SUPPORTING INFORMATION

Synthetic oral mucin mimic from polymer micelle networks

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Synthesis of amphiphilic diblock copolymers (mPEG-PLA). Methoxy-poly(ethylene glycol-block-lactic acid) (mPEG-PLA) was synthesized by ring opening polymerization (ROP) of D-lactide with mPEG (molecular weight (MW) ~5,000 Da) using stannous octoate (1% w/w) as a catalyst; adopting the procedure from Simone et al. (Figure S1(a)). The reactant mixture (mPEG and D-lactide) was preheated (140°C, for 2 h, under N₂ atmosphere) to remove any traces of water. The catalyst was added upon reduction of reactant temperature to 120°C and ROP was allowed to proceed for 6 h.

Diblock copolymers of different polymer amphiphilicities were synthesized by altering the hydrophobic (PLA) block molecular weight (target PLA MW were ~10,000 Da, 20,000 Da, 32,000 Da, 50,000 Da, 60,000 Da and 90,000 Da), while keeping the hydrophilic (mPEG) block (MW ~5000 Da) constant. This was achieved by altering the stoichiometric molar ratio of mPEG:D-Lactide used in the ROP reaction above. Diblock copolymers of different PLA block MWs, and therefore different amphiphilicities were used to synthesize filamentous or spherical micellar morphologies. In our current studies a diblock polymer with low PLA MW of ~20,000 Da (mPEG5k-PLA20k) was used to synthesize spherical micelles. On the other hand, a diblock polymer of relatively high PLA MW of ~50,000 Da (mPEG5k-PLA50k) was used to synthesize filamentous micelles.

Purification and Characterization of mPEG-PLA copolymers. The synthesized diblock copolymers were purified by dissolving in dichloromethane (DCM) and re-precipitation twice in cold diethyl ether. Upon purification, the polymer molecular weights were determined using gel permeation chromatography (GPC). GPC showed an increase in polymer MW with increasing molar ratio of mPEG:D-Lactide (Figure S1(b)). The purified diblock copolymers were characterized using Fourier transform infrared (FTIR) spectroscopy. The presence of mPEG and
PLA blocks was confirmed from their respective methylene and carbonyl peaks in FTIR spectra. The peaks near 2700-3100 cm\(^{-1}\) and 1450 cm\(^{-1}\) (\(-\text{CH}\) stretching from \(-\text{CH}_2\) groups) were contributions from methylene groups of the PEG. The peak at 1750 cm\(^{-1}\) were contributions by the carbonyl stretching in the ester groups of the PLA block (Figure S1(c)). The polymers were further characterized using proton nuclear magnetic resonance (\(^1\text{H}-\text{NMR}\)) spectroscopy, where the proton contributions from PLA block (-\(\text{CH}\) ~5.2 ppm and -\(\text{CH}_3\) ~1.5 ppm) and PEG block (-\(\text{CH}_2\) ~3.6 ppm) (Figure S1(d)). Therefore, from GPC results in conjunction with FTIR and GPC characterization verified the formation of the diblock copolymer.

**Synthesis of biotin functionalized diblock copolymer (biotin-PEG-PLA).** Biotinylated poly(ethylene glycol-block-lactic acid) (biotin-PEG-PLA) was synthesized by covalently coupling biotinylated poly(ethylene glycol)-amine (biotin-PEG-NH\(_2\), MW ~5,000 Da) with acid-terminated poly(lactic acid) (PLA-COOH, MW ~18,000-24,000 Da) (Figure S2(a)). The reactant polymers (biotin-PEG-NH\(_2\) and PLA-COOH) were dissolved in DCM (polymer concentration ~2% w/v) and kept sealed under a nitrogen atmosphere. N-N’-dicyclohexylcarbodiimide (DCC) was added dropwise (PLA:DCC molar ratio ~1:2) to the polymer solution and the reaction was allowed to proceed for 24 hours.

**Purification and Characterization of biotin-PEG-PLA copolymer.** The reaction mixture was filtered to remove by-products (like dicyclohexylurea) formed during the course of reaction, followed by precipitation twice in cold diethyl ether. Any trace solvent residue in the precipitated product was removed by rotary evaporation. Further, to remove any unreacted biotin-PEG-NH\(_2\) chains from the desired biotin-PEG-PLA product, the obtained polymer was dissolved in acetone and precipitated twice in cold deionized water. The precipitate was centrifuged to collect the
product, followed by lyophilization to remove any residual water. The purified product (biotin-PEG-PLA) was characterized using GPC and $^1$H-NMR.

The presence of biotin was quantified using the 2-(4-hydroxyphenylazo) benzoic acid (HABA)-avidin assay. To the HABA-avidin working solution prepared as outlined by Shuvaev et al., the biotin-PEG-PLA copolymer was added. The replacement of weak HABA-avidin intermediate into a more stable biotin-avidin complex results in release of free HABA, measured from decrease in absorbance ($\lambda$=500 nm) using a spectrophotometer (Cary WinUV). From a biotin calibration curve of known concentrations, the extent of biotin conjugation onto diblock copolymers can be calculated.

GPC MW characterization of biotin-PEG-PLA showed a subtle change in weight-average MW from PLA-COOH (Figure S2(b)). $^1$H-NMR more readily verified the conjugation of biotin-PEG with PLA-COOH from the proton contributions from PEG (-CH$_2$ ~3.5 ppm) and PLA blocks (-CH ~5.2 ppm and -CH$_3$ ~1.4 ppm) on purified biotin-PEG-PLA copolymer Figure S2(c). The PEG peak verifies the presence of biotin in the diblock, as the peak contributions from the small biotin molecules were not clearly resolvable through $^1$H-NMR. Biotin accessibility and activity in diblock copolymer was quantified through the colorimetric HABA-avidin assay (Figure S2(d), which confirmed that nearly all the PLA chains were conjugated with biotin-PEG (~100% conjugation).

**Radiolabeling streptavidin.** Protein streptavidin was radiolabeled ($^{125}$I-Streptavidin) using Iodogen® iodination reagent. Iodogen® (1,3,4,6-tetrachloro-3$\alpha$-6$\alpha$-diphenylglycouril) was added to a glass tube (2 mg/ml in chloroform, 200 $\mu$l) and was evaporated off using dry nitrogen gas to form a thin coat over the tube wall. To the Iodogen® coated test tube, streptavidin (1mg/ml in PBS, 100 $\mu$l) and radioactive iodine ($^{125}$I) were added (~30 $\mu$Ci) and incubated for at least 5
minutes enabling iodination of tyrosyl groups in streptavidin. The free iodine was removed from radiolabeled streptavidin \((^{125}\text{I}}\text{-Streptavidin}) by centrifugation of the solution through gel filtration columns (Biorad, Hercules, CA, Biospin® 6 Tris columns).

To estimate free iodine and thereby the amount of radiolabel incorporated in protein streptavidin, a trichloroacetic acid (TCA) precipitation method was used. To the radiolabeled streptavidin (1 mg/ml in PBS, 2 μl), TCA (20% w/v in DI water, 1 ml) and bovine serum albumin (BSA) (5% w/v in PBS, 1 ml) were added and incubated for at least 10 minutes. TCA addition readily precipitates the protein (BSA and radiolabeled streptavidin) from supernatant containing free iodine. The precipitate was pelletized by centrifugation and the supernatant was collected. By measuring radioactive gamma counts (Perkin Elmer 2470 Wizard2) from the supernatant and precipitate separately, the extent of free iodine and the amount of radiolabel incorporated was determined.

**Evaluation of non-specific network growth from biotin-micelle depositions without complementary streptavidin additions.** By monitoring biotin-micelle depositions without streptavidin would help determine whether biotin moieties by themselves would cause intermicelle complexation and grow into networks. To examine such a possibility and to see if non-specific interactions significantly affect network formation, LBL networks were grown using biotinylated spherical micelles (biotin-SM) without streptavidin. These networks were formed by adopting a similar procedure as described under micelle-LBL network formation, but without providing intermittent streptavidin additions. Biotin-SM (without streptavidin additions) showed poor ability to form networks without the affinity-based crosslinker streptavidin (Figure S6). This observation was visually confirmed via fluorescence imaging (Figure S7), suggesting an insignificant impact of non-specific interactions on network formation.
Evaluating non-specific crystal violet staining on micelle-LBL networks without any bacterial growth. A similar procedure was adopted as described under bacterial growth quantification using crystal violet assay. 7, 10, and 14 layered micelle-LBL networks (SM-LBL, FM-LBL), which possess relatively higher network-surface coverage was used. These micelle-LBL were subjected to crystal violet staining without addition of bacteria to evaluate the possibility of non-specific staining of the micelles. The staining of these FM-LBL and SM-LBL networks were compared to those of control substrate surfaces (polystyrene surface) with and without any bacterial growth (Figure S8). The extent of crystal violet staining was measured via absorbance at wavelength, λ = 600 nm (BioTek Synergy Mx, Gen5 2.0, Winooski, VT). The control substrates with bacterial growth (no-networks) showed a significantly higher crystal violet staining. SM-LBL and FM-LBL networks without bacteria showed a slightly elevated level of staining compared to blank substrates (also without bacteria). Although, this effect was statistically insignificant, it was accounted for in the % bacterial growth calculation, as given below,

\[ \% \text{ Bacterial Growth} = \frac{(\text{Abs}_{\text{LBL(bacteria)}} - \text{Abs}_{\text{LBL(no-bacteria)}})}{(\text{Abs}_{\text{Substrate(bacteria)}} - \text{Abs}_{\text{Substrate(no-bacteria)}})} \times 100 \]
SUPPORTING FIGURES

a) \[
\begin{align*}
\text{mPEG} & \quad \text{+} \quad \text{Lactide} \quad \xrightarrow{\text{Sn(Oct)}_2 \quad 120^\circ \text{C}} \\
mPEG-b-PLA
\end{align*}
\]

b) 

![Graph showing molecular weight distribution](image)

| Diblock Polymer | Mn | Mw | PDI |
|-----------------|----|----|-----|
| mPEG5k          | 7619| 7962| 1.045|
| mPEG5k-PLA10k   | 14457| 19006| 1.315|
| mPEG5k-PLA20k   | 19483| 32056| 1.65 |
| mPEG5k-PLA50k   | 33343| 50884| 1.53 |

d) 

- mPEG5k-PLA20k
- mPEG5k-PLA50k

- C-H Stretching from CH₂ group (from mPEG block)
- Carbonyl peak in ester (from PLA block)
- Methylene group (from mPEG block)
d) $^1$H NMR (CDCl$_3$, 400 MHz)

mPEG

mPEG$_5k$-PLA$_20k$

PEG block (-CH$_2$)

PLA block (-CH$_2$)

Lactide

mPEG$_5k$-PLA$_50k$

PEG block (-CH$_2$)

PLA block (-CH$_3$)
**Figure S1.** Synthesis and characterization of amphiphilic mPEG-PLA diblock copolymers (a) Reaction scheme for mPEG-PLA diblock copolymer synthesis, via ring-opening polymerization (ROP) of D-Lactide with mPEG (MW~5,000 Da) using catalyst stannous 2-ethylhexanoate (b) GPC MW characterization of synthesized mPEG-PLA diblock copolymers. GPC plot shows increase in polymer molecular weight with increasing PLA block length modifying the diblock amphiphilicity. (c) FTIR characterization of mPEG-PLA polymers confirming the presence of both PEG and PLA blocks by detecting methylene and carbonyl groups respectively. (d) $^1$H-NMR characterization of mPEG-PLA corroborates the formation of diblock copolymer, from the PEG peaks (-CH$_2$ protons) and PLA peaks (-CH$_3$ and –CH protons).
a) Biotin-Poly(ethylene glycol)-NH₂ + Poly(lactic acid) (Carboxylic acid terminated) → Biotin-Poly(ethylene glycol)-Poly(lactic acid) (Biotin-PEG-PLA)

d) Calibration

| Polymer   | Mn  | Mw  | PDI |
|-----------|-----|-----|-----|
| Biotin-PEG-PLA | 17958 | 28887 | 1.56 |
| PLA       | 17547 | 27206 | 1.55 |
| Biotin-PEG | 6654  | 6966  | 1.05 |

Retention time (min)
Figure S2. Synthesis and characterization of biotin-functionalized diblock copolymers (a) Reaction scheme for biotin-PEG-PLA diblock copolymer synthesis, showing DCC assisted
coupling of carboxylic acid groups of PLA-COOH (MW ~17,500 Da, as per GPC) with amine groups of biotin-PEG-NH₂ (MW ~5,000 Da) to form biotin-PEG-PLA (b) GPC MW characterization of synthesized biotin-PEG-PLA copolymer. Changes in PLA MW from biotin-PEG coupling can only be seen as a subtle change in weight-average molecular weight. (c) ¹H-NMR characterization of biotin-PEG-PLA verifies the formation of diblocks from the PEG peaks (-CH₂ protons) and PLA peaks (-CH₃ and –CH protons). Contributions from biotin protons could not be readily deduced, a result of overwhelming PEG and PLA protons contribution present in the polymer. (d) Detection and quantification of biotin in biotin-PEG-PLA copolymer using the HABA-avidin colorimetric assay. A near ~100% conjugation was estimated which indicates absence of any free PLA chains (PLA without biotin).

**Figure S3.** Visualization of nile red-loaded filomicelles using fluorescence microscopy under TRITC channel using a 20x objective.
Figure S4. Evaluating wettability of FM-LBL networks using sessile drop-contact angle measurement. A significantly lower contact angle (°) was observed when the surface was modified with a FM-LBL network. Also, the contact angle plateaued at ~29° for number of layers ≥ 5.
Figure S5. Confocal laser scanning micrographs of micelle-LBL networks made with curcumin-loaded filomicelle and is visualized for its network homogeneity.
Figure S6: Micelle-LBL network growth using spherical micelle (SM-LBL). Deposition of biotinylated-spherical micelle (Biotin-SM) and the protein streptavidin resulted in layer-by-layer network formation. On the contrary, layer-by-layer deposition of Biotin-SM without its affinity-based crosslinker streptavidin could not form networks. Similarly, layer-by-layer deposition of streptavidin and spherical micelles without biotin moieties showed inability to form networks. These control systems illustrate the significant role played by the affinity-based biotin-streptavidin crosslinkers for network growth.
**Figure S7:** Fluorescence micrographs showing the effect of affinity-based moieties (biotin, streptavidin) and number of layer additions (NoL) performed during micelle-LBL network formation process on polystyrene substrates. Control layer-by-layer (LBL) depositions of Biotin-SM without streptavidin, and micelles without biotin moieties with streptavidin additions were performed separately. These controls lacked either biotin or streptavidin affinity moieties, hence insignificant network formation trends were observed even with increasing NoL. This is unlike the LBL depositions of biotin-SM and streptavidin that showed significant LBL growth because of their ability to effectively crosslink via the biotin-streptavidin affinity linkages.
Figure S8: Crystal violet staining of LBL networks of spherical (SM-LBL) and filamentous (FM-LBL) micelles without bacteria, to determine the possibility of non-specific crystal violet staining of the micelles. 7, 10, and 14 layered LBL networks that possess relatively higher network-surface coverage were used. SM-LBL and FM-LBL networks without bacteria showed a slightly elevated level of staining compared to blank substrates (also without bacteria). However, blank substrates with bacterial growth showed a significantly higher staining compared to networks and blank substrate without bacteria. This clearly indicates the specificity of crystal violet for staining bacterial components. A 2-sample t-test performed using a statistical software (Minitab), found differences between networks and substrate (without bacteria) to be insignificant, while between systems (with and without bacterial growth) to be significant. The differences were considered significant (*) only when p-value < 0.05.
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http://www.piercenet.com/instructions/2160485.pdf