

From Microtornadoes to Facial Rejuvenation: Implication of Interfacial Water Layers

Andrei P. Sommer* and Dan Zhu

Institute of Micro and Nanomaterials, University of Ulm, 89081 Ulm, Germany

Received January 18, 2008; Revised Manuscript Received September 5, 2008

ABSTRACT: Crystalline interfacial water layers have been observed at room temperature on both hydrophobic and hydrophilic surfaces – in air and subaquatically. Their implication in biology (and evolution) was postulated in a visionary paper in 1971 by Szent Györgyi. Today, they are believed to play a fundamental role in protein folding. A recent X-ray diffraction study reports on their presence on crystals in contact with their growth solution. Their subaquatic persistence on hydrophobic solids was reported in 2007. Their relevance in nanoscale phenomena is reflected by the multidisciplinary focus in their study. In the course of a systematic exploration of interfacial water layers on solids we discovered microtornadoes, found a complementary explanation to the surface conductivity on hydrogenated diamond, and arrived at a practical method to repair elastin degeneration using light. The result was rejuvenated skin, reduced wrinkle levels, juvenile complexion, and lasting resilience.

Conforming to an extreme sensibility of interfacial water layers to direct observation techniques, a major part of the existing data stems from computer models. Experimentally, interfacial water layers have been studied by X-rays and neutron scattering,^{1,2} atomic force microscopy (AFM),^{3–5} near-field scanning optical microscopy (NSOM),⁶ atomic force acoustic microscopy (AFAM),⁷ drop evaporation experiments,⁸ and recently on hydrogen-terminated nanocrystalline diamond.⁹ A synoptic analysis of all these perspectives provides an increasingly clear picture of the nature of interfacial water: The central and probably generalizable result seems to be that the structural difference between water layers on a solid surface exposed to air, and water layers that prevail at the interface between the same solid surface and bulk water, is less pronounced than that between water layers on a hydrophobic and a hydrophilic surface, studied under identical conditions. The practical importance of this insight is enormous: Extracting information on water structures in air is simpler than probing water layers at the interface between a solid and bulk water, and we are justified to use the structural information (molecular order, density, or viscosity of the water) acquired in simple systems for modeling complex systems. In practice, this means that structural information obtained for instance by AFM in air on a certain model substrate could be transferred for modeling partial aspects in biological systems. The relevance of the “partial aspect” approach becomes clear from realizing that biological surfaces are inhomogeneous: Their dynamic nature, with nanoscopic patches varying in polarity and topography, exclude a macroscopic perspective and complicate distinctive experimental insight. Thus, the requirement for model surfaces, which permit us to mimic specific biosystem aspects, is clear. Obviously, biocompatible materials based model surfaces are the best choice here. The themes investigated have one aspect in common: The substrates impose their order to the interfacial water layer – in other words the water layers may be looked upon as informational blueprints. The recently reported synthesis of high density carbon nanocrystals¹⁰ might enrich our perception of interfacial water layers. In explaining the synthesis, the authors arrived at the conclusion that the new nanocrystals were formed in a process in which the crystalline interfacial water imposed its order to the metastable (solidifying) carbon nanocrystals. Clearly, this possibility implies evidence for an active function of interfacial water. In the era of *think small* the realization of this aspect deserves our attention and merits further study.

Subaquatic Interfacial Water Layers on Nanocrystalline Diamond. We are currently using nanocrystalline diamond to

extract information on interfacial water layers. Minimal corrosion, extensive biocompatibility, and a precisely adjustable nanostructure promote this material to an ideal model platform for getting structural insight into water layers contacting biological surfaces. Specifically on hydrogen-terminated diamond (hydrophobic), we found that interfacial water is crystalline, and thereby proton conductive – both in air⁹ and subaquatically. Figure 1 (left panel) illustrates the principle of the experiment designed to probe interfacial water layers subaquatically. It shows the electrodes of an Ohmmeter on hydrogen-terminated diamond, one in contact with the substrate, the other in intermediate contact, that is, immersed in a 20 μL drop of ultra pure water. For the constellation cathode in drop – anode on dry substrate, we obtained a representative value of 5.63 M Ω . For the reverse situation, the resistance was up to 21.29 M Ω . These values were determined by the use an Ohmmeter equipped with platinum electrodes (purity 99.99%). For both electrodes in contact with the dry substrate the resistances were significantly lower (1.30 M Ω). We interpret the enhanced conductivity on the dry substrate in terms of proton hopping, facilitated by a crystalline water layer on the hydrogenated diamond. The premise is that the higher the order of the water molecules in the water layer, the better the conductivity. Conversely, the relatively low resistance of 5.63 M Ω compared to 21.29 M Ω can be explained by noting that the bulk water in the drop covering the cathode is involved in reducing the degree of crystallinity in the interfacial water layer interconnecting cathode and diamond. Furthermore, when the cathode is immersed into the drop, reduction in the order by the coexisting bulk water is less effective due to the interfacial H₂O molecules aligned between cathode and hydrogen. Figure 1 (right panel) shows chains of polarized H₂O molecules, bridging the gap between hydrogen atoms and the cathode, thus facilitating the transfer of excess protons in the water layer masking the substrate. Hydrogenated diamond recommends itself as a unique platform to study interfacial water layers both in air and subaquatically, including their potential implication in the recently reported cell–cell communication involving protons.¹¹ Our results represent a strong challenge for the established paradigm of surface conductivity in hydrogenated diamond.^{12–14}

Microtornadoes – Extrapolated Implications. Microtornadoes formed in the laboratory under conditions similar to those implied in the genesis of supercell tornadoes: A column of humid air forms between a layer of cold air moving over warm, humid air on the ground. Wind shear makes the column start to spin and form a high-speed vortex that extends from the cloud cover to the ground. The interplay of the factors participating in this real-world scenario could be analyzed in microtornadic systems generated under the

* To whom correspondence should be addressed. E-mail: samoan@gmx.net.

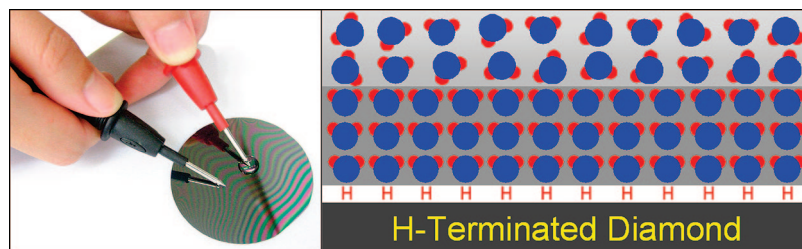


Figure 1. Left: Principle of surface conductivity measurement on hydrogenated nanocrystalline diamond exposing the crystalline order of the subaqueous interfacial water layers (disc $\varnothing = 5$ cm). Right: Schematic of array of proton-conducting H_2O chains, polarized by the hydrogenated site. Protons are easily pulled along the chains aligned between hydrogen atoms on the diamond and immersed cathode.



Figure 2. Light microscopy photographs display microvortex profiles (arrows) in nanocrystalline igloos grown under identical conditions on different hydrophobic surfaces: Polystyrene (left) and nanocrystalline diamond (right). As a general tendency, microtornadoes develop more frequently on polystyrene than on nanocrystalline diamond.

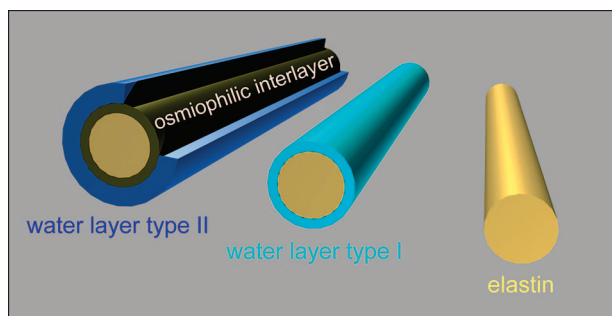


Figure 3. Elastin fibers (right) are hydrophobic, pH-insensitive, and withstand 100°C . Natively, the elastic fibers and the surrounding matrix are separated by ordered interfacial water layers (middle), predicted to determine the contact interaction of nanoscale objects with their environment,²³ instrumental in maintaining structural integrity and stretchability. During the process of physiological aging elastin fragmentation and disintegration are probably induced by osmiophilic groups (polar aminoacids, fatty acids and calcium salts). Hydrophilic conversion and reduction in polarity contrast due to the osmiophilic interlayer further encourages the encapsulation of the fibers by a viscous interfacial water layer (left).^{3–5}

roof of nanocrystalline igloos.^{15,16} One relevant result from the laboratory experiments was the insight that with a temporal and spatial coincidence of tornadogenic events, tornadoes necessarily form. Figure 2 shows two microvortices on prominent biomaterials, polystyrene (cover of Petri dish) and nanocrystalline diamond, both hydrophobic. Remarkably, polystyrene favored the emergence of microtornadoes. A novel element in the anatomy of microtornadoes is the violence by which they cut their characteristic profile into the nanocrystalline igloos, removing their building blocks with the precision of a water jet cutter. We cannot exclude that microtornadoes occur naturally in evaporative systems, for instance, under nanocrystalline films with a layered architecture, which provide the geometry necessary for the formation of a transient temperature gradient normal to the surface.¹⁵ From our study we conclude that the probability of microtornadic activity increases with decreasing

surface roughness. Because of an anticipated corrosive potential and a possible impact on processes involving surfaces that are smooth on the nanoscale, microtornadic activity deserves attention. The minimum microvortex diameter registered so far was $20\ \mu\text{m}$ (cf. Figure 2). Biosurfaces of that dimension that are smooth on the nanoscale are virtually inexistent, at least in mammals. Therefore, it is improbable that microtornadoes here play a substantial role.

Interfacial Water Layers in Biological Aging. The surfaces employed by us to study interfacial water layers were hydrophobic or hydrophilic.^{6–9} Normally, they preserved their original surface polarities during the period of the measurement, even in water containing nanoparticles.⁸ Apparently, their presence had no effect on the prevalent interfacial water structure. The record for laboratory experiments demonstrating the crystallinity of interfacial water layers separating sessile drops from hydrophobic surfaces reached 191 h. The experiments eventually led to the discovery of the microtornadoes.^{15,16} In the experiments performed on model materials, interfaces could always be clearly defined: air–solid or liquid–solid, whereby the surface polarities of the solids forming an interface with the water were conserved. In biological systems the predominant liquid is also water but classification of surface polarities and definition of interfaces, specifically of liquid–solid interfaces are generally in no way trivial. The principal factor complicating a clear physicochemical description of biological interfaces is its diffuse character, mostly with a minimal polarity contrast. Presumably, the interface proximal to elastin – a protein of the extracellular matrix, which gives elasticity to the walls of our arteries, heart, lungs, intestines and skin, and maintains tissue architecture by providing strength and resilience to tissues – is the only exception where the physicochemical situation is clear, at least partially. The reason for the exception is derived from the interplay between the function and hydrophobic nature of this unique protein: Independent of the actual interfacial partner (solid or liquid), the pronounced stretchability of the elastic fibers excludes immobilization by the surrounding tissue matrix and implies longitudinal mobility. Since the fibers operate under physiological conditions, that is, an environment whose dominating fraction is



Figure 4. Photographs show wrinkle levels before (left) and after irradiation (right) with WARP 10, a device originally developed for self-aid administration under severe battlefield conditions, for instance, pain alleviation.²⁵ Less pronounced wrinkle levels with shorter wrinkle valleys after 9 weeks. The change in wrinkle levels after 10 months of consecutive irradiation is displayed in the table of contents image.

water, we are justified in assuming that most of the mobile molecules surrounding the elastic fibers are water molecules. This picture leaves room for an energy minimizing argument, related to the possible organization of the interfacial water on elastic fibers performing their function: From previous work we concluded that crystalline water layers immobilized on hydrophobic substrates are instrumental in reducing dissipative effects at the solid–liquid interface during evaporative processes.¹⁷ We exploited the effect in practice for Q-factor amplification by hydrophobic conversion of conventional optical elements (quartz glass) used in shear-force mode NSOM,¹⁸ and imaged thereby for the first time cell organelles in a live cell in culture medium.¹⁹ With fading polarity contrast to the surrounding medium, the functional performance decreases in both systems NSOM tip and elastic fiber. For the latter this eventually results in its integration into the surrounding tissue matrix and a complete loss of stretchability. Indeed, histological studies indicate that the initial process by which elastin loses its elasticity is the accretion of an osmiophilic interlayer comprising polar aminoacids, fatty acids and calcium salts.^{20,21} Its effect is hydrophilic conversion, which necessarily results in a differently structured interfacial cladding: In a process of progressive maturation, as portrayed in Figure 3, the crystalline water layer enveloping the elastic fibers is successively replaced by a viscous interfacial water layer. The resulting polarity contrast and virtual swelling of the elastic fibers will further diminish their biologically intended function. Consequently, in skin enforced by elastic fibers, a surface-covering progression of this process is likely to initiate wrinkle formation. Whereas decreased skin elasticity is considered as a factor that promotes wrinkle formation, the relationship between decreased skin elasticity and wrinkle formation is not fully understood. It is believed that the elastic fibers play an important role in maintaining skin elasticity.²²

On the basis of the simple physicochemical picture (condensed in Figure 3) on the one hand, and ample experimental evidence on the tunability of interfacial water layers by visible light – both on hydrophobic and hydrophilic surfaces^{6–8} – on the other hand, we designed a program to restore elastin maturation in vivo. For this one of us irradiated the skin around the corner of the eyes with light delivered by an array of LEDs (WARP 10, Quantum Devices, Inc. WI). Operating in the range 600–720 nm (central wavelength 670 nm, 50% relative spectral output 660–680 nm), it covers a 10 cm² area with an integral light intensity of 728 W m⁻². To exclude adverse effects, that is, inhibition of cellular functions,²⁴ irradiation times were adjusted to doses around 4×10^4 J m⁻², known to temporally increase blood circulation. The representative photographs of the selected facial zones in Figure 4 show the wrinkle levels before and after their daily irradiation for nine consecutive weeks, respectively.

Earlier we demonstrated that 670 nm laser light, applied in the same intensity range, depleted interfacial water layers in air,^{6,7} and transformed their crystalline structure subaquatically.⁸ From an analysis of the prevalent molecular situations at the relevant interfaces (loss of one degree of freedom due to unilateral restriction

in mobility) we predicted interfacial water densities superior to that of bulk water.^{7,8} Results provided by recent AFAM experiments support this picture,⁷ and high-energy X-ray transmission-reflection methods indicated for interfacial water in contact with hydrophilic surfaces a density increase of 1.2.²⁶ Interestingly, for interfacial water in contact with hydrophobic surfaces molecular dynamics predicted besides a density deficit gap of 4 Å a similar tendency (Pavel Jungwirth, personal communication). From the aforementioned physicochemical analysis, it is thus reasonable to assume that the action of the light consists of transiently stripping the elastic fibers of their water claddings. Apparently the denser, otherwise protective claddings are sealing and stabilizing the osmiophilic interlayer on elastic fibers (cf. Figure 3). Their transient removal (or destabilization), concomitant with a synchronized induction of metabolic processes by activation of cellular functions,²⁴ is likely to facilitate a gradual dissolution of the osmiophilic interlayers, thereby reversing elastin rigidity. Prior to utilizing the WARP 10, we irradiated the same facial zones according to the same protocol for a consecutive period of three months using a home-built 660 nm LED array, delivering an intensity of 70 W m⁻². Importantly, the irradiation was ineffective, presumably because the intensity was simply too small to produce a substantial change in the interfacial water organization. Recently, we started complementary experiments to explore light induced phase transitions in interfacial water layers on a variety of substrates by monitoring their rheological properties. We found clear observational evidence for the existence of light intensity thresholds, that is, the capacity of the light to change the order of interfacial water. It may be noted that the WARP 10 operates in the wavelength window which has been used for more than 40 years in wound healing, far from both ultraviolet and infrared radiation. According to recent research²⁷ high doses ($360\text{--}720 \times 10^4$ J m⁻²) of infrared radiation (760–1440 nm) possibly contribute to photoaging.

Conclusions. We showed that consideration of crystalline interfacial water layers leads to progress in a variety of fields including but not limited to chemistry, physical chemistry, physics, material sciences, nanotechnology, proteomics, meteorology, and biomedicine. The principal discovery described in this work is that by targeting water layers on elastin, facial wrinkle levels could be significantly reduced by irradiation of the skin with visible light, which was found to interact with interfacial water layers on model substrates. Besides its function in the skin, elastin provides elasticity to the heart and blood vessels. Here the aqueous quality of the elastin environment is more pronounced than in the skin. Therefore, we are justified in believing that our approach can be easily converted to deep body rejuvenation programs.

References

- (1) Reedyk, M. F.; Arsic, J.; Hollander, F. F. A.; de Vries, S. A.; Vlieg, E. Liquid Order at the Interface of KDP Crystals with Water: Evidence for Icelike Layers. *Phys. Rev. Lett.* **2003**, *90*, 066103.

- (2) Michalarias, I.; Beta, I.; Ford, R.; Ruffe, S.; Li, J. C. Inelastic neutron scattering studies of water in DNA. *Appl. Phys. A: Mater. Sci. Process.* **2002**, *74* [Suppl.], S1242–S1244.
- (3) Goertz, M. P.; Houston, J. E.; Zhu, X. Y. Hydrophilicity and the Viscosity of Interfacial Water. *Langmuir* **2007**, *23*, 5491–5497.
- (4) Jinesh, K. B.; Frenken, J. W. M. Capillary Condensation in Atomic Scale Friction: How Water Acts like a Glue. *Phys. Rev. Lett.* **2006**, *96*, 166103.
- (5) Li, T. D.; Gao, J.; Szoszkiewicz, R.; Landman, U.; Riedo, E. Structured and Viscous Water in Subnanometer Gaps. *Phys. Rev. B* **2007**, *75*, 115415.
- (6) Sommer, A. P.; Franke, R. P. Modulating the Profile of Nanoscopic Water Films with Low Level Laser Light. *Nano Lett.* **2003**, *3*, 19–20.
- (7) Sommer, A. P.; Caron, A.; Fecht, H. J. Tuning Nanoscopic Water Layers on Hydrophobic and Hydrophilic Surfaces with Laser Light. *Langmuir* **2008**, *24*, 635–636.
- (8) Sommer, A. P.; Pavlath, A. E. The Subaquatic Water Layer. *Cryst. Growth Des.* **2007**, *7*, 18–24.
- (9) Sommer, A. P.; Zhu, D.; Brühne, K. Surface Conductivity on Hydrogen-Terminated Nanocrystalline Diamond—Implication of Ordered Water Layers. *Cryst. Growth Des.* **2007**, *7*, 2298–2301.
- (10) Liu, P.; Cui, H.; Yang, G. W. Synthesis of Body-Centered Cubic Carbon Nanocrystals. *Cryst. Growth Des.* **2008**, *8*, 581–586.
- (11) Beg, A. A.; Ernststrom, G. G.; Nix, P.; Davis, M. W.; Jorgensen, E. M. Protons Act as a Transmitter for Muscle Contraction in *C. elegans*. *Cell* **2008**, *132*, 149–160.
- (12) Maier, F.; Riedel, M.; Mantel, B.; Ristein, J.; Ley, L. Origin of Surface Conductivity in Diamond. *Phys. Rev. Lett.* **2000**, *85*, 3472–3475.
- (13) Chakrapani, V.; Angus, J. C.; erson, A. B.; Wolter, S. D.; Stoner, B. R.; Sumanasekera, G. U. Charge Transfer Equilibria Between Diamond and an Aqueous Oxygen Electrochemical Redox Couple. *Science* **2007**, *318*, 1424–1430.
- (14) Nebel, C. E. Surface-Conducting Diamond. *Science* **2007**, *318*, 1391–1392.
- (15) Sommer, A. P. Microtornadoes under a Nanocrystalline Igloo: Results Predicting a Worldwide Intensification of Tornadoes. *Cryst. Growth Des.* **2007**, *7*, 1031–1034.
- (16) Sommer, A. P.; Zhu, D. Microtornadoes under a Nanocrystalline Igloo. 2. Results Predicting a Worldwide Intensification of Tornadoes. *Cryst. Growth Des.* **2007**, *7*, 2373–2375.
- (17) Sommer, A. P. Controlling Arrangement of 60 nm Nanospheres in Evaporating Sessile Drops with Low Level Laser Light. *Cryst. Growth Des.* **2005**, *5*, 559–563.
- (18) Sommer, A. P.; Franke, R. P. Hydrophobic Optical Elements for Near-Field Optical Analysis (Noa) in Liquid Environment—A Preliminary Study. *Micron* **2002**, *33*, 227–231.
- (19) Sommer, A. P.; Franke, R. P. Near-Field Optical Analysis of Living Cells in Vitro. *J. Proteome Res.* **2002**, *1*, 111–114.
- (20) Stadler, R.; Orfanos, C. E. Maturation and Aging of Elastic Fibers. *Arch. Derm. Res.* **1978**, *262*, 97–111.
- (21) Fujimura, T.; Haketa, K.; Hotta, M.; Kitahara, T. Loss of Skin Elasticity Precedes to Rapid Increase of Wrinkle Levels. *J. Dermatol. Sci.* **2007**, *47*, 233–239.
- (22) Sommer, A. P.; Gheorghiu, E. Proteobionics: Biomimetics in Proteomics. *J. Proteome Res.* **2006**, *5*, 611–618.
- (23) Sommer, A. P. Limits of the Impact of Gravity on Self-Organizing Nanospheres. *J. Phys. Chem. B* **2004**, *108*, 8096–8098.
- (24) Sommer, A. P.; Pinheiro, A. L.; Mester, A. R.; Franke, R. P.; Whelan, H. T. Biostimulatory Windows in Low-Intensity Laser Activation: Lasers, Scanners, and NASA’s Light-Emitting Diode Array System. *J. Clin. Laser Med. Surg.* **2001**, *19*, 29–33.
- (25) Mester, A. R.; Sommer, A. P. *Proceedings of the Second International Conference on Near-field Optical Analysis: Photodynamic Therapy & Photobiology Effects*; Johnson Space Center, May 2001, Houston, TX; NASA Publication, CP-2002-210786, pp 11–13.
- (26) Reichert, H. X-ray Investigations of the Structure of Water and Ice at Interfaces. ESF-Conference Water Interfaces in Physics, Chemistry and Biology: A Multi-Disciplinary Approach, Obergurgl, Austria, December 10, 2007.
- (27) Schroeder, P.; Lademann, J.; Darvin, M. E.; Stege, H.; Marks, C.; Bruhnke, S.; Krutmann, J. Infrared Radiation-Induced Matrix Metalloproteinase in Human Skin: Implications for Protection. *J. Invest. Dermatol.* **2008**, *128*, 2491–2497.

CG8000703