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Review Article

Nanocrystallinity, Chemical Surface Modification and Light-Tuning of Diamond Layers for Improved Cell Growth

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Abstract

Currently, there exists no biomaterial with an innovative potential comparable to that of (ultra)nanocrystalline diamond. Ulm University produced the first nanocrystalline "diamond Petri dish" in 2013. Tests performed on them with human sperm cells revealed a cell performance improved by 300% in comparison to that of conventional polystyrene Petri dishes. Observational evidence indicates that under culture conditions, diamond neutralizes the build-up of layers of reactive oxygen species (ROS) at the culture dish surfaces/cell interface. In contrast, the glue-like nanoscopic interfacial water layers on the hydrophilic side of the polystyrene Petri dishes typically used to culture cells act as a trap for ROS, such as H_2O_2. As a consequence, cells on polystyrene are exposed to both endogenous and exogenous ROS and are thereby inadequate for extended cell culture experiments.

Nanocrystalline diamond Petri dishes permit the prolongation of cell culture experiment times without interfering with the cells. The interpretation of the difference between nanodiamond and polystyrene led to a new understanding of biocompatibility, thereby promoting nanodiamond as a "gold standard" in biomedicine.

Furthermore, nanocrystalline diamond Petri dishes are sufficient for the complete in vitro fertilisation (IVF) routine, the preservation of oocytes and zygotes – known to be extremely sensitive to oxidative stress - in incubators, and the preservation and growth of stem cell cultures. Moreover, the biocompatibility of the diamond layers can be selectively improved by biomimetic structuring (3D patterns), by varying the chemical surface termination and by irradiating with nondestructive levels of 670 nm laser or light emitting diode (LED) light.

Keywords: Nanocrystalline diamond; Polystyrene; Petri dish; ROS; ATP; 670 nm light; Sperm cells

Introduction

Synthesis of (ultra) nanocrystalline diamond layers

Materials science has left behind the traditional routes of engineering and is developing in just about all the areas of the fundamental and applied sciences. One such area, predicted to have the most profound impact on daily life, is biomaterials - the development of new substrates for engineering and medicine [1]. Extended in vitro tests in general and permanent implants in

particular demand chemical inertness and bio durability, which are the most prominent biorelevant properties of a biomaterial. This brings nanocrystalline diamond naturally into the focus of our attention. Laboratory experiments using different biological cells have shown that nanocrystalline diamond is an incomparable biomaterial with unusual biocompatible properties [2], which are not explicable solely on the basis of its chemical and biological inertness. Furthermore, we demonstrated that the physical and chemical determinants of this biocompatibility, including information on nanoscopic interfacial water layers, can only reasonably be defined on diamond substrates. On the basis that diamonds are produced by nature, and by considering the biomimetic definition by Vincent, "The abstraction of good design from nature" [3], we may interpret variations of synthetic diamond, for instance, nanocrystalline diamond, as biomimetic materials. Due to the unique properties of diamond and the possibility of synthesizing and depositing homogenous films of nanocrystalline diamond via chemical vapor deposition (CVD) technologies on different substrates with sophisticated topographies, the fabrication of biomimetic surface layers becomes viable. The formation of CVD diamond films and layers involves different steps, including heterogeneous nucleation and the growth of primary diamond nuclei, which starts when individual carbon atoms on the substrate surface begin to form sp^3-tetrahedral lattices. The nucleation process can be induced by manual abrasion of nano-/micron-sized diamond particles on the substrate or by ultrasound agitation where the substrate is immersed in an aqueous suspension of nanodiamond crystallites with sizes typically between 5 and 20 nm produced through a detonation process [4,5]. The ultrasonic agitation method (nanoseeding [6-8]) facilitates the deposition of diamond films on substrates with complex shapes and surface topographies. A typical result of a seeding experiment is shown in Figure 1.

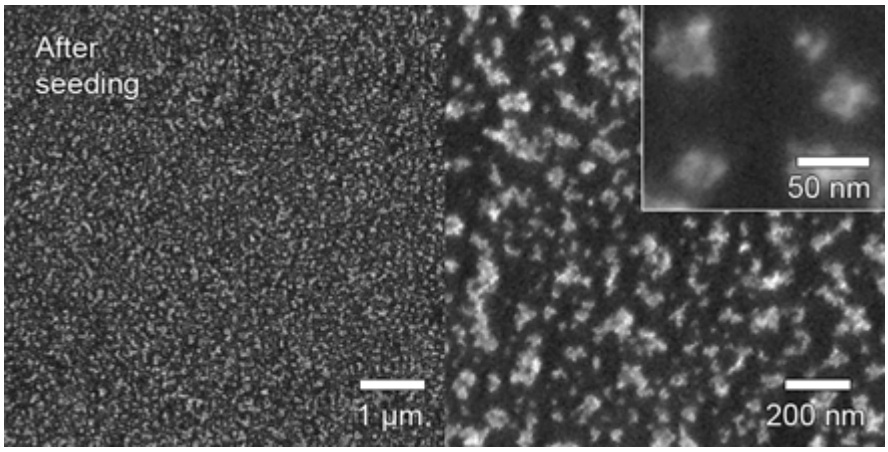

Figure 1: Homogeneous and distinct aggregation of nanodiamond clusters on a single-crystal silicon substrate at different magnifications after nucleation (SEM micrograph) [9].

Diamond micromechanics and chemical surface termination

Nanocrystalline diamond substrates allow us to implement the complete set of the eight determinants of biocompatibility as one hierarchical biomimetic model. Special attention should be given to substrates with moderate hydrogenation that are covered with a nanoscopic layer of interfacial water that is surprisingly stable in air [10] as well as in water [11]. Notably, hydrogenation is stable in water [12]. On the macroscale, surface hydrogenation is known to enhance the hydrophobic character of materials. On the nanoscale, however, hydrogenation is instrumental in binding a nanoscopic water layer to a diamond, which apparently acts as a soft viscoelastic blanket for cells during events of first contact [10]. The functional interplay between the stability and the molecular order of the nanoscopic interfacial water layer covering a diamond surface controls the actual biocompatibility of that surface. This can be further modified and tuned by chemical surface termination employing a plasma process, which is standard in microelectronic fabrication. Thus, chemical surface termination by O, H and F is achieved with different degrees of biocompatibility. Figure 2 illustrates that the chemical surface termination of nanocrystalline diamond has dramatic effects on cell behaviour and provides valuable insight for the fabrication of cell selective surface patterning.

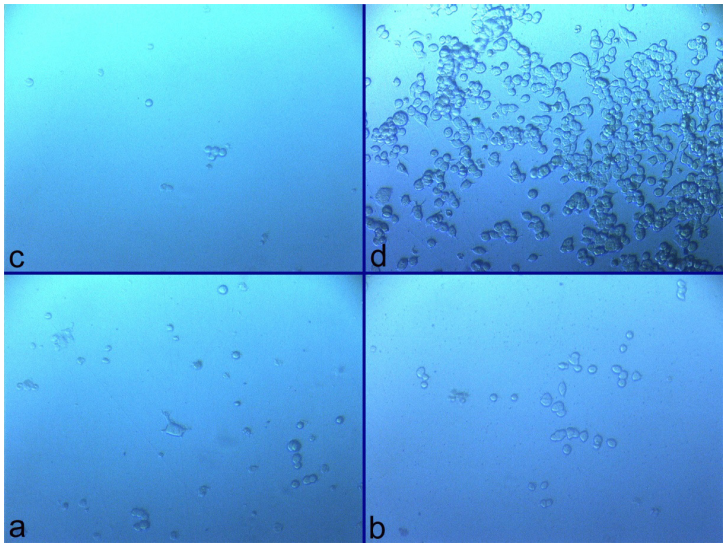

Figure 2: Light microscopy images of rat neuronal PC 12 cells on differently terminated nanocrystalline diamond: (a) untreated, (b) terminated with hydrogen, (c) with fluorine and (d) with oxygen.

In the subsequent CVD process, the thermal dissociation of the molecules constituting the gas phase, typically hydrogen and methane, as well as the heating of the substrate to the deposition temperature for diamond growth, is achieved by a suitable heat source, for instance, a hot filament tungsten wire. Depending on the initial seeding method and specific growth conditions in the CVD chamber, nanocrystalline diamond films with different surface morphologies and mechanical performance can be produced [13]. Examples of micromechanical components with complex geometries, such as the microgears used in mechanical watches, are displayed in the literature [9]. Importantly, this technology can be employed to deposit homogeneous films of nanocrystalline diamond on large silicon surfaces. Silicon with a melting point of ca. 1400 °C is the most suitable substrate for nanocrystalline diamond deposition (the substrate temperature is approximately 700 °C). Figure 3 displays an arrangement of micromechanical components with a delicate structure using lithographic techniques that allow scale-up and mass production. The components are homogeneously coated with a thin film of nanocrystalline diamond. The synoptic panel in Figure 4 is a representative overview of the intrinsic surface properties of the films. Importantly, the same technology can be applied to fabricate innovative laboratory equipment on a mass production scale for applications in life sciences, for instance, 3D cell culture dishes or microfluidic devices.

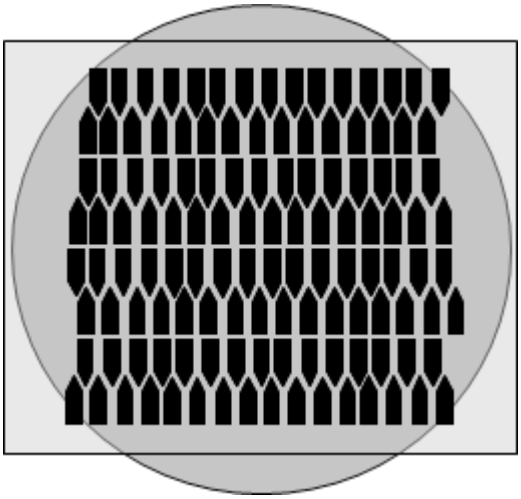

Figure 3: Top view of a multitude of cutting edges of ultrasharp scalpels designed by CAD methods and located on a photolithography mask (courtesy GFD).

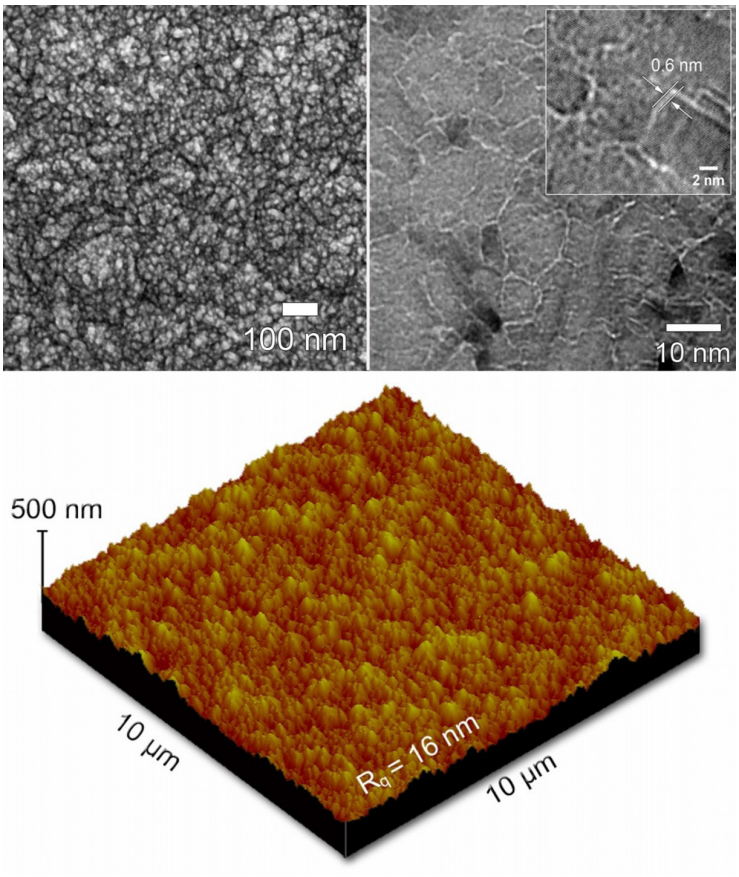

Figure 4: SEM image of the nanocrystalline diamond film shows a homogenous distribution of diamond grains within a size range of approximately 10 nm (top left). TEM image of the nanocrystalline diamond film. Using an out-of-focus mode, grain boundaries and hence grain structures become visible (top right). The high-resolution TEM image in the inset highlights the grain boundaries and the atomic planes of the diamond crystallites. AFM analysis of a 5-µm-thick nanocrystalline diamond film on a (100) silicon wafer. The topography of the 10 × 10 µm2 scan shows a minimal surface roughness with a root mean square roughness (Rq) of 16 nm (bottom).

Surface Modification by 3D Tailoring

Subsequently, arrays of cylindrical microcavities have been designed by CAD and produced on silicon surfaces and coated with films of nanocrystalline diamond (Figure 5, a-h). The cavities were etched into silicon wafers by the following procedure: A photoresist was spin-coated onto the silicon wafers (a), followed by a soft bake. The photoresist was patterned using contact lithography, i.e., via exposure to UV light, conforming to patterns in a chromium layer on a glass mask. Nonexposed photoresist was removed using a developer solution (b). Next, an electron beam evaporator was used for the deposition of a metal layer serving as an etch mask (c). The remaining photoresist was removed using an organic solvent (d). Cavities with diameters of approximately 100 µm or 200 µm (or other geometries) were etched into the silicon wafers by deep reactive ion etching (RIE) (e). The etch mask was chemically removed with a wet chemical (f). After ultrasonication in a nanodiamond solution, serving as a seeding medium (g), the nanocrystalline diamond films were deposited by a hot filament CVD process using a gas mixture containing hydrogen, methane oxygen and nitrogen, with process parameters optimised for each individual application (h). As such, photolithography and etching techniques can be used to produce microstructures in silicon devices. Alternatively, small-scale intricate parts can be produced by the micro metal injection moulding of metals [14] or by hot embossing technology using a micromachined embossing master, a low-cost method for generating microstructures on polymer substrates [15].

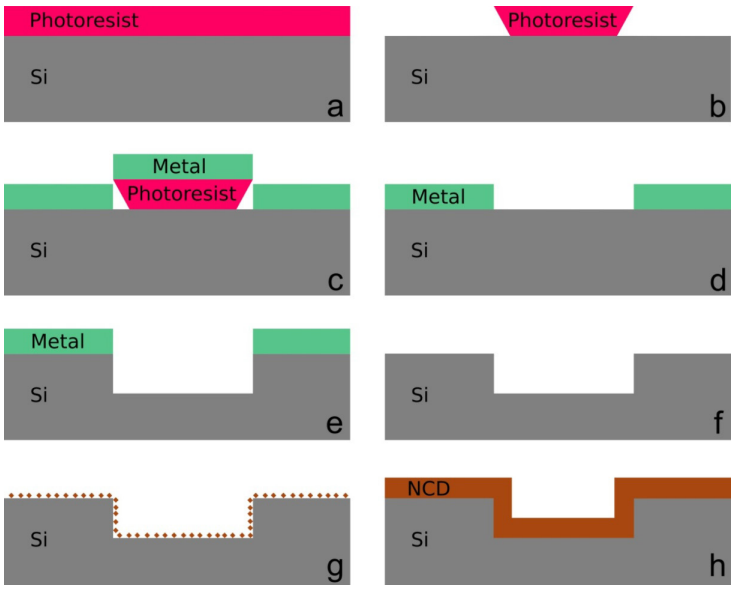

Figure 5: Fabrication steps of systematically and deliberately patterned nanocrystalline diamond films on microstructured silicon wafers by photolithography and reactive ion etching (RIE).

Biocompatibility of Biomimetic Surfaces

Observations during experiments with embryoid bodies formed from P19 cells led us to challenge a major cell biological paradigm, namely, that cancer cells are not social. To better understand the motivation of our approach, it is instructive to recapitulate the principal results, which eventually brought moderately hydrogenated nanocrystalline diamond substrates into the focus of our attention. One fascinating feature of embryoid bodies produced by P19 cells is that under favourable conditions of inducing differentiation, the bodies start to beat with a constant frequency of ~1 Hz. As seen under a light microscope, the beating process very much resembles that of a heart. However, P19 cells are initially tumour cells. Chen et al. suggested that the root cause of the periodic beating is to pump nutrients to nutrient-deprived cells situated in the core of the spheroid [16]. Using this intuitive picture, we interpreted the process of collective cell beating in embryoid bodies as a manifestation of social behaviour, a concept that turned out to be extremely fruitful, particularly in predictive modelling. This concept challenges and complements the pioneering work of Abercrombie et al. that explored the social behaviour of fibroblasts relative to tumour cells in culture. On the level of earlier methodologies, the authors observed that in fibroblasts cocultured with tumour cells, contact inhibition prevented the formation of multilayers, whereas the tested tumour cells formed cellular multilayers [17,18]. When multilayers are formed, cells in sublayers, for instance, at the bottom of a Petri dish, are cut off from accessing nutrients by the top layers. Today, there exists a growing body of observational evidence indicating that tumour cells are social [19]. Traditionally, the in vitro tests employed in the design and development of biocompatible materials focus only on the interaction between the individual cells and the biomaterial. Research paradigms of social cell behaviour focus concurrently on cell-cell and cell-biomaterial interactions, thereby facilitating new ways to assess the biocompatibility of biomaterials.

Starting from the premise that tumour cells are indeed social, we were interested in the possibility to systematically study social cell behaviour in vitro. For this, we designed an experimental scenario in which, after the formation of a confluent monolayer at the bottom of a culture dish, the rapidly proliferating tumour cells must differentially act between two possibilities: to form additional layers on top of the first one or to occupy new space by climbing on biomaterial substrates placed vertically on the ground monolayer [20]. Consistent with the aforementioned scenario, the prevalence of multilayer formation instead of the colonization of vacant space would indicate nonsocial cell behaviour. Remarkably, the cells displayed a significant difference in their climbing performance, with a strong dependence on the substrate material. Surprisingly, cells exposed their best and worst climbing performance on moderately hydrogenated nanocrystalline diamond and polystyrene (specimen cut from Petri dish used for cell culture), respectively (Figure 6).

The insight derived from the cell performance experiment shown in Fig. 6 initiated a series of discoveries, which put us in the position to systematically innovate the Petri dish using biomimetic principles. The central question, which is self-evident by the results of the P19 experiment, is the following: Is the relatively low cell performance observed on polystyrene relative to moderately hydrogenated nanocrystalline diamond a singular finding, only valid for P19 cells, or could it play a role with regard to a wider population of cells? In a first attempt to interpret the low cell performance on polystyrene relative to that on diamond, it is tempting to exclude the possibility of implicating the long-term determinants of biocompatibility (establishing the basis of

the biomimetic triangle [21]) in the reported difference. When the effect was identified first, a comparative analysis, based on the hierarchical structure of the biomimetic triangle, led us to consider a possible surface softening of the plastic material as an intrinsic cause of the low cell performance on polystyrene [21]. As shown in Section 8, we confirmed the surface softening experimentally and concluded that the effect impacts the structure of the nanoscopic interfacial water layer (top determinant of the biomimetic triangle).

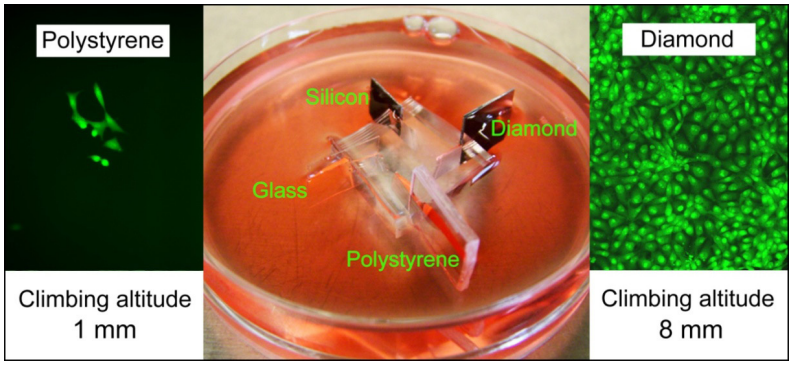

Figure 6: Method for quantifying cell performance in vitro: P19 cells climbing substrates placed vertically on a confluent cell layer. The maximum climbing altitudes of the cells for polystyrene and diamond, after a culturing time of 26 hours, are indicated in the image – determined via fluorescence microscopy [20].

Bottom Up Versus Top Down

Virtually all biomaterial and pharmacological tests designed to interrogate cells begin in a Petri dish, that is, in vitro. When used for in vitro fertilisation (IVF), the Petri dish serves as the environment for the beginning of human life. Petri dishes have been made of the translucent materials glass and polystyrene for 134 [22] and 65 years [23], respectively. During laboratory experiments, which showed that the performance of cells was better on nanocrystalline diamond than on polystyrene, the question arose: Is there a way to coat Petri dishes with nanocrystalline diamond? Considering that nanocrystalline diamond films are synthesized at substrate temperatures on the order of 600-800 °C, it was immediately clear that polystyrene cannot be coated with nanocrystalline diamond. Instead, commercially available Petri dishes that are made of quartz glass, a transparent material with a melting point above 1500 °C, appeared to be possible candidates. In contrast to the array of microcavities shown in Figure 7, the nanocrystalline diamond-coated Petri dish had to be transparent – otherwise, it is virtually useless for biological applications, such as the inspection of cells under inverted light microscopes, conventionally used in life science laboratories. Fortunately, quartz glass is transparent. The translucency of nanocrystalline diamond-coated quartz glass Petri dishes (diameter, 50 mm) is documented in ref. [24] with a photograph of a representative prototype of a series of nanocrystalline diamond-coated quartz glass Petri dishes - to the best of our knowledge, the first diamond-coated Petri dishes in the world. The expressive title of a 2008 press release of the American Chemical Society – Diamonds May Have Been Life's Best Friend on Early Earth [25] - served as inspiration for a new direction in our research and led to the first biological tests performed in nanodiamond-coated Petri dishes. The results of the tests, which explored the performance of human sperm cells in nanodiamond-coated Petri dishes, confirmed the previously observed tendency [20]: Sperm performance was approximately 20% better on nanodiamond (Petri dish generation III) than on polystyrene [26]. The anticipated potential of the experimental findings to improve the Petri dish and discriminate cell performances in vitro and their expected impact on life sciences in general, and IVF in particular, was reflected by the resonant coverage in the media [27-30]. Considering that the Petri dish, the temporary home to embryos grown in the laboratory, is used today in more than 99.9 % of IVF procedures [31], the motivation to extend the research in the direction of what might be called the design and development of a super biocompatible Petri dish with 3D features based on translucent quartz glass substrates, which can be coated with nanocrystalline diamond, is clear.

Materials that are similar in scale to biological molecules and systems and allow for the materialization of architectures on biorelevant scales, including the nanoscale [32], microscale [33] and macroscale [34], open the doors to exciting new applications in biomedical engineering and nanomedicine. Specifically, chemically inert substrates based on nanocrystalline diamond with grain sizes matching cell receptors, microcavities adjusted to the dimension of oocytes or embryoid bodies and substrates with diameters up to six inches allow us to cover the complete spectrum of biomimetic hierarchies.

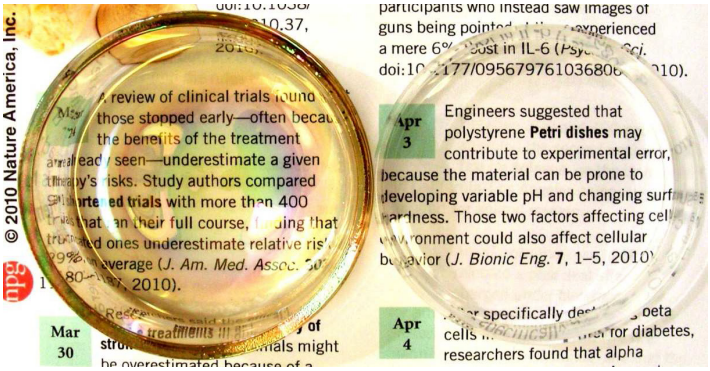

Figure 7: Quartz glass coated with nanocrystalline diamond still transparent after coating (left), polystyrene - transparent but softening during the experiments (right) [26].

Nanocrystalline Diamond for In Vitro Fertilisation (IVF)

The previously reported beneficial effect of nanocrystalline diamond-coated quartz Petri dishes on cell performance [24,26] was further validated in a new study that used human sperm cells to assess the effect of diamond-coated Petri dishes on the motility of human sperm cells. The parameter motility was chosen because it reflects the level of the cell's internal energy supply, the effects of ROS result in an immediate decrease in motility. ROS limit sperm cell motility: ROS production in cells involves electron leakage from mitochondria. The result of mitochondrial ROS generation is damage to these organelles and the initiation of an apoptotic cascade; as a result, sperm cells lose their motility [35]. We focused on sperm cells with progressive motility because it is believed that these cells have fewer chromosomal abnormalities and the best DNA integrity. The increase in motility on nanocrystalline diamond-coated quartz Petri dishes relative to that on controls (polystyrene Petri dishes used in IVF) was 300% [36]. Figure 8 displays the results of the preliminary test. The results are statistically significant.

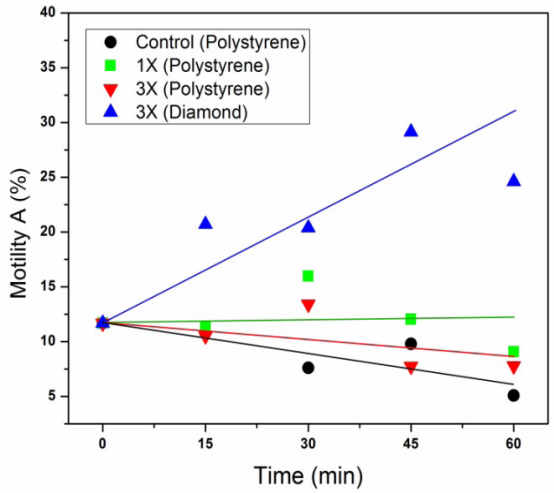

Figure 8: Motility data determined with polystyrene Petri dishes and in comparison, nanocrystalline diamond-coated Petri dishes, as a function of time.

Measurements were carried out at different points in time. The black line (--●--) represents the control (polystyrene). The green line (--■--) represents sperm cells irradiated with 670 nm LED light at a dose (1X) known to boost mitochondrial levels of adenosine triphosphate (ATP). The cells responded with a clear increase in motility. Very interesting are the results for irradiation at a triple dose (3X) known to induce an increase in intracellular ROS, manifesting itself as a decrease in sperm motility. Indeed, the red line (--▼--) reflects such behaviour for the cells irradiated in polystyrene Petri dishes. Surprisingly, even at detrimental doses (3X), the cells in the diamond Petri dishes (--▲--) responded with an enormous increase in motility. To gain insight into the root cause of this effect, the sperm motilities were measured in diamond Petri dishes without laser irradiation. Here, we also observed a giant increase in motility, comparable to the samples irradiated in the diamond Petri dish. To explain the boost in motility, we are led to

assume that nanocrystalline diamond is not only virtually ROS-free but possesses even the capacity to neutralize the effects of oxidative stress (ROS), as can be concluded from the comparison with relatively low-motility on polystyrene [red line (--▼--)] where the laser treatment had a detrimental impact on sperm motility. The inescapable conclusion is that nanocrystalline diamond acts, in principle, as a scavenger for ROS, in particular for ROS molecules originating from the sperm cells themselves, such as H_2O_2, where the diamond surface is instrumental in the neutralization of the ROS. Additional experiments are necessary to understand the ROS scavenging effect of nanocrystalline diamond and to assess the implication of the nanoscopic water layer at the diamond/cell interface in the ROS scavenging process.

Towards the Superbiocompatible Petri-Type Dish

Previously, we used moderately hydrogenated 3D structured nanocrystalline diamond surfaces to form embryoid bodies in vitro [37]. Notably, the substrate for the diamond film was silicon, a material that is not transparent to light. As a consequence, inspection of the embryoid bodies was only possible with a light microscope operating in reflection mode. Considering that life-science laboratories are usually equipped with optical microscopes that illuminate the samples (i.e., Petri dishes) from below, it is clear that nontransparent 3D Petri dishes represent a serious handicap in regard to the live inspection of the cells forming embryoid bodies. Another major limitation of nontransparent substrates in general is that such substrates, when irradiated with moderate intensities, i.e., intensities on the order of terrestrial solar intensity (~1000 Wm^{-2}) of red laser light, will heat up, causing thermal damage to the exposed cells. Considering the recently discovered beneficial effects of red light on cell performance (with an increase in ATP production in response to light treatment [38]), the possibility of synergistically combining 3D Petri dishes coated with nanocrystalline diamond (carbon-based biomaterial exhibiting biocompatibility due to chemical inertness, with no release of toxic compounds and absolute stability in liquid environments) becomes an attractive challenge that promises to revolutionize the life sciences.

Three-dimensional array-structured culturing systems are designed (Figure 9) and fabricated to grow spherical cell aggregates (embryoid bodies) and thus act as a 3D patterned chemically inert Petri dish. This now allows the implementation of the complete set of determinants of biocompatibility [20] as one biomimetic model, inspired by the characteristic periodic seed niche topography on the surface of strawberries. The 3D strawberry structure provides a setting for reproducibly culturing spherical cell aggregates in suspension. The spheroids are standardly used to study embryogenesis and tumorigenesis in vitro. Routinely, embryoid bodies are produced by the hanging drop method and cultured in polystyrene Petri dishes.

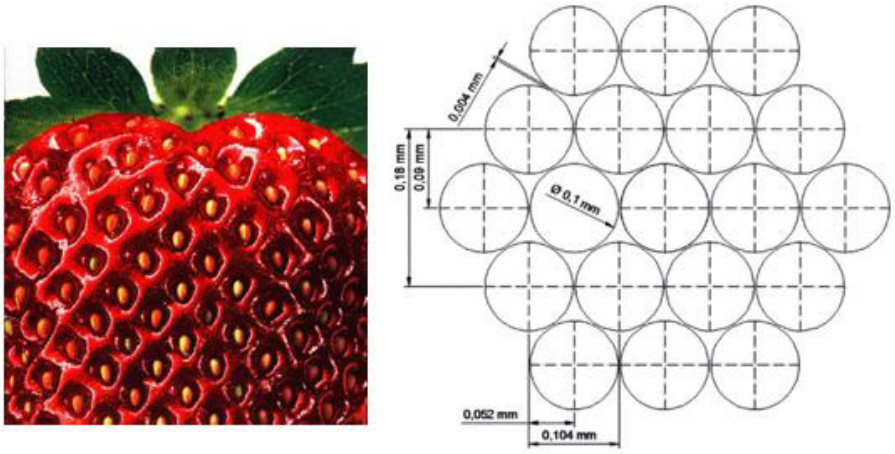

Figure 9: Characteristic niche topography on the strawberry surface (left); schematic model of the corresponding array structure (right).

A prototype of the 3D Petri dish is displayed in Figure 10 (left). Nanoscopic interfacial water layers masking the moderately hydrogenated diamond surfaces prevent the attachment of the predominantly adherent cells to the substrate during events of first contact, thereby accentuating their binding to each other - a condition for the formation of embryoid bodies. A precondition for their formation is a certain critical cell concentration below which cells tend to attach to the surfaces of the microcavities where they form monolayers (Figure 10). Embryoid bodies start to form at higher cell concentrations, i.e., where competitive binding favours cell - cell attachment relative to cell–substrate attachment. The latter situation is presented in Figure 11 [37].

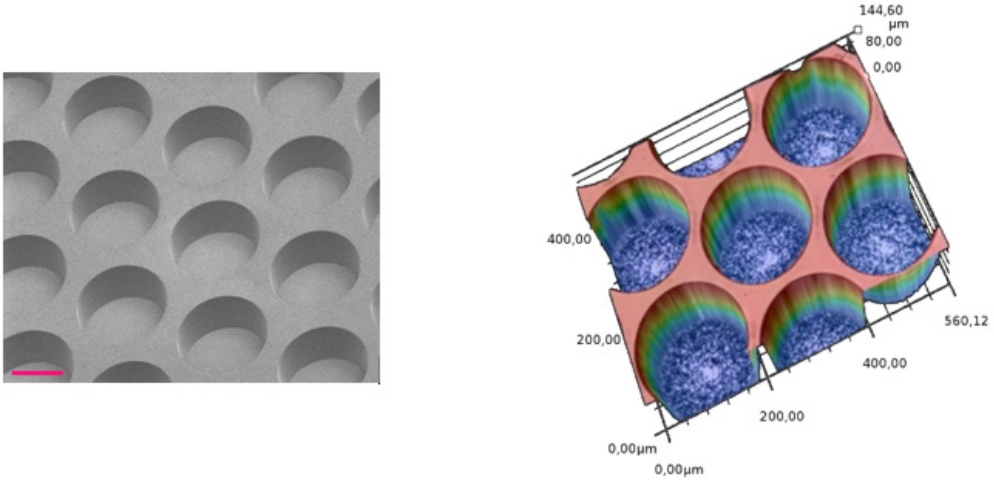

Figure 10: SEM (left) and digital microscope images (right) with nanocrystalline diamond deposited at the bottom of the cavities with a diameter of 200 µm.

The confocal laser scanning microscopy (CLSM) image shows monolayers of P19 cells at the bottoms of the diamond-coated cavities. The diameter of the cavities is ~200 µm (see Figure 11). More recently, the effect of diamond-coated Petri dishes on the formation of spherical aggregates of pluripotent stem cells (embryoid bodies) has been investigated. Notably, the diamond surfaces promoted embryoid body formation, whereas embryoid bodies did not form on polystyrene.

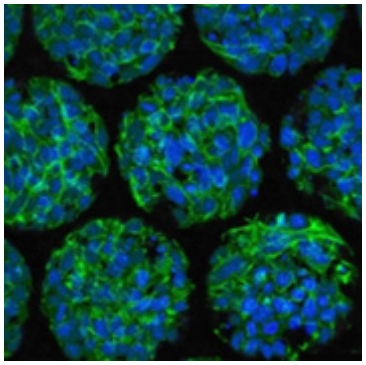

Figure 11: CLSM image shows monolayers of P19 cells at the bottoms of the diamond-coated cavities.

One major advantage of the microcavity method (3D Petri dish), when compared with the conventional "hanging drop method", is that the spheroids are immobilised in the cavities. In this state, it is possible to change the cell culture medium (adjusting the correct pH and safeguarding constant nutrient supply, which is essential for growth) without disturbing the embryoid bodies or washing them away before maturity, i.e., the desired harvest size.

As such, embryoid body formation on nanocrystalline diamond-coated microcavities is now a simple and straightforward process; it protects against potentially oxidative stress caused by centrifugation or by the substrate itself, and as such, microstructured NCD is becoming the "gold standard" for investigations of biomedical cell cultures with far-reaching results [37] and consequences.

The Petri dish is used today in more than 99.9% of IVF procedures [31]. The data presented motivate the extension of the corresponding research towards the design and development of a superbiocompatible Petri dish with parameters suitable for application as 3D structured biomimetic features that could be used for the mass fertilisation of oocytes and to study cell spheroid development - scenarios that also demand the possibility of monitoring cells via a light microscope. These applications are expected to interest the entire life science community. The inspection and manipulation of rare or precious cells, such as oocytes and pluripotent stem cells, demand partial immobilisation under ROS-free conditions, as is possible with nanocrystalline diamond-coated microcavities. With chemical terminations, in particular H and O, nanocrystalline diamond can be used in single-cell experiments (microcavities of the dimension of a cell) and to study the formation of embryoid bodies (microcavities of the dimension of the cell spheroids). The 3D structures are ideal for the production of multicellular tumour spheroids mimicking early

events in metastasis and for their interrogation by pharmacological compounds. The prospect of totally undisturbed (i.e., by the substrate) cell–cell communication and signalling, which are critical for cell function, are direct benefits of the 3D Petri dish. Likewise, 3D culture systems are better for mimicking pathological conditions, such as cancer. For example, cancer cells grown in 2D can easily be killed by low doses of anticancer drugs. However, in 3D, the same cells are resistant to the same doses of anticancer drugs, similar to the in vivo situation. Therefore, cells grown in 3D are more suitable for testing new drugs. Moreover, microtissues cultured in 3D dishes are promising for the repair of damaged or diseased tissue in regenerative medicine. These aspects are summarised in the form of a visual synopsis in Figure 13.

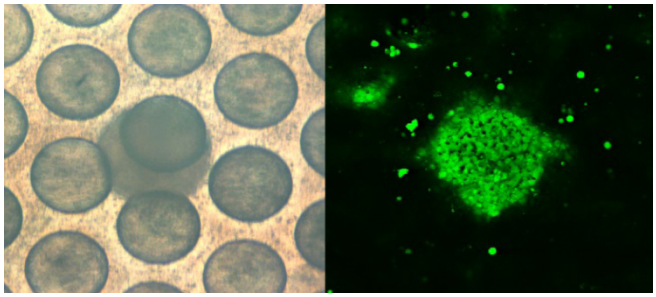

Figure 12: Light microscopy image of an embryoid body formed by mouse embryonal carcinoma P19 cells on strawberry-patterned diamond (left) and CLSM image of embryoid bodies stained with calcein (right).

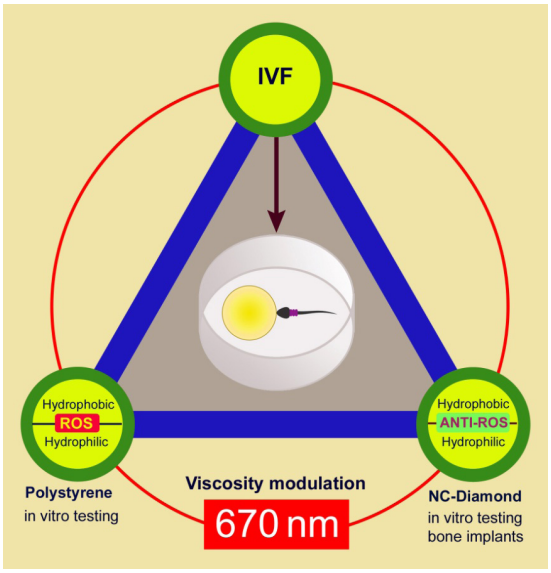

Figure 13: Visual summary of the difference between conventional polystyrene and nanocrystalline diamond substrates for Petri dish geometries with particular emphasis on potential IVF routines.

Effect of Laser Light on Interfacial Water Viscosity and Cells

Atomic force acoustic microscopy (AFAM) in combination with 670 nm laser light - a method that can be used to quantitatively and qualitatively analyse nanoscopic interfacial water layers on substrates - revealed the presence of a stable viscoelastic water layer on hydrogenated diamond and a comparably soft water layer on hydrophilic species [39]. Whereas the AFAM experiments were performed under ambient conditions, their relevance for realistic conditions mimicking extra-/intracellular interfaces was validated by similar experiments using a quartz crystal microbalance (QCM), proving the subaquatic persistence of the nanoscopic water layers [40]. The mechanism of interaction between the 670 nm laser light and the nanoscopic interfacial water layers was recently elucidated by using soft X-ray absorption spectroscopy [41]. It is important to note that the same laser light that was used in the aforementioned AFAM experiment to modulate the nanoscopic interfacial water layers bound to test surfaces is instrumental in reducing interfacial water viscosities and the viscous friction between hydrophilic surfaces and the diamond tip of a nano indenter, mediated by nanoscopic interfacial water, as well as in stimulating ATP production in oxidatively stressed cells [38]. Thus, 3D structured quartz glass Petri dishes coated with a translucent

film of nanocrystalline diamond would allow us to fully exploit the synergistic interplay between the cell-friendly properties of nanocrystalline diamond, its potential to facilitate the growth of embryoid bodies and the use of red laser or LED light to enhance cell performance and accelerate cell growth.

Originality of the Approach and Potential of the Anticipated Results

Nanocrystalline diamond-coated Petri dishes possess intrinsic qualities that bring them into the focus of life science research. The first biological results obtained are expected to lead to an extension of our current understanding of biocompatibility. ROS-free biomaterial surfaces with antibacterial properties are recommended for both the production of culturing devices and the design and development of implants. For example, the reported sperm experiments indicate that nanocrystalline diamond has a further exciting property, namely, to act as a ROS scavenger under physiological conditions. Whereas TiO_2 surfaces are known to respond to irradiation by forming ROS, nanocrystalline diamond surfaces respond differently. There is observational evidence indicating that the high viscosity (glue-like) nanoscopic interfacial water layers on hydrophilic substrates are involved in extending the residence times of ROS. Since 670 nm laser light was found to reduce the viscosity of the interfacial water layers, it is postulated that 670 nm light can be used to modulate the ROS capturing capacity of biomaterial surfaces and bio surfaces and thereby also to fine tune biocompatibility. The newly developed method for recording changes in interfacial water viscosity by combining nanoindentation with laser irradiation [38] will allow us to assess the viscosity reductions due to laser irradiation under relevant cell culture conditions and different pH values and temperatures.

In an attempt to understand the ROS scavenging effect, we suggest that the ROS molecules, known to have very short lifetimes in bulk liquid but apparently having longer lifetimes on the surfaces of polystyrene Petri dishes – a result which can be understood by analogy with the lifetime extension of certain fluorescent dyes on surfaces - are trapped in the viscous nanoscopic water layer at the bottom of the polystyrene Petri dish. This explains their detrimental effect on sperm cells on polystyrene Petri dishes (c.f. Figure 8). Considering that nondestructive levels of 670 laser light have the potential to dramatically reduce the viscosity of the nanoscopic interfacial water layers [38], we suggest that irradiation with such laser light could counteract the detrimental effects of the ROS by simply reducing the viscosity and thereby the trapping capacity of the nanoscopic water layers in the relevant interfacial zone.

In 2010, it was suggested that polystyrene Petri dishes soften when exposed to aqueous liquids, an effect facilitating the establishment of a nanoscopic layer of reactive oxygen species (ROS) [21]. The effect has been discovered by testing cell performance and using different living cells, including mouse embryonal carcinoma cells P19, murine fibrosarcoma cells L929 and human cervical cancer cells HeLa, as ROS-sensitive probes on polystyrene in comparison with fully inert and ultrasmooth nanodiamond layers. Nature Medicine commented on the research: "Polystyrene Petri dishes may contribute to experimental error because the material can be prone to developing variable pH and changing surface hardness. Those two factors affecting cellular environment could also affect cellular behaviour." [27,42]. The first experimental proof of this nanomechanical softening was provided in 2012. We designed and developed a measuring chamber allowing us to perform extended nanoindentation measurements in a liquid milieu. With its assistance, we provided clear evidence for the nanomechanical/physicochemical effect, extending the catalogue of the factors determining the cellular stress landscape in vitro. The discovery of the nanoscale surface mechanical properties and their impact on cell behaviour is of highest relevance to both the life sciences and the manufacturers of cell culture devices. In 2013, we produced the first nanocrystalline "diamond Petri dish" employing dedicated hot filament CVD methods together with an oxygen-based reactive ion etching technique. The first cell tests performed on these nanodiamond layers revealed unexpected properties, with a cell performance improved by 300% compared to that of conventional polystyrene Petri dishes. However, the mechanism is not fully understood, and there is converging observational evidence suggesting that nanodiamond acts in cell culture conditions as a reducer of ROS.

Complementary, in vitro experiments with human sperm cells, serving as bioprobes that are highly sensitive to ROS, confirmed the previously observed trend: cell viability on nanocrystalline diamond-coated quartz Petri dishes was 20% superior to that on polystyrene Petri dishes. This and further tests indicated that nanocrystalline diamond surfaces are practically ROS-free. This is particularly important because when used for IVF, the Petri dish serves as the environment for the beginning of human life. Science commented on these new results [43]. However, further tests indicate that nanocrystalline diamond surfaces are not only ROS-free in a passive sense but have even the capacity to actively neutralize the effects of ROS. Virtually all biomaterial and pharmacological tests designed to interrogate cells begin in Petri dishes, i.e., in vitro. Earlier, Chen et al. reported that the average density of adherent neural stem cells on nanocrystalline diamond was twice as good as that on polystyrene [44]. Furthermore, Jakubowski et al. [45] investigated the susceptibility of biomaterial medical steel, titanium and medical steel coated with nanocrystalline diamond to bacterial colonization. The comparison with titanium is of particular interest because of the prominent bactericidal properties of irradiated TiO_2. Surprisingly, the nanocrystalline diamond surface exhibited the highest resistance to bacterial colonization, significantly higher than that of titanium. The resistance of medical steel was very poor [45]. The presence/absence of bacteria is not only important for in vitro testing; during the process of implantation, it is a crucial indicator for the failure or success of the event. The presence of a thin layer of bacteria between the implant and bone often triggers inflammatory processes and initiates the long-term destabilization of the bone/implant interface.

More recently, the effect of diamond-coated Petri dishes on the formation of spherical aggregates of pluripotent stem cells (embryoid bodies) has been investigated. Notably, the diamond surfaces promoted embryoid body formation, whereas embryoid bodies did not form on polystyrene. One promising

method for producing embryoid bodies in the laboratory is to use 3D-structured surfaces, i.e., surfaces covered with arrays of microcavities allowing the cells to assemble into spheroids to grow. Such biomimetic structures are ideal for the systematic production of multicellular tumour spheroids mimicking early events in metastasis in vitro and for the interrogation of cell spheroids by pharmacological compounds. Previously, we showed that cavities coated with moderately hydrogenated nanocrystalline diamond films promoted the formation of cell–cell bonds, facilitating the establishment of embryoid bodies [37]. One major advantage of the microcavity method (3D Petri dish), when compared with the conventional "hanging drop method", is that the spheroids are immobilized in the cavities. In this state, it is possible to change the cell culture medium (adjusting the correct pH and safeguarding constant nutrient supply, which is essential for growth) without disturbing the embryoid bodies or washing them away before maturity, i.e., the desired harvest size. Embryoid body formation on nanocrystalline diamond-coated microcavities is a simple and straightforward process; it protects against oxidative stress caused by centrifugation or by the substrate itself. Our results motivate the design of a hybrid material that will concurrently fulfil the four basic requirements of the ideal cell culture device: biocompatibility safeguarding maximum cell performance, preselected polarity, full translucency and bio durability (stability under relevant cell culture conditions). With the available chemical terminations, nanocrystalline diamond promises extraordinary advantages in cell culture, single-cell experiments and the study of embryoid bodies. Especially in Germany, where research with human embryos is prohibited, embryoid bodies play a central role in clinical research. Nanodiamond-based 3D structures are an ideal solution for the undisturbed production of multicellular spheroids mimicking early events in cell assemblies in vitro and for their interrogation by pharmacological compounds. Likewise, they can be used for the mass fertilisation of oocytes and embryonic development. The undisturbed (i.e., by the substrate) chemical communication and proximity of the cells in the 3D Petri dish also facilitates adhesion molecules and surface receptors on one cell to better bind to the adhesion molecules and surface receptors on a proximal cell. Proximity is necessary for cell–cell communication and signalling, which are in turn critical for cell function. One benefit of the 3D Petri dish over the 2D culture systems is that it mimics pathological conditions, such as cancer. For example, cancer cells grown in 2D can easily be killed by low doses of anticancer drugs. However, in 3D, the same cells are resistant to the same doses of anticancer drugs, similar to the in vivo situation. Therefore, cells grown in 3D are more suitable for testing new drugs. Moreover, microtissues cultured in 3D Petri dishes are promising for directly repairing damaged or diseased tissue in regenerative medicine applications.

A further parameter that is crucial to both culturing device and implant fields is surface polarity, expressed as hydrophobicity or hydrophilicity. In an animal model, Kloss et al. demonstrated that bone-implant contact was better on oxygen-terminated diamond coated implants (hydrophilic) than on titanium- or hydrogen-terminated species (hydrophobic) [46]. Furthermore, we also explored the tribological properties of nanocrystalline diamond surfaces by atomic force acoustic microscopy (AFAM) and investigated the bonding stability of the water molecules on both hydrogenated and nonhydrogenated nanocrystalline diamond surfaces of identical surface roughness. The principal difference was the presence of an extremely stable interfacial water layer on the surface of the hydrogen-terminated species. The presence of this lubrication film – explaining the extremely low friction between hydrogenated diamond species – promoted the hydrogen terminated nanocrystalline diamond surfaces to ideal pairings in tribological settings [47]. Applications in the future will include but will not be limited to diamond-on-diamond implant surfaces and micromechanical components.

Special attention should be given to nanocrystalline diamond substrates with moderate hydrogenation, which promote the formation of a nanoscopic interfacial water layer that is surprisingly stable in air as well as in water. On the macroscale, hydrogenation enhances the hydrophobic character of materials. On the nanoscale, however, hydrogenation is instrumental in binding a nanoscopic water layer to a diamond, which apparently acts as a soft viscoelastic blanket for cells during events of first contact. By employing a plasma process that is standard in microelectronic fabrication, the surface termination can be further modified and tuned to different degrees of biocompatibility via chemical surface termination by O, H, N and F. These options of chemical surface variability are complemented by the possibility of predictably adjusting the crystalline structures of nanodiamonds in general, as well as their mechanical properties [48,49], and of industrially relevant nanodiamond coatings in particular. By this tunability of their chemo structural properties, nanodiamonds advance to the status of a veritable "designer material" with an incomparable functional spectrum.

Conclusion

For cells adhering to biomaterial surfaces, the initial contact is mediated by nanoscopic interfacial water layers acting as informational blueprints during cellular recognition. The data presented herein leave room for the possibility that certain material surfaces could convert interfacial water to interfacial ROS. In contrast to the cell-friendly diamond, the polystyrene surfaces used in cell culture experiments apparently facilitate the formation of a nanoscopic interfacial layer of ROS, transiently trapped in the viscous interfacial water film formed on the hydrophilic polystyrene. This ROS layer presumably induces oxidative stress and is therefore probably the intrinsic cause for the reduced cell performance on polystyrene relative to that on nanodiamond. In biomaterial and pharmacological tests, the classical routine typically includes three parts - preclinical (in vitro and animal) and clinical studies - where potential biomaterials (pharmaceutical agents) are tested by trial and error, which is rather costly. Improving the biocompatibility of the Petri dish will inevitably improve the reliability and predictive value of these in vitro tests, thereby contributing to an overall cost reduction of not only the in vitro parts of testing procedures but also the parts comprising animal experiments. In this book chapter, we described some progress regarding the transition from basic research to a biomedical product: a biomimetically designed

Petri-type experimental dish, which promises to revolutionize life sciences.

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Conflict of Interest

No conflict of interest.

References

1. HJ Fecht, K Brühne, P Gluche (2014) Carbon-based Nanomaterials and Hybrids - Synthesis, Properties and Commercial Applications. Jenny Stanford Publishing, Singapore.
2. P Ball (2013) Four decades of materials developments transform society. MRS Bulletin 38(11): 873-885.
3. J Vincent (1995) Borrowing the best from nature, in Encyclopaedia Britannica Yearbook.
4. A Krüger, F Kataoka, M Ozawa, T Fujino, Y Suzuki, et al. (2005) Unusually tight aggregation in detonation nanodiamond: Identification and disintegration. Carbon 43(8): 1722-1730.
5. OA Shenderova VV Zhirnov, DW Brenner (2002) Carbon nanostructures. Critical Reviews in Solid State and Materials Sciences 27(3-4): 227-356.
6. OA Williams, O Douhéret, M Daenen, K Haenen, E Osawa, et al. (2007) Enhanced diamond nucleation on monodispersed nanocrystalline diamond. Chemical Physics Letters 445(4-6): 255-258.
7. M Daenen, O Williams, JD Haen, K Haenen, M Nesládek (2006) Seeding, growth and characterization of nanocrystalline diamond films on various substrates. Physica Status Solidi (A) Applications and Materials 203(12): 3005-3010.
8. A Kromka, O Babchenko, B Rezek, M Ledinsky, K Hruska, et al. (2009) Simplified procedure for patterned growth of nanocrystalline diamond micro-structures. Thin Solid Films 518(1): 343-347.
9. M Wiora (2013) Characterization of nanocrystalline diamond coatings for micro-mechanical applications (Doctoral Dissertation). Germany.
10. AP Sommer, D Zhu, A Mester, H Försterling, M Gente, et al. (2009) Interfacial water an exceptional biolubricant. Cryst. Growth Des 9(9): 3852-3854.
11. AP Sommer, D Zhu, R Franke, H Fecht (2008) Biomimetics: Learning from diamonds. Journal of Materials Research 23(12): 3148-3152.
12. D Zhu, L Zhang, R Ruther, R Hamers (2013) Photo-illuminated diamond as a solid-state source of solvated electrons in water for nitrogen reduction. Nature Materials 12: 836-841.
13. M Wiora, K Bruehne, A Floeter, P Gluche, T Willey, et al. (2009) Grain size dependent mechanical properties of nanocrystalline diamond films grown by hot-filament CVD. Diamond and Related Materials 18(5-8): 927-930.
14. T Hartwig, G Veltl, F Petzoldt, H Kunze, R Scholl, et al. (1998) Powders for metal injection molding. Journal of the European Ceramic Society 18(9): 1211-1216.
15. H Becker, U Heim (2000) Hot embossing as a method for the fabrication of polymer high aspect ratio structures. Sensors and Actuators 83(1-3): 130-135.
16. Q Chen, S Harding, N Ali, A Lyon, A Boccaccini (2008) Biomaterials in cardiac tissue engineering: Ten years of research survey. Materials Science and Engineering: R 59(1-6): 1-37.
17. M Abercrombie, J Heaysman (1954) Invasiveness of sarcoma cells. Nature 174(4432): 697-698.
18. M Abercrombie, J Heaysman, H Karthauser (1957) Social behaviour of cells in tissue culture. III. Mutual influence of sarcoma cells and fibroblasts. Exp Cell Res 13(2): 276-291.
19. E Ben-Jacob, D Coffey, H Levine (2012) Bacterial survival strategies suggest rethinking cancer cooperativity. Trends in Microbiology 20(9): 403-410.
20. AP Sommer, D Zhu, M Wiora, H Fecht (2008) The top of biomimetic triangle. Journal of Bionic Engineering 5(2): 91-94.
21. AP Sommer, D Zhu, T Scharnweber, H Fecht (2010) On the social behaviour of cells. Journal of Bionic Engineering 7(1): 1-5.
22. Petri RJ (1887) A small modification of the Koch plate method. Central Journal for Bacteriology and Parasite Science 279: 230.
23. R Fisk (1956) Disposable culturing device. U.S. Patent Patent 2(874): 091.
24. AP Sommer, D Zhu, F Gagsteiger, H Fecht (2013) Petri dish generation III," On-line Journal of Nano and Advanced Materials Workshop and Fair, pp. 44-45.
25. M Woods (2008) American Chemical Society's Weekly PressPac -- July 23, 2008, American Chemical Society.
26. Sommer AP, D Zhu, F Gagsteiger, H Fecht (2013) Sperm performance better on diamond than on polystyrene. Materials Research Society symposia proceedings. Materials Research Society.
27. (2010) News in Brief. Nature Medicine 16: 502-503.
28. (2013) Random Sample. Diamonds are a sperm's best friend Science 339(6121): 743-744.
29. N Fleming (2013) IVF sperm do better in a diamond dish. New Scientist 217(2906): 16.
30. J Ruttimann Oberst (2013) Diamonds in the rough. Endocrine News, pp. 20-21.
31. J Cohen (2006) Growing embryos in a world that is flat: A short history of embryo culture in Petri dishes. Fertility Magazine 6: 12-24.
32. Y Champion, H Fecht (2005) Nano-Architectured and Nanostructured Materials. Fabrication, Control and Properties, Wiley-VCH, pp. 166.
33. H Fecht, M Werner (2004) The Nano-Micro Interface: Bridging the Micro and Nano Worlds. Wiley-VCH.
34. G Jeronimidis (2008) Bioinspiration for engineering and architecture: Materials-Structures-Function in Proceedings of the 28th Annual Conference of the Association for Computer Aided Design in Architecture (ACADIA), Minneapolis.
35. R Aitken, K Jones, S Robertson (2012) Reactive oxygen species and sperm function - in sickness and health. J Androl 33(6): 1096-1106.
36. AP Sommer, S Jaganathan, M Maduro, K Hancke, W Janni, et al. (2016) Genesis on diamonds II: contact with diamond enhances human sperm performance by 300%. Ann Transl Med 4(20): 407.
37. AP Sommer, D Zhu, T Scharnweber, H Fecht (2009) Body building on diamonds. Journal of Bionic Engineering 6(1): 14-17.
38. AP Sommer, M Haddad, H Fecht (2015) Light Effect on Water Viscosity: Implication for ATP Biosynthesis. Scientific Reports 5: 12029.
39. AP Sommer, A Caron, H Fecht (2008) Tuning Nanoscopic Water Layers on Hydrophobic and Hydrophilic Surfaces with Laser Light. Langmuir 24(3): 635-636.
40. AP Sommer, D Zhu, HD Försterling, T Scharnweber, A Welle (2008) Crystalline water at room temperature – Under water and in air. Cryst Growth Des 8: 2620-2622.
41. AP Sommer, K Hodeck, D Zhu, K Lange, H Fecht, et al. (2011) Breathing Volume into Interfacial Water with Laser Light. J Phys Chem Lett 2(6): 562-565.

42. Sommer, N Lotan (2010) Controls needed to reduce problem of plastic contamination. Nature 465: 289.

43. M Price (2017) Diamond dishes could boost IVF success rates. science.

44. YC Chen, DC Lee, CY Hsiao, YF Chung, HC Chen, et al. (2009) The effect of ultra-nanocrystalline diamond films on the proliferation and differentiation of neural stem cells. Biomaterials 30(20): 3428-3435.

45. W Jakubowski, G Bartosz, P Niedzielski, W Szymanski, B Walkowiak (2004) Nanocrystalline diamond surface is resistant to bacterial colonization. Diamond and Related Materials 13(10): 1761-1763.

46. FR Kloss, R Gassner, J Preiner, A Ebner, K Larsson, et al. (2008) The role of oxygen termination of nanocrystalline diamond on immobilisation of BMP-2 and subsequent bone formation. Biomaterials 29(16): 2433-2442.

47. P Sommer, D Zhu, AR Mester, HD Försterling, M Gente, et al. (2009) Interfacial Water an Exceptional Biolubricant. Cryst Growth Des 9(9): 3852-3854.

48. V Mitic, H Fecht, M Mohr, G Lazovi, L Kocic (2018) Exploring fractality of microcrystalline diamond films. AIP Advances 8(7): 075024.

49. P Németh, K McColl, LAJ Garvie, CG Salzmann, M Murri, et al. (2020) Complex nanostructures in diamond. Nature Materials 19: 1126-1131.