Click Chemistry Functionalizes Self-Assembling Peptide Hydrogels

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MOTIVATION

Self-assembling peptide (SAP) hydrogels provide a fibrous microenvironment to cells that mimics the structure of *in vivo* extracellular matrix (Fig. 1). This structure regulates cell adhesion, proliferation, migration, and drug response.

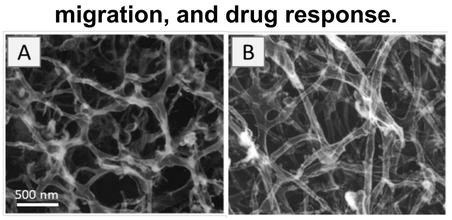


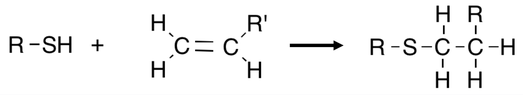
Figure 1. SAP hydrogel architecture. Transmission electron microscopy images show that **(A)** SAP hydrogel has a fibrous structure similar to **(B)** Type I collagen[1].

Functionalizing SAP during peptide synthesis:
Limits options
Increases costs
Prohibits spatial and temporal patterns

SAPs could be functionalized with:
Cellular adhesion ligands
Growth factors
Drugs
"Smart" biosensors

New methods for SAP functionalization are needed to circumvent these limitations and enable new scientific inquiries

Proposed Solution: Thiol-ene Click Chemistry
Efficient
Cytocompatible[2]
Initiated by UV light



METHODS

The following SAP sequences were synthesized for this study (Biomer Technology):

SAP Type	Sequence
KFE	(acetyl)-FKFEFKFE-$CONH_2$
KFE-RGD	(acetyl)-GRGDSP-GG-FKFEFKFE-CONH2
KFE-RDG	(acetyl)-GRDGSP-GG-FKFEFKFE-CONH2
KFE-Alloc	(alloc)-KGGFKFEFKFE-CONH2

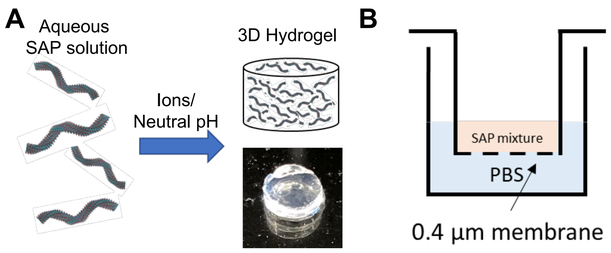


Figure 2. Schematic of SAP hydrogel formation. (A) In the presence of ionic solutions or neutral pH, SAPs assemble into fibers that form a 3D hydrogel. **(B)** SAP mixture is placed in a permeable cell culture insert and submerged in PBS or media for 30 minutes.

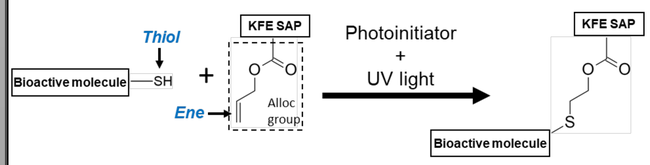


Figure 3. Schematic of the thiol-ene reaction. UV light can be used to covalently attach a molecule with a thiol group to a SAP molecule with an "ene"-containing group (e.g. an alloc group).

RESULTS

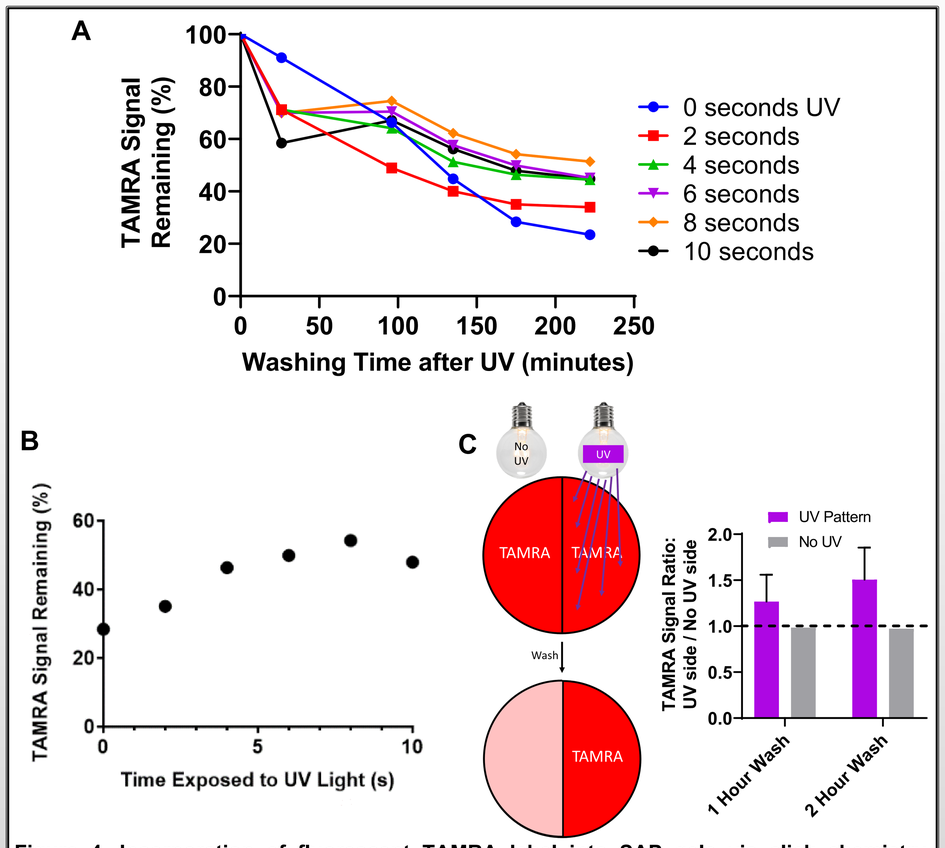


Figure 4. Incorporation of fluorescent TAMRA label into SAP gels via click chemistry. TAMRA fluorophore was functionalized with a free cysteine group and attached to SAP hydrogels via click chemistry. **(A)** Gels were exposed to UV light for different amounts of time and washed in PBS to remove unbound TAMRA. Fluorescence was measured at the center of the gel repeatedly. **(B)** Fraction of original TAMRA signal remaining after 3 hours of washing. **(C)** Mean ± SD of N=3 TAMRA gels exposed to UV on one half of the gel only and washed, compared to N=1 control gel that was not exposed to UV. TAMRA signal was measured on both sides and compared after 1 and 2 hours of washing away any unbound TAMRA.

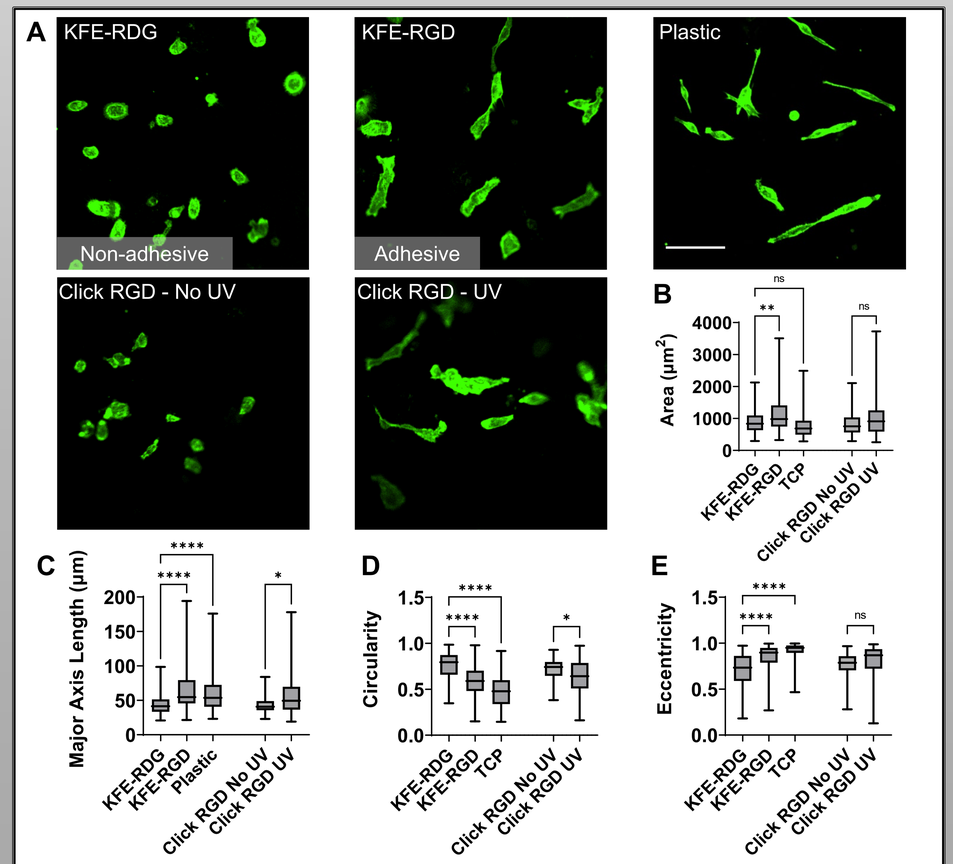


Figure 5. Incorporation of RGD into SAP gels via click chemistry promotes cancer cell spreading. (A) Representative images of HT1080 fibrosarcoma cells cultured for 48 hours on 2D SAP gels with non-adhesive KFE-RDG, adhesive RGD-KFE, tissue culture plastic, RGD incorporated with the thiol-ene reaction (click RGD) with and without UV initiation. Actin cytoskeleton labeled with fluorescent phalloidin. Scale bar = 100 µm. **(B-E)** Plots indicate maximum, minimum, median, and quartiles for the indicated cellular morphological measurements on each type of gel. N>75 cells per group.* p<0.05, ** p<0.01, **** p<0.0001 via one-way ANOVA with Tukey multiple comparisons post-test. Not all significances shown.

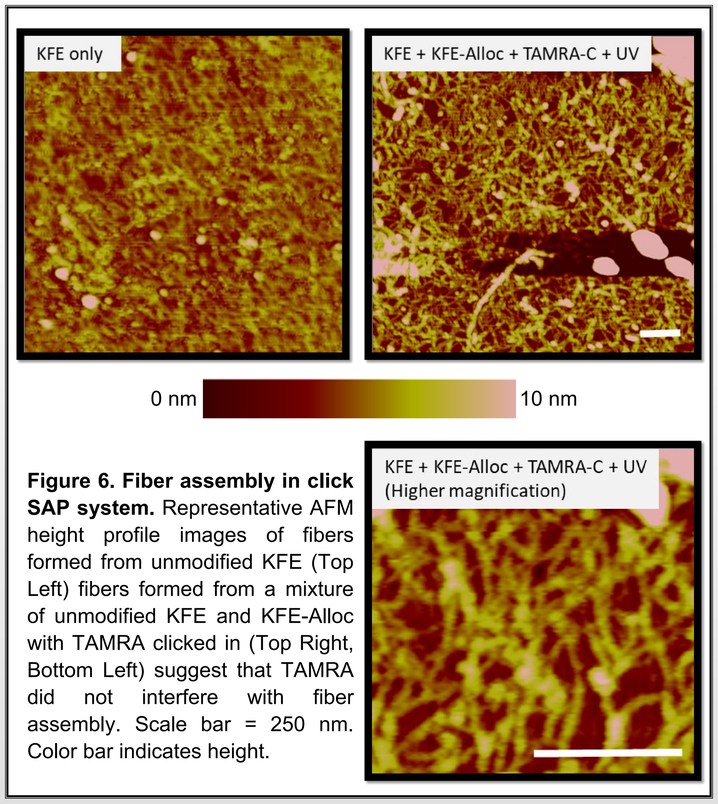


Figure 6. Fiber assembly in click SAP system. Representative AFM height profile images of fibers formed from unmodified KFE (Top Left) fibers formed from a mixture of unmodified KFE and KFE-Alloc with TAMRA clicked in (Top Right, Bottom Left) suggest that TAMRA did not interfere with fiber assembly. Scale bar = 250 nm. Color bar indicates height.

CONCLUSIONS

- **The thiol-ene click reaction is effective for binding and patterning fluorescent labels and cell-adhesive peptides in SAP hydrogels, without losing their biological activity.**
- **Modifying KFE SAP with an alloc group and binding additional molecules via click chemistry does not interfere with SAP fiber formation.**
- **In summary, a simple modification allows SAP gels to be functionalized by cytocompatible click chemistry, without interfering with the fibrous matrix structure.**

FUTURE DIRECTIONS

- **The ability to incorporate bioactive molecules in a 3D matrix via click chemistry will be tested by encapsulating cells within SAP hydrogels.**
- **Patterns of biochemical cues such as RGD in SAP gels will be generated using click chemistry to mimic those that occur *in vivo*.**
- **A smart fluorescent biosensor to detect MMP activity will be incorporated in the SAP gel using click chemistry.**

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REFERENCES

1. Sieminski et al. *J of Biom Mat Res* 2008
2. Sawicki & Kloxin. *Biomat Sci* 2014