Supplementary information

Methodology

mRNA extraction

The strained cells were centrifuged at 1300 rpm and the supernatant was replaced by 1 ml of TRIzol™ (Thermofisher Scientific). 15 µl of Proteinase K (Thermofisher Scientific) was added to each vial and samples were incubated in a ThermoMixer c (Eppendorf) at 35 °C and 1550 rpm for 15 min. 300 µl chloroform was then added to each sample, the vials were shaken vigorously and incubated at room temperature for 5 min, followed by centrifugation at 12000 rpm for 30 min at 4 °C. The upper aqueous phase was the carefully transferred to a new vial and 200 µl chloroform was added. The vials were shaken vigorously, incubated for 3 min at room temperature, and then centrifuged at 12000 rpm for 15 min at 4 °C. The upper aqueous phase was the carefully transferred to a new vial, 500 µl 99 % (v/v) isopropanol was added to each sample, vials were vigorously shaken as and incubated at room temperature for 5 min, followed by centrifugation at 15000 rpm for 10 min at 4 °C. The supernatant was then replaced by 1 ml 75 % (v/v) ethanol solution and the samples were vortex for 10 s, followed by centrifugation at 15000 rpm for 5 min at 4 °C. Samples were twice more washed with 75 % (v/v) ethanol solution as elaborated above. Finally, the supernatant was removed, and the obtained pellets were dried and suspended in DEPEC water (Thermofisher Scientific) and incubated at 55 °C for 10 min at 1000 rpm in the a ThermoMixer c (Eppendorf). The amount and quality of the purified nucleic acid was determined using NanoDrop One^{c} Microvolume Spectrophotometer (Thermo Scientific). Samples were stored at -80 °C until the next step.

cDNA synthesis

A volume of each sample containing 1 µg RNA was transferred to each well of the PCR plate and completed to 6 µl with DEPEC water (Thermofisher Scientific). 14 µl of the Master Mix, containing Random Primer Mix, Enzyme Mix and Reaction Mix was added to each well (ProtoScript II First Strand cDNA Synthesis Kit; New England Biolabs). The samples were kept on ice throughout the experiments. cDNA synthesis was performed using qTower^{3} qRT (Analytik Jena) using the following reaction protocol: priming for 5 min at 25 °C, reverse transcription for 1 h at 42 °C, reverse transcription inactivation for 5 min at 80 °C, optional hold at 4 °C. After reaction, 30 µl PCR grade water was added to each sample, the plate was centrifuged at 1500 rpm for 5 min, and the samples were pipetted into new RNAse free vials and stored at -20 °C until the next step.

Real-Time qPCR

8 µl of diluted cDNA (dilution factor 1:20) was added to each well of a PCR plate. 5 µl of Luna® Universal qPCR Master Mix (New England Biolabs) and 0,5 µl of each reverse and forward primer (5 µM; Table S-1) was added to the designated wells. Following controls were also considered in the experiments:

- Reverse transcription negative control: Samples with primers but without master mix
- Non-template negative control to check for primer-dimer formation and contamination: PCR grade water (no sample) with each primer set and Master Mix.
- Porcine genomic DNA control to verify no specific amplification with the primers: Samples with Master Mix and primers for the housekeeping gene

The Syber Green Standard Protocol was used. Following initial denaturation at 95 °C for 5 min, samples were exposed to of 45 cycles comprised of denaturation at 95 °C for 10 s, annealing at 60 °C for 10 s, and extension at 72 °C for 10 s. Finally, a melting curve was obtained in the range of 60 °C to 95 °C within 15 s with ∆T= 1 °C.
Gene expression was calculated using the $\Delta\Delta Ct$ method as follows:

$$\Delta Ct = Ct\ gene\ of\ interest - Ct\ House\ keeping\ gene$$

$$\Delta\Delta Ct = \Delta Ct\ treated - \Delta Ct\ untreated$$

Gene expression = $2^{-\Delta\Delta Ct}$

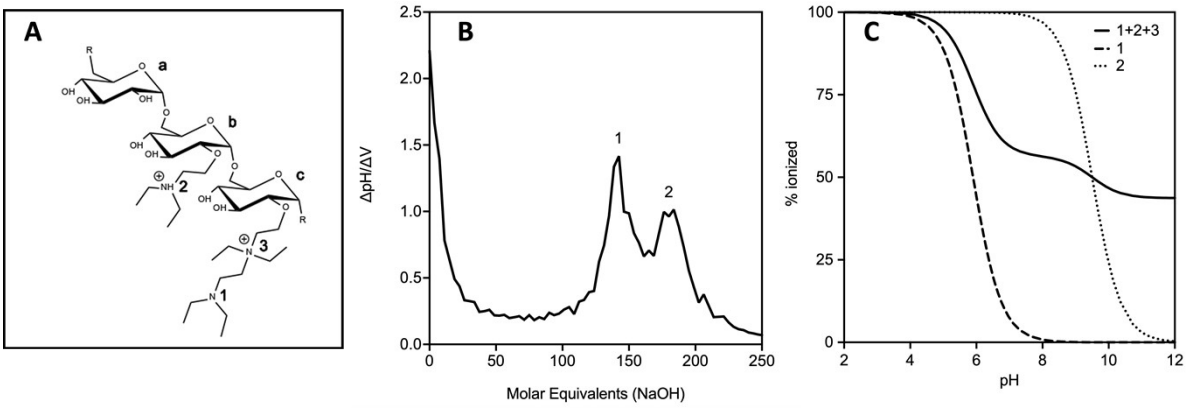
Sup. Tab. 1. *List and sequence of the primer sets used in the experiments*[1,2]

Gene Symbol	5'-3'	Tm (°C) calculated
TNF-α	ATGAGCACTGAGAGCATGATCCG CCTCGAAGTGCAGTAGGCAGA	63
IL-1β	GAGCATCAGGCAGATGGTGT CAAGGATGATGGGCTCTTCTTC	59
IL-6	GCTGCTTCTGGTGATGGCTACTGCC TGAAACTCCACAAGACCGGTGGTGA	67
IFN-γ	CCTCAGATGTACCTAATGGTGG GCTTGATCACATCCATGCTCC	59
TBPI (House keeping)	AACAGTTCAGTAGTTATGAGCCAGA AGATGTTCTCAAACGCTTCG	60

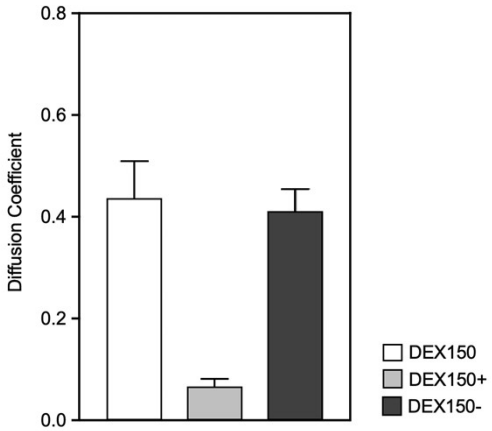
[1] Nygard, AB., Jørgensen, C.B., Cirera, S. *et al.* Selection of reference genes for gene expression studies in pig tissues using SYBR green qPCR. *BMC Molecular Biol* **8**, 67 (2007). https://doi.org/10.1186/1471-2199-8-67

[2] Gao, H., Zhang, Q., Chen, J., *et al.* Porcine IL-6, IL-1β, and TNF-α regulate the expression of pro-inflammatory-related genes and tissue factor in human umbilical vein endothelial cells. *Xenotransplantation* **25**, 5 (2018). https://doi.org/10.1111/xen.12408

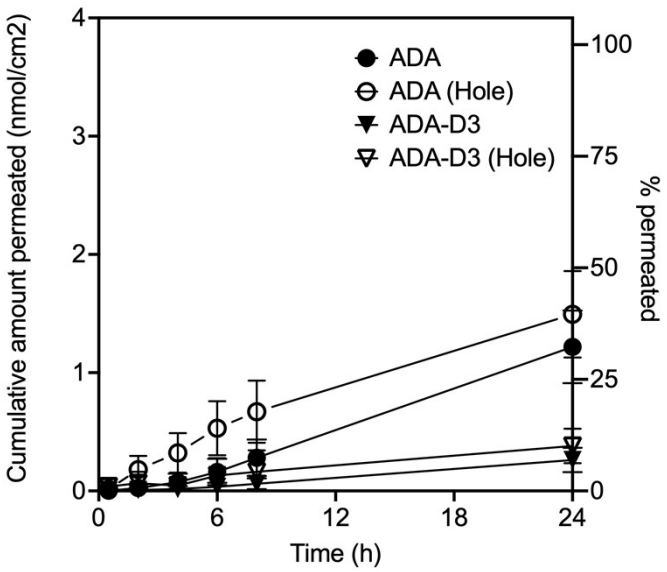
Results



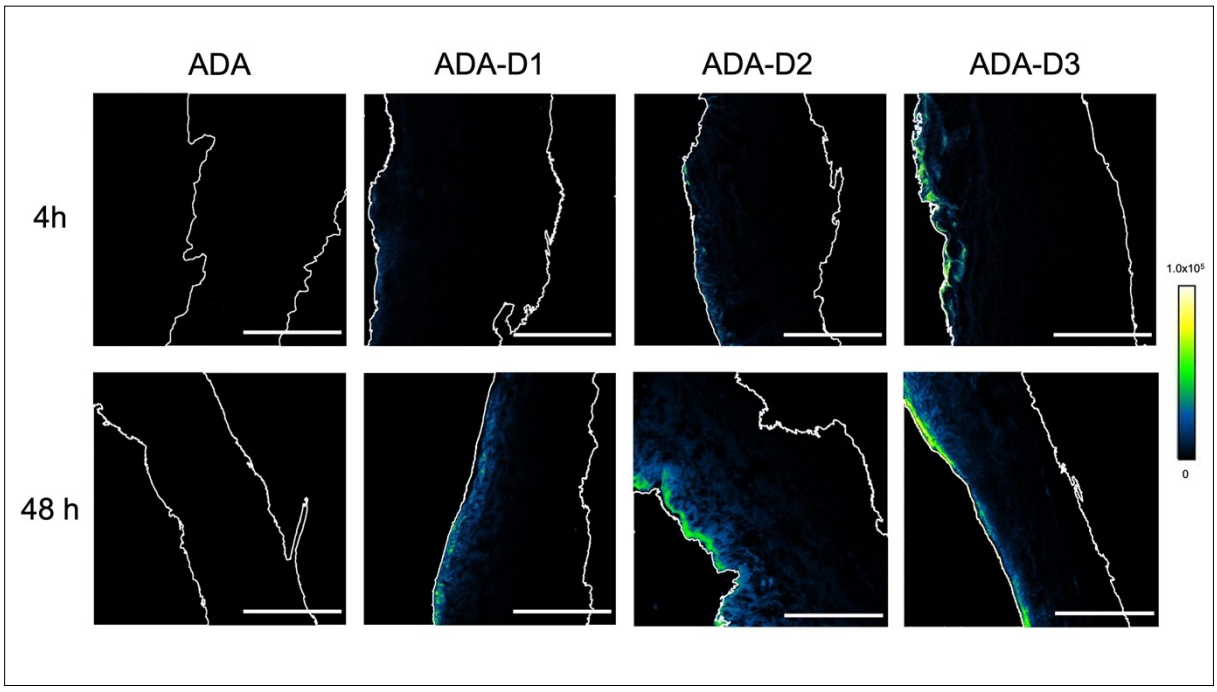
Sup. Fig. 1 (A) Chemical structure of DEAE-dextran which consists of three different monomers (a, b, c) where the substituents include two tertiary amines (1, 2) and a quaternary amine. (3). **(B)** As determined by potentiometric titration, the plot of ΔpH/ΔV vs. molar equivalents of NaOH, shows the point of complete deionization of each of the two tertiary amines (1,2). **(C)** Proportion of ionized tertiary amines (1,2) at different pH values, as well as of the total molecule including the permanent charged quaternary amine group (1+2+3). At physiological pH, 58% of the amines are positively charged, corresponding to a total net charge of approximately +187 for the 150 kDa DEAE-dextran.



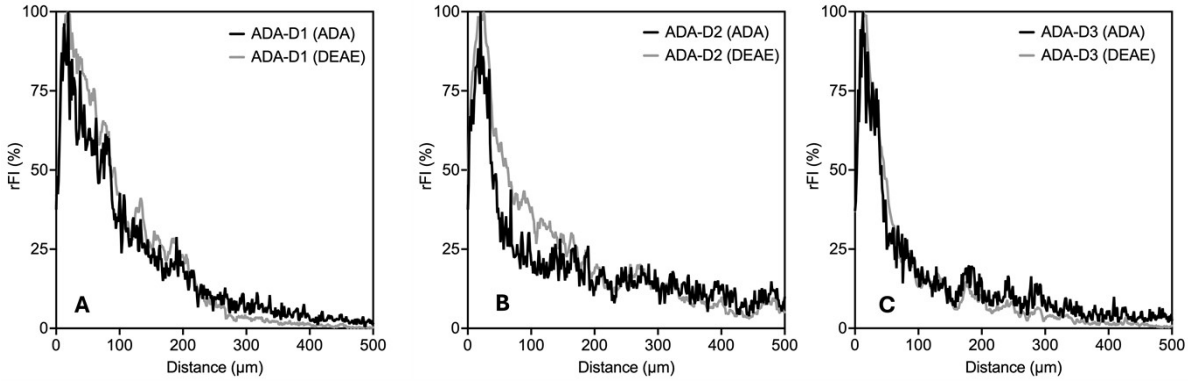
Sup. Fig. 2. Diffusion coefficient of dextran (MW 150 kDa) with different surface charges (anionic (Dex 150-), neutral (Dex 150), and cationic (Dex 150+) in in artificial synovial fluid measured using FRAP technique at CLSM.



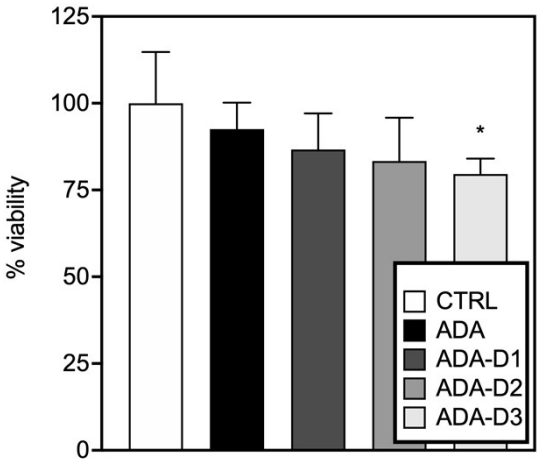
Sup. Fig. 3: Comparison of permeation of ADA as a solution and in complex across an untreated and a cannula punctured synovial membrane. Mean ± SD; n=6.



Sup. Fig. 4: CLSM images of *ex-vivo* synovium after 4 h as well as 48 h showing the retention of FITC-labeled DEAE-dextran in cationic ADA-nanocomplexes with different molar equivalents of cationic DEAE-dextran. Direction of diffusion from left (luminal) to right (basolateral); Scale bar: 500 µm.



Sup. Fig. 5: Plot profiles generated from CLSM images of the measured fluorescence intensities of rhodamine-labeled ADA and FITC-labeled DEAE-dextran from ADA-D1 **(A)**, ADA-D2 **(B)**, and ADA-D3 **(C)** nanocomplexes (n=3) after 48 h proving simultaneous diffusion and thus stability of the complexes in the tissue. Error bars were omitted for clarity reasons.



Sup. Fig. 6: % viability of synovial tissue in presence of nanocomplex formulations in different molar ratios (ADA-D1-3) after 48 h of incubation at 37° C as determined by MTT-assay. Concentration of the formulations has been selected to match that used in the Franz-cell experimental setup (0.1 mg/mL ADA equivalent to 0.1, 0.2 and 0.3 mg/mL DEAE-Dextran in ADA-D1, ADA-D2 and ADA-D3, respectively). Mean ± SD; n=5; ANOVA+ Dunnett's multiple comparison test; significant differences: none of the formulations was significantly different compared to ADA alone (ADA); '*' ($p < 0.05$) compared to compared to freshly dissected tissue.