Characterization of Multi-Domain Peptide with mimetic sequences

Ayaka Hatano[1,2], Nicole Carrejo[3], Jeffrey D. Hartgerink[3,4]

[1]Department of Applied Chemistry, Tokyo Institute of Technology, Tokyo, Japan, [2]Nakatani RIES: Research & International Experiences for Students,
[3]Department of Chemistry, Rice University, Houston, TX, USA, [4]Department of Bioengineering, Rice University, Houston, TX, USA

Background

About MDP

Multidomain peptides (MDPs) are capable of self-assembling to form hydrogels[1].

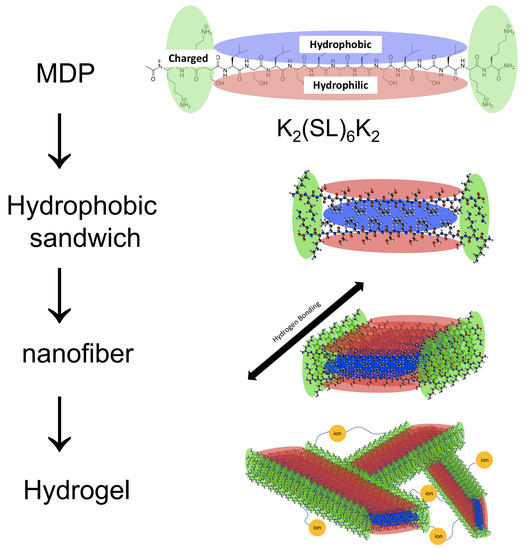


Figure 1. MDPs form hydrogel by self-assembling.

Mimic's function

- Composed of short sequences of amino acids mimicking growth factors
- Deliver growth factors or drugs
- Promote cellular function
  - Cell growth
  - Cell proliferation
  - Cell differentiation

Problem & Solution

Problem

Mimics were used in a mouse diabetic wound healing model.

- No differences on wound closure between K2 and K2 mimics
- A large immune response of K2 that covers up the effect of the mimics

solution

We can instead use E2 mimic.

- E2 does not have a large immune response.
- The response we see will be due to the mimic.

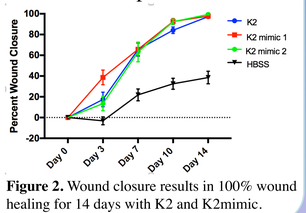
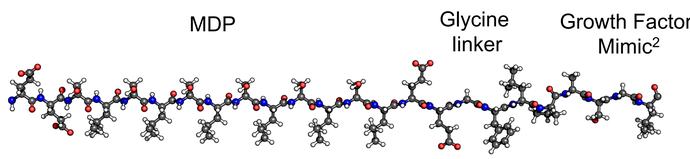
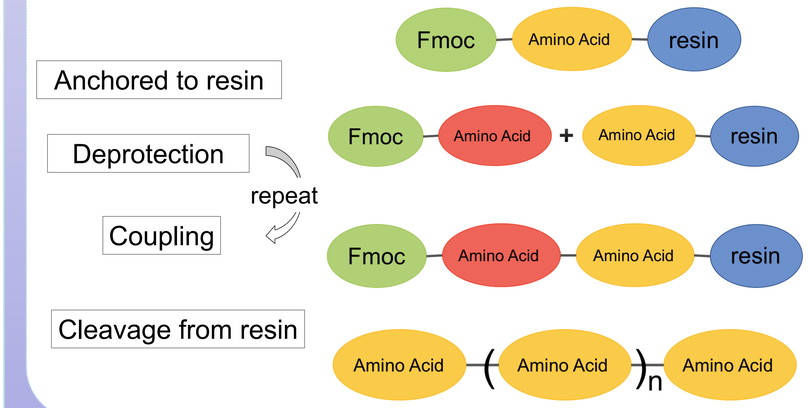


Figure 2. Wound closure results in 100% wound healing for 14 days with K2 and K2mimic.

Peptide Synthesis of E2 mimic

E E S L S L S L S L S L E E G F L P A S G L





E2 mimic was manually synthesized using solid-phase peptide synthesis with an Fmoc protected amino acids.

① The C-terminus must be anchored to a resin (beads).
② The amino acid is deprotected confirmed by Ninhydrin test.
③ Coupling connects the next amino acid by amide bonding (peptide bonding).
④ Repeat synthesizing one by one
⑤ A free peptide is cleaved from the resin.
⑥ Dialyze it to remove impurities.
⑦ Dry it into the powder
⑧ Adding HBSS + sucrose results in a hydrogel.

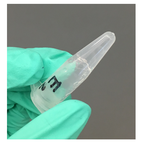


Figure 3. The picture of hydrogel

Characterization of K2, K2 mimic, E2 and E2 mimic

Mass Spectrometry

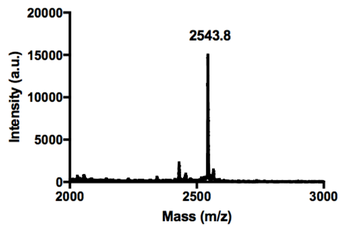


Figure 4. MALDI mass spec results in a peak at 2543.8. The mass of what the peptide is 2519.8. The gap is expected to be caused by the attachment of Na^+.

Circular Dichroism Spectroscopy

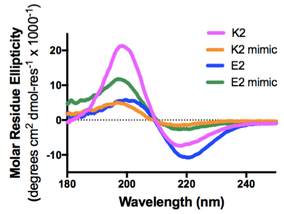


Figure 5. Circular dichroism spectroscopy shows the characteristic peaks for β-sheet secondary structure including a minimum near 216 nm and a maximum near 195 nm.

Infrared Spectroscopy

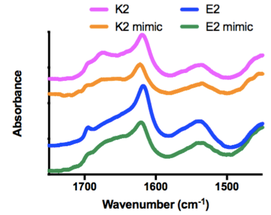


Figure 6. Infrared spectroscopy shows a peak near 1620 cm^{-1} and a peak near 1695 cm^{-1} indicating anti-parallel β-sheet secondary structure.

Rheology

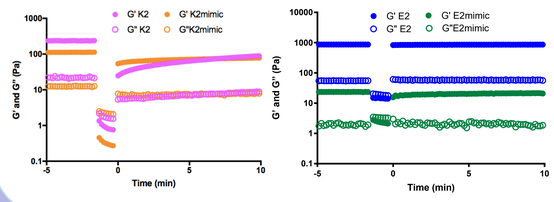


Figure 7. G' indicates the solid like properties of the hydrogel while G'' indicates the liquid like. The ratio of G' and G'' must be at least 10. At first, the G' is larger than the G''. Then a shearing force is applied that causes the hydrogel to turn into a liquid as shown by the inversion of G' and G''. After the shearing force is removed at time=0 min, the hydrogel is able to recover.

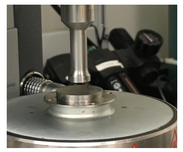


Figure 8. The picture of Rheology

Conclusions

- Successfully synthesized E2 mimic peptide as shown by MALDI (Figure 4).
- Confirmed E2 mimic has a β-sheet secondary structure using CD and IR (Figure 5,6).
- Determined that E2 mimic forms a hydrogel by Rheology (Figure 7).

Future work

Future work will include subcutaneous injections of E2 mimic (Figure 9). When K2 is injected into mice it is densely infiltrated by cells (Figure 10). However, when E2 is injected into mice, no cells are seen in the hydrogel (Figure 10).
If subcutaneous injections of E2 mimic result in pictures like K2, then we know that the mimic would be creating the bioactive response since E2 alone results in no cellular infiltration.

Figure 9. Cartoon of subcutaneous injections.

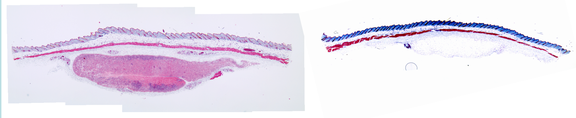


Figure 10. K2 (left) and E2 (right) subcutaneous injection tissue sections stained after removal from mouse.

Acknowledgement & References

This research project was conducted as part of the 2017 Nakatani RIES Fellowship for JP Students with support from the Nakatani Foundation. For more information on the Nakatani program, see http://nakatani-ries.rice.edu/. Special thanks to Prof. Jeffrey Hartgerink and Nicole Carrejo for their excellent research guidance and to Prof. Junichiro Kono, Sarah Phillips, Kenji Ogawa, and Utako Endo for their support and making this program possible.

1 Dong, H., et al. *J. Am. Chem. Soc.* **129**, 12468-12472 (2007).
2 Vaz, E. R. *et al. PLoS One* **10**, e0136116 (2015).