Electronic Supplementary Material (ESI) for Biomaterials Science.


## Supplementary Tables and Figures

**TableS1.** Elemental analysis of PEM in various preparations analyzed by energy-dispersive x-ray spectroscopy (EDS).

| Element | PEM, Weight (%) (Average±SD) | | | | |
|---|---|---|---|---|---|
| | **<15µm** | **<53µm** | **53-104µm** | **104-381µm** | **Intact membranes** |
| **Carbon** | 68.30±9.25$^{a}$ | 60.72±4.55$^{ab}$ | 55.09±3.96$^{b}$ | 70.36±4.65$^{a}$ | 64.13±5.74$^{ab}$ |
| **Nitrogen** | 12.61±8.45$^{ab}$ | 18.65±3.42$^{ab}$ | 21.36±2.82$^{a}$ | 9.17±3.34$^{b}$ | 13.82±6.07$^{ab}$ |
| **Oxygen** | 16.67±1.72$^{ab}$ | 18.29±3.47$^{ab}$ | 21.13±2.98$^{a}$ | 13.36±3.64$^{b}$ | 19.87±1.29$^{a}$ |
| **Magnesium** | 0.01±0.02 | 0 | 0 | 0 | 0 |
| **Sulfur** | 2.21±0.06$^{b}$ | 2.25±0.55$^{b}$ | 2.34±0.87$^{b}$ | 6.88±2.58$^{a}$ | 2.15±1.07$^{b}$ |
| **Calcium** | 0.08±0.11$^{ab}$ | 0.07±0.05$^{b}$ | 0.06±0.06$^{b}$ | 0.22±0.06$^{a}$ | 0.01±0.03$^{b}$ |

Values represent Mean ± SD from three independent measurements. Values with different superscript letters (Tukey multiple means comparison) are significantly different (ANOVA; $P < 0.05$).

**Table S2.** Calcium content of manually processed PEM measured by calcium colorimeter with the associated kit (Hanna HI 758, ITM Instruments Inc., Canada)

| **Type of ESM processing** | **$CaCO_3$ (ES) content (wt %) in 0.2-1 g** |
|---|---|
| Manually processed- 381-504µm | 0.39±0.16 |
| Manually processed-104-381µm | 0.42±0.22 |
| Manually processed-53-104µm | 1.55±1.15 |
| Manually processed-<53µm | 0.49±0.29 |
| Manually processed-<15µm | 0.37±0.20 |
| Industrially processed | 2.64±0.12 |

Values represent Mean ± SD from 2 independent samples each done in triplicate (n = 3).

**Table S3.** Amino acid composition of PEM in various size (Hospital for Sick Children, Peter Gilgan Centre for Research & Learning, SPARC Biocentre, Toronto, ON)

| Amino acid | mole% | | | | | | |
|---|---|---|---|---|---|---|---|
| | PEM (Unsieved) | PEM (104-381 µm) | PEM (53-104 µm) | PEM (<53µm, R1) | PEM (<53µm, R2) | PEM (Emulsiflex) | Mean± Std. Dev |
| Asx (Asp+Asn) | 7.5 | 7.6 | 7.7 | 7.6 | 7.5 | 7.7 | 7.6±0.1 |
| Glx (Glu+Gln) | 9.9 | 10.2 | 10.3 | 10.1 | 10.0 | 10.2 | 10.1±0.1 |
| OH-Pro | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9±0.0 |
| Ser | 6.5 | 6.6 | 6.8 | 6.7 | 6.5 | 6.7 | 6.6±0.1 |
| Gly | 9.8 | 9.9 | 10.0 | 10.0 | 9.8 | 10.0 | 9.9±0.1 |
| His | 3.1 | 3.2 | 3.2 | 3.2 | 3.1 | 3.2 | 3.2±0.0 |
| Arg | 5.0 | 5.1 | 5.2 | 5.1 | 5.0 | 5.2 | 5.1±0.1 |
| Thr | 5.9 | 6.0 | 6.1 | 6.0 | 5.9 | 6.1 | 6.0±0.1 |
| Ala | 3.5 | 3.5 | 3.6 | 3.6 | 3.5 | 3.6 | 3.5±0.0 |
| Pro | 9.6 | 9.7 | 9.9 | 9.7 | 9.6 | 9.8 | 9.7±0.1 |
| Tyr | 1.3 | 1.3 | 1.3 | 1.4 | 1.3 | 1.4 | 1.3±0.0 |
| Val | 7.7 | 7.7 | 7.8 | 7.7 | 7.6 | 7.7 | 7.7±0.1 |
| Met | 3.3 | 3.3 | 3.4 | 3.3 | 3.3 | 3.3 | 3.3±0.0 |
| Ile | 3.1 | 3.1 | 3.2 | 3.2 | 3.1 | 3.1 | 3.1±0.0 |
| Leu | 4.3 | 4.3 | 4.4 | 4.4 | 4.3 | 4.4 | 4.4±0.1 |
| OH-Lys | 0.4 | 0.4 | 0.4 | 0.5 | 0.4 | 0.4 | 0.4±0.0 |
| Phe | 1.7 | 1.7 | 1.7 | 1.7 | 1.7 | 1.7 | 1.7±0.0 |
| Lys | 3.1 | 3.2 | 3.1 | 3.2 | 3.1 | 3.1 | 3.1±0.0 |
| Cys A | 13.2 | 12.3 | 11.1 | 11.6 | 13.4 | 11.6 | 12.2±0.9 |

**Table S4.** Amino acid composition of the PEM powder (WP-1) as compared to the industrial ESM

| Amino acid | mole% | | |
|---|---|---|---|
| | PEM | I-ESM industrial dry Batch 2 | Reference values* |
| Asx (Asp+Asn) | 7.6±0.1 | 2.3 | 7.9±0.4 |
| Glx (Glu+Gln) | 10.1±0.1 | 6.3 | 10.5±0.5 |
| OH-Pro | 0.9±0.0 | 0.9 | 1.1±0.2 |
| Ser | 6.6±0.1 | 8.2 | 6.7±0.5 |
| Gly | 9.9±0.1 | 11.7 | 10.4±0.5 |
| His | 3.2±0.0 | 3.7 | 3.1±0.3 |
| Arg | 5.1±0.1 | 6.0 | 5.2±0.2 |
| Thr | 6.0±0.1 | 6.7 | 6.1±0.3 |
| Ala | 3.5±0.0 | 5.5 | 4.0±0.1 |
| Pro | 9.7±0.1 | 10.5 | 10.6±0.8 |
| Tyr | 1.3±0.0 | 1.9 | 1.3±0.2 |
| Val | 7.7±0.1 | 9.2 | 7.5±1.1 |
| Met | 3.3±0.0 | 3.9 | 3.2±0.4 |
| Ile | 3.1±0.0 | 4.1 | 3.2±0.4 |
| Leu | 4.4±0.1 | 6.1 | 4.7±0.2 |
| OH-Lys | 0.4±0.0 | 0.9 | 0.2±0.2 |
| Phe | 1.7±0.0 | 2.3 | 1.4±0.3 |
| Lys | 3.1±0.0 | 2.9 | 3.2±0.2 |
| Cys A | 12.2±0.9 | 6.9 | 9.9±0.7 |

* Average of 5 references **1.** Leach RM, Jr., Rucker RB, Van Dyke GP (1981) Egg shell membrane protein: a nonelastin desmosine/isodesmosine-containing protein. Arch Biochem Biophys 207: 353-359; **2.** Baumgartner S, Brown DJ, Salevsky E, Jr., Leach RM, Jr. (1978) Copper deficiency in the laying hen. J Nutr 108: 804-811; **3.** Salevsky E, Leach RM (1980) Studies on the Organic-Components of Shell Gland Fluid and the Hens Eggshell. Poultry Science 59: 438-443; **4.** Crombie G, Snider R, Faris B, Franzblau C (1981) Lysine-Derived Cross-Links in the Eggshell Membrane. Biochimica et Biophysica Acta 640: 365-367; **5.** Ahmed TAE, Suso HP, Maqbool A, Hincke MT (2019) Processed eggshell membrane powder: Bioinspiration for an innovative wound healing product. Mater Sci Eng C Mater Biol Appl; 95:192-203

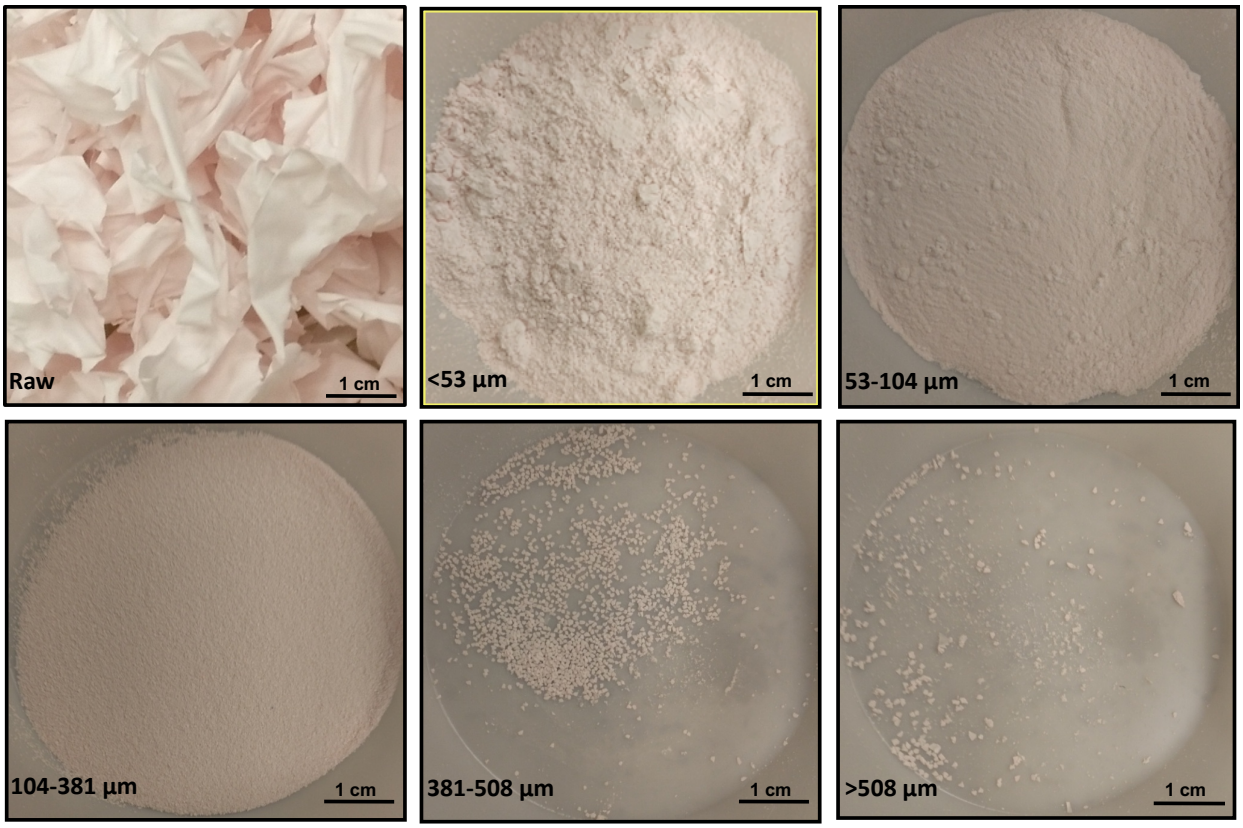



**Figure S1**. Macroscopic appearance of processed PEM after cryogenic grinding and sieving.

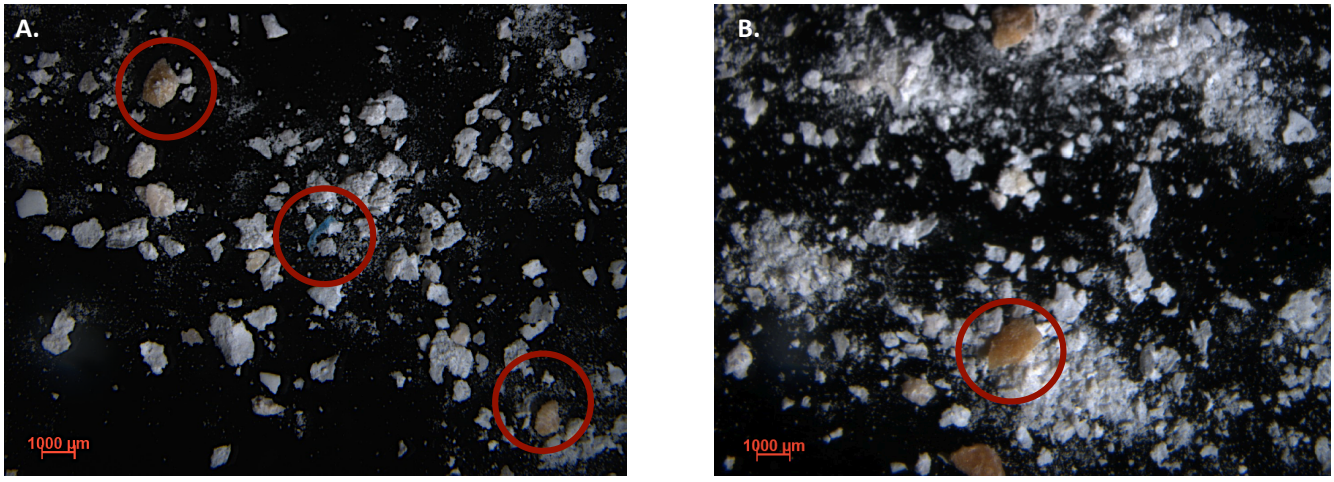
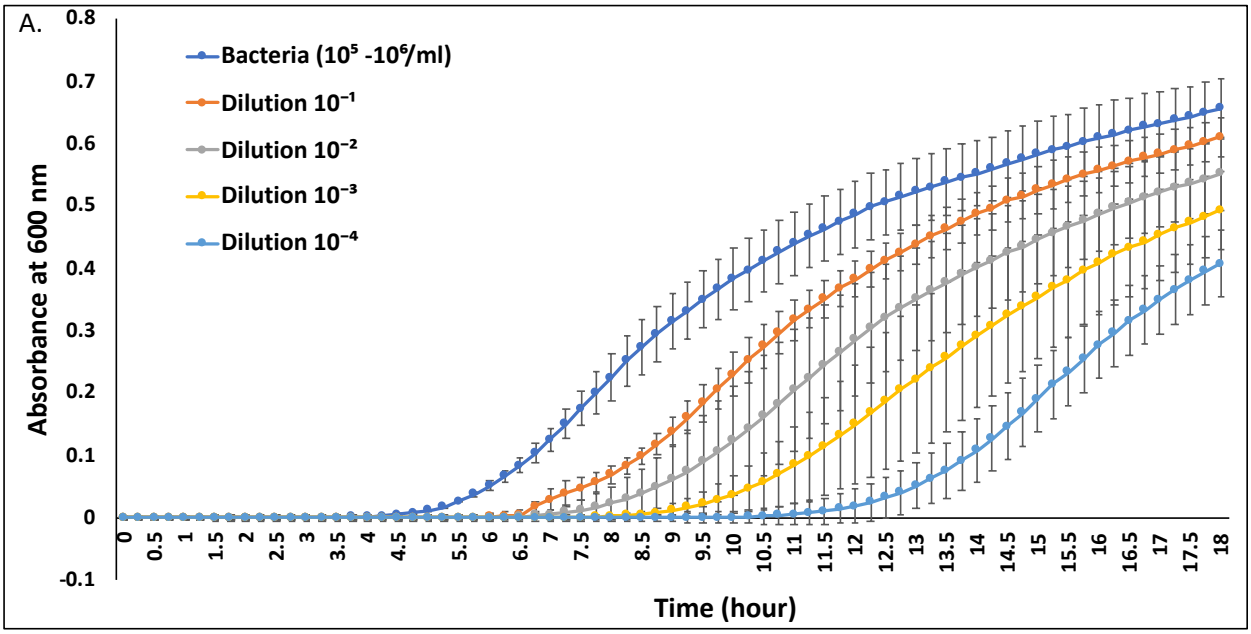



**Figure S2**. Dry industrial ESM (I-ESM) visualized by stereomicroscopy showing contamination with **A.** Egg shell and environmental debris **B.** Yolk at 7.5X magnification.



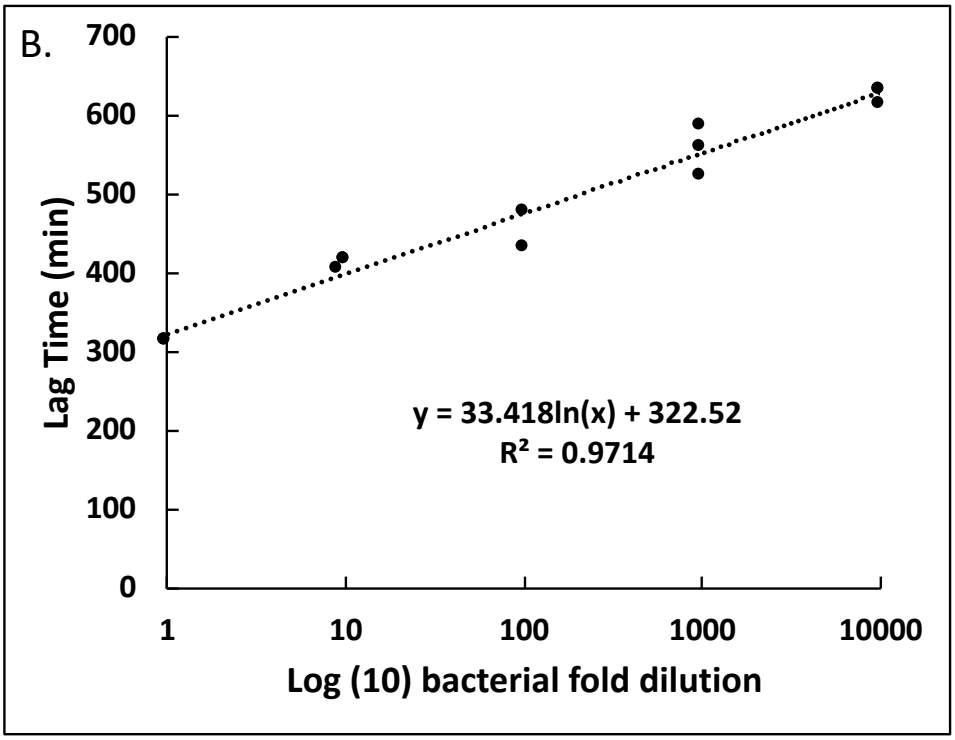




**Figure S3**. The antimicrobial activity associated with PEM treatment was measured as bacterial growth inhibition calculated from growth curves**. A.** Serially ten-fold dilution series of *S. aureus* obtained from the uninhibited control. Most concentrated bacterial solution (cell density =10^5-10^6) was serially diluted in a microplate (in triplicate) and bacterial growth was monitored by measuring the optical density at 600 nm every 15 min for 18 hours. **B.** A standard curve was

generated to establish a correlation between the number of viable bacterial cells in the inoculum and bacterial growth lag time. This standard curve was used to determine bacterial growth inhibition.