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# Supporting Information

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# Electrically Conductive and 3D-printable Oxidized Alginate-Gelatin Polypyrrole:PSS Hydrogels for Tissue Engineering

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((Supporting Information can be included here using this template))

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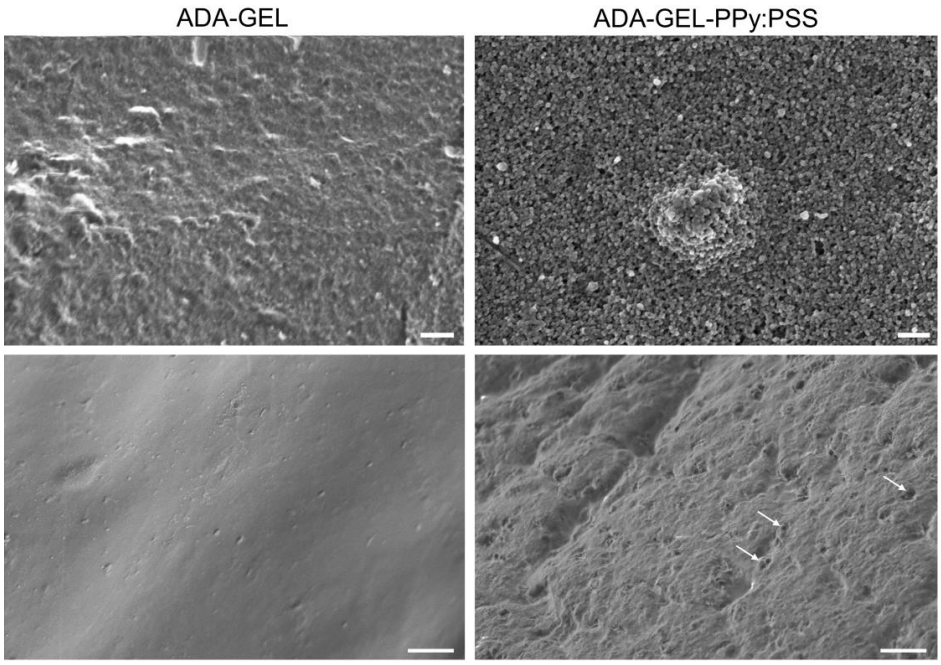


**Figure S1.** SEM images of ADA-GEL (left) and ADA-GEL-PPy:PSS (right). Top row: Hydrogel imaged from the top. Scale bars: 400 nm. The formation of a dense, globular PPy layer can be observed. Bottom row: SEM images from the inside of the hydrogels. Scale bars: 20 µm. A dense hydrogel structure can be observed for the as-prepared hydrogels, as found for 3.75%/7.5% ADA-GEL before.[56,84] White arrows indicate the presence of polypyrrole globules surrounded by a dense ADA-GEL matrix.

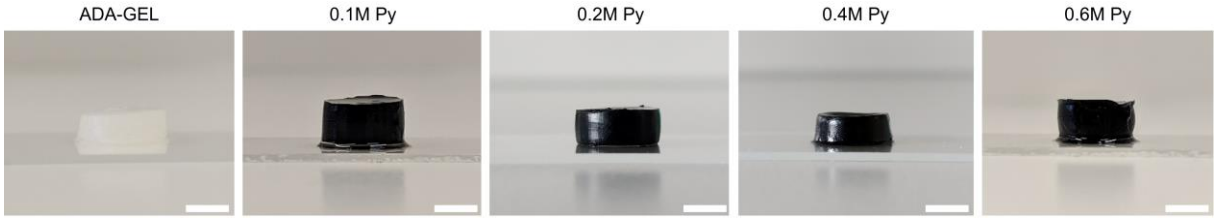


**Figure S2.** Hydrogel cylinders of ADA-GEL-PPy:PSS and ADA-GEL fabricated using different Py molarities. From left to right: ADA-GEL, 0.1M Py, 0.2M Py, 0.4M Py, and 0.6M Py. Scale bars: 5 mm.

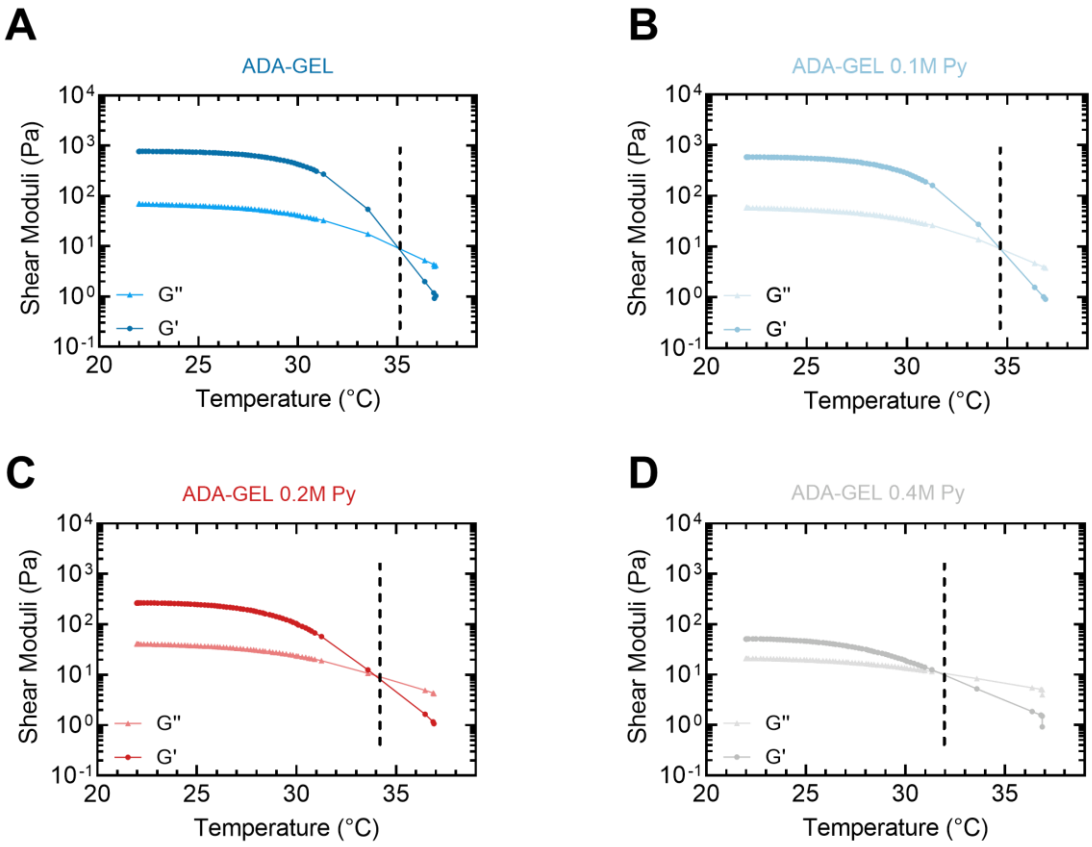


**Figure S3.** Shear moduli G' and G'' of ADA-GEL and ADA-GEL containing 0.1M Py, 0.2M Py, and 0.4M Py. A shift of the solid-to-liquid state (dashed line) to lower temperatures is observed with increasing Py content.

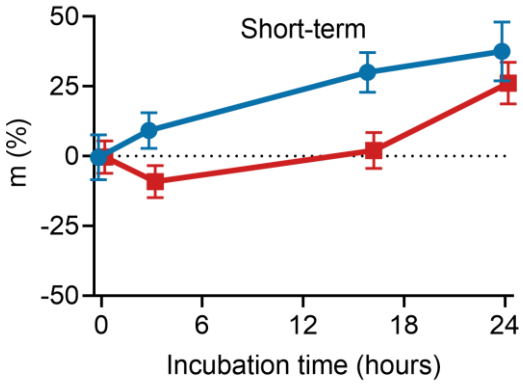


**Figure S4.** Short-term swelling behavior (< 24h) of ADA-GEL (blue) and ADA-GEL-PPy:PSS (red). Data are shown as mean ± SD (n = 6)**.**

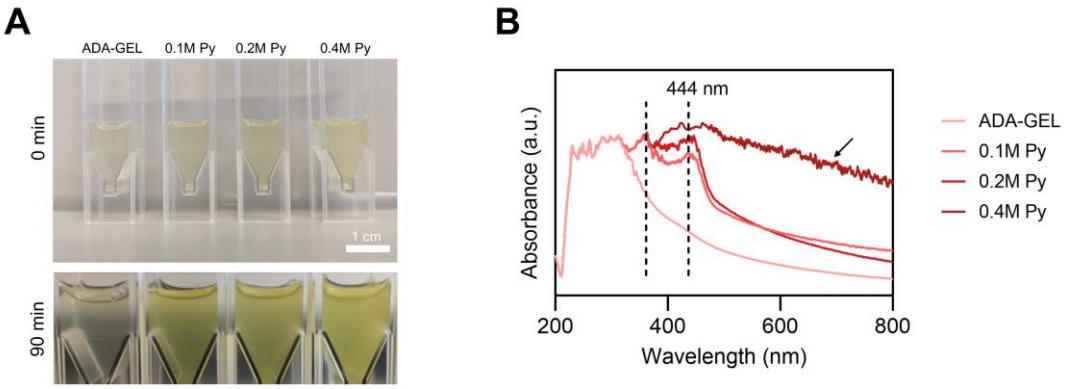


**Figure S5.** Aging of Py-containing ADA-GEL biomaterial ink. A) Macroscopic images showing the aging of the precursors over time, highest for the highest Py-containing precursor. A similar color change was observed for samples stored in the dark in the 3D-printing cartridge compartment. B) UV-vis spectra after 60 min of storage at room temperature (22°C, air atmosphere, not protected from light). Absorbance peaks forming at $\lambda$ = ~365 - 460 nm (dashed lines) for Py-containing ADA-GEL observed, indicative of bi-polaron absorbance and suggesting PPy formation in the hydrogel. In addition, 0.4M Py composition shows broad absorption in the range of $\lambda$ = 400 – 800 nm (arrow), indicative of dispersed hydrophobic Py droplets inside the aqueous ADA-GEL, leading to increased initial turbidity (A, 0.4M) and broad absorbance after 60 min.

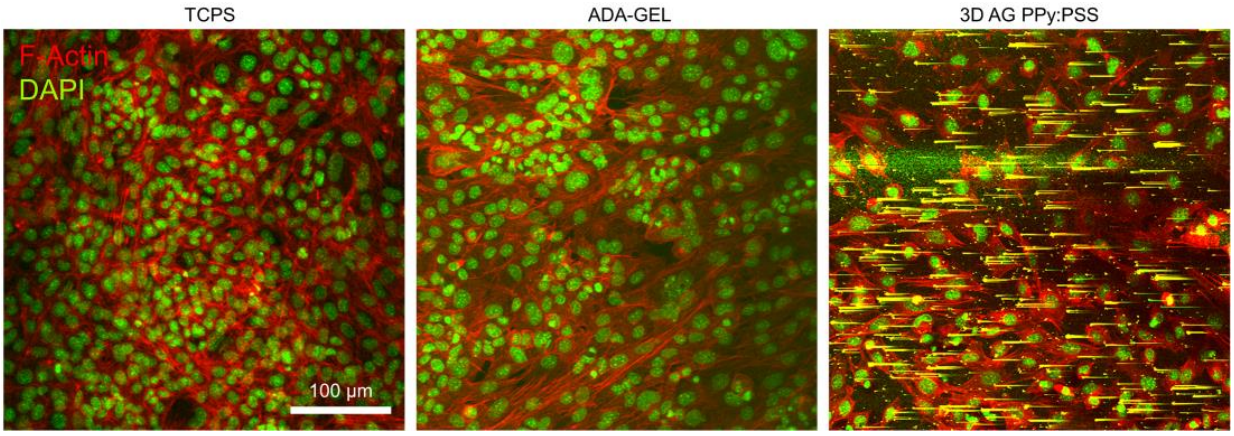


**Figure S6.** Fluorescence microscopy images of ATDC-5 cells grown for seven days on tissue culture polystyrene (TCPS, flat surface), hydrogel cylinders (ADA-GEL, almost flat surface), and 3D-printed hydrogel scaffold (ADA-GEL-PPy:PSS, 3D-printed morphology). Red: F-Actin. Green: Nuclei (DAPI, green colored to allow for higher contrast).

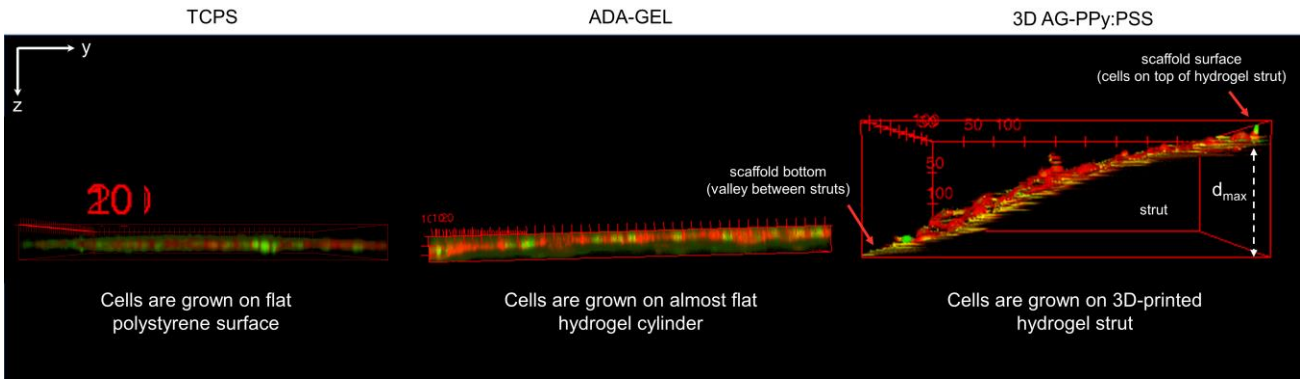


**Figure S7.** Multiphoton fluorescence microscopy images of ATDC-5 cells grown for seven days on tissue culture polystyrene (TCPS, flat surface), hydrogel cylinders (ADA-GEL, almost flat surface), and 3D-printed hydrogel scaffold (ADA-GEL-PPy:PSS, 3D-printed morphology). $d_{max}$ describes the depth in z-direction in which cells could be detected on the overall scaffolds. Cells were growing on the scaffold top as well as in grooves between scaffold struts. Red: F-Actin. Green: Nuclei (DAPI, green colored to allow for higher contrast).

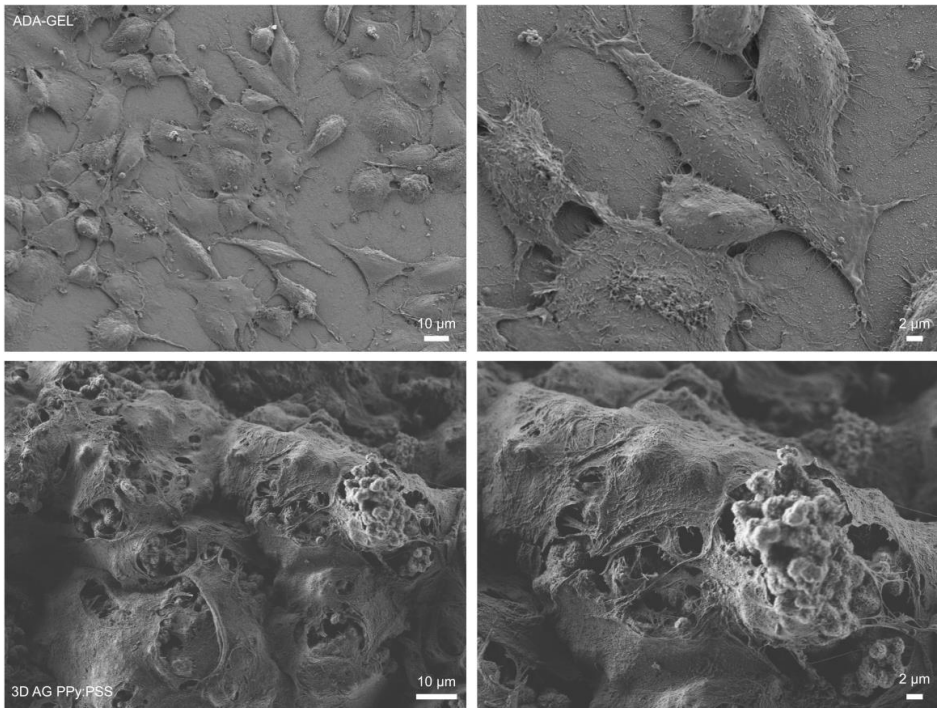


**Figure S8.** Scanning electron microscopy images of ATDC-5 cells grown for seven days on ADA-GEL and 3D-printed ADA-GEL PPy:PSS hydrogels (3D AG PPy:PSS).

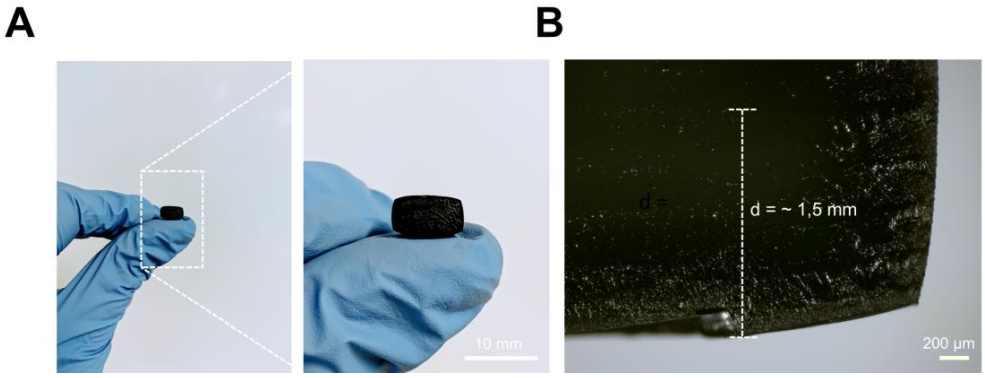


**Figure S9.** Cross section images (A) of an ADA-GEL-PPy:PSS (0.1M PPy) hydrogel cylinder indicating homogenous formation of black polypyrrole throughout the hydrogel (B), reaching a depth (d) of polymerization > 1.5 mm inside the gel.

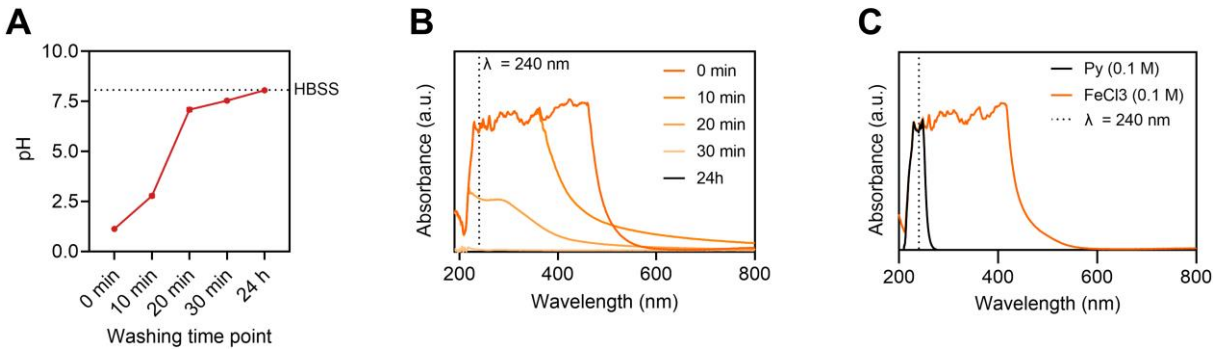


**Figure S10.** pH monitoring and UV-vis measurement of washing supernatant during ADA-GEL-PPy:PSS hydrogel washing using HBSS after oxidation to prepare the hydrogels for cell-culture. HBSS was analysed after 10 min, 20 min, 30 min, and 24 h of washing. Each washing time point consisted of removal of supernatant and replacing with fresh HBSS solution (4 ml). Data are shown as mean ± SD (n = 4). A) pH was used to monitor $H^+$ removal which is a byproduct of the polypyrrole synthesis. After 24h, the washing solution surrounding the hydrogel adapted to the pH of fresh HBSS stock solution, indicating the removal of $H^+$. B) UV-vis was utilized to ensure removal of Fe-based byproducts (λ = ~250 - 450 nm) and the absence of un-converted pyrrole (λ = ~210 - 260 nm). After the last washing step (24h), the data suggest complete removal of Py and $FeCl_3$ during washing. C) UV-vis of $FeCl_3$ and Py aqueous solutions (0.1M, in HBSS) which served as controls.

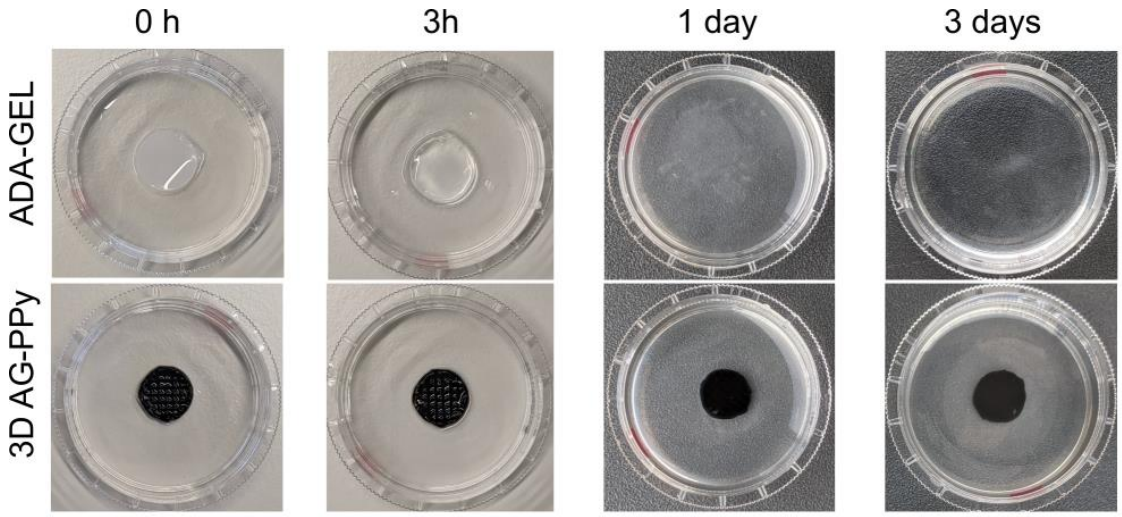


**Figure S11**. Enzymatic degradation of ADA-GEL-PPy:PSS and ADA-GEL mediated by incubation of hydrogel samples (n = 4) in solution containing 1 U/ml of collagenase type II at 37°C (in 35 mm petri-dish, Sarstedt, Germany), as performed previously.[30]

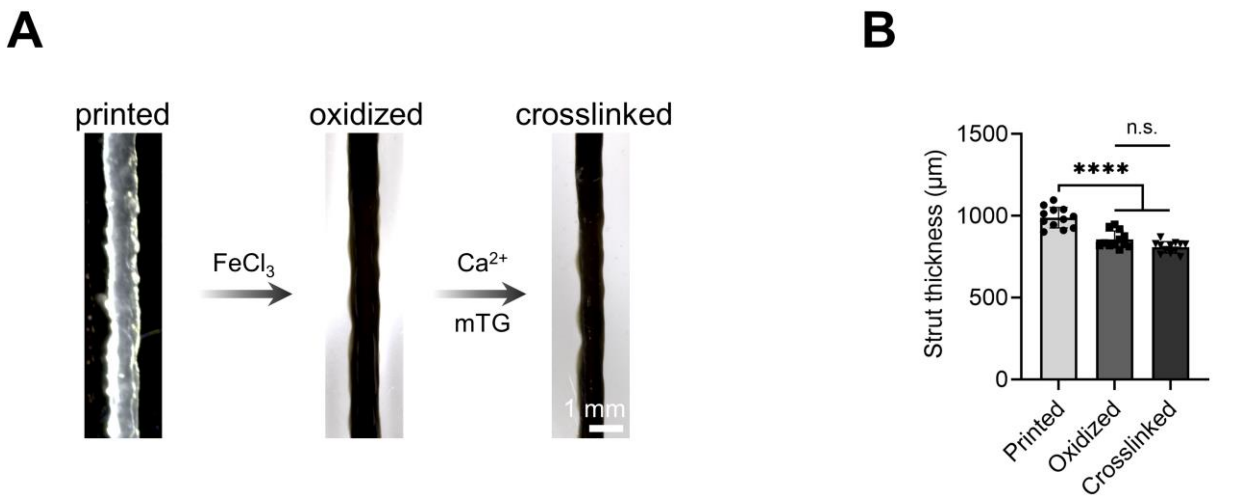


**Figure S12.** Strut thickness of printed, oxidized, and crosslinked ADA-GEL-PPy:PSS (n = 3, four measurements each print). Struts were printed with a diameter = 410 µm extrusion needle (Nordsen EFD, US). A) Light microscopy images. B) Quantification of strut thickness. Data are shown as mean ± SD. A decrease in strut thickness from as-printed (~ 990 ± 62 µm) to oxidized (~ 860 ± 50 µm) scaffolds was observed ($P < 0.0001$), analyzed via one-way ANOVA analysis and Bonferroni post-hoc test.

**Table S1.** Simplified molecular-input line-entry system (SMILES) notations for species analyzed.

| **Chemical** | **SMILES notation** |
|---|---|
| **$FeCl_3$** | **[Fe](Cl)(Cl)Cl** |
| **Pyrrole (Py)** | **C1=CC=CN1** |
| **Polystyrene sulfonate (PSS)** | **CCC(C)C1=CC=C(C=C1)[S-](=O)(=O)=O** |
| **Polypyrrole (PPy)** | **C1(=CC=C(N1)C2=CC=C(N2)C3=CC=C(N3)C4=CC=C(N4)C5=CC=C(N5)C6=CC=C(N6)C7=CC=C(N7)C8=CC=C(N8)C)C** |