Molecular Weight Tuning Optimizes Poly(2-Methoxyethyl Acrylate) Dispersion to Enhance the Aging Resistance and Anti-Fouling Behavior of Denture Base Resin

– Supplemental Appendix –

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1. Experimental section

1.1. Synthesis of rhodamine-labeled PMMA (PMMA-Rh)

In a round bottom flask, methacryloyl thiocarbamoyl rhodamine-B (0.01mol%), methoxy methacrylate, chain transfer agent i.e., methyl mercaptopropionate (MMP) and AIBN were dissolved in acetonitrile to make the concentration of monomer ~2M. The reaction mixture was sealed and purged with nitrogen gas for 45mins and then was immersed in an oil bath at 70ºC. The reaction was allowed to stir at 70ºC for 16 h after which, the reaction was cooled in dry ice/acetone bath to stop the polymerization and the reaction was exposed to the air. The solvent was evaporated, and the viscous solution was added dropwise in to rapidly stirring cold hexane. Purification was carried out by redissolving the precipitated polymer in dichloromethane and adding it to cold hexane three times. The polymer was dried under high vacuum overnight to yield pure product.

PMMA-Rh (representative polymer) **^{1}H NMR ($CDCl_3$):** ð 3.7 (s,3H C*H*$_3$OCOCH$_2$CH$_2$S-), ð 3.65 (b,120H C*H*$_3$OCO-), ð 3.0-2.5 (m, 7H chain transfer agent and end group), ð 2.1-1.7 (br, 80H, -C*H*$_2$C(CH$_3$)- backbone), ð 1.1-0.8 (br, 120, -CH$_2$C(C*H*$_3$)- backbone)

1.2. Protein adsorption

The initial protein adsorption on resin surfaces was evaluated according to previous studies.[1, 2] The samples (disc-shaped, diameter: 15 mm, thickness: 2 mm) were soaked into PBS at room temperature for 1 h. Then the samples were immersed into bovine serum albumin (BSA) broth (2 mg of protein/mL of PBS, 100 µL) and incubated at 37 °C in 5% CO_2 for 4 h. After that, the samples were washed twice with PBS to remove unadhered protein. Then, micro-bicinchoninic acid (200 µL; Micro BCA™ Protein Assay Kit) was added to samples and incubated at 37 °C for 30 min to measure the amount of protein adhered to resin surfaces. Use a micro-plate reader (Epoch) to measure the absorbance at 562 nm to evaluate the total amount of adhered protein.

2. Result

2.1. Polymer synthesis and characterization

PMEA-1 ^{1}H NMR ($CDCl_3$): ð 4.24 (br, 18H, -COOC*H*$_2$CH$_2$OCH$_3$), ð 3.69 (s, 3H C*H*$_3$OCOCH$_2$CH$_2$S-), ð 3.58 (br, 17H, -COOCH$_2$C*H*$_2$OCH$_3$), ð 3.36 (br, 26H COOCH$_2$CH$_2$OC*H*$_3$), ð 3.0-2.5 (m, 7H chain transfer agent and end group), ð 2.5-2.2 (br, 9H, -CH$_2$C*H*- backbone), ð 1.7 (m, 21, C*H*$_2$CH- backbone).

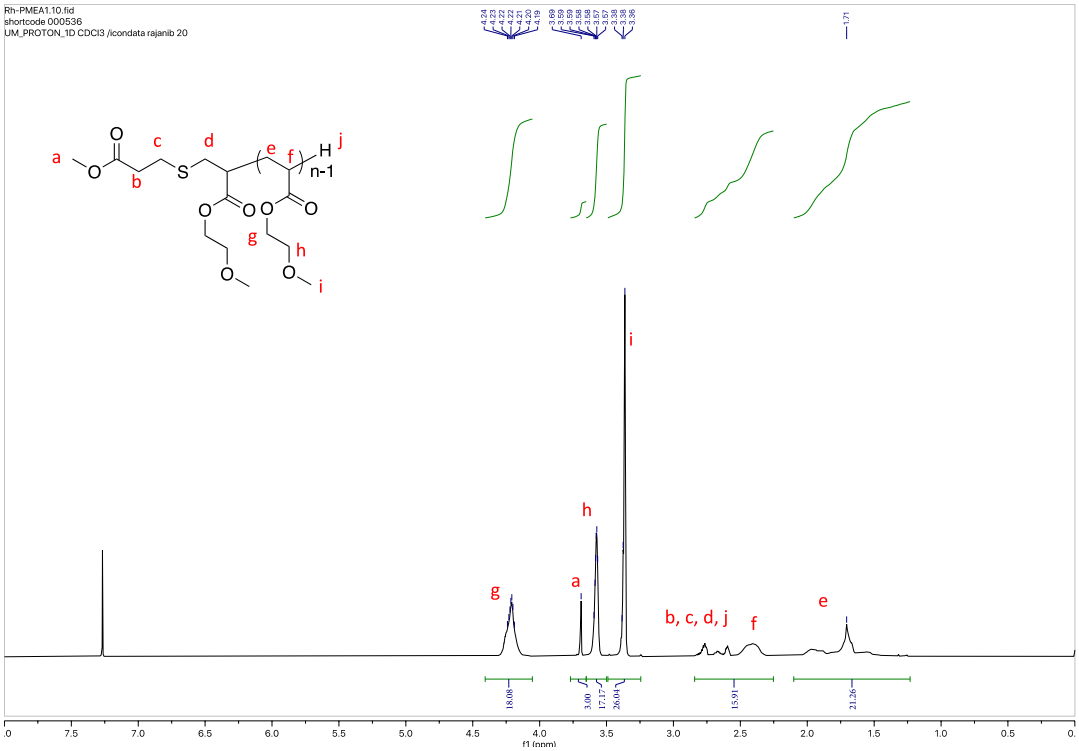

Figure S1. ^{1}H NMR spectrum of PMEA-1 in $CDCl_3$**.** The degree of polymerization was determined by comparing the integrated areas from the peak from OC*H*$_3$ of the chain transfer agent (MMP) (a, 3.67ppm) and the COOC*H*$_2$CH$_2$OCH$_3$ protons of the polymer (g, 4.24ppm).

2.2. Mechanical properties

Table S1. Flexural strength of resins with PMEA

	3%	5%	10%
Control	**78.12 ± 1.40^{d}**	**78.12 ± 1.40^{c}**	**78.12 ± 1.40^{c}**
MEA	**77.27 ± 4.01^{d}**	**74.97 ± 5.43^{bc}**	**67.33 ± 4.53^{b}**
PMEA-1	**76.38 ± 2.00^{bc}**	**75.59 ± 0.99^{bc}**	**65.83 ± 2.37^{b}**
PMEA-2	**72.75 ± 1.26^{b}**	**70.67 ± 1.11^{b}**	**31.18 ± 1.19^{a}**
PMEA-3	**55.24 ± 1.74^{a}**	**43.49 ± 1.92^{a}**	-
PMEA-4	**56.42 ± 1.13^{a}**	**44.17 ± 2.35^{a}**	-
***P* value**	**0.000**	**0.000**	**0.000**

Different letters indicate a significant difference between different materials of the same content

Table S2. Elastic modulus of resins with PMEA

	3%	5%	10%
Control	**1998.27 ± 61.27^{b}**	**1998.27 ± 61.27^{b}**	**1998.27 ± 61.27^{c}**
MEA	**2374.87 ± 116.28^{c}**	**2317.82 ± 71.45^{c}**	**2088.67 ± 111.36^{c}**
PMEA-1	**1926.84 ± 68.43^{b}**	**1934.29 ± 35.98^{b}**	**1657.38 ± 86.86^{b}**
PMEA-2	**1935.61 ± 65.79^{b}**	**1989.06 ± 43.75^{b}**	**1389.13 ± 97.83^{a}**
PMEA-3	**1771.40 ± 49.85^{a}**	**1613.38 ± 91.02^{a}**	-
PMEA-4	**1868.93 ± 27.18^{ab}**	**1611.47 ± 56.88^{a}**	-
***P* value**	**0.000**	**0.000**	**0.000**

Different letters indicate a significant difference between different materials of the same content

Table S3. Vickers hardness of resins with PMEA

	3%	5%	10%
Control	**11.87 ± 0.19^{cd}**	**11.87 ± 0.19^{d}**	**11.87 ± 0.19^{b}**
MEA	**13.43 ± 0.37^{e}**	**13.17 ± 0.27^{e}**	**12.67 ± 0.22^{c}**
PMEA-1	**12.03 ± 0.64^{d}**	**12.57 ± 0.73^{e}**	**11.88 ± 0.49^{b}**
PMEA-2	**11.26 ± 0.41^{bc}**	**11.02 ± 0.27^{c}**	**7.77 ± 0.31^{a}**
PMEA-3	**10.65 ± 0.84^{b}**	**9.20 ± 0.47^{b}**	-
PMEA-4	**9.26 ± 0.27^{a}**	**7.77 ± 0.35^{a}**	-
***P* value**	**0.000**	**0.000**	**0.000**

Different letters indicate a significant difference between different materials of the same content

Table S4. Flexural strength of pre- and post-thermocycled resins

	Control	MEA	PMEA-1	PMEA-4	*P* value
Before aging	**78.13 ± 1.41^{b}**	**77.27 ± 4.01^{b}**	**76.39 ± 2.00^{b}**	**56.42 ± 1.14^{a}**	**0.000**
Aging-thermocycling	**71.08 ± 1.01^{b}**	**70.81 ± 3.05^{b}**	**69.86 ± 3.05^{b}**	**57.30 ± 2.67^{a}**	**0.000**

Different letters indicate significant differences between different materials

Table S5. Elastic modulus of pre- and post-thermocycled resins

	Control	MEA	PMEA-1	PMEA-4	*P* value
Before aging	**1198.28 ± 61.30^{a}**	**2374.87 ± 116.28^{b}**	**1926.84 ± 68.43^{a}**	**1868.93 ± 27.19^{a}**	**0.000**
Aging-thermocycling	**2202.97 ± 75.36^{ab}**	**2306.64 ± 44.77^{b}**	**2170.04 ± 139.44^{ab}**	**2042.61 ± 108.82^{a}**	**0.008**

Different letters indicate significant differences between different materials

Table S6. Vickers hardness of pre- and post-thermocycled resins

	Control	MEA	PMEA-1	PMEA-4	*P* value
Before aging	**11.86 ± 0.20^{b}**	**13.44 ± 0.37^{c}**	**12.04 ± 0.64^{b}**	**9.26 ± 0.27^{a}**	**0.000**
Aging-thermocycling	**12.61 ± 0.46^{b}**	**13.78 ± 0.59^{c}**	**12.49 ± 0.71^{b}**	**11.09 ± 0.75^{a}**	**0.000**

Different letters indicate a significant difference between different materials

2.3. Saliva derived biofilm

Table S7. Biofilm thickness of resins before and after static immersion

	Control	MEA	PMEA-1	PMEA-4	*P* value
Before aging	**141.04 ± 6.86^{d}**	**121.36 ± 6.86^{c}**	**63.98 ± 6.89^{a}**	**104.96 ± 13.47^{b}**	**0.000**
Aging-static immersion	**134.48 ± 4.49^{c}**	**126.28 ± 7.33^{bc}**	**60.70 ± 9.38^{a}**	**114.80 ± 10.04^{b}**	**0.000**

Different letters indicate a significant difference between different materials

Table S8. Biofilm biomass of resins before and after static immersion

	Control	MEA	PMEA-1	PMEA-4	*P* value
Before aging	**74.64 ± 5.58^{c}**	**66.79 ± 7.44^{c}**	**27.61 ± 5.55^{a}**	**48.38 ± 4.97^{b}**	**0.000**
Aging-static immersion	**79.40 ± 5.12^{c}**	**71.94 ± 4.95^{c}**	**27.09 ± 8.04^{a}**	**57.14 ± 8.75^{b}**	**0.000**

Different letters indicate a significant difference between different materials

2.4. SEM images of fractured surfaces

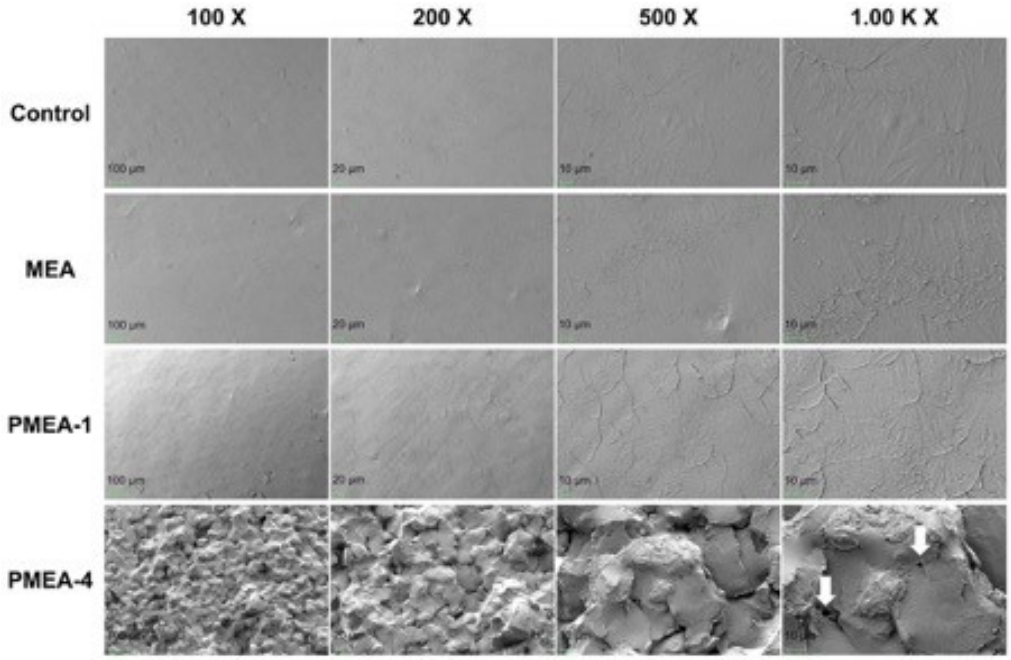

Figure S2. Scanning electron microscopy (SEM) images of fractured surfaces of specimens with different magnifications. White arrow indicates pores.

2.5. Protein adsorption

Here we investigated protein adsorption on the resin surfaces as a measure of antifouling activity. We selected BSA for this initial experiment because albumin is abundant in saliva. The resin with PMEA was incubated with BSA solution for 4 h at 37 °C and showed about 20% reduction in BSA adsorption as compared to the control while there is no statistically significant difference between PMEA-1 and PMEA-4. The adsorption behaviors of resins appear to reflect the contact angle and surface energy rather than CAH. To get insight into the mode of BSA adsorption, we examined the wettability of pristine resin surfaces using BSA solution as a reference liquid. Although there is no substantial difference in contact angle, the surface energy values decreased significantly for all the resins. This is likely due to surface

adsorption of BSA, and reduced SA might indicate that the adsorbed BSA denatured and exposed the hydrophobic domains. The CAH values also increased, but the trend is similar to that without BSA. This may reflect the increased hydrophobicity, but the BSA adsorption might not change the surface chain mobility of the resin surface.

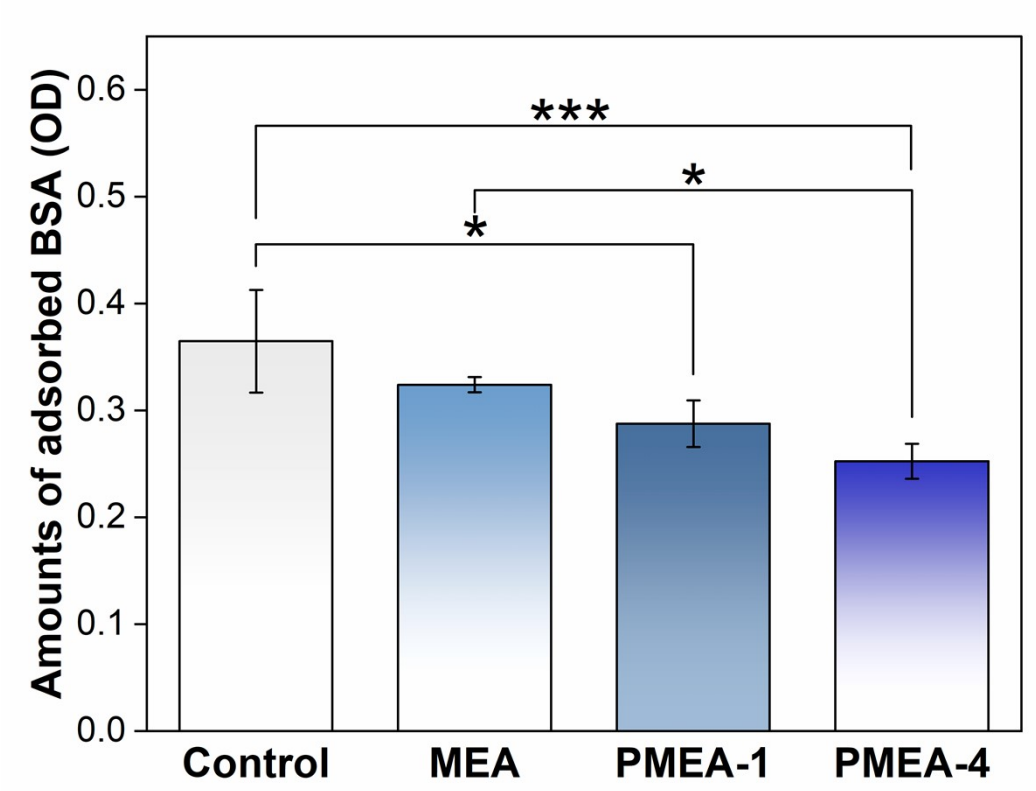

Figure S3. Relative amount of bovine serum albumin adsorbed onto resins. The amount of BSA was determined by the colorimetric BCA protein assay as the optical density at 562 nm. The "b-series" PMEA lots were used for biological experiments (Table 2). Asterisks indicate statistical significance (* $P < 0.05$ and *** $P < 0.001$).

Supplement References

1. M. J. Lee, J. Y. Kim, J. Y. Seo, U. Mangal, J. Y. Cha, J. S. Kwon and S. H. Choi, *Nanomaterials (Basel)*, 2020, **10**.
2. M. Lee, H. Kim, J. Seo, M. Kang, S. Kang, J. Jang, Y. Lee and J.-H. Seo, *Applied Surface Science*, 2018, **427**, 517-524.