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# Supporting information

# Acoustic monitoring of changes in well-defined hyaluronan layers exposed to chondrocytes

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**Experimental Section:**

*Cell expansion*

Surplus chondrocytes from patient undergoing Autologous Chondrocyte Implantation (ACI) were cultured in DMEM/F12 (Invitrogen, Paisley, UK) supplemented with 0.1 mg/mL L-ascorbic acid (Apotekets produktionsenhet Umeå, Sweden), 1x Penicillin-Streptomycin (PAA Laboratories, Pasching, Austria), 2 mM L-Glutamine (Invitrogen) and 10% human serum, at 37°C in 7% $CO_2$ and 90% relative humidity. Medium was changed two to three times per week. Subculture was made with trypsin-EDTA solution (0.125% trypsin (Invitrogen) diluted in 0.1 M PBS with 0.2 g/L EDTA) when the cells reached 80% confluence. Cells in passage 3 were used for QCM-D experiments.

*Chemicals*

Oligo ethylene glycol (OEG) disulfides with terminal hydroxyl (SS-OEG, structure: -(S-$CH_2$-($CH_2$-O-$CH_2$)$_7$-$CH_2$-OH)$_2$, MW: 771.0 Da) and biotin groups (SS-OEG-biotin, structure: -(S-$C_2H_4$-CO-NH-($CH_2$-O-$CH_2$)$_9$-NH-CO-$C_4H_8$-Biotin)2, MW: 1539.9 Da) were purchased from Polypure, Norway. All chemicals, unless otherwise stated, were purchased from commercial sources. Phosphate buffered saline (PBS) buffer were made from tablets (NaCl 137 mM, KCl 2.7 mM, phosphate 10 mM). Streptavidin, hyaluronidase and GAG derivatives were aliquoted

in water in and stored at -20 °C. Water was purified and deionized to a resistivity of >18.2 MΩ·cm with a MilliQ system (Millipore, France). End-on biotinylated hyaluronan (b-HA, MW = 23 kDa) was obtained from INNOVENT, Jena, Germany.

*Preparation of biotinylated sensors*

For the QCM-D measurements on biotinylated end-on HA, Au coated, AT-cut quartz crystals with a fundamental frequency of 5 MHz from Q-Sense (Västra Frölunda, Sweden) were used. Prior to thiol incubation all sensor surfaces were cleaned in a 5:1:1 solution of water, 25 % ammonia and 30 % hydrogen peroxide at 80 °C for 10 min to remove organic contaminants. The surfaces were rinsed repeatedly with water, dried under nitrogen and then incubated for >12 h in the ethanolic thiol solution (99% SS-OEG and 1% SS-OEG-biotin) prior to use. Subsequently, the surfaces were rinsed in ethanol and ultra sonicated at 25 °C for 3-5 min to remove non-covalently bound thiols. The surfaces were again rinsed in ethanol and dried under nitrogen prior to mounting the surfaces in the QCM-D instrument.

*QCM-D*

The principle of QCM-D relies on the sharp and stable oscillation resonance when an electric AC field is applied over the electrodes to the quartz crystall. The surface of the disk-shaped sensor performs a shear oscillation, i.e., with periodic motion back and forth parallel to the sensor surface. The resonance frequency of this oscillation decreases when mass adsorbs to the surface and increases when mass is detached. Recording of this shift of the resonance frequency, $\Delta f$, thus gives information about the attached/detached mass to the sensor surface. The second measured quantity in QCM-D is the energy dissipation, $\Delta D$, (or damping) of the oscillator. Formally $\Delta D$ is defined as the fraction of the energy dissipating during one oscillation cycle and the total energy stored in the oscillator. Practically the $\Delta D$ factor senses the viscoelastic properties of the mass coupled to the oscillator.

QCM-D experiments were performed using a Q-Sense E1 unit (BiolinScientific / Q-Sense, Göteborg, Sweden) equipped with a window module that allows for simultaneous visualization of platelets on the sensor surface. A polarized light microscopy (Leica DM4000M) equipped with a 10x objective was used for imaging.

All measurements were carried out at 37 ºC. Human derived chondrocytes were injected at a concentration of 750.000 cells/mL.

Frequency and dissipation shifts were measured at the 3$^{rd}$ harmonic and the frequency shifts were normalized to the fundamental frequency by dividing the values by 3.

*Statistics*

Mean values and sample standard deviation were calculated and given in the result section and Figures. Experiments were repeated 3-5 times to ensure reproducibility.

**Control experiments:**

*Streptavidin control*

Control experiments were performed on streptavidin coated sensor surface. After immobilization of streptavidin on the sensor surface coated with a biotinylated SAM, chondrocytes were added (Figure S1). No cell attachment was observed, neither with the QCM-D nor with the light microscope. Rinsing the surface with medium after 145 minutes removed all chondrocytes.

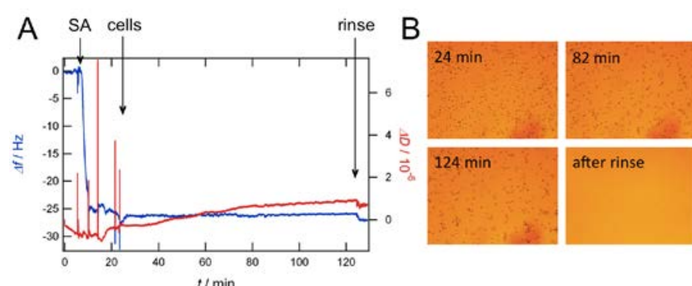


**Figure S1:** (**A**) Frequency (*Δf*, blue) and dissipation (*ΔD*, red) shifts for the addition of chondrocytes to a streptavidin (SA) coated QCM-D sensor. **(B)** Light microscopy image taken during the QCM-D experiment.

*Supernatant control*

Further control experiments were performed incubating the HA-coated sensor surface with supernatant form chondrocytes (Figure S2). Supernatant was obtained by spinning down cells at 7000 rpm for 5 min at 22 ºC.

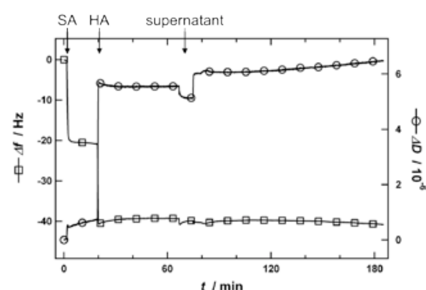


**Figure S2: A)** Frequency (*Δf*, square) and dissipation (*ΔD*, circle) shifts for the addition of supernatant to a HA coated QCM-D sensor.