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AUTHOR(S)

Robert Murphy, Elena Bobbi, Fernando CS de Oliveira, Sally-Ann Cryan, Andreas Heise

Gelating polypeptide matrices based on the difunctional N-carboxyanhydride (NCA) diaminopimelic acid cross-linker

Robert D. Murphy,[1] Elena Bobbi,[1] Fernando C. S. de Oliveira,[1] Sally-Ann Cryan[2,3,4] and Andreas Heise[1,4,5*]

[1]Department of Chemistry, Royal College of Surgeons in Ireland, Dublin 2, Ireland. [2]Drug Delivery & Advanced Materials Team, School of Pharmacy, RCSI, Dublin 2, Ireland. [3]Trinity Centre for Bioengineering, Trinity College Dublin (TCD), Dublin 2, Ireland. [4]Centre for Research in Medical Devices (CURAM), RCSI, Dublin 2 and National University of Ireland, Galway, Ireland. [5]Advanced Materials and Bioengineering Research Centre (AMBER) RCSI and TCD, Dublin 2, Ireland.

Correspondence to: Andreas Heise (E-mail: *andreasheise@rcsi.ie*)

((Additional Supporting Information may be found in the online version of this article.))

ABSTRACT

This work reports the synthesis of a new difunctional *N*-carboxyanhydride (NCA) monomer, namely diaminopimelic acid (DAP), and its use in the one-pot preparation of an organogelating copolypeptide. The organogel is formed *in situ* through ring opening polymerization (ROP) of DAP NCA from helical poly(ε-carbobenzyloxy-L-lysine) (PZLL) blocks in a mixture of DMF/chloroform. Gelation occurs by immobilizing the solvent through core crosslinking and are stabilized through physical intermolecular conformations. After removal of the carbobenzyloxy (cbz or Z) protecting groups, the network remains intact in exceedingly high aqueous concentrations (99.5%). FTIR is used to characterize the secondary structure, revealing the conformational arrangements that contributed to these stabilized gel networks with their relative mechanical properties determined via real-time rheological assays. DAP core crosslink of the random coil forming polypeptoid poly(sarcosine) (PSar), is also resulting in networks but is devoid of any stabilized physical interactions, thus yielding significantly weaker gels as a result.

INTRODUCTION

Gelators are used to fabricate an important class of three-dimensional networks with a range of different physico-chemical domains. Composed of polymeric sequences that bear permeable molecular crosslinking junctions, these materials function as a swellable matrix permitting the uptake and encapsulation of water or organic solvents. Polymeric hydrogels are a particularly interesting class of mimetic matrices due to their biological relevance.[1,2] They are composed of hydrophilic regions which are capable of entrapping high concentrations of water with an interconnected polymeric network analogous to that of the extracellular matrix, rendering them suitable as augmented biomaterials.[3,4] Macromolecular engineering inspired by native protein interactions has led to synthetic polypeptide based hydrogels that can be modulated by optimizing the chemistry of amino acid side groups.[5] The inherent self-assembly of polypeptide amphiphiles has been explored by many groups as a basis for gelation. Native amino acids have been utilized as polymeric blocks in these supramolecular gelators, contributing to material with tailored mechanical properties due to control of conformational assemblies.[6,7] The physical bonding contributing to gelation usually emanates from the polypeptide chain

composition via electrostatic interactions,[8] hydrophobic interactions,[9] hydrogen bonding,[10] π–π stacking,[11] and van der Waals forces.[12] The resulting hydrogels possess relatively moderate mechanical strength in addition to unique properties as a consequence of their reversible non-covalent bonding character. In chemically crosslinked hydrogels, the framework consists of covalent networks formed through stepwise orthogonal chemical ligation methods. A number of these reactions have been used in the generation of polypeptide organogels or hydrogels such as thiol-ene click,[13,14] schiff-base,[15] enzyme induced crosslinking,[16,17] radical polymerization[18] and the recently utilized triazolinedione (TAD) click reaction.[19, 20]

Covalent crosslinks evident in nature are limited to the spontaneously occurring disulphide bond of native cystine residues.[21] Cystine, the endogenous dimerized version of oxidized cysteine, is the only difunctional amino acid found in ribosomal derived proteins. Therefore in polypeptide synthesis, it represents the most common difunctional amino acid feedstock that has been used in NCA preparation and synthesis.[22- 24] In particular, cystine NCA has been used in the development of a series of multifunctional polypeptides known as core crosslinked stars (CCS) or nanogels, which can be successfully designed using this crosslinker in the arm-first method.[25,26] Chen and coworkers have reported the use of a PEG-NH_2 initiated copolymerization of γ-benzyl-L-glutamate (BLG) NCA or ε-carbobenzyloxy-L-lysine (ZLL) NCA with L-cystine NCA, which formed nanogels post-deprotection.[27] The nanogels demonstrated a promising release profile of doxorubicin (DOX) molecular cargo in both reductive and acidic conditions. A more recent example of the monomers' use in material design was reported by Jing *et al.*[28] A novel near infrared (NIR) cyanine dye was loaded into a cystine containing copolypeptide nanogel, with a reduction stimulus inducing the disassembly of the nanogel and promoting the release of the hydrophobic cyanine dye inside HepG2 carcinoma cells.

Herein, we propose a one-pot approach to synthesize organogels based on a core crosslinked copolypeptide and its conversion to mechanically robust hydrogels using a novel difunctional amino acid NCA derived from diaminopimelic acid (DAP). Diaminopimelic acid (DAP) is a non-proteinogenic amino acid which is an essential constituent of the peptide linkages that form the cell wall of certain bacteria.[29] DAP is a vitally important intermediate in the biosynthesis of lysine within bacterial organisms, particularly in gram-negative species.[30] With its structure being the analogous epsilon-carboxy derivative, the pathway for conversion to lysine is only native to that of bacteria, due to the presence of non-mammalian enzymes.[31] As such DAP represents an easily sourced, naturally occurring amino acid which holds the potential to be utilized as an alternative crosslinker in the development of biomaterials such as hydrogels or micelles. Two separate polymer sequences composed of the random coil forming polypeptoid poly(sarcosine) and the α-helical polypeptide poly(Z-L-lysine) were initially synthesized and then chain extended via ROP of the DAP monomer, forming core crosslinked networks and stiff organogels. A real time gelation profile of the materials was determined via rheological assays, demonstrating the emergence of enhanced mechanical properties during the course of the DAP NCA polymerization with each respective polymer. Acid hydrolysis of Z protecting groups was conducted to assess the uptake in aqueous conditions, which afforded mechanically stable hydrogels. To the best of our knowledge, this is the first reported case of the synthesis and use of the difunctional DAP NCA monomer in the development of polymeric materials.

EXPERIMENTAL

Materials

All chemicals were obtained from Sigma-Aldrich unless otherwise stated. DL-diaminopimelic acid was purchased from Fluorochem. The NCAs of ε-carbobenzyloxy-L-lysine and sarcosine were

synthesized with a slight deviation of literatures procedures (detailed in Supporting Information).

Methods

^{1}H-NMR spectra were recorded on a Bruker Avance 400 (400 MHz) spectrometer at room temperature with TFA-d as a solvent. Attenuated total reflection (ATR) FTIR was recorded using a Thermo Scientific iS10 spectrometer in the region of 4000–400 cm^{-1}. A background measurement was initially performed before analyzing the sample. Sixteen scans were completed using a resolution of 2 cm^{-1}. Size exclusion chromatography (SEC) was conducted in hexafluoroisopropanol (HFIP) using an PSS SECurity GPC system equipped with a PFG 7 µm 8 × 50 mm pre-column, a PSS 100 Å, 7µm 8 × 300 mm and a PSS 1000 Å, 7µm 8 × 300 mm column in series and a differential refractive index (RI) detector at a flow rate of 1.0 mL min-1. The systems were calibrated against Agilent Easi-Vial linear poly(methyl methacrylate) (PMMA) standards and analyzed by the software package PSS winGPC UniChrom. Rheological measurements of hydrogels were completed on a MCR 301 digital rheometer (Anton Paar). All experiments were conducted at room temperature using a conical plate (CP50-1, Anton Paar) consisting of a 50 mm diameter geometry and a gap length of 0.1 mm. The use of a protective hood was employed to prevent evaporation. For the real-time gelation study, the solution (for P1:PZLL + DAP NCA; for P2:PSar + DAP NCA or for P3: allylamine + ZLL NCA + DAP NCA) was placed onto the rheometer plate and analyzed immediately using the 'fast moving profile'. The measurements were carried out at 20 °C and were also under a protective hood to prevent evaporation.

Preparation of diaminopimelic acid NCA

DL-diaminopimelic acid (4 g, 21.03 mmol) and α-pinene (5.73 g, 42.06 mmol) were suspended in 130 mL of anhydrous THF in a three-neck flask and heated to reflux. Then triphosgene (4.99 g, 16.82 mmol) dissolved in 50 mL anhydrous THF was added dropwise. The mixture was allowed to stir for 24 hours at which point a clear solution was observed. The solution was bubbled with nitrogen for one hour then filtered and reduced under vacuum to yield an oil. After multiple precipitations in hexane an off yellow oil was obtained (2.3 g, yield 45%). ^{1}H-NMR (Figure 1)

Synthesis of poly-Z-L-lysine-b-poly-diaminopimelic acid

The NCA of ε-carbobenzyloxy-L-lysine (430 mg, 1.40 mmol) was dissolved in a 25 mL mixture of anhydrous $CHCl_3$ and DMF (5:1) in a Schlenk flask under a N_2 atmosphere. The flask was then placed in a thermostatically controlled water bath containing 5M NaCl at 0°C. Allylamine (1.34 mg, 1.00 × 10-2 mmol) was solubilized in 2.5 mL of anhydrous $CHCl_3$ and added via syringe to the Schlenk flask. The flask was evacuated under vacuum to liberate the CO_2 generated, with stirring allowed for 18 hours at 0°C. FTIR was used to confirm total consumption of the ZLL NCA monomer. The NCA of diaminopimelic acid (85.01 mg, 3.51×10^{-1} mmol) was dissolved in 2.5 mL of DMF and quickly added via syringe to the Schlenk flask. The solution was allowed to stir at 0°C until full conversion of the DAP NCA monomer was confirmed by FTIR. The gelled polymer was then precipitated into excess diethyl ether thrice and dried in a vacuum oven to afford the polymer (370 mg, yield 82%). 1H-NMR (Figure S6) *poly(sarcosine)-*b*-poly(diaminopimelic acid) was prepared by substituting ZLL NCA for sarcosine NCA, while in the random copolymer both NCAs were mixed before adding in the allylamine initiator.

Deprotection of poly(Z-L-lysine)-b-poly(diaminopimelic acid)

The dried polymer (350 mg) was then dissolved in 10 mL trifluoroacetic acid and allowed to stir until fully dissolved. Then, 2 mL of HBr (33 wt% in acetic acid) was added drop wise to the solution in a six-fold molar excess (with respect to ε-carbobenzyloxy-L-lysine protecting groups) and the solution was allowed to stir for 18

hours. The polymer was precipitated thrice into diethyl ether (40 mL) and centrifuged. After a final wash with diethyl ether, the material was dried under vacuum in a desiccator. The dried polymer was then immersed in deionized water at which point swelling was observed. The pH was adjusted to 7.5 using NaOH and dialysis was performed against deionized water for 3 days using a 10 kDa MWCO membrane. The polymer was then lyophilized (125 mg, yield 70%).

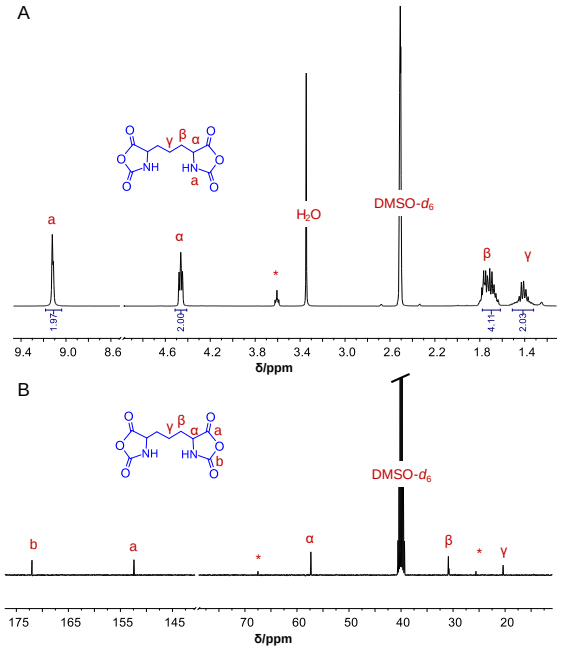

FIGURE 1. ^{1}H-NMR (A) and ^{13}C-NMR spectra (B) of DAP NCA of DAP NCA, both in DMSO-d^6 (*THF).

RESULTS AND DISCUSSION

DAP NCA was synthesized by a modified literature method using triphosgene.[23] The monomer was then purified by multiple precipitations in hexane yielding an off-white oily residue. The molecular structure was characterized, and its purity confirmed by FTIR, ^{1}H and ^{13}C NMR spectroscopies. Characteristic anhydride stretching bands from the NCA rings are present at 1841 and 1775 cm^{-1} in the FTIR spectrum (Figure S3). Two NCA rings are clearly observed after conversion of the difunctional amino acid (Figure S4) in the ^{1}H-NMR spectrum with integrated peaks summing to the total number of protons in the molecule in addition to amide protons from the NCA rings which can be seen at 9.25 ppm (Figure 1A). The ^{13}C-NMR spectrum confirms the presence of equivalent NCA anhydride carbonyls, which could be assigned to the peaks at 152 and 172 ppm, respectively (Figure 1B).

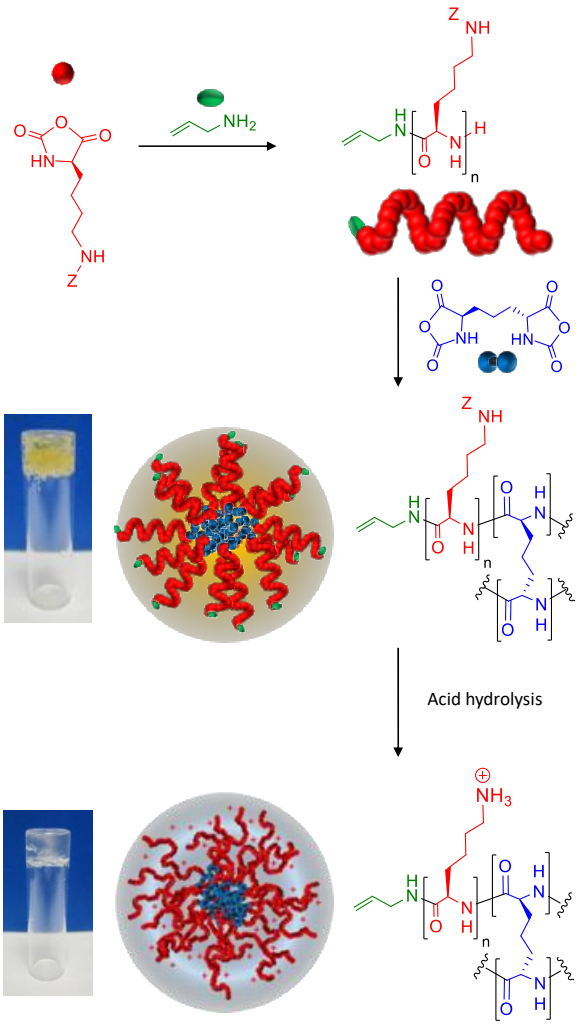

FIGURE 2. Illustration of sequential ROP of ZLL NCA and DAP NCA forming core crosslinked networks. Note that the schematic structures do not imply core-crosslinked micelles but simply illustrate the solvophobic and hydrophobic collapse of PDAP chains in the solvent or in water resulting in swelling.

In the following step, core cross-linking of the α-helical polypeptide poly(Z-L-lysine) (PZLL) and of a random coiling polypeptoid, non-ionic

poly(sarcosine) (PSar) was attempted through the ROP of DAP NCA from the respective macroinitiators. Allylamine initiated ROP of NCA ZLL at 0 °C (same procedure for the NCA of sarcosine) was carried out to generate the corresponding homopolypeptides (Figure 2) in a mixture of chloroform and DMF (5:1) at a monomer to initiator ratio of 80:1 until full monomer conversion was reached (monitored by FTIR). Size Exclusion Chromatography (SEC) analysis of samples withdrawn from the reaction mixture at this time point confirmed the monomodal distribution of both polymers with a dispersity $Đ_M < 1.2$ (Figure 3).

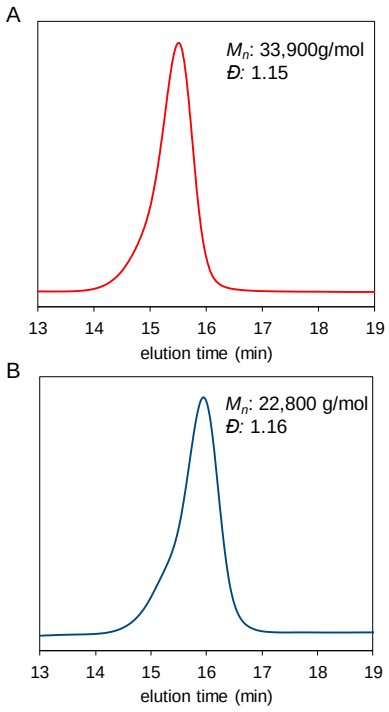

FIGURE 3. SEC traces with molecular weight and dispersities for initial blocks consisting of A) $PZLL_{80}$ and B) $PSar_{80}$.

DAP NCA was then added directly to the polymerization mixture at a monomer ratio of 80 (ZLL or PSar) to 20 (DAP) (Figure 2). The addition of the DAP NCA to the PZLL homopolypeptide in solution had no immediate effect on polymer solvation. However, after 2 days the entire $PZLL_{80}$-b-$PDAP_{20}$ (P1) solution was immobilized and would no longer stir (Figure S5). It was difficult to obtain well resolved ^{1}H-NMR spectra for the copolypeptide as it gelled in all commonly used NMR solvents. Initial attempts resulted in spectra with suppressed proton signals from the characteristic PZLL sequence due to the highly crosslinked DAP core (Figure S6) as a result of reduced mobility.[32] Interestingly, even while maintaining the DAP NCAs' stoichiometric ratio, switching the initial PZLL sequence for a PSar sequence failed to induce any *in situ* organogelation, rather the solution turned opaque and slight precipitation was observed. Since PSar is a predominately random coil forming sequence, it is devoid of the structural organization of α-helical forming PZLL, which is a conformation known to facilitate solvent gelation at sufficiently high concentrations.[33] Thus, for *in situ* gelation, maintaining the PZLL as the initial sequence was necessary in order to promote organogelation through subsequent macroinitiation of the difunctional NCA monomer. Although after precipitation of $PSar_{80}$-b-$PDAP_{20}$ (P2) polymer, it was found to gelate at higher polymer concentrations in both DMF and water. In order to investigate the impact of the polymer structure on the gelation, ZLL and DAP NCA were copolymerized simultaneously yielding a random copolymer $PZLL_{80}$-co-$PDAP_{20}$ (P3). An opaque solution was observed within a few hours, yielding a stiff organogel, signifying a fully cross-linked network. This would be expected when considering the sequential growth of the polymer varying between ZLL and DAP repeat units versus the core crosslinking approach with PZLL-b-DAP.

After removing the entrapped solvent from each of the copolypeptides, the solid samples were trialed for uptake of a range of different organic solvents showing a particularly high efficacy for polar aprotic solvents. Each of the polymers; $PZLL_{80}$-b-$PDAP_{20}$ (P1), $PSar_{80}$-b-$PDAP_{20}$ (P2) and $PZLL_{80}$-co-$PDAP_{20}$ (P3) formed stable organogels in toluene, chloroform, dichloromethane, tetrahydrofuran (THF), ethyl acetate, dimethyl sulphoxide (DMSO),

dimethylformaide (DMF), hexafluoro-isopropanol (HFIP) and acetonitrile; while ethanol, methanol, isopropanol, hexane, petroleum ether and diethyl ether had no solubilizing or swelling effect on the materials. For both $PZLL_{80}$-b-$PDAP_{20}$ and $PZLL_{80}$-co-$PDAP_{20}$, the Z protecting group of the PZLL copolymers was removed by acidic treatment (TFA/HBr 33 wt%) revealing the pendant ionised ammonium groups of poly(lysine) (PLL), promoting water solubility within the polymer architecture (Figure 2). After removal of the excess acid with dialysis followed by lyophilization of the materials, the hydrogelation ability of the now amphiphilic copolypeptides was examined using the "vial inversion" method (Figure S7). There was uptake of water for P1, forming a transparent hydrogel while interestingly P3 did not appear to uptake any water and was ultimately insoluble after deprotection. For the sarcosine containing copolymer, P2, swelling in water was evident although at higher polymer concentration. The gels formed from P2 had a swelling ratio of 5.62 after an incubation period of 2 hours in water. Prompting this, further screening of spectroscopic and rheological properties was focused on P1.

FTIR spectroscopy was used to deduce backbone arrangements of the dried and swollen P1 organogel and hydrogel samples. Based on the orientation of the DAP monomer after ROP, a multitude of turning and twisting conformation of this crosslinked peptide core could possibly be observable using FTIR, akin to those turns found from β-conformers in small peptides.[34] The homopolypeptide composed purely of PZLL is known to convey characteristic bands in the amide I region (1650 cm^{-1}) correlating to the peptide backbone νC=O, with the ε-amide Z protecting group $\nu_{C=O}$ stretch appearing above 1700 cm^{-1}. Interestingly, the dried P1 sample shows the split of this band into a multitude of peaks (Figure 4A). The bands at 1649/1652 cm^{-1} denote helical conformation, with random coiling observed at 1642 cm^{-1} and mixed β-motifs at 1632 and above 1660 cm^{-1}, probable cause of DAP core crosslinking.

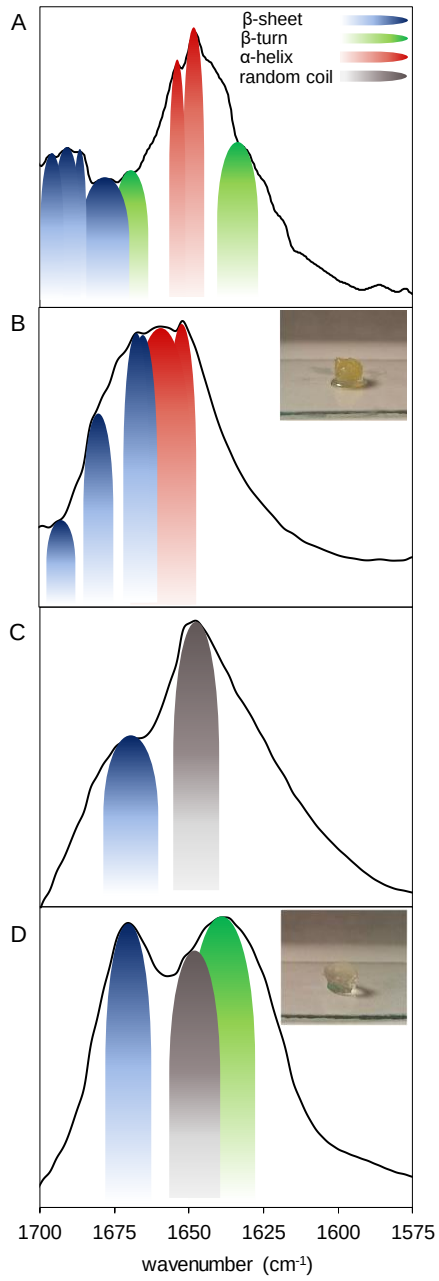

FIGURE 4. FTIR spectra of A) P1 dried organogel, B) P1 swollen organogel (in DMSO), C) lyophilised hydrogel and D) swollen hydrogel (in D_2O).

After P1 was swollen in a small volume of DMSO, FTIR spectra of the resulting organogels displayed a broadened signal in the amide I region (Figure 4B). A strong band at 1658 cm^{-1} appeared, indicative of a mixed α-helix/random coil conformation, denoting the disruption of

the hydrogen bonding associated with helix stabilization. The β-motif bands had receded, although peak shouldering was evident. For the deprotected P1 copolypeptide, the spectra display two absorptions at 1647 and 1672 cm^{-1}, signifying expected random coiling of PLL and a characteristic β-turn amide stretch (Figure 4C). This correlates since the DAP crosslinker causes the polymer backbone to orientate into multiple patterns, which is particularly true for the PZLL block of P1 (Figure S8) with subsequent turning and twisting of the peptide backbone due to the dense hydrophobic core. Importantly, after swelling P1 in D_2O, the gel exhibited a significant shift in intensity of the β-sheet and the β-turn bands, with reduction of the random coil peak (Figure 4D). This potentially confirms the conformational state of DAP crosslinking in the core since no intermolecular H-bonding contributes to the gelation.

The influence of difunctional peptide crosslink on the stability of the hydrogel network was ascertained by real-time evaluation of the mechanical properties. Initially, the effects of DAP NCA crosslinking on P1-P3 polymeric systems were determined using an oscillatory time sweep, maintaining the same stoichiometric ratios of PZLL (or PSar) to monomer DAP NCA (P1, P2) and ZLL NCA to DAP NCA (P3) as used in the development of gelators (Figure 5A). Reaction mixtures containing either PZLL/DAP NCA, PSar/DAP NCA or allylamine/ZLL NCA/DAP NCA solubilized in DMF were placed directly on the rheometer plate and analyzed immediately. P3 shows the most significant change in viscoelastic properties with a rapid increase in modulus observed from 10 minutes onwards. Considering this pure monomer:monomer system, the polymerization of the ROP of both ZLL and DAP appear to be simultaneously occurring, with fast chain propagation forming a fully crosslinked system which rapidly gelates DMF. Similarly, for P1, the solvated linear PZLL sequence appears to rapidly crosslink within 20 minutes (with bubbles observed from evolved CO_2) after the addition of the DAP NCA monomer. This suggests the formation of a network comprising mixed physical and chemically crosslinked hyperbranched polymers. (Figure 5B). While pure core-crosslinked micelles have been observed by others following a similar approach using cystine NCA,[22] we hypothesize that the higher feed ratio of ZLys NCA to DAP NCA employed here tips the balance towards a system where no micellization occurs due to increased hydrophobic content. P2 does not undergo the same gelation profile of both P1 and P3 as it lacks the conformation of PZLL residues, which can stabilize these organogels in DMF due to their innate α-helical nature. Some solution gelation for P2 is observed but it is inferior in terms of gel strength in comparison to its counterparts.

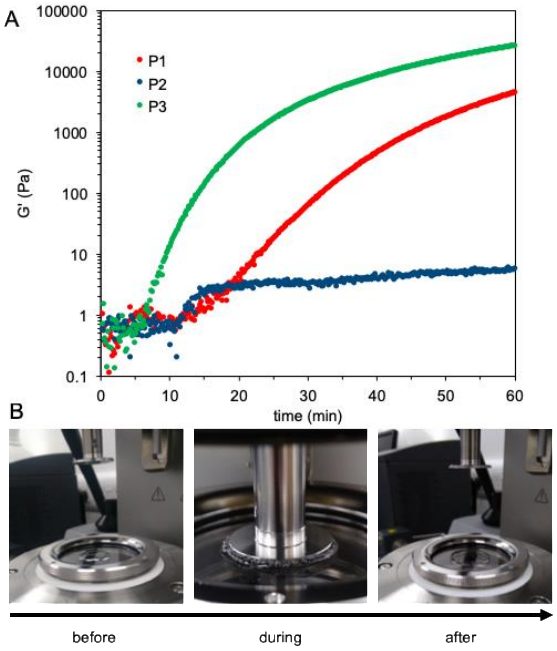

FIGURE 5. (A) Real time oscillatory sweep tracking organogel formation in P1, P2 and P3 monomer systems in 10 wt% in DMF. (B) P1 (PZLL and DAP NCA monomer in solution) before, during (bubbles from CO_2 evolution) and after (gel formed) one-hour polymerization time.

For the P1 hydrogel, rheological amplitude and frequency sweeps were conducted to directly elucidate the G' (storage modulus) and G'' (loss modulus) under different shear/strain parameters. At 2.0 wt%, P1 appears to take on a stiff gel state with an unusually high G' of 2680 ± 9 Pa, comparative to 80 ± 6 Pa for commonly used gelatin hydrogels in tissue engineering.[35] The crosslinked copolypeptide could maintain a gel network at very low material concentrations in a range below 2.0 wt% (Figure 6A and B). Surprisingly, even at 0.5 wt% P1 maintained its assembling character with G' at 758 ± 10 Pa and G'' at 70 ± 11 Pa comparable to the mechanical strength of hydrogels assembled from diblock star copolypeptides.[11] An interesting concentration dependent property was observed in the amplitude sweep at 0.5 wt% (Figure S9A). The reduction in material concentration appeared to constitute a more robust gel network as it appears to have a higher strain induced breaking point (29.3 %) than for the 2.0 wt% gel (5.2 %) under the same conditions (Figure S9B). This could be a consequence of the diluted polymer assemblies undergoing a conformational shift preventing shear-based effects' deforming the network.

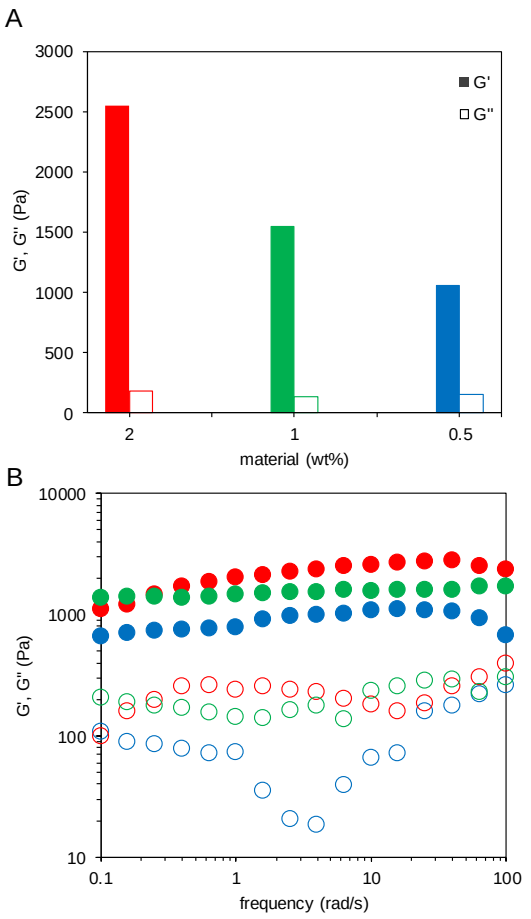

FIGURE 6. A) Compilation of rheological properties of P1 hydrogel at different polymer concentrations and B) frequency sweeps of P1 hydrogel at different polymer concentrations.

CONCLUSIONS

In conclusion, organogels were formed from *in situ* ring opening polymerization of an α-helical polypeptide (PZLL) with a novel difunctional NCA monomer (DAP), immobilizing the solvent through core crosslinking and stabilized through physical intermolecular conformations. The DAP monomer could also core crosslink the random coil forming polypeptoid (PSar), although resulting networks were devoid of any stabilized physical interactions, thus yielding significantly weaker gels as a result. Ensuing cleavage of Z protecting groups of PZLL segments afforded stiff hydrogels with high mechanical stability. The hydrogels remained

intact at aqueous concentrations as high as 99.5 % and were mechanical strong as determined by rheological measurements and could hold potential as matrices in regenerative medicine.

ACKNOWLEDGEMENTS

Financial support from a Science Foundation Ireland (SFI) Principle Investigator Award 13/IA/1840(T) is gratefully acknowledged. F.C.S.d.O. acknowledges funding from the Brazilian Government-CAPES Grand Number No. 13695/2013-04.

REFERENCES AND NOTES

1. D. Seliktar, *Science* **2012**, *336*, 1124
2. X. Du, J. Zhou, J. Shi, B. Xu, *Chem. Rev*. **2015**, *115*, 13165
3. A. K. Gaharwar, N. A. Peppas, A. Khademhosseini, *Biotechnol. Bioeng.* **2014**, *111*, 441
4. M. W. Tibbitt, K. S. Anseth, *Biotechnol. Bioeng*. **2009**, *103*, 655
5. X. Zhou, Z. Li, *Adv. Healthcare Mater*. **2018**, *7*, 1800020
6. A. P. Nowak, J. Sato, V. Breedveld, T. J. Deming, *Supramol. Chem*. **2006**, *18*, 423
7. Z. Li, T. J. Deming, *Soft Matter* **2010**, *6*, 2546
8. G. Ma, W. Lin, Z. Yuan, J. Wu, H. Qian, L. Xua, S. Chen, *J. Mater. Chem. B* **2017**, *5*, 935
9. A. P. Nowak, V. Breedveld, L. Pakstis, B. Ozbas, D. J. Pine, D. Pochan, T. J. Deming, *Nature* **2002**, *417*, 424
10. C. Vacogne, S. Brosnan, A. Masic, H. Schlaad, *Polym. Chem*. **2015**, *6*, 5040
11. R. Murphy, T. Borase, C. Payne, J. O'Dwyer, S. A. Cryan, A. Heise, *RSC Adv*. **2016**, *6*, 23370
12. K. N. Sill, B. Sullivan, A. Carie, J. E. Semple, *Biomacromolecules* **2017**, *18*, 1874
13. C. C. Ahrens, M. E. Welch, L. G. Griffith, P. T. Hammond, *Biomacromolecules* **2015**, *16*, 3774
14. A. M. Oelker, S. M. Morey, L. G. Griffith, P. T. Hammond, *Soft Matter* **2012**, *8*, 10887
15. M. T. Popescu, G. Liontos, A. Avgeropoulos, E. Voulgari, K. Avgoustakis, C. Tsitsilianis, *ACS Appl. Mater. Interfaces* **2016**, *8*, 17539
16. Y. Sun, Y. Hou, X. Zhou, J. Yuan, J. Wang, H. Lu, *ACS Macro Lett*. **2015**, *4*, 1000
17. K. X. Ren, H. T. Cui, Q. H. Xu, C. L. He, G. Li, X. S. Chen, *Biomacromolecules* **2016**, *17*, 3862.
18. R. Murphy, D. P. Walsh, C. A. Hamilton, S. A. Cryan, M. in het Panhuis, A. Heise, *Biomacromolecules* **2018**, *19*, 2691
19. S. B. Hanay, B. Ritzen, D. Brougham, A. A. Dias, A. Heise, *Macromol. Biosci*. **2017**, *17*, 1700016
20. S. B. Hanay, J. O'Dwyer, S. D. Kimmins, F. C. S. de Oliveira, M. G. Haugh, F. J. O'Brien, S.-A. Cryan, A. Heise, *ACS Macro Lett.* **2018**, *7*, 944
21. R. C. Fahey, J. S. Hunt, G. C. Windham, *J. Mol. Evol*. **1977**, *10*, 155.
22. A. Sulistio, A. Widjaya, A. Blencowe, X. Zhang, G. G. Qiao, *Chem. Commun*. **2011**, *47,* 1151
23. J. Ding, S. Fenghua, C. Xiao, L. Lin, L. Chen, C. He, X. Zhuang, X. Chen, *Polym. Chem*. **2011**, *2*, 2857.
24. E. D. Raftery, E. G. Gharkhanian, N. G. Ricapito, J. McNamara, T. J. Deming, *Chem. Asian J.* **2018**, *13*, 3547
25. A. Sulistio, J. Lowenthal, A. Blencowe, M. N. Bongiovanni, L. Ong, S. L. Gras, X. Zhang, G. G. Qiao, *Biomacromolecules* **2011**, *12*, 3469
26. A. Sulistio, A. Blencowe, A. Widjaya, X. Zhang, G. G. Qiao, *Polym. Chem*. **2012**, *3*, 224
27. F. Shi, J. Ding, C. Xiao, X. Zhuang, C. He, L. Chen, X. Chen, *J. Mater. Chem*. **2012**, *22,* 14168
28. T. Jing, L. Fu, L. Liu, L. A. Yan, *Polym. Chem.,* **2016**, *7,* 951.
29. T. L. Born; J. S. Blanchard, *Curr. Opin. Chem. Biol*. **1999**, *3*, 607

30. D. L. Dewey, E. Work, *Nature* **1952**, *169*, 533
31. G. Scapin; J. S. Blanchard, *Adv. Enzymol. Rel. Areas of Mol. Biol.* **1998**, *72*, 279
32. K. Y. Baek, M. Kamigaito, M. Sawamoto, *Macromolecules* **2001**, *34*, 215
33. C. D. Vacogne, M. Schopferer, H. Schlaad, *Biomacromolecules* **2016**, *17*, 2384.
34. M. Hollosi, Z. Majer, A. Z. Ronai, A. Magyar, K. Medzihradszky, S. Holly, A. Perczel, G. D. Fasman, *Biopolymers* **1994**, *34*, 177
35. N. I. zur Nieden, C. C. Turgman, X. Lang, J. M. Larsen, J. Granelli, Y. J. Hwang, J. G. Lyubovitsky, *ACS Appl. Mater. Interfaces* **2015**, *7*, 10599.

GRAPHICAL ABSTRACT

Robert D. Murphy, Elena Bobbi, Fernando C. S. de Oliveira, Sally-Ann Cryan and Andreas Heise

Gelating polypeptide matrices based on the natural difunctional N-carboxyanhydride (NCA) diaminopimelic acid cross-linker

The synthesis of hydrogelling polypeptides is achieved by chain extension of polypept(o)ides with a difunctional N-carboxyanhydride yielding core-crosslinked structures. In particular, polylysine-based polymers form strong gels at low concentration in organic solvents and, after deprotection, in water. Weaker gels are obtained by replacing the polylysine block by polysarcosine due to the absence of intermolecular interaction and stabilization.

GRAPHICAL ABSTRACT FIGURE ((Please provide a square image to be produced at 50 mm wide by 50 mm high. Please avoid graphs and other figures with fine detail due to the relatively small size of this image.))