonstrated the use of copper spirals in plastic IUDs.[29,30] These Cu wires locally excrete low concentrations of copper ions in the uterus and inhibit sperm survival after entering the female reproductive tract.[31] It is now proven to be an effective, non-hormonal, reversible female contraception method and is being widely used since 1970s.[32,33] Safety of such devices is evaluated *via* apoptosis levels in surrounding tissues. These showed no increase in endometrial tissue after exposure to Copper IUDs and is thus considered safe.[34] Disadvantages include disruptions in the menstrual cycle and post-surgical pain and bleeding and implantation involves a small surgery. Therefore, this method is not widely recommended for all women.[24]

Due to its compelling effectiveness and the absence of hormonal residues and metabolites in waste water, the use of Cu in male contraception also gained experimental implications.[35] The group of Kapur and Laumas developed the intravasal Cu (IVDs) which are inserted in the vas deferens and can be used as effective and reversible method for male contraception.[36,37] Through many other research programs, the implantation of copper devices in the male reproductive system also included: the lumen of the ductus deferens, epididymis, scrotum, etc.[26] The biggest concern is the direct implantation of these Cu-IVDs in the scrotum and vas deferens created toxicological effect on the tissues.[38] The development of copper in effective and non-invasive male contraceptive is yet to be accomplished.

Micro- or nanoscience has evolved as a remarkable tool in biomedical applications.[39,40] The use of micro- or nanoparticles is more advantageous than their bigger conjugates due

to their high surface-to-volume ratio and their additional intrinsic properties.[41] The photothermic or magnetothermic effect of metal or metal-oxide nanoparticles has been explored as a reversible, non-surgical method for male contraception.[42,43] However, all these methods involve intravenous administration and testicular hyperthermia might damage the testicles and permanently suppress the spermatogenesis process.[44,45]

Besides a variety of catalytic applications,[46] copper micro- or nanoparticles are being used as a biocidal agents for the reduction and elimination of microbes.[47]

In this manuscript, the contraceptive effect of copper microparticles on bovine spermatozoa is explored. Copper particles of three different sizes are synthesized according to a recently developed strategy[46] and their effect on the overall sperm motility and velocity is investigated (see Figure 1). To demonstrate a dependence on concentration, the release of Cu^{2+} ions is estimated by spectrophotometric and ICP-OES methods.

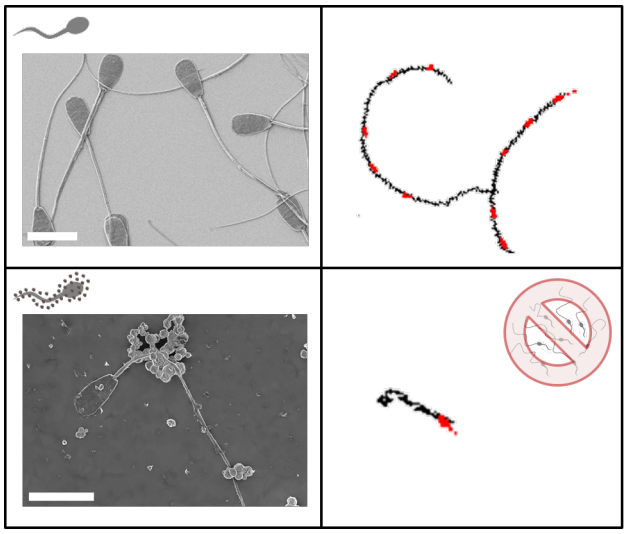

Figure 1: Exemplary SEM images of bovine spermatozoa in the absence and presence of copper microparticles (Cu_2 particles) with the following example track is shown in the upper and lower panel respectively. The tracks represent the motion of sperm cells in sperm medium (control) and in presence of $0.1\,\mathrm{g\,L^{-1}}$ Cu_2 particles in sperm medium, incubated for 15-30 minutes. The tracks are tracked for 220 frames (5.5 s). Scale bar: 10 µm.

Experimental Section

Materials and methods

TL-Sperm (Caisson Labs, USA), sodium pyruvate (100 mM, Gibco, Thermofisher, Germany), gentamicin sulfate (Cassion labs, USA), bovine serum albumin (Sigma Aldrich, Germany) was purchased and used without any purification. Straws of cryo-preserved bovine semen (Masterrind GmbH, Meißen, Germany) was stored in liquid N_2 until use.

$CuSO_4$ (Reagent Plus, ≥99 %), H_3BO_3 (≥99.5 %), H_3PO_4 (85 %), and HNO_3 (65 %) was obtained from Sigma Aldrich, Germany. CH_3COOH (100 %) was obtained from VWR, Germany and polyethylenimine (M.Wt - 10000) from Polysciences, Germany.

We selected three different sized copper particles in the range 0.1 to 0.3 µm labeled as $Cu_{0.2}$, 1 to 2 µm labeled as Cu_2 and 6 to 7 µm labeled as Cu_7 (see Figure 2). The preparation of $Cu_{0.2}$ and Cu_7 is shown elsewhere.[46] Cu_2 particles are formed by solvothermal synthesis described in detail in SI. The particles were characterized using XRD (Bruker D2 phaser diffractometer) and SEM (Zeiss DSM 982 GEMINI electron microscope).

Preparation of sperm medium (SP-TALP)

SP-TALP is a sperm specific medium, it is a tyrode's modified albumin lactate pyruvate medium. In a preparation of 10 mL SP-TALP, 9.5 mL of TL-Sperm is taken and it is supplemented with 500 µL of sodium pyruvate (100 mM), 50 µL of gentamicin sulfate and 60 mg of bovine serum albumin. The medium was kept at 37 °C prior to the motility assay.

Sperm preparation

A straw of bovine semen was taken out from cryogenic storage and quickly thawed in a water bath (at 37 °C) for 2 minutes. The straw was emptied in a 1.5 mL eppendorf tube and to it 500 µL of SP-TALP was added. The sperm sample was then washed by centrifuging at 100 g for 7 minutes. The supernatant was removed and the washing was continued two more

times. Finally, the sperm pellet was resuspended in 500 µL of SP-TALP and kept in the incubator at 37 °C.

Sperm-particle interaction

The synthesized Cu particles were added in SP-TALP in a concentration of 2 mg mL^{-1} (stock Cu particle solution). The samples were sonicated for 10 minutes to properly disperse the particles. They were kept in the incubator at 37 °C for at least 30 minutes before starting the experiments. The stock solution of Cu particles (2 mg mL^{-1}) was diluted with sperm cells to make 0.1 mg mL^{-1}, 0.5 mg mL^{-1} and 1 mg mL^{-1} solutions. A control sample was also prepared without any Cu particles. The dilutions are shown in Table 1.

Table 1: Dilution table for preparation of required concentrations of Cu particles

Concentration of particles (mg mL^{-1})	Amount of stock Cu particle solution (µL)	Amount of Sperm (µL)	Amount of SP-TALP (µL)
0	0	50	50
0.1	5	50	45
0.5	25	50	25
1.0	50	50	0

Sperm motility assay

To understand the influence of the particles on sperm cells, the total amount of motile cells (motility) and velocity of the sperm cells were characterized using an inverted microscope (Axio observer, Carl Zeiss Microscopy GmbH) and recorded with an attached Zeiss camera (Axiocam 702 Mono). The movies were recorded at a frame rate of 40 fps for at least 200 frames with 10x magnification. After incubating the Cu particles with spermatozoa, videos were taken after every 15 minutes time range for 3 distinct intervals marked as Ist, IInd and IIIrd. The time range for Ist, IInd and IIIrd time intervals are 0 - 15 minutes, 15 - 30 minutes and 30 - 45 minutes respectively. The data is obtained for every case by evaluating 4-5 videos

with at least 200 sperm cells. Statistical treatment was performed on the motility with a one-way ANOVA with Bonferroni method (in Origin). To maintain an equivalency within the results, control samples were correspondingly measured in parallel to every particle inhibition case and from the same straw of semen.

Motion analysis

The videos were analyzed using ImageJ software with CASA plugin.[48] With the help of this plugin, we calculated the motility of the sperm cells which is given as the ratio of number of motile sperms to the total number of sperms:

$$\%\text{Motility} = \frac{\text{Number of motile sperms (N)}}{\text{Total number of sperms } (N_0)} * 100\,\% \tag{1}$$

The velocity of the sperm cells is calculated in multiple modes.[49] Here we took into account the three different velocities: The curvilinear velocity (VCL), the straight line velocity (VSL) and the average path velocity (VAP) obtained directly from the plugin.[48] Statistical treatment on VCL for Figure 4 was performed with a one-way ANOVA with Bonferroni method (in Origin).

Detection of Cu^{2+} ions by spectrophotometry

The spectrophotometric determination of cupric ions was commenced following the literature.[50]

Preparation of samples:

$Cu_{0.2}$, Cu_2, and Cu_7 were each added to a vial, containing either DI-water or SP-TALP to make the final concentration to $5\,\mathrm{g\,L^{-1}}$. The mixture was sonicated for 30 minutes and kept overnight to make the dissolved ions be in equilibrium with the medium. On the following day, the sample mixture was centrifuged and the supernatant was taken for further use. Next, the supernatant was diluted with PEI, buffer, water and SP-TALP to make a final

concentration of $0.1\,\mathrm{g\,L^{-1}}$.

General procedure for analysis:

Stock solutions of 0.1 M copper (II) sulfate, $9.4\,\mathrm{mg\,mL^{-1}}$ Polyethylenimine (PEI) and Britton–Robinson (BR) buffer of pH 6 were prepared. The buffer solution was prepared by mixing 0.04 M each of H_3PO_4, H_3BO_3 and CH_3COOH, the pH was then adjusted by 0.2 M NaOH solution. The analysis was done in DI-water and SP-TALP for each case separately.

For water, 30 µL of PEI was added from the stock to make the final concentration of the solution $0.094\,\mathrm{mg\,mL^{-1}}$, then 600 µL of BR buffer was added. Subsequently different volumes of 0.1 M $CuSO_4$ were added to make the Cu concentration 0 to 500 µM. The mixture was diluted with DI-water to make the final volume to 3 mL. For SP-TALP, the same procedure was followed, just the volume of the measuring samples is adjusted with SP-TALP.

The UV absorption spectra are measured using Cary 50 Scan UV-Visible spectrophotometer in the range 200 to 800 nm using a 1 cm path length quartz cuvette.

Detection of Cu^{2+} ions by ICP-OES method

Inductively coupled plasma optical emission spectrometry (ICP-OES) (iCAP 7400 from Thermo Scientific, Waltham, USA) was used to determine the copper concentration. Initially, 8 standards were prepared by diluting a solution containing $10\,000\,\mathrm{mg\,L^{-1}}$ copper in 2 M HNO_3 (Bernd Kraft, Duisburg, Germany). The respective copper concentrations of the standards were $500\,\mathrm{mg\,L^{-1}}$, $100\,\mathrm{mg\,L^{-1}}$, $50\,\mathrm{mg\,L^{-1}}$, $10\,\mathrm{mg\,L^{-1}}$, $5\,\mathrm{mg\,L^{-1}}$, $1\,\mathrm{mg\,L^{-1}}$, $0.5\,\mathrm{mg\,L^{-1}}$, and $0.1\,\mathrm{mg\,L^{-1}}$. From the fivefold measurement of all standards, a calibration curve was constructed. The given detection limit was calculated by the software QtegraTM from Thermo Scientific with $0.02\,\mathrm{mg\,L^{-1}}$. The concentration of every sample was determined fivefold.

For the experiment, 8 mg of Cu particles ($Cu_{0.2}$, Cu_2, and Cu_7) were taken in a vial and 7.68 mL of DI-water or SP-TALP was added to each of them. The mixture was sonicated for 30 minutes and kept overnight for maximum dissolution of Cu^{2+} in solution. After that, the

sample was centrifuged and the supernatant was collected. Next, 1.32 mL of conc. HNO_3 was added to the supernatant, to make the final concentration 890 mg L^{-1}. The solution was further filtered with 2 µm nylon syringe filter to obtain a clean solution without any turbidity. Similarly, two control samples of just DI-water and SP-TALP were also measured.

For both these methods, the detected amount of Cu^{2+} ions is evaluated by the percentage of Cu^{2+} ions released in the medium from the initial starting concentration, elaborately detailed in SI (section S5).

Results & Discussion

Characterization of copper particles

To study the sperm inhibition effect by differently sized particles, we synthesized copper particles of three different size ranges *via* an assisted polyol reaction approach: smaller particles of 0.2 µm ($Cu_{0.2}$), medium-sized particles (Cu_2) and larger particles (Cu_7) (Figure 2A, B, and C).

An additive-facilitated polyol method is employed to synthesize the $Cu_{0.2}$ and Cu_7 particles where simultaneous competing nucleation leads to the formation of copper microparticles in different size ranges.[46] Cu_2 particles are synthesized by a simple hydrothermal method using ascorbic acid as a stabilizer and reducing agent.

The X-ray diffraction pattern (Figure 2D) of these particles exhibit similar reflexes attributing to metallic copper. These reflexes comply very well with the standard face-centered cubic phase of copper (JCPDS No. 040836) and the sharp reflexes depict the crystalline nature of samples.

Although these copper particles have positive surface charges (see Table S1), we did not observe any significant charge based interaction with the sperm cells like positively charged Fe_2O_3 particles,[51] or IRONSperm.[52]

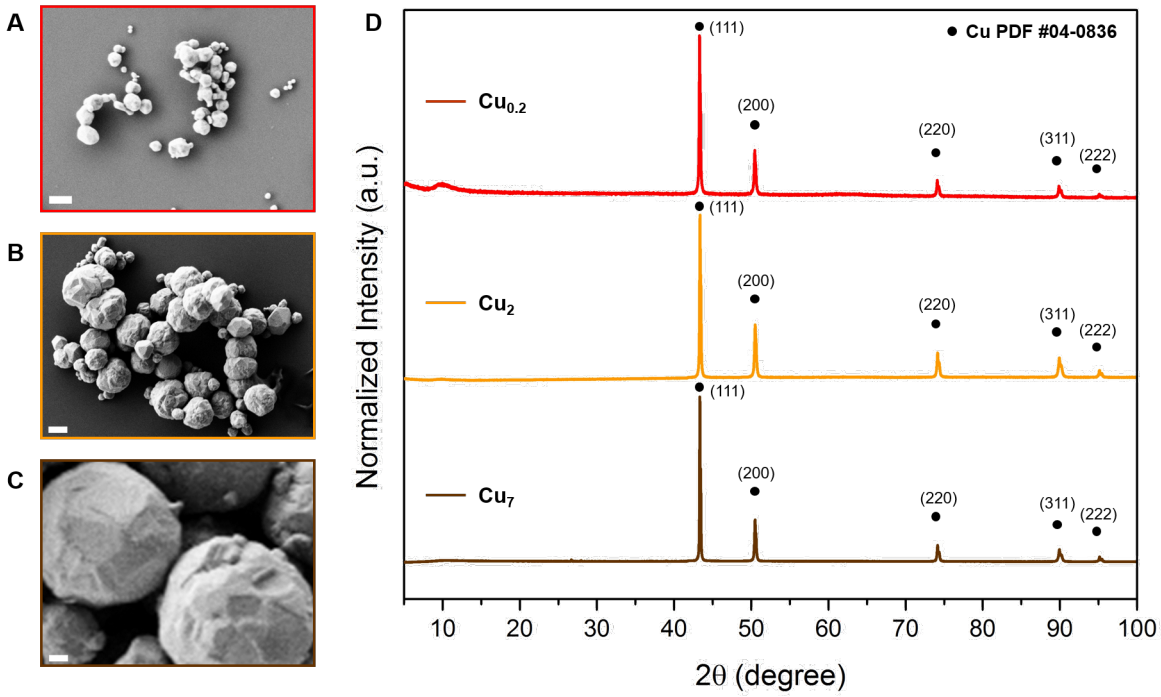

Figure 2: Characterization of the copper particles: SEM images of (A) $Cu_{0.2}$ (B) Cu_2 and (C) Cu_7 particles. Scale bar: 1 µm. (D) XRD reflexes of $Cu_{0.2}$, Cu_2 and Cu_7 particles, compared the standard FCC phase of Cu.

Amount of copper ions released in aqueous media

As mentioned earlier, Cu^{2+} ions cause alterations in metabolic properties and are responsible for the immobilization of sperm cells.[53,54] At higher concentrations, copper ions decrease the mitochondrial activity of the sperm cells,[23] and can further inhibit the the acrosomal reaction between the male and female gametes.[55]

The toxic action of these cupric ions on the sperm cells is extensively influenced by the concentration of ions in the sample solution.[23,54] Therefore, the amount of Cu^{2+} ions released from differently sized colloidal microparticles is investigated which supported the size-dependent effect on sperm cells.

To quantify and compare the amount of Cu^{2+} ions liberated by these differently sized particles, spectrophotometry and ICP-OES as two different approaches were used (detailed in the experimental section). From both methods, the percentage of Cu^{2+} ions released from a known amount of copper particles was calculated and compared for DI water and sperm

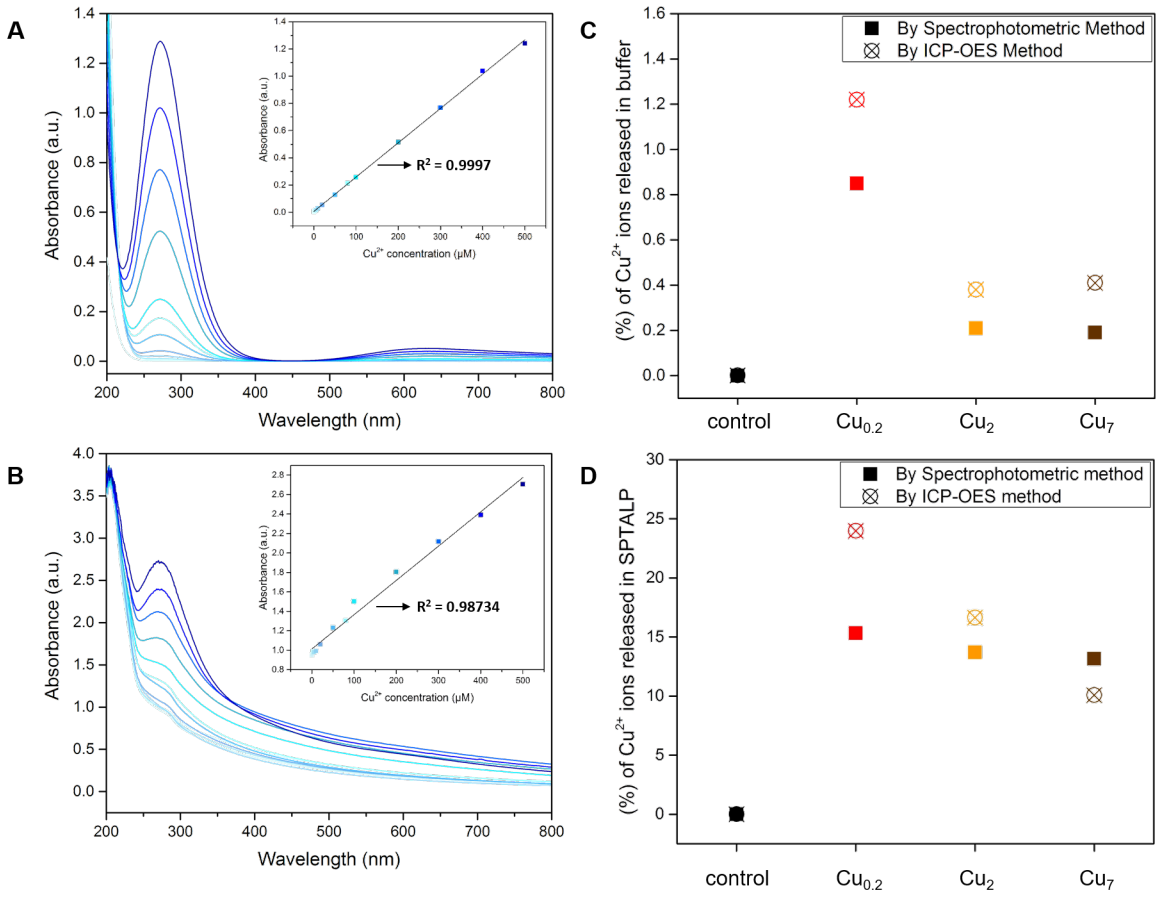

Figure 3: Detection of Cu^{2+} ions: Absorbance spectra of PEI in presence of different concentration Cu^{2+} ions (0 to 500 µM) for spectrophotometric determination in (A) DI-Water and (B) SP-TALP. The inset displays the Cu^{2+}-PEI complex absorbance vs Cu^{2+} ions concentration (0 to 500 µM). The percentage of cupric ions released from differently sized copper samples by spectrophotometry and ICP-OES is compared in (C) DI-Water and (B) SP-TALP.

medium (SP-TALP).

In the spectrophotometric method, a complexation reaction between a cationic polymer polyethyleneimine (PEI) and Cu^{2+} ions was utilized.[50,56] The resulting Cu-PEI moiety showed an absorbance peak at 275 nm and a significantly lower absorbance at 630 nm in aqueous medium. Initially, the absorbance spectra were plotted with a known concentration of Cu^{2+} ions (0 to 500 µM) in water (Figure 3A) and SP-TALP (Figure 3B). The absorbance at 275 nm for these spectra were plotted against Cu^{2+} ions concentrations (µM) and a calibration curve was obtained in both, water and SP-TALP (see inset of Figure 3A and B).

For both these media, the absorption intensity shows a linear positive correlation with Cu^{2+} ions concentration. But, in the case of SP-TALP, the transition from lower to higher wavelength, and the attained absorbance maxima were much greater than that observed in the case of DI water. As the SP-TALP is composed of different bases, phosphates, hydrocarbons, proteins, and minerals, there is a possibility of complexation of these medium constituents with the cationic polymer PEI, leading to these absorption discrepancies.

A quantitative measurement of the copper ion concentration for different sized particles was obtained from ICP-OES analysis.

Since the starting concentrations (in g/L) of the particles in both methods were different, we estimated the percentage of Cu ions released from a given amount of particles to make a uniform comparison (see SI, section S5). Figure 3C and D show the percentage of Cu^{2+} ions released in aqueous and SP-TALP respectively for different size ranges of copper particles. The trend in the amount of Cu^{2+} ions released by both methods was found to be similar. However, it is interesting to note that the amount of Cu^{2+} ions released in water is much lower compared to SP-TALP, for instance in case of $Cu_{0.2}$ particles, by ICP-OES method a maximum of 1.22 % was determined in water as compared to 23.95 % release in SP-TALP. This is probably due to the presence of proteins and bases in the SP-TALP which favors dissolution by complexation of metal ions. As expected, a clear decrease in the amount of Cu^{2+} ions released with a surge in particle size is observed. This reduction is not so prominent in case of SP-TALP, when quantifying the Cu^{2+} ions using spectrophotometry. Whereas a significant decrease of $Cu_{0.2}$ ions percentage is observed using the ICP-OES. This discrepancy can be explained by the possible complexation of PEI and SP-TALP and the resulting error associated with the spectrophotometric analysis in SP-TALP. The ICP-OES gives more accurate and reliable results.

Evaluation of spermatozoa motility in presence of Cu particles in SP-TALP

The effect of differently sized copper particles in the inhibition of sperm cells is studied by evaluating the motility and the velocity of the sperm cells under varying particle concentrations and incubation time. Particle concentrations of $0.1\,\mathrm{g\,L^{-1}}$, $0.5\,\mathrm{g\,L^{-1}}$ and $1\,\mathrm{g\,L^{-1}}$ were used and these tests were conducted over three time intervals of 15 minutes each (see "sperm motility assay" in experimental section). As time progressed, the number of motile sperm cells decreased, so to maintain homogeneity in the measurement results, a control sample (without the Cu particles) was also measured at every interval. This incubation period was estimated over time intervals and not exact time (in minutes) because, as there is a control sample alongside the Cu incubated sample, studying the motion of one sample in microscope changes the incubation time for the other, so it is preferable to indicate a time interval within which these studies were conducted.

Figure 4 A, B, and C shows the average overall motility in percentage of sperm cells after incubating with $0.2\,\mu\mathrm{m}$, $2\,\mu\mathrm{m}$, and $7\,\mu\mathrm{m}$ particles respectively. Similarly, in Figure 4D, E, and F the average curvilinear velocity (VCL) is plotted for the same.

The mean percentage of motile spermatozoa is compared by altering the incubation conditions. The motility of sperm cells in presence of $Cu_{0.2}$ particles within the I^{st} interval (0-15 minutes) drops to around 11 % (89 % of the sperm population was immotile) from its control value with particle concentration as low as $0.1\,\mathrm{g\,L^{-1}}$. The motility further drops to less than 5 % from its control value in the following interval (within 15-30 minutes). The percentage of motile sperm finally drops to zero at the higher interval (30-45 minutes). For higher concentrations, no motile sperms were found and just to indicate the experimental existence, a column bar in the negative direction is plotted (see Figure 4A)

With the increasing Cu particle size (see Figure 4B), the effect on sperm motility changes. Analogous to the $Cu_{0.2}$ particles, the motility of the sperm cells decreases with rise in the concentration of the Cu_2 particles but the rate of decrease is much slower compared to

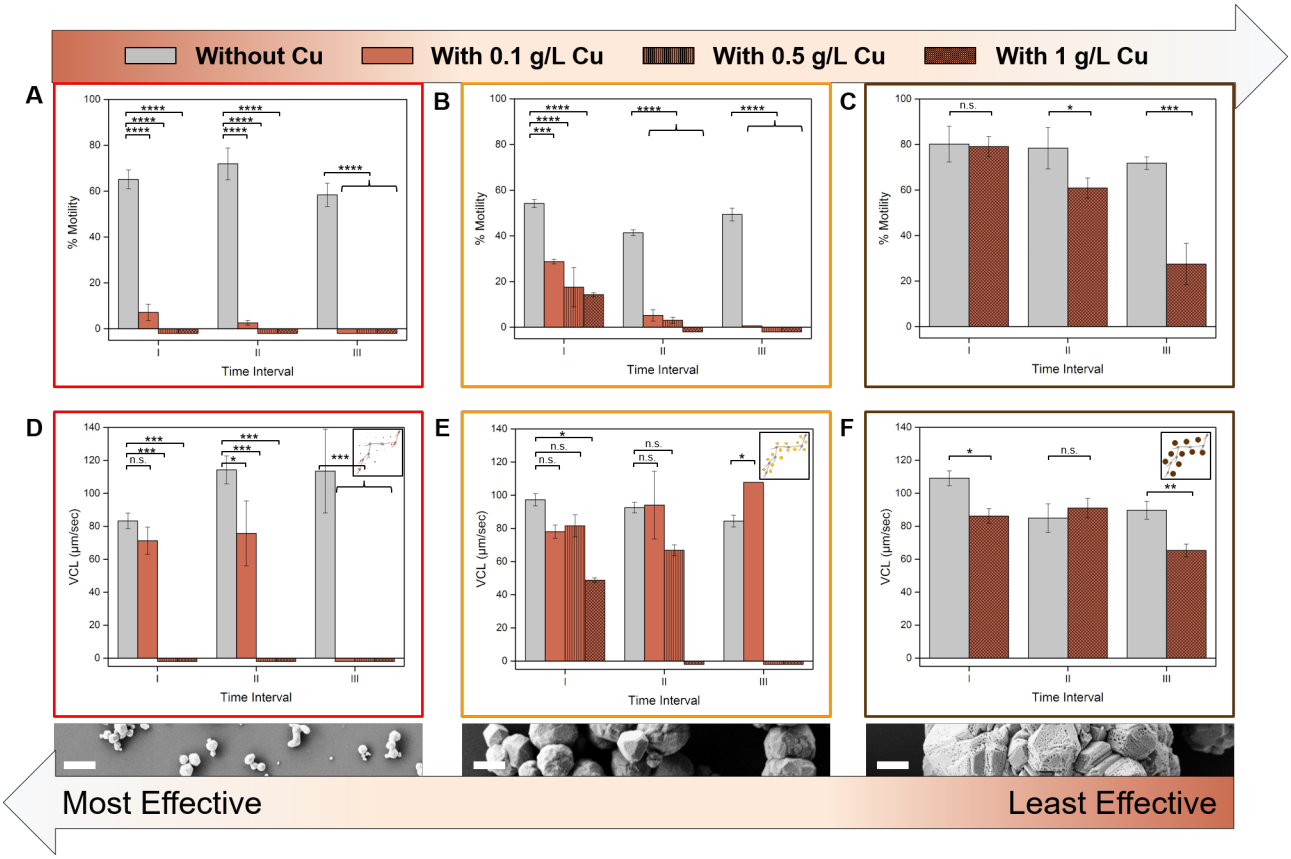

Figure 4: Average motility and curvilinear velocity (VCL) of sperm cells in comparison to a control post-incubation over three time intervals with (A & D) $Cu_{0.2}$, (B & E) Cu_2, and (C & F) Cu_7 particles. The SEM image of $Cu_{0.2}$, Cu_2 and Cu_7 particles are shown below for reference. The inset shows a schematic representation for estimation of motility (in A, B, and C) and VCL (in D, E, and F), respectively. The significance level was set at: **** (P <0.0001); *** (P <0.001); ** (P <0.01); * (P <0.05); n.s (statistically non-significant). Scale bar: 2 µm

the smaller Cu particles. The cells are comparatively more motile at similar concentration ranges. The motility drops to 53 % at 0.1 g L^{-1} concentration of Cu, to 32 % at 0.5 g L^{-1} and to 26 % at 1 g L^{-1} concentration compared to the control during the I^{st} interval. In the II^{nd} interval, the motility gets further reduced to 12 % at 0.1 g L^{-1}, to 7 % at 0.5 g L^{-1}, and completely diminishes at 1 g L^{-1} concentration of Cu. In the III^{rd} interval, the sperm sample with the lowest concentration of Cu show some motility but it is not even 1 % compared to its control.

With Cu_7 particles, a not so prominent decrease in the sperm motility was noted. At

the highest Cu concentration ($1\,\mathrm{g\,L^{-1}}$) and highest incubation time (III^{rd} interval) still 38 % sperm cells were found to be motile compared to the control (see Figure 4C). As there was not such a drastic decrease in sperm motility even at the highest Cu concentration, the experiments with lower concentrations were not performed.

The effectiveness of the $Cu_{0.2}$ over Cu_2 and Cu_7 particles can be correlated to the amount of Cu^{2+} ions liberated by the Cu particles in the SP-TALP. As the size of the particle decreases, more amount of Cu^{2+} ions are released in the medium (see "Amount of copper ions released in aqueous media" in results and discussion section) and it is more effective in inhibiting sperm viability.

The curvilinear velocities (VCL) of the sperm cells lie in the range of $60\,\mu\mathrm{m\,s^{-1}}$ to $120\,\mu\mathrm{m\,s^{-1}}$. With the Cu particles of different size ranges and concentrations, the motile sperm maintained their velocity within this velocity range, a convincing trend is hard to predict. There was a slight decrease in the velocity compared to the control, for instance, in the case of $Cu_{0.2}$ particles, (see Figure 4D), the velocity decreases by 15 % in I^{st} interval and by 34 % in the II^{nd} interval compared to the control. A similar decreasing trend was observed for Cu_2 particles (see Figure 4E) in the I^{st} interval and for Cu_7 particles in I^{st} and III^{rd} interval (see Figure 4F). Although this decrease was not consistent and the presence of copper particles might not influence the velocity of the motile sperms. The VAP (average path velocity) and VSL (straight line velocity) show a similar trend to the VCL and are plotted in SI Figure S2.

Conclusion

Copper particles of three different sizes were synthesized by assisted polyol method.[46] When these particles were incubated with bovine spermatozoa they showed inhibiting effects on sperm motility, which was studied *in vitro*. The inhibition efficiency varied with concentration and incubation time with the sperm cells and a strong size-dependent inhibition of

sperm cell motility was noted, with the smaller particles being more effective inhibitors compared to the larger ones. The Cu^{2+} ions are known to be responsible for the immobilization of sperm cells,[25] thus the extent of release of Cu^{2+} ions is correlated to the particle size by spectrophotometry and ICP-OES in both, water and SP-TALP. Additionally, SP-TALP favors an increased dissolution of metallic microparticles, releasing more Cu^{2+} ions, which is probably due to the presence of complexing proteins and bases in the medium. Therein, spectrophotometry was found to be less reliable for quantification of copper ions in complex media compared to ICP-OES.

Nano/microparticles are characterized by a high surface to volume ratio, which favors a rapid dissolution of ions from the copper particles. This is one of the main advantages of using nano/microparticles compared to macroscopic copper structures. Besides this size-dependent effect, another large benefit compared to traditional hormonal contraceptives is the reduced burden on waste water systems. It is well studied that the influx of hormones and their metabolites have drastic effects on aquatic systems[9,11] and eventually human lives.[13]

The immobilization of bovine spermatozoa by copper microparticles has been demonstrated and such particles prove to be very useful components for prospective use in lubricants or as spermicidal agents for male contraception. In future studies, besides the biocompatibility of this approach, the compatibility of the here presented materials needs to be evaluated in combination with lubricants and potentially with latex, before proceeding to clinical tests.

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Supporting Information Available

The supporting info is available online and contains

- Supporting video
- Synthesis of Cu_2 particles.
- Zeta-potential measurement.
- Average (VAP) and linear velocity (VSL) of the sperm cells under different experimental conditions.

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