Supporting information

Discovery and optimization of glycosidic activity in readily industrialized metallic implants for localized synthesis of antibacterial drugs

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**Experimental section**

Unless stated otherwise, experiments were performed using PBS (10mM or 100mM), containing 150mM NaCl.

**Metals.** Custom manufactured 35N LT® grooved wire at a diameter of 200µm was manufactured using conventional and bulk- replicable methods at Fort Wayne Metals Research Products Corp (Fort Wayne, IN USA). In addition, 254µm 316L annealed round wire was processed using similar methods as reported elsewhere. Cold rolled Nitinol and 35N LT® strip were manufactured using standard cold forming methods, and rolled to a thickness of 152µm. Strips were cut using advanced laser cutting techniques performed by MeKo (Sarstedt, Germany), producing S-Wire forms to mimic laser cut stent segments. S-Wire forms were then coated using cold spray sputter deposition technology at Thinfilms, Inc. (Hillsborough Township, NJ USA). Alloy chemical compositions are based on ASTM specifications as follows: Nitinol (ASTM F2063), 316L (ASTM A276), 35N LT® (ASTM F562). Scanning electron microscopy was performed via a Hitachi S-3400N. Wires were incubated in PBS at 37°C up to 24 hours and imaged at various time points.

**Fluorogenic enzyme substrates.** Enzyme-like catalysis was studied using the two fluorescent enzyme-substrates resorufin β-D galactopyranoside and resorufin β-D glucuronide (Sigma-Aldrich). Unless stated otherwise the concentration was 5 mg/L, and a volume of 200µl was used for each experiment. Wires were incubated with these substrates up to 24h and fluorescence was evaluated using an Infinite 200Pro platereader (Tecan Group). pH was measured using a Hanna instruments pH210 microprocessor pH meter with a HI 1083 probe.

**Synthesis of antibiotic prodrugs.** The prodrugs were synthesized as described elsewhere. In short: synthesis started with converting D-(+)-glucurono-6,3-lactone into the methyl ester and subsequent acetylation in pyridine delivered the fully protected glucuronic acid intermediate. Next steps involved the formation of the glycosyl bromide which was glycosylated with 4-hydroxy-benzaldehyde to deliver the linker precursor. Reduction and activation, followed by conjugation to the drugs delivered the fully protected antibiotic prodrugs. Zemplen deacetylation, followed by methyl ester hydrolysis delivered the antibiotic prodrugs of cipro- and moxifloxacin.

**HPLC.** Analytical HPLC was performed on a Shimadzu LC-2010A HT equipped with an Ascentis® Express Peptide ES-C18 column with 2.7 µm particles, a length of 150 mm and an internal diameter of 3.0 mm from Supelco Analytical. Mobile phase A was ultrapure H2O supplemented with 0.1 % TFA (v/v) and mobile phase B acetonitrile (MeCN) supplemented with 0.1 % TFA (v/v).

**Antibiotic prodrug hydrolysis.** Prodrugs were incubated at a final concentration of 200 µM in PBS (10 mM) in presence or absence of wires (35N LT®/Fe, nitinol/Fe) for 24 h. Wires were removed and the samples were analyzed via analytical HPLC (Setup 1), elution starting...
from mobile phase B 5% to mobile phase B 100% over 15 min, hold 5 min. Retention times \( t_r \) for ciprofloxacin and moxifloxacin were 7.76 and 8.75 min and the corresponding prodrugs 10.10 and 11.29 min, respectively.

**In vitro zonal inhibition.**

Agar plugs were prepared by casting one mL tryptic soy broth (TSB) agar (Sigma-Aldrich, St. Louis, MO, USA) into each well in 12-well cell culture plates (TC Plate 6 Well, Sarstedt, Nümbrecht, Germany). Prodrug of moxifloxacin or ciprofloxacin dissolved in TSB broth was added to the agar plugs to a final concentration reaching 100 times the minimal inhibitory concentration (90 \( \mu \text{M}/39 \text{ mg L}^{-1} \) for moxifloxacin prodrug and 15\( \mu \text{M}/9 \text{ mg L}^{-1} \) for ciprofloxacin prodrug) and allowed one hour to diffuse into the agar. A single bacterial colony of *Staphylococcus aureus* DSM20231 or *Escherichia coli* K12 DSM498 was added to four mL saline (0.85 %), vortexed thoroughly and adjusted to OD600 = 0.1. Bacteria were spread on the agar surface by dipping a sterile cotton swab into the bacterial solution and streaking evenly onto the agar. Moxifloxacin prodrug was used to inhibit growth of *S. aureus* ciprofloxacin prodrug to inhibit growth of *E. coli*. After 10 minutes incubation at 37°C, S-Wire forms of Nitinol and 35N LT® spray coated with Fe (0.1, 0.5 or 1.5 µm) were placed in the center of each well and incubated for 24 h at 37°C. Subsequently, bacterial growth was imaged using a digital camera.

**Data analysis.** Unless stated otherwise, the numerical data are presented as mean ± SD and calculated based on at least 3 independent experiments. Data was plotted and analyzed in GraphPad Prism 5. Statistical analysis was conducted using a Student’s t-test or one way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test to compare all data or Dunnet’s multiple comparison test to compare outcomes to a control in GraphPad Prism 5. Statistical significance was defined as \( P < 0.05 \) (*), \( P < 0.01 \) (**), and \( P < 0.001 \) (***).
Figure S1. (A) Representative SEM images of the surface of the coated nitinol wires after incubation in PBS at 37°C at various time points. Scale bar represents 200µm; (B) Energy-dispersive X-ray spectroscopy qualitative chemical maps of iron, nickel, cobalt and chromium of the 35N LT® iron coated wires (0.1 and 0.5 µm) after 24 hours in PBS and subsequent air-drying. The complimentary Fe-deficient and Co-, Ni-, Cr-rich chemical maps demonstrate microscale windows of exposure to the underlying 35N LT® due to corrosion. Scale bar represents 25µm. (C-E) Glycosidic catalysis mediated by the wire alloys sputter-coated with Fe. Activity was quantified upon a 24 incubation of the wires in 140 mM NaCl saline buffered to pH 7.4 using 10 mM (C,D) or 100 mM (E) sodium phosphate. Statistical evaluation in panels C and D refers to the comparison within the corresponding groups, in panel E – relative to the non-catalyzed reaction.