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## COMMUNICATION

 $\beta$ -Peptide coatings by surface-initiated polymerization†Li Chen,<sup>ab</sup> Yong Lei,<sup>ab</sup> Abbas G. Shilabin,<sup>†ab</sup> James D. Delaney,<sup>c</sup> George R. Baran<sup>\*b</sup> and Scott McN. Sieburth<sup>\*a</sup>

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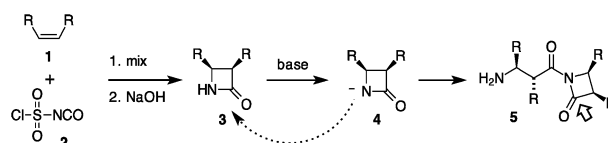
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Homopolymers of  $\beta$ -lactams can be grown by surface-initiated polymerization. These surface-linked  $\beta$ -peptides are living polymers with the potential to be utilized as tunable, protease-resistant interfaces in multiphase structural composites where the characteristics of the interface influence bulk properties.

Biopolymers play many roles, including formation of the structural components of organisms. They can be the structure (cell walls<sup>1</sup>), can guide structure formation (diatom frustule<sup>2</sup>) or can be a minor but critical component of a composite. This last case is exemplified by substances such as seashell nacre where polypeptides present on surfaces of aragonite platelets comprise less than 5% of the composite by weight, and yet contribute to an increase in toughness by several orders of magnitude over the toughness of aragonite alone.<sup>3</sup> Polypeptides can also perform as biocompatible<sup>4,5</sup> or antibiotic surfaces.<sup>6,7</sup> Polypeptides are, however, readily degraded *in vivo* by ubiquitous proteolytic enzymes.

$\beta$ -Peptides, homologs of the naturally occurring  $\alpha$ -peptides, are polymers of  $\beta$ -amino acids that incorporate an additional carbon between the amine and the carbonyl group of the monomers.  $\beta$ -Peptides form all of the secondary structures found with the  $\alpha$ -peptides, yet are highly resistant to proteolytic enzymes.<sup>8,9</sup> We report here the base-catalyzed growth of  $\beta$ -peptide homopolymers on glass and silica particles, by surface-initiated polymerization of readily available  $\beta$ -lactams.

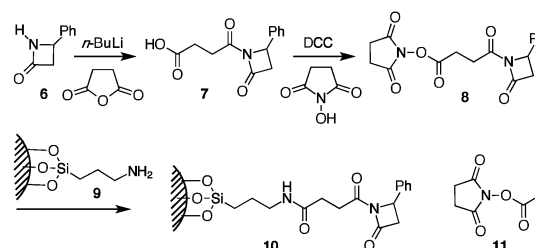
Surface-attached polymers can be formed by linking a pre-formed polymer (“grafting to”) or by growing a polypeptide from a polymerization initiating group secured to the surface (“grafting from”). This latter method, surface-initiated polymerization, is a powerful process for creating a densely coated surface.<sup>10–12</sup> In the case of  $\beta$ -peptide homopolymers,

Scheme 1  $\beta$ -Lactam formation and base-catalyzed polymerization.

the “grafting to” method cannot be used because they undergo self-association to give insoluble oligomers, making grafting impossible. The self association of  $\beta$ -peptides has been a long standing obstacle to the study of these polymers.<sup>13,14</sup> Use of surface-initiated polymerization has the potential to circumvent this solubility issue.

Polymerization of  $\beta$ -lactams **3** directly yields  $\beta$ -peptides without the need for additional activation of the monomer and without byproducts.  $\beta$ -Lactams are readily prepared by cycloaddition of chlorosulfonyl isocyanate **2** with alkenes, Scheme 1.<sup>15</sup> Under basic conditions, attack of deprotonated lactam **4** on another monomer yields *N*-acylated  $\beta$ -lactam **5**. Subsequent attack of **4** on activated  $\beta$ -lactam **5** (arrow) leads to  $\beta$ -peptide formation. Growth of  $\beta$ -peptides under basic catalysis occurs almost exclusively from *N*-acylated  $\beta$ -lactams such as **5**. Gellman<sup>16</sup> and others<sup>14,17</sup> have demonstrated that an added *N*-activated  $\beta$ -lactam can act as an efficient initiating group, leading to low polydispersity. Based on this precedent, we have prepared an activated  $\beta$ -lactam initiating group **10** to study surface-initiated polymerization of  $\beta$ -lactam monomers, Scheme 2.

Treating  $\beta$ -lactam **6**<sup>18</sup> with *n*-butyllithium followed by succinic anhydride gave acid **7**. Condensation of **7** with *N*-hydroxy succinimide then gave bifunctional **8**. While reaction at either terminus would provide a polymerization initiation site,<sup>16</sup> when **8** was treated with a primary amine the hydroxysuccinimide was cleanly displaced. Treatment of



Scheme 2 Preparation of surface-linked polymerization initiating group.

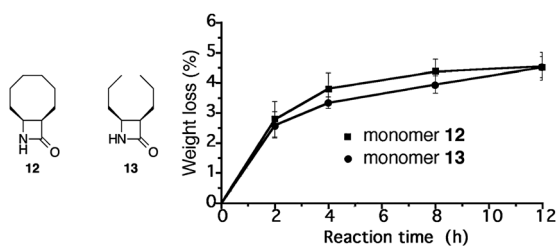
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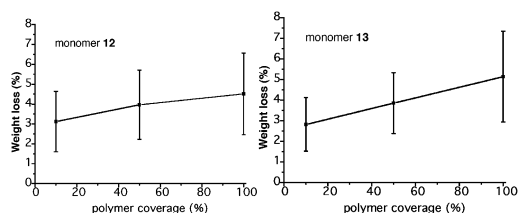


**Fig. 1** Monomers and polymerization weight gain (TGA) with reaction time.

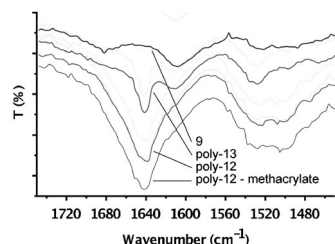
aminopropylsilane coated glass particles **9** (surface area  $10 \text{ m}^2 \text{ g}^{-1}$ , amine coverage<sup>19</sup>  $7.8 \mu\text{mol g}^{-1}$ ) led to polymerization substrate **10**. Reanalysis of surface amine density indicated that  $>90\%$  had reacted.

Two  $\beta$ -lactams were selected for initial studies, **12** and **13**, Fig. 1. Cyclooctane-fused **12** was found by Gellman to give cyclooctane  $\beta$ -peptides that retained solubility, presumably because of limited intermolecular associations. The closely related *cis*-3,4-di-*n*-propyl azetidinone **13** lacks the conformational constraints of the fused cyclooctane ring and its *cis* stereochemistry was expected to yield *n*-octane  $\beta$ -peptide oligomers with conformations suitable for  $\beta$ -sheet interactions.<sup>20,21</sup>

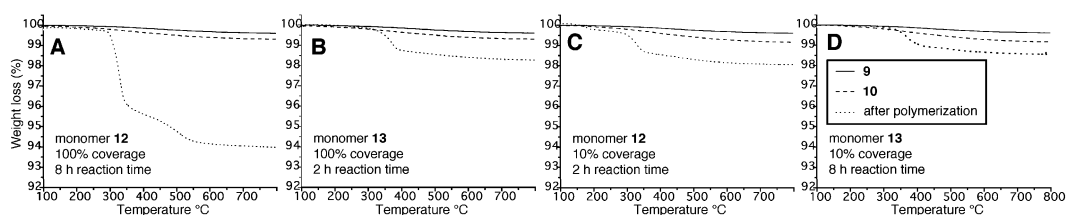
Suspensions of initiator-linked glass particles **10**, combined with  $\beta$ -lactam **12** or **13** (100 equiv., 0.2 M in DMF) at  $0^\circ\text{C}$ , were treated with lithium hexamethyldisilazide (15 equiv., 1 M in THF) and then quenched after a predetermined time with sat.  $\text{NH}_4\text{Cl}$ . The treated glass was washed three times each with THF, water, acetone, chloroform and acetone, and then dried overnight at ambient temperature under vacuum.



**Fig. 2** Weight gain as a function of surface coverage.



**Fig. 3** IR spectra of aminosilylated silica (**9**) and after polymerization of **12** and **13**.



**Fig. 4** TGA analysis of four experiments surveying monomers **12** and **13**, reaction time and surface density of the  $\beta$ -peptide.

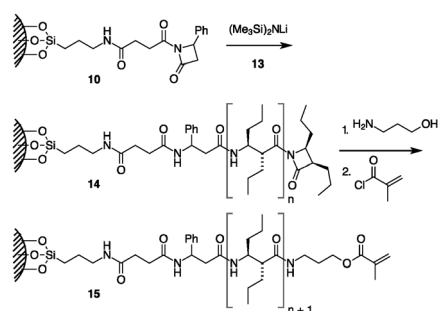
Reactions run in triplicate for 2, 4, 8 and 12 h were analyzed by TGA and the results are shown in Fig. 1. Extensive washing of the product was undertaken to ensure that only covalently attached polymer was retained. Polymers of **12** are known to have good solubility properties,<sup>16,22</sup> while **13** would be expected to have limited solubility. Nevertheless, the average weight gain with reaction time increased similarly for both monomers.

Treatment of the glass particles with the initiator linking unit **8** diluted by succinimidyl acetate **11** (**8/11** 1 : 1 and 1 : 9) gave 50% and 10% initiator coverage, respectively.<sup>10</sup> Polymerization of monomers **12** and **13** was then conducted with the resulting glass using 8 h reaction times, under conditions identical to those described above. The results are shown in Fig. 2. For these reactions, run in triplicate, polymerization of monomer **12** gave an average of 5.2% weight gain for the silica particles without dilution of the initiator. At 50% and 10% coverage the weight gains were 4.8% and 4.5%, respectively. The change in weight gains for monomer **13** at 100%, 50% and 10% coverage were 5.1%, 3.9% and 2.8%, respectively. In each case, the same quantities of monomer and base were used, e.g., 200 equiv. and 1000 equiv. of monomer relative to initiator for 50% and 10% initiator coverage, respectively. The nonlinear polymer weight gains with decreasing initiator coverage may be due to an increase in the length of the polymer chains with an increase in the monomer/initiator ratio. This would be consistent with the work of Gellman, *et al.* in their solution studies of monomer **12** polymerization, where diminishing quantities of initiator gave a corresponding increase in polymer length.<sup>16,17</sup>

The carbonyl region of four infrared spectra are shown in Fig. 3 using identically treated silica (*vide infra*). Little absorption is seen for the aminosilylated silica **9**. After attachment of the initiator and polymerization of monomers **12** and **13**, significant absorptions are seen for characteristic amide I (*ca.*  $1640 \text{ cm}^{-1}$ ) and amide II ( $1520\text{--}1300 \text{ cm}^{-1}$ ). Little change was noted after derivatization with methacrylate. This is unsurprising as the amides far outnumber the esters and have a larger extinction co-efficient.<sup>23</sup>

It is notable that the carbonyl absorbances for poly-**13** are much less broad than poly-**12**. This is consistent with poly-**13** having extensive hydrogen bonding interactions, whereas poly-**12** would not.<sup>16</sup>

The reaction parameters were surveyed using commercial aminopropyl silane coated silica (surface area  $4.5 \text{ m}^2 \text{ g}^{-1}$ , amine coverage  $6.5 \mu\text{mol g}^{-1}$ ) and a fractional factorial experimental design, Fig. 4. In these four experiments, TGA of the commercial aminopropyl silane coated silica **9** found *ca.* 0.3% organic material. A small increase in the volatiles was observed after derivatization of the surface amino groups with



**Scheme 3** Elaboration of the living polymers.

**8** and **8/11** mixtures. With cyclooctane monomer **12**, 100% initiating group coverage and 8 h polymerization time, Graph A, an additional 5.4% surface organics resulted from base-catalyzed polymerization. When the initiator coverage was reduced to 10% and the reaction time was reduced to 2 h, Graph C, the added cyclooctane polymer was lowered to 1.1%. Switching to monomer **13**, with 100% initiator coverage and 2 h reaction time, Graph B, the additional polymer was 1% of the silica. Returning to 8 h reaction time, with 10% initiator coverage, Graph D, polymerization of monomer **13** gave 0.7% polymer weight.

The polymerization reaction is depicted in Scheme 3. A suspension of particles coated with initiator (**10**) is treated with a large excess of monomer **12** or **13** and a catalytic amount of strong base (lithium hexamethyldisilazide). In the case of **13**, attack of the  $\beta$ -lactam monomer anion on the activated  $\beta$ -lactam in **10** leads to ring opening and generation of a new activated lactam terminus. A second monomer anion will attack the terminal carbonyl and generate **14** ( $n = 1$ ). Termination of the polymerization by depletion of the monomer or by quenching the process with mild acid will leave a reactive terminal  $\beta$ -lactam **14**.

A variety of nucleophiles can be used to open the  $\beta$ -lactam in **14**. Scheme 3 illustrates the use of 3-aminopropanol. Reaction of the nucleophilic amine followed by capping of the resulting terminal alcohol with methacryloyl chloride leads to an acrylate-terminated  $\beta$ -peptide. Treatment of the silica- $\beta$ -peptide particles in this way leads to additional weight as determined by TGA, of about 0.3%. The incorporation of these silica- $\beta$ -peptide-methacrylate particles into composites and the resulting properties of these composites will be reported elsewhere.

$\beta$ -Peptides are outstanding surrogates for naturally occurring  $\alpha$ -peptides, forming secondary and tertiary structures that effectively mimic the natural products but are also stable towards proteolytic enzymes. We have found that homopolymers of  $\beta$ -lactams can be grown from surfaces functionalized with

$N$ -acylated  $\beta$ -lactam initiating groups. It appears that utilizing this “grafted from” technology can overcome the propensity of  $\beta$ -peptides to precipitate as insoluble matter.

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## Notes and references

§ In this example, lactam **13** and its polymer are depicted as a single enantiomer for simplicity. While it is very likely that the details of polymerization and the properties of the polymer derived from the racemic monomer will be different from that derived from a single enantiomer, the ability of the polymer produced from 3,4-*cis* disubstituted  $\beta$ -lactams such as **13** to adopt a  $\beta$ -sheet compatible conformation should be similar in both cases. The use of racemic  $\alpha$ -amino acids to prepare  $\beta$ -sheets has been addressed theoretically<sup>24</sup> and experimentally.<sup>25</sup>

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