

Microwave-assisted synthesis of norbornene-functionalized macromers for hydrogel crosslinking and 3D bioprinting

Jonathan Bryan and Chien-Chi Lin

Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN, 47907

Statement of Purpose: Biorthogonal click reactions are commonly used for hydrogel crosslinking in 3-dimensional (3D) cell culture and bioprinting applications. For example, norbornene-functionalized macromers (e.g., gelatin-norbornene (GelNB), poly(ethylene glycol)-norbornene (PEGNB), etc.) afford fast, selective, and adaptable hydrogel crosslinking. One bottleneck for 3D bioprinting techniques, including extrusion-based, digital light processing (DLP), or volumetric bioprinting, is the need for high volume of hydrogel precursor solutions to ensure proper formation of the desired structure. Hence, there exists a critical need to improve polymer synthesis efficiency and yield, while reducing the costs and solvent consumption. We reason that microwave-assisted chemical reaction can accelerate the synthesis of norbornene-functionalized macromers while reducing organic solvent use. This work explores the use of a benchtop microwave reactor to reduce reaction time and solvent volume in GelNB and PEGNB synthesis and their applications in modular hydrogel crosslinking and DLP bioprinting.

Method: Norbornene modified gelatin (GelNB), and PEG-norbornene-carboxylate (PEGNBC) were synthesized via reacting with inexpensive and odorless carbic anhydride (Fig. 1) following our published protocols but with synthesis parameters adapted for a microwave reactor (CEM Discover 2.0) [1, 2]. A range of reaction times were used to tune the degree of functionalization (DoF) for GelNB, which was determined via fluoroldehyde assay. PEGNBC synthesis was further optimized via design of experiments methodology for maximizing DoF. Additionally, a new aqueous synthesis route was developed for synthesizing norbornene-functionalized PEG from reacting carbic anhydride with amine-terminated PEG, yielding PEGaNBC. ^1H NMR was used to quantify norbornene substitution on PEG. GelNB and PEGNBC hydrogels were crosslinked with 4-arm PEG-thiol and dithiothreitol (DTT) respectively. The photoinitiator Lithium phenyl2,4,6-trimethylbenzoylphosphine (LAP) was used with tartrazine for photoabsorbance in DLP printing.

Results: Every increment of 10 °C will roughly double reaction rate based on the Arrhenius equation. Using this information, existing protocols were adapted to reduce reaction time. A range of 45% to 82% GelNB DoF was achieved by varying reaction time at 90 °C (Fig 2A). When crosslinked, microwave synthesized GelNB achieved G' of ~2 to 6.25 kPa at fixed macromer and crosslinker contents (Fig 2B). Synthesis via conventional direct heating at 40 °C for between 2 and 16 hours showed no time dependent DoF tunability (Fig 2C). SDS-PAGE of both heating conditions showed a slight decrease in gelatin molecular weight with microwave heating (data not shown). Molecular weight differences may explain why direct heating gel G' was relatively higher (Fig 2D). Similar PEGNBC DoF was achieved using microwave and conventional heating (Fig 2E) while microwave heated

reactions were 35.5 hours shorter and used 40% less organic solvent. Hydrogels with macromers synthesized via both heating methods had similar G' (Fig 2F). Aqueous synthesis of PEGaNBC was achieved. Hydrogels formed with PEGaNBC had rapid gelation (< 10 s) and stiffness tunable from 1-15 kPa by varying polymer concentration and crosslinker functionality (data not shown). PEGNBC was readily printed via DLP with protocol similar to our published work (data not shown) [3].

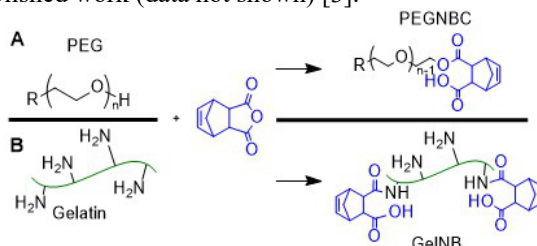


Figure 1. Reaction scheme of (A) PEGNBC synthesized from reacting PEG-OH with carbic anhydride and (B) GelNB synthesized from reacting gelatin with carbic anhydride.

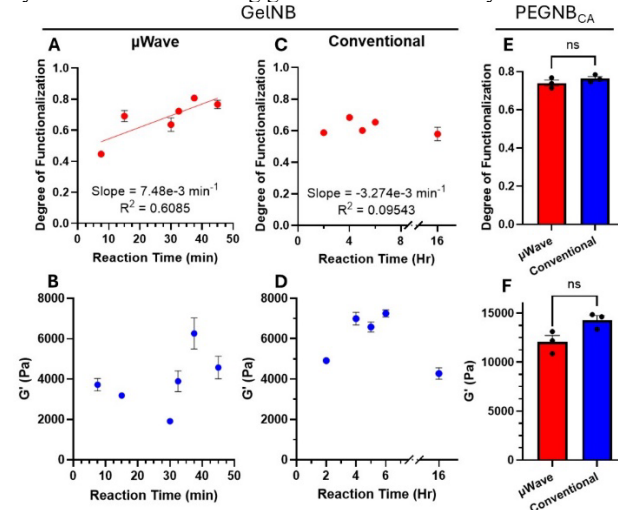


Figure 2. DoF (A, C), and G' (B, D) of corresponding GelNB hydrogels with microwave or direct heating. DoF (E) and G' (F) of corresponding PEGNBCA Hydrogels. All conditions use $n = 3$. Error bars represent SEM.

Conclusions: Natural (GelNB) and synthetic macromers (PEGNBC) were successfully functionalized with norbornene using microwave heating, which afforded more tunable DoF while substantially reducing reaction time compared to direct heating. Materials synthesized via microwave were amenable to DLP bioprinting. Future work will focus on fully optimize the synthesis protocols and produce macromers for DLP bioprinting applications.

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References: [1] Kim et al., ACS Biomater Sci Eng. 2021(7):4196. [2] Lin et al., ACS Macro Letters 2021(10):341. [3] Kim et al., ACS Appl. Mater. Interfaces. 2023(15)2737.