UNDER PRESSURE – FROM TEAR FILM TO EMBRYONIC DEVELOPMENT: IMPORTANT INSIGHTS BASED ON THE MEASUREMENT OF THE SURFACE TENSION

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Abstract

The properties of biosurfaces encompass a broad scientific field, which includes molecular biology, developmental biology, and modern medicine. Multiple approaches exist to address the need to better understand the fundamental interactions between cells, extracellular matrix, and environment – often they require a range of expensive equipment, consumables, and highly qualified specialists.

Our experience in studying the properties of the tear film allows us to put in use a broad spectrum of analytical approaches in that field that could be achieved by using a relatively simple but robust biophysical approach – measuring the surface tension at the aforementioned interfaces. For instance, parameters like tissue interfacial surface tension, elastic modulus, viscosity and overall fluidity, could be obtained by cavitation rheology or even by tissue surface tensiometry (TST). Those measurements could subsequently be used to elucidate the amount and distribution of the adhesion energy between developing tissues in an embryo, as well as for the tissues undergoing invasion.

***Keywords*:** *tear film, embryonic development, tissue differentiation and invasion, surface pressure*

While the traditional definition of biosurfaces is constraint to an interface between biological systems and synthetic or natural materials (Luckham & Hartley, 1994), broader approach would greatly deepen our understanding of the boundaries of living organisms and of the forces acting on a molecular scale on these boundaries. One of the best studied biosurfaces is the tear film. Each of its three layers – the mucin layer, adhering to the cornea, the aqueous layer and the uppermost thin lipid layer, was evaluated using a plethora of methods and techniques, resulting in an elucidation of the role of its components (Petar Eftimov et al., 2020; G. A. Georgiev, Eftimov, & Yokoi, 2017; Georgi As Georgiev, Eftimov, & Yokoi, 2019). That precise comprehension led to better understanding of the pathologies of the tear film, such as dry eye syndrome (Petar Eftimov, Yokoi, Tsuji, Peev, & Georgiev, 2022; Willcox et al., 2017; Yokoi & Georgiev, 2019) and to a more fluent workflow for evaluation of eye-drop formulations (Daull et al., 2020; Petar Eftimov et al., 2022; Petar Eftimov et al., 2024) and material properties of artificial vision aids, such as contact lenses (P. Eftimov, Yokoi, Peev, & Georgiev, 2019; P. B. Eftimov, Yokoi, Peev, Paunski, & Georgiev, 2021).

But what are the methods that underpin these achievements? Though varying from infrared spectroscopy (Borchman et al., 2011) to cell culturing of specific ocular lines (Petar Eftimov, Stefanova, Lalchev, & Georgiev, 2015), one of the most valuable parameters giving information on the tissue interfaces overall fluidity, viscosity and elasticity is the existing surface tension at the interfaces between the cells, extracellular environments and the materials coming in contact with them. Meaningful results could be obtained by simple experiments, like measuring the speed of the spreading molecules on a surface that mimics tear film composition due to the Marangoni effect. Relying on a simple mathematical expression, one can easily calculate the surface pression of a tested solution (γ_s), putting it in contact with a liquid with known surface tension (γ_w):

$$u \approx \frac{(\gamma_w - \gamma_s)^{\frac{2}{3}}}{(\mu\rho r)^{\frac{1}{3}}} \quad (1),$$

by measuring the effective spread radius (r) and the speed of the spread (u), as illustrated on fig. 1.

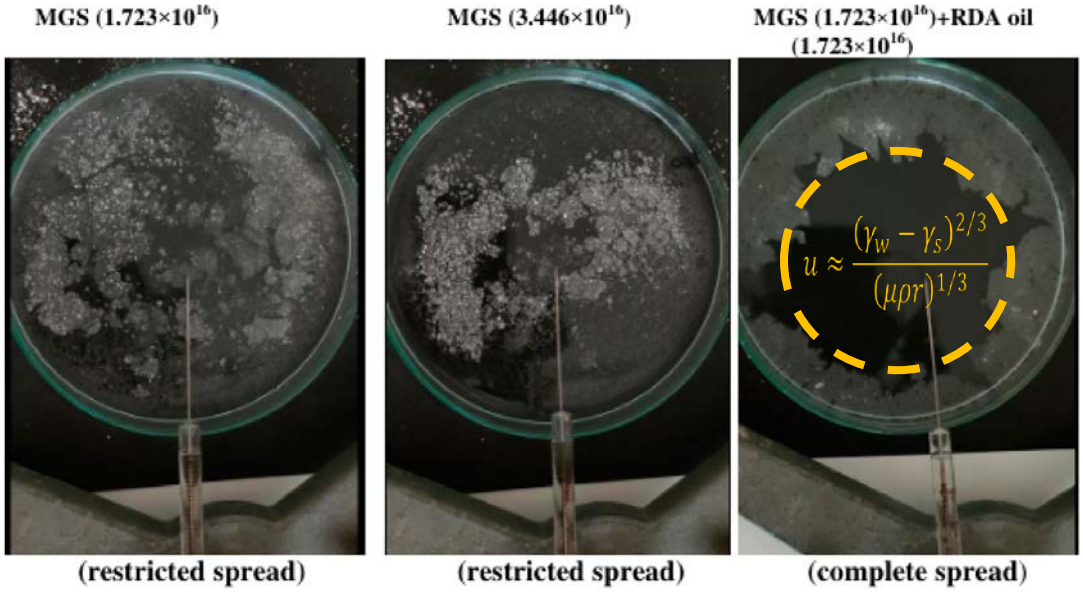

Figure 1. Measurement of the surface tension in a crucial tear film component – meibomian gland secretion of a patient in two different concentrations (left and middle panel), compared with the spread of solution, substituted with commercial solution (modified from Eftimov et al. 2022 (Petar Eftimov et al., 2022))

Another potent approach is the axisymmetric drop or bubble shape analisys (AD(B)SA), in which a drop or bubble is put in contact with a surface (biosurface or material) and the contact angle is measured at the air/water/material interface (see fig. 2).

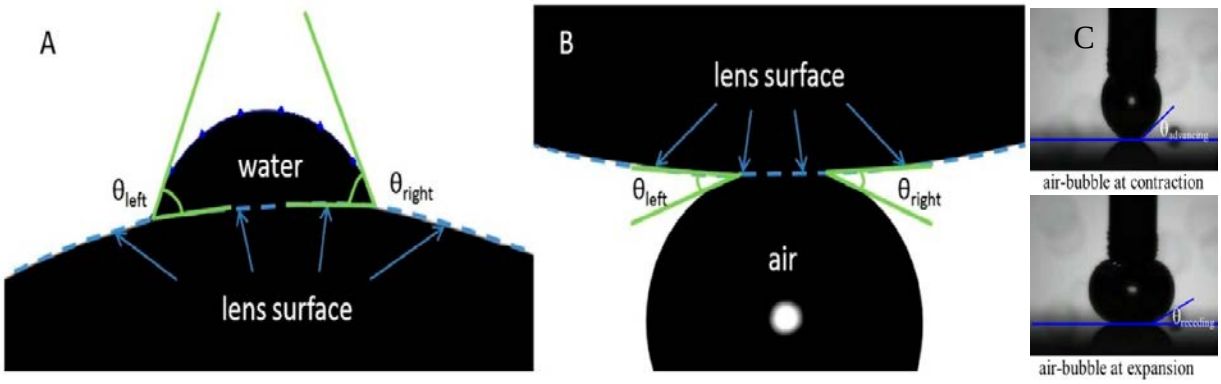

Figure 2. Measuring the contact angle on the surface of a contact lens (panel A, B – from Eftimov et al, (P. Eftimov et al., 2019)) and on a surface of CIRC cell line coated glass plate (panel C – from Eftimov et al, (Petar Eftimov et al., 2015)).

The Young-Laplace equation, which describes the shape of a liquid interface under the influence of gravity and surface tension, could be integrated numerically to generate theoretical drop profiles and optimize them to match the experimental drop shape, thus determining the surface tension at the interface.

$$\Delta P = \gamma \left(\frac{1}{R_1} + \frac{1}{R_2} \right) \quad (2)$$

The measurements required are relatively simple compared to the obtained deep understanding of the examined biosurface at the level of intermolecular interactions, Van der Waals and London forces (Kwok, 1999; Kwok & Neumann, 1999), as well as subsequent analysis of the amount and distribution

of the adhesion forces on the interfaces of interest. This approach can be applied when studying differential adhesion phenomena on the interface of developing tissues during the embryogenesis – another challenging and complex system, which normally requires significant investment in time and equipment. Currently, there are three main theoretical approaches toward biophysical explanation of the early embryonic cellular differentiation.

1. Differential adhesion hypothesis (**DAH**) - a concept introduced by Steinberg in 1966 (Steinberg, 1996), based on cellular separation experiments by Philip L. Townes and Johannes Holtfreter (Townes & Holtfreter, 1955). The basic assumption is that cells in the developing embryo have varying degrees of surface adhesion, which causes them to reorganize spontaneously to minimize their interfacial free energy (Foty & Steinberg, 2005). Mixing cells with different surface tensions would lead to their separation and regrouping in a manner that can be predicted and precisely measured. For example, if limb bud tissue with a surface tension of 20 dyne/cm is combined with pigment epithelium with a surface tension of 12 dyne/cm, the pigment cells with the lowest surface tension remain on the surface of the aggregate (Bécam & Huynh, 2007). Although this hypothesis gives a very straightforward explanation and a reliable link between cellular behavior, measured surface pressure and the type of expressed adhesive molecules (i.e. cadherins) it doesn't appear to recognize the role of extracellular matrix and the cytoskeleton involvement.
2. Differential contraction hypothesis (**DCH**) – a buildup of Steinberg assumptions, made by Harris in 1976, which recognizes the active role of the cytoskeleton in cell adhesion and movement (Harris, 1976). Later calculations of the cortical tensile force to adhesion energy showed that the latter is significantly lower, which raised questions on the assumptions of DAH (Maître et al., 2012).
3. Differential interfacial tension hypothesis (**DITH**) – a new, refined approach, that questions the molecular origin of the forces responsible for cell movement, rearrangement and differentiation, which aims to consolidate the two aforementioned hypothesis. The authors (Stirbat et al., 2013), emphasize on the importance of cell to cell vs. cell to media communications and on the role of particular molecules – α-catenins as a catalyser for the remodeling of the actin cytoskeleton.

What makes the common ground of these three hypotheses is the recognition of the surface tension, as a driving force for cellular differentiation and migration, thus making the development of methods for its quick and reliable measurement a very important task. Not surprisingly, a lot of techniques had been implemented in this regard – a short list includes external and internal methods (Boot, Koenderink, & Boukany, 2021). We will focus on external methods, which measure the surface tension and other mechanical properties without penetrating the cell surface, like atomic force microscopy (**AFM**) (Schiele et al., 2015), micropipette aspiration (**MPA**) (Hochmuth, 2000) and tissue surface tensiometry (**TST**) (Foty, Pfleger, Forgacs, & Steinberg, 1996). AFM is very precise, but requires expensive equipment, highly trained professionals and special preparation of samples, which significantly lowers its value in evaluating dynamic development of embryonic tissues. While it is easier to perform, TST and MPA both have a disadvantage to apply external tensile force on the examined sample, which can interfere with the normal development, as shown by the DCH and DITH.

What we propose is to use a well-established method – AD(B)SA for measuring the surface pressure and to implement a new set of analytical approaches for the subsequent calculation of the adhesion forces acting on a cell to cell and cell to media interfaces. The method can be used on live cell cultures (Petar Eftimov et al., 2015), without staining or other modifications, and is relatively non-invasive, which allows a continuous follow-ups during a selected period of time. Required equipment is unexpensive and the workflow could be automated to a greater extent, which contributes to more concise results. This approach is useful as well in predicting the behavior of tissues invasion during normal development or neoplastic growth, making it even more important as a possible input into a broad

diagnostic instrumentation dedicated to this ever-changing field. The link between measured contact angles and Gibbs free energy (see fig. 3) is very useful to elucidate the energy, chemistry and dynamics of the solid biosurfaces.

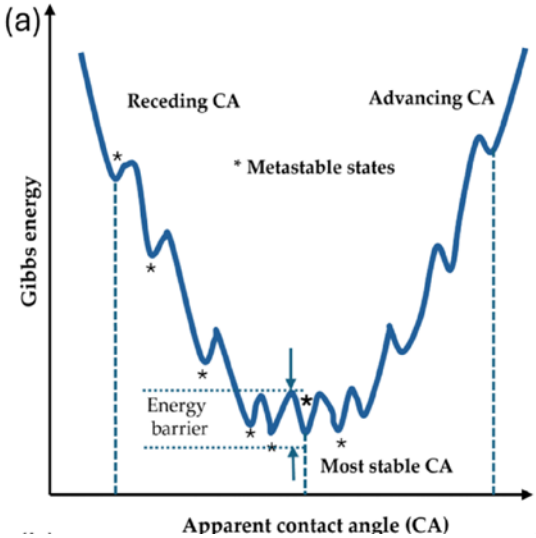

Figure 3. Link between Gibbs free Energy and the advancing, receding and equilibrium contact angle of Young (modified from (Georgi As Georgiev, Baluschev, Eftimov, Bacheva, & Landfester, 2024) .

The mathematical formalism used to calculate SFE from the contact angle values is given by the Tadmor equation (Tadmor, 2004):

$$\theta = arccos\left(\frac{\Gamma_A cos\theta_A + \Gamma_R cos\theta_R}{\Gamma_A + \Gamma_R}\right) \quad (3),$$

where $\Gamma_R \equiv \left(\frac{\sin^3\theta_R}{2-3\cos\theta_R+\cos^3\theta_R}\right)^{1/3}$ and $\Gamma_A \equiv \left(\frac{\sin^3\theta_A}{2-3\cos\theta_A+\cos^3\theta_A}\right)^{1/3}$

Precise measurement of SFE allows the elucidation of what type of functional groups (dipoles, donors or acceptors of H-bonds) are present at the solid surface and the prediction of the (bio)adhesion of coatings and (bio)materials to cells, tissues, implants and other relevant interfaces(Georgi As Georgiev et al., 2024).

Using biophysical and analytical methods, often provides robust, reliable and non-expensive alternative in achieving crucial insights in fields like developmental biology, histopathology and diagnostics of invasive processes.

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