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Self-assembly of Crown Ether/Peptide Conjugates for Biological Applications: The Role of Crown Ether Size in the Hydrogelator

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Introduction [1, 2]

This work presents a new design of dipeptide hydrogelator system based on crown ether, where, the applications of crown ether-based compounds on hydrogelation are very limited in biology. In this study, we developed crown-ether based hydrogels and their potential biomedical applications were explored by using phenylalanine amino acid [12] as supramolecular hydrogelators and we used these compounds for biological studies to discover the biocompatible material. These hydrogelators synthesized based on following postulates(i) the materials can be self-assembled into hydrogel if it has both a hydrophobic part FF-peptide and at N-terminal hydrophilic part crown ether which possesses a hydrophobic ring surrounding and a hydrophilic cavity, which allows them to form a hydrogel when attached with FF-peptide, and (ii) to investigate the effect of ring size and hydrophilicity of crown ethers on cell adhesion properties.

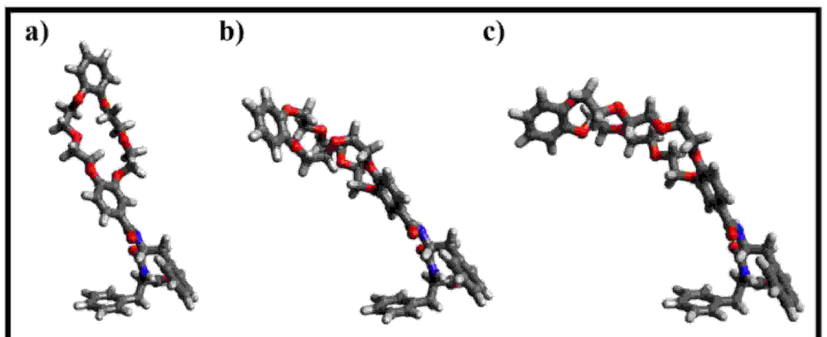

Fig. 1. Schematic representation for synthesis of crown Ether/Peptide conjugates; a) DB18C6FF, b) DB21C7FF, and c) DB24C8FF using solid phase peptide synthesis (SPPS) [3]

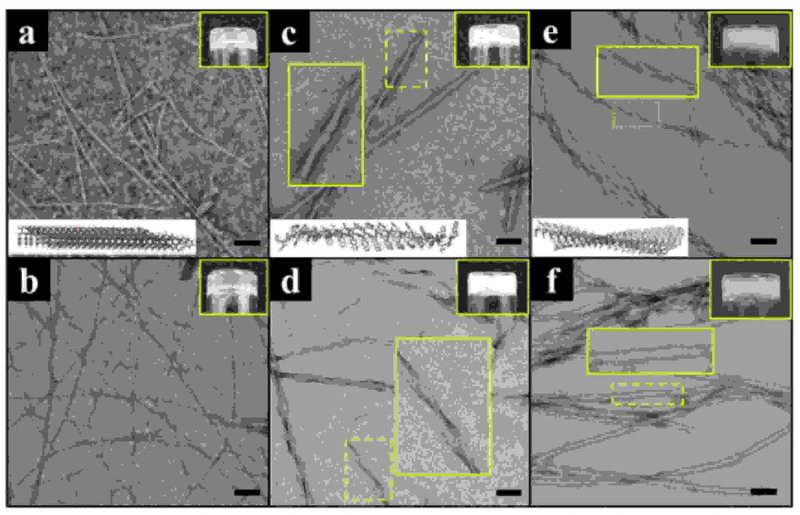

Fig. 2. Dissolving of crown-ethers dipeptides at a concentration of 2 wt% with a pH range from 12-7 in aqueous solution led to the formation of hydrogels. Moreover, the TEM analysis revealed that the hydrogels of a) $DB18C6F_LF_L$ and b) $DB18C6F_DF_D$ showed nanofibers with random morphology with almost the same diameter 11±1 nm but c) $DB21C7F_LF_L$, and e) $DB24C8F_LF_L$ showed left-handed twisted nanofibers with diameters 19±2 and 21±2 respectively. d) $DB21C7F_DF_D$ and f) $DB24C8F_DF_D$ showed right- handed twisted nanofibers with diameters 16±2 and 19±2 respectively (scale bar=100 nm).

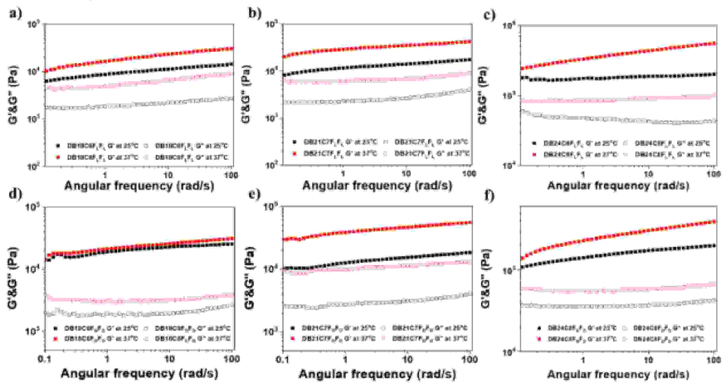

Fig. 3. The elastic properties of the hydrogels were determined by the rheological analysis, all hydrogels showed the storage modulus G′higher than loss modulus G″, the storage modulus are higher than $2x10^5$ Pa at 37°C, whereas, the minimum storage modulus which is necessary to support the mass of cell is around 100 Pa indicating that all hydrogels will be very useful for tissue engineering and biomedical applications..

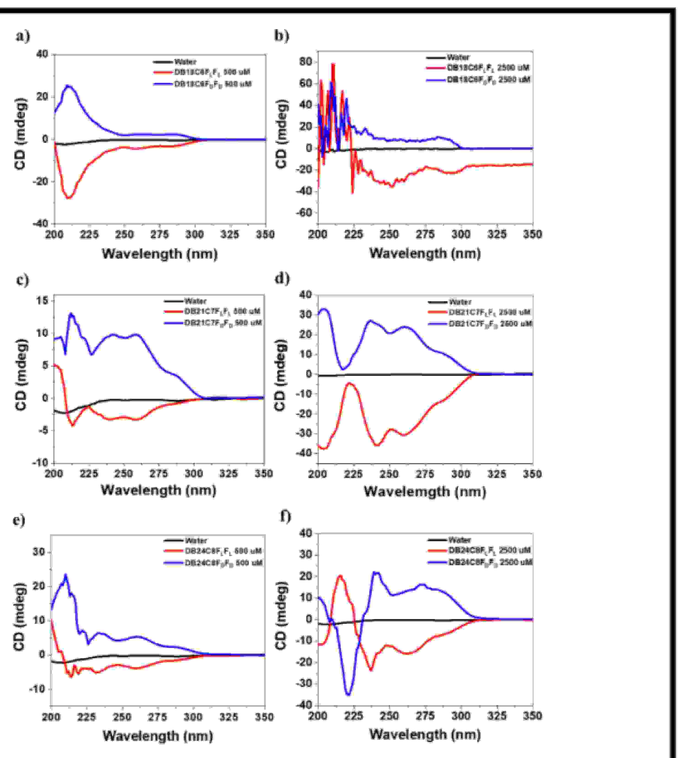

Fig. 4.Circular dichrosim study for a) DB18C6FF at 500 uM, b) DB18C6FF at 2500 uM, c) DB21C7FF at 500 uM, d) DB21C7FF at 2500 uM, e) DB24C8FF at 500 uM, and c) DB24C8FF at 2500 uM concentration to understand the secondary structures of the self-assembled peptides in water. Peptide DB18C6FF at the concentration of 500mM shows strong negative signal at 209 nm. In addition, a shoulder peak appreared at around 220 nm which is the indication of superhelical arrangement. The peptide also showed peaks in the range of 260 and 290 which is attributed to π-π* transition of crown ether aromatic groups perturbed by dissymmetric surrounding. D- form of DB18C6FF exhibited only sign-inverted signals under the same condition. The chiral helicity is completely collapsed at higher concentration (> 2500 mM) and only weak signals were noticed which is due to precipitation of the some monomers in the CD-cuvette. In the case of DB21C7FF and DB24C8FF the superhelical structures were maintain even at higher concentrations

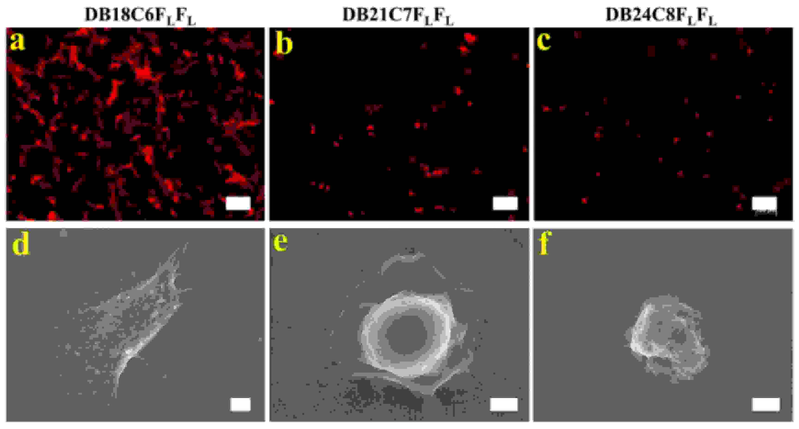

Fig. 5. Fluorescence microscopy under 10X magnification (a, b, and c) for $DB18C6F_LF_L$, $DB21C7F_LF_L$, and $DB24C8F_LF_L$ respectively of 3A6-RFP cells after culture for 24 h. (scale bar: 100 µm). (d, e, and f) SEM images of 3A6-RFP cells grown on hydrogel coated surfaces for 24 h for $DB18C6F_LF_L$, $DB21C7F_LF_L$, and $DB24C8F_LF_L$ respectively. (Scale bar of (d) = 3 µm; Scale bar = 1 µm).

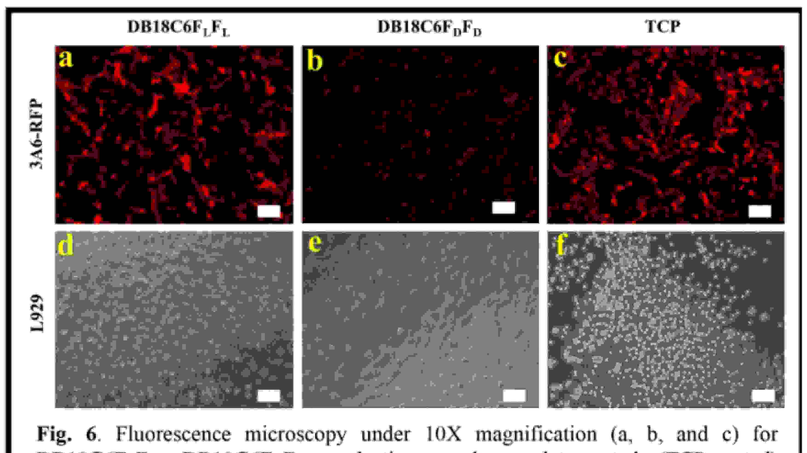

Fig. 6. Fluorescence microscopy under 10X magnification (a, b, and c) for $DB18C6F_LF_L$, $DB18C6F_DF_D$, and tissue culture plate-coated (TCP-coated) respectively of 3A6-RFP cells after culture for 24 h. Optical microscopy under 10X magnification (a, b, and c) for $DB18C6F_LF_L$, $DB18C6F_DF_D$, and tissue culture plate-non coated (TCP-non coated) respectively of L929 cells culture for 24 h. (scale bar: 100 µm).

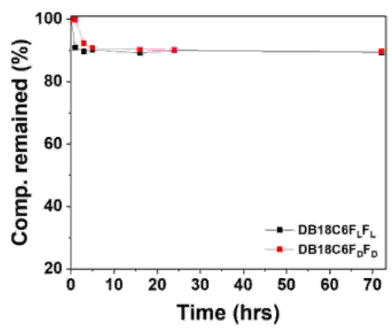

Fig. 7. The bio-stability of $DB18C6F_LF_L$ and $DB18C6F_DF_D$ hydrogelators through incubation with proteinase K, a powerful protease that hydrolyzes a wide range of peptide molecules, unexpected results from L- enantiomer gave bio-stable resistance to proteinase K which is quite different from what is published before. The result suggests that $DB18C6F_LF_L$ have a longer in vivo lifetime which is useful for using in the in vivo study, whereas, both $DB18C6F_LF_L$ and DB18C6FDFD have similar bio-stability toward proteinase K (around 10% degradation) within 72 hr.

Conclusion: The crown peptides hydrogels showed the size and morphological dependent cell adhesion properties. $DB18C6F_LF_L$ is amongst the smallest peptides used in this study which shows an enhanced cell adhesion and significant cellular viability for 3A6RPF cells and L929 cells with almost similar result in L- and D-form, remaining four crown dipeptide hydrogels $DB21C7F_LF_L$, $DB21C7F_DF_D$, $DB24C8F_LF_L$, and $DB24C8F_DF_D$ cells grown on these substrates are failed to attach and displayed anti adhesion like properties.

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