The sperm stewing in its own ROS—in the plastic Petri dish

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Endogenously produced reactive oxygen species (ROS) are essential to life, being involved in many different biological functions. However, when overproduced, or when the levels of antioxidants become severely depleted, these reactive species become highly harmful, causing oxidative stress through the oxidation of biomolecules (1). Oxidative stress is implicated in the etiology of several diseases and in aging. In their paper entitled Genesis on diamonds II: contact with diamond enhances human sperm performance by 300% (2). Sommer *et al.* portray an unprecedented biological scenario: the concurrent internal and external action of endogenously produced ROS on cells and their modulation with near infrared (NIR) light. The conclusion derived from their interplay could enhance the predictive potential of *in vitro* experiments, extend our understanding of biocompatibility, innovate biomaterial design and, in accord with previous results indicating that the adenosine triphosphate (ATP) output of the mitochondrial rotary motor (ATP synthase) is controlled by the viscosity of the interfacial water in mitochondria (3), institutes a radical paradigm shift in low level light therapy (LLLT) (4) where current theory assumes that precondition for ATP synthesis is absorption of the NIR light by cytochrome c oxidase (5). The discrimination was possible by quantifying differences in the behavior of ROS-sensitive cells (spermatozoa) on a flat glass surface (Makler Counting Chamber) following contact with substrates (Petri dishes) as different as plastic (polystyrene) and diamond (nanocrystalline diamond coated quartz), and initial exposure to various doses of NIR light (LED, central wavelength 670 nm).

The polystyrene cell culture dish (Petri dish) is the most basic tool in life science laboratories (6). The glass version, hence *in vitro*, was introduced in 1887 by Petri (7). Except geometrical modifications and hydrophilic conversion of the hydrophobic plastic, the polystyrene Petri dish remained virtually unchanged for 130 years. Today, the polystyrene Petri dish stands at the beginning of pharmacological and biomaterial tests (cell culture, animal model, clinical trial), where it is used to interrogate cells, but also at the beginning of human life, i.e., *in vitro* fertilization (IVF). Previous experimental work challenged the biodurability of polystyrene Petri dishes: Sommer *et al.* showed that when in contact with aqueous media, the polystyrene becomes soft, an effect facilitating the establishment of a nanoscopic alkaline layer which in concert with entrapped ROS has a detrimental effect on cells (8).

One principal source of cellular ROS are mitochondria—microscopic organelles which reside inside the cell and are involved in the production of ATP. Under conditions of cellular stress, mitochondrial ROS are released into the cytoplasm, but are supposed not to escape from the cell (9): the lifetime of the ROS molecules generated by the mitochondria is extremely short (typically 10^{-6} to 10^{-3} sec), in agreement with their reactive nature. However, intracellular ROS can undergo conversion into other types of ROS which have a longer lifetime and eventually escape the cell, usually in the form of hydrogen peroxide molecules (H_2O_2). Prevalence of this conversion process is believed to

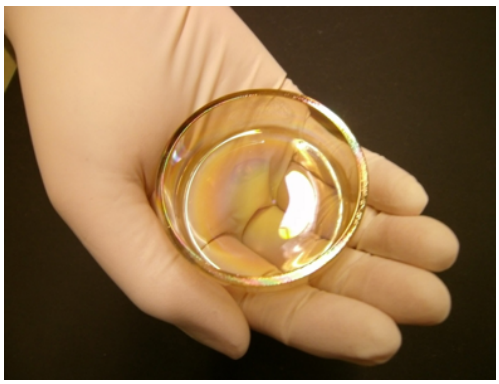

Figure 1 ROS-free microenvironment?—The diamond Petri dish.

be crucial for any extended examination of cells *in vitro*, in general, and, in particular, IVF.

Sperm cells are susceptible to ROS-induced damage because their plasma membranes are rich in polyunsaturated fatty acids and their cytoplasm contains low concentrations of the ROS-scavenging enzymes. Many clinical and research institutes are investigating antioxidant supplementations to prevent infertility problems caused by impaired sperm function, including decreased viability and motility (10). ROS generation takes place at the electron transport chain (ETC) located at the inner membrane of the mitochondria during the process of oxidative phosphorylation. Impressive evidence that human sperm mitochondria have the potential to generate ROS—and that mitochondrial ROS converted to H_2O_2 escapes to the outside of the cell—was obtained by using mitochondrial electron transport inhibitors to disrupt the electron flow through the ETC, and a chemiluminescence system to detect H_2O_2 in the extracellular space (11). This short introduction is instrumental for a better understanding and interpretation of the results published by Sommer *et al.* in ATM (2).

Instead of commenting on the original data presented in the *ATM* paper, we find it instructive to provide a detailed explanation of the title of our paper. For this we include here a brief excerpt recently published in *Science* (12):

"*During IVF procedures, sperm is introduced to an egg in a Petri dish. If the egg is successfully fertilized, the resulting zygote is implanted into the woman's uterus. The critical fertilization stage usually takes place on polystyrene, a plastic from which almost all Petri dishes are made. Sperm, like most cells, exude harmful, cell-disrupting molecules known as ROS. Inside the body, these ROS last only fractions of a second and are quickly neutralized as they bind with nearby molecules. But polystyrene naturally forms a thin, gluelike nano-layer of water on its surface, which traps the ROS. "The sperm is stewing in its own ROS", says Andrei Sommer, a physicist who led the study… "This longer exposure is highly, highly, highly destructive to the cell". The upshot is that many sperm cells exposed to polystyrene quickly lose their motility, turning from "guided missiles" into barely moving or immobile cells incapable of fertilizing an egg. Generally, the more highly motile sperm cells a sample contains, the more likely it is that an IVF procedure will result in pregnancy, though a host of other factors like sperm count and egg quality also play a role. (Eggs produce less ROS and therefore are less susceptible to oxidative damage than are sperm cells.) Building on previous work by his lab, Sommer and colleagues wondered whether keeping sperm cells on a material like diamond, which forms a slick, not sticky, surface layer of water, would protect them from ROS. The researchers coated quartz Petri dishes with a super-thin layer of diamond less than a micron thick (Figure 1), and put human sperm cells on them. They assigned the cells one of four grades ranging from A (rapidly moving and the most likely to fertilize) to D (completely immobile and incapable of fertilization). Then they did the same thing for sperm cells taken from the same sample but placed on traditional polystyrene Petri dishes.*

After an hour, the diamond-coated Petri dishes contained 300% more Grade A sperm cells than did the polystyrene dishes… That's likely because ROS can't stick to the diamond surface in the same way that they do to polystyrene, Sommer says. "The ROS, when landing on diamond, have no chance to harm the cells."

In a separate set of experiments, the team tested how light affects sperm cells. Previous studies have shown that exposing cells to a wavelength of light, 670 nanometers, toward the red end of the visible spectrum, causes their energy-producing mitochondria to boost production of adenosine triphosphate, which powers the cells. When the researchers exposed sperm cells to this wavelength, they found about twice as many Grade A cells after 30 minutes than were in control groups. Used in conjunction, diamond-coated Petri dishes and near-infrared light might make sperm cells intended for IVF procedures more energetic and longer lasting, increasing the chance of a successful fertilization, Sommer says, although more testing is needed before any of these techniques could be used in the clinic (12)."

It is worth noting that a powerful inverse relationship between mitochondrial ROS generation and sperm motility has already been reported (10): specifically, inhibition of sperm motility was observed in the presence of a glycolytic substrate, glucose. Possibly, glucose a material of pronounced hydrophilicity was instrumental in the process

of amplifying the scavenging and immobilization of ROS molecules, so eloquently described in *Science* (12).

In normal IVF, a Petri dish containing oocytes and sperm cells is placed into an incubator. Whereas oocytes are protected against ROS by cumulus cells, sperm cells are susceptible to extracellular ROS (13). A Google search for intracellular ROS (Mai 2017) produces ten times more hits than that for extracellular ROS. Biological effects of intracellular ROS, known to play a crucial role in both physiological and pathological processes in living organisms, and ranging from beneficial to a "coup de grâce" to the cell (14) are relatively well understood, when compared the effects of extracellular ROS. For instance, monocyte/macrophage use ROS as a defense against invading microorganisms but themselves are hypersensitive to ROS, undergoing excessive apoptosis (15).

In summary, the data and their conclusive interpretation presented by Sommer *et al.* (2) represent an attractive challenge to both theory and experimental side. They demand for the consideration and implementation of new concepts in the design of *in vitro* experiments. In view of the significant increase in both sperm vitality (16) and motility (2) on diamond, in comparison to the limitations inherent to plastic labware, they may even inspire the design of advanced culturing systems which allow us to overcome disadvantages of current microfluidic systems (17), where pulses of NIR light (18) could be used to induce metabolic processes into cells. Finally, in conjunction with previous work (19) which provided a clear understanding for the use of intermittently applied NIR light to modulate the balance between mitochondrial ATP and ROS production, on the one hand, and the energy consumption by the cells, on the other hand, the new results and their explanation help us to understand the biological effects offered by LLLT, thereby leading to better therapies.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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