Experimental report

11/04/2024

Proposal: 9-13-1100 **Council:** 4/2023

Title: Using rheo-SANS to monitor the structure of in situ forming peptide-like hydrogel drug delivery implants.

Research area: Engineering

This proposal is a new proposal

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Samples: Peptoid-D-peptide control no drug
Negative control
Peptoid-D-peptide + MIV-150
Peptoid-D-peptide + cabotegravir
Peptoid-D-peptide + etonogestrel

Instrument	Requested days	Allocated days	From	To
D22	3	2	28/08/2023	30/08/2023

Abstract:

We are developing an in situ forming hydrogel implant made of peptide-like molecules to deliver HIV and contraceptive drugs over a prolonged period (greater than 28days). This will remove the need for patients to comply with complex drug regimens, improving adherence to medicines. The implant forms after injection under the skin in response to a phosphatase enzyme trigger. The objective of this project is to use Rheo-SANS on D22 to study fundamental mechanism of gel formation over time and see how the presence of drug relates to changes in the microscopic structure and rheological/mechanical properties important for in situ forming drug-releasing implants. We also wish to see how the underlying structure of the gel responds to increasing levels of shear stress, until the gel breaks, providing information as to how the hydrogel will exist after implantation under the skin. This data will support our existing rheology, microscopy, SANS, DOSY, QENS, spectroscopy and drug release data. This should allow us to tailor diffusion within the gels to drug release kinetics for sustained delivery by modifying the chemical structure and/or formulation of our hydrogel forming peptide.

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Background: The focus of this work is a promising peptide-mimetic hydrogel material composed of a low molecular weight peptoid-D-peptide molecule that responds to the presence of physiological enzymes (phosphatases) to form a hydrogel *in situ* upon injection. The ultimate goal is to provide HIV preventative and contraception protection to women in a discretely administered platform for ~84 days. Several researchers have previously studied how peptides form hydrogels *in situ* in response to an external trigger, such as pH change. This allows the relationship between the underlying structures formed before, during and after gel formation, and then their response to shear to be studied.[1] It is possible to monitor and correlate these over time using a combination of rheology and small angle neutron scattering (SANS).[2]

Aims/Objectives: In this study our aim was to:
1) Determine how the presence of drug in our peptoid-D-peptides influences gel formation, its microscopic structure and the underlying mechanism of gel formation i.e. monitor structures (size, shapes) of pre-gel solution to the final gel with and without drug attached.
2) Study the level of rheological shear stress/strain will deform/break the gel, providing insight as to how the implant may behave in the body.

Experimental: Rheo-SANS with D22 at ILL was utilised in an attempt to link the microscopic structures formed (SANS) to the changing mechanical properties (rheology) after the addition of phosphatase enzyme (the gel trigger) and throughout the gelation process for drug attached peptoid-D-peptides. Each peptoid-D-peptide-drug (drugs = MIV-150, cabotegravir, etonogestrel) was tested at a concentration of 5% w/v within a Couette cell, alongside a peptoid-D-peptide (no drug) control. A neutron wavelength of λ= 6 Å was employed (Q range = ~0.002 – 0.4 $Å^{-1}$), and a temperature of 310 K. Rheological analysis was be conducted in a titanium concentric cylinder (0.5mm gap, 30mm diameter Ti cell). At the time-sweep experiment (~4 hours), strain and frequency sweeps (totalling ~1 hour) were performed. Simultaneously using SANS provided insight as to how gels respond to external stress e.g. within body after injection, and whether the peptoid-D-peptide systems breaks down into individual fibres, small pockets of gel or exist as a bulk gel network.[3] Data reduction and analysis was performed using ILL software tools.

Preliminary Results and On-going Progress: We were able to follow the gelation process for ~4 hours upon addition of phosphatase enzyme, simultaneously collecting data relating to SANS and rheological properties. Our peptoid-D-peptides form gels that are proven to effectively scatter neutrons and data obtained can be used determine structure of fibrous hydrogel networks.[4] Detailed analysis is currently being performed in order to link SANS data to rheological properties over time. In this context, the large amount of data generated is currently being processed, including linking time sweeps and gel formation to *in situ* scattering data collected during the gelation i.e. the trend in the scattering data with time. It will be important to plot scattering intensity versus time as compared to tan δ (rheology) for each peptoid-D-peptide drug and controls. *In situ* scattering data has been collected during the strain sweep, it will be important to establish whether there is a change in the scattering as the gel becomes broken. This data will form the basis of a research paper the experimental team will submit to a high quality journal.

Next Steps: We intend to use rheo-SANS to study multi-drug peptoid-D-peptide formulation to decipher whether there is a difference in microscopic structure and the underlying mechanism of gel formation and/or gel deformation for peptoid-D-peptide long-acting injectable products for combined HIV prevention and contraception e.g. peptoid-D-peptide + MIV-150 + cabotegravir; peptoid-D-peptide + MIV-150 + etonogestrel; peptoid-D-peptide + cabotegravir + etonogestrel; peptoid-D-peptide + all three drugs.

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References: 1. McAulay, K et al. Organic Materials 2020, 02, 108-115. DOI: 10.1055/s-0040-1708832. **2.** Draper, E. R et al. Langmuir 2017, 33 (9), 2387-2395. DOI: 10.1021/acs.langmuir.7b00326. **3.** Yan, C et al. Soft Matter 2010, 6 (20), 5143-5156, 10.1039/C0SM00642D. DOI: 10.1039/C0SM00642D. **4.** Coulter, S. M et al. Advanced Healthcare Materials, 2023, 12, 2203198. DOI: 10.1002/adhm.202203198