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Integrated Antimicrobial and Non-fouling Hydrogels to Inhibit the Growth of Planktonic Bacterial Cells and to Keep the Surface Clean

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Supplemental Materials

Synthesis of *N*,*N*-Dimethyl-*N*-(ethylcarbonylmethyl)-*N*-[2-(methacryloyloxy)ethyl]ammonium salicylate (CBMA-1 C2 SA): Sodium salicylate (1.64 g, 10 mmole) was dissolved in DI water (10 mL), and the sodium salicylate solution was added to a solution of *N*,*N*-Dimethyl-*N*-(ethylcarbonylmethyl)-*N*- [2-(methacryloyloxy)ethyl]ammonium bromide (3.24 g, 10 mmole) in DI water (10 mL). The reaction was stirred at 25 ^{o}C for 24 hours. The product was extracted by chloroform and dried in vacuum and analyzed. ^{1}H NMR (300 MHz, D_2O) (Figure S-1): δ7.0-7.67(m, 1H), 7.35-7.28(m, 1H), 6.86-6.79(m, 2H), 6.11 (s, 1H), 5.65(s, 1H), 5.03(s, 2H), 4.66 (t, 2H, *J* = 3.0 Hz), 4.42 (t, 2H, *J* = 3.0 Hz), 4.20(q, 2H, *J* = 7.2Hz), 3.76 (s, 6H), 1.92(s, 3H), 1.29(t, 3H, *J* =6.0 Hz).

***In vitro* Salycilate Release and HPLC Analysis Experiments:** Small 10mm disks (10 mm biopsy punch, Acuderm Inc., FL) were cut from fully-hydrated hydrogels. pCBMA-2 hydrogel disks were initially soaked in water containing 25mg/ml of sodium salicylate for 2 hours, and then rinsed with DI water. Hydrogel disks of pCBMA-2 encapsulating salicylate, and pCBMA-1 C2 SA were placed in vials containing 10 ml of prewarmed DI water at 37◦C with constant stirring. At predetermined time points, 1 mL of fluid was removed and replaced with 1mL of prewarmed DI water. The samples was filtered through a Acrodisc CR 13mm syringe filter with 0.2 µm PTFE membrane (PALL Corp., USA) and analyzed for salicylate content.

The amount of salicylate released was monitored using a high performance liquid chromatography system (HPLC) (Waters, MA) consisting of a separation module (Model 2695) and a UV/Visible Detector (Model 2489). All separations were performed on an Econosil C18 5µ column (4.6mm X 250mm) (Alltech, USA) using a mixture of 60% acetonitrile and 4% water. The flow rate of the mobile phase was 0.5ml/min. The elution was monitored at 280 nm. The cumulative fractional release at time t was then calculated based on a freshly prepared calibration curve (R^2>0.999).

Figure S-2 shows the release of salicylate (SA) from hydrogels as a function of time. Over 90% of salicylate initially encapsulated in pCBMA-2 hydrogel was released within 10 minutes. pCBMA-1 C2 SA hydrogel showed a controlled release of salicylate,

releasing 90% of salicylate over 8 days. Due to their strong hydration and excellent biocompatibility, hydrogels have been extensively studied as controlled release drug delivery vectors. The drug release from hydrogels is mainly controlled by diffusion or breaking of chemical linkages.*[1, 2]* In diffusion-controlled hydrogels, macromolecules encapsulated in the hydrogels and are gradually released, whereas small hydrophilic drugs are quickly released due to their high diffusion coefficient. Chemically-controlled release of drugs from hydrogels requires the hydrolytic or enzymatic cleavage of the polymer backbone or the linkage between the side chain of polymer matrices and the tethered drug. In pCBMA-1 C2 SA hydrogels, salicylate is bound to the hydrogel as its counter ion. Thus, the release of salicylate from pCBMA-1 C2 SA in water is controlled by the hydrolysis of the ester bond between carboxybetaine and the leaving group. In contrast, unrestricted salicylates that were encapsulated in pCBMA-2 hydrogel were rapidly released.

[1] Lin, CC.; Metters, AT. *Advanced Drug Delivery Reviews* **2006**, *58*, 1379.
[2] Peppas, NA.; Hilt, JZ.; Khademhosseini, A.; Langer, R.; *Advanced Materials* **2006**, *18*, 1345.

Figure S-1. ^{1}H NMR of CBMA-1 C2 SA at 300 MHz, D_2O.

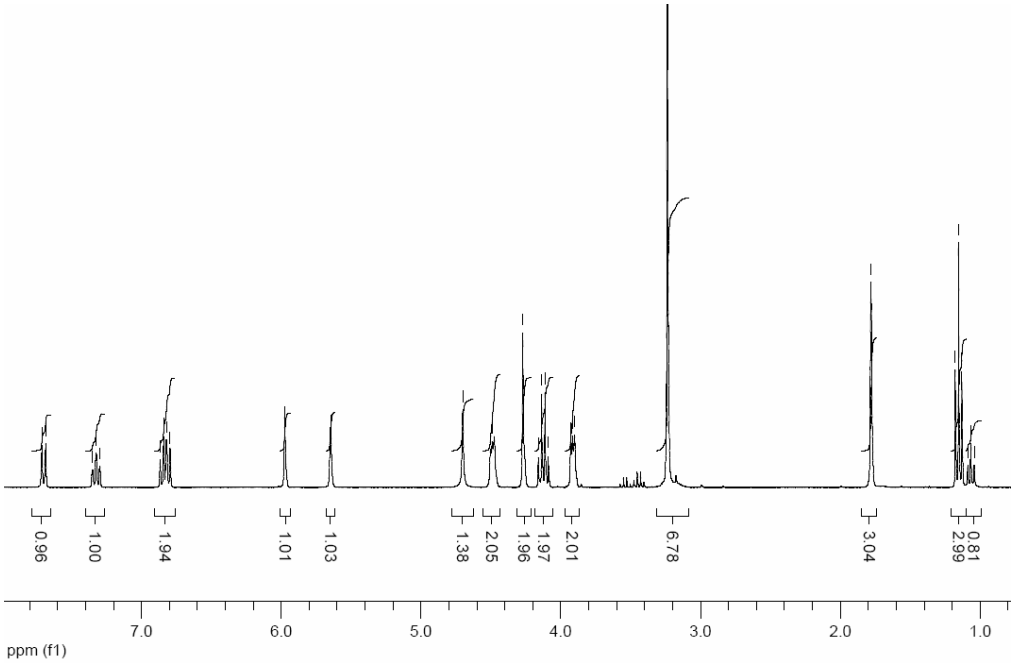

Figure S-2. *In vitro* SA release from pCBMA-2 hydrogels (▲) and pCBMA-1 C2 SA hydrogels (■) as a function of time at 37°C in water. SA release was measured by a high performance liquid chromatography (HPLC). The total loaded salicylate in pCBMA-2 and pCBMA-1 C2 SA were 2.9mg/disc and 1.7mg/disc. The results are averaged from three replicates.