Click Chemistry and Implantable Biomaterial for Local Enhancement of Systemic Small Molecules

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Purpose: This study presents a novel drug delivery platform aimed to optimize the local concentrations of systemic drugs through click chemistry and an implantable biomaterial. As a therapeutic proof of concept, we apply our discoveries to the construction of an antibiotic agent based on vancomycin.

Background: Click chemistry technology allows two reaction partners to interact exclusively with each other and ignore other possible reagents present in nature. Our published studies have shown that the local concentration of a systemic radioprobe containing a click chemistry reagent can be increased by ten times to an area previously injected with an implantable biomaterial (alginate) modified with its click reaction partner (trans-cyclooctenes, TCO) (Ref. 1). Now we present a platform that does not only lead to localization but to subsequent release at the desired location (Fig. 1).

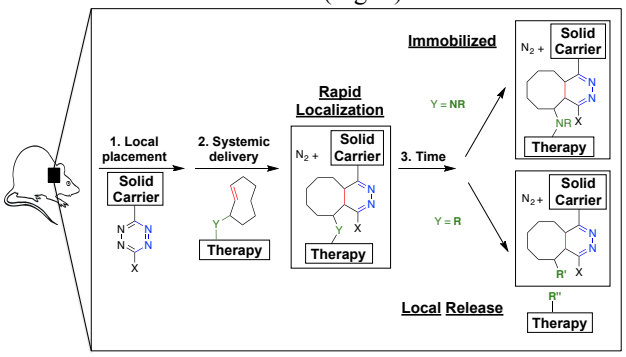

Figure 1. Hypothesis for approach

Methods: We chemically synthesized the following molecules: an alginate modified with tetrazine (Tz-gel), as well as multiple agents modified with *trans*-cyclooctene (TCO): a releasable fluorophore (TCO-R-F), a non-releasable flourophore (TCO-NR-F) and a releasable vancomycin (TCO-R-Vanco). *Animal Model*: After IACUC approval, *in-vivo* real-time biodistribution studies of fluorescence were carried out in nu/nu mice (n=2 per condition) by injecting either nothing or a type of alginate (control vs Tz-Gel). Then subjects received a tail-vein injection of TCO-R-F or TCO-NR-F. The negative controls were: 1. No gels with TCO-R-F (Negative control, mouse 1); 2. control alginate with TCO-R-F (Gel control, mouse 4). The two experimental groups were Tz-gel and either TCO-R-F (Released protocol, mouse 2) or TCO-NR-F (Immobilized protocol, mouse 3). Fluorescence was measured with an IVIS Spectrum (Perkin Elmer, MA) and reported in radiance. *Minimum Inhibitory Concentration (MIC) of Releasable Vancomycin*: We mixed Vancomycin or TCO-R-Vanco with either a regular alginate gel or Tz-gel overnight. The following day luminescent methicillin sensitive Staph. aureus (MSSA, Xen 29, Perkin Elmer, MA) in broth were added to the mixture and allowed to grow for 24 hours (n=3). Then luminescence was measured with an IVIS Spectrum (Perkin Elmer, MA) and reported in radiance.

Protocol:	**Neg. control**	**Released**	**Immobilized**	**Gel control**
Mouse:	**1**	**2**	**3**	**4**
Gel Implant:	None	Tz-gel	Tz-gel	Alg
Fluorophore:	TCO-**R**-F	TCO-**R**-F	TCO-**NR**-F	TCO-**R**-F

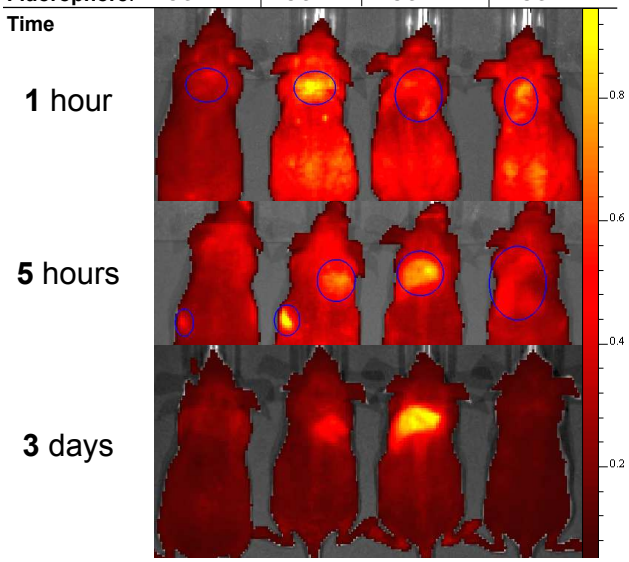

Figure 2. *in-vivo* proof of concept of platform

Results: *Animal Model*: The *released* protocol showed the largest amount of radiance at 1hr then decreased over 5 hours. The *immobilized* protocol showed a large amount of radiance at 1 hr, 5 hr and 3 days. The gel control showed a small amount of radiance at 1hr that decreased promptly. The negative control did not show any focal radiance. *MIC of releasable vancomycin*: The MIC for vanco was 0.5 nmoles/mL (not shown), for TCO-R-vanco with Tz-gel was 2.0, for TCO-R-vanco with control alginate >4.0 (units: nmoles/mL).

Protocol	Gel Implant	Antibiotic	Concentration (nmoles/mL) 2	1	0.5
Experimental group	Tz-gel	**TCO-R**-Vanco			
Negative **control**	Alginate	**TCO-R**-Vanco			

Figure 3. MIC of luminescent MSSA

Conclusions: We present a system to chemically modify an implanted biomaterial with small molecules. This approach enables: 1. systemic small molecules to be immobilized at a pre-implanted material and then locally released through a cascade reaction in a mouse model. 2. a modified antibiotic construct that releases vancomycin and inhibits growth of luminescent bacteria (MSSA).

References: 1. Mejia Oneto JM. Acta Biomat. 2014, doi: 10.1016/j.actbio.2014.08.019