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RPPR Final Report
as of 02-Mar-2022

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Proposal Number: 75666LSRIP **Agreement Number: W911NF-20-1-0047**
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Results Dissemination: See attached final report.

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Nothing to Report

RPPR Final Report
as of 02-Mar-2022

Partners

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I certify that the information in the report is complete and accurate:
Signature: Betty A. Rasmussen
Signature Date: 2/28/22 1:22PM

Analysis of Photopolymerization Induced Biostasis via MALDI Imaging Mass Spectrometry: Final Report

Type of equipment: Matrix Assisted Laser Desorption Ionization Mass Spectrometer (MALDI-MS)

Manufacturer of equipment and model number: Shimadzu. MALDI 7090

Cost of Equipment: $366,826.43

Quantity: One

Special circumstances surrounding any change from the Grantee's proposal: Receipt of instrument was delayed due to multiple COVID-associated shutdowns of the production facility in the UK. An instrument with some similar capability was provided on loan (MALDI 8020) allowing work to proceed until the MALDI 7090 instrument was received.

A concise discussion of the use of the equipment including (a) any research work described in the proposal, and (b) any other research of interest to DoD:

The labs of Drs. Christopher Bowman, Kristi Anseth and Sabrina Spencer are recipients of a grant from DARPA (W911NF-19-2-0024) to induce and reverse biostasis in living cells and tissues via intracellular crosslinking and degradation of hydrophilic polymers. Following cellular uptake of these polymers, an exogenous trigger causes them to form a network architecture that diminishes the diffusion of biomacromolecules (e.g. proteins, nucleic acids) and thereby mitigate the performance of cellular processes including those that might be deleterious (e.g. apoptosis, necrosis, differentiation) to biomedical interventions. An orthogonal trigger is subsequently employed to degrade the network, returning the cells to their prior state, effectively reversing biostasis. It is envisioned that this approach may be used to stabilize biological-based therapeutics at room temperature, mitigating the dependence of such materials on the cold chain for storage and transport. Moreover, this technology could be used to stabilize injury at the site of occurrence for subsequent movement to and treatment of patients at appropriate medical facilities.

The characterization of these polymers both outside of and within biological contexts is key to accomplishing and progressing the objectives of this project and MALDI is a powerful tool in that respect. While COVID caused shutdowns at the Shimadzu factory in the UK, since acquisition of the instrument, it has been applied to the characterization of novel degradable polymers and their functionalized analogues with previously unachievable mass resolution, elucidating polymerization mechanisms and coupling efficiencies that would otherwise not be possible.

To aid in the accomoplishment of these aims, the acquired instrument was used to identify synthesized precursors for polymer core structures that degrade via radial immolation with application of long-wavelength ultraviolet light (Figure 1a). These core structures can be difficulty to characterize via HNMR and HPLC when multiple norbornene functional groups are present, creating many endo-exo stereoisomers and complicating chromatographic separation and proton peak identification. Product validation via mass spectroscopic techniques is preferred, therefore. The soft ionization of MALDI is especially advantageous, avoiding undesired fragmenting of the highly cleavable, photolabile bonds.

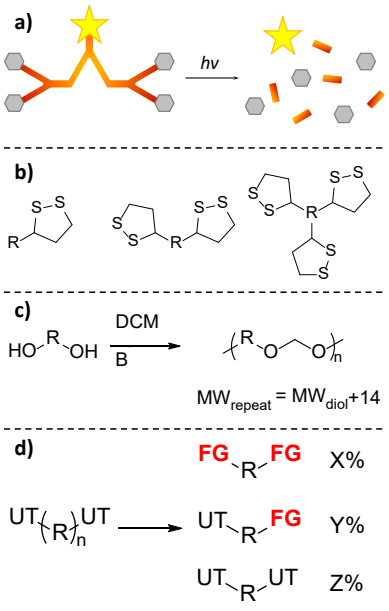

Figure 1. a) Generalized scheme of immolative dendrimer core whereupon absoprtion by chromophore (star) results in radial degradation of structure releasing terminal functional groups and any conjugated species (hexagons). b) Generalized structures of photocurable mono, di, and trifunctional dithiolanes currently being studied for photoresponsive, covalent adaptable materials. c) Polymerization of diols to form methylene-containing polymers. Molecular weight of repeat unit is the molecular weight of the diol plus that of one carbon and two hydrogens. d) Functionalization of polymer end-groups results in mixture of monofunctionalized, difunctionalized and unfunctionalized species (UT represents unmodified termini; FG represents desired terminal functional groups). MALDI allows determination of relative fractions of functionalized polymers.

The MALDI instrument has been invaluable for identification for otherwise difficult to characterize reaction products. One research direction that has arisen from the DARPA biostasis project but has since found broader application is the photocuring of multifunctional dithiolanes (cyclic disulfides, Figure 1b). These reactions can be performed in the absence of initiator and result in networks with dynamic covalent bonds that can rearrange in response to reduction and oxidation triggers.

Efforts to create additional, controllable sonication labile and hydrolytically degradable materials has led to the discovery of a novel polymerization that yields methylene-linked monomer units (Figure 1c). This was done by reacting alchohols together in dichloromethane in the presence of a strong base. Unlike complementary molecular mass determination techniques (e.g. Gel permeation chromatography, multi-angle light scattering, etc.), MALDI data provides the molecular weight of polymer repeat units, allowing direct validation of the polymerization products and support of proposed polymerization mechanism.

The MALDI instrument was also used to evaluate the end-group modification strategy for these same methylene-containing polymers. While H^1NMR can provide average functionality calculations, it cannot distinguish among functionalization distributions. MALDI allows researchers to determine distributions of termini substitution rather than average substitution alone (Figure 1d).

As the instrument has been made available to any researcher at the University of Colorado in Boulder, it has also been used in support of other research projects on campus. Professor Ryan Hayward has a grant from the Department of Energy (DE-SC0020982) investigating the domain coarsening of cocontinuous microphase separated polymer networks. The ability to tune domain size post-synthetically has valuable implications in a variety of transport processes, including membrane, electrical, and photonic applications. The MALDI instrument funded by the DURIP was used to assess end-group fidelity of telechelic polystyrene and poly(ethylene glycol) that are used to form such networks. One issue with relying on H^1NMR for end-group analysis of polymers is the problem of peak broadening and consequent difficulty of peak integration. Currently, poly(dimethylsiloxane) is also being investigated as an alternative and characterization via MALDI remains a vital technique in this evaluation.

As the global coronavirus pandemic prevented a timely receipt of the instrument, some planned applications of the instrument have had to be postponed. Nevertheless, experiments are currently underway to characterize degradation of hydrogels via MALDI as proposed in the DURIP application. MALDI measurements are anticipated to occur in early March of 2022. Early results from MALDI imaging of spatial distribution of synthetic and biomacromolecular species of tissues and organoids placed in induced biostatic state is expected in the summer of 2022.

Short term plans for use of the instruement also extend to research outside of the DARPA biostasis project. Among these is the evaluation of the polymerization of alkynes and the polymerization of maleimides, allowing the distinction between multiple mechanisms via the analysis of repeat unit masses. These research objectives are part of grants funded by the National Institutes of Health (R01 RHL132353 and R01 HL142935). Future plans also include analysis of thioamide-thiol reactions. Spectral difference following exposure to light indicates a reaction taking place; MALDI, with its soft ionization technique, once again is ideally suited to elucidating the nature of this reaction.

The MALDI funded by the DURIP award has had substantial positive effect on the research of the awardees as well as the broader academic community at the University of Colorado. The availability of this instrument has made possible characterization and analyses previously impossible at this institution.