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A Combinatorial Library of Photocrosslinkable and Degradable Materials**

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Photocrosslinkable and degradable polymers are finding a broad range of applications as drug-delivery vehicles, tissue-engineering scaffolds, and in the fabrication of microdevices.[1–3] However, the synthesis of multifunctional macromers that form these degradable networks commonly involves multiple functionalization and purification steps, which makes the development of large numbers of polymers with diverse properties difficult. Here, we develop the first combinatorial library of degradable photocrosslinked materials. A library of acrylate-terminated poly(β-amino ester)s was synthesized in parallel via a condensation reaction that combines primary or secondary amines with diacrylates. This library of macromers was then photopolymerized to form degradable networks, with a wide range of degradation times (<1 day to minimal mass loss after three months), mass-loss profiles, and mechanical properties (~4 to 350 MPa). We believe this library approach will allow for the rapid screening and design of degradable polymers for a variety of applications.

The spatial and temporal control afforded during photoinitiated polymerizations has motivated their wide application in the general field of biomaterials.[1,2] For example, photocrosslinkable hydrogels are used for the delivery of cells to injured tissues,[4–8] for the encapsulation and controlled delivery of biological molecules,[9–11] and for controlled fluid flow and cell confinement in microfluidics.[12,13] Additionally, highly crosslinked photopolymers are currently used in dentistry[14] and are being developed as bone-replacement materials[15,16] and for the fabrication of microdevices.[17] Many of these applications are only possible owing to the controlled nature of this type of polymerization. For example, photoinitiated control of polymerization allows for their application as injectable biomaterials[18,19] with a non-cytotoxic polymerization process.[20] Additionally, through use of masks and lasers, the spatial control of the polymerization process allows for unique patterning and construction of complex materials.[21]

Numerous photopolymerizable and degradable materials have been developed, including polyanhydrides, poly(propylene fumarates), poly(ethylene glycol), and polysaccharides,[8,15,16,18] all utilizing multiple reaction and purification steps for synthesis of the photopolymerizable precursors. Despite this work, it has proven challenging to predict specific desirable properties (e.g., degradation and mechanics) from known chemical and structural details of the network precursors. These properties are essential in the design of degradable polymers. For instance, it may be desirable to synthesize a very hard material for some applications (e.g., orthopaedics), whereas a soft material is advantageous for other applications (e.g., tissue adhesive).[22,23] One potential solution to the inability to predict physical behavior is the generation of a higher-throughput approach to rapidly synthesize and screen photopolymerizable libraries of biomaterials. Combinatorial polymer synthesis has been previously performed by numerous investigators[24–27] and has led to the identification of polymers with unique properties. However, this has not been previously performed for the synthesis of photoreactive macromers that form crosslinked and degradable polymers.

To this end, we have synthesized degradable, photocrosslinkable macromers through the conjugate addition of primary or bis(secondary) amines to diacrylates (Fig. 1) to form functionalized poly(β-amino ester)s. Polymerization of the macromer occurs by a step-growth mechanism and the resulting linear macromers contain both esters and tertiary amines in their backbones.[28] Side-chain functionalized polymers can be synthesized by incorporation of functionalized amines or diacrylates (e.g., R_2 or R_3 in Fig. 1). By altering the ratio of the diacrylate to amine, poly(β-amino ester)s with a wide range of molecular weights and end groups can be synthesized. To form crosslinked networks, acrylate terminated poly(β-amino ester)s were readily obtained by performing synthesis with an excess of diacrylate (amine molar ratio of

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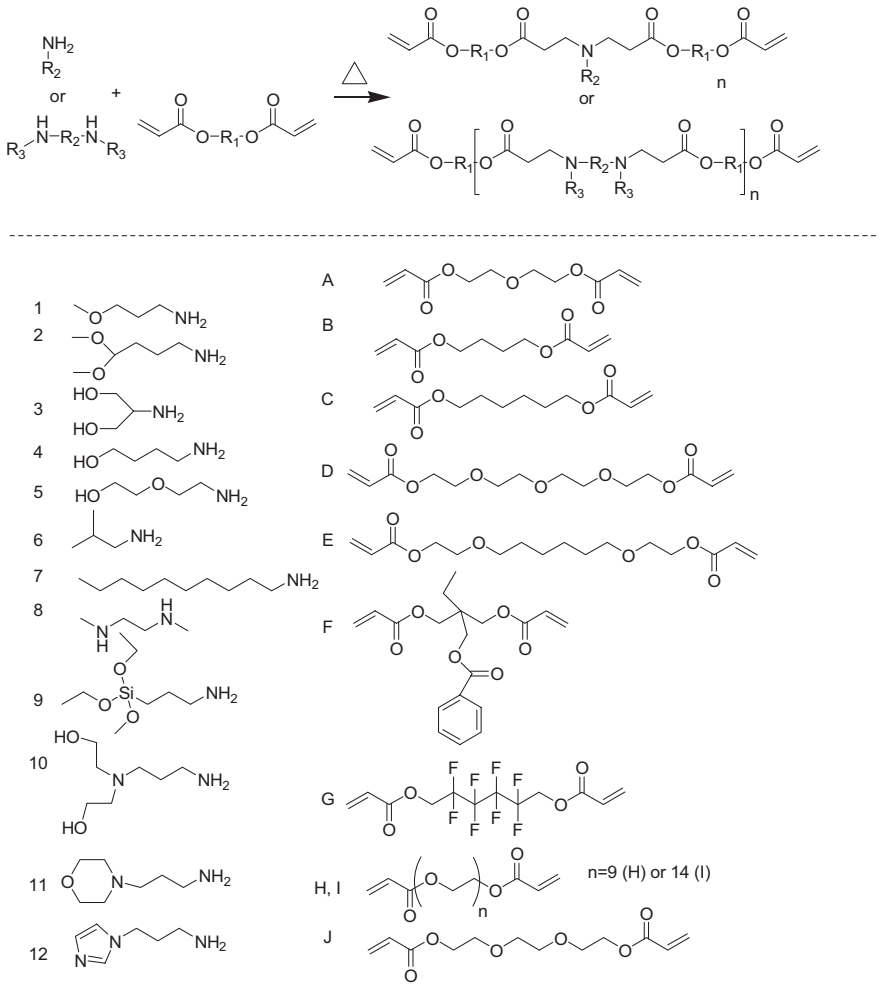

Figure 1. General polymerization scheme and chemical structures. Diacrylated macromers were synthesized by the condensation polymerization of an amine with a diacrylate (top). The various monomers used included 12 amines and 10 diacrylates (bottom) to produce a library of 120 photopolymerizable macromers. The macromers were crosslinked into polymers with exposure to ca. 10 mW cm^{-2} UV light (365 nm) for 5 min.

1.2). After photocrosslinking, the poly(*β*-amino ester) networks degrade under physiological conditions via hydrolysis of their backbone esters to yield small molecule bis(*β*-amino acid)s, diol products, and poly(acrylic acid) kinetic chains. In addition to the simplicity of synthesis, the benefits of this system are: i) amine and diacrylate monomer reagents are inexpensive and commercially available, ii) polymerization can be accomplished without the need for additional protection/deprotection schemes because amines participate directly in the bond-forming processes in these reactions, iii) no byproducts are generated during synthesis, which eliminates the need for purification steps, and iv) the conjugate addition reaction is generally tolerant of additional functionality such as alcohols, ethers, and tertiary amines, which further expands the available amines and diacrylates available for the library.

The library of 120 diacrylate terminated poly(*β*-amino ester) macromers (twelve amines and ten diacrylates reacted at a diacrylate to amine molar ratio of 1.2) was synthesized using the reagents shown in Figure 1. These reagents were chosen to provide chemical diversity, including variations in hydrophobicity.[29] The synthesis of representative macromers was verified using NMR spectroscopy (see Supporting Information, Fig. S1) and gel-permeation chromatography (GPC, see Supporting Information, Fig. S2). The NMR results illustrate the disappearance of the amine protons during macromer synthesis and the prevalence of acrylate components in the final macromer. The GPC results indicate that macromer molecular weights are ca. 2–3 kDa (1 Da = 1.661×10^{-27} kg) with polydispersities of ca. 1.5. Eighty-nine liquid macromers from this library were polymerized into crosslinked and degradable networks of approximately 200 mg, and the degradation behavior was monitored over several months in triplicate (see Experimental). (Several macromers crosslinked during synthesis and were not investigated further; see Experimental for more details.) We characterize degradation as the ability to cleave ester linkages in the polymer networks, which releases network components (i.e., crosslinks, kinetic chains) when immersed in 150 mM phosphate-buffered saline (PBS) while rotating at 37 °C.

The distributions of polymer mass loss at two time points (24 h and 57 days) are shown in Figure 2. The polymers exhibited a wide range of degradation behavior with mass loss of 100 % within 24 h for some networks, while others lost little mass even after 57 days of immersion. As seen in Figure 2a (data included as Supporting Information, Table S1), many of the polymers that had degraded within 24 h (such as D, H, I, and J) were synthesized from diacrylates containing hydrophilic ethylene glycol units. Mass loss was much slower when a more hydrophobic amine (e.g., number 7 in Fig. 1, which contains a long aliphatic chain) was incorporated into the macromer. After 57 days (shown in Fig. 2b), a number of other polymers completely degraded, while others lost only a small amount of their initial mass. These results show a wide distribution of mass loss at these two time points and show that chemical versatility, through unique combinations of amines and diacrylates, plays a role in polymer-degradation behavior. Since variations in polymer-degradation behavior are desired depending on the application, these results indicate that the polymer library could be useful for applications

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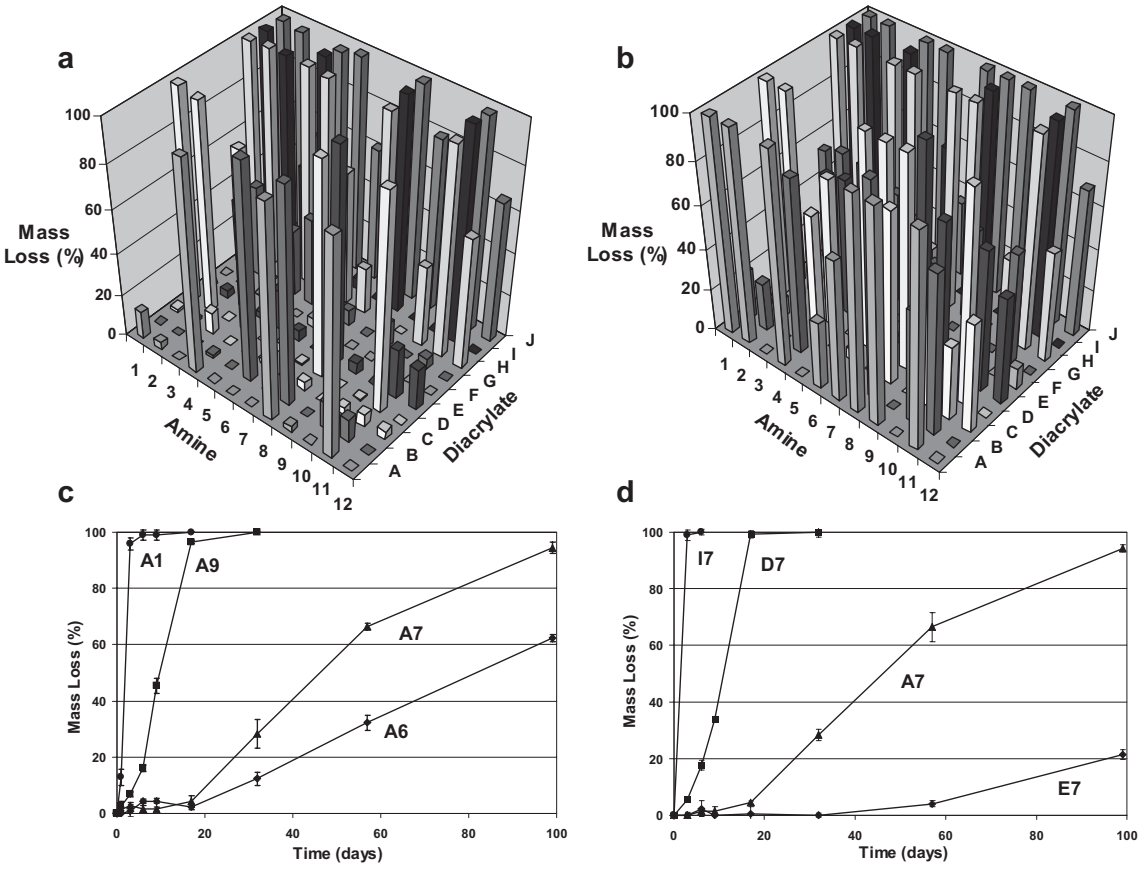

Figure 2. Degradation behavior of polymers fabricated from the macromer library. The mass loss after a) 1 and b) 57 days for the polymers formed from the macromer array synthesized with 12 amines (1–12) and 10 diacrylates (A–J). Additionally, the mass-loss profiles are reported for macromers synthesized with c) one diacrylate (A) and four amines (1, 6, 7, and 9) and d) one amine (7) and four diacrylates (A, D, I, E). Degradation was performed by immersing the samples in PBS on an orbital shaker at 37 °C. These results illustrate the breadth of degradation profiles that are obtained using the macromer library.

where polymers completely degrade very quickly to applications where little mass loss is desirable for extended periods.

The degradation behavior for one specific diacrylate (A) polymerized with several amines and photocrosslinked is shown in Figure 2c. Here, one chemical component was held constant and the other was altered to control the degradation profiles. In this example, polymers degraded in approximately one week (A1), three weeks (A9), three months (A7), and greater than three months (A6). This trend cannot be inferred easily from the chemical structure of the amines indicated in Figure 1, underscoring the advantage of a combinatorial approach for this application. Additionally, one amine (7) was polymerized with several diacrylates and photocrosslinked. The degradation behavior is shown in Figure 2d and illustrates polymers that degrade in approximately one week (I7), two weeks (D7), three months (A7), and greater than three months (E7). This result follows the general trend that polymers formed from the more hydrophilic macromers (e.g., I and D) degraded faster than polymers A (fewer ethylene glycol repeat units) and E (longer aliphatic chain). The overall degradation profiles range from relatively linear mass loss to systems where mass loss is slow at early times and accelerates as network-degradation proceeds. These variations, achieved through simple chemical modifications, illustrate the versatility of this polymer library in tuning or choosing polymers with specific degradation properties. This may prove useful in the identification of degradable polymers for tissue engineering and drug delivery. For example, polymer matrices for tissue engineering ideally degrade slowly enough for sufficient tissue formation on the 3D scaffold, yet rapidly enough so that tissue development is not physically impeded. The release rate of encapsulated drugs from delivery systems is commonly controlled by affecting the degradation rate of encapsulating polymers. We expect this diversity in polymer-degradation times could prove useful in tuning encapsulated drug-release kinetics to a target profile, an important factor in optimizing tissue regeneration.[30]

As the mechanical properties of biomaterials are typically important for medical applications, the library was mechanically characterized. The elastic modulus (E) was determined for ca. 80 members of the polymer library (see Figure 3) using a rapid nanoindentation technique.[31] Within this library sub-

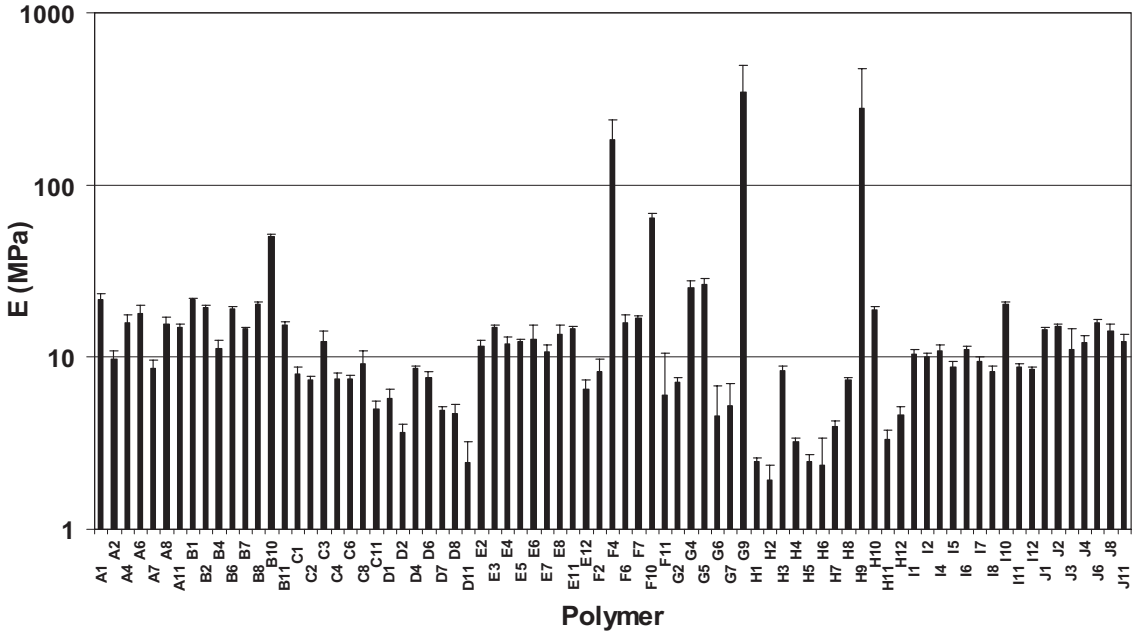

Figure 3. Mechanical behavior of polymers fabricated from the macromer library. The elastic modulus (*E*), determined with a nanoindentation method, is reported for 79 of the candidate polymers from the macromer library. These polymers exhibit a range of *E* ranging from ca. 4 to 350 MPa (note the log scale on the *y*-axis). Error bars represent standard deviation.

set, *E* varied from ca. 4 to 350 MPa with an average modulus of 21.2 MPa (standard deviation of 5.3 % among experiments on an individual polymer). Approximately 95 % of the polymers exhibited *E* within the range of 4 to 25 MPa, which is on the order of moduli for elastomers and non-biodegradable polyurethanes. However, several polymers (e.g., F4, G9, H9) exhibited significantly greater *E*, on the order of moduli for nylon and high-density polyethylene.[32,33] Although it would have been difficult to predict a priori that these specific polymers would exhibit superior elastic stiffness, especially since polymers with similar chemistry had moduli that were much lower, this property may be desirable for certain load-bearing or stress-matching applications. Importantly, mechanical stiffness does not correspond directly to the degradation rate, demonstrating the potential to derive materials from this library with optimal stiffness and degradation behavior independently.

At this point, diversity in polymer mechanics and degradation kinetics has been investigated based on the amines and diacrylates chosen for the macromer library. The available degradation and mechanical properties of the library could be further expanded by adjusting the ratio of diacrylate to amine during macromer synthesis. In this work, the ratio was held at 1.2 for the diacrylate to amine, leading to acrylate-terminated macromers. By decreasing the amount of diacrylate, the macromer molecular weight will increase, since the polymerizing chains will not terminate as quickly with more reactive amines present. Additional flexibility could also be attained by copolymerizing various macromers within the polymer library.

In summary, we have synthesized and characterized the first library of degradable photocrosslinked materials. The large diversity in degradation profiles and elastic moduli demonstrates the potential of this approach in the rapid optimization of material properties. Since crosslinking is radically initiated, these materials may also find non-medical uses as degradable plastics. The chemical diversity presented by these materials could offer other advantages, including potential for specific cellular interactions,[34] modification of toxicity, and the facilitation of drug delivery.[35,36] We believe this combinatorial approach will provide a new method for identification and optimization of degradable and photopolymerized materials.

Experimental

Macromer Synthesis and Characterization: Macromers were synthesized in parallel by mixing the amine and diacrylate in a 10 mL scintillation vial. The vial was reacted while stirring at 90 °C overnight. Samples were stored at 4 °C prior to analysis. The chemical structures and molecular weights of several polymer systems were verified using GPC and ^{1}H NMR spectroscopy (see Supporting Information).

Polymerization and Degradation: The macromers were mixed with the photoinitiator 2,2-dimethoxy-2-phenylacetophenone (DMPA, Sigma, dissolved in 10 wt % methylene chloride) and placed in a vacuum desiccator overnight for solvent removal. The macromer/initiator mixture was placed between two glass slides separated with a 1 mm spacer and polymerized with exposure to ca. 10 mW cm^{-2} UV light (Blak-Ray UV lamp, 365 nm) for 5 min. Polymer slabs (~0.8 cm × 1.2 cm, three per macromer) were cut from the samples, weighed, and placed in tissue-culture cassettes. The cassettes were submerged in 150 mM NaCl PBS and placed on an orbital shaker in a 37 °C incubator for degradation. At each time point, samples were removed, dried, and weighed to determine the mass loss. Samples A3, A5, A10, A12, B3, B9, B12, C5, C7, C9, D3, D5, D9, D10, D12, E10, F3, F5, F8, F9, G3, G8, G10, G12, I3, I9, J5, J7, J9, J10, and J12 cross-

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linked during the polymerization process and were not evaluated in the degradation study.

Mechanical Properties Characterization: Macromers were dissolved at a 1:2 (v:v) ratio in tetrahydrofuran containing 2 wt % DMPA and a spot volume of 10 μL was pipetted onto the surface of an epoxy monolayer-coated glass slide (Xenopore XENOSLIDE E, Hawthorne, NJ) (~18 spots per slide). The tetrahydrofuran was allowed to evaporate for 30–60 min at room temperature. The deposited macromers were then polymerized by exposure to long-wave UV light (Blak-Ray) for 10 min in the presence of argon. They were again vacuum desiccated for at least seven days prior to analysis. Polymer-spot thickness was analyzed via contact profilometry (Tencor P10 Surface Profilometer, San Jose, CA) and was greater than 200 μm for all spots. Nanoindentation was conducted on a pendulum-based nanoindenter (force resolution: 1.5 μN, displacement resolution: 0.1 nm, force maxima: 30 mN, displacement maxima: 4 μm) equipped with a scanning stage (NanoTest600 NT1 and NT0, Micro Materials, Wrexham, UK) and fitted with a spherical indenter of radius $R = 500$ μm. For this contact-based approach it was necessary that polymers adhered well to the underlying slide substrate; polymers not meeting this criterion upon photocrosslinking were excluded from this analysis. Indentations were conducted in load control at a rate of 5 μN s^{-1} to a maximum depth of 600 nm, resulting in maximum loads ranging from 20 to 800 μN and contact strains less than 1 %. This method was previously described in detail in the literature [31]. Load-depth responses were analyzed for E via the method of Field and Swain [37]. Each of the 79 polymers was synthesized and analyzed in triplicate, with three indentations conducted per spot or a total of nine indentations per polymer.

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