ΔIn Vivo Chemistry and Implantable Biomaterial for Targeting Therapeutics

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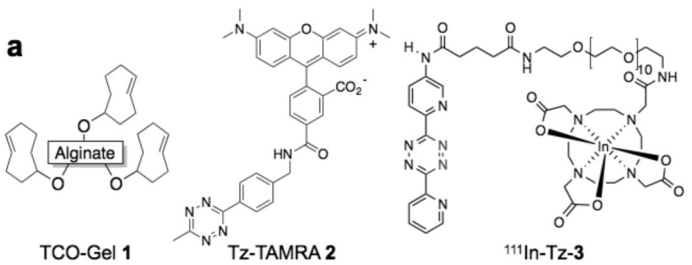
Purpose: This study is designed to evaluate a novel drug delivery system aimed to optimize local drug concentrations of systemic medications. Prior studies have shown that through in vivo chemistry a systemic molecular payload containing tetrazine (Tz) can be localized to an area previously tagged with an antibody modified with trans-cyclooctenes molecules (TCO). To explore the potential of this method for the treatment of focal orthopaedic infections, we set out to quantify the size of the molecular payload that can be delivered and to establish that a molecular payload could be delivered in vivo to TCO covalently bonded to an alginate gel.

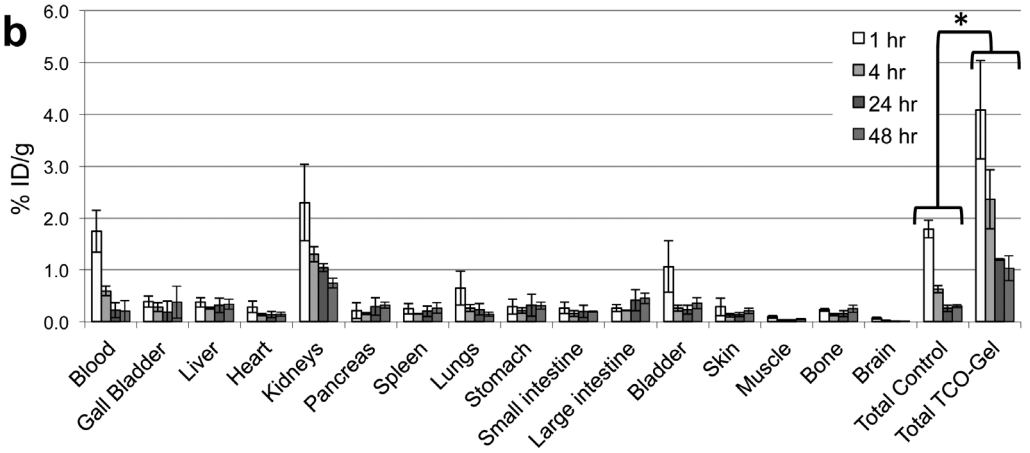


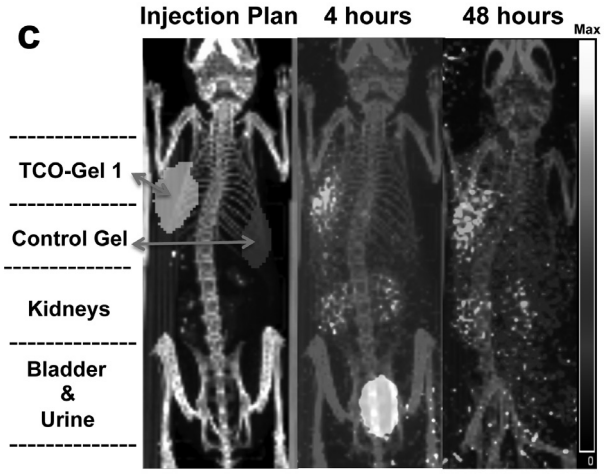
Methods: Investigators synthesized the desired molecules through organic chemistry: a modified alginate (TCO-Gel **1)**, a fluorescent probe (Tz-TAMRA **2**), and a radioactive probe (^{111}In-Tz-**3**) (Figure a). To quantify the maximum amount of a molecular payload that can be delivered to the biomaterial, TCO-Gel **1** was exposed to fluorescent Tz-TAMRA **2** (ex. [excitation] 555 nm, em. [emission] 580 nm), and then the supernatant was removed. Fluorescence images of the supernatant were taken and fluorescence outputs were quantified digitally. Control alginate was treated identically and compared. After Institutional Animal Care and Use Committee approval, in vivo biodistribution studies were carried out by injecting either control or TCO-Gel **1** subcutaneously at each flank area of BALB/c mice. 3 to 4 hours later the subject received a tail vein injection of ^{111}In-Tz-**3** in normal saline (mean dose 1.63 MBq). Mice (n = 3) were euthanized at 1, 4, 24, and 48 hours. Organs, bodily fluids of interest, and gels were harvested and washed. Radioactivity was measured using a gamma counter, corrected for isotope decay and presented as percent injected dose per gram (%ID/g). A similar approach was used for in vivo imaging studies, except with a larger dose of ^{111}In-Tz-**2** (38.8 MBq). At 4 and 48 hours, the mouse was anesthetized and imaged with a SPECT (single photon emission computed tomography)/CT imaging station.

Results: Our in vitro studies revealed that a molecular payload of 29.9 nmoles can be delivered per mL of 2.0% (w/v) alginate solution in ddH_2O containing Dulbecco's PBS (phosphate-buffered saline) and calcium sulfate ions as cross-linkers. Our in vivo studies revealed that we can deliver more than 4% ID/g to the subcutaneous space of a murine model at 1 hour compared to < 0.3% ID/g delivered to musculoskeletal areas. The radioactivity level is maintained above 1% ID/g at the TCO-Gel **1** even after 48 hours. The difference between the groups is statistically significant at all time points (Figures b and c).

Δ OTA Grant

- The FDA has not cleared this drug and/or medical device for the use described in this presentation (i.e., the drug or medical device is being discussed for an "off label" use). For full information, refer to page 600.





Conclusions: We present a simple and modular method to modify a biomaterial with small molecules after in vivo implantation. This approach enables a hydrogel to enhance the spatial location of systemic small molecules through in vivo delivery by an order of magnitude. Further studies are required to assess this methodology with therapeutics molecules that are relevant to orthopaedic challenges.

See pages 99 - 147 for financial disclosure information.