4D-printed multifunctional microcarriers for passive and active cargo delivery

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Abstract

Nowadays, remote controllable soft and smart microrobots have facilitated less-invasive medical operations due to their unique capability to adapt and perform multiple functions. Microrobots are categorized based on their propulsion mechanisms into the chemical, physical, or biohybrid ones.[1] Biohybrid microrobots are the ones who propel by means of motile cells or microorganisms as they take advantage of the taxis and cell-interaction abilities of the living components.[2]

From the wide range of motile cells and microorganisms, bacteria and sperm cells are shown to be efficient moving in complex fluids and environments, even against flow, being suitable for various in vivo medical operations.[3-5]

For example, genetically modified Magnetococcus marinus “MC-1”, known as magnetotactic bacteria (MTB), has been widely used for targeted drug delivery.[6-8] They not only can be steered by external magnetic fields because of existing the magnetosomes but also can be tracked via magnetic resonance imaging (MRI).[9]

On the other hand, sperm cells are optimized to swim in various viscous environments inside the female reproductive tract without inducing any immunoreaction or inflammation. Moreover, recently, studies showed that sperm have high drug loading-capability for targeting various gynecological cancers, in particular cervical and ovarian cancer.[10,11] Naturally, sperm cells respond to different physical and chemical changes in their surroundings. For example chemotaxis (tendency to swim toward a higher concentration of extracellular signals), thermotaxis (tendency to swim towards a temperature gradient), thigmotaxis (tendency to swim near the surface), and rheotaxis (swimming against the fluid flow), which can be considered together with the additional functionalities provided by the synthetic microstructures for engineering sperm-based microrobots to be able to target the disease location by either local taxis or external control.[12-14]

Our group was the pioneer of sperm-hybrid micromotors in both development of in vitro/in vivo assisted fertilization applications and targeted drug delivery approaches.[15-18] Primarily, the capture, guidance and release of single sperm cell was successfully demonstrated.[11] Then, the influence of different parameters like sperm morphology and tail beating amplitude, as well as, the temperature and viscosity of the surrounding fluid on sperm-hybrid microrobots’ performance was investigated.[15,16] To improve sperm release different strategies have been developed such as using either thermo-responsive rolled-up microtubes, or by bending arm microstructures.[17,18]

The aim of the current work is to assist sperm cells reaching the fertilization site in a case of oligospermia (when the sperm count is <20 million sperm per mL). Currently, there are two different ways to treat this male infertility problem in the clinics. One is in vitro fertilization (IVF) and the other is intracytoplasmic sperm injection (ICSI). In IVF the preselected sperm cells (millions of them) are introduced with the retrieved oocytes and in ICSI only one sperm cell is picked up and directly injected into the oocyte for the fertilization. Although, the fertilization rates are quite high, there is a challenge to increase the embryo implantation rates.[19] This problem might be caused by the oxidative stress on gametes outside the natural environment (female reproductive tract) during retrieval, washing, selection, and incubation which affect their quality. To address this challenge, here, we introduced 4D-printed multifunctional sperm-hybrid microcarriers to transport a high number of sperm cells inside female reproductive tract while assisting them by local capacitation and facilitating the digestion of the cumulus complex that surrounds the oocyte.

4D-printed microcarriers are fabricated with a non-stimuli-responsive polymer (IPS photoresist) and a thermo-responsive hydrogel poly(N-isopropylacrylamide) (PNIPAM) via two-photon polymerization (TPP).[20] Thermo-responsive hydrogels like PNIPAM are undergo reversible phase transition from a swollen hydrophilic state at temperatures below its lower critical solution temperature (LCST) to a collapsed hydrophobic state at temperatures above its LCST, which can be beneficial to make an adoptive gates in our microcarriers.[21-23] Hereby, PNIPAM not only used for triggering sperm cell release at specific temperature but also considered to expel heparin (specific protein caused sperm hyperactivation) at the same time, for future in vivo assisted fertilization scenarios. Moreover, microcarriers are functionalized with hyaluronidase-loaded polymersomes (HYAL-Psomes) to locally mediate the hyaluronic acid (HA) degradation found in the oocyte-cumulus complex.[24]

Conclusively, our presented sperm-hybrid microcarriers are able to: (i) capture, transport and release multiple motile sperm cells, to increase the chances of fertilization, (ii) release heparin at the certain time to in-situ capacitate/hyperactivate sperm cells, and (iii) mediate degradation of HA (found in the cumulus complex matrix, one of the natural biological barrier) to assist sperm penetration into the oocyte, by the HYAL-Psomes previously immobilized on the microcarrier's surface **(Figure 1)**.[25]

References

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Figures

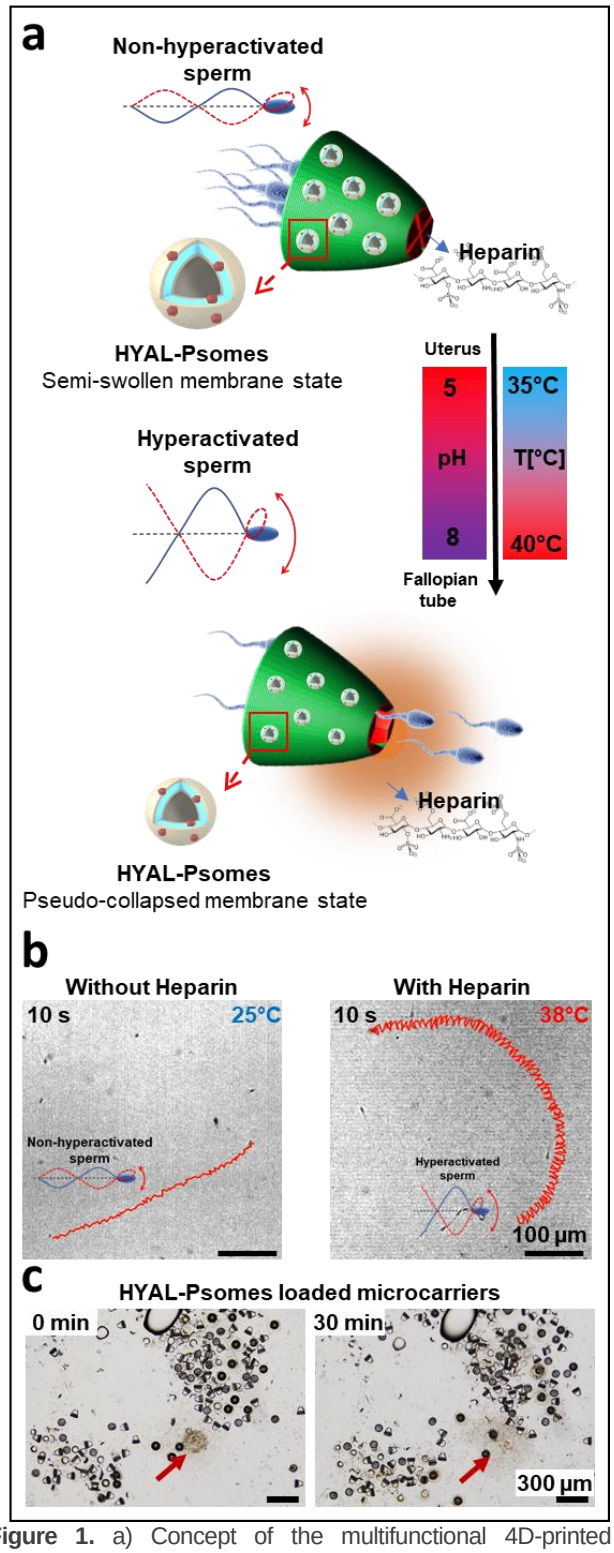

Figure 1. a) Concept of the multifunctional 4D-printed microcarrier with heparin (used for sperm hyperactivation) and hyaluronidase-loaded polymersomes (used for cumulus cell removal), and the expected natural stimuli (pH and temperature varying from the uterus to the fertilization site) for active (motile sperm cell) and passive (heparin and hyaluronidase) cargo release. b) Sperm hyperactivation in presence of heparin with circular motion pattern. c) Locally cumulus cell removal by HYAL-Psomes loaded microcarriers, the arrow indicates the location of oocyte surrounded by cumulus cells. Adopted from [25].