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Synthesis of ultra-high molecular weight (UHMW) polymers by controlled production of initiating radicals

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

ABSTRACT

This study demonstrates that the gradual and slow production of initiating radicals (i.e. hydroxyl radicals here) is the key point for the synthesis of ultra-high molecular weight polymers *via* controlled radical polymerization. Hydrogen peroxide (H_2O_2) and ferrous iron (Fe^{2+}) react *via* Fenton redox chemistry to initiate reversible addition-fragmentation chain transfer (RAFT) polymerization. This work presents two enzymatic – mediated (i.e., Bio-Fenton-RAFT and Semi Bio-Fenton-RAFT) and one syringe pump–driven Fenton-RAFT polymerization processes in which the initiating radicals are carefully and gradually dosed into the reaction solution. The "livingness" of the synthesized UHMW polymers is demonstrated by chain extension and aminolysis experiments. Zimm plots obtained from static light scattering (SLS) technique are used to characterize the UHMW polymers. This Fenton-RAFT polymerization provides access to polymers of unprecedented UHMW ($M_w \sim 20 \times 10^6$ g mol^{-1}) with potential in diverse applications. The UHMW polymers made via the controlled Fenton-RAFT polymerization by using a syringe pump shows that it is possible to produce such materials through an easy-to-set up and scalable process.

KEYWORDS: Fenton; hydroxyl radicals; RAFT polymerization; SLS; UHMW polymers.

INTRODUCTION

The development of reversible deactivation radical polymerization (RDRP) techniques has resulted in an explosive level of novel polymeric materials with wide-ranging industrial and academic relevance.[1] In particular, the unrivalled control afforded by RDRP processes enables access to predefined polymer structures, compositions, and functionalities for a wide range of applications.[2-5] However, undertaking controlled radical polymerizations for the synthesis of ultra-high molecular weight (UHMW, defined as molecular weight equal or greater than 1×10^6 g mol^{-1}) polymers remains a formidable challenge. The synthesis of the UHMW polymers is of great interest of materials specialists because the materials fabricated from UHMW polymers show excellent mechanical strength. The synthesis of UHMW polymers via RDRP techniques was initially accomplished under high pressure and heterogeneous conditions.[6-13] Sumerlin and co-workers later reported the synthesis of UHMW poly(dimethylacrylamide) (PDMA) via a UV–mediated reversible addition – fragmentation chain transfer (RAFT) polymerization using trithiocarbonate (TTC) and xanthate chain transfer agents.[14] Recently, enzyme-mediated RAFT polymerization has been reported to be successful in synthesizing UHMW homo- and co-polymers in aqueous media.[15,16]

Balancing multiple competing chemical transformations in water and in the presence of oxygen is a role performed well by nature's catalysts – enzymes. Enzymes accelerate biochemical reactions with unrivalled rate enhancement and orthogonality under mild aqueous conditions, and offer excellent inspiration as catalysts in RDRP processes.[17] Among them, glucose oxidase (GOx) shows significant promise as both an industrial catalyst

and for mediating RDRP reactions.[18-21] In the presence of molecular oxygen, GOx catalyzes the production of hydrogen peroxide (H_2O_2) while oxidizing β-D-glucose to gluconic acid (Fig. S1, ESI).[22,23] This effect has been exploited as a highly efficient means of GOx-mediated oxygen removal (degassing) in a traditional RAFT polymerization.[20,21]

In this study, we report the importance of controlled production of hydroxyl radicals (HO$^\bullet$) via the Fenton reaction for the controlled synthesis of UHMW polymers in aqueous media (Scheme 1). Following our previous work describing a Bio-Fenton RAFT procedure,[24] we aimed to form UHMW polymers. As depicted in Scheme 1, the H_2O_2 generated by GOx is employed in a Fenton redox pairing with the iron ions of either hemoglobin (Hb, via Bio-Fenton RFAT) or ammonium ferrous sulphate (via Semi Bio-Fenton RAFT), thereby generating HO$^\bullet$. The highly reactive HO$^\bullet$ can attack the monomer and initiate polymerization in reaction vessels open to air. Propagation of the growing polymer is controlled via a RAFT mechanism in the presence of a symmetric TTC chain transfer agent (*bis*-TTC), leading to polymers with low dispersities and well-defined chain lengths. Structures of the RAFT agent and monomers employed in this study are shown in the dashed box. While avoiding the need for traditional deoxygenation protocols and with extremely low catalyst loadings (nM), GOx–mediated Fenton RAFT polymerization (either Bio-Fenton RAFT or Semi Bio-Fenton RAFT) allows for the preparation of controlled UHMW polymers with quantitative monomer conversion values in the presence of air, including, to the best of our knowledge, the largest well-defined polymer ever prepared via controlled radical polymerization ($M_w \sim 2 \times 10^7$ g mol^{-1}). We then show that UHMW polymers can be formed within 1-2 hours through a

continuous Fenton-RAFT process when hydroxyl radicals are carefully controlled by using a

syringe pump.
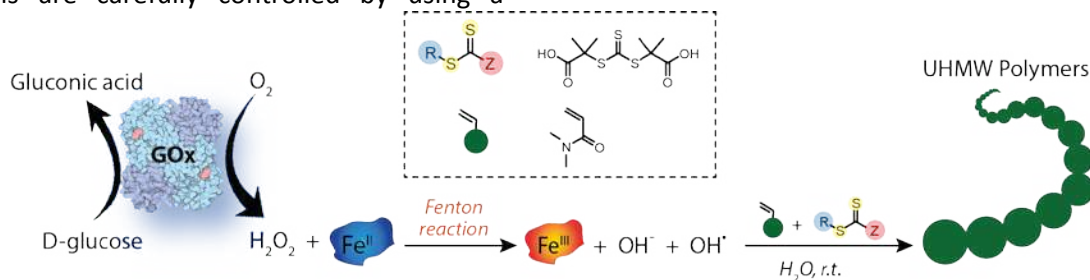
Scheme 1 Schematic illustration of the Semi/Bio-Fenton RAFT polymerization employed for the synthesis of UHMW polymers in the presence of air. H_2O_2 is slowly generated by GOx in the presence of dissolved oxygen, and then reacts with Fe^{2+} via the Fenton chemistry enabling the synthesis of UHMW polymers.

EXPERIMENTAL

Materials

Glucose oxidase (GOx) from Aspergillus niger and hemoglobin from bovine blood (Hb; H2500) were purchased from Sigma-Aldrich as lyophilized powder and used as received. Monomer *N,N*-dimethylacrylamide (DMA, Sigma-Aldrich, 99%) was purchased from Sigma-Aldrich and deinhibited by passing over inhibitor removers and/or basic alumina prior to use. 2-Hydroxyethyl acrylate (HEA, Sigma-Aldrich, 96%) was purified as described previously.[25] D-glucose (99.5%, BDH), hydrogen peroxide (H_2O_2, 30 wt.%, Chem-supply Co.), ammonium ferrous sulphate hexahydrate ((NH_4)$_2$Fe(SO_4)$_2$.6H_2O, Uni Lab Co.) and 3,3',5,5'-tetramethylbenzidine (TMB, 99%, Sigma) were employed as received. *S,S'*-Bis(α,α'-dimethyl-α''-acetic acid)-trithiocarbonate (*bis*-TTC) was synthesized according to a previously published procedure[26] and characterized via ^{1}H and ^{13}C NMR spectroscopy. Solvent *N,N*-dimethylacetamide (DMAC, Sigma-Aldrich, 99%) was employed for SLS analysis as received.

Characterization

Nuclear Magnetic Resonance (NMR) Spectra. NMR spectroscopy was carried out on a Varian

Unity 400 MHz spectrometer operating at 400 MHz. Deuterated solvent ($CDCL_3$ or D_2O) was employed as reference and sample concentrations were approximately 10 mg mL^{-1}. *Size Exclusion Chromatography (SEC).* The water-soluble polymerization mixtures were analyzed directly by aqueous SEC using an eluent of Milli-Q water with 0.1 vol% *trifluoroacetic acid* (TFA). The system was operated at a flow rate of 1 mL min^{-1} at 25 °C. A Shimadzu Liquid Chromatography system was utilized, equipped with a Shimadzu RID-10 refractometer (λ = 633 nm) and Wyatt 3-angle light scattering (LS) detector (λ = 690 nm), with three Waters Ultrahydrogel columns in series ((i) 250 Å porosity, 6 μm diameter bead size; (ii) linear, 10 μm diameter bead size; (iii) Ultrahydrogel 2000 column) for separation up to molecular weights of 10×10^6 g mol^{-1}). The dn/dc value of polydimethyl acrylamide (PDMA) in the above eluent was calculated to be 0.190 mL g^{-1} at 25 °C.[27] Molecular weight and dispersity values were then calculated using the Wyatt ASTRA SEC/LS software package. All SEC samples filtered through 0.45 μm filters.
Static Light Scattering (SLS). To calculate the weight averaged molecular weight (M_w) of synthesized UHMW polymers we employed SLS. SLS was carried out on an ALV-5022F light

scattering spectrometer with samples in cylindrical cuvettes of 8 mm path length. Samples were illuminated with a HeNe laser (623 nm wavelength), and measurements were made at five concentrations as a function of scattering angle. Data were analyzed over the range 30–150° in 10° increments with varied concentrations of polymers, i.e. 0.5-2.0 mg mL^{-1}. Each measurement was 10 s, with 3 repeats at each angle. Measurements with intensity spikes due to dust were rejected. Zimm analysis was conducted using the built-in software. The Zimm analysis highlights where the concentration is too high (so that aggregation occurs), or where the concentration is too low (so there is insufficient scattering), for which the samples were not included in the analysis. Only samples with at least 3 measurable concentrations were used in the analysis. For SLS measurements, the UHMW PDMAs were dissolved in DMAC with 50 mM LiCl solution (dn/dc measured to be 0.06 mL g^{-1}).

Methods

OH-radical detection test. Measurements were carried out in 1 mL water solution containing different concentrations of Hb (12.5 and 25 μM), D-glucose (100mM), GOx (250 nM) and TMB (0.38 mM) at 25 °C for 10 min. The absorbance spectra were observed using a UV-vis spectrometer at 652 nm. The detection also repeated for different concentration of H_2O_2 (10 to 1000 μM) instead of GOx and D-glucose.

H_2O_2 detection test. The concentration of H_2O_2 generated by GOx has been also monitored using peroxidase-like activity technique. In this method, solution of D-glucose (100mM) and GOx (250 nM) was incubated in Milli-Q water at 25 °C for a predefined time period. 0.2 ml of sample were finally mixed with 0.8 mL of Hb

(15.6 μM) and TMB (0.475 mM) and analysed via UV/Vis spectroscopy. The Absorbance at 652 nm was converted to H_2O_2 concentration using the standard curve.

Bio-Fenton RAFT polymerization of DMA open to air. Different stock solutions of the reagents, i.e. GOx, D-glucose, and Hb (iron source), were prepared. A separate standard solution of DMA and *bis*-TTC was also prepared. A typical procedure involved mixing all reagents (except Hb) (19.40890 mmol DMA, 0.00194 mmol *bis*-TTC, 0.97044 mmol D-glucose, and 2.42610×10^{-6} mmol GOx) together in a 50 mL round-bottom flask, keeping the total reaction volume at 9.7 mL. Finally, a stock Hb solution was added to initiate the Bio-Fenton RAFT polymerization of DMA open to air and mark t =0. Samples were extracted for ^{1}H NMR and SEC analysis to monitor monomer conversion and polymer formation. These were diluted immediately with a ~200 mM hydroquinone solution in D_2O (or H_2O) for quenching the reaction.

Semi Bio-Fenton RAFT polymerization of DMA open to air. Different stock solutions of the reagents, i.e. GOx, D-glucose, and ammonium ferrous sulphate (iron source), were prepared. A separate standard solution of DMA and *bis*-TTC was also prepared. A typical procedure involved mixing all reagents (9.70440 mmol DMA, 0.00097 mmol *bis*-TTC, 0.97044 mmol D-glucose, and 2.42610×10^{-6} mmol GOx) except ammonium ferrous sulphate together in a 50 mL round-bottom flask and keeping total reaction volume as 9.7 μL. An aliquot of a separately non-degassed Fe (II) solution (9.70442×10^{-3} mmol) was added to initiate the GOx-mediated Fenton-RAFT polymerization of DMA in the presence of air and mark t = 0. Samples were taken after various reaction times, i.e. 5, 15, 30, 45, 60, and 75 minutes, for

4

^{1}H NMR and SEC analyses to monitor monomer conversion and polymer formation, respectively. These were diluted immediately with ~200 mM hydroquinone solution in D_2O (or H_2O), which was found to be sufficient for quenching the reaction.

Synthesis of UHMW polymers with different chain lengths via Semi Bio-Fenton RAFT. To access different chain lengths, the initial molar ratio of monomer to RAFT agent was modified. The ratio of the Fenton reagents (i.e., ammonium ferrous sulphate and GOx/D-glucose/O_2) was fixed relative to the monomer concentration. These UHMW PDMAs were synthesized at the same dual-catalyst system concentrations without stirring and pre-polymerization incubation time and were performed in 50 mL round bottom flasks in reaction volume scaled of 9.7 mL.

Chain extension experiment in the presence of air. For the chain extension experiment, half of the reaction solution of PDMA with 1×10^6 g mol^{-1} (Table S2, entry 3) was taken, and then 8.7 mL DI water and 500 μL DMA (4.85 mmol) were added into it, which led to monomer conversion values above > 95% when left overnight. No more catalysts, i.e. GOx and Fe^{2+}, were needed. The other half was taken for SLS analysis.

Aminolysis experiment. To determine the existence of the trithiocarbonate moiety in the UHMW polymer chains, 50 μL of ethanolamine was added to the reaction mixture (the solution was split to two batches) of the polymer with targeted DPs = 200,000 & 400,000 (Table 1, entries 5 & 7) after a polymerization time of 6 h. The cleavage reaction was left overnight. The obtained aminolyzed PDMA was isolated by the dialysis, followed by freeze-drying before SLS analysis.

Mimicking GOx-mediated Fenton RAFT polymerization via a Syringe pump. To synthesize an UHMW polymer with molecular weight of ~ 1×10^6 g mol^{-1} via the Fenton RAFT polymerization[27] using a syringe pump, 3 μL of the H_2O_2 solution (30 wt %) was diluted to 1 mL. By employing a degassed 1 mL syringe, this diluted solution was injected into a degassed 50 mL round-bottom flask consisting of monomer, RAFT agent, solvent (i.e. DI water), and ammonium ferrous sulphate. The polymerization time was taken as t=0 when the syringe pump was turned on. It should be noted that ammonium ferrous sulphate was added to the flask after 6 min when first drop of H_2O_2 fell into the reaction mixture. Samples were taken through degassed syringes after various reaction times, i.e. 15, 30, 45, and 60 minutes, for ^{1}H NMR and SEC analyses to monitor monomer conversion and polymer formation, respectively. These were diluted immediately with D_2O (or H_2O), which was found to be sufficient for quenching the reaction.

RESULTS AND DISCUSSION

Preparation of UHMW Polymers *via* Bio-Fenton RAFT Polymerization open to air

Inspired by recent reports,[14-16] we were motivated to push the limits of our recent work (i.e., Bio-Fenton RAFT technique[24]) to prepare molecular weights higher than previously achieved via RDRP with an optimized set of reaction conditions (i.e. $[GOx]_0$ = 250 nM and $[Hb]_0$ = 25 μM) in the presence of air. Access to ultra-high molecular weight polymers via RDRP is a formidable task requiring a careful balance between rates of chain propagation and termination in order to maintain control over polymerization homogeneity.[14] Initially, we

5

carried out the synthesis of PDMA with increasing targeted degrees of polymerization (DP) from 10,000 to 150,000 repeat units (corresponding to theoretical molecular weights of $1 \times 10^6 - 1.5 \times 10^7$ g mol^{-1}) via the Bio-Fenton RAFT system in reaction vessels open to air. This is readily achieved by using different molar ratios of monomer to RAFT agent. For these experiments, we maintained the biological catalyst system (i.e., GOx/Hb) constant relative to the concentration of monomer, i.e. 2 M (Table S1 for details, ESI).

Well-controlled UHMW polymers with high monomer conversions (ca. 90%) were achieved in all cases after 48 h (Fig. 1a). Due to the large size of the UHMW polymers, very low sample concentrations (< 1 mg mL^{-1}) were required for SEC/LS analysis, resulting in differential refractive index (DRI) signals of limited resolution (Fig. S2, ESI) and so light scattering chromatograms are depicted here (Fig. 1a). These polymers exceed the molar mass upper limit of our SEC/LS detector; therefore, static light scattering (SLS) was employed to determine their weight-averaged molecular weight (M$_w$).[28-30] The M$_w$ of the polymers was determined by SLS at 623 nm between angles 30°-150° with various concentrations of each polymer prepared (0.2-2.0 mg mL^{-1}). The M$_w$ and radius of gyration (R$_g$) were determined via a Zimm plot analysis (Table S1, ESI). Fig. 1b illustrates the close agreement of experimentally observed *M*$_w$ values with the theoretical values (*M*$_n$) determined via ^{1}H NMR, further highlighting the well-controlled nature of the Bio-Fenton RAFT process (Fig. 1b). In the Zimm plot Kc/ΔR$_θ$ is plotted against sin^2 (θ/2) + kc, where k is a constant. Fig. 1c and Fig. 1d illustrate the Zimm plots of the UHMW polymers with M$_w$ = 4.010 × 10^6 g mol^{-1} (R$_g$ = 96 nm) and 8.023 × 10^6 g mol^{-1} (R$_g$ = 141 nm),

respectively. The M$_w$ of the UHMW polymers is the intercept of the two lines at c = 0 and θ = 0 in the Zimm plot, obtained as 1/M$_w$. The radius of gyration is determined at zero concentration. Importantly, a M$_w$ value of 1.22 x 10^7 g mol^{-1} was observed for the highest targeted DP material prepared via the Bio-Fenton RAFT, representing ca. 130,500 repeat units.
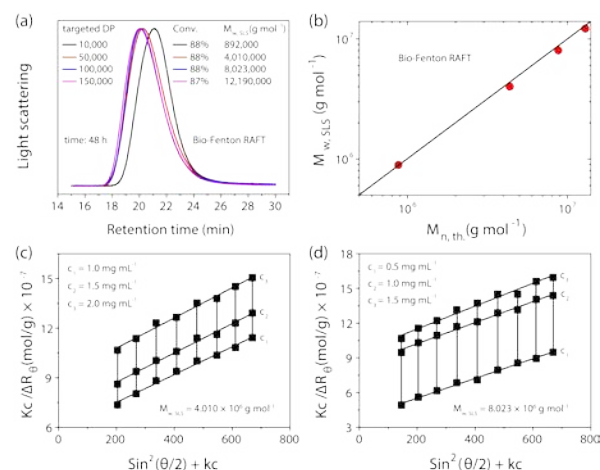

Figure 1 (a) SEC chromatograms of the UHMW PDMAs synthesized in the reaction vessels open to air with different targeted DPs ranging from 10,000 to 150,000 detected by light scattering (LS) detector. (b) Experimental M$_w$ values of the UHMW polymers analyzed by SLS versus theoretical values. (c) Zimm plot of PDMA with M$_{w, SLS}$ = 4.010 × 10^6 g mol^{-1}. (d) Zimm plot of PDMA with M$_{w, SLS}$ = 8.023 × 10^6 g mol^{-1}.

We believed that the slow generation of H$_2$O$_2$, and accordingly OH radicals, by GOx is the key parameter in the synthesis of UHMW polymers via the Bio-Fenton RAFT. In our initially reported Fenton initiation system we were unable to reach DPs higher than 500.[27] To investigate this, we employed a colorimetric assay to determine the rate of hydroxyl radical production in the Hb catalyst system. It should be noted that hydroxyl radicals are the sole source of initiating species in these systems, and hence are the determining factor for the rate of RAFT

6

polymerization. To this end, 3,3',5,5'-tetramethyl benzidine (TMB) was employed as a chromogenic probe, as it has high specificity towards hydroxyl radicals. When GOx with D-glucose was employed to generate H_2O_2 gradually *in situ*, the hydroxyl radical generation via Hb catalysis was seen to be increasing within the reaction time (Fig. 2a). In contrast, at various concentrations of H_2O_2, the production of hydroxyl radicals via Hb catalysis was observed with a saturation/stabilization effect seen within relatively short time scales (Fig. 2b). These observations provide evidence that the slow generation of H_2O_2 by GOx will result in the presence of initiating radicals (i.e. HO•) for longer times, leading to the synthesis of UHMW polymers via Bio-Fenton RAFT polymerization. In fact, due to the lower radical concentration/flux in Bio-Fenton RAFT, the termination reactions are minimized. While, in the normal Fenton-RAFT process,[27] the ultra-fast reaction led to increased termination when the polymer chain lengths became long (DP > 500). With the straightforward preparation of these UHMW materials under ambient, aqueous conditions, we believe that the routine synthesis of UHMW polymers with even higher DPs via the Bio-Fenton RAFT system is foreseeable. However, synthesis of such UHMW PDMAs takes quite a long time (i.e. 48 h) via the Bio-Fenton RAFT system. Therefore, to reduce polymerization time Hb was replaced with ammonium ferrous sulphate, i.e. a synthetic source of Fe^{2+}. With the inorganic source of iron, the GOx–mediated polymerization is termed as Semi Bio-Fenton RAFT.
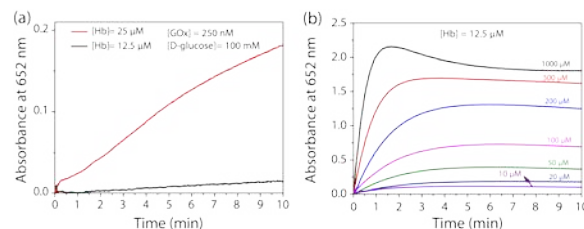

Figure 2 UV/Vis spectroscopic analysis of TMB oxidation by hydroxyl radicals generated via Hb-catalyzed Fenton reaction at λ_{max} = 652 nm: (a) when H_2O_2 is slowly generated by GOx and its concentration gradually increases within the reaction time, and (b) when H_2O_2 loading concentration varies between 10 to 1000 μM.

Preparation of UHMW Polymers *via* Semi Bio-Fenton RAFT Polymerization open to air

UHMW PDMAs with M_n ~ 1×10^6 g mol^{-1} were successfully synthesized via a Semi Bio-Fenton RAFT technique in reaction vessels open to air. At fixed GOx and D-glucose concentrations, different concentrations of ammonium ferrous sulphate (0–10 mM) were attempted to initiate polymerization of DMA ($[DMA]_0$ = 1 M). Pleasingly, with this catalyst system quantitative monomer conversions were observed in less than 2 hours when $[Fe^{2+}]$ was 1 and 3 mM (Fig. 3a; Table S2, entries 3 & 4, ESI), although the latter resulted in polymers of higher dispersity. Pseudo first-order kinetics with regard to monomer conversion was observed as shown in Fig. 3a, and SEC chromatograms corresponding to each polymerization time with clear shift is illustrated in Fig. 3b. The linear trend of ln $[M]_0/[M]_t$ versus reaction time indicates that the concentration of initiating radicals is constant during polymerization time. ^{1}H NMR peaks of PDMA at polymerization times as 5 and 15 minutes and the formula used for the calculation of monomer conversion are included as Figure S3 in the ESI. The Semi Bio-Fenton RAFT system led to polymers with low dispersity values (< 1.1) at higher monomer

conversion values, with experimental molecular weights close to theoretical M_n values (Fig. 3c). With the lowest and highest Fe^{2+} concentrations (i.e., 0.1 and 10 mM) polymerization was limited to 35% and 10% monomer conversion, respectively (Table S2, entries 2 & 5, ESI). While the former continued slowly, eventually reaching ca. 85% conversion, the latter completely ceased when left overnight. When $[Fe^{2+}]$ was 10 mM, $Fe(OH)_3$ salts were seen to precipitate out after 10 minutes, resulting in a lack of Fe^{2+} ions in the reaction mixture, which is thought to result in the low conversion. In the absence of ammonium ferrous sulphate no polymer was obtained (Fig. 3a), demonstrating that Fe^{2+} is required to catalyze the GOx-mediated RAFT polymerization as expected.
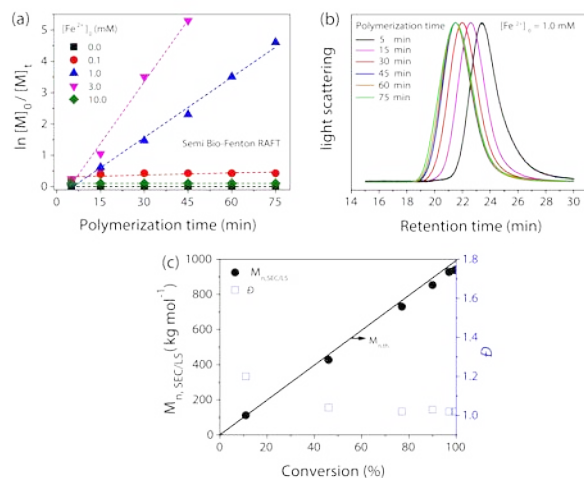

Figure 3 Synthesis of UHMW PDMA with M_n ~ 1000 kg mol^{-1} via the Semi Bio-Fenton RAFT process in the reaction vessels open to air with [GOx] = 250 nM and [D-glucose] = 100 mM. (a) $\ln([M]_0/[M]_t)$ plot against polymerization time at different Fe^{2+} concentrations. (b) SEC chromatograms of the synthesized polymers within polymerization time when $[Fe^{2+}]$ = 1 mM. (c) Plot of M_n and dispersity values vs monomer conversion when $[Fe^{2+}]$ = 1 mM.

To probe the "livingness" of the synthesized UHMW polymers a chain extension experiment was conducted, whereby the prepared UHMW PDMA was secondarily reacted with further monomer. Pseudo-triblock copolymers of PDMA–*b*–PDMA–*b*–PDMA were attained by adding subsequent portions of DMA (and solvent) directly to the PDMA reaction mixtures once the monomer conversion had reached >99% (Table 1, entry 1), again proving the role of gradual production of initiating radicals in the formation of UHMW polymers. The chain extended polymer achieved full monomer conversion after a further 20 h (Table 1, entry 2), and displayed a shift to higher molecular weight species and a symmetrical peak shape when analyzed by SEC (Fig. 4a & b). The Zimm plots of both PDMA and PDMA–*b*–PDMA–*b*–PDMA are illustrated in Fig. 4c. The SLS method showed that molecular weight of the chain extended polymer was 2.160×10^6 g mol^{-1}, which was slightly higher than the theoretical value but still in relatively good agreement (Table 1, Entry 2). It is important to note that unlike the synthesis of multiblock copolymers via traditional RAFT and many other RDRPs,[31-33] no additional initiator or catalyst was required for the chain extension step – i.e., the addition of fresh monomer and solvent was sufficient to continue chain growth. This important finding is due to the efficient catalytic behavior of the GOx/Fe^{2+} system resulting in the continuous supply of initiating radicals in the presence of D-glucose and O_2, compared with the degradative nature of thermal free radical initiators which are depleted throughout the course of a given reaction.
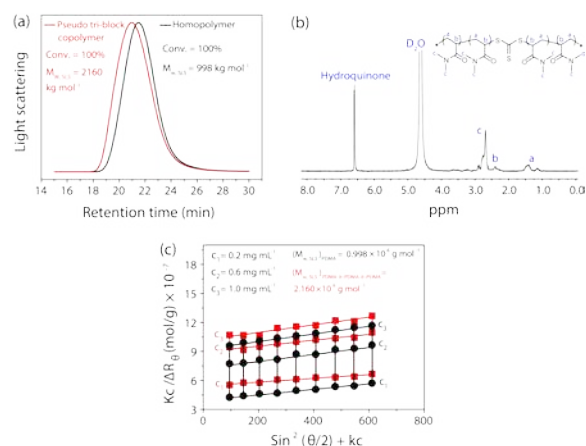
Figure 4 (a) SEC peaks of the homo – and triblock co – polymers synthesized via the Semi Bio-Fenton RAFT polymerization. (b) ^{1}H NMR peak of the chain extended PDMA without any sign of monomer peaks. (c) Zimm plots of the homopolymer and pseudo triblock copolymer obtained from SLS.

Since the synthesis of an UHMW PDMA with M_w ~ 1×10^6 g mol^{-1} via the Semi Bio-Fenton RAFT process were successful, we aimed to push the limits of this polymerization system to make polymers with molecular weights as high as possible. Therefore, polymers with targeted DPs ranging from 10,000 to 400,000 were synthesized, each reaching full monomer conversion within 6 h (Fig. 5a). These were then isolated and characterized via SLS to determine the molecular weights. Fig. 5b and Fig. 5c illustrate the Zimm plots of the UHMW polymers with $M_w = 4.660 \times 10^6$ g mol^{-1} and 1.975×10^7 g mol^{-1}, respectively. The M_w and R_g of the UHMW polymers are reported in Table 1. The obtained experimental results exhibit that molecular weight starts deviating from the theoretical values (towards lower M_w values) when the DP was higher than 200,000 (Table 1, entry 7), indicating an upper limit of our strategy at M_w values around ~ 2×10^7 g mol^{-1}, or chain lengths of 200,000 repeat units. We believe that this break-down may result from

the extremely low RAFT agent concentrations being insufficient to exert control over the chain-growth. Moreover, the high viscosities of the resulting reaction mixtures limits the potential to increase monomer/RAFT agent concentrations. To investigate this, a control experiment in the absence of RAFT agent was performed (Table 1, entry 9). After 4 hours, ^{1}H NMR analysis showed quantitative monomer conversion. SEC/LS analysis displayed almost identical chromatographic peaks for polymers obtained with and without the added RAFT agent when targeting these high DPs (Figure S4, ESI), strongly indicating that both reactions were proceeding via a free radical mechanism only under these conditions. The symmetrical nature of our chosen RAFT agent means it will be embedded at the mid-chain position of the formed UHMW polymers. To investigate this, chain cleavage via aminolysis was carried out on the synthesized polymer with targeted DP = 200,000 ($M_{w, SLS} = 1.975 \times 10^7$ g mol^{-1}). The obtained molecular weight for the cleaved polymer was almost half of the original PDMA (9.440×10^6 g mol^{-1}), proving the presence of TTC at the mid-chain position (Table 1, entry 6; Fig. 5d). This is quite remarkable, given that other researchers have reported difficulties in the use of this "embedded" RAFT agent structure due to steric hindrance.[34] However, aminolysis of the polymer with targeted DP of 400,000 showed a M_w similar to the original polymeric product, as well as almost identical SEC/LS chromatograms (Table 1, entry 8; Fig. S5), further indicating absence of the TTC at the mid-chain position. This straightforward, sustainable, and highly robust technique shows significant potential for the preparation of UHMW designer polymers by controlling the concentration of initiating radicals.

Table 1 Characterization data of the UHMW polymers synthesized via the Semi Bio-Fenton RAFT polymerization.

Entry[a]	Targeted DP	Time (h)	Conv. (%)[b]	$M_{n, th.}$ (g mol^{-1})[c]	$M_{w, SLS}$ (g mol^{-1})	R_g (nm)
1	10,000	2	> 99	992,000	998,000	44
2[d]	Chain extension	20	> 99	1,984,000	2,160,000	65
3	50,000	5	> 99	4,957,000	4,660,000	90
4	150,000	6	> 99	14,870,000	---	---
5	200,000	6	> 99	19,826,000	19,750,000	292
6[e]	aminolysis of entry 5	---	---	---	9,440,000	124
7	400,000	6	> 99	39,652,000	4,528,000	84
8[e]	aminolysis of entry 7	---	---	---	4,489,000	85
9[f]	N/A	4	> 99	N/A	4,512,000	80

[a][DMA] = 1 M, [GOx] = 250 nM, [D-glucose] = 100 mM, and [Fe^{2+}] = 1 mM in all Semi Bio-Fenton RAFT reactions. Reaction volume was set as 9.7 mL. [b]Determined via ^{1}H NMR analysis. [c]Calculated from conversion values using the following formula: $M_{n-th.}$ = DP × Conv. × MW$_{DMA}$ + MW$_{bis\text{-}TTC}$. [d]Chain extended polymer synthesized after adding only DMA and DI water to the reaction solution of entry 1. [e]Ethanolamine was used to cleave the UHMW PDMAs from mid-chain position in the entries 5 and 7. [f]Control experiment without bis-TTC, which led to a free radical polymerization.
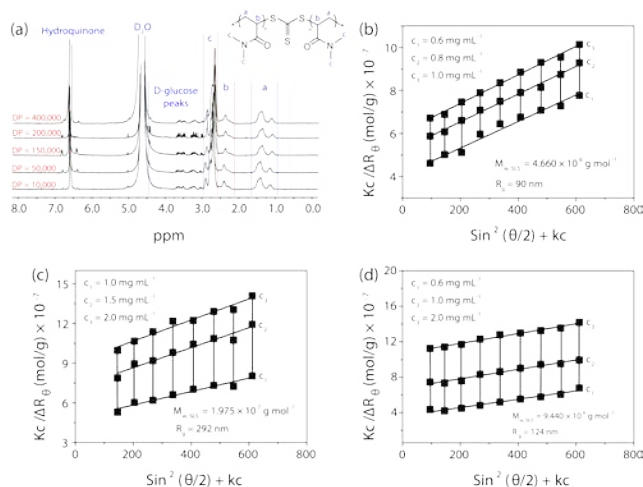

Figure 5 (a) ^{1}H NMR peaks of UHMW PDMAs formed via the Semi Bio-Fenton RAFT polymerization. No sign of monomer peaks was observed. (b) Zimm plot of PDMA with $M_{w, SLS}$ = 4.660 × 10^6 g mol^{-1}. (c) Zimm plot of PDMA with $M_{w, SLS}$ = 1.975 × 10^7 g mol^{-1}. (d) Zimm plot of aminolyzed PDMA with targeted DP = 200,000 ($M_{w, SLS}$ = 1.975 × 10^7 g mol^{-1}) synthesized via the Semi Bio-Fenton RAFT polymerization, which shows $M_{w, SLS}$ = 9.440 × 10^6 g

mol^{-1} for the cleaved polymer.

Preparation of UHMW Polymers *via* continuous Fenton-RAFT Polymerization

To further demonstrate that the controlled production of H$_2$O$_2$, and consequently OH radicals, by GOx during the polymerization is the key to the successful synthesis of UHMW polymers via this technique, we designed an experiment with a syringe pump that injected H$_2$O$_2$ gradually into a *degassed* reaction solution, thus mimicking the function of GOx in the system.[35] To determine the required rate of addition we first monitored the concentration of H$_2$O$_2$ generated by GOx within 120 min (Fig. 6a; Table S3, ESI). The obtained results exhibit that the oxidation process of D-glucose (100 mM) with GOx (250 mM) in the presence of oxygen can produce H$_2$O$_2$ at a rate of approximately 1.5 mM/h. In the absence of GOx, a H$_2$O$_2$ solution (30 wt %) was injected into the polymerization reaction mixture at a rate of 3.64 μmol every 15 min over 1 h in order to synthesize PDMA with M_w ~ 1 × 10^6 g mol^{-1}.[27] Similar to the Semi Bio–Fenton RAFT system, near quantitative monomer conversion was obtained after 60 min (Table S4, ESI). We observed pseudo first-order kinetics for the polymerization with respect to monomer conversion (Fig. 6b), with SEC chromatograms at different polymerization times showing clear shifts towards higher molecular weights (Fig. 6c) with a close agreement between the experimental and theoretical molecular weight values (Fig. 6d). Since the monomer conversion was 4% after 15 min, the bimodality of SEC peak could be from uneven initiation at the beginning of the reaction. The dispersity of the synthesized polymer was ~ 1.2 at the end of reaction. This demonstrates that the core Fenton initiation system can be exploited for

the synthesis of UHMW polymers provided the H_2O_2 and OH radical concentrations are regulated carefully, an effect that is inherent in enzyme cascade reactions such as those employing GOx.
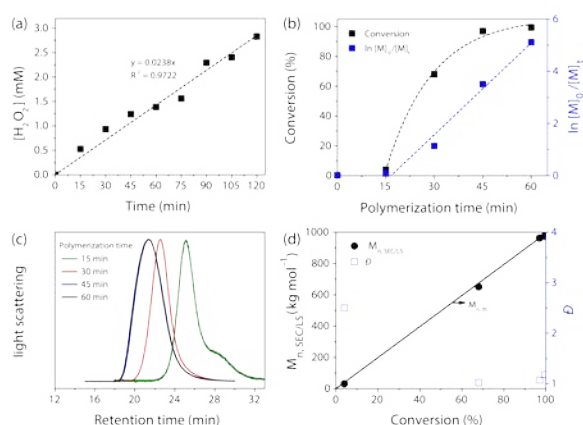

Figure 6 (a) Concentration of H_2O_2 generated by GOx/D-glucose in the presence of oxygen within 120 min.; (b) Monomer conversion and semi-log plot of conversion versus polymerization time via the Fenton RAFT polymerization.; (c) SEC peaks of the synthesized PDMAs at different polymerization times.; (d) Molecular weight and dispersity values of the synthesized polymers at different conversions.

CONCLUSIONS

We have demonstrated the exceptional properties of the Fenton reaction in combination with RAFT for the preparation of unprecedentedly large, well-controlled UHMW synthetic polymers ($M_w \sim 20 \times 10^6$ g mol^{-1}). Several variations of both the iron and hydrogen peroxide sources were investigated, with the excellent results obtained found to be predicated on a controlled generation of the redox reagents, either via enzymatic production or dosing via an external syringe pump. The facile formation of UHMW polymers by using a syringe pump makes this process cheaper and more scalable. Chain extension and chain

cleavage experiments show the high retention of the RAFT agent in the formed polymers, which may be considered somewhat surprising given the "embedded" nature of the trithiocarbonate moiety and the extremely long chain lengths. The slowly controlled addition of initiating radicals could offer unique access to predefined, UHMW polymeric materials via other RAFT systems such as azo initiators – or redox pair – driven processes.

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12

Graphical Abstract Text and Image

Amin Reyhani, Stephanie Allison-Logan, Hadi Ranji-Burachaloo, Thomas G. McKenzie, Gary Bryant, and Greg G. Qiao

Synthesis of ultra-high molecular weight (UHMW) polymers by controlled production of initiating radicals

This research exhibits the importance of the controlled production of initiating radicals in the synthesis of ultra-high molecular weight (UHMW) polymers. Herein, an enzyme (i.e. GOx) and a syringe pump are employed to gradually generate initiating radicals (i.e. hydroxyl radicals here) via Semi/Bio-Fenton-RAFT and continuous Fenton-RAFT polymerizations, respectively. Use of syringe pump as a straightforward and robust approach shows significant potential for the synthesis of UHMW polymers by controlling the concentration of initiating radicals.
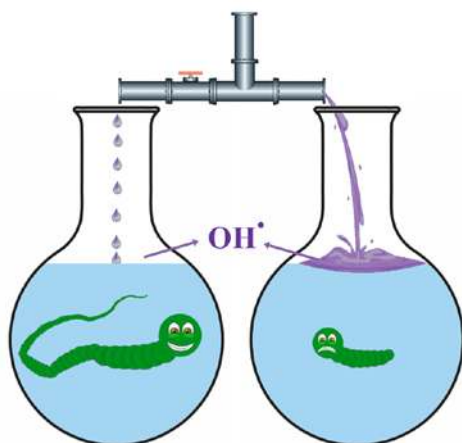
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