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In vitro adhesion measurements between skin and micropatterned poly(dimethylsiloxane) surfaces

E.J. De Souza[*1], M. Kamperman[1], G. Castellanos[1], E. Kroner[1], V. Armbruester[2], M.S. Romann[2], B. Schick[2] and E. Arzt[1]

Abstract— **Micropatterned adhesive surfaces may have potential in reconstructive surgery. The adhesion performance of mice ear skin to micropatterned poly(dimethylsiloxane) (PDMS) was investigated, under in vitro conditions, and compared to flat substrates. No significant difference in separation force *F* was observed between flat substrates and micropatterned surfaces with pillar arrays. However, the energy necessary for separation of the substrate from the skin was sensitive to the topography. Furthermore, our results show that the force-displacement curves depended on the wetness of the skin: Highest force values were obtained for fresh skin while the forces decreased as the skin dried out. The results are encouraging for further studies on the potential of patterned PDMS in biomedical applications.**

I. Introduction

In otology closure of tympanic membrane defects is a main issue in order to improve hearing and to separate the middle ear cavity from the external auditory meatus. Modern concepts of tympanoplasty include placement of a graft at the site of the tympanic membrane perforation to achieve defect closure [1]. Mainly collagen grafts like temporalis fascia or perichondrium are used hereby as graft materials. Stable attachment of the graft to the remnant tympanic membrane is crucial for successful defect closure. During wound healing process the squamous epithelium of the remnant tympanic membrane is migrating from the defect border onto the graft resulting in defect closure as far as an additional epithelium graft has not been used. One major concern after tympanoplasty is graft displacement resulting in a persistent tympanic membrane defect. Thus availability of grafts with improved adhesion capability would contribute to improve surgical success. In addition, the need for harvesting an appropriate graft is a further motivation to search for new materials being suited for closure of tympanic membrane defects. Even more, new graft materials with improved adhesion capabilities may allow reducing surgical morbidity by changing surgical concepts in the future.

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[*]Corresponding author: e-mail: emersonjose.desouza@inm-gmbh.de
[1] INM, Leibniz-Institut für Neue Materialien gGmbH, Campus D2, 2D-66123 Saarbrücken, Germany, phone: 0049(0)681-9300-500, fax: 0049(0)681-9300-503
[2] Klinik und Poliklinik für HNO, Universität des Saarlandes, Kirrberger Straße, 66424 Homburg/Saar, Germany, phone: 0049(0)6841-1622983

Recent work inspired by the attachment system of gecko feet that exhibit attachment organs with long micro and nanosized pillars [2]-[3], has shown that patterned surfaces with pillar arrays can exhibit enhanced adhesion compared to planar surfaces [4]. The adhesion forces of the pillar arrays are attributed to van der Waals and capillary forces between the pillar and substrate surfaces [5]. Biomimetic gecko adhesives have been fabricated from polymers [4] as well as carbon nanotubes [6], but mainly tested on stiff and flat substrates.

As the eardrum is a living and complex viscoelastic material system, it is an interesting challenge to study the adhesion performance of biomimetic gecko adhesives on eardrums. In this paper, we investigate for the first time the adhesion between patterned poly(dimethylsiloxane) (PDMS) elastomer and skin of mice ear under in vitro conditions.

Skin of the ears of hairless mice was used as a model system for eardrums, because the systems exhibit comparable thickness and mechanical properties. Skin was selected over actual eardrums to facilitate sample preparation and adhesion measurements. PDMS is a biocompatible polymer used e.g. for contact lenses [7] and as tissue for cell adhesion [8]-[11] but little is known about its adhesion performance to skin. Despite a large body of literature on adhesion and mechanical properties of skin [12], to the best of our knowledge no studies have been performed of pillar arrays on skin. The adhesion performance of the pillar arrays was investigated over time with varying wetness and compared with flat substrates.

I. Materials and Methods

A. Materials

Sylgard 184 Silicone, a two-component PDMS elastomer, was purchased from Dow Corning. PDMS substrates were fabricated using a base to curing agent ratio of 10:1 (by weight). The agents were thoroughly mixed by hand and degassed under vacuum until all air bubbles were removed. The prepolymer was poured onto flat and micropatterned silicon wafers and subsequently cured at 75°C for 48 h to ensure full curing. Both silicon wafers were silanized in perfluorooctyltriethoxysilane vapour [13] to allow the separation of the cured PDMS without breaking the structures. A more detailed description of the fabrication of the micropatterned PDMS structures can be found in [4].

Cured PDMS samples were removed from the silicon wafers and punched into 1 mm x 1 mm pieces. The structures were characterized by white light interferometry (Figure 1). The fabricated PDMS surfaces were composed of pillars with radius of 2.5 µm, height of 10 µm and spacing between pillars of 5 µm. Earlier we have shown that arrays with these pillar dimensions lead to enhanced adhesion when tested against sapphire [4].

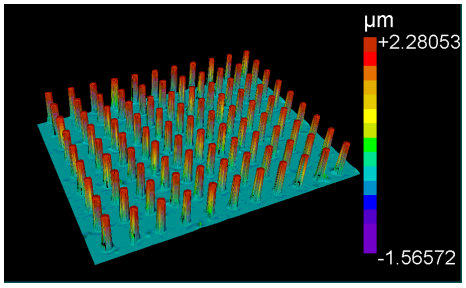

Figure 1. White light interferometry image of PDMS with pillar arrays. The radius of the pillars is 2.5 μm and the aspect ratio length/diameter is 2.

Pieces of ear-skin of mice were obtained from Saarland University Hospital, Homburg and were kept at 37°C in a cell culture solution (DMEM high Glucose,10% FCS, 1% Penicillin/Streptomycin), purchased from (PAA). Under these conditions, the skin could be kept alive for roughly one week. If the skin or the serum changed its color within this time, we considered the skin not adequate for use in the adhesion measurements. A solution of 1xDulbecco`s PBS without Ca & Mg purchased from PAA The Cell Culture Company, was used during the experiments to support the skin with humidity. Typical dimensions of the skin were roughly 1cmx1cmx0.5mm as shown in Figure 2.

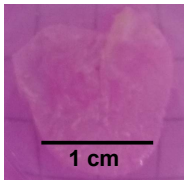

Figure 2. Mouse ear skin sample.

B. Adhesion Measurements

The adhesion measurements were performed with a home-built apparatus as shown in Figure 3. The set-up consists of a laser interferometer (SP 120 from SIOS, Ilmenau, Germany) and a hexapod nanopositioning stage (F-206 from Physik Instrumente, Karlsruhe, Germany) similar to the one described in [4]. A thin glass cantilever with dimensions 110x15x0.4 mm (Length x Width x Thickness) was used as shown in Figure 3. With a cantilever stiffness [14]-[15], k, of 19.46 ± 0.22 N/m, forces between (9.73 ± 0.11) and (973.18 ± 11.14) µN could be measured. The force F is simply obtained from F=k.D, where D is the cantilever deflection. A laser reflection enables measurement of the cantilever deflection.

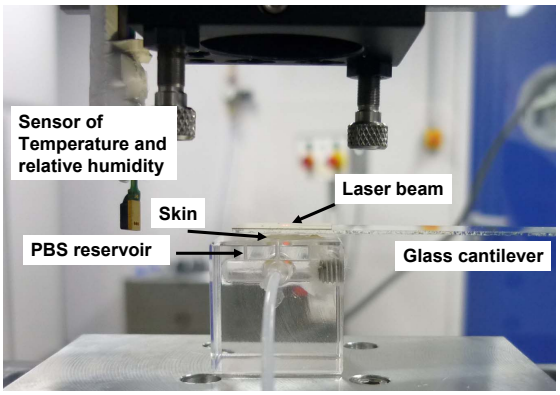

Figure. 3. Image of equipment for measuring the adhesion between skin and PDMS.

The skin samples were placed on a box made of PMMA with five channels to a reservoir of PBS. This system was used to keep only the bottom of the skin wet during the experiments, thereby minimizing the effect of the fluid on the adhesion measurements. A humidity sensor and a thermometer were placed 15 mm away from the skin. The PDMS sample sticks by self adhesion to the glass cantilever, while the skin adheres to the PMMA box through an interfacial layer of PBS.

The measurements were performed as follows: The skin sample is moved with a constant velocity in the direction of the cantilever. After a certain distance, the skin sample will make contact with the PDMS, after which it is pressed to full contact until a certain preload is reached. Then, the direction is reversed and the skin sample is moved to its original position. Figure 4 shows an adhesion curve between two hard, flat substrates to illustrate the results of a typical measurement.

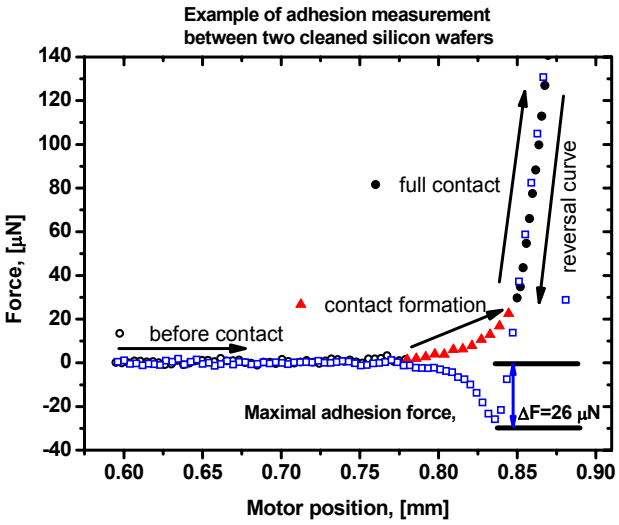

Figure 4. Example of an adhesion measurement. The precision of our equipment is demonstrated in this graph, which allows us to measure low forces with high precision even for hard materials like silicon.

II. Results

A. Adhesion between PDMS and Skin

To investigate the "gecko effect" [5] on skin, we measured the separation force of flat and micropatterend PDMS against skin, see Figure 5.

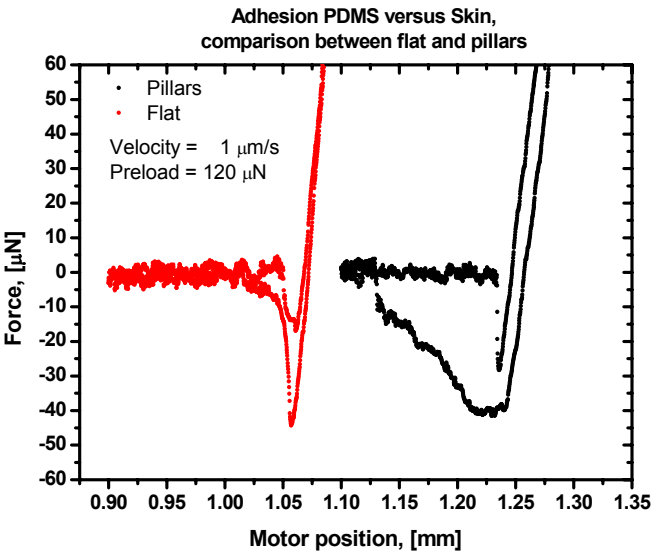

Figure 5. Force-displacement curves between PDMS and skin. The contact for flat specimens occurs at ~ 1.05 mm and the contact for patterned surfaces at ~ 1.20 mm. The maximal force is insensitive to topography.

The curves shown in Figure 5 display an average curve of at least five experiments repeated immediately after each other. However, since the skin gets dry, these results cannot be compared to different states of the skin.

The shift in displacement between the two graphs indicates a difference in thickness of the PDMS samples, but plays no role in the adhesion performance. During contact formation, the PDMS and skin deform, pushing the cantilever down until full contact is reached and preloading starts. Under identical conditions (rate, preload, humidity and temperature), similar maximal separation forces were obtained for the flat and micropatterned substrates. On the other hand, the hystereses of both cases differ significantly, the importance of which will be discussed below.

B. Effect of drying

During our measurements we observed that the force decreased significantly as the skin became dry. At t = 0 in Fig. 6, a series of measurements was started. We repeated the measurements automatically and observed how long it took until the force dropped to immeasurable values. The results displayed in Fig. 6 clearly demonstrate how the wetness affected the adhesion between PDMS and skin. We performed experiments for flat and structured PDMS and observed the same behavior for all wetting states, reproducing the results shown in the previous section. After the measurement, we wetted the skin externally with the PBS solution and repeated the measurements. Again we observed the same trends. If the skin became dry over several days, the adhesion became immeasurable within the force resolution of our equipment. The situation of "no adhesion" was also observed with flat and patterned substrates.

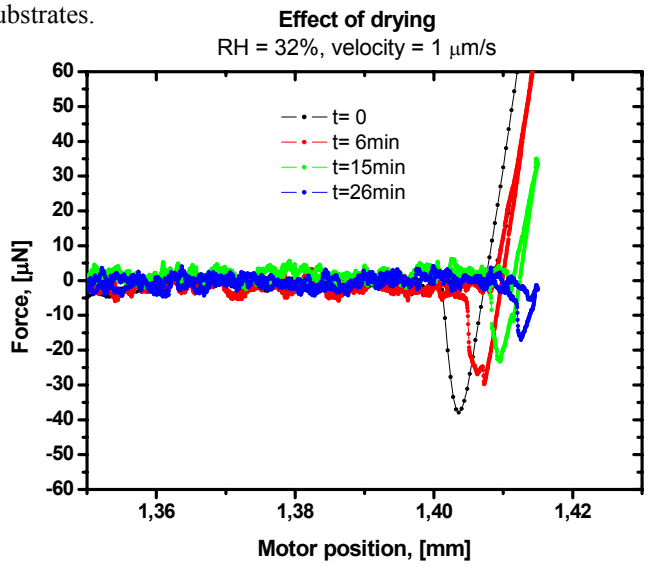

Figure 6. Wetness-dependent force-displacement curves. Rate, preload and environmental conditions are the same as in fig.5. The force decreases by a factor of 2 after 30 min.

While the measurements on wet skin were strongly sensitive to preload, we observed no preload dependence for dead skin. Rarely, we could measure forces at the limit of our resolution about 10 μN by changing the position of contact. On the other hand, external wetting of the skin which was dried for several days, led to similar trends and values.

For fresh skin, the maximum force increased to significantly higher values than those displayed for t=0 in Fig. 5 and Fig. 6. "Fresh" means that experiments were performed immediately after receiving the skin from the hospital. Keeping the skin alive in the cell culture helped to control but could not avoid the aging effect observed in our measurements. The maximum force values for "extra fresh" samples even exceeded the limits of our cantilever set-up.

III. Discussions

A. Complexity of measurements

Besides the time dependent effects shown above, we observed that the adhesion force and the complete force-displacement curves strongly depended on the preload, measurement velocity, topography and chemistry homogeneity of the skin surface, its initial thickness and the dependence of all these parameters on time. The adhesion on

skin is therefore a complex phenomenon which requires more detailed investigation.
Since the skin is wetted by the PBS, one might intuitively think that the adhesion was due to capillary forces. However, based on our earlier investigations of forces due to real liquid bridges on hard substrates [14]-[15], we can state that the effects observed here are quite different. The typical force-displacement curves due to capillary forces were not reproduced. The bulk skin contained water but this may not be true for the surface state.

The forces measured in our experiments fall mainly into three classes: a) F >1000 μN for "extra fresh skin", b) F ~ 200-600 μN for intermediate conditions and c) F ~ 10-40 μN for dry skin. These values can be compared with the force necessary to fixate a piece of PDMS with the dimensions (1x1x0.1) mm (Length x Width x Thickness). Such dimensions are realistic, for example, for application of PDMS in ear surgery. Based on the density of roughly 1 g/cm^3 for PDMS, a force on the order 1 μN is obtained for the minimal value required to hold these two surfaces against the gravitational force. Comparing this value to our results, even the forces for the dry state are 10 times larger. This comparison clearly demonstrates the realistic potential of PDMS in biomedical situations where adhesion is required.

The different hysteresis shown in Figure 5 indicates that the value for the work of separation observed for the situation of pillar arrays is larger than that for flat substrates. This effect could be beneficial for adhesion systems where long time performance is required. However, we considered **it** difficult to give an absolute value for the energy because of the limited reproducibility caused by the time dependent behavior of the skin as mentioned above. Nevertheless, it may be interesting to investigate this effect on model systems and to emphasize the role of energy hysteresis besides the role of maximal force.

Further investigations of structures with different shapes [16]-[18], aspect ratio and elasticity modulus are warranted to obtain a more complete picture. In particular, it needs to be verified that the effect of topography is lost in adhesion to skin. We cannot rule out at present that patterned surfaces with smaller or larger feature sizes do produce better adhesion than flat samples. This would have important implications on the design of future adhesive materials for possible use in surgery.

IV. Conclusion

Our results revealed new perspectives for the biomedical application of PDMS, highlighted the critical properties of skin and suggested new investigations for better understanding of adhesion between soft materials.

Acknowledgment

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