

SI - Monitoring Tissue-Level Remodelling during Inflammatory Arthritis Using a Three-dimensional Synovium-on-a-Chip with Non-invasive Light Scattering Biosensing

Mario Rothbauer^{a,£,*}, Gregor Höll^{a,£}, Christoph Eilenberger^a, Sebastian R.A. Kratz^a, Bilal Farooq^a, Patrick Schuller^a, Isabel Olmos Calvo^b, Ruth A. Byrne^b, Brigitte Meyer^b, Birgit Niederreiter^b, Seta Küpcü^c, Florian Sevelda^d, Johannes Holinka^d, Oliver Hayden^e, Sandro F. Tedde^f, Hans P. Kiener^b, and Peter Ertl^{a,*}

Affiliations:

^a Faculty of Technical Chemistry, Vienna University of Technology, Vienna, Austria

^b Division of Rheumatology, Department of Medicine III, Medical University Vienna, Vienna, Austria

^c Institute of Synthetic Bioarchitectures, Department of Nanobiotechnology, University of Natural Resources and Life Sciences, Vienna, Austria

^d Department of Orthopedic Surgery, Medical University of Vienna, Vienna, Austria

^e Heinz-Nixdorf-Chair of Biomedical Electronics, Department of Electrical and Computer Engineering, TranslaTUM, Campus Klinikum rechts der Isar, Technical University of Munich, 81675 Munich, Germany

^f Siemens Healthcare GmbH, Technology Center, TI TC BMT, 91058 Erlangen, Germany

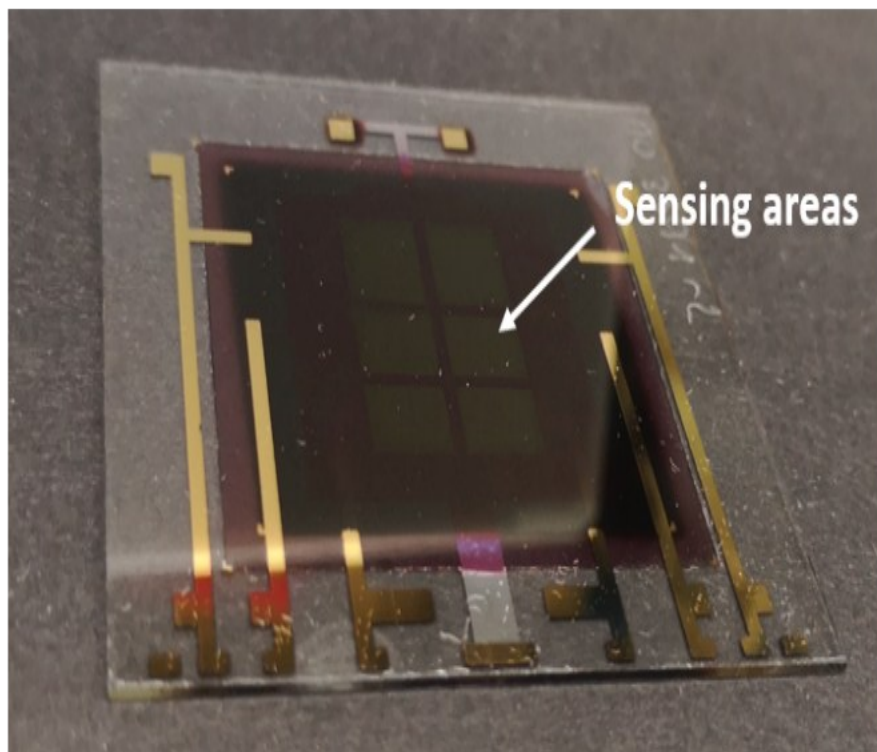
£ These authors contributed equally.

* corresponding authors: mario.rothbauer@tuwien.ac.at; peter.ertl@tuwien.ac.at

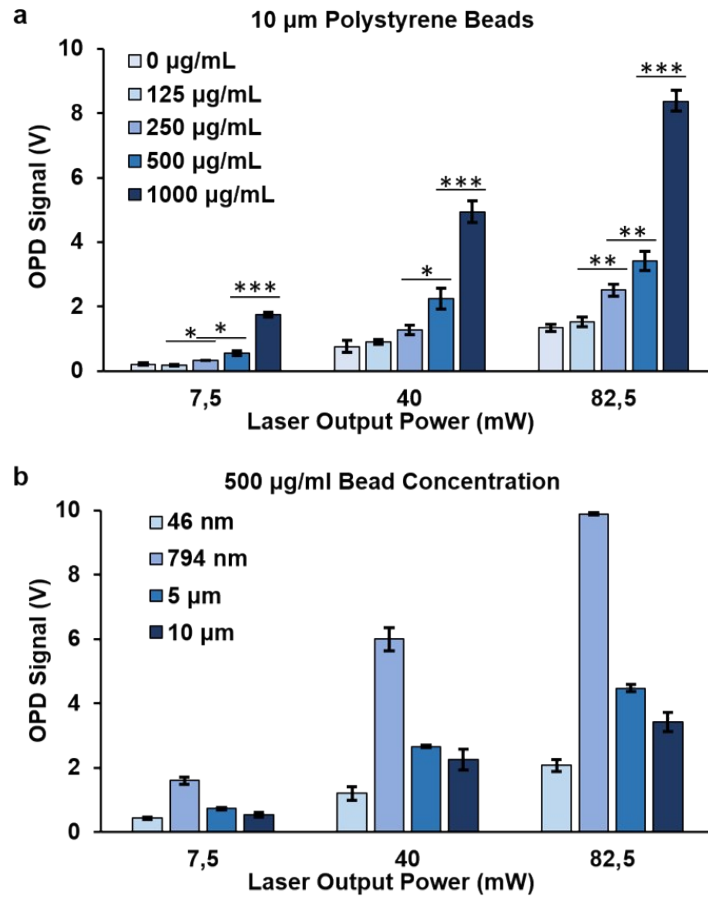
Patient ID	Patient Age [years]	Patient Gender	Diagnosis	Operated Joint	Medication
13	27	female	Chronic Polyarthritis	Left Articulatio composita	Enbrel
14	60	female	Chronic Polyarthritis	Right Articulationes metacarpophalangeae II	MTX/ETX & Humira
15	44	female	Chronic Polyarthritis	Left Articulatio composita	Arava
16	70	female	Chronic Polyarthritis	Right Articulationes metacarpophalangeae II	none
68	44	female	Chronic Polyarthritis	Right Articulationes metacarpophalangeae II	Arava

SI Table 1. Pseudonymized clinical data for patient FLS cell lines

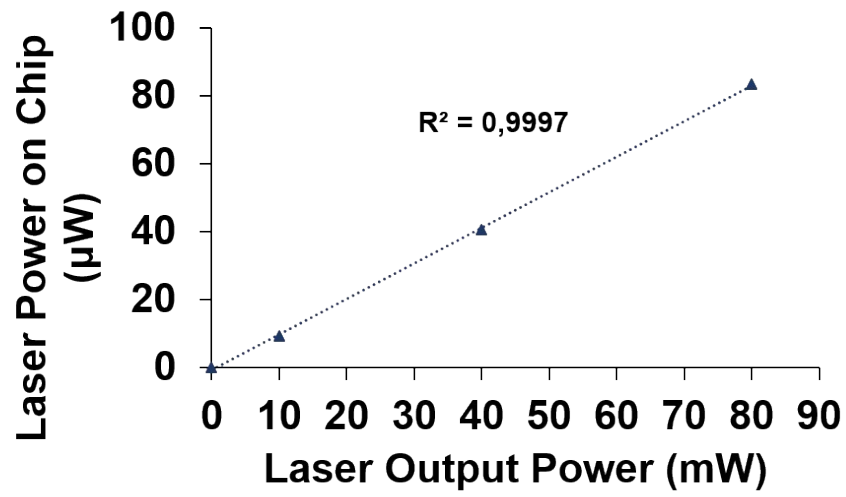
FIGURES



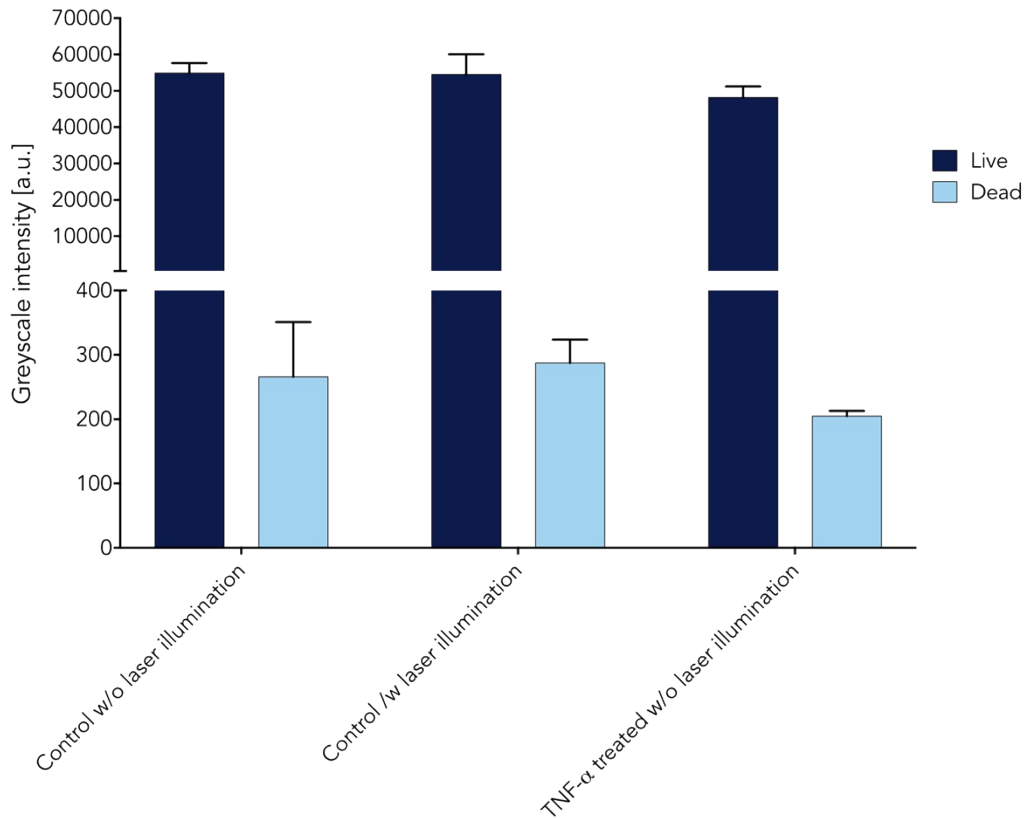
SI Fig. 1 Top front view image of organic photodiode designed as read out for scattered laser light. The individual OPD sensing area has a length and width of 50 mm each.



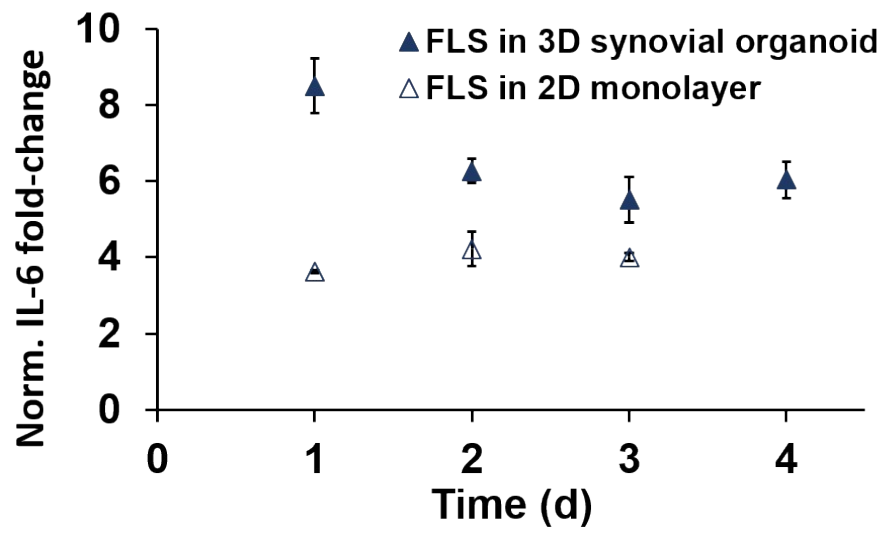
SI Fig. 2 Physical characterization of light scatter analysis using micro- and nanoparticles as artificial cells and cell organelles. Influence of a) particle concentration and b) particle size on the output voltage of the light scatter analysis. The significance of the data was determined by using a 2-way ANOVA. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. b, Light scatter measurement of different sized polystyrene beads in PBS at a concentration of 500 $\mu\text{g ml}^{-1}$ ($n=3$).



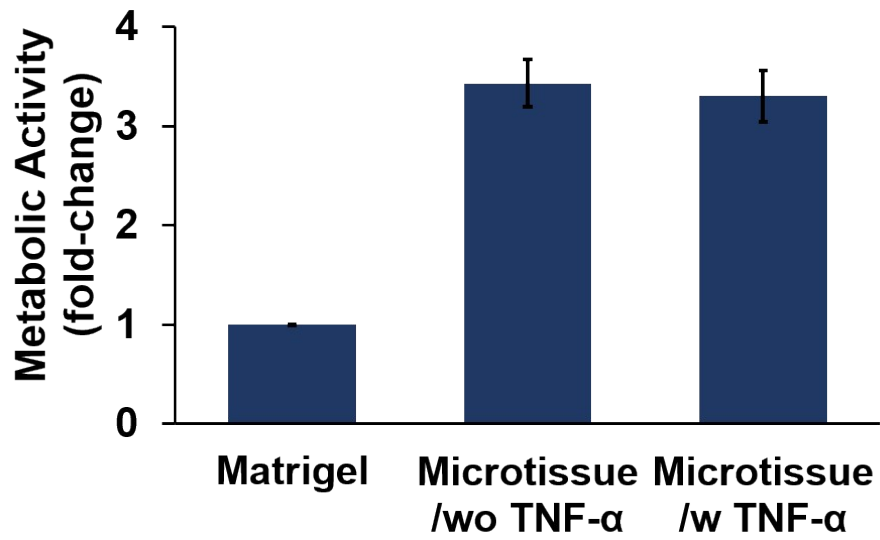
SI Fig. 3 Correlation of laser power on chip and laser output power. Comparison of the set output laser power of the 488 nm sapphire laser with the laser power on chip, measured between collimator and chip by a handheld power meter.



SI Fig. 4. Influence of laser beam illumination on cell viability. Image analysis of a Live/Dead Cytotoxicity assay using 2 μM Calcein AM and 4 μM Ethidium-homodimer at day 8 was calculated by using FIJI to measure the mean grey scale value of the synovial organoids. The organoids were either switched once a day from the lightscatter station to an incubator and vice versa (/w laser illumination) or were kept in an incubator (w/o laser illumination) for the whole duration of 8 days. Semi-continuous light scatter analysis of two chips was conducted by using 50 msec laser pulses every 30 sec at approximately 37 μW laser power on chip. A student t-test showed no significant differences ($p > 0.05$) neither in the greyscale intensities between the organoids in the incubator or the ones in the lightscatter station nor in the greyscale intensities between organoids cultured with and without TNF- α (10ng ml⁻¹). (patient sample ID FLS #13, n=2)



SI Fig. 5 IL-6 secretion of patient-derived two-dimensional monolayers of FLS maintained over a culture duration of three days and of three-dimensional Matrigel FLS organoids maintained over a culture duration of four days in the presence and absence of the inflammatory cytokine TNF- α at a concentration of 10 ng ml⁻¹. (2D: n=2(technical replica), 3D: n=3, patient sample ID FLS #68)



SI Fig. 6 Metabolic activity of untreated and TNF- α stimulated synovial organoids at day 4 post-seeding in the light scattering biochip array using Presto blue assay. (patient sample ID FLS #16)