Response to "Comment on 'A method to measure cellular adhesion utilizing a polymer micro-cantilever'" [Appl. Phys. Lett. 104, 236103 (2014)]

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This reply is in response to a Comment posed by Eibl on our recently published Letter[1] as mentioned in the title. While the primary focus of this paper was to present a new adhesion measurement tool and the results[1] are preliminary in nature as reiterated in the text several times (p. 2, paragraph 1, column 1 and p. 5, last paragraph, column 1), we would be happy to address these concerns. We agree with the author of the Comment that additional work is required after functionalizing the micro-cantilever surface with cellular adhesion receptor proteins to achieve optimal physiological measurements as we also indicated in our manuscript[1] (page 5, 1st paragraph, column 2).

As we discussed[1] (p. 4, paragraph 2, column 1) 3,3′-dithiobis[sulfosuccinimidylpropionate] (DTSSP) coated cantilever surface adheres covalently to the surface proteins of the cell membrane.[2–4] We interpreted the differences observed in cells lines as an evidence reflecting their unique cell properties and our studies confirm widely accepted results.[5–11] The main point of criticism of author of the Comment is that "such measurements… may not accurately reflect physiological aspects of cell-cell adhesion." We would like to emphasize the fact that all *in vitro* studies involving cell culture are always limited in their physiological significance. However, we believe that meaningful results can be inferred by chemical cross-linking as explained below.

In the Comment, the author states that we "assume" that "all the covalent bonds between DTSSP and membrane proteins rupture." We did not make such an assumption. There are four possible rupturing point scenarios (as alluded to also by the author) when the cantilever is retracted from the cell membrane: (a) The linkage between the disulfide group of the DTSSP and the gold surface of the cantilever breaks requiring energy equivalent to (∼45 kcal/mol).[12] (b) The bond between DTSSP and the NH_2-groups on cell transmembrane proteins breaks (C-N bond: requiring a bond energy of approx. 175 kcal/mol).[13] (c) The transmembrane proteins binding within the phospholipid bilayer of the cellular membrane is disrupted. Finally, (d) the binding between the cell membrane with the substratum or reciprocally with either the cell membrane of neighboring cells or the adjacent extracellular matrix (ECM) is compromised. The first two events outlined above are highly unlikely because of the inherent requirement of very strong bond energies.

In our experimental setup, we suspect that retraction of the cantilever may have extracted some of the transmembrane proteins, as suggested by outcome (c) above, since they require much less energy than breaking covalent bonds. In relevant studies, cross-linkers[14–17] such as DTSSP and Bis[2-(Sulfosuccinimidooxycarbonyloxy)ethyl] (sulfo-BSOCOES) have been used to quantify cell adhesion molecules (CAMs, generally transmembrane proteins) by covalently anchoring the CAM to an external substrate using cross-linkers and then lysing the rest of cells by using surfactants. This way the authors[14–17] were able to estimate the protein density on the cell's surface and used that information to determine relative quantity of CAMs and thereby cell adhesion strength.

It is also possible that upon cantilever retraction we replicated outcome (d), but in the absence of detailed analysis, it is not possible to speculate further. Significant to this discussion, cross linkers have been used to attach cells to cantilevers for cell-cell adhesion measurements[18,19] and the fact that epithelial cells exhibit greater adhesive strength compared to mesenchymal cells.[6,20]

In summary, we are thankful that the author of the Comment acknowledges the fact that our cantilever approach offers advantages over the conventional laser-based AFM probes and agree that further improvements are required to be ideally suited for measurements of so-called "physiological cellular adhesion properties."

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