3D Gelatin-Chitosan hybrid Hydrogels combined with human Platelet Lysate highly support human Mesenchymal Stem Cell proliferation and osteogenic differentiation

Federica Re$^{1,2}$, Luciana Sartore$^3$, Vladimira Moulisova$^{4,5}$, Marco Cantini$^5$, Camillo Almici$^6$, Andrea Bianchetti$^6$, Clizia Chinello$^7$, Kamol Dey$^3$, Silvia Agnelli$^3$, Cristina Manferdini$^8$, Simona Bernardi$^{1,2}$, Nicola F. Lopomo$^9$, Emilio Sardini$^9$, Elisa Borsani$^{10,11}$, Luigi F. Rodella$^{10,11}$, Fabio Savoldi$^{12,13}$, Corrado Paganelli$^{12}$, Pierangelo Guizzi$^{14}$, Gina Lisignoli$^8$, Fulvio Magni$^7$, Manuel Salmeron-Sanchez$^5$, Domenico Russo$^{1*}$

$^1$Department of Clinical and Experimental Sciences, University of Brescia, Bone Marrow Transplant Unit, ASST Spedali Civili, Brescia, Italy,

$^2$CREA (Centro di Ricerca Emato-Oncologica AIL), ASST Spedali Civili di Brescia, Brescia, Italy,

$^3$Mechanical and Industrial Engineering Department, University of Brescia, Brescia, Italy,

$^4$Biomedical Center, Faculty of Medicine, Charles University, Pilsen, Czech Republic,

$^5$Centre for the Cellular Microenvironment, Division of Biomedical Engineering, School of Engineering, University of Glasgow, Glasgow, United Kingdom,

$^6$Laboratory for Stem Cells Manipulation and Cryopreservation, Department of Transfusion Medicine, ASST Spedali Civili, Brescia, Italy,

$^7$Department of Medicine and Surgery, Clinical Proteomics and Metabolomics Unit, University of Milano-Bicocca, Vedano al Lambro, Italy,

$^8$IRCCS Istituto Ortopedico Rizzoli, Laboratorio di Immunoreumatologia e Rigenerazione Tissutale, Bologna, Italy,

$^9$Department of Information Engineering, University of Brescia, Brescia, Italy,

$^{10}$Department of Clinical and Experimental Sciences, Division of Anatomy and Physiopathology, University of Brescia, Brescia, Italy,

$^{11}$Interdipartimental University Center of Research "Adaptation and Regeneration of Tissues and Organs - (ARTO)", University of Brescia, Brescia, Italy,
12Department of Orthodontics, Dental School, University of Brescia, Brescia, Italy,

13Dental Materials Science, Discipline of Applied Oral Sciences, Faculty of Dentistry, The University of Hong Kong, Hong Kong,

14UO of Orthopedic and Traumatology, ASST Spedali Civili of Brescia, Brescia, Italy

Corresponding author: *Domenico Russo, Department of Clinical and Experimental Sciences, University of Brescia, Bone Marrow Transplant Unit, ASST Spedali Civili, Brescia, Italy. Tel: +39 0303996812; Fax: +39 0303996811; E-mail: domenico.russo@unibs.it

Supplementary material
**Supplementary Figure S1** Live/Dead staining of BM-hMSCs cultivated for 21 days in G-CH1 and G-CH2 hydrogels in the complete medium FBS or complete medium hPL. Scale bar: 100 µm.
Supplementary Figure S2 (A) SEM micrographs of BM-hMSCs cultivated in G-CH1 scaffold and G-CH2 scaffold in complete medium hPL w/o osteogenic differentiation stimuli at 21 days culture. Scale bar: 5 µm.
(B) SEM micrographs of calcium phosphate deposition in G-CH1 and G-CH2 with BM-hMSCs at day 21 with osteogenic differentiation stimuli and hPL. Scale bars: 20 µm and 5 µm.
**Supplementary Figure S3** Evaluation of calcium phosphate deposition with SEM-EDX of G-CH1 in OM with hPL, G-CH1 in complete medium hPL, G-CH2 in OM with hPL and G-CH2 in complete medium hPL at day 21. EDX spectra of treated sample area were reported.
Supplementary Figure S4 Enrichment in KEGG pathways (A) and networks (B) based on STRING annotation tool (https://string-db.org/) applied on the list of 59 protein specific of only hPL independently from sample preparation method. Network edges are based on molecular action (Kmeans clustering n=6). FDR=False Discovery Rate.
Supplementary Figure S5 List of significant protein differences obtained comparing hPL and PPP in not depleted samples, based on Peaks Studio, applying the following filters: Fold change≥1.5; pANOVA<0.05; ≥2unique peptides, in at least 4 of 12 replicates.

| UNIPROT Accession | Description | Protein Avg Mass (Da) | protein coverage (%) | Unique peptides | Average Normalized Area NOT depleted hPL | NOT depleted PPP | RATIO (hPL/PPP) |
|-------------------|-------------|-----------------------|-----------------------|----------------|-----------------------------------------|-----------------|----------------|
| P02775|CXCL7_HUMAN | Platelet basic protein | 13894 | 19 | 2 | 1.65E+06 | 7.38E+04 | 22.4 |
| P68871|HBB_HUMAN | Hemoglobin subunit beta | 15998 | 32 | 2 | 1.81E+06 | 2.57E+05 | 7.0 |
| P69905|HBA_HUMAN | Hemoglobin subunit alpha | 15258 | 30 | 3 | 6.93E+06 | 1.14E+06 | 6.1 |
| P0DOX2|IGA2_HUMAN | Immunoglobulin alpha-2 heavy chain | 48935 | 32 | 2 | 3.95E+05 | 1.63E+05 | 2.4 |
| P02751|FINC_HUMAN | Fibronectin | 262622 | 26 | 47 | 3.24E+06 | 5.04E+06 | 0.6 |
| P02671|FIBA_HUMAN | Fibrinogen alpha chain | 94973 | 53 | 79 | 1.51E+07 | 2.84E+07 | 0.5 |
| P01593|KVD33_HUMAN | Immunoglobulin kappa variable 1D-33 | 12848 | 38 | 2 | 8.54E+04 | 2.01E+05 | 0.4 |
**Supplementary Figure S6** List of significant protein differences obtained comparing hPL and PPP in depleted samples, based on Peaks Studio, applying the following filters: Fold change≥1.5; pANOVA<0.05; ≥2unique peptides, in at least 4 of 12 replicates.

| UNIPROT Accession | Description                                      | Protein Avg Mass (Da) | protein coverage (%) | Unique peptides | Average Normalized Area NOT depleted hPL | NOT depleted PPP | RATIO (hPL/PPP) |
|-------------------|--------------------------------------------------|-----------------------|----------------------|-----------------|------------------------------------------|-----------------|-----------------|
| P02775 | CXCL7_HUMAN | Platelet basic protein | 13894             | 40                  | 4              | 3.58E+06 | 8.59E+04 | 41.7  |
| P07936 | TSP1_HUMAN | Thrombospondin-1 | 129383             | 3                   | 2              | 1.02E+06 | 4.57E+04 | 22.3  |
| P68871 | HBB_HUMAN | Hemoglobin subunit beta | 15998             | 50                  | 4              | 5.16E+06 | 8.50E+05 | 6.1   |
| P69905 | HBA_HUMAN | Hemoglobin subunit alpha | 15258             | 36                  | 6              | 9.66E+06 | 2.59E+06 | 3.7   |
| P06681 | CO2_HUMAN | Complement C2 | 83268             | 7                   | 6              | 1.56E+06 | 5.69E+05 | 2.7   |
| P0C0L5 | CO4B_HUMAN | Complement C4-B | 192750            | 36                  | 2              | 3.78E+05 | 1.42E+05 | 2.7   |
| P59666 | DEF3_HUMAN | Neutrophil defensin 3 | 10245             | 19                  | 2              | 3.89E+05 | 1.54E+05 | 2.5   |
| P21333 | FLNA_HUMAN | Filamin-A | 280737             | 5                   | 7              | 1.11E+06 | 4.52E+05 | 2.5   |
| P15814 | IGLL1_HUMAN | Immunoglobulin lambda-like polypeptide 1 | 22963             | 11                 | 2              | 2.28E+06 | 9.48E+05 | 4.4   |
| P15169 | CBPN_HUMAN | Carboxypeptidase N catalytic chain | 52286             | 4                   | 2              | 3.95E+05 | 1.88E+05 | 2.1   |
| O00187 | MASP2_HUMAN | Mannan-binding lectin serine protease 2 | 75702             | 6                   | 3              | 2.03E+05 | 1.00E+05 | 2.0   |
| P07360 | CO8G_HUMAN | Complement component C8 gamma chain | 22277            | 30                  | 5              | 1.16E+06 | 6.43E+05 | 1.8   |
| P01860 | IGHG3_HUMAN | Immunoglobulin heavy constant gamma 3 | 41287             | 28                  | 2              | 4.05E+06 | 2.39E+06 | 1.7   |
| P02748 | CO9_HUMAN | Complement component C9 | 63173             | 34                  | 15             | 3.39E+06 | 2.02E+06 | 1.7   |
| P22792 | CPN2_HUMAN | Carboxypeptidase N subunit 2 | 60557             | 6                   | 2              | 1.24E+06 | 7.49E+05 | 1.7   |
| Q9Y490 | TLN1_HUMAN | Talin-1 | 269765             | 2                   | 4              | 1.85E+06 | 1.14E+06 | 1.6   |
| P26927 | HGFL_HUMAN | Hepatocyte growth factor-like protein | 80320             | 11                  | 4              | 5.45E+05 | 3.50E+05 | 1.6   |
| P01034 | CYTC_HUMAN | Cystatin-C | 15799             | 19                  | 2              | 1.90E+05 | 1.23E+05 | 1.5   |
| P00742 | FA10_HUMAN | Coagulation factor X | 54732             | 6                   | 3              | 6.97E+05 | 1.19E+05 | 0.6   |
| P05160 | F13B_HUMAN | Coagulation factor XIII B chain | 75511             | 15                  | 8              | 5.81E+05 | 1.06E+05 | 0.5   |
| Q9YK7 | FGBP_HUMAN | IgGFe-binding protein | 572021            | 1                   | 4              | 1.90E+05 | 3.50E+05 | 0.5   |
| Q8IU80 | TMPSE_HUMAN | Transmembrane protease serine 6 | 90000             | 4                   | 2              | 1.40E+06 | 2.67E+06 | 0.5   |
| Q15485 | FCN2_HUMAN | Ficolin-2 | 34001             | 8                   | 2              | 3.74E+05 | 7.38E+05 | 0.5   |
Supplementary Figure S7 Enrichment in biological processes (A) and networks (B) based on STRING annotation tool (https://string-db.org/) applied on proteins resulted significantly varied in their abundance by label-free relative quantification. Network edges are based on molecular action (Kmeans clustering n=6).
Supplementary Table 1 Enrichment in biological process based on STRING annotation tool (https://string-db.org/) applied on the list of 59 proteins specific of only hPL independently from sample preparation method.