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Spermbots: A Promising Futuristic Innovation in Assisted Reproductive Technology

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ABSTRACT

Spermbots are robotic sperms formed out of sperm cells conjugated to artificial microstructures, having potential applications ranging from biomedical processes, drug delivery systems, *in situ* real time imagery and assisted reproduction. The robotic sperm can act as an exploratory microdevice in biological networks. Incorporation of a biological entity like sperm into microstructures under the environment of magnetic or electric fields helps in shape templating and carrying chemotherapeutic agents to target sites. Besides its role in drug delivery systems, spermbots can potentially contribute in combating infertility, especially in oligo-zoospermia and necro-zoospermia in males. Numerous checkpoints may impede sperm cells to reach the oocyte *in vivo*. Spermbots bypass these sites and carry sperm to oocyte. Targeted delivery always requires interventions of natural functional aspects of living systems. Sperm flagellum, being a biological motor in nature, can be harnessed as a driving force in spermbots to ensure delivery of fertile sperm cells with impaired motile machinery to the target. Moreover, the technology has a potential to unravel sperm migration patterns and understanding the processes *in vivo*. After a review of documented literature on possible use of spermbot technology in assisted reproduction, we hereby discuss the application of this new and innovative technology in humans and animals. The paper also highlights certain shortfalls in the widespread application of this cargo delivery technology in assisted reproduction.
Key words: Assisted reproduction, cargo, robotics, spermbot, micromotors.

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INTRODUCTION

Robotic designs have a tremendous applications ranging from clinical surgical procedures to the targeted drug delivery systems in biological systems. For its use in assisted reproduction, miniaturized and biologically compatible sperm robotic designs have generated. These novel devices besides being biocompatible can generate propulsive force under an external electro-magnetic field and can be actuated yet precisely controlled. Spermatozoa have their own

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propulsion mechanism-flagella and sperm itself is biocompatible with biological tissues. Strong autonomous propulsion (Magdanz *et al.,* 2017), chemotaxis (Chen *et al.,* 2017) and versatility shown by mammalian sperm cells help to use them as biological carriers or bionic robotic templates. Hence, sperm cells have been used to form robots for targeted drug delivery as also for assisted reproduction. In assisted reproductive technology, controlled actuation of a sperm cargo towards a desired location opens up a myriad possibility. The assisted reproduction techniques such as *in vitro* fertilization and intracytoplasmic sperm injection still appear to have low outcomes, probably due to non-availability of a quality sperm for fertilization. Thus, it is desired by infertility clinics and research laboratories all over the world to ensure most fertile spermatozoa reach the fertilization site. Among the latest techniques of sperm selection, microfluidics has been most successful in bring a fertile sperm (highly motile with high DNA integrity) through migration and propulsive mechanisms via very minute channels (Nosrati *et al.,* 2017). However, Spermbot technology takes lead in delivery of a competent sperm to the target oocyte, thus may potentially serve as an alternative to assisted reproduction technology with promising impact. In this technology, invitro fertilization of oocytes by defective sperm can be bypassed by guiding the selected sperm cells to the oocyte and hence mimicking the natural environment, *in vivo* (Koleoso *et al.,* 2020). This way, the innovative technique of sperm robotics can be a promising tool to improve outcomes of assisted reproduction.

Spermbot design and application

Two types of spermbots have been demonstrated- tubular and helical, both guided by weak magnetic fields (Mariana *et al.,* 2016). The tubular spermbot is formed out of single sperm cell, trapped inside a microtube. The driving force for functioning of the spermbot is the tail (Magdanz *et al.,* 2013). The helical spermbot entraps and transports single immotile cells. There is a rotating magnetic field which drives the helix in a screw-like motion. Spermbots hold promise for potential application in single cell manipulation and assisted reproduction, but also for targeted drug delivery. Artificial flagella have also been constructed to form microbots but to make these flagella responsive to magnetic fields, they are coated with magnetic nanoparticles of Nickel or Iron (Singh *et al.,* 2013)

Micro motor operated flagellum, a feature of micro swimmers-bacteria, microalgae and spermatozoa has been associated with formation of robotic structures (Astbury and Saha, 1953; Bacette and Afzelius, 1976; Macnab, 1999). Therefore, micromotor activity of sperm flagella forms basis for formation of robotic sperms in combination with microstructures. For propulsion, spermbots utilize flagellar movements. Constructed artificial flagella can be directly coupled with micromachines to form micro/nanorobots, also described as biohybrid microrobots.

Weibel *et al.* (2005) designed first biohybrid microbot with microalgae which was first of its kind. The first sperm driven magnetic microtube was formed by Magdanz *et al.* (2013). His team encapsulated a bovine spermatozoa head inside a microtube fabricated from a square iron sheet having 50 µm dimensions. They used cryopreserved bovine sperm cells after thawing and added 100 magnetic microtubes (50 µ m long, 5–8 µ m in diameter) to the petri dish containing 2 mL of sperm-TALP (Tyrode Albumin Lactate Pyruvate) medium with suspended cells. The random coupling of microtubes with spermatozoa was allowed. Khalil *et al.* (2014) reported a first sperm shaped design which was named synthetic magnetic microbot. Madina-sanchez (2016) reported magnetic microhelices formed after coupling of microhelical structures with spermatozoa and Doxorubicin. This was followed by another breakthrough by Xu et al in 2018, when the authors reported sperm hybrid micromotor for drug delivery. Later, Khalil *et al.* (2019) and Magdanz *et al.* (2020) described hydrodynamic modelling of spermbots, followed by self-assembly of nanoparticles with sperm cells. For making spermbots more applicable, Ridzewski *et al.* (2020) combined spermatozoa with gelatin microstructures while Xu *et al.* (2020) combined sperm with heparin loaded lipososmes. Again a report by Khalil *et al.* (2020) describes coupling of natural micro-swimmers with synthetic microstructures to form biological robots and spermbot was suggested as one such biological robot with wide biological applications.

Many techniques have been used for assembling biological entities into robotic designs, one such interesting device is IRON Sperms. The design was reported by Magdanz *et al.* (2020) which was an electrostatic self-assembly technique to fabricate with soft magnetic microswimmers, emulating the motion of motile sperm cells- called IRONSperms. They have been called as sperm-templated soft magnetic microrobots. In IRONSperms, fabrication is done using a suspension of rice grain–shaped maghemite nanoparticles (100-nm diameter) and bovine sperm cells (~60 µm long). The particles in the suspension are driven under electrostatic forces (van der Waals and Coulomb forces). These robotic structures have been reported to help in delivery systems. But, their application in assisted reproduction remains to be revealed, comprehensively. The authors demonstrated that the flagellum of the sperm cell helps in propelling the microtube forward and iron membrane aids in steering with magnetic field. In this technology, electromagnetic coils with feedback from optical microscope can be used for closed loop, for the targeted delivery to a selected reference point. Further microtube radius, sperm cell penetration and temperature affect the speed of spermbots. There is more penetration of sperm with increase in microtube radius (Magdanz *et al.,* 2013). Spermbots having more penetration power show decreased speed owing to increased confinement of the flagella. Moreover, speed was increased on increasing the temperature (Singh *et al.,* 2020). Also, performance of sperm was reported to be more when sperm cells were allowed to bind hollow space of microtubes using Fibronectin (Fn) protein and Caffeine has been added to the environment to boost the motility of the cells (Diaz *et al.,* 2007). Owing to its ferromagnetic nature, external magnetic fields, like a compass needle, are desired to set orientation in spermbot structures. This may ensure highly controlled direction of propulsion of a coupled spermbot with an external magnetic field generated either by permanent magnets or by electromagnets. In fact, this is required in the devices, as the propulsion of an uncoupled sperm cell is known to be random (Singh *et al.,* 2020). The comprehensive studies and replication of experiments are still awaited in literature. This offers a wide research area to be pursued and investigated by researchers across the globe.

Spermbot: cargo delivery and assisted reproduction

Many modified tubular spermbots have been used for delivery of cancer drugs. One such example is the use of sperm cell loaded with doxorubicin for its target delivery (Xu *et al.,* 2017). These systems are run by an artificial microstructure fabricated by two-photon nanolithography which aids in capturing the drug-loaded sperm cell. In the drug delivery process, the sperm cell acts as source of actuation for the magnetic microstructure and finally propels it to cancer site (spheroid of cancer cells *in vitro*). Finally, the drug- sperm conjugate is released by a spring mechanism in the target tissue. In affected tissues the sperm cell delivers the drug to the cancerous mass/cells.

Although bearing many challenges, there is a good potential of the sperm-driven micro-biorobot in assisted reproductive technologies. Spermbot may bypass lengthy and cost associated steps in assisted fertilization through guiding the sperm to the target oocytes (Magdanz *et al.,* 2014). However, the spermbot speed considerably decreases initial spermatozoa speed. It is well established that low sperm motility is one of the main causes of infertility in males. In fact, a condition where the sperm is healthy but unable to swim effectively to make it to an egg for fertilization decrease the outcomes of *in vitro* fertilization and other techniques. Spermbot may have potential role to assist in transport of such type spermatozoa to the oocyte for binding *in vivo or in vitro*. This may be helpful for the infertile couples and animals having necro-zoospermic or oligo-zoospermic males.

It is well established that in routine IVF procedure, gametes and embryos are subjected to multiple washing and handling steps, imposing an immense stress to gametes. Contrary to this, using spermbots, oocytes and spermatozoa may be kept in their usual microenvironment during fertilization, minimizing stress and maximizing pregnancy outcomes (Magdanz and Schmidt, 2014). As on date, the journey of the sperm towards ovum and fertilization *in vivo* is still poorly understood. The spermbot technology might also help in gaining a better understanding of the journey of the spermatozoa and the obstacles they face on their way. This area demands investigations for better applicability in assisted reproductive technologies to improve their outcomes.

Spermbot and infertility

The microtube of a spermbot is responsive to a magnetic field. It traps/envelopes the cell and helps in tracking sperm *in vivo* without affecting sperm activity (Singh *et al.,* 2020) and has a potential to act as an explorative device. Spermbots can thus help to explore *in-vivo* pathways for spermatozoa and its migration pattern through the female reproductive tract. Spermatozoa are held back after being encountered by obstacles at many locations in the way for its journey to oocyte but use of a spermbot can potentially overcome these hurdles while traveling to its destiny. This can also aid in better understanding of *in-vivo* migration of sperm to oocyte and different causes of female infertility. Migration pattern of spermatozoa is an important process in nature which is again least understood. Obstacles that spermatozoa face on their way need comprehensive investigations. Important progress can be made in assisted reproductive technologies for combatting increasing infertility in humans and animals. Spermbot can treat certain forms of infertility particularly male infertility arising out of poor semen quality, low sperm counts or defects in the chemotactic response of sperm (Medina-Sánchez *et al.,* 2016). Medina Sanchez and his team used micromotors for carrying sperms to ensure that fertile sperm was available to oocyte aiding in *in-vitro* fertilization of infertile couples.

CONCLUSIONS

Spermbots- a recent advance in cargo delivery systems may have a great potential in assisted reproduction. Some of the challenges like reliability, compatibility and fabrication of spermbots demands deep insights into this innovative field. Continued innovation and development are needed to unlock the full potential of this field. Most of the applications like targeted drug delivery, in situ imaging, system exploration as well as assisted reproduction have been demonstrated through proof-of-concept studies. The real-world applications of these micro-robots needs to be validated and mainstreamed before they can be used for therapeutic and diagnostic purposes in humans as well as animals.

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