How to make a spermbot

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This essay is a thought experiment on making a spermbot - a microrobot technology created by coupling genetically modified sperm cells with various types of payloads for targeted deliveries in a living system. Although this bot doesn't yet exist, there is a chance that it will become a reality in the next decade. When it does, it may significantly improve medicine and health care.

Why do we need a spermbot? The delivery of therapeutic and diagnostic agents to areas in the body that are difficult to access using traditional approaches (e.g., passive diffusion through circulation) is the subject of intensive research. How can the drug/biological agent be transported over a long distance and be specifically targeted to a population of cells within an organ/tissue has become a million-dollar question. This question has become increasingly more important as we are ushering in a new era of gene therapies. For example, we only want the gene editing reagents such as CRISPR (clustered regularly interspaced short palindromic repeats) to enter cells that need to be fixed without messing up the genomes of healthy cells.

Luckily, nature has already provided an answer – the sperm. Sperm are designed with a single purpose in mind – delivering the paternal genetic and epigenetic information safely to the egg (*Fig. 1*). Sperm flagella are propulsion and locomotion systems with incredible energy-efficiency. The shape of sperm is hydrodynamically optimized to allow sperm to travel over a long distance through the female reproductive tract. Their protein and sugar-coated cell surfaces are both a sensor for navigation and a shield that protects the sperm from immune attacks in the female body. A healthy human sperm can live up to 3-5 days (!) in the female reproductive tract following ejaculation. Once reaching its destination, sperm undergoes a chain of biochemical reaction called capacitation, penetrates the egg coat, and then releases its cargo - mostly DNA - into the cytoplasm of the egg via a carefully choregraphed membrane fusion event.

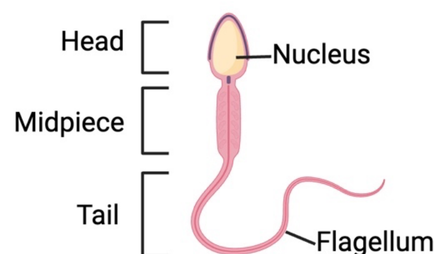

Figure 1. The structure of a human sperm. Figure created with BioRender.com.

The concept of building a nature-inspired spermbot for either improved reproductive outcome or targeted drug delivery has been discussed previously. I would be remiss if I did not mention a body of pioneering work that aimed to make a spermbot or something alike (to name a few: [1-8]). However, we are still far away from the goal of making a robust sperm-based drug delivery system. What currently available are largely hybrid micromotors that combine metal parts or scaffolds with natural sperm to enhance certain aspects of sperm functions such as sperm motility or payload-carrying capabilities.

In this essay, I propose a design that would generate a fully functional spermbot in a living system (hereafter referred to as *FFS*). An FFS does not contain any metal or other artificial materials to avoid toxicity and to ensure biocompatibility. It is powered by the same energy sources that fuel a sperm, and its movement is guided through a non-invasive technology. Although speculative, this design is mostly based on a combination of existing technologies.

1. Starting materials

The raw materials for producing FFS come from the patient who will later receive the FFS (*Fig. 2*). This makes sure that once the FFS enters the patient body, it will not be treated as a foreign object and cleared out by the patient's immune system. This resembles a chimeric antigen receptor (CAR)-T cell therapy in which T cells from a cancer patient are isolated, genetically modified, and then reintroduced to the same cancer patient to kill cancer cells. In the case of FFS, skin fibroblast cells collected from the patient who will later receive the FFS are cultured in a dish and converted to induced pluripotent stem cells (iPSCs) using a well-established protocol [8, 9]. The iPSCs are subject to a series of genetic manipulations through CRIPSR-mediated genome editing (detailed in the following sessions). The genetically modified iPSC is then differentiated into an FFS using in vitro sperm production protocols that have been proven feasible in rodents [10-12]. It is worth mentioning that iPSCs can derive from both male and female somatic cells [12, 13], meaning that FSS can be produced in vitro using cells from both the male and female. It is also possible that in vitro sperm production will be established in the human in the near future [14] (assuming ethical approval will be granted).

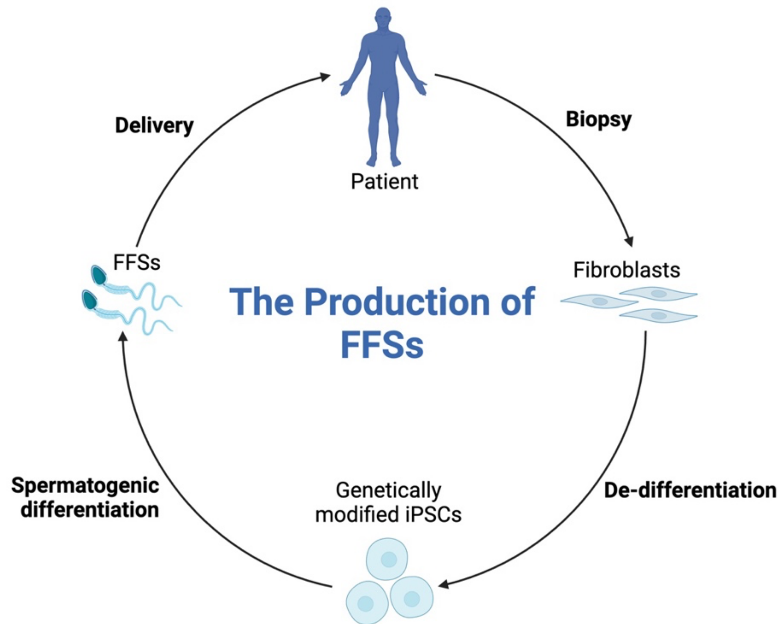

Figure 2. The production cycle of FFSs. Figure created with BioRender.com.

2. Cargo loading

Like natural sperm [15], an FFS can actively take up circular plasmid DNA under physiological conditions. Small molecule drug and protein can be packaged in lipid-based nanoparticles and loaded into an FFS when incubated together [16].

3. The energy source and propulsion system

Since an FFS is essentially a genetically modified sperm, it uses the same energy sources as the natural sperm (e.g., fructose) and uses the flagellum as the propulsion system.

4. The navigation system

To direct a spermbot to a target location in a living body, previous work proposed to use an externally controlled magnetic field. Although non-invasive, this approach would require coupling a sperm with a metal casing or metal nanoparticles, posing the risk of toxicity in the living body. The FFS will use ultrasound as an alternative non-invasive navigation system. Ultrasound can manipulate cells with high spatiotemporal precision via acoustic radiation force (ARF). Recent pioneering work has generated genetically modified cells with enhanced acoustic properties by introducing gas vesicles (GVs) - a unique class of gas-filled protein nanostructures – into the cells [17-19]. Because of their lower density and higher compressibility relative to water, these GVs show strong ARF with opposite polarity to most other materials. At the iPSC stage of the

FFS production (*Fig. 2*), the transgene encoding the GV protein is inserted in-frame at the 3' end of a spermatid-specific gene such as protamine 1 (*PRM1*) via CRISPR. This allows the generation of GVs only at the last stage of sperm development, thus minimizing any potential negative impact of the GVs on the health of the FFS. Once expressed inside an FFS, GVs invert the cell's acoustic contrast and amplify the magnitude of its ARF, allowing it to be selectively manipulated with sound waves (*Fig. 3*).

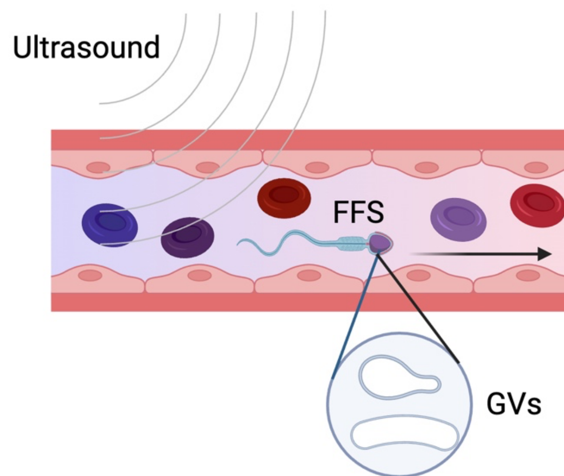

Figure 3. Ultrasound-guided FFS. Figure created with BioRender.com.

5. The docking system

Once arriving at the target location, the FFS needs to specifically bind to target cells before its cargo can be released. A docking system mediated by ligand-receptor protein interaction is in place to achieve this goal (*Fig. 4*), resembling the CAR-T cell therapy. In the CAR-T cell therapy, specific receptor proteins (i.e., CARs) expressed on the surface of the engineered T cells facilitate the recognition of and binding to specific proteins, or antigens, on the surface of cancer cells. Similarly, transgenes that encode surface receptor proteins which specifically bind to the surface proteins of the target cells are inserted into one of the safe harbor loci of the FFS genome during the iPSC stage. This allows the specific docking of the FFS to the target cells without interfering other cells in the same organ/tissue.

6. The cargo release system

Like sperm, the FFS releases its cargo by inducing cell fusion. A synthetic circuit is in place to achieve a programmed fusion event between the FFS and the target cell (*Fig. 4*). In this circuit, the binding of the receptor proteins on the surface of the FFS to the surface proteins of the target cells as described above triggers the cleavage of a transcription activation domain (TAD) off the inner cell membrane of the FFS via a synNotch design [20]. The cleaved TAD relocates

to the cell nucleus to drive the transcription of the vesicular stomatitis virus G protein (VSV-G) gene integrated in one of the safe harbor loci of the FFS genome. The VSV-G protein drives cell fusion events between FFS and its bound target cells at a low pH condition [21]. Cancer cells tend to have a lower extracellular pH than normal cells [22], making VSV-G-mediated cell fusion feasible. For non-cancer target cells, an additional circuit that knocks down the expression of membrane H^{+} transporters and $HCO3^{-}$ transporter genes in FFS upon FFS-target cell docking is needed to lower cellular pH [23].

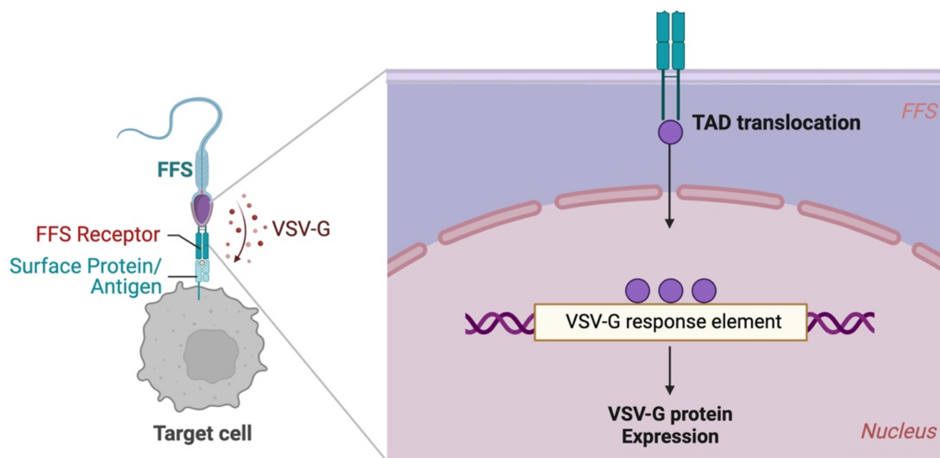

Figure 4. The FFS docking and cargo release system. Figure created with BioRender.com.

In summary, this essay presents a new solution to the targeted drug delivery problem. The proposed spermbot represents a nature-inspired design that builds upon years of hard work of countless scientific minds working in the field of reproductive biology, gene regulation, synthetic biology, and bioengineering. It is the hope that this essay may invite more attention to and discussions on this topic, which may eventually drive progress forward.

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