Eggshell (ES) and eggshell membrane (ESM) is a significant byproduct of the egg producing industry (Ahmed et al., 2019). Many studies have been undertaken to utilize ES waste for potential value added applications (Cordeiro and Hincke, 2011). Described here are the datasets from our evaluation of processed eggshell membrane powder (PEP) as a wound healing product using the mouse excisional wound splinting model (Ahmed et al., 2019). PEP biomaterial was characterized by proteomics using various extraction and solubilization strategies including moderate (lithium dodecyl sulphate (LDS) and urea/ammonium bicarbonate) and harsh conditions (3-mercaptopropionic acid (3-MPA) and NaOH/dimethylsulfoxide) in order to progressively overcome its stable, insoluble nature (Ahmed et al., 2019, Ahmed et al., 2017). Analysis of proteomic data allowed the relative abundance of the
main PEP protein constituents to be determined. The efficacy of PEP for promotion of wound healing was assessed using the mouse excisional wound splinting model, and well-established semi-quantitative histological scoring. (More details about the PEP biomaterial characterization and its in vivo evaluation can be found in the related research article (Ahmed et al., 2019)).

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### Specifications Table

| Subject area                        | Materials Science |
|-------------------------------------|-------------------|
| More specific subject area          | Biomaterials      |

| Type of data | Tables and figures. |
|-------------|--------------------|
| How data was acquired | Agilent 1200 nanopump (Reversed-phase (RP) nanoscale capillary liquid chromatography (nanoLC), Agilent Technologies Canada Inc., Ontario, Canada) connected to mass spectrometer 5600 with a nanoelectrospray ion source (ES-MS/MS, AB Sciex, MA, USA), LOGOS microwave hybrid tissue processor (Milestone, MI, USA). Leica microtome (Leica Biosystems Inc., ON, Canada). Zeiss Mirax Midi whole slide digital scanner (Carl Zeiss Canada Ltd, ON, Canada). |

| Data format | Raw and analyzed |
|-------------|-----------------|
| Experimental factors | **Mass spectrometry:** Eggshell membrane collected at the egg breaking unit was processed (washed, milled, sieved, and γ sterilized) into a micronized powder (<100 μm), which was designated “Processed Eggshell Membrane Powder” (PEP). For proteomics, PEP samples were subjected to various extraction and solubilization strategies including moderate (via lithium dodecyl sulphate (LDS) and urea/ammonium bicarbonate (NH₄HCO₃)) and harsh conditions (via 3-mercaptopropionic acid (3-MPA) and NaOH/dimethylsulfoxide) conditions. Samples prepared by 3-MPA, NaOH/DMSO, and LDS/DTT treatment were subjected to in-gel digestion, while in the case of urea/NH₄HCO₃ extraction, in-solution digestion was performed. The protein constituents of PEP were identified using LC/MS/MS analysis, with a false discovery rate (FDR) of 1% and at least two unique peptides. Keratins were discarded from the identified protein inventory. In addition, any protein identified with only one unique peptide (according to the Scaffold software interface) was discarded from the final protein inventory. **Tissue processing:** The processed wound samples were cut into two halves (Upper and lower halves) and then embedded in paraffin (Leica Biosystems Inc., ON, Canada). PEP (50 mg) was suspended in PBS and centrifuged. The resultant pellet was centered in pre-embedding media and processed with the LOGOS tissue processor. **Digital scanning:** Stained tissue and PEP sections were scanned with Zeiss Mirax Midi whole slide digital scanner (12 slides/scan and 40X objective lens). Exposure time was 10-100 ms (bright field) and the specimen threshold level of 40–45. |

| Experimental features | A complete protein inventory for PEP was created by merging the proteins identified by LC/MS/MS analysis after various extraction and solubilization strategies. Relative abundances of proteins identified in the PEP biomaterial were determined using Scaffold proteome software. The effect of PEP on wound healing was evaluated in the mouse excisional wound splinting model using the macroscopic planimetric timecourse (30–38 mice) and a histological scoring system (4 mice each at time points 3, 10, and 17). Various histological parameters related to wound healing were scored for all stained section. The absence of stainable collagen in the PEP biomaterial was confirmed using Masson’s trichrome staining of the PEP pellet. |

| Data source location | MS/MS spectrometry was conducted in the Proteomics Platform Of Québec Genomics Center, CHU de Québec Research Center (Laval, QC, Canada). In vivo experiments were carried out in the animal care and veterinary service facility (ACVS), Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada. Wound tissue sample processing, embedding, sectioning, staining, and scanning was performed in the Histology Core Facility, Department of Pathology and Laboratory Medicine, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada. |

| Data accessibility | The data are available within the article. |
| Related research article | Ahmed TAE, Suso HP, Maqbool A, and Hincke MT. Processed Eggshell Membrane Powder: Bioinspiration for an Innovative Wound Healing Product, Mater Sci Eng C Mater Biol Appl. 95 (2019) 192–203. |
1. Data

The presented data demonstrates the utilization of various extraction strategies (moderate to harsh conditions) \cite{1-4} to identify the protein constituents of PEP by proteomics. The proteomic approach allows the estimation of relative abundances of the main protein constituents of PEP biomaterial. The data demonstrates the use of animals (C57BL/6J mice) for the planimetric timecourse and histological assessment of healing of the splinted excisional wound after application of a biomaterial (PEP). The data describes an established histological scoring system used to assess the effect of PEP on various histological parameters critical to assess wound healing promotion. The histological processing of PEP biomaterial via pelleting and pre-embedding in agar-formalin media provides researchers with a strategy to process powdered biomaterials and even cells.

Table 1
Various extraction conditions used for the in-solution and in-gel digestion-based proteomic analysis of PEP.

| Extraction strategy | In-solution digestion\(^a\) | In-gel digestion |
|---------------------|-----------------------------|-----------------|
| Digestion buffer   | urea 8 M/ammonium bicarbonate 100 mM, sonication (2 x 15s on – 1min off on ice), centrifugation (16,000 x g, 10min, 4 °C) | 3-mercaptopropionic acid (1.25 M), 1.7 M acetic acid, 24 hours, 80 °C, shaking water bath. |
|                     |                             | NaOH (5% w/v), DMSO, 4 hours, 50 °C, hot plate stirrer. |
|                     |                             | LDS (73mM)/DTT (50 mM), NuPAGE sample buffer only, 30 minutes, 70 °C, Heat block. |

\(^a\) Moderate extraction conditions.
\(^b\) Harsh solubilization conditions.

Fig. 1. Venn chart showing a comparison of the PEP proteome to the recently published ESM proteome (Ahmed et al., 2017) \cite{4}. Twenty four (24) of the PEP proteins were not previously identified in the ESM proteome. Micronization to prepare PEP facilitated the identification of a greater number of proteins by increasing the efficiency of the in-solution digestion approach.
### Table 2
Inventory of the PEP proteome, as compared to the ESM (hand-peeled and flakes) proteome.

| No. | Protein name                                      | Gene Symbol | Gene ID     | PEP | ESM |
|-----|---------------------------------------------------|-------------|-------------|-----|-----|
| 1   | Actin, γ1                                         | ACTG1       | 415296      | YES | YES |
| 2   | ADAM metallopeptidase with thrombospondin type 1 motif, 5 | ADAMTS5     | 427971      | YES | NO  |
| 3   | A-kinase anchoring protein 12                     | AKAP12      | 421634      | NO  | YES |
| 4   | Albumin                                           | ALB         | 396197      | YES | YES |
| 5   | Aminopeptidase N, Alanyl (membrane) aminopeptidase. | ANPEP       | 395667      | NO  | YES |
| 6   | Angiopoietin like 3                               | ANGPTL3     | 100189558   | YES | NO  |
| 7   | Annexin A2                                        | ANXA2       | 396297      | YES | NO  |
| 8   | Antigen identified by monoclonal antibody Ki-67   | MKI67       | 423963      | NO  | YES |
| 9   | Apolipoprotein A-1                               | APOA1       | 396536      | YES | YES |
| 10  | Apolipoprotein B                                 | APOB        | 396535      | YES | YES |
| 11  | Apolipoprotein D                                 | APOD        | 424893      | YES | YES |
| 12  | Apolipoprotein H (β-2-glycoprotein I)            | APOH        |            |     |     |
| 13  | Aponevotellenin 1                                | APOV1       | 396476      | YES | YES |
| 14  | ash1 (absent, small, or homeotic)-like           | ASH1L       | 425064      | NO  | YES |
| 15  | ATPase H+ transporting accessory protein 2        | ATP6AP2     | 418573      | YES | NO  |
| 16  | ATP-binding cassette, sub-family A (ABC1), member 4 | ABCA4      | 424490      | NO  | YES |
| 17  | Avian β-defensein 9                               | AvBD9       | 414343      | YES | NO  |
| 18  | Avian β-defensein 10                              | AvBD10      | 414341      | YES | NO  |
| 19  | Avian β-defensein 11                              | AvBD11      | 414876      | YES | NO  |
| 20  | Avidin                                            | AVD         | 396260      | YES | YES |
| 21  | BPI fold containing family C, member B            | BPIFCB      | 771461      | NO  | YES |
| 22  | Breast cancer 2                                   | BRCA2       | 374119      | NO  | YES |
| 23  | Bromodomain containing 8                         | BRD8        | 416219      | YES | NO  |
| 24  | BTB domain containing 7                          | BTBD7       | 423424      | YES | NO  |
| 25  | Ca+ + dependent secretion activator 2            | CADPS2      | 417756      | YES | NO  |
| 26  | Cadherin 1, type 1, E-cadherin (epithelial)       | CDH1        | 415860      | YES | YES |
| 27  | Cadherin, EGF LAG seven-pass G-type receptor 3    | CELSR3      | 107054381   | NO  | YES |
| 28  | Calcium channel, voltage-dependent, T type, α 1H subunit | CACNA1H   | 416526      | YES | NO  |
| 29  | Calcium/calmodulin-dependent protein kinase II β  | CAMK2B      | 374174      | YES | NO  |
| 30  | Calmodulin 2                                      | CALM        | 395855      | YES | YES |
| 31  | Carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 6 | CHST6     | 770257      | YES | NO  |
| 32  | Carboxypeptidase E                                | CPE         | 422424      | YES | NO  |
| 33  | Cathepsin B                                       | CTSB        | 396329      | YES | YES |
| 34  | Cathepsin E-A-like                               | CTEAL       | 417848      | YES | NO  |
| 35  | Cell division cycle 20B                          | CDC20B      | 426169      | YES | NO  |
| 36  | Centriolin                                        | CNTRL       | 417121      | YES | NO  |
| 37  | Centrosomal protein 152kDa                       | CEP152      | 415437      | YES | NO  |
| 38  | Chondroitin sulphate proteoglycan 4              | CSPG4       | 425524      | YES | NO  |
| 39  | Chromosome 1 open reading frame, human C12orf35   | C1H12ORF35  | 418136      | YES | NO  |
| 40  | Clusterin                                         | CLU         | 395722      | YES | YES |
| 41  | Coagulation factor II (thrombin)                  | F2          | 395306      | YES | NO  |
| 42  | Cochlin                                           | COCH        | 395779      | YES | NO  |
| 43  | Collagen III (α1 chain)                          | COL3A1      | 396340      | NO  | YES |
| 44  | Collagen IV (α1 chain)                           | COL4A1      | 395530      | NO  | YES |
| 45  | Collagen IV (α2 chain)                           | COL4A2      | 424797      | NO  | YES |
| 46  | Collagen IV (α6 chain)                           | COL4A6      | 422350      | NO  | YES |
| 47  | Collagen V (α2 chain)                            | COL5A2      | 423986      | NO  | YES |
| 48  | Collagen VII (α1 chain)                          | COL7A1      | 427584      | YES | NO  |
| 49  | Collagen VIII (α1 chain)                         | COL8A1      | 418378      | YES | NO  |
| 50  | Collagen X (α1 chain)                            | COL10A1     | 1000858979  | YES | YES |
| 51  | Collagen XI (α chain)                            | COL11A1     | 374046      | YES | NO  |
| 52  | Collagen XII (α chain)                           | COL12A1     | 395875      | NO  | YES |
| 53  | Collagen XXII (α1 chain)                         | COL22A1     | 420315      | NO  | YES |
| 54  | Complement component 3                           | C3          | 396370      | YES | NO  |
| 55  | Contactin                                        | CNTN5       | 395317      | YES | NO  |
| 56  | Cortactin binding protein 2                      | CTNBP2      | 417766      | YES | NO  |
| 57  | Corticotropin releasing hormone                  | CRH         | 404297      | YES | NO  |
| 58  | CREMP (cysteine rich ESM protein)                | CREMP       | 776923      | YES | YES |
| 59  | CREMP1                                           | N/A         | N/A         | NO  | YES |
| 60  | CREMP2                                           | N/A         | N/A         | NO  | YES |
| 61  | CREMP3                                           | N/A         | N/A         | YES | YES |
| No. | Protein name                                      | Gene Symbol | Gene ID  | PEP | ESM |
|-----|--------------------------------------------------|-------------|----------|-----|-----|
| 62  | CREMP4                                           | N/A         | N/A      | NO  | YES |
| 63  | CREMP5                                           | N/A         | N/A      | YES | NO  |
| 64  | CREMP6                                           | N/A         | N/A      | NO  | YES |
| 65  | CTS telomere maintenance complex component 1     | CTC1        | 418324   | NO  | YES |
| 66  | CUB and Sushi multiple domains 2                 | CSMD2       | 419640   | NO  | YES |
| 67  | Cystatin C                                       | CST3        | 396947   | YES | NO  |
| 68  | Dedicator of cytokinesis 1                       | DOCK1       | 423960   | NO  | YES |
| 69  | Deleted in malignant brain tumors 1 protein-like (EW135). | DMBT1L | 426826 | YES | NO  |
| 70  | DENN/MADD domain containing 4C                   | DENND4C     | 427236   | NO  | YES |
| 71  | Desmoplakin                                       | DSP         | 420869   | NO  | YES |
| 72  | Dickkopf homolog 3                               | DKK3        | 396023   | YES | NO  |
| 73  | di-N-acetyl-chitobiase                            | CTB5        | 424535   | NO  | YES |
| 74  | DnaJ heat shock protein family (Hsp40) member C7 | DNAJC7      | 428312   | NO  | YES |
| 75  | Dynein, axonemal, heavy chain 1                  | DNAH1       | 415943   | NO  | YES |
| 76  | Dynein, axonemal, heavy chain 12                 | DNAH12      | 416004   | NO  | YES |
| 77  | Dynein, axonemal, heavy chain 9                  | DNAH9       | 417314   | NO  | YES |
| 78  | Dynein, cytoplasmic 2, heavy chain 1             | DYNC2H1     | 418979   | NO  | YES |
| 79  | Dystrophin                                        | DMD         | 396236   | NO  | YES |
| 80  | EGF containing fibulin-like extracellular matrix protein 1 | EFEMP1 | 428543 | YES | NO  |
| 81  | EGF-like repeats and discoidin I-like domains 3  | EDIL3       | 427326   | YES | NO  |
| 82  | Enolase 2 (γ, neuronal)                          | ENO2        | 395689   | NO  | YES |
| 83  | Enolase 3                                        | ENO3        | 396016   | NO  | YES |
| 84  | EPH receptor B3                                  | EPHB3       | 396179   | NO  | YES |
| 85  | Eukaryotic translation elongation factor 1 al    | EEF1A1      | 373963   | NO  | YES |
| 86  | Family with sequence similarity 20, member C      | FAM20C      | 416445   | YES | NO  |
| 87  | Family with sequence similarity 21, member A      | FAM21A      | 423772   | NO  | YES |
| 88  | F-box and WD repeat domain containing 8          | FBXW8       | 417024   | NO  | YES |
| 89  | Fibrinogen γ chain                               | FGG         | 395837   | YES | NO  |
| 90  | Fibronectin 1                                    | FN1         | 396133   | YES | YES |
| 91  | Flightless I homolog                             | FFLI        | 416515   | NO  | YES |
| 92  | Folate receptor 1 (adult)                        | FOLR1       | 395638   | NO  | YES |
| 93  | G protein-coupled receptor kinase interactor 1    | GIT1        | 417584   | NO  | YES |
| 94  | G protein-coupled receptor kinase interactor 2    | GIT2        | 374035   | NO  | YES |
| 95  | Galactosylceramidase                             | GLAC        | 423394   | YES | NO  |
| 96  | Gastrokine 2                                     | GKN2        | 419515   | YES | NO  |
| 97  | Glutamine and serine rich 1                      | QSER1       | 421599   | NO  | YES |
| 98  | Glutathione peroxidase 3                         | GPX3        | 427638   | YES | NO  |
| 99  | Glutathione S-transferase α 3                     | GSTA3       | 414896   | NO  | YES |
| 100 | Golgi glycoprotein 1                             | GLG1        | 396492   | YES | NO  |
| 101 | Group-specific component (vitamin D binding protein) | GC         | 395696   | NO  | YES |
| 102 | Heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa) | HSPA5 | 396687 | YES | NO  |
| 103 | Heat shock 70kDa protein 8                       | HSPA8       | 395853   | NO  | YES |
| 104 | Hemoglobin, α 1                                  | HBAα        | 416652   | YES | NO  |
| 105 | Hemoglobin, γ G                                  | HBG2        | 396845   | YES | NO  |
| 106 | Hemopexin                                        | HPX         | 415976   | NO  | YES |
| 107 | HEP21 protein                                    | HEP21       | 395192   | YES | NO  |
| 108 | Heterogeneous nuclear ribonucleoprotein A2/B1    | HNRNPAB2B1  | 420627  | NO  | YES |
| 109 | Heterogeneous nuclear ribonucleoprotein A3 homolog 1 -like | HNRNPA3 | 100859627 | NO | YES |
| 110 | Heterogeneous nuclear ribonucleoprotein D-like   | HNRNPDL     | 422601   | NO  | YES |
| 111 | Hexosaminidase B (β polypeptide)                 | HEBXB       | 427204   | YES | NO  |
| 112 | Histone H1.11L                                   | HIST1H11L   | 427892   | NO  | YES |
| 113 | Histone H1.11R                                   | HIST1H11R   | 427896   | NO  | YES |
| 114 | Histone H2A                                      | HIST1H2A4   | 404299   | NO  | YES |
| 115 | Histone H2B                                      | HIST1H2B    | 427886   | YES | NO  |
| 116 | Histone H3 family 3C                            | HIST1H3C    | 417950   | YES | YES |
| 117 | Histone H4                                      | HIST1H4     | 417950   | YES | YES |
| 118 | Histone H5                                      | HIST1H5     | 693250   | NO  | YES |
| 119 | Hyaluronan and proteoglycan link protein 3       | HAPLN3      | 415495   | YES | NO  |
| 120 | Ig heavy chain                                  | N/A         | N/A      | NO  | YES |
| 121 | Ig heavy chain variable region                   | N/A         | N/A      | NO  | YES |
| 122 | Ig J polypeptide, linker protein for Ig a and α polypeptides | IgJ | 374117 | NO  | YES |

(continued on next page)
| No. | Protein name                                      | Gene Symbol | Gene ID | PEP | ESM |
|-----|--------------------------------------------------|-------------|---------|-----|-----|
| 123 | Ig light chain variable region                   | N/A         | N/A     | NO  | YES |
| 124 | Ig mu chain C region                              | N/A         | N/A     | YES | YES |
| 125 | Ig α heavy chain                                  | N/A         | N/A     | YES | YES |
| 126 | Ig γ chain                                       | N/A         | N/A     | YES | YES |
| 127 | Ig light chain                                    | N/A         | N/A     | NO  | YES |
| 128 | Ig λ-like polypeptide 1                           | IGLL1       | 416928  | YES | YES |
| 129 | Immunoglobulin like domain containing receptor 1  | ILDR1       | 418358  | NO  | NO  |
| 130 | Junction plakoglobin                               | JUP         | 429710  | NO  | NO  |
| 131 | Kinesin family member 21B                         | KIF21B      | 421178  | NO  | NO  |
| 132 | Kinesin family member 26A                         | KIF26A      | 423489  | NO  | NO  |
| 133 | La ribonucleoprotein domain family, member 4B     | LARP4B      | 420457  | NO  | NO  |
| 134 | LDL receptor-related protein 11                   | LRPR1       | 421629  | NO  | NO  |
| 135 | Lectin, mannose-binding 2                         | LMAN2       | 100859676 | NO | NO |
| 136 | Leucine zipper protein 1                          | LUZP1       | 428210  | NO  | NO  |
| 137 | Lipocalin 8, extracellular fatty acid-binding protein | LCN      | 396393  | YES | YES |
| 138 | Lymphocyte antigen 86                            | LYB6        | 420872  | YES | YES |
| 139 | Lysyl oxidase-like 2                              | LOXL2       | 419533  | YES | YES |
| 140 | Mediator complex subunit 15                       | MED15       | 416941  | NO  | NO  |
| 141 | Melanoma inhibitory activity family, member 3     | MIA3        | 421337  | NO  | NO  |
| 142 | Milk fat globule-EGF factor 8 & protein (lactadherin isofrom 2) | MFGED8   | 415494  | YES | YES |
| 143 | Mucin 6 oligomeric mucus/gel-forming (ovomucin, β subunit) | MUC6      | 414878  | YES | YES |
| 144 | Mucin-5AC-like                                    | LOC100859676 | 100859676 | YES | YES |
| 145 | Myeloid/lymphoid or mixed-lineage leukemia 2      | ML2         | 425846  | NO  | NO  |
| 146 | Myeloid/lymphoid or mixed-lineage leukemia 3      | ML3         | 420437  | NO  | NO  |
| 147 | Myosin, heavy chain 10, non-muscle                | MYH10       | 396465  | NO  | NO  |
| 148 | Myosin, heavy chain 9, non-muscle                 | MYH9        | 396469  | NO  | NO  |
| 149 | N-acetylglucosamine-1-phosphate transferase, a and β subunits | GNPTAB    | 418096  | NO  | NO  |
| 150 | Neuron navigator 2                                | NAV2        | 422977  | NO  | NO  |
| 151 | Neuron navigator 3                                | NAV3        | 417869  | NO  | NO  |
| 152 | Neurotensin Y                                    | NPY         | 396464  | NO  | NO  |
| 153 | Neurotrin                                        | NTM         | 395450  | NO  | NO  |
| 154 | Olfactomedin 4, tiarin-like                       | OLFM4       | 418826  | YES | YES |
| 155 | Ovalbumin                                         | SRRBIN14    | 396058  | YES | YES |
| 156 | Ovalbumin-related protein X                       | SERPINB14C  | 420898  | YES | YES |
| 157 | Ovalbumin-related protein Y                       | SERPINB14B  | 420897  | YES | YES |
| 158 | Ovalomucin, β subunit                             | MUC5B       | 395381  | YES | YES |
| 159 | Ovocleidin 116 (matrix extracellular phosphoglycoprotein) | MEPE    | 395256  | YES | YES |
| 160 | Ovocalyxin G2 (Ten)                               | OTEN        | 100313508 | YES | YES |
| 161 | Ovocalyxin 32 (Retinoic acid receptor responder 1) | RARRRES1  | 395209  | YES | YES |
| 162 | Ovocalyxin 36 (BPI fold containing family B, member 3) | BPIFB3    | 419289  | YES | YES |
| 163 | Ovostatin                                        | OVTST       | 396151  | YES | YES |
| 164 | Ovotransferrin (transferrin)                      | OVTSL       | 425757  | NO  | NO  |
| 165 | P21 protein (Cdc42/Rac)-activated kinase 3       | PKA3        | 422342  | NO  | NO  |
| 166 | Porphobilinogen synthase 5                        | PGM5        | 427215  | NO  | NO  |
| 167 | Phospholipase B domain containing 1               | PLBD1       | 417967  | NO  | NO  |
| 168 | Piccolo (presynaptic cytomatrix protein)          | PCLO        | 395319  | NO  | NO  |
| 169 | Pgp-120 (PEM1)                                    | PGT1        | 395364  | YES | YES |
| 170 | Polycystic kidney and hepatic disease 1 (autosomal recessive) | PKHD1    | 422044  | NO  | NO  |
| 171 | Polymeric immunoglobulin receptor                 | PIGR        | 419848  | NO  | NO  |
| 172 | Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 | PLOD1     | 419485  | YES | YES |
| 173 | Programmed cell death 6                           | PDCD6       | 420988  | NO  | NO  |
| 174 | Prolyl 4-hydroxylase, β polypeptide               | F4H8        | 374091  | YES | YES |
| 175 | Prolactin                                      | PRLX1       | 395802  | NO  | NO  |
| 176 | Prostaglandin D2 synthase 21kDa                   | PGD2S       | 374110  | YES | YES |
| 177 | Prostate stem cell antigen                        | PSCA        | 420302  | YES | YES |
| No. | Protein name                                               | Gene Symbol | Gene ID     | PEP | ESM |
|-----|-----------------------------------------------------------|-------------|-------------|-----|-----|
| 185 | Prostatic acid phosphatase-like                           | LOC428451   | 428451      | YES | YES |
| 186 | Protein 0-fucosyltransferase 2                           | POPUT2      | 395112      | YES | NO  |
| 187 | Protein phosphatase, Mg2+/Mn2+ dependent, 1J             | PPM1J       | 419873      | NO  |     |
| 188 | Protein tyrosine phosphatase, receptor type, A           | PTPRA       | 396060      | NO  |     |
| 189 | Protocadherin 1                                          | PCDH1       | 416194      | NO  |     |
| 190 | Quescin Q6 sulphhydril oxidase 1                         | QSOX1       | 373914      | YES |     |
| 191 | Retbindin (Riboflavin-binding protein)                   | RTBDN       | 396449      | YES |     |
| 192 | Retinoic acid receptor responder 2                       | RARRES2     | 420366      | YES |     |
| 193 | Rho guanine nucleotide exchange factor (GEF) 17          | ARHGEF17    | 777518      | NO  |     |
| 194 | Ribosomal protein L36                                    | RPL36       | 373936      | NO  |     |
| 195 | Ring finger protein 17                                    | RNF17       | 418961      | NO  |     |
| 196 | Rootletin, ciliary rootlet coiled-coil                    | CROCC       | 428191      | NO  |     |
| 197 | RPE-spondin-like                                         | LOC771089   | 771089      | NO  |     |
| 198 | Salivary amylase, z/A                                    | AMY1A       | 414139      | NO  |     |
| 199 | Sal-like 4                                                | SALL4       | 769286      | NO  |     |
| 200 | Secretoglobulin family 1C member 1 -like                 | LOC101749303| 101749303   | NO  |     |
| 201 | Secretory trypsin inhibitor                               | SPINK1      | 101749216   | NO  |     |
| 202 | Semadomain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3G | SEMA3G     | 415945      | YES |     |
| 203 | Serine peptidase inhibitor, Kazal type 2 (acrosin-trypsin inhibitor) | SPINK2     | 770729      | YES |     |
| 204 | Serine peptidase inhibitor, Kazal type 5, (Ovoinhibitor) | SPINK5     | 416235      | YES |     |
| 205 | Serine peptidase inhibitor, Kazal type 7 (ovomucoid)     | SPINK7      | 416236      | YES |     |
| 206 | Serine/threonine kinase 38                               | STK38       | 428260      | NO  |     |
| 207 | Serpin peptidase inhibitor, clade B (ovalbumin), member 1 | SERPINB1   | 420894      | NO  |     |
| 208 | Serpin peptidase inhibitor, clade B (ovalbumin), member 5 | SERPINB5   | 420900      | NO  |     |
| 209 | Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2 | SERPINE2   | 424805      | YES |     |
| 210 | Serpin peptidase inhibitor, clade F (z-2 antiplasmin, pigment epithelium derived factor), member 2 | SERPINF2   | 100857105   | YES |     |
| 211 | Shroom family member 3                                   | SHROOM3     | 422636      | NO  |     |
| 212 | Similar to arf-GAP with Rho-GAP domain of Zebrafish      | N/A         | N/A         | NO  |     |
| 213 | Similar to CREB binding protein b of Zebrafish           | N/A         | N/A         | NO  |     |
| 214 | Similar to cadherin 4 of Zebrafish                       | CDH4        | N/A         | NO  |     |
| 215 | Similar to Calumenin A of Zebrafish                      | N/A         | N/A         | YES |     |
| 216 | Similar to IgGf-binding protein-like of wild turkey.     | ZAN         | N/A         | NO  |     |
| 217 | Similar to Kunitz-like protease inhibitor                | LOC771972   | 771972      | YES |     |
| 218 | Similar to metastasis associated 1 of Zebrafish          | MTA1        | N/A         | NO  |     |
| 219 | Similar to Septin 4a of Zebrafish                        | N/A         | N/A         | NO  |     |
| 220 | Similar to transcription factor EB Zebrafish             | TFEB        | N/A         | NO  |     |
| 221 | Similar to zinc finger ZZ-type and EF-hand domain-containing protein 1 of wild turkey | ZZEF1      | 100541118   | NO  |     |
| 222 | Sperm repeat containing, nuclear envelope 1              | SYNE1       | 421640      | YES |     |
| 223 | Spermin, β, non-erythrocytic 5                           | SPTBN5      | 423225      | NO  |     |
| 224 | Sperm associated antigen 16                              | SPAG16      | 424009      | NO  |     |
| 225 | Stromal cell derived factor                              | SDF4        | 419423      | YES |     |
| 226 | Syndecan binding protein (syntenin)                      | SDCBP       | 421136      | NO  |     |
| 227 | TATA box binding protein like                            | TBPL2       | 776269      | NO  |     |
| 228 | Tenascin C                                               | TNC         | 396440      | YES |     |
| 229 | Teneurin transmembrane protein 3                         | TENM3       | 422557      | NO  |     |
| 230 | Tetratricopeptide repeat domain 3                        | TTC3        | 418518      | NO  |     |
| 231 | Thyroid hormone receptor interactor 11                   | TRIP11      | 423414      | NO  |     |
| 232 | TIMP metallopeptidase inhibitor 3                        | TIMP3       | 396483      | YES |     |
| 233 | Titin                                                    | TTN         | 424126      | NO  |     |
| 234 | transcobalamin 2                                         | TCN2        | 429737      | NO  |     |
| 235 | Transient receptor potential cation channel, subfamily M, member 1 | TRPM1     | 427494      | NO  |     |
| 236 | Transient receptor potential cation channel, subfamily V, member 2 | TRPV2     | 417603      | NO  |     |
| 237 | Transthyretin.                                           | TTR         | 396277      | YES |     |
| 238 | Tsukushi, small leucine rich proteoglycan                | TSUK1       | 419088      | YES |     |
| 239 | Tumor necrosis factor receptor superfamily, member 6b, decoy | TNFRSF6B  | 395086      | YES |     |
| 240 | Tumor necrosis factor superfamily member 10              | TNFSF10     | 378894      | NO  |     |
| 241 | Ubiquitin B                                              | UBB         | 396190      | NO  |     |

(continued on next page)
the main protein constituents of PEP biomaterial (Table 3). The kinetics of wound healing (with and without PEP) in the mouse splinting excisional wound model was determined using a macroscopic planimetric strategy with histological scoring (Table 4). The histological scoring system was established to assess various histological parameters including degree of angiogenesis, collagen deposition, fibroblast infiltration, macrophage infiltration, polymorphonuclear cells (PMN) infiltration, fibrin clot formation, epidermal differentiation and indentation along with the presence of multinucleated giant cells (Table 5). Finally, PEP was stained with Masson’s trichrome to confirm the absence of stainable collagen using an innovative pre-embedding histological approach (Fig. 2).

2. Experimental design, materials, and methods

2.1. Proteomic analysis

Processed eggshell membrane powder (PEP, <100 μm) [3] was subjected to various extraction and solubilization strategies as utilized previously for ESM proteomics [4] (Table 1). A complete protein inventory for PEP was created by merging the proteins identified after application of moderate extraction [lithium dodecyl sulphate/dithiothreitol (LDS/DTT) or urea/ammonium bicarbonate (NH₄HCO₃)] and harsh solubilization conditions [3-mercaptopropionic acid (3-MPA) or sodium hydroxide/dimethylsulfoxide (NaOH/DMSO)] (Fig. 1 and Table 2). Conditions of in-gel (3-MPA, NaOH/DMSO, and LDS/DTT) or in-solution [urea/NH₄HCO₃] tryptic digestion were applied and the resultant
peptides were analyzed using the 5600 mass spectrometer with a nanoelectrospray ion source connected to Agilent 1200 nanopump (ES-MS/MS) [3,4].

2.2. Relative abundance of PEP protein constituents

MS/MS peak lists were generated using ProteinPilot (Version 4.5) and analyzed using Mascot (Version 2.4.0) and X!Tandem (CYCLONE version), both programmed to search the TAX_GallusGallus_9031_20141114 database (unknown version, 222,250 entries). Validation of MS/MS based peptide and protein identification was performed using Scaffold Proteome software (version 4.3.4).

| Gene symbol | Average total spectral count | % abundance |
|-------------|-------------------------------|-------------|
| LOXL2       | 33.3                          | 28.0        |
| CREMPs      | 31.2                          | 27.0        |
| LYZ         | 13.8                          | 12.0        |
| COL10A1     | 11.5                          | 10.0        |
| SERBIN14    | 7.3                           | 6.0         |
| MEPE        | 4.0                           | 3.0         |
| TF          | 3.0                           | 3.0         |
| CLU         | 2.0                           | 2.0         |
| HAPLN3      | 2.0                           | 2.0         |
| OC-17       | 2.5                           | 2.0         |
| GKN2        | 1.0                           | 0.8         |
| NUCB2       | 1.0                           | 0.8         |
| ORM1        | 1.0                           | 0.8         |
| QSOX1       | 1.0                           | 0.8         |
| SERPINB14B  | 1.0                           | 0.8         |
| SERPINB14C  | 1.0                           | 0.8         |
| VTG2        | 1.0                           | 0.8         |

Table 4
Number of mice used for the in vivo study.

| Purpose of the study     | Number of C57BL/6J mice evaluated |
|--------------------------|-----------------------------------|
|                          | Day 0  | Day 3  | Day 7  | Day 10 | Day 14 | Day 17 |
| Wound closure curve      | 38     | 38     | 34     | 34     | 30     | 30     |
| Histology                | 0      | 4      | 0      | 4      | 0      | 4      |
| Total                    | 38     | 38     | 34     | 34     | 30     | 30     |

Table 5
Scoring scheme for the different histological parameters to assess wound healing.

| Histological parameter                          | Score    |
|------------------------------------------------|----------|
|                                               | 0        | 1        | 2        | 3        | 4        | 5        |
| Angiogenesis                                  | Absent Scanty | Low | Moderate | Marked | Profound |
| Collagen deposition                           | Absent Scanty/ disorganized | Low/ fragmented | Moderate/ separated | Marked/ organized | Profound/ Restored |
| Fibroblast infiltration                       | Absent Scanty | Low | Moderate | Marked | Profound |
| Macrophage infiltration                       | Absent Scanty | Low | Moderate | Marked | Profound |
| PMN infiltration                              | Absent Scanty | Low | Moderate | Marked | Profound |
| Fibrin clot                                   | Absent Scanty | Low | Moderate | Marked | Profound |
| Epidermal differentiation and indentation     | Absent Scanty | Low | Moderate | Marked | Profound |
| Multinuclear giant cells                      | Absent Scanty | Low | Moderate | Marked | Profound |
spectra were searched against the Uniprot and NCBI chicken databases. The relative abundance of the PEP protein constituents was estimated by averaging the total spectral count of each identified protein using the aforementioned Scaffold Proteome software (Table 3).

2.3. In vivo study

All in vivo experiments were conducted following the approved animal protocol (CMM 2108) by the University of Ottawa Animal Care committee and according to the guidelines of the Canadian Council on Animal Care (CCAC). All animal protocols are in compliance with the NIH Guide for Care and Use of Laboratory Animals (Animal Welfare Assurance # A5043-01). Capacity of PEP for promotion of wound healing was assessed using the well-established mouse excisional wound splinting model [5] and the subsequent macroscopic planimetric timecourse [6] and histological scoring. A total of 38 C57BL/6J male mice (10–12 weeks old, Jackson Laboratories, USA) were used for the entire study (Table 4).

2.4. Histological assessments

PEP (50 mg) was suspended in 1 mL PBS and centrifuged for 5 minutes at 13,000 rpm. The resultant pellet was centered in a base mould; pre-mounting media composed of 2% agar and 10% formalin was poured gently over the pellet and left for few minutes to solidify. The resulted PEP block was processed using the LOGOS tissue processor, embedded in paraffin and then sectioned using a Leica microtome. PEP sections were stained using Masson trichrome to confirm the absence of stainable collagen in the PEP biomaterial (Fig. 2). For evaluation of wound healing, histological scoring system was established to assess parameters that represent wound healing [7–9], including degree of angiogenesis, collagen deposition, fibroblast infiltration, macrophage infiltration, polymorphonuclear cells (PMN) infiltration, fibrin clot formation, epidermal differentiation and indentation along with presence of multinucleated giant cells. Every parameter was given a score of 0–5 based on its graded level of abundance. Score 0 indicates complete absence, while score 5 indicates profound manifestation of the assessed parameter. Scoring of collagen deposition was based, not only on the degree of abundance (i.e. absent,
scanty, low, moderate, profound, restored), but also on the degree of organization (disorganized, fragmented, separated, organized) (Table 5).

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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