**Supplementary information**

# Combining QCM-D with live cell imaging reveals the impact of serum proteins on the dynamics of fibroblast adhesion on tannic acid-functionalised surfaces

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**Abbreviations**
Human gingival fibroblasts (hGF), fetal bovine serum (FBS), titanium (Ti), tannic acid (TA) coatings formed at pH 7.8 (TA78) and pH 6.8 (TA68),

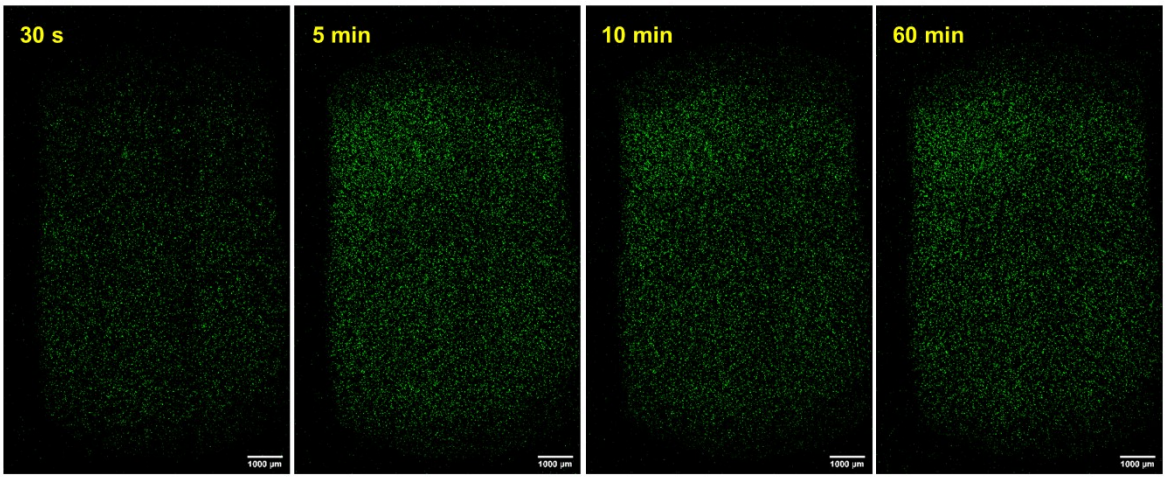


**Figure S1. Cell distribution over the titanium sensor surface after cell injection into the QCM-D chamber.** Cell suspension of 0.5 × $10^6$ cells/ml was injected into a QCM module at 300 µl/min for 30 sec. Cells were labelled with a CellTracker™ Green CMFDA fluorescent dye (green). The tile stacks were acquired with a laser scanning confocal microscope equipped with a 5×/0.15 dry HC PL FLUOTAR objective. Scale bar: 1000 µm.

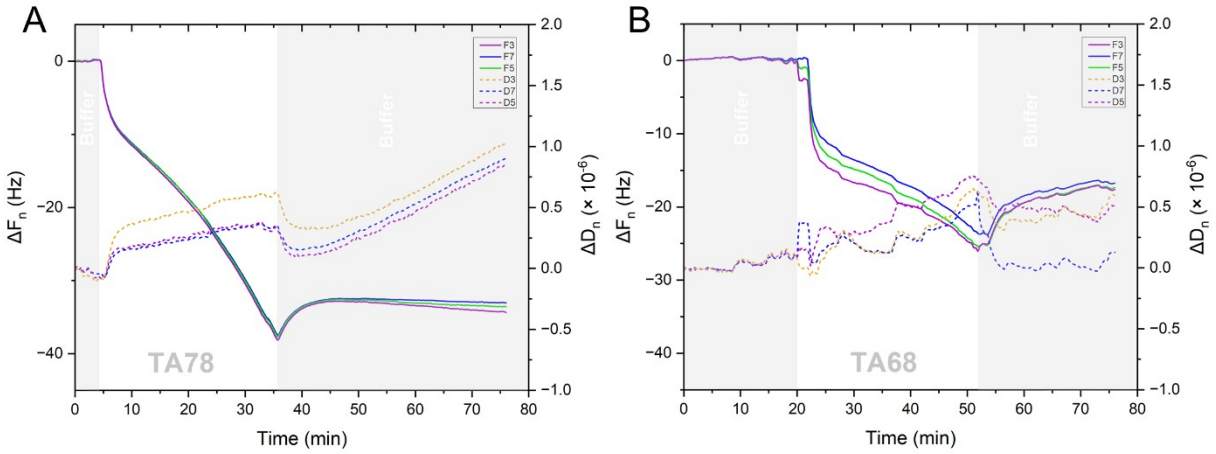


**Figure S2. TA deposition onto titanium surfaces at pH = 7.8 (A) and pH = 6.8 (B). Representative progression of frequency (ΔF) and dissipation (ΔD) shifts of the 3rd, 5th, 7th and 9th QCM-D harmonic (*n*) as a function of time.** Sections 1, 2, and 3 represent equilibration in a buffer, 30-minute deposition of TA, and washing with a buffer; 100 µl/min flow rate. The experiment was performed at 37 °C.

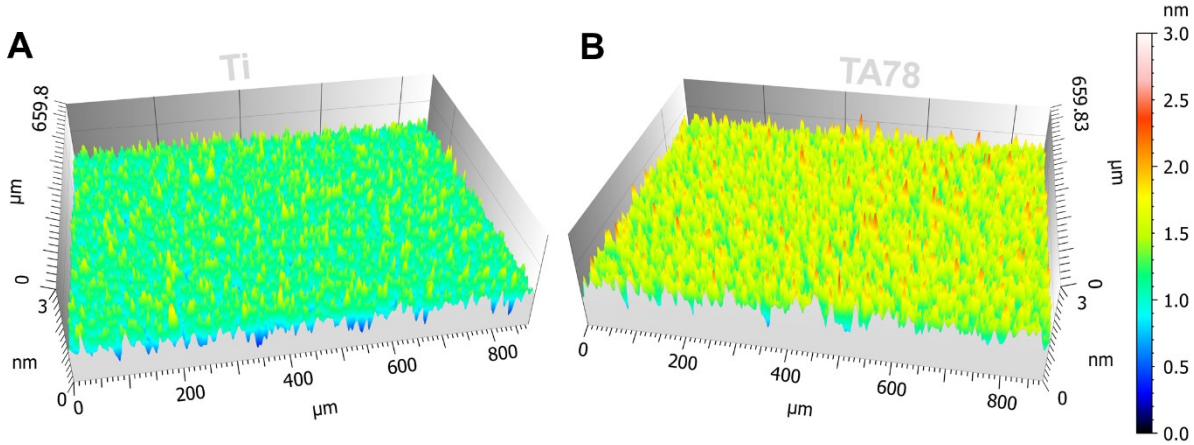


**Figure S3. 3D-profilometry images of uncoated (A) and TA-coated (B) titanium QCM-D sensor crystals.** TA was deposited at a flow rate of 100 µl/min at 37 °C for 30 minutes, followed by washing with a coating buffer and rinsing with water. The images were captured using a Sensofar S neox optical profilometer equipped with a 20× objective in an interferometric scan mode. The average surface roughness ($S_a$) calculated from the profilometry data was 0.19 ± 0.01 nm for Ti and 0.20 ± 0.02 nm for TA78, with measurements taken from two different sensors and five readings per sensor.

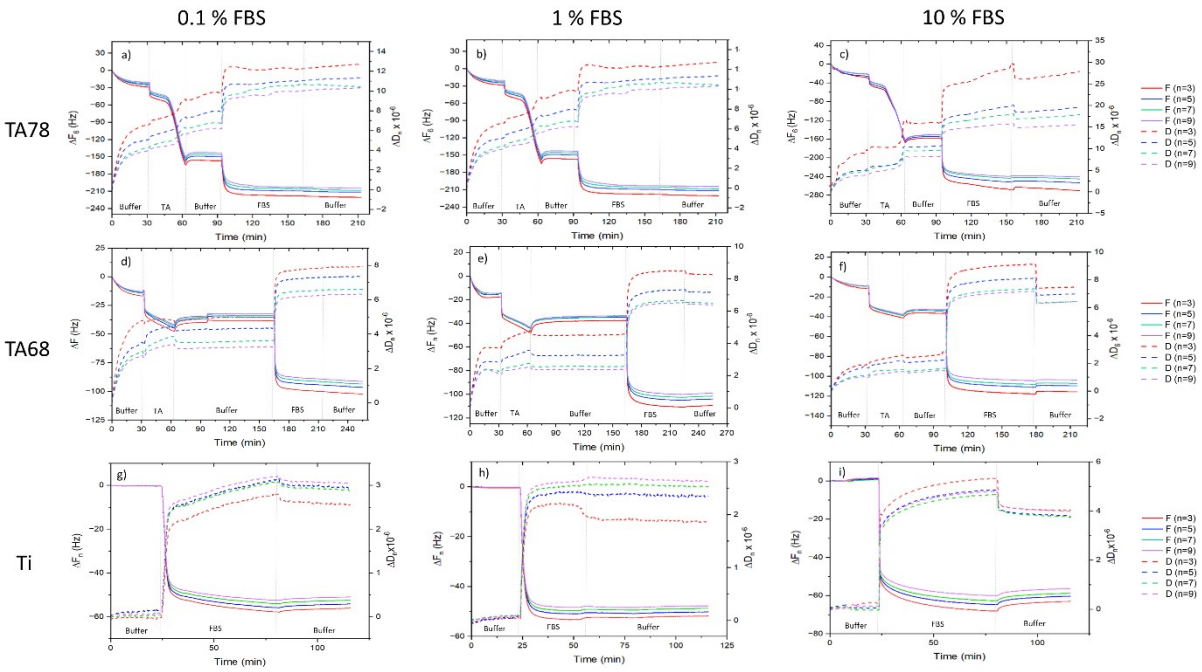


**Figure S4. Adsorption of FBS proteins onto TA nanocoatings and uncoated titanium surface followed by QCM-D.** The QCM-D data are presented as representative normalised frequency and dissipation shifts for 3rd, 5th, 7th and 9th harmonics over time. The sensors were pre-coated with 1 mg/ml TA for 30 minutes, 100 µl/min. The corresponding FBS solution was deposited for 60 minutes, 100 µl/min. In the beginning of the experiment and after each step, a buffer (100 mM HEPES, 600 mM NaCl buffer, pH 6.8 or 7.8) was introduced into the chamber. The experiments were performed at 21 °C. The data is divided into three sets: adsorption onto (A-C) TA78 coating, (D-F) TA68 nanocoating and (G-I) uncoated titanium. These sets correspond to different concentrations of FBS: A, D and G correspond to 0.1% FBS; B, E, and H represent 1% FBS; lastly C, F, and I show 10% FBS. The thickness of the TA68 and TA78 layers was 5.2 ± 0.1 nm and 26.3 ± 0.4 nm, respectively (Sauerbrey).

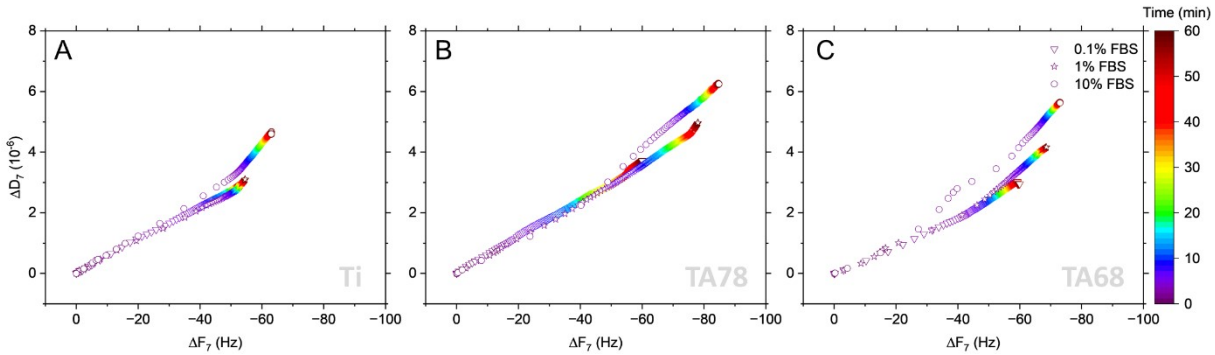


**Figure S5. Average DF plots for 7th harmonic of adsorption of FBS proteins onto uncoated titanium (A), tannic acid nanocoatings TA78 (B) and TA68 (C).** The sensors were pre-coated with 1 mg/ml TA for 30 minutes, 100 µl/min. The corresponding FBS solution was deposited for 60 minutes, 100 µl/min. In the beginning of the experiment and after each step, a buffer (100 mM HEPES, 600 mM NaCl buffer, pH 6.8 or 7.8) was introduced into the chamber. The experiments were performed at 21 °C.

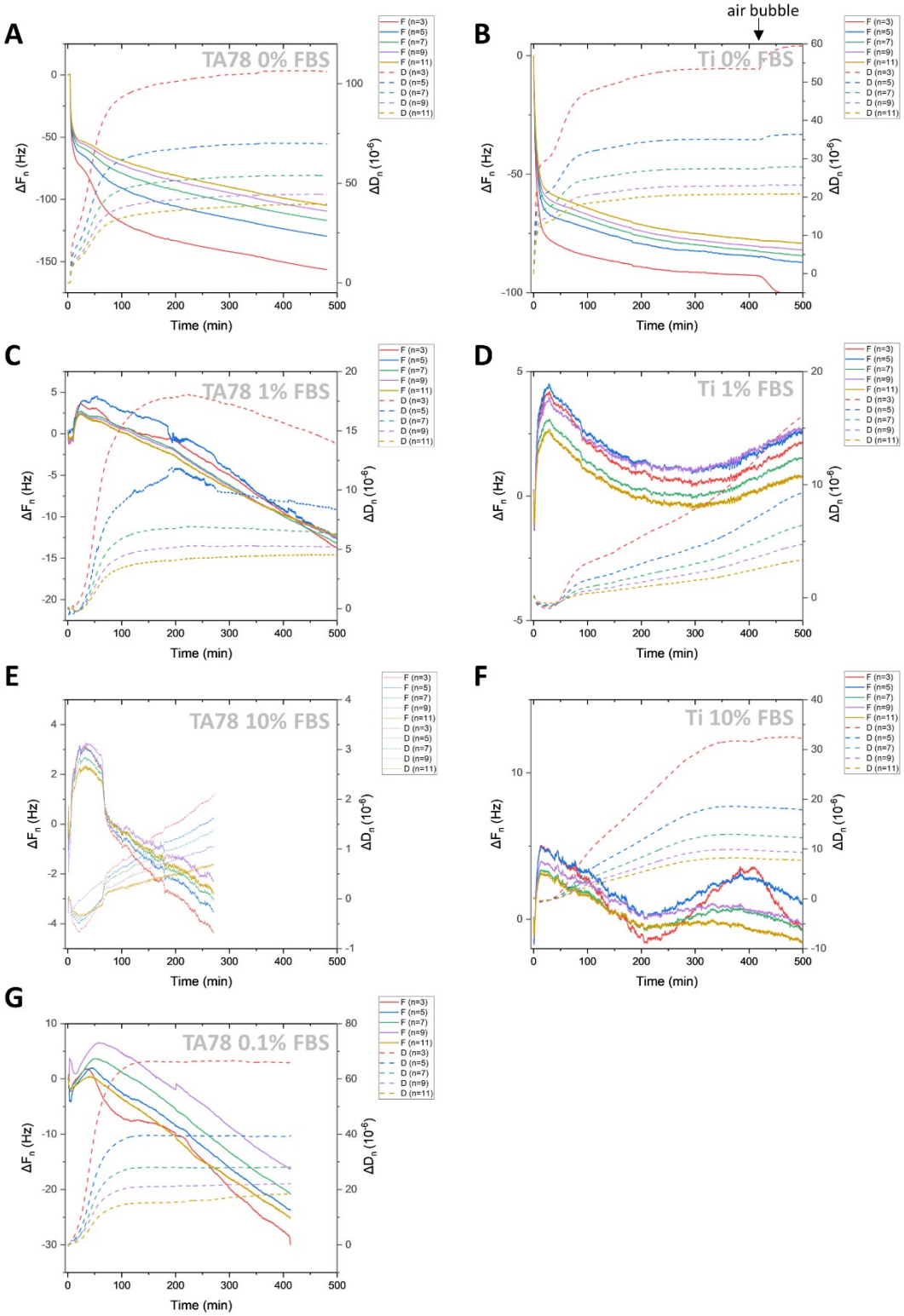


**Figure S6. Representative frequency and dissipation shifts at 3rd, 5th, 7th and 9th harmonics (n) as a function of time after hGF injection in medium containing 0-10% FBS.** Left panel (A,C,E,G) is TA78 coated titanium surface. Right panel (B,D,F) is uncoated bare titanium surface. Cells were cultured in serum-free medium (A-B) and medium supplemented with 0.1% FBS (G), 1% FBS (C-D), 10% FBS (E-F). Arrow shows an air bubble entering the QCM-D chamber.

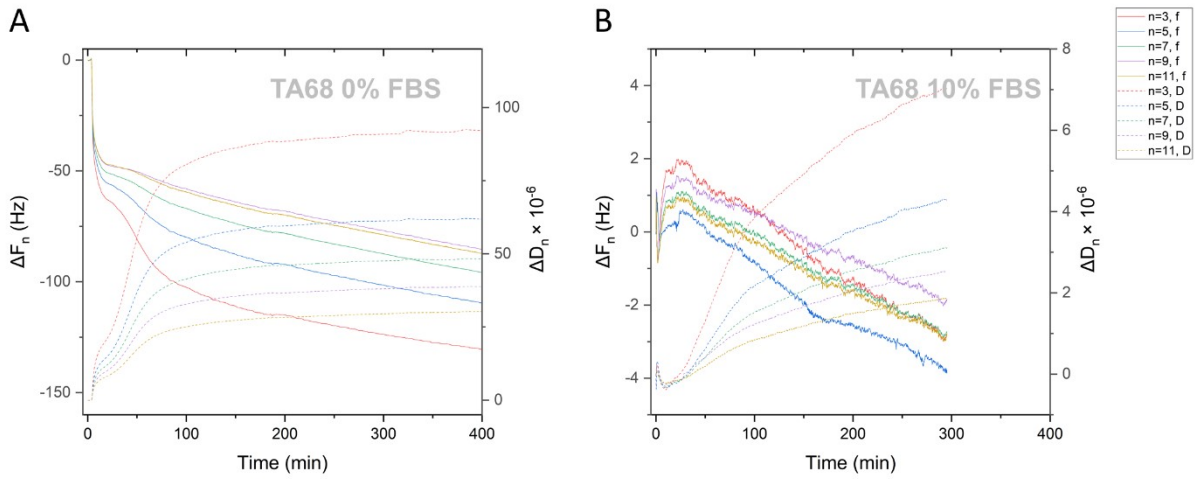


**Figure S7. Representative frequency and dissipation shifts at 3rd, 5th, 7th and 9th harmonics (n) on TA68 coatings as a function of time after hGF injection in medium containing (A) 0 %FBS and (B) 10% FBS.**

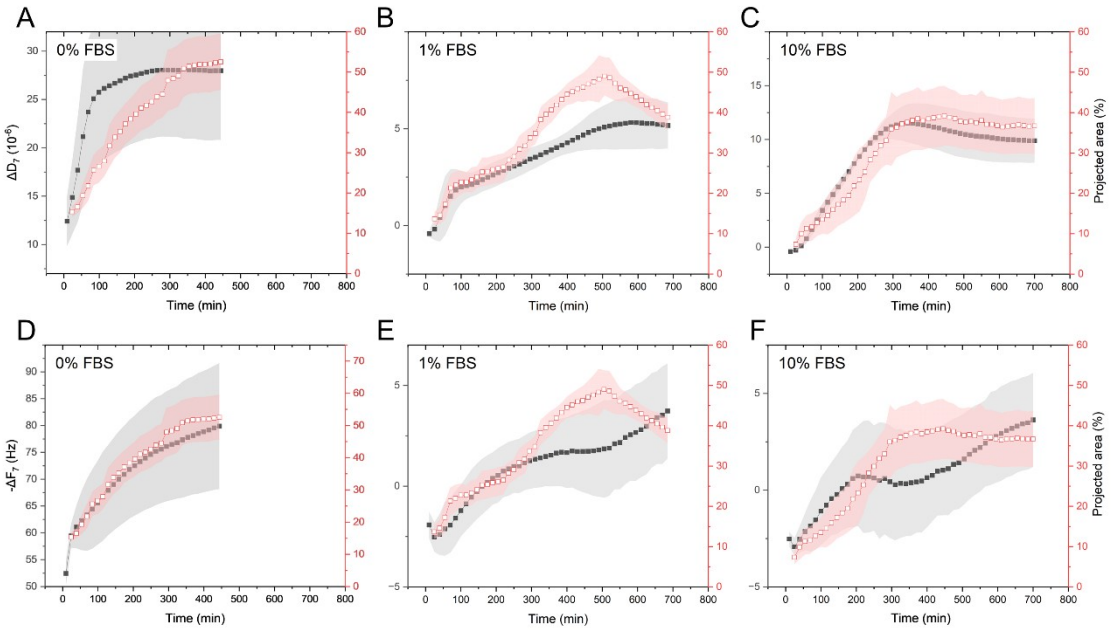


**Figure S8. Relationship between projected cell area and changes in dissipation (A-C) and frequency (D-F) over of time** for hGFs cultured on uncoated Ti surfaces in medium containing 0% FBS (A, D), 1% FBS (B, E), or 10% FBS (C, F). Empty red rectangles represent projected cell area. Filled black rectangles represent ΔD (A-C) or ΔF (D-F). Values are mean ± SD.

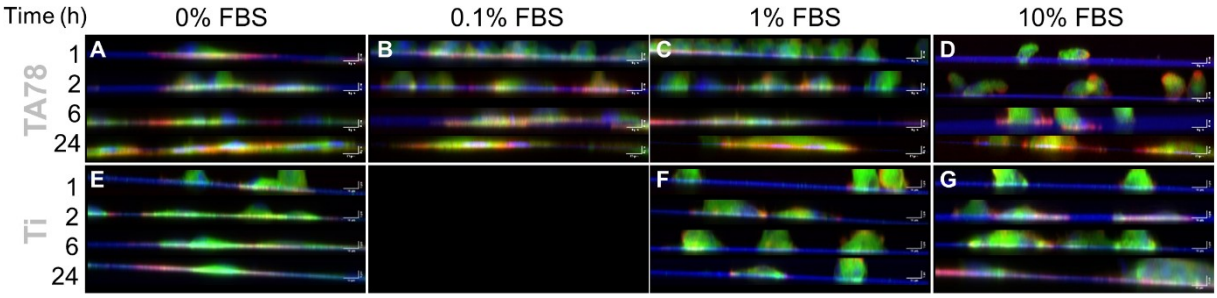


**Figure S9. Representative XY-projections of hGFs cultured on TA78-coated (A-D) and uncoated (E-G) titanium disks in medium containing 0% FBS (A, E), 0.1% FBS (B), 1% FBS (C, F), or 10% FBS (D, G).** Cells were cultured for 1, 2, 6 or 24 hours. The images were acquired with the upright CLSM equipped with 63× objective. Typical z-stack had 10 slices with 1 µm interval. Immunostaining of F-actin (red), vinculin (green) and nuclear DNA (blue). The blue reflection from the disk surface is an artifact signal. Scale bar: 10 µm width and 5 µm height.

**Table S1. Slopes of the ΔD/ΔF versus time curves presented in Figure 2B,D.**

| Surface | Slope of the ΔD/ΔF versus time curve ($10^{-11}$) | | | |
|---|---|---|---|---|
| | **0% FBS** | **0.1% FBS** | **1% FBS** | **10% FBS** |
| **TA78** | 9.78 | 9.87 | 5.65 | 0.37 |
| **Ti** | 5.53 | - | 1.3 / 0.22* | 1.3 |

* - two phases