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**Short Visit Grant**  **or** **Exchange Visit Grant**

***(please tick the relevant box)***

**Scientific Report**

**The scientific report (WORD or PDF file – maximum of eight A4 pages) should be submitted online within one month of the event. It will be published on the ESF website.**

***Proposal Title****:* Gauging cell-cell tension biosensors and monolayer stress microscopy.

***Application Reference N°:*** 4872

1. **Purpose of the visit**

Tensional forces are produced by the contractile actomyosin cytoskeleton coupled to adhesions between neighboring cells and between cells and the extracellular matrix. Tensional forces are sensed by specific proteins complexes at these adhesions and appropriate intracellular biochemical responses are turned-on by these complexes. This process is termed mechanotransduction. Integrin complexes are the main mechanotransducers at cell-ECM adhesions and cadherin complexes are emerging as mechanotransducers at cell-cell adhesions. Crucial in the investigation of mechanotransduction are the tools to quantitatively measure forces within tissues and correlate cell behavior with mechanical input. Traction force microscopy is the current standard to measure tensile forces at the basal cell surface. Recently, the Trepat lab (host for this proposed exchange visit) has developed a technique called monolayer stress microscopy which extends the use of traction force microscopy to include the calculation of cell-cell tension.

Complementary to traction force microscopy which measures global system tension, genetically encoded tension-sensing FRET probes have been developed that measure tension across specific proteins. A small, elastic repetitive peptide from spider silk, (called TSMod, when flanked by donor and acceptor fluorophores), has been inserted into several adhesion complex proteins to allow the measurement of tension across them (as a decrease in FRET due to physical separation of the fluorophores). This has led to the surprising findings that the E-cadherin complex is under tension also outside of cell-cell junctions4 and that closely co-localized junctional proteins (PECAM and VE-Cadherin) experience opposite changes in tension in shear stressed endothelial cells. It also showed that indeed specific proteins can experience a range of tensions within tissues.

The applicant has expressed the E-cadherin TSMod tension sensor in DU145 prostate carcinoma cells and in DLD1 colon carcinoma cells and has determined that their viability and overall behavior in tissue culture was not severely changed. He has also developed and tested an alpha-catenin TSMod tension sensor and a sensor in which the TSMod module is inserted in an E-cadherin-alpha-catenin fusion protein (which forces interaction between E-cadherin and F-actin directly and likely increases the relative amount of probe that experiences tension). He has established that these probes respond (with an increase in FRET efficiency) to the release of tension by pharmacological inhibition of MyosinII, which acutely inhibits actomyosin contractility. The Trepat lab has established the protocols to perform monolayer stress microscopy on the epithelial cell lines in which we express these FRET tension sensors and to perform monolayer stress microscopy in parallel to FRET imaging of the CFP/YFP variants used in the above probes. We will therefore use the experimental setups and protocols established in the host lab (under their expert guidance) to directly compare changes in FRET efficiency of the E-cadherin complex tension sensors with changes in global cell-cell tension measured by monolayer stress microscopy.

By using this approach we will: 1) Establish whether or not the specific tension on the E-Cadherin complex and global cell-cell tension in a monolayer are correlated in space and time. 2) Investigate whether we can pinpoint the E-Cadherin complex as the primary tension transmitter in epithelial tissues. If this is the case, the E-cadherin tension sensors can be established as bonafide proxies for tissue tension and therefore can be used as read-outs for tissue tension in 3D culture and model organisms that are not amenable to traction force microscopy. If E-cadherin and cell-cell tension do not closely correlate, this will pose new and interesting questions about which protein complex is then the primary transmitter of tension between neighboring cells in epithelial tissues (or whether the TSmod sensors are maybe not measuring tension accurately).

1. **Description of the work carried out during the visit**

First a suitable process in which to measure fluctuations in forces across E-Cadherin junctions was investigated. As has been described before (Serra-Picamal et al. 2012), epithelial cells grown to confluency while confined by a PDMS micropattern will exhibit colective migration movements once said pattern is removed. The specific PDMS pattern used in that study was rectangular, facilitating the analysis of a relatively uniform bidirectional migration along a single axis by only imaging the epithelial layer in the middle of the rectangle. However this previous experiment was performed using a 10x low magnification objective, which we deemed not appropriate for imaging the FRET probe. We therefore chose to use a different micropattern, consisting of circlular patterns with a diameter of 200 micrometers such that the use of a higher manification (40x objective) was possible.

The applicant learned to make polyacrylamide (PAA) gels containing fluorescent (red) beads as is routinely done in the lab of Dr. Xavi Trepat. The PAA gels were subsequently coated with collagen. Then the PDMS micropatterns were placed on top, after which the cells expressing the E-CadherinTSMod were seeded, thus enabling the attachment of cells only in the microislands. The microislands were grown to a high density overnight. The next day prior to imaging, the PDMS micropatterns were removed and extraneous cells were carefully washed away. Then the cells were put on an inverted widefieldmicroscope with appropriate filters for imaging the E-CadherinTSMod FRET probe, as well as for the red fluorescent beads inside the PAA gel. Multiple positions were sought out after which the cells were imaged overnight with a 5-10 minute interval to capture the migration of the cell monolayer. The next day the cells were trypsinized to relax the forces on the PAA gel and the beads were imaged.

In addition to the "main" experiment we wanted to confirm whether the cell lines expressing the E-CadherinTSMod seeded on PAA gels, would behave similarly in short-timescale FRET experiments as is routinely done in the lab of Dr. Johan de Rooij back in the Netherlands. In short cells expressing the E-CadherinTSMod are imaged at intervals of 30 seconds. After 5 minutes of baseline signal, actomyosin contractility was inhibited (and thus presumebly the direct force on the E-cadherin complex) using the ROCK inhibitor Y-26732.

The analysis of bead displacement and the calculation of the Traction forces/Monolayer Stresses were performed by the Trepat Lab. The FRET calculations were performed using FIJI in combination with a custom written macro. In short, FRET Ratio images were calculated on a pixel-by-pixel basis and different ROIs were chosen to analyze different regions in the migrating monolayer. The ROIs make use of a tresholding function in order to segment the junctions, thus enabling the analysis of the FRET ratios only in junctions.

1. **Description of the main results obtained**

First we investigated whether the cell lines expressing the E-CadherinTSMod would be able to form a monolayered microisland. The epithelial cell lines tested were DU145, MDCK and DLD1 cells. However due to difficulties growing the cells and unexpected low expression, the DLD1 cells were dropped. Both DU145 and MDCK cells are able to form round monolayers using the micropatterns and both cell lines are able to migrate outward. We noticed however that some cells aggregate on top of the monolayer and this phenomonen was observed in both cell lines. This problem was not alleviated by using a higher PAA gel stiffness (9 or 12 kPa).

The measured traction forces are highest along the leading edge of the monolayer as expected. In addition the calculated monolayer stresses are also highest in the areas with the highest migration. Preliminary data of the FRET analysis of migrating MDCK monolayers suggests that we can measure a stress increase on E-Cadherin during migration. This stress increase can be rapidly observed at the leading edge of the migrating monolayers. Furthermore it seems that the increase in stress is of a lower magnitude and is measured at a later timepoint in cells just behind the leading edge. We have not yet been able to integrate these two datasets to compare the FRET ratio and calculated monolayer stresses in the exact same ROI. Our current preliminary analysis suggests that the local tension on E-Cadherin does not correlate directly with the calculated monolayer global stresses in the areas investigated.

Some results obtained suggest that the used technical setup of the microscope was insufficient for a reliable FRET analysis. The reason is currently unknown and is being investigated. This could explain why we were unable to replicate our findings of the short timescale experiments. Thus we still have to optimize this assay.

1. **Future collaboration with host institution (if applicable)**

While we did not meet our initial goals, the applicant has now learned the techniques required to set up the imaging of the FRET probes in combination with the PAA gels in Utrecht. The data obtained from the PAA gels will then be analyzed by the Trepat Lab. We will also further develop the analysis methods together with the Trepat Lab to incorporate both FRET data and Traction Force/Monolayer Stress data in a single analysis. Thus we will continue to collaborate on this project.

1. **Projected publications / articles resulting or to result from the grant *(ESF must be acknowledged in publications resulting from the grantee’s work in relation with the grant)***

If the technical difficulties can be worked out, we intent to use the results of this collaboration for a manuscript.

1. **Other comments (if any)**