

# Diamonds are for men, too

Andrei P. Sommer

Ulm, Germany

Correspondence to: Andrei P. Sommer, Ulm, Germany. Email: andrei.sommer@uni-ulm.de or samoan@gmx.net.

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Response to: Försterling HD, Pavláth AE, Mester ÁR, et al. The sperm stewing in its own ROS—in the plastic Petri dish. *Ann Transl Med* 2017;5:366. Batsanov SS, Batsanov AS. Editorial on “Genesis on diamonds II: contact with diamond enhances human sperm performance by 300%”. *Ann Transl Med* 2017;5:407.

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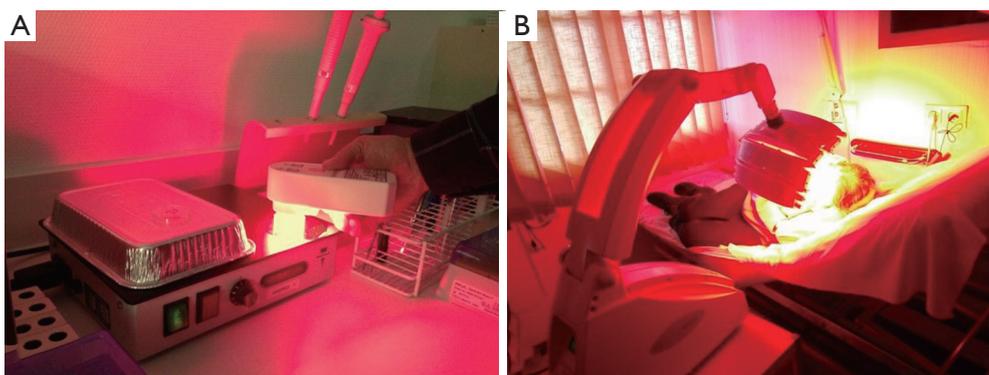
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## Introduction

The results of a simple *in vitro* experiment presented 2016 in an editorial (1) were commented in *Science* (2) and later in three editorials (3-5). What makes those results to be interesting? Is it the finding that the progressive motility of human spermatozoa increased by 300% after one hour contact with diamond Petri dishes, when compared to standard polystyrene Petri dishes? Is it the additionally reported beneficial effect of near infrared (NIR) light on the performance of the spermatozoa? Undoubtedly, the findings (1) suffice to revolutionize *in vitro* fertilization (IVF) routines, one day: (I) by safeguarding a critical microenvironment—the interfacial zone between cell and Petri dish—which on diamond is virtually free of a reactive oxygen species (ROS) and in which spermatozoa have a higher probability to fertilize oocytes and (II) by using NIR light to fill up mitochondrial adenosine triphosphate (ATP) reservoirs. However, the results presented in (1) are in no way restricted to IVF. Probably, their validity can be extended to *in vitro* tests in general and thus practically exploited in life sciences and medicine.

For a justification of the aforementioned general validity postulate, it is instructive to review the basic cellular components and physiological properties in the three editorials (3-5) whose interplay is considered by their authors to be relevant for the discussion/interpretation of the *in vitro* experiments performed in the diamond and polystyrene Petri dishes, respectively (1). Batsanov *et al.*: interfacial water viscosity, mitochondria,

electrons, ATP, ROS. Lanzafame: water, mitochondria, ATP, ROS, cytochrome c oxidase. Försterling *et al.*: interfacial water viscosity, mitochondria, ATP, ROS. Clearly, these components/properties are not typical for spermatozoa. Actually, they are so fundamental that there is no cell containing mitochondria where their interaction would not control elementary biological processes, including cell survival and apoptosis. Let us consider a typical *in vitro* scenario where cells deprived of their native milieu experience oxidative stress concomitant with excess ROS production in mitochondria. In accordance with a negative polarity of ROS molecules their growth inevitably results in a spontaneous increase in the viscosity of the interfacial water in mitochondria. An immediate result of the increase in interfacial viscosity is a drop in the production of ATP, associated with a reduced efficiency of the mitochondrial rotary motor (ATP synthase). The reciprocity between overproduction of mitochondrial ROS and ATP synthesis is well documented in the literature. Importantly, it is possible to intervene in this relationship with light. Irradiation of cells with biostimulatory levels of NIR light (6) counteracts the destructive effect of ROS by reducing the viscosity of the interfacial water in mitochondria. As a result, the mitochondrial nano-turbine can rotate easier. For the cell this means normalization of its ATP production, as suggested by the results of recent laboratory experiments performed on model surfaces (7,8). Indeed, there is observational evidence that exposure to NIR light



**Figure 1** NIR light treatment of human spermatozoa *in vitro*. (A) A gold mirror is used to prevent absorption of the photons by the black heater surface. After irradiation Petri dishes are kept on the heater and covered to protect sperm cells from light; (B) NIR light therapy to accelerate the wound healing in a patient, immediately after surgery. NIR, near infrared.

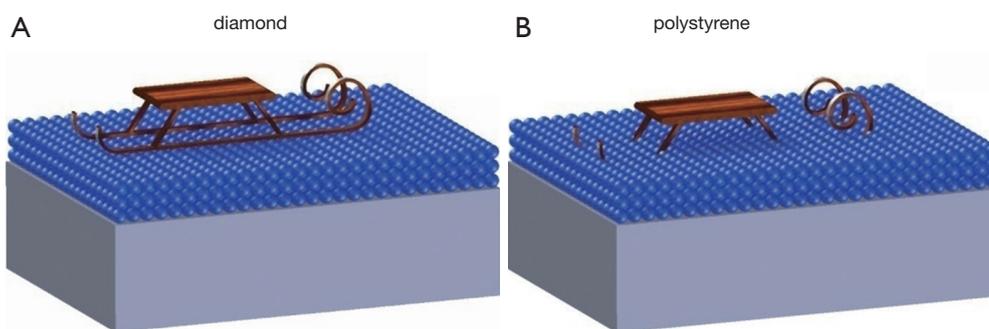
delivered at biostimulatory levels stimulated proliferation in cells as different as fibroblasts (9), endothelial cells (10) and neuroblastoma cells (11). *Figure 1A* illustrates the irradiation procedure in a typical *in vitro* experiment.

Importantly, the beneficial effect of NIR light irradiation is not restricted to cells *in vitro*. The same effect can be exploited for various *in vivo* scenarios, where cell vitality and performance are low due to inflammatory processes in association with a spontaneous increase in ROS, for instance, for the treatment of open wounds. Other conditions where irradiation with NIR light shows promising potentials include, but are not limited to, the amelioration of retinal function after light-induced photoreceptor degeneration (12) and transcranial treatment of the ischemic penumbra in stroke, or traumatic brain injuries affecting in particular military personnel (13). Despite of extensive applications in clinical practice (*Figure 1B*), there is still a considerable gap between current theory and practice: The predominant theoretical model, which explains the interaction of NIR light with cells, assumes that the photons are absorbed by the enzyme cytochrome c oxidase (14). In our opinion this mechanism of interaction is not satisfactory: The absorbance of cytochrome c oxidase for NIR photons is minimal—it might contribute to the synthesis of ATP but not to the expected extent. Although it is clear that more research is necessary, the experimental confirmation that interfacial water viscosity is instantly reduced by NIR light (7) puts us in the position to provide a satisfactory explanation to *in vitro* results and clinical data documenting the biological effect of NIR light. With the understanding of a basic mechanism progress in field is possible.

### Implications for high resolution microscopy techniques

It is worth noting that the light-induced viscosity modulation is a basic physical phenomenon, therefore it is reasonable to assume the validity of similar processes for cells during extensive microscopical inspection and analysis, which will inescapably change the native state of the targeted system. Here, we consider high spatiotemporal resolution fluorescence microscopy techniques, which provide unprecedented insight into molecular processes in live cells. During their observation the cells are illuminated with extreme laser intensities reaching  $1 \text{ TWm}^{-2}$ . Earlier we reported that laser intensities as low as  $4 \text{ kWm}^{-2}$  were sufficient to significantly reduce the viscosity of interfacial water (7). Remarkably, a drop in viscosity could only be measured on hydrophilic surfaces. The viscosity of these interfacial water layers, comprising 2–3 monolayers of water molecules, reaches values up to one million times higher than that of bulk water, comparable to the glue-like properties of molasses, and increases both with hydrophilicity and confinement. The elevated interfacial viscosity on hydrophilic surfaces and the possibility to reduce it with light is of considerable biological interest.

The intracellular space is known to be densely packed with cellular constituents—organelles, membranes and macromolecules—whose surfaces present a pronounced hydrophilicity, thus are masked with a glue-like hydration layer. For proteins this picture justifies the principle of the master-slave concept (15), where protein hydration layers are supposed to dictate protein folding and function. Moreover, based on the picture of a multitude



**Figure 2** Artist's view of molecular picture at the bottom of the Petri dish. On diamond ROS molecules are prevented from anchoring to the bottom by a compact ice-like water layer (blue spheres) attached to the diamond. Therefore, here ROS molecules escaping from stressed cells are instantly neutralized by the large fraction of bulk water prevalent in the cell culture liquid (A). On hydrophilic polystyrene, however, ROS molecules are transiently immobilized by the viscous interfacial water layer attached to the bottom where they impose supplementary stress on the cells (B). ROS, reactive oxygen species.

of hydrophilic surfaces in a densely crowded intracellular space, it can be safely assumed that the laser light used today in many laboratories for live cell observation and analysis will interfere with both stationary states and dynamic processes in the interior of cells, for instance transcription factor residence times on DNA (16), simply by reducing interfacial water viscosity. In this context we note that 670 nm is not the only wavelength which interacts with interfacial water layers. Actually, we demonstrated that the effect is not restricted to NIR light (17). The special advantage of the wavelength 670 nm consists in its good tissue penetration without heating the fraction of bulk water in biological tissue, as opposed to infrared light.

### The bottom of the Petri dish

The beauty of the interfacial water viscosity model considered here consists in the fact that its validity is not restricted to the intracellular space; it is also applicable to explain the processes taking place between cells *in vitro* and the Petri dish. Whereas interfacial water layers masking the hydrophobic diamond surface are compact and strongly bound to the diamond surface due to the hydrogen atoms connected to the nanocrystalline diamond surface, the interfacial water on the hydrophilic polystyrene is viscous and glue-like. This picture is consistent with the results of numerous experiments performed on model surfaces. In our editorial (1) we concluded that on hydrophilic polystyrene (Petri dish) the interfacial water layer acts as a scavenger for ROS molecules escaping from oxidatively stressed cells (Figure 2). Conforming to the viscous consistency

of the interfacial water at the bottom of the plastic, ROS molecules were predicted to stick to the glue film, thereby experiencing an extension of their lifetime. ROS molecules accumulating in this way are likely to exert additional stress on cells *in vitro*. Absence of a ROS scavenger film on diamond provides a plausible explanation to the 300% gain in sperm motility observed on the diamond Petri dishes (1).

An interesting alternative model attempting to explain the 300% gain in progressive sperm motility on diamond, compared to the continuous loss in motility on polystyrene Petri dishes, is proposed by the Batsanov group (3): "Can it be related to diamond surface having a unique property of negative electron affinity (18), thus readily releasing electrons into water?". While the question appears to be simple, it is highly intriguing. By the special process of fabrication, a certain amount of hydrogen atoms is bound to the surface of nanocrystalline diamond. Their prevalence is involved in attracting and binding water molecules to the diamond surface. This form of diamond is a perfect electrical insulator. However, when the nanocrystalline diamond is treated with hydrogen, the resulting diamond species becomes electrically conductive (19). For this special diamond variation it is assumed (18) that electrons, which are liberated from the diamond subsurface, are taken up by a water layer on the diamond surface. Because ROS usually carry a negative electric charge, the authors of (3) suggest logically that the electrons released by the diamond surface will repel the negatively charged ROS molecules. The model ignores the aforementioned function of the interfacial water layer on the diamond surface but arrives to the same result, namely absence of ROS molecules at the

substrate/cell-interface. It would not have been surprising from the evidence of recent work (18) on the conductivity of nanocrystalline diamond if the root cause of the 300% increase in progressive sperm motility was electrical repulsion between electrons and ROS molecules. But the repulsion did not take place in this way. A large number of tests showed that the culturing side of the diamond Petri dishes is practically not electrically conductive, what might be considered a very unfavorable condition for release of electrons. This is reassuring, though it does not, of course, prove that electrons were totally absent in the process under consideration. Nevertheless, the mechanism proposed by the Batsanov group is compelling. Although the validity of the model explaining the surface conductivity phenomenon on hydrogen-terminated diamond (18) was challenged by experiment (19) the release of electrons from the surface of hydrogen-terminated diamond can be regarded as ascertained. Therefore, the hypothesis put forward by the Batsanov group holds the promise for a systematic amplification of the anti-ROS quality of the diamond Petri dish. For this the diamond surface will have to be additionally terminated with hydrogen—an innovative element towards the design of the super-biocompatible Petri dish.

### ROS and hydrophilicity

Destructive effects of extensive ROS bombardment are today in the focus of intensive research efforts. What has been overlooked so far is the impact of ROS on both intra- and extracellular hydrophilicity. On the nanoscale an increase in hydrophilicity accentuates the sticky nature of surfaces, thereby slowing down intracellular functions and contributing to a lifetime extension of extracellular ROS, as it is exemplarily described for the polystyrene Petri dish. Presumably, a synergistic interplay of both effects—simultaneous increase in intra- and extracellular ROS, is one of the most important elements in the factors causing biological aging. While effects of excess ROS are transient, the accompanying surface hydrophilization could induce permanent changes, especially in the extracellular matrix where an increase in hydrophilicity encourages the accumulation and durable deposition of osmiophilic groups (polar amino acids, fatty acids and calcium salts)—a progressive encapsulation process, which is believed to promote both a loss of elastic function and formation of fibrous tissue (20,21). Insight into the mechanism of formation of a variety of age related pathological

conditions, including but not limited to, skin wrinkling (22), limitation of normal penis function (23) and benign prostatic hyperplasia (24), a condition where chronic prostatitis is a frequent forerunner, could help to inspire appropriate therapies, for instance, the use of NIR light. The basic principles of laser biostimulation were discovered by Professor Endre Mester (25) using the first NIR laser in history. Since then the Mester group saved more than 1,000 patients from limb amputation, most of them with *ulcera cruris*. Insight derived from this series of editorials invite to new research.

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### Footnote

*Conflicts of Interest:* The author has no conflicts of interest to declare.

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