Supplementary Material for

Influence of Diabetes on the Foreign Body Response to Nitric Oxide-Releasing Implants

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Synthesis of N-diazeniumdiolate-modified mesoporous silica nanoparticles

Mesoporous silica nanoparticles (MSNs) functionalized with *N*-diazeniumdiolate NO donors were synthesized using a variant of the Stöber method, as reported previously [40]. Bare mesoporous silica particles were first prepared by adding TEOS (1.395 mL) to a solution of water (162 mL), EtOH (175 mL), NH_4OH (11.8 mL), and CTAB (280 mg). The silicate solution was allowed to stir for 2 h until MSN formation was complete, after which DET (1.31 mL) was added dropwise over 1 min to initiate cation exchange between CTAB and the aminosilanes. The reaction was stirred overnight (~18 h). Secondary amine-modified particles were subsequently collected via centrifugation (6540*g*, 4 ^{o}C, 5 min). Residual CTAB in the MSN mesopores was removed via ion exchange with H^+ ions by sonicating the particles in an ethanolic HCl solution (9:1 v/v EtOH:HCl; 3×20 min). The amine-modified MSNs were then washed with EtOH (2×) and dried under reduced pressure.

Secondary amines on the MSNs were converted to *N*-diazeniumdiolate NO donors by reaction with gaseous NO. The DET particles were initially dispersed in 9:1 (v/v) DMF:MeOH at 5 mg mL^{-1} prior to adding 5.4 M methanolic NaOMe (5 µL per 3 mg MSN) as a catalyst for NO donor formation. Glass vials containing the particle solutions were equipped with stir bars, placed in a stainless-steel Parr hydrogenation vessel, and connected to an in-house NO reactor. The Parr bottle was flushed with Ar (3×short, 3×10 min) to remove oxygen from the reaction solution prior to pressurizing the vessel with pure (>99.5%) NO gas (10 bar) for 3 d. Of note, the NO gas was scrubbed over solid potassium hydroxide for at least 4 h prior to the *N*-diazeniumdiolate formation reaction. After 3 d the NO gas was vented and the vessel again flushed with Ar. The NO donor-modified particles were collected via centrifugation, washed with EtOH (3×), and dried under reduced pressure. The resulting NO-releasing particles were stored

in a vacuum-sealed Mylar bag at -20 ^{o}C until further use. Control (i.e., non-NO-releasing) DET MSNs were treated similarly with NaOMe but without the *N*-diazeniumdiolate formation process. Characterization of the NO-releasing DET particles is provided as supplementary data (Table S1).

Synthesis of S-nitrosothiol-modified mesoporous silica nanoparticles

Thiol-based MSNs were synthesized using a mercaptosilane/alkoxysilane co-condensation method adapted from reported procedures for nonporous, thiol-modified particles [39]. A silane precursor solution was initially prepared by mixing MPTMS (1.32 mL) and TEOS (1.19 mL) in a glass vial. The silane mixture (2.28 mL) was added to a stirring solution of water (210 mL), EtOH (84 mL), NH_4OH (9.6 mL), and CTAB (240 mg). The reaction was allowed to proceed for 2 h. The thiol-modified particles were collected and purified using the same washing and CTAB removal steps described above for the DET MSNs. *S*-nitrosothiol (RSNO) NO donors were formed on the thiol groups in a subsequent nitrosation step. The MPTMS MSNs (200 mg) were dispersed in a mixture of MeOH (4.00 mL) and 5 M HCl (2.00 mL) and stirred on ice. A solution (2.00 mL) of sodium nitrite (2.27 M) and diethylenetriaminepentaacetic acid (DTPA; 500 μM) in water was added dropwise to particle dispersion and stirred on ice for 1 h. The MSNs were collected via centrifugation, washed with cold MeOH (3×), and dried under reduced pressure. The RSNO-modified particles were used immediately thereafter. Characterization of the NO-releasing RSNO particles is provided as supplementary data (Table S1).

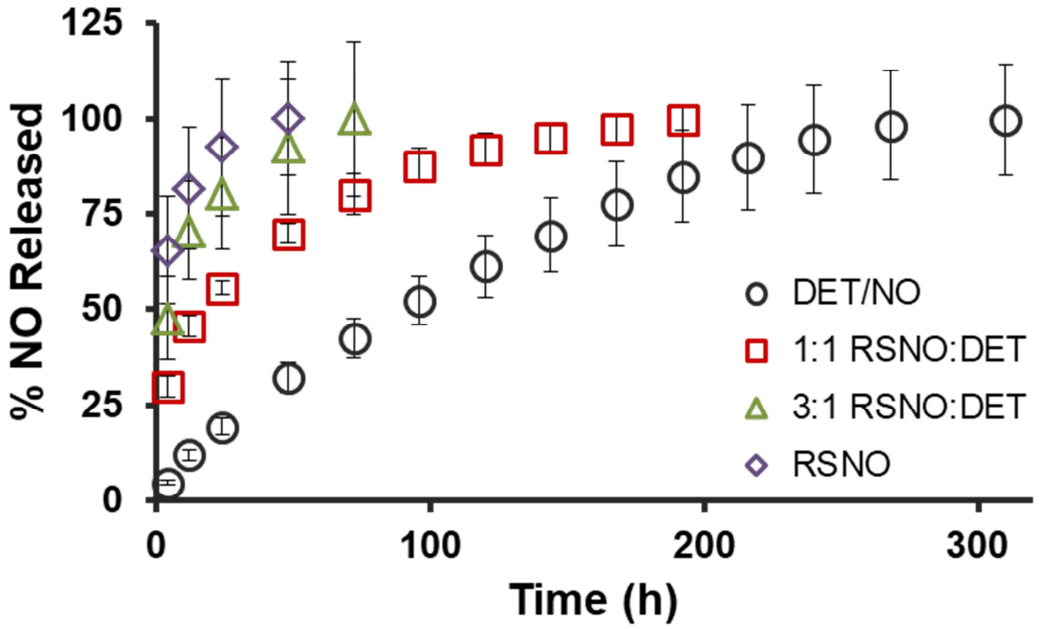

Figure S1. Graph of temporal NO release expressed as a percentage of the total NO released. Total amounts of NO released were calculated by integration of the NO flux vs. time curves to be 1.37±0.20 (RSNO), 1.44±0.29 (3:1 RSNO:DET), 5.55±0.12 (1:1 RSNO:DET), and 2.72±0.25 (DET/NO) μmol NO per cm^2 implant surface area.

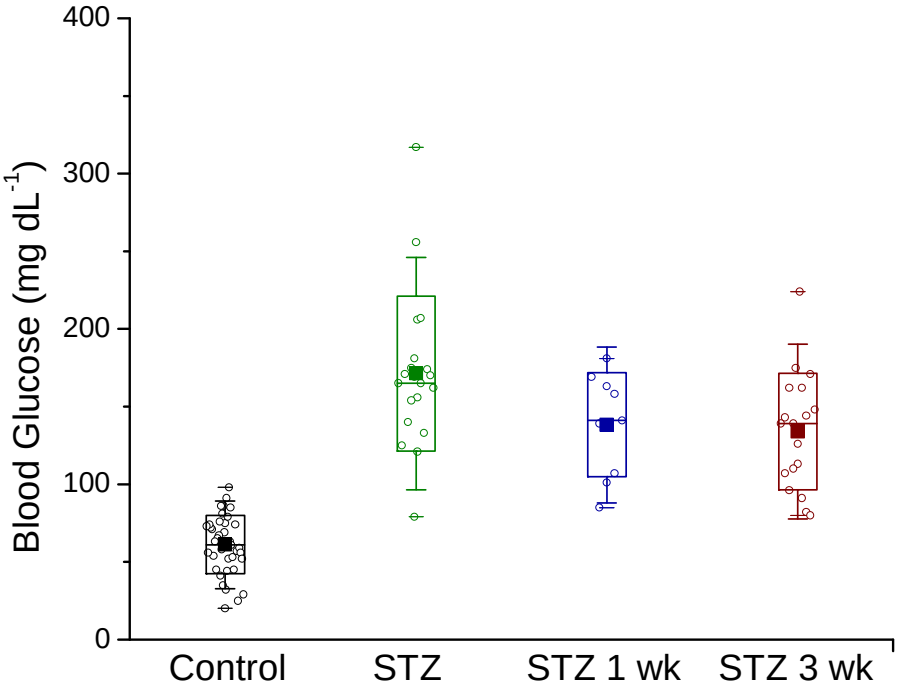

Figure S2. Box and whisker plot of post-prandial blood glucose values determined by a Freestyle Lite glucometer before and after STZ administration. Blood glucose values approximately ~1 (1 wk) and 3 (3 wk) weeks after implantation are also shown.

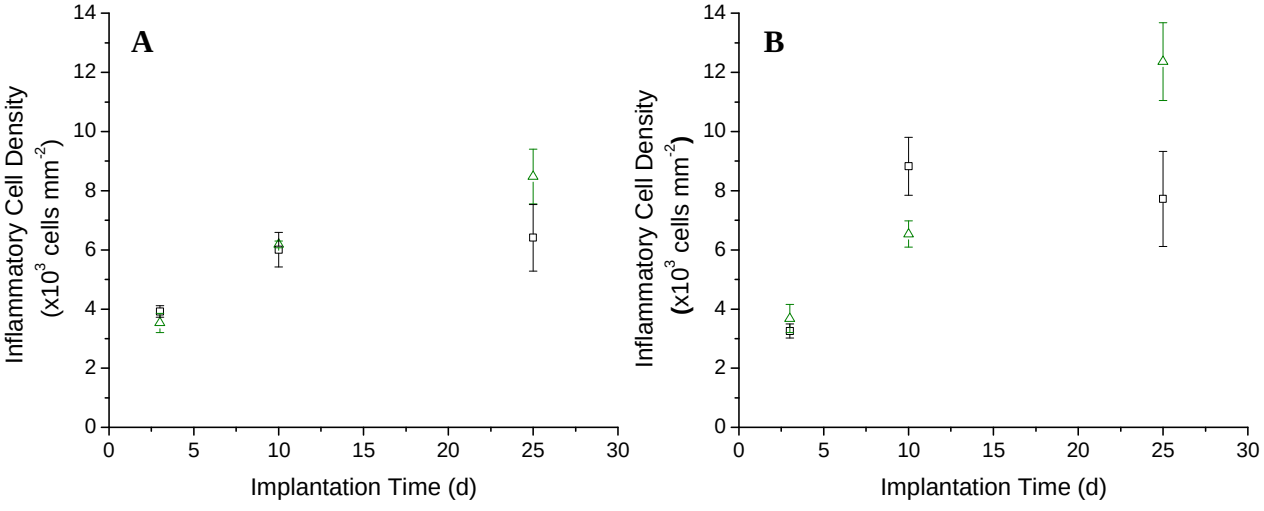

Figure S3. Inflammatory cell densities at RSNO control (A) and DET control (B) implants in the healthy (black square) and STZ-treated pig models (green triangle). Inflammatory cell densities are expressed as the average value ± standard error of the mean.

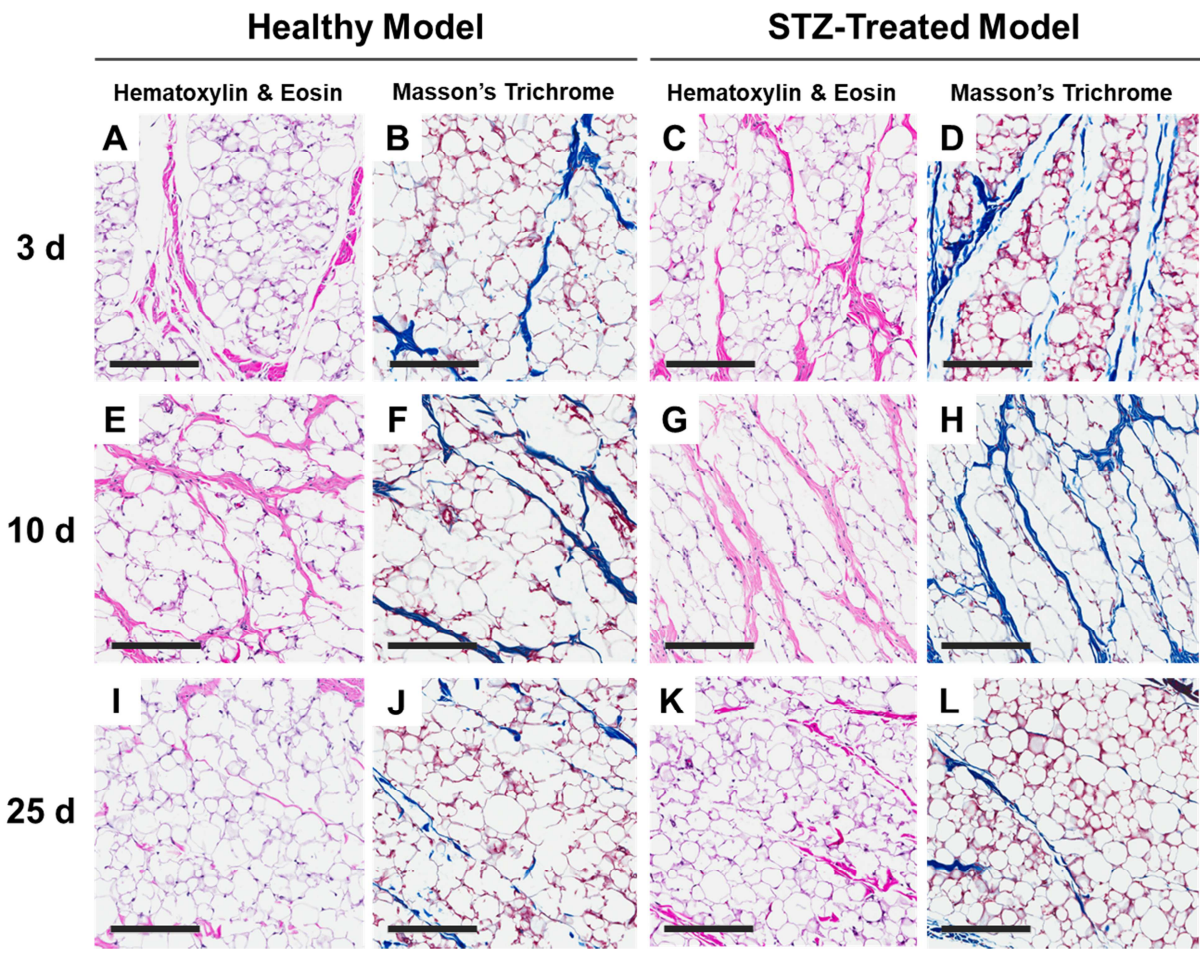

Figure S4. Photomicrographs of H&E and MT stained tissues >10 mm away from DET control implants, representative of unperturbed subcutaneous tissue. The scale bar at the bottom-left corner of each image represents a distance of 200 µm.

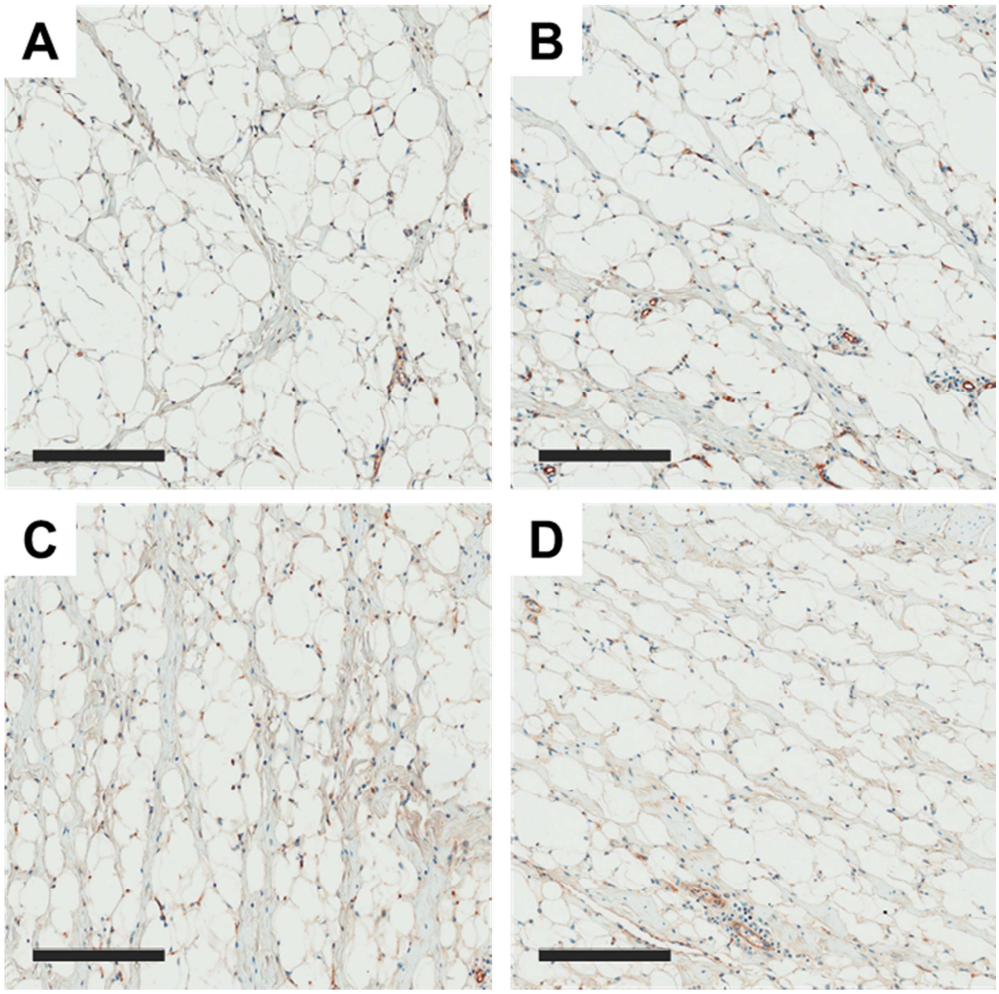

Figure S5. Photomicrographs anti-CD31 and hematoxylin stained tissues >10 mm away from DET/NO (A,C) and DET Control (B,D) implants in healthy (A,B) and diabetic (C,D) pigs. These tissue regions represent of unperturbed subcutaneous tissue. The scale bar at the bottom-left corner of each image represents a distance of 200 µm.

Table S1. Characterization of NO donor-modified silica nanoparticles.[a]

NO Donor Modification	Particle Diameter[b] (μm)	$[NO]_t$ (μmol mg^{-1})	$t_{1/2}$ (min)
S-nitrosothiol (RSNO)	1.76±0.07	2.29±0.47	128.8±2.3
N-diazeniumdiolate (DET)	0.87±0.17	1.61±0.17	35.7±7.5

[a]Results are expressed as average values ± standard deviation of $n \geq 3$ separate experiments.[b]Geometric particle diameter estimated via scanning electron microscopy.[c]Total NO storage per mg of particles.[d]Half-life of NO release.