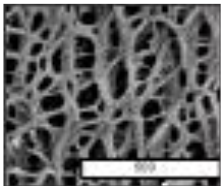
Kevin Mchugh, Department of Bioengineering at Rice University
Leveraging dynamic covalent bonding to create a LA Ganfeborole formulation: PK and efficacy for TB

"We see [dynamic covalent bonding] as a flexible tool"

Overview of hydrogels

Hydrogel composition and nanostructure.



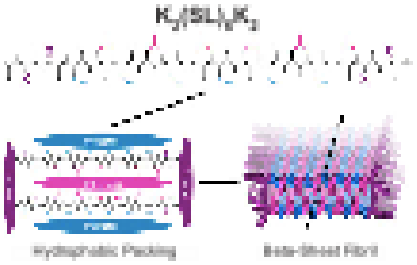
- Up to 99% water and 1% material of interest.
- Natural (peptides) or synthetic (polymers) materials can be used.
- Fibrous nanostructure enables the potential to retain bioactive agents. (hydrogel encapsulates the drug of interest).

Application for TB treatment.

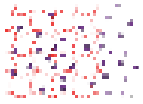
- We are using interesting chemistry to develop a LA hydrogel formulation of Ganfeborole [GFB], a TB drug candidate that requires QD oral dosing for >8w.

Engineering peptide hydrogels

Multidomain peptide (MDP) primary structure.



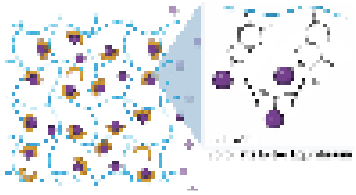
- The "right" amino acid sequence self-assembles into hydrogel-forming nanofibers that can load and release small-molecule drugs.
- General MDP design.
  - Core: Alternating hydrophilic and hydrophobic residues drive Beta-sheet formation.
  - Termini: A pair of charged residues enables non-covalent cross-linking of nanofibers.
- Key MDP characteristics.

Valuable characteristics	Limitation
Biocompatible Biodegradable (enzymatic degradation to aa). Injectable (via 25G needle or smaller) Mild preparation conditions (aq. salt solution) Easy and inexpensive to produce	Small-molecule drugs are rapidly released over hours via diffusion or weak electrostatic forces. 

  - MDPs are injectable due to shear thinning and recovery. The thixotropic material "liquifies" under shear stress (i.e., while passing through a small-bore needle) and "re-gels" on the other side (i.e., once the shear stress is removed).

Drug-nanofiber interactions can tailor drug release.

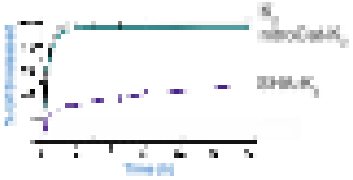
- Various mechanisms bond the drug to the hydrogel (e.g., covalent linkage, electrostatic interaction, and hydrophobic association).
- Dynamic covalent bonding is a "traceless" mechanism with potential for long-lasting release of bioactive drug.
  - Specific chemistry forms a reversible, slightly energetically favored covalent bond between the unmodified drug and hydrogel.
  - The equilibrium between bound and soluble drug is shifted towards the bound state.
  - Only the smaller proportion of soluble drug is free to diffuse out, extending the release.



Leveraging dynamic covalent bonding for LA/ER formulations

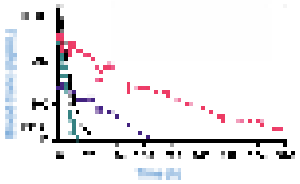
MDP modification with boronate esters – catechol (Cat-K_2), nitrocatechol (nitroCat-K_2), or salicylhydroxamic acid (SHA-K_2) hydrogels.

- Functionalized MDPs retain key MDP functions: Self-assembly; Beta-sheet formation; Hydrogel integrity; and Shear recovery.
- Boronate ester groups form dynamic covalent bonds with boronic acid in BA-containing drugs (i.e., GFB), which could extend drug release.
- *in vitro* studies confirm delayed release of small-molecule BA drugs mixed with modified (Cat-, nitroCat-, SHA-K_2) vs unmodifed MDPs (K_2).
  - SHA modification significantly delayed the release of all four drugs (Ixazomib, Bortezomib, Tavaborole, and GFB).
  - Only SHA-K_2 significantly delayed GFB release and advanced to *in vivo* studies.



PK and efficacy of GFB+hydrogel SC in BALB/c mice (TB test case in collaboration with Eric Nuermberger).

- Single-dose PK in uninfected mice (SHA-K_2+GFB SC vs GFB SC).
  - SHA-K_2 extended the release of GFB.
  - SHA-K_2+GFB (600mcg) sustained conc > ED50 for ≥3w; Increased AUC 2.8-fold.



- Single-dose efficacy in mice with acute Mtb infection (SHA-E_2+GFB SC vs GFB SC x1 vs QD oral GFB x2w).
  - SHA-E_2 outperformed the equivalent QD oral dose and single soluble injection.
  - SHA-E_2+GFB suppressed Mtb growth for 2w (Growth at 3w due to depot depletion).

Expanding drug flexibility

Drug modification with phenylboronic acid (PBA).

- Enables dynamic covalent bonding between functionalized MDPs and drugs that do not contain BA (Only five BA-containing drugs are FDA-approved).
- Modified hydrogels extend the release of PBA-modified small molecules and biologics; bioactivity is not significantly altered.

Summary

- **Developing a library of functionalized hydrogels** capable of dynamic covalent bonding with BAs for extended release.
- **Demonstrated extended release** of BA-containing drugs and PBA-modified drugs and proteins**.**
- **Demonstrated preclinical efficacy** of LAI GFB for TB treatment.
- **Future directions:** Chronic infection studies; Optimize SHA-modified hydrogel; Model PK in humans; Combination therapy with new small-molecule drugs and protein therapeutics.