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Research Article

Human Sperm for Basic Embryo Research And 3D Treatment of Patient-Representative Ovarian Cancer Cells

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ABSTRACT

Cellular micromotors are appealing for locally delivering high concentrations of medications and non-invasively focusing on disease sites like cervical cancer and early ovarian cancer lesions that are difficult to reach. Specialized micromotors known as spermatozoa are used to ascend the female reproductive system. In fact, micromotors created from cow sperm can be used to treat gynecological cancers. However, due to the stark molecular differences between human and bovine sperm, translating this concept to human material poses a significant translational challenge for advancing this technology closer to the clinic. Here, the anticancer drug doxorubicin (DOX) is successfully used to treat 3D cell cultures of ovarian and cervical cancer. In addition, we use high-resolution optical microscopy to pinpoint the chemotherapeutic drug's subcellular localization in human sperm. Using sperm samples from patients undergoing assisted reproduction and healthy donors, we also evaluate the long-term effects of medications on sperm viability and motility. In contrast to DOX, which is hydrophobic and not suitable for direct loading into sperm, camptothecin (CPT) is shown to be loaded into sperm microcaps as we demonstrate the guidance and release of human drug-loaded sperm onto cancer tissues. Future targeted combinatorial drug therapies will be made possible by this co-drug delivery strategy.

Key words: Human sperm, Basic embryo research, 3D treatment, Ovarian cancer cells, Public health.

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INTRODUCTION

Today, generic chemotherapies are used for the majority of cancer treatments, but they can have unfavorable side effects like infection, anemia, fatigue, and nausea. Chemotherapeutic drugs frequently deliver *via* passive carriers that depend on the body's circulatory system,[1] which presents significant challenges to their use in long-distance transport and targeting. These constraints can be overcome, and new non-invasive targeted therapies enabled by biohybrid micromotors[2] and engineered motile eukaryotic cells and microorganisms that combine cellular and synthetic components. This is because both their motion and their functionality are susceptible to both internal and external stimuli.[3] Due to their superior capacity for drug protection through intracellular encapsulation, targeted transportation through particular migration (eg: They can target more successfully due to their chemo- and aerotaxis-sensitive reactions to their surroundings. For example, the artificial parts in bio-hybrid micromotors can support motion in the opposite direction. g. using propulsion or guidance from an external physical actuation, such as magnetic[4] or ultrasound fields. By using reporters (such as. g. the use of radioactive isotopes, absorber molecules, and infrared labels) to improve their visibility in deep tissue. Ovarian cancer is the fifth-leading cause of cancer-related deaths in women, is the most frequently diagnosed cancer, has the highest mortality rate of all gynecological cancers, and is the most common cancer among women material.[5]

This issue is significant because human and animal sperm have vastly different chemical compositions. Sperm from different species may have different anatomical features and membrane compositions, which could affect how drugs are transported.[6] Furthermore, the DNA of bovine sperm is compressed by a single packaging protein called protamine P1, unlike human sperm, which contains two distinct protamines, P1 and P2, along with some residual histone packaging. Chromatin becomes noticeably more stable as a result. Given the numerous ways in which human sperm nuclei differ from those of many other species, it is also unclear how consistently and effectively this method could be used to analyze sperm collected from different people. Additionally, earlier studies only used cervical cancer HeLa cells, which have undergone significant genetic and phenotypic drift over a long period of time,[7] leading to discernible differences between batches of cell lines and the original cancer. The ability of spermbots to target clinically significant cancer cells is still unknown. One illustration of this variation is the way that various cell lines respond to chemotherapeutic agents. So, it's critical to know if spermbots can successfully eradicate cancer cells that still contain significant traces of the original cancer. Engineering of human sperm is highly desired to treat more patient-representative reproductive tract cancer models.[8] Due to its unique etiology inside the fallopian tube, ovarian cancer, in particular HGSOC, is the reproductive cancer with the highest unmet need. This makes it particularly alluring in this context. To create a trustworthy pipeline for anticancer drug loading (DOX, model drug) in human sperm, we look into the mechanism of DOX loading and incorporate[9] the system into a flexible enhancement platform suitable for concentrating on early ovarian cancer lesions. This is the first instance of a human sperm-based drug delivery system that is fully operational.[10] By running numerous factorial

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optimization experiments, we were able to optimize the loading of DOX into human sperm while preserving sperm motility for upcoming experiments. It was determined that 25 °C was the ideal loading temperature. In addition, we looked into how a chemotherapeutic drug interacted with human sperm and where it was located in the cell's subcellular region. Finally, we used early-passage ovarian cancer HGSOC patient samples and 3D cancer cell cultures of the reproductive system to assess this system's anticancer efficacy (Figure 1). Regarding potential *in vivo* applications, another technical challenge that free-swimming sperm must overcome is how to effectively reach the target and avoid the accumulation in undesirable tissues in order to prevent toxic effects on healthy cells. Because somatic-cell fusion abilities for sperm have been previously reported drug-loaded sperm traveling randomly could unselectively fuse with cells they came across along the way. Healthy tissues may be harmed by this. A precise guidance system for aiming drugged human sperm will therefore be needed for clinical application. We designed a magnetic, streamlined microcap to transport up to three human sperm with the smallest energy loss and the simplest release mechanism.

In order to make the cap functional, we added an additional anticancer drug (CPT). In order to functionalize a second drug onto the sperm microcap, we developed a micro-precipitation technique that increased the drug's stability and loading capacity in comparison to physical absorption. Due to CPT's hydrophobicity, this was accomplished. Dual drug loading into both sperm and their coupled microcaps is a crucial step in spermbot's targeted combinatorial cancer therapy. Less than 30 percent of women have a disease that has a survival rate of more than ten years. Early ovarian cancer lesions have been found to predominantly originate in the fallopian tubes in high-grade serous ovarian cancer (HGSOC), the most common and aggressive type of ovarian cancer.[11] On the other hand, the fallopian tubes are small, deeply buried, and notoriously difficult to access structures, making it difficult to examine or manipulate them outside of the body. We urgently need new technologies that can treat or remove these lesions in the fallopian tubes that are cancer precursors. Recent research has led to the development of micromotors based on bovine sperm that are useful for biomedical applications. Sperm must travel in a highly specialized, self-propelled manner in order to ascend the female reproductive system, including the fallopian tube.[12] The serous tubal intraepithelial carcinoma (STIC) lesions, also known as early pre-invasive HGSOC lesions (occurring in the fallopian tube with restricted access), are among the best candidates for treatment with these micromotors, which were developed by modifying sperm to include new functionalities. In fact, one of our teams recently investigated the potential for magnetically and flagellally propelled bovine sperm-hybrid micromotors to swim for a variety of applications, including drug delivery and assisted fertilization. The knowledge of sperm hybrid micromotors that is currently known to science was only obtained through studies using cow sperm. However, due to the delicate mucosal immune system of the female fallopian tube, injecting bovine sperm may result in serious immunoreactions and inflammation in patients. This demonstrates how crucial it is to adapt the procedure to work with a human sperm-bearing system. Given that the ultimate goal of this technology is to translate sperm-based drug delivery to human patients, the success of the ensuing steps in this technology heavily depends on the transferability to humans. The idea of injecting bovine sperm into human fallopian tubes must also take into account possible patient acceptance as well as ethical issues.

METHOD

HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 10% (v/v) fetal bovine serum (FBS), 100 U mL penicillin, and 100 g mL streptomycin at 37 °C in a moist environment with 5% CO2. As mentioned previously, HeLa spheroids were produced. A total of 14 HeLa cells were removed, stored for two weeks, and then used. Equal amounts of HeLa cells (2 105 cells resuspended in 4 mL) were added to 16 3 point 5 cm cell-repellent dishes (Greiner bio-one) after trypsinization and PBS washing to create homogenous spheroids. Spheroids were divided into four groups and each group received the treatments described in the corresponding text passages after maturation had taken place for two days.

Spheroids and cell cultures Briefly, red blood cells from ascites were lysed, the remaining cellular fraction was obtained by centrifugation, and the cells were maintained in OCMI media74 at 37 °C in a humid environment while being exposed to a mixture of 5% O2 and 5% CO2. White blood cells were eliminated, and tumor fractions were separated

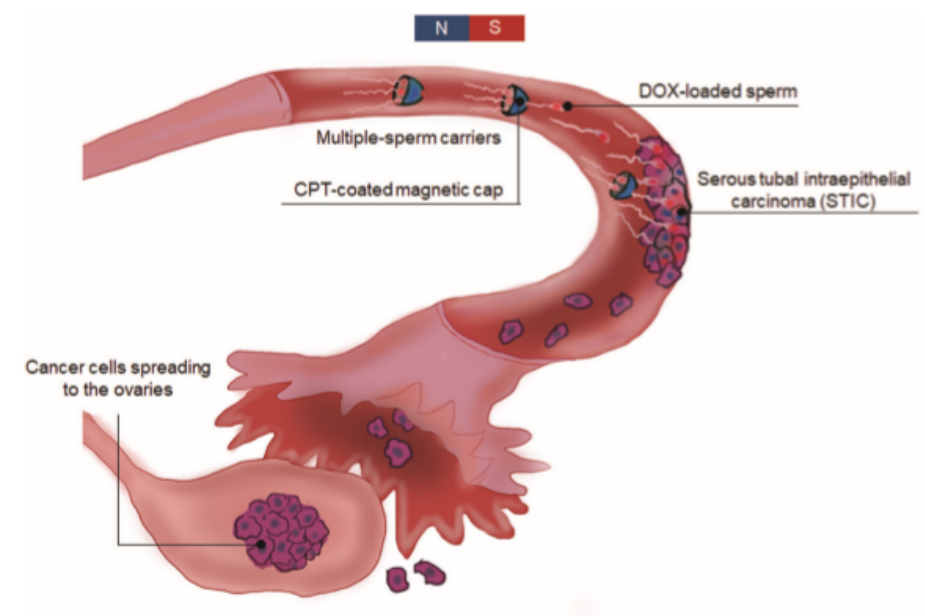

Figure 1: Target early ovarian cancer STIC lesions with a drug delivery system based on human sperm. For the purpose of controlling future drug dosages, the inset displays a model for carrying numerous drug-loaded sperm. STIC stands for serous tubal intraepithelial carcinoma.

from stromal cells using serial passaging and selective detachment. Early passage numbers (p20s) were utilized in the experiments. It OCM. Drug sensitivity has been mentioned in relation to the 66-1 culture before. 46 Stephen Taylor (16_STTA_01) has been given a license by the MCRC Biobank for the endeavor. OCM. Following the instructions for HeLa cells (see above), spheroids 66-1 were created.

The samples were then suspended in G-IVFTM PLUS medium after being cleaned with SPERMRINSETM (Vitrolife). The G-Series Manual states that progressively motile sperm was removed using SPERMGRADTM (Vitrolife). Sperm samples were cleaned and kept at room temperature until they could be used. To display the sperm samples, videos with a fast frame rate were taken. Using ImageJ, the videos were examined to ascertain sperm concentration based on haemocytometer counting and motility rates.

Co-incubating human sperm with DOX-HCl allowed for drug loading, as was mentioned in our earlier research. 6 106 sperm were briefly diluted in SP-TALP. The sperm solution was gently blended with 1 mL of a 100 g mL-1 DOX-HCl solution, and the mixture was then incubated for 1 h at room temperature before going through two rounds of purification (Fig. S1a†). The CASA system (AndroVision®, Minitube GmbH) was used to monitor the movement of 200 sperm in 10 fields at 37 °C in order to assess sperm motility. At 1 and 8 hours, four replicates of each of six sperm samples were examined to determine how they would evolve over time. A patient sample contained immotile sperm. Two techniques were employed to assess the effectiveness of drug loading. A preliminary indirect method was used, as was the case in our earlier research. In this instance, the sperm sample was centrifuged at 300 g for 5 min to obtain the supernatant for concentration analysis. The con was discovered using a varioskan lux fluorescence spectrometer the quantity of DOX-HCl.

Second, we applied a direct technique in accordance with a previously described protocol. The DOX-HCl-loaded sperm underwent three rounds of purification before being redispersed in SP-TALP. To fully lyse the cells, the dispersion was incubated with Triton-X 100 (4%) at 37 °C for 30 min. Isopropanol that had been acidified (0-point 75 N HCl) was added, diluting it ten times. It was then abandoned for 12 hours at 4 °C in the dark. The mixture was then centrifuged at a speed of 12000 g for 10 minutes. The total loading could be calculated thanks to the supernatant measurement. As a control, an unloaded reference sample created following the same steps was used.

Two-photon lithography was used to create arrays of microcaps (Photonic Professional GT, Nanoscribe). Simply put, a half-ellipsoid shell with a semi-major axis of 10 m and a semi-minor axis of 5 m was used to construct the microcap. A 780 nm laser and a 5-mW exposure were used to polymerize dip-in photoresist (IP-DIP). After drying in a critical point dryer, the sample underwent processing using the mr-Dev 600 (Micro Resist) chip. The microcap was subsequently coated with 10 nm Fe and 2 nm Ti using an e-beam metal evaporator (Edwards auto 500, Moorfield). To load CPT onto microcaps, the microprecipitation technique previously discussed was condensed. Initially, 1 mg of CPT was dissolved in a 5:5:1 solution of chloroform, methanol, and DMSO. Following that, 3 10 5 mL of CPT solution was used to disperse the microcaps. To precipitate CPT onto the microcaps, the dispersion was slowly mixed with a precipitation reagent (a water/DMSO mixture at a specific ratio). Fluorescence microscopy (Cellobserver; excitation: 350 nm; emission: 435 nm) was used to observe CPT-loaded microcaps. Spectrometry was used to assess the effectiveness of the drug loading process. In brief, microcaps containing CPT were subjected to three washes of the magnet's pull. To counteract the impact of solvent evaporation on the measurement of CPT concentration, the loaded CPT was subsequently redissolved in DMSO as the measurement solvent. A fluorescence spectrometer (SpectraMax, excitation: 350 nm; emission: 435 nm) was used to measure the amount of CPT in DMSO. We used pure DMSO as a blank control. ImageJ conducted more analysis. Cancer cell spheroids were created as previously described, and each one was then individually supplemented with 100 L SP-TALP containing 104 sperm that had been loaded with DOX-HCl, 100 L SP-TALP containing 104 sperm that had not been loaded with DOX-HCl, 100 L SP-TALP containing 104 unloaded sperm, 100 L cell medium, 100 L SP-TALP, or 100 L DO. Spheroids were trypsinized, broken down, and then reseeded into 10 cm dishes after 96 hours of treatment. Adherent cells were counted after 12 hours, and a ratio between reattached cells in each group and those in the blank control treated with cell medium only was calculated. After being stained for an hour with Alexa Fluor 488-conjugated WGA (AF488-WGA), the outer sperm membranes were fixed for 20 minutes at room temperature in PBS containing 1% paraformaldehyde. Using confocal laser scanning microscopy on an LSM880 Zeiss microscope equipped with an Airyscan super-resolution module based on 32 individual detector elements collecting photons at high signal to noise ratio, superresolution fluorescence images in two distinct channels were obtained. Two-channel Airyscan super-resolution imaging with a Zeiss alpha PlanApochromat 100 NA1.46 oil objective lens was carried out with a 458 nm excitation laser and LP 645 nm emission filter for DOX-HCL and a 514 nm excitation laser and 495-550 nm emission filter for Alexa Fluor 488-conjugated WGA. In the standard super-resolution Airyscan mode, the pinhole was configured. Lower magnification of the image in Figure 1: In experiment S1b, a Zeiss PlanApochromat 20 NA 0-point 8 objective lenses was employed. Using Thermo Fisher Scientific's EVOS microscopy system, movies of sperm attached to streamlined caps were recorded. ImageJ was then used to edit the movies. 2-photon lithography (Nanoscribe) was used to create streamlined caps. In a nutshell, the Describe (Nanoscribe) software was used to write the CAD codes before the program was executed by the 3Dlithography nano-printer. Following crucial development and drying, Fe (10 nm) and Ti (2 nm) were coated onto the caps with a 15-degree tilt by e-beam deposition (Edwards). HeLa cells were used to examine how cells expanded in the presence of microcaps. Two sets of four 3 point 5 cm Petri dishes with 3 mL of cell medium each, along with 20 L of cell culture medium that was either empty (empty group) or contained 3200 microcaps (microcap group), were used for each group. Permanent magnets were positioned beneath the Petri dishes to draw the microcaps down to the substrate. There were 105 HeLa cells seeded into each dish. Three days of culture later, cells were counted and trypan blue stained. The reattachment rate was calculated as the ratio of the number of alive cells to the total number of cells.

RESULT

Figure 1 depicts a human sperm-based drug delivery system for STIC lesions associated with early ovarian cancer.

On figure 2, you can see high-resolution photos and a timeline of the human sperm's motility while it was being drugged.

On figure 4, proposed 3D-nanoprinted carriers for the local delivery of a single or multiple human sperm to cancer lesions are depicted.

DISCUSSION

To study how well human sperm can carry drugs, the broad-spectrum chemotherapeutic DOX-HCl was employed as a model drug. These structures were located in different parts of each sperm. Therefore, in addition to its advantages as a therapeutic molecule, DOX-HCl has the potential to be used as a dye to further characterize sperm nuclei in living and motile sperm cells in the future.

Early cancer lesion cells must adhere to and move from the fallopian tube to the ovaries and other sites in order for ovarian cancer to

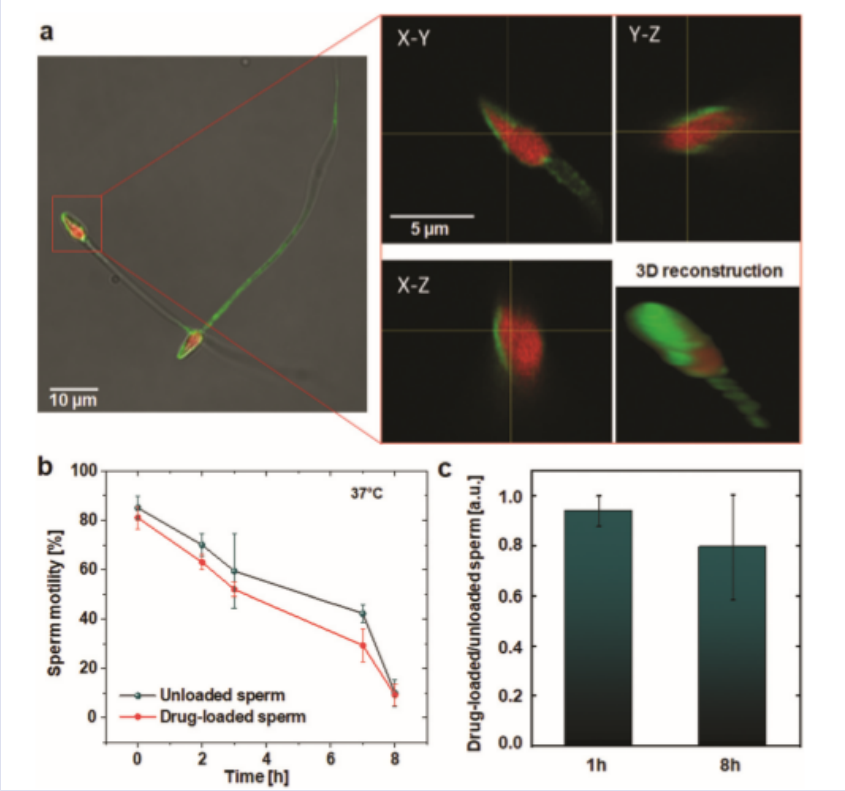

Figure 2: Monitoring the movement of human sperm that has been drugged over time with high-resolution images. (a) Fluorescence and Airyscan images of two human sperm that have been loaded with DOX-HCl; (c) the ratio of motile sperm that have been loaded with DOX-HCl compared to sperm that have not; (d) donor samples; data represent means and standard deviations. The chemical name for doxorubicin is DOX-HCl.

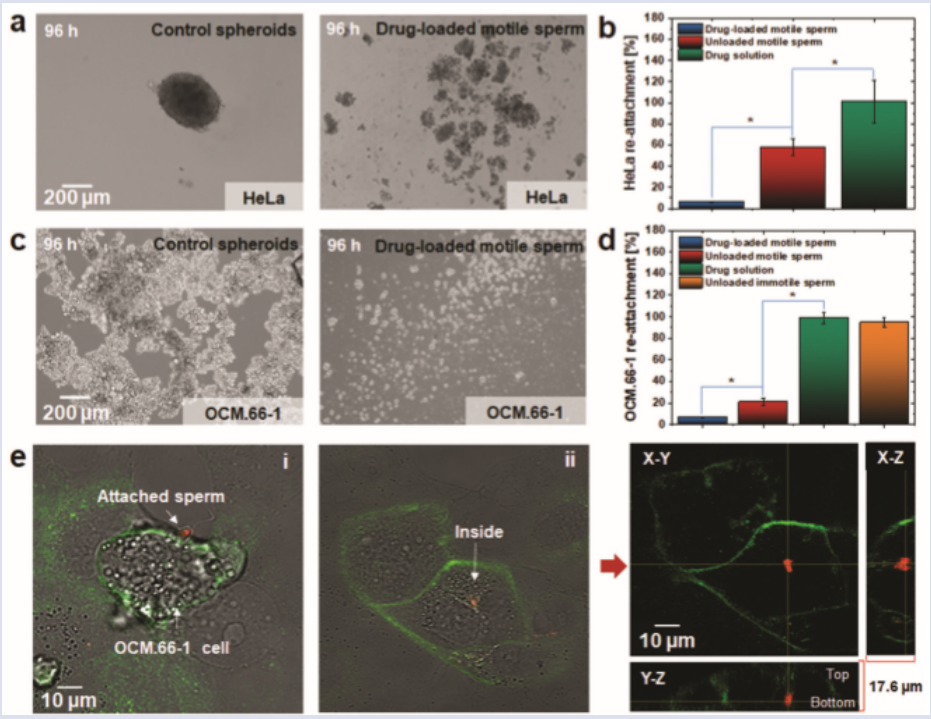

Figure 3: Human sperm with DOX-HCl loading has anticancer properties. The ratio of attached cells in the group-of-interest to those in the control group, where only cancer cell medium was added to the cell spheroids, was used to calculate the cell re-attachment rate as a proxy for critical aspects of metastatic ability. (a) Images taken using optical microscopy of HeLa cell spheroids before and after receiving 96 hours of treatment from human sperm that has been drugged. (b) The percentage of HeLa spheroid-derived cells that reattached after 96 hours of treatment in comparison to control samples (c) OCM optical microscopy images. Before and after 96 hours of treatment, 66-1 cell spheroids, and (d) the rate of OCM reattachment. 96 hours of treatment with human sperm that has been DOX-loaded resulted in 66-1 spheroid cells as compared to control samples.

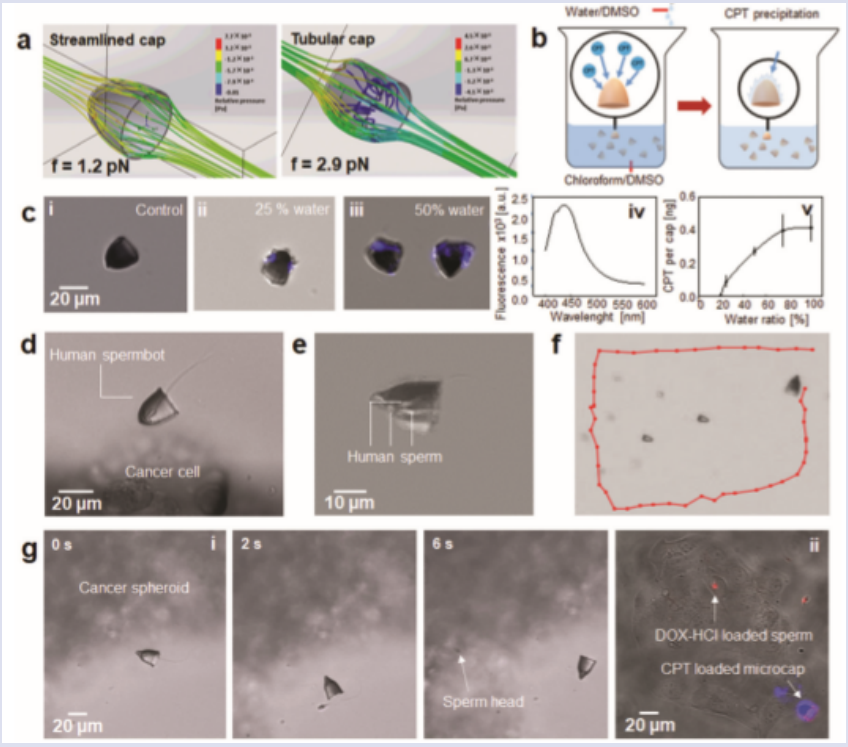

Figure 4: Proposed 3D-nanoprinted carriers for the close delivery of a single or several human sperm to cancerous lesions. Using the same diameter and height, (a) flow simulations of a streamlined and a tubular sperm cap ((b) CPT loading mechanism on the microcaps. (c) CPT precipitation while water was introduced (d) A drug-loaded spermobate was approaching the OCM. Ovarian cancer cell spheroid 66-1. (e) Using the suggested streamlined magnetic cap to transport up to three human sperm. (f) Magnetic guidance of a human sperm embryo. (g) The direction of a human sperm to OCM. cells of ovarian cancer and sperm/drug release close to the cancer cells, 66-1.

spread. Inhibiting cell adhesion could stop the spread of cancer cells because it's crucial for the formation of ovarian cancer metastases. Any variations in cell number in the following experiments can be attributed to the anticancer properties of human sperm and/or the chemotherapeutic drugs they carried because the sperm medium had no discernible impact on cell proliferation. It is possible that the partial spheroid disintegration and cell damage caused by the sperm's hyaluronidase reaction and tail beating contributed to this sperm-specific effect that was unrelated to DOX-HCl. This intriguing aspect of the sperm's micromotor system needs more study in the future. Previous research has demonstrated that the external mechanical beating induced by rotating microdiscs can harm the DNA and plasma membranes of cancer cells. This much force can damage the integrity of 40 cells. Sperm can fuse with cancer cells very quickly, as shown by the drug solution group, which contained the same overall amount of DOX-HCl as the drug-loaded sperm. DOX, however, must relocate into the nuclei of cancer cells after cell fusion in order to carry out its known functions of interfering with DNA-based mechanisms. HeLa cells, which were created in the 1950s and later became the first *in vitro* cancer model system and immortalized cancer cell line. HeLa cells have undergone numerous passages since the cell line's inception, causing them to develop a number of de novo traits that differ between different HeLa batches and their cervical cancer of origin, despite the fact that significant progress has been made and is being made using this cell line. It is therefore difficult to predict how cancers will behave at the cellular and molecular level as well as how patients will respond *in vivo*, such as how they will respond to drugs, using HeLa cells and other well-known overpassaged cancer cell lines.[13]

Because it has the highest unmet need among all gynecological cancers, ovarian cancer is of particular interest to the spermbot technology described in this work. It is now well known that HGSOC, the most dangerous and common type of ovarian cancer, develops STIC lesions inside the fallopian tube, an area of the reproductive tract that is currently impossible to access for molecular analysis using non-invasive technologies. This contradicts the widespread belief that ovarian cancer develops in the ovaries. Sperm-based drug delivery to and elimination of pre-invasive HGSOC lesions is an extremely desirable and quick tactic in light of the recent paradigm shift in the ovarian cancer etiology.[14]

Sperm that has been infected with DOX-HCl can be used to treat unmet medical needs in the treatment of gynecological cancers, a novel approach that has a lot of promise. The method fuses the benefits of chemical medication with the biological characteristics of sperm, such as active motion, the potential for somatic cell fusion, and mechanical tail beating. The use of sperm to deliver drugs may also have advantages for drug dosage, encapsulation, and transportation. Functional drugs (DOX-HCl) can be shielded by membrane encapsulation from enzymatic degradation and dilution by bodily fluids. Furthermore, the chromosomes of the sperm head may offer a number of options for the intracellular storage of DNA-binding medicines like DOX-HCl. Sperm are advantageous for safely delivering drugs over long distances and inside the gynecological tract because of their ability to propel themselves and the peristaltic activity of female reproductive organs. It is intriguing to consider the possibility that sperm's previously understood capacity to fuse with somatic cells may improve drug uptake *via* cell-to-cell transmission. It is noteworthy that sperm can combine with a wide range of cells and that the resulting chimeric cells can remain stable in culture for more than 50 passages.

Despite the fact that randomly injecting human sperm with DOX-HCl has a promising therapeutic effect on the cancer cells examined in this study, being able to target the drug-loaded sperm to the cancer spheroids would allow for more effective dosage and prevent unwanted drug accumulation. These two characteristics would be very

beneficial for the upcoming *in vivo* applications of the technology. A proof-of-concept sperm bot's sperm head was loaded with DOX, and CPT was immobilized on its microcap. Due to its distinct affinity for topoisomerase I and its unique cytotoxicity to cancer cells in the S phase, CPT is frequently employed as an anticancer model drug. Its low water solubility, difficulty in delivery, and difficulty in encapsulation make the creation of an appropriate preparation difficult. This method would increase the treatment's overall efficacy by allowing one sperm to simultaneously carry a hydrophilic medication inside of its head and a hydrophobic medication on its cap. The hydrodynamic resistance increased as a result of the rising CPT agglomeration on the cap 53,54, despite improvements in the loading efficiency of CPT and the water-to-precipitation-reagent ratio. The efficiency of the mechanical coupling between caps and sperm depends on their relative concentrations, the fit of the cap, and the conditions of incubation. For instance, coupling rates can reach 62% when sperm and microcaps are co-incubated at concentrations of 3 104 sperm per L and 100 caps per L for 10 min at 37 °C. Coupled spermbots can also be further enriched by extraction or guidance out of the chamber, enabling the introduction of new caps that maintain coupling efficiency by allowing more sperm to be trapped inside de novo provided caps. Alternative designs, created by some of the authors, employ single sperm micromotors that resemble trains or numerous sperm microstructures that capture them for later transport and release. Preclinical studies will enable real-time *in vivo* imaging of sperm-based micromotors within the reproductive system because the required spatial resolution is ca. If sperm-based micromotors can be developed in sizes between 100 and 200 m, this range could be reached. 100-150 μm. A straightforward mechanism that involves swinging the cap and altering the magnetic field's orientation can also be used to release sperm effectively. This method allows for the hydrodynamic release of various components from an artificial support structure, such as single cells or particles. 56 The sperm was magnetically attached to an OCM and then fastened to a stylish microcap. A cancer cell's spheroid (66-1). Theoretically, a 90° swerve angle is necessary to decouple the cap from the sperm if there is no friction on the contact surface between the sperm and the cap. The sperm's tail-beating behavior, which must be balanced by the sperm head's wiggling angle inside the cap, which is approximately 57°, is not taken into account by this swerve angle. Theoretically, 147° of swerve angle was needed to successfully release the sperm from the cap as a result of this. The sperm was successfully released when the cap was rotated by 147 degrees using the external magnet. The optimized microcap performance provided by the spermbot system is therefore the result of a strong trade-off between release reliability and swimming stability. The sperm managed to break free of the cap and move in the direction of the cancer cell spheroid where it was ready to release and deliver its internalized drug cargo to the intended cancer cells *via* cell-to-cell fusion and/or other mechanisms. The drug was able to be released by passive diffusion because the CPT-functionalized cap stayed close to the cancer cells. Due to CPT's low water solubility, the CPT-loaded microcap allows for a slow drug release after being delivered to the target, which may aid in facilitating sustained anticancer effects. An active lactone form and an inactive open ring form coexist in equilibrium in 57 CPT. The pH of the environment has an effect on the equilibrium ratio. Low pH levels (between 5 and 6) present in tumor microenvironments may aid CPT's transition to the active lactone form (58). Due to this pH-sensitive transformation, the CPT-precipitation loaded microcap has a tumor-selective ability, which may enhance its therapeutic effects on tumor tissues while concurrently reducing its toxicity toward nearby untransformed cells. It is significant that CPT did not affect sperm motility because it is consistent with the drug's known mechanism of action, which, like DOX, is based on interfering with DNA-based processes necessary for highly proliferating cancer cells to replicate their DNA. One DNA-unrelated function that CPT is not expected to affect is the beating of the flagella of non-diverging sperm cells. Spermbots' precise swimming direction is controlled by external magnetic fields to enable targeting. The spermbot is extremely magnetic field sensitive due to the ferromagnetic layer of the microcap. The primary cause of departures, which are kept below 3 m by precision guidance in that way, is the sperm head wriggling. During natural fertilization, oocyte targeting is accomplished by sperm rheotaxis and chemotaxis. Other studies using animal sperm have demonstrated the ability to successfully guide sperm-motors against blood and oviduct fluid flow. In order for the spermbot to successfully swim upstream in the fallopian tube and hit its cancer target, sperm rheotaxis must be applied correctly. As it approaches the targeted cancer lesion, the spermbot's magnetic coating and steering-capable design enable precise guidance to the targeted cells.

CONCLUSION

We have developed a novel drug delivery system that combines human sperm with various anti-cancer compounds with the aim of potentially treating female gynecologic diseases, particularly untreated ovarian and cervical cancers in humans. It will be interesting to learn precisely how sperm delivers medications at the molecular level and whether the process(es) involved are/are particular to certain cancer cells and embryo transfer. Sperm may be able to deliver their cargo into the cytoplasm of cancer cells through processes known as membrane fusion.

REFERENCES

1. Saw WS, Anasamy T, Foo YY, Kwa YC, Kue CS, Yeong CH, *et al*. Delivery of nanoconstructs in cancer therapy: challenges and therapeutic opportunities. Adv Therap. 2021;4(3):2000206.
2. Gonzales WV, Mobashsher AT, Abbosh A. The progress of glucose monitoring—A review of invasive to minimally and non-invasive techniques, devices and sensors. Sensors. 2019;19(4):800.
3. Mazrad ZAI, Lee K, Chae A, In I, Lee H, Park SY. Progress in internal/external stimuli responsive fluorescent carbon nanoparticles for theranostic and sensing applications. J Materials Chem. 2018;6(8):1149-78.
4. Quashie D, Benhal P, Chen Z, Wang Z, Mu X, Song X, *et al*. Magnetic bio-hybrid micro actuators. Nanoscale. 2022;14(12):4364-79.
5. Ha HI, Chang HK, Park SJ, Lim J, Won YJ, Lim MC. The incidence and survival of cervical, ovarian, and endometrial cancer in Korea, 1999-2017: Korea Central Cancer Registry. Obstetr Gynecol Sci. 2021;64(5):444-53.
6. Bulkele EA, Foutouhi A, Wigney K, Santistevan AC, Collins C, McNabb B, *et al*. Effects from disruption of mitochondrial electron transport chain function on bull sperm motility. Theriogenology. 2021;176(1):63-72.
7. Xu H. Custom-Designed Biohybrid Micromotor for Potential Disease Treatment. 2020.
8. Schmidt CK, Medina-Sánchez M, Edmondson RJ, Schmidt OG. Engineering microrobots for targeted cancer therapies from a medical perspective. Nature Commun. 2020;11(1):5618.
9. Chavda VP, Pandya A, Kumar L, Raval N, Vora LK, Pulakkat S, *et al*. Exosome nanovesicles: A potential carrier for therapeutic delivery. Nano Today. 2023;49(1):101771.
10. Shah P, Shende P. Biotherapy using Sperm Cell-oriented Transportation of Therapeutics in Female Reproductive Tract Cancer. Curr Pharm Biotechnol. 2022;23(11):1359-66.
11. Karst AM, Levanon K, Drapkin R. Modeling high-grade serous ovarian carcinogenesis from the fallopian tube. Proc Nat Acad Sci. 2011;108(18):7547-52.
12. Whitaker ED, Baccolini R. Heroes and Helpmeets: Visions of Gender in Italian Instructional Resources on Gametes, Reproductive Systems, and Human Evolution. Sci Edu. 2022;1-26.

13. Murphy SV, Atala A. Regenerative Medicine Technology: On-a-Chip Applications for Disease Modeling, Drug Discovery and Personalized Medicine. CRC Press. 2016.

14. Xu H, Medina-Sánchez M, Zhang W, Seaton MPH, Brison DR, Edmondson RJ, *et al*. Human spermbots for patient-representative 3D ovarian cancer cell treatment. Nanoscale. 2020;12(39):20467-81.

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