

MIT Open Access Articles

Comprehensive proteomic characterization of stem cell-derived extracellular matrices

The MIT Faculty has made this article openly available. **Please share** how this access benefits you. Your story matters.

Citation: Ragelle, Héloïse et al. "Comprehensive proteomic characterization of stem cell-derived extracellular matrices." *Biomaterials* 128 (June 2017): 147-159 © 2017 Elsevier Ltd

As Published: <http://dx.doi.org/10.1016/j.biomaterials.2017.03.008>

Publisher: Elsevier BV

Persistent URL: <https://hdl.handle.net/1721.1/126846>

Version: Original manuscript: author's manuscript prior to formal peer review

Terms of use: Creative Commons Attribution-NonCommercial-NoDerivs License



1 Comprehensive proteomic characterization of stem cell-derived extracellular matrices

2
3
4 Héloïse Ragelle^{1*}, Alexandra Naba^{1*‡}, Benjamin L. Larson¹⁻³, Fangheng Zhou¹, Miralem Prijic¹,
5 Charles A. Whittaker¹, Amanda Del Rosario¹, Robert Langer¹⁻⁴, Richard O. Hynes^{1,5}, Daniel G.
6 Anderson¹⁻⁴

7
8 ¹ David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of
9 Technology, Cambridge, MA 02142, USA

10 ² Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of
11 Technology, Cambridge, MA 02139, USA

12 ³ Institute for Medical Engineering and Science, Massachusetts Institute of Technology,
13 Cambridge, MA 02139, USA

14 ⁴ Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA
15 02139, USA

16 ⁵ Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, MA
17 02138, USA

18 [‡] Present address: Department of Physiology and Biophysics, University of Illinois at Chicago,
19 Chicago, IL 60612, USA

20
21 * These authors contributed equally to the work

22 Corresponding author: dgander@mit.edu

23 24 Abstract

25
26 In the stem-cell niche, the extracellular matrix (ECM) serves as a structural support that
27 additionally provides stem cells with signals that contribute to the regulation of stem-cell function,
28 via reciprocal interactions between cells and components of the ECM. Recently, cell-derived
29 ECMs have emerged as *in vitro* cell culture substrates to better recapitulate the native stem-cell
30 microenvironment outside the body. Significant changes in cell number, morphology and function
31 have been observed when mesenchymal stem cells (MSC) were cultured on ECM substrates as
32 compared to standard tissue-culture polystyrene (TCPS). As select ECM components are known
33 to regulate specific stem-cell functions, a robust characterization of cell-derived ECM proteomic
34 composition is critical to better comprehend the role of the ECM in directing cellular processes.
35 Here, we characterized and compared the protein composition of ECM produced *in vitro* by bone
36 marrow-derived MSC, adipose-derived MSC and neonatal fibroblasts from different donors,
37 employing quantitative proteomic methods. Each cell-derived ECM displayed a specific and
38 unique matrisome signature, yet they all shared a common set of proteins. We evaluated the
39 biological response of cells cultured on the different matrices and compared them to cells on
40 standard TCPS. The matrices lead to differential survival and gene-expression profiles among the
41 cell types and as compared to TCPS, indicating that the cell-derived ECMs influence each cell
42 type in a different manner. This general approach to understanding the protein composition of
43 different tissue-specific and cell-derived ECM will inform the rational design of defined systems
44 and biomaterials that recapitulate critical ECM signals for stem-cell culture and tissue engineering.

45
46
47 **Keywords:** extracellular matrix; mesenchymal stem cell; proteomic analysis; stem-cell derived
48 matrices; ECM cell-culture substrate; tissue engineering

49 50 51 1. Introduction

1
2 In the body, cells are surrounded by a complex three-dimensional microenvironment, termed the
3 extracellular matrix (ECM), which provides cells with many chemical and biophysical signals
4 required for cell function. Both specific components and biophysical properties of the ECM
5 coordinate intracellular signaling and downstream biological responses through bidirectional
6 interactions with the cells, regulating numerous physiological processes such as cell survival,
7 migration, proliferation and differentiation [1–3]. In the stem-cell niche, cell-matrix interactions
8 influence and modulate stem-cell self-renewal and differentiation. That is, the ECM operates *in*
9 *vivo* not only as a cellular support but also directs cell fate through coordinated physical and
10 biochemical cues [4–6].

11
12 During mammalian stem-cell culture, stem cells are removed from their native microenvironment
13 (*e.g.*, the bone-marrow niche for bone-marrow-derived mesenchymal stem cells (MSC), adipose
14 tissue for adipose-derived mesenchymal stem cells) and need to adapt to a relatively foreign
15 environment, that is, tissue-culture polystyrene (TCPS), which is fully synthetic and does not
16 present standard ECM signals. Evidence in the literature has shown that TCPS biases MSC
17 function resulting in lower proliferation rates and a loss of stemness over sequential passages [7–
18 9]. Therefore, ECM-based cell-culture substrates have been developed in an attempt to better
19 recapitulate *in vitro* the native cellular microenvironment [10–14]. MSC are able to deposit an ECM
20 on TCPS over the course of two weeks and this ECM can be used, after decellularization, as a
21 culture substrate for a new batch of MSC. It has been reported that the culture of MSC on *in vitro*
22 cell-derived ECM induces significant biological changes in MSC function compared to standard
23 culture conditions [10,11,15,16]. As ECM components are key players in the regulation of cellular
24 processes, it is critical to gain a better knowledge of ECM composition to decipher how ECM
25 components regulate cell function. In addition, a comprehensive understanding of cell-matrix
26 interactions will provide further insight into the rational design of ECM-mimicking substrates for
27 tissue engineering and regenerative medicine [17,18].

28
29 Despite several reports on the development of cell-derived ECMs for MSC culture, detailed data
30 about their molecular composition is limited. Traditional biochemical analysis of ECM is
31 challenging on account of the insolubility and complexity of ECM components [19]. To address
32 these issues, we have applied a proteomic approach initially described for the analysis of tumoral
33 ECM [20]. The method consists of a sequential digestion of the ECM proteins followed by tandem
34 mass spectrometry and bioinformatic analyses, yielding a detailed inventory of the ECM and ECM-
35 associated proteins (termed the “matrisome”). By coupling this method to label-based quantitative
36 proteomics, we were able to characterize and compare the molecular composition of cell-derived
37 ECM produced by different cell types *in vitro*, specifically bone- marrow-derived human
38 mesenchymal stem cells (Bm MSC), adipose-derived MSC (Ad MSC) and human neonatal dermal
39 fibroblasts (NHDF), as well as to evaluate the ECM produced by cells from different donors.
40 Proliferation and mRNA transcriptomic profiling of the cells cultured on the different ECM were
41 performed and compared to standard culture conditions.

42
43 We observe that, in addition to a set of common proteins, each cell-derived ECM contains cell-
44 type-specific proteins. Quantitative proteomic analysis reveals a specific matrisome signature for
45 each type of ECM. The matrices lead to differential cell growth and gene expression among the
46 cells as compared to TCPS culture, indicating that the ECM signatures influence each cell type in
47 a differential fashion.

48 49 50 51 **2. Materials and methods**

2.1. Preparation of *in vitro* cell-derived ECM

ECM plates were provided by StemBioSys (San Antonio, Texas) and prepared according to a published protocol [16]. Briefly, human mesenchymal stem cells derived from bone marrow (Bm MSC, Lonza) or from adipose tissue (Ad MSC, Life Technologies), or neonatal dermal fibroblasts (NHDF, Life Technologies) were seeded onto a 75 cm²-cell culture flask coated with human fibronectin (1 h at 37°C) at a cell density of 3,500 cells/cm² and cultured in α -MEM medium supplemented with 15% fetal bovine serum (FBS) and 1% penicillin-streptomycin for 14 days. The medium was refreshed the day after initial seeding and then every 3 days. At day 7, ascorbic acid 2-phosphate (A2P, Sigma) was added to the medium at a final concentration of 50 μ M, and A2P-supplemented medium was used until the end of ECM production, with medium changes every other day. At day 14, plates were decellularized using 0.5% Triton in 20 mM ammonium hydroxide for 5 min, rinsed two times with Hank's Balanced Salt Solution containing both calcium and magnesium (HBSS +/+), and once with ultra-pure H₂O. Plates coated with cell-derived ECM were stored dry at 4°C until use for cell culture. *In vitro* ECM produced by commercially available Ad MSC (two different 35- and 45-year-old female donors), Bm MSC (six different 19- to 22-year-old males and female donors) and NHDF (two different new born male donors) are designated as Ad ECM, Bm ECM and Der ECM, respectively. The donor characteristics listed above were provided by the companies.

2.2. Proteomic analysis of ECM samples

The ECM was mechanically detached from the 75 cm²-cell culture flask using a cell scraper in 2 ml of HBSS +/+, centrifuged at 16,000x g for 5 min, washed with 1 ml of HBSS +/+, centrifuged, and dried in a Speed-Vac (Savant) for 15 min. The ECM pellet was then processed as described previously [20,21]. Briefly, the ECM pellet was resuspended and reduced in a solution of 8 M urea, 100 mM ammonium bicarbonate, and 10 mM dithiothreitol at pH 8 under agitation at 37°C for 2 h. After cooling, cysteines were alkylated by adding iodoacetamide at a final concentration of 25 mM for 30 min. The ECM sample was then diluted to 2 M urea, 100 mM ammonium bicarbonate (pH 8), and deglycosylated with PNGaseF (2000 U, New England BioLabs, Ipswich, MA) for 2 h under agitation at 37°C, followed by digestion with Lys-C (Wako Chemicals USA, Richmond, VA), at a ratio of 1:100 enzyme:substrate, under agitation at 37°C for 2 h. Final digestion was done using trypsin (Sequencing Grade, Promega, Madison, WI), at a ratio of 1:50 enzyme:substrate, under agitation at 37°C overnight, followed by a second aliquot of trypsin, at a ratio of 1:100 enzyme:substrate, and an additional 2 h of incubation. Digests were acidified and desalted using 30mg HLB Oasis Cartridges (Waters Corp., Milford, MA) eluted with 50% acetonitrile with 0.1% trifluoroacetic acid (TFA), followed by concentration in a Speed-Vac.

2.3. Analysis by mass spectrometry (LC-MS/MS)

Each sample was separated by reverse-phase HPLC using an EASY-nLC1000 liquid chromatograph (Thermo Fisher Scientific, Waltham, MA) over a 140-minute gradient before nano-electrospray using a Q Exactive mass spectrometer (Thermo Fisher Scientific). The mass spectrometer was operated in a data-dependent mode. The parameters for the full-scan MS were: resolution of 70,000 across 350-2000 m/z; AGC 3e6; and maximum IT 50 ms. The full MS scan was followed by MS/MS for the top 10 precursor ions in each cycle with a normalized collision energy (NCE) of 28 (unlabeled samples) or 32 (labeled samples) and dynamic exclusion of 30 s. Raw mass spectral data files (.raw) were searched using Proteome Discoverer (Thermo Fisher Scientific) and Mascot version 2.4.1 (Matrix Science) using the SwissProt *Homo sapiens* database (SwissProt_2016_02, *Homo sapiens* 20199 sequences) containing 20,199 entries. Mascot search

1 parameters were: 10 ppm mass tolerance for precursor ions; 0.8 Da for fragment-ion mass
2 tolerance; 2 missed cleavages of trypsin; fixed modifications were carbamidomethylation of
3 cysteines and for the quantitative experiments: Tandem Mass Tag (TMT) 6-plex modifications of
4 lysines and peptide N-termini; variable modifications were oxidized methionines, deamidation of
5 asparagines, pyro-glutamic acid modification at N-terminal glutamines; and hydroxylation of
6 prolines and lysines. Only peptides with a Mascot score greater than or equal to 25 and an isolation
7 interference less than or equal to 30 were included in the quantitative data analysis. The average
8 false discovery rate was 0.0080 (ranging from 0.0033- 0.0107). Proteins were identified as being
9 ECM-derived or not using the human matrixome annotations as previously described [22,23].

10 11 2.4. Label-based quantitative proteomics

12
13 Peptide labeling with Tandem Mass Tag (TMT) reagents (Thermo Fisher Scientific) was performed
14 according to the manufacturer's instructions. Lyophilized samples were dissolved in 70 μ l ethanol
15 and 30 μ l of 500 mM triethylammonium bicarbonate (pH 8.5) and the TMT reagent was dissolved
16 in 30 μ l of anhydrous acetonitrile. The solutions containing peptides and TMT reagents were
17 vortexed and incubated at room temperature for 1 h. Samples were labeled using the TMT 6-plex
18 channels as follows:

19 *Study of ECMs produced by bone marrow-derived MSC from different donors:* Replicate 1: donor
20 1: TMT-129N, donor 2: TMT-127N, donor 3: TMT-126, donor 4: TMT-130C, donor 5: TMT-128C,
21 donor 6: TMT-128N. Replicate 2: donor 1: TMT-126, donor 2: TMT-127, donor 3: TMT-128, donor
22 4: TMT-129, donor 5: TMT-130, donor 6: TMT-131.

23 *Study of ECMs produced by different cell types:* Replicate 1: Bm ECM: TMT-126, Der ECM: TMT-
24 127, Ad ECM: TMT-128. Replicate 2: Bm ECM: TMT-126, Der ECM: TMT-128, Ad ECM: TMT-
25 127.

26 An initial LC-MS/MS analysis was performed on each sample, and the total number of peptides
27 identified and the sum of the intensity of the precursor ions were used as initial metric to determine
28 equivalent peptide amount and normalize the samples. Equal amounts of samples labeled with
29 the different isotopic TMT reagents were combined and concentrated to completion in a vacuum
30 centrifuge, resuspended in 100 μ l of 0.1% formic acid, and analyzed by LC-MS/MS as described
31 above. TMT quantification was isotopically corrected according to the manufacturer's instructions,
32 and the values were normalized to the median of each channel.

33 The protein expression data were analyzed as described in [24]. Fold changes were expressed in
34 the \log_2 scale relative to the average of all samples. To account for biological variation, observed
35 proteins with TMT \log_2 fold change ratios below -1 were considered reduced in expression and
36 proteins with TMT \log_2 fold change ratios above 1 were considered increased in expression. The
37 coefficients of variation across the biological replicates were calculated for all proteins as the
38 standard deviation divided by the mean.

39 The raw mass spectrometry data have been deposited to the ProteomeXchange Consortium via
40 the PRIDE partner repository with the dataset identifier PXD005521 and 10.6019/PXD005521
41 [25,26]. The raw mass spectrometry data will become publicly available upon acceptance of the
42 paper. During the evaluation of the manuscript, the reviewers can access the raw mass
43 spectrometry by login to the [PRIDE website](http://www.ebi.ac.uk/pride/archive/login) (<http://www.ebi.ac.uk/pride/archive/login>) using the
44 following credentials: username: reviewer66584@ebi.ac.uk /Password: njYYGIKR.

45 46 47 48 49 2.5. Cell culture

1 Bone-marrow-derived human mesenchymal stem cells (Bm MSC, Texas A&M University System
2 Health Science Center), adipose-derived human MSC (Ad MSC, Thermo Fisher Scientific) and
3 human neonatal dermal fibroblasts (NHDF, Lonza) were seeded on each substrate (*i.e.*, Bm ECM,
4 Ad ECM, Der ECM or TCPS) at a cell density of 3,500 cells/cm². Cells were cultured in α -MEM
5 medium supplemented with 20% FBS and 1% penicillin-streptomycin. The medium was changed
6 the day after seeding and every 3 days thereafter. For the cell growth experiment, cells were
7 harvested and counted using a hemocytometer 72 h after seeding. After fixation with 3.2%
8 paraformaldehyde (PFA) solution, actin filaments were stained with Alexa Fluor[®] 488 Phalloidin
9 (Life Technologies, Carlsbad, CA) for 30 min and cell nuclei was stained with DAPI for 15 min.
10 Samples were imaged using an Evos fluorescent microscope. For flow cytometric analysis, a
11 number of 10⁵ Bm MSC were incubated with the desired antibodies *i.e.*, FITC-conjugated CD36
12 antibody (Biolegend), FITC-conjugated ITGAV antibody (Thermo Fisher), APC-conjugated ITGB8
13 antibody (Thermo Fisher) or APC-conjugated FGFR2 antibody (R&D Systems) following
14 manufacturer's instructions, prior to flow cytometric analysis using a FACS Calibur flow cytometer
15 (Beckman Coulter, Brea, USA). Data was analyzed using the FlowJo software (Three Star,
16 Ashland, USA).

17

18 2.6. Immunostaining of the ECM

19

20 For confocal imaging, each ECM was prepared on glass-bottom dishes using the same protocol
21 as above to generate ECM-coated plates. ECM was fixed with 3.2% PFA for 15 min at room
22 temperature and then incubated with donkey serum (0.5% in PBS) at 4°C for 1 h. Anti-Collagen I
23 antibody (Abcam, 1:100 in 1% BSA) was then added to the sample. After three rinses with 1%
24 BSA solution, Alexa Fluor[®] 555 donkey anti-rabbit secondary antibody was added for 1 h at 4°C
25 (1:1000 in 0.5% donkey serum). The sample was washed 5 times with 1% BSA and incubated
26 with an anti-fibronectin antibody (Rabbit monoclonal F1 Alexa Fluor[®] 647, Abcam, 1:100 1 h 4°C).
27 Cell nuclei were stained with DAPI for 15 min. Olympus FV1200 confocal microscope was used
28 for imaging and image reconstruction was done using Image J.

29

30 2.7. Electron microscopy

31

32 The protocol for electron microscopy was adapted from [16]. Briefly, ECM samples were hydrated
33 for 1 h with PBS at 37°C and fixed with 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH
34 7.4) at room temperature for 1 h. Following 30 min incubation with 0.1 M sodium cacodylate buffer
35 at room temperature, the samples were dehydrated in ascending concentrations of ethanol (35%,
36 70%, 95%, 100%), and coated with 30 nm gold-palladium. After coating, ECM samples were
37 imaged using a scanning electron microscope (Zeiss Merlin High-resolution SEM).

38

39 2.8. Transcriptomic analysis

40

41 Total RNA was extracted using RNeasy Mini Kit (Qiagen, Hilden, Germany) from NHDF, Ad MSC,
42 and Bm MSC after 3 days of culture on TCPS, Ad ECM, Bm ECM or Der ECM, in triplicate. Total
43 RNA was amplified and labeled using Gene Chip 3' IVT Express Kit (Affymetrix) and hybridized to
44 Human Genome Microarrays (Affymetrix). Microarray data were analyzed using Spotfire. 310
45 genes with differential expression ($\text{abs log}_2\text{FC} \geq 1$, $p\text{-value} \leq 0.05$) in at least one of the 3
46 comparisons (Der ECM *versus* TCPS, Ad ECM *versus* TCPS, Bm ECM *versus* TCPS) for at least
47 one of the 3 cell types (NHDF, Ad MSC, Bm MSC) were selected for self-organizing map (SOM)
48 clustering. Replicates were averaged and row normalized with the GenePattern module
49 PreprocessDataset so that the average of each row is set to 0 with a variance of 1 [27]. These
50 row-centered data were then clustered using the GenePattern module SOMclustering. A range of
51 cluster numbers was tested and the final number of clusters to consider was identified using elbow

1 analysis where the last increase in cluster number explaining an additional 1% of the variance in
2 the data was selected. In this experiment, this corresponded to 13 clusters that explained about
3 81% of the variance in the data. Gene Set Enrichment Analysis (GSEA) was performed to analyze
4 the gene sets and obtain the enrichment sets [28,29]. All microarray data are publicly available
5 through the Gene Expression Omnibus archives under accession number GSE94667.

6 The same RNA was also used for real time Reverse Transcriptase-Polymerase Chain Reaction
7 (RT-PCR) to confirm microarray results. Real-time RT-PCR was performed with validated primers
8 and Cells-to-CT Taqman reagents (Applied Biosystems, Foster City, CA) on a StepOnePlus
9 thermocycler (Applied Biosystems). GAPDH was used as a loading control for each sample.

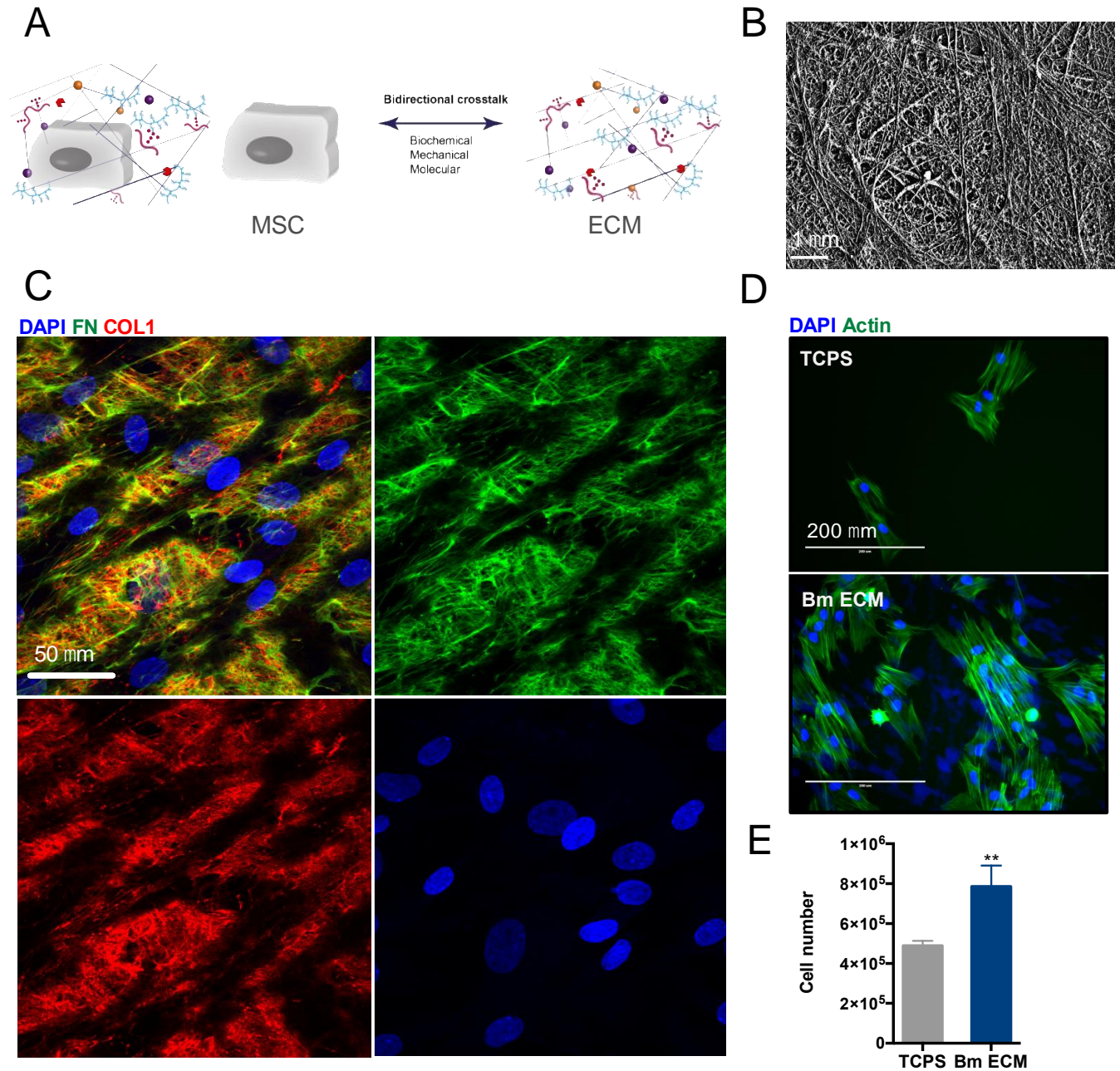
10 2.9. Statistical analyses

11 Data are presented as mean \pm standard deviation (SD) and were analyzed using GraphPad Prism
12 6 (GraphPad Software Inc, La Jolla). Statistically significant differences were assessed by
13 unpaired t-test or, for multiple comparisons, by an analysis of variance (ANOVA) at a 0.05
14 significance level and Tukey's post test, with * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

15 3. Results

16 3.1. Bm ECM improves the growth of mesenchymal stem cells *in vitro*

17 MSC produced an insoluble ECM after 14 days in culture in ascorbic acid-supplemented medium
18 (Fig 1B-C). This cell-derived ECM obtained after decellularization was used as a substrate for the
19 culture of a new batch of MSC. The ECM produced by bone-marrow-derived MSC (Bm MSC)
20 displayed a dense fibrillar structure as imaged by electron microscopy (Fig 1B). Immunostaining
21 showed that the cells were embedded in complex networks of fibronectin and collagen I (Fig 1C).
22 We compared the growth of Bm MSC on TCPS and on their own matrix, Bm ECM, 72 h after
23 seeding. MSC on Bm ECM grew significantly more than those on TCPS, indicated by an increase
24 in total cell number (Fig 1D and E).
25
26
27
28
29



1
2
3
4
5
6
7
8
9
10
11
12

Figure 1: ECM produced by Bm MSC *in vitro*. (A): Bidirectional interactions between MSC and ECM regulate cell function. (B): Electron microscopy of Bm ECM reveals a fibrillar structure. Scale bar is 1 μ m. (C): Immunostaining of Bm MSC on their ECM after 14 days using fibronectin antibody (green, top right picture), collagen I antibody (red, bottom left picture) and DAPI (cell nuclei, blue, bottom right picture). The overlay is shown in top left picture. Scale bar is 50 μ m. (D): Fluorescence microscopy images of Bm MSC on TCPS (top) or Bm ECM (bottom) after 3 days of culture. Actin cytoskeleton was stained with Alexa Fluor[®] 488 Phalloidin and cell nuclei with DAPI; scale bar is 200 μ m. (E): Number of Bm MSC after 3 days of culture on Bm ECM (blue bar) or TCPS (grey bar). Results show mean \pm SD of three independent experiments performed in triplicate. Statistics were done using unpaired t-test (** $p < 0.01$).

3.2. Proteomic characterization of *in vitro* cell-derived Bm ECM

As we and others have observed changes in MSC cell behavior on cell-derived ECM as compared to standard TCPS culture, we were motivated to develop a comprehensive characterization of the Bm ECM molecular composition.

3.2.1. Proteomic approach to characterize *in vitro* cell-derived ECM

Proteomic characterization of ECM is challenging on account of the complexity and biochemical properties of ECM components, which are mainly large, insoluble, highly cross-linked and glycosylated proteins. The primary technique for proteomic characterization, mass spectrometric analysis, requires proteins to be solubilized and digested into peptides. Traditional approaches to overcome ECM biochemical intractability employ solubilizing agents in concentrations that are incompatible with mass spectrometers [19]. This technical issue, along with the difficulty to functionally annotate ECM components accurately, have impeded the development of a global characterization of stem-cell ECM and explain why only partial characterizations of stem-cell matrices have been reported so far. Recently, Naba *et al.* have overcome this hurdle by developing a global proteomic approach that consists of the sequential solubilization and digestion of ECM proteins into peptides, without the need for centrifugation, minimizing material loss. This multistep process is comprised of ECM denaturation, reduction, alkylation, deglycosylation and protease digestion, followed by liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) [20,21], bioinformatic analysis and filtering through MatrisomeDB, an inventory of all human matrisome genes and proteins, to specifically identify matrisome proteins (*i.e.*, the proteins that constitute the ECM) [22,23,30].

Here, Bm ECM produced *in vitro* by bone marrow-derived MSC was characterized using this proteomic approach. The total number of proteins detected in Bm ECM across the donors was 420 on average (range 340 to 499 proteins), of which 20% were expressly identified as ECM or ECM-associated proteins (corresponding to between 64 and 84 proteins). The remaining proteins, designated as non-ECM proteins, consisted of insoluble intracellular components that remained after the decellularization step and were, for the most part, proteins associated with the actin cytoskeleton or with intermediate filaments (Supplementary Table III). Despite their number, the non-ECM-associated proteins represented only a small fraction of the proteomic content, *i.e.*, approximately 25% of the precursor-ion MS signal intensity, which is representative of peptide abundance and the matrisome proteins were detected in large abundance (Supplementary Fig 1).

Fibronectin pre-coating was used in this study to help prevent ECM detachment from the culture dish during ECM production, most likely by providing a more adhesive surface for the cells [11]. To verify that the fibronectin pre-coating did not affect the final ECM composition, and more particularly, did not contribute to an overestimation of the fibronectin amount in the ECM, we performed mass spectrometry analysis of ECM deposited on pre-coated TCPS and compared this ECM deposited on uncoated TCPS. Results showed that fibronectin was the most abundant protein, and was detected in similar amounts, in both conditions. In addition, the pre-coating did not affect ECM composition, as shown by the distribution of the 10 most abundant ECM proteins (Supplementary Fig 2). Finally, to estimate the contribution of bovine serum proteins to the composition of cell-derived ECM, mass spectrometry data were searched for bovine proteins and results showed that they represent less than 2% of the total protein content (Supplementary Fig 3). Some peptide sequences are common to both human and bovine species, and were considered as human in this study.

3.2.2. ECM produced by bone marrow-derived MSC displays a specific and consistent matrisome signature

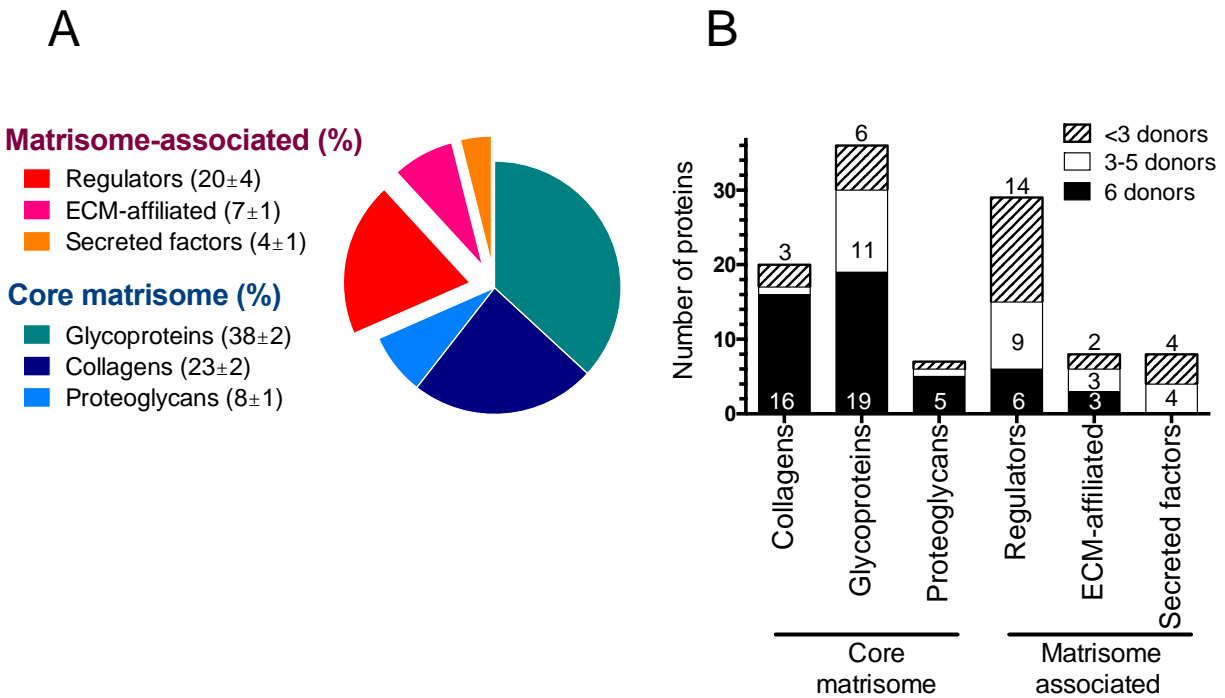
1
2 To gain a thorough understanding of the ECM produced by Bm MSC *in vitro*, we characterized
3 the ECM produced by MSC from six different donors. All proteins, even those detected with only
4 one peptide, were included in the analysis to provide an exhaustive proteomic composition of Bm
5 ECM. The detailed individual composition for each donor is listed in Supplementary Table I.
6

7 The ECM produced by Bm MSC in culture over the course of 14 days presents a relatively complex
8 composition. Indeed, across the six donors, 108 ECM and ECM-associated proteins were
9 identified, of which 62 proteins were present in more than five donors (listed in Table I) and 49
10 proteins were consistently found in all six donors (Fig 2B). The matrisome proteins of the common
11 set were present in large amounts, as indicated by the precursor-ion MS intensity and peptide
12 number, while the proteins found in fewer than three donors were detected with only a few
13 peptides, indicative of their low abundance (Supplementary Table I). As the most abundant
14 peptides are preferentially selected for fragmentation and identified with LC-MS/MS, proteins in
15 low amounts are thus less likely to be consistently detected, partly explaining why some proteins
16 are only detected in some of the donors. The common proteins were detected consistently across
17 donors, as indicated by the similar numbers of peptides and peptide intensities, suggesting that
18 ECM produced by Bm MSC isolated from different donors present a relatively consistent
19 composition.
20

21 Matrisome proteins can be classified in two distinct categories: those proteins that constitute the
22 core matrisome (*i.e.*, collagens, glycoproteins, and proteoglycans) and those proteins that are
23 associated and interact with the core matrisome proteins, designated as matrisome-associated
24 proteins (*i.e.*, regulators, secreted factors, and other ECM-affiliated proteins). Figure 2A shows
25 the matrisome signature of Bm ECM as a segmentation of the matrisome proteins into each sub-
26 category. Core matrisome proteins were dominant; they represented around 60% of the total
27 number of Bm ECM matrisome proteins and were also very abundant (75% of the precursor-ion
28 MS signal intensity and 50% of the number of peptides, Supplementary Fig 1). The proteins of the
29 core matrisome are structural proteins that confer mechanical properties to the ECM, and also
30 play roles in cell adhesion and signaling predominantly via integrin binding [31]. Some of the most
31 abundant glycoproteins detected by LC/MS-MS were fibronectin, tenascin-C, fibulin-1, emilin-1
32 and thrombospondin-1. Collagens I, VI and XII as well as perlecan were identified as the most
33 abundant collagens and proteoglycans, respectively (Table I). Components of the core matrisome
34 were detected with a similar number of peptides and in comparable abundance across the 6
35 donors, suggesting that Bm ECM composition is consistent across biological replicates
36 (Supplementary Table I).
37

38 In addition to the core matrisome proteins, the ECM serves as a reservoir for regulators (*i.e.*, ECM-
39 remodeling enzymes, cross-linkers and protease inhibitors), secreted factors and other ECM-
40 affiliated proteins. These proteins fall into the category of matrisome-associated proteins and are
41 less abundant than the core matrisome proteins (Fig 2A). Although more variation in the qualitative
42 composition of matrisome-associated proteins was observed among the donors, a common set of
43 matrisome-associated proteins was consistently found and includes cross-linkers such as
44 transglutaminase-2 and LOXL1/2, the ECM-remodeling enzymes cathepsin-B and matrix
45 metalloproteinase MMP-2, as well as the protease inhibitor serpine H1. These enzymes participate
46 in ECM arrangement by controlling the fine balance between stability and remodeling [32,33]. In
47 addition to these regulators, annexin A2 and galectins 1 and 3 were identified as ECM-affiliated
48 proteins. These proteins are associated with structural ECM proteins and regulate diverse cell
49 functions through downstream signaling. For example, the binding of annexin A2 to the
50 glycoprotein tenascin-C is believed to promote cell migration and mitogenesis through the loss of
51 cell adhesion [34].

1
 2 Finally, the ECM serves as a reservoir for numerous secreted factors that bind to core matrisome
 3 proteins, including proteoglycans as well as ECM-associated proteins, and trigger various
 4 signaling pathways [1]. On account of their small size, low abundance and higher solubility, they
 5 may be partially lost during decellularization and their detection is more variable among the
 6 donors. Thus, we have chosen to include the secreted factors detected in more than 3 donors in
 7 Table I and they include the chemokine CXCL12 that is constitutively expressed in the bone
 8 marrow, and responsible for bone-marrow stem-cell homeostasis regulation [35], as well as the
 9 calcium-binding proteins S100A6/A10 implicated in various cellular processes such as cell
 10 proliferation and differentiation through interactions with annexin A2 [36].
 11



12
 13
 14 **Figure 2: Proteomic characterization of ECM produced by Bm MSC from different donors.**
 15 (A): Matrisome signature of Bm ECM. Pie chart represents the distribution of Bm ECM proteins by
 16 percentage of total number for each matrisome protein sub-category. The average percentage ± SD for
 17 each sub-category was calculated for the 6 donors and is presented in the parentheses. (B): Protein
 18 distribution among donors. Columns represent the number of proteins for each matrisome sub-category
 19 detected in all donors (black bars), 3 to 5 donors (white bars) or fewer than 3 donors (hatched bars). Proteins
 20 represented by at least 1 peptide are included. Results were collected from two technical replicates of two
 21 independent experiments.

22
 23
 24

Table I: Bm ECM signature. The list shows the proteins identified in at least 5 donors or in at least 3 donors for the secreted factors.

CORE MATRISOME PROTEINS

COLLAGENS

Fibril forming		Network forming		FACIT		Beaded filament forming	
<i>Gene symbol</i>	<i>Protein</i>	<i>Gene symbol</i>	<i>Protein</i>	<i>Gene symbol</i>	<i>Protein</i>	<i>Gene symbol</i>	<i>Protein</i>
COL1A1/2	Collagen I α 1/2	COL4A1/2/5	Collagen IV α 1/2/5	COL12A1	Collagen XII α 1	COL6A1/2/3	Collagen VI α 1/2/3
COL2A1	Collagen II α 1	COL8A1	Collagen VIII α 1	COL14A1	Collagen XIV α 1		
COL3A1	Collagen III α 1			COL16A1	Collagen XVI α 1		
COL5A1/2	Collagen V α 1/2						
COL11A1	Collagen XI α 1						

GLYCOPROTEINS

Major known		Growth factor binding protein		Vascular ECM proteins		Other	
<i>Gene symbol</i>	<i>Protein</i>	<i>Gene symbol</i>	<i>Protein</i>	<i>Gene symbol</i>	<i>Protein</i>	<i>Gene symbol</i>	<i>Protein</i>
EFEMP2	Fibulin 4	LTPB1	Latent TGF- β binding protein 1	FGB	Fibrinogen β	AEBP1	AE-binding protein 1
EMILIN1	Emilin 1			FGG	Fibrinogen γ	EDIL3	EGF-like repeats and discoidin I-like domains 3
FBN1/2	Fibulin 1/2			VTN	Vitronectin		
FBLN1/2	Fibrillin 1/2					MFGE8	Milk fat globule-EGF factor 8 protein
FN1	Fibronectin					MXRA5	Matrix-remodeling associated 5
MFAP2/5	Microfibrillar-associated proteins					PXDN	Peroxidasin homolog
POSTN	Periostin					TGFBI	TGF β -induced protein
THBS1/2	Thrombospondin 1/2					VWA1	von Willebrand factor A domain containing protein 1
TNC	Tenascin C						

PROTEOGLYCAN

Leucine-rich repeat (LRR)		LINK		Complex		Hyalactans (LINK/CLEC/CCP)	
<i>Gene symbol</i>	<i>Protein</i>	<i>Gene symbol</i>	<i>Protein</i>	<i>Gene symbol</i>	<i>Protein</i>	<i>Gene symbol</i>	<i>Protein</i>
BGN	Biglycan	HAPLN1	Hyaluronan and proteoglycan link protein 1	HSPG2	Perlecan	VCAN	Versican
DCN	Decorin						

MATRISOME ASSOCIATED PROTEINS

REGULATORS

Proteases		Protease inhibitors		Crosslinkers	
<i>Gene symbol</i>	<i>Protein</i>	<i>Gene symbol</i>	<i>Protein</i>	<i>Gene symbol</i>	<i>Protein</i>
CTSB	Cathepsin B	SERPINH1	Serpin peptidase inhibitor, H1	LOXL1/2	Lysyl oxidase-like 1/2
HTRA1	HtrA serin peptidase 1	PZP	Pregnancy zone protein	TGM2	Transglutaminase 2
MMP2	Matrix metalloproteinase 2				

ECM-AFFILIATED PROTEINS

<i>Gene symbol</i>	<i>Protein</i>
ANXA2/5	Annexin A2/A5
LGALS1/3	Lectin galactoside-binding soluble 1/3 (Galectins)

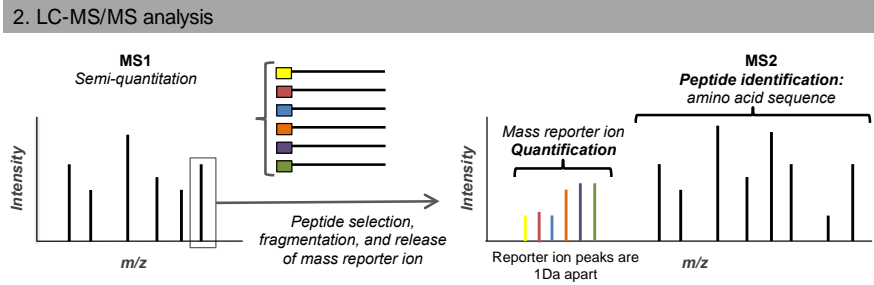
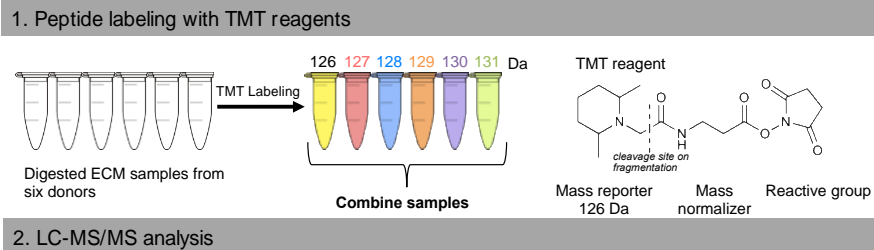
SECRETED FACTORS

<i>Gene symbol</i>	<i>Protein</i>
S100A6/10	S100 calcium binding protein A6/10
CXCL12	Chemokine C-X-C motif ligand 12

1 *3.2.3. Quantitative proteomic characterization of ECM produced by Bm MSC from different donors*

2
3 Quantitative proteomic approaches enable an accurate measure of relative protein amounts
4 between different samples [37,38]. Isobaric mass tag labeling or Tandem Mass Tag (TMT) labeling
5 was performed to evaluate the relative abundance of ECM proteins produced by Bm MSC from
6 different donors and thus gain a better understanding of Bm ECM composition consistency. This
7 method allows the measurement of relative expression levels of proteins in ECM derived from
8 different donors after labeling the peptides of each sample with a different chemical tag [39]. On
9 fragmentation during MS2, each tag releases a different mass fragment (see color code in Fig
10 3A), and quantification of these released fragments is exploited as the basis of the relative
11 quantification approach. Results are presented in Figure 3B in the format of a heat map, where
12 the colors indicate the relative protein expression level for each donor relative to the average
13 across the six donors (red are increased; blue are decreased). The coefficients of variation for
14 protein expression level across the different donors were 5.7%, 5.8%, 6.6%, 7.6%, 6.5% and 7.1%
15 for donor 1 to donor 6, respectively (average of 6.5%). All protein ratios were within a \log_2 fold
16 change range of -0.6 to 0.65 indicative of a relatively consistent protein profile across the six
17 donors. Thus, the TMT labeling suggests that Bm MSC isolated from different donors produce
18 similar ECMs, both qualitatively and quantitatively.

A



B

	Proteins	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6
GLYCOPROTEINS	EMLIN1						
	FBN1						
	FN1						
	FBLN1						
	POSTN						
	TNC						
	TGFBI						
THBS1							
COLLAGENS	COL1A1						
	COL2A1						
	COL3A1						
	COL4A1						
	COL5A1						
	COL6A1						
	COL8A1						
	COL11A1						
	COL12A1						
	COL16A1						
	COL1A2						
	COL4A2						
	COL5A2						
	COL6A2						
COL6A3							
PROTEOGLYCANS	HSPG2						
	BGN						
REGULATORS	TGM2						
	SERPINH1						
	LOXL1						
ECM-AFFILIATED	LOXL2						
	LGALS1						

Log₂

1.5

1

0

-1

-1.5

1
2
3 **Figure 3: Quantitative proteomic analysis of the ECM produced by Bm MSC from six**
4 **donors.** (A): TMT labeling experimental workflow. Each peptide solution is labeled with a specific TMT
5 reagent, samples are combined, and peptides are separated based on their mass-to-charge m/z ratios
6 (MS1). On MS2, peptides of particular m/z are selected, fragmented, and the different mass fragments are
7 released, enabling relative quantification. (B): Relative quantitative comparison among the six donors.
8 Proteins represented by at least 2 peptides are presented. Results show the fold-change (FC) in protein
9 detection levels normalized to the mean of all samples in log₂ scale. Data were collected from two technical
10 replicates of two independent experiments.

3.3. Comparison of the composition of ECMs produced *in vitro* by different cell types

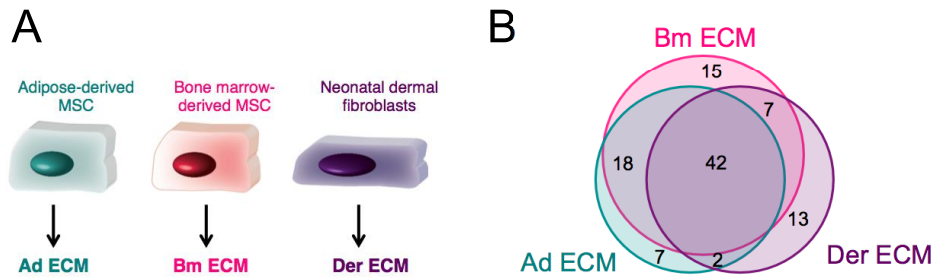
As Bm MSC from different donors produce an ECM with a defined and consistent proteomic composition, we investigated whether this ECM composition was unique to MSC originating from the bone marrow or universal among different cell types. Adipose-derived MSC (Ad MSC) were chosen in order to compare ECM produced by mesenchymal stem cells from a different tissue of origin. Neonatal dermal fibroblasts (NHDF) were chosen as a different primary cell type from a different tissue of origin (Fig 4A). ECM from Ad MSC and NHDF (Ad ECM and Der ECM, respectively) were produced using the same experimental procedure as for Bm ECM and their protein composition was compared, first qualitatively, then quantitatively, following the same approach as described above.

The number of matrisome proteins detected for each sample was in the same range, despite a lower peptide intensity signal for Der ECM compared to Ad ECM and Bm ECM, that might be explained by the lower total mass of insoluble matrix produced by NHDF after 14 days (Supplementary Fig 3). Qualitative analysis based on matrisome protein lists showed that ECM from the cells originating from different tissue types contain a large set of common proteins that represents 40% of the total protein number (Fig 4B). The overlap includes all collagens previously found in Bm ECM, except for collagen 14 α 1. The predominant glycoproteins detected in Bm ECM were also identified in Ad ECM and Der ECM, as well as most of the proteoglycans.

Quantitative TMT labeling approach was applied to compare the relative protein abundance in the three ECM samples, and significant tissue-type variations in the composition of the ECM were observed (Fig 4C). Only proteins represented with at least two peptides were taken into account for the analysis to guarantee more reliability, and this explained why 32 proteins were represented on the TMT heat map (Fig 4C) while 42 proteins were qualitatively detected in all three matrices (Fig 4B).

Der ECM was the most diverse of the three ECMs and a key observation was that Der ECM possessed the lowest abundance of collagens, compared to ECM from both types of MSC. More precisely, the amounts of collagens II and VIII were more than two times lower (\log_2 FC of approximately -1.35), as well as for collagens XI and V whose quantities were halved (\log_2 FC of approximately -1). Another observation was the differential levels of fibrillin-2 (FBN2) between the three samples: high in Der ECM and low or very low in Bm ECM and Ad ECM, respectively. In addition, fibulin-2 (FBLN2) and vitronectin (VTN) were three and two times less abundant in Bm ECM. Some variations in FBLN2, COL8A1, COL4A1 and, to a lesser extent, VCAN expression were also noted between Ad ECM and Bm ECM. In addition to the quantitative differences in the common proteins, some proteins were detected in one ECM and not in the others (Table II). For example, tenascin-X, a glycoprotein that is primarily expressed in loose connective tissues was solely detected in Ad ECM [34]. The detailed composition of Ad ECM and Der ECM is available in Supplementary Table II.

These data highlight that ECMs from different cell types are not identical but not totally discrete: while sharing a large set of common proteins, each ECM presents some unique components that may relate to the specialized functions of the tissue of origin.



C

	Proteins	Bm ECM	Ad ECM	Der ECM
GLYCOPROTEINS	FBLN2	Dark Blue	Light Blue	Light Red
	VTN	Dark Blue	Light Blue	Light Red
	FBN2	Light Blue	Dark Blue	Light Red
	MXRA5	Light Blue	Light Red	Light Red
	FBN1	Light Blue	Light Red	Light Red
	EMILIN1	Light Blue	Light Red	Light Red
	THSB1	Light Blue	Light Red	Light Red
	POSTN	Light Blue	Light Red	Light Red
	TGFBI	Light Blue	Light Red	Light Red
	FN1	Light Blue	Light Red	Light Red
	TNC	Light Blue	Light Red	Light Red
COLLAGENS	COL2A1	Light Red	Light Red	Dark Blue
	COL8A1	Light Red	Light Red	Dark Blue
	COL11A1	Light Red	Light Red	Light Blue
	COL5A2	Light Red	Light Red	Light Blue
	COL1A2	Light Red	Light Red	Light Blue
	COL5A1	Light Red	Light Red	Light Blue
	COL4A2	Light Red	Light Red	Light Blue
	COL1A1	Light Red	Light Red	Light Blue
	COL3A1	Light Red	Light Red	Light Blue
	COL4A1	Light Red	Light Red	Light Blue
	COL12A1	Light Red	Light Red	Light Blue
	COL6A2	Light Red	Light Red	Light Blue
	COL6A3	Light Red	Light Red	Light Blue
	COL6A1	Light Red	Light Red	Light Blue
PROTEOGLYCANS	VCAN	Light Blue	Light Red	Light Red
	HSPG2	Light Blue	Light Red	Light Red
	BGN	Light Blue	Light Red	Light Red
REGULATORS	TGM2	Light Blue	Light Red	Light Red
	SERPINH1	Light Blue	Light Red	Light Red
ECM-AFFILIATED	ANXA2	Light Blue	Light Red	Light Red
	LGALS1	Light Blue	Light Red	Light Red

Log₂

1.5 (Dark Red)

1 (Light Red)

0 (White)

-1 (Light Blue)

-1.5 (Dark Blue)

1
2
3 **Figure 4: Characterization of ECM produced by different cell types.** (A): ECM from Bm MSC
4 (Bm ECM), Ad MSC (Ad ECM) and NHDF (Der ECM) were produced and characterized. (B): Venn diagram
5 of the overlap of protein numbers. (C): Quantitative proteomics using TMT labeling. Results show the fold-
6 change (FC) in protein detection levels normalized to the mean of all samples in log₂ scale. Results were
7 collected from two technical replicates of two independent experiments and proteins identified with at least
8 2 peptides were considered.
9

1 **Table II: Source-specific protein lists for ECM derived from the three cell types.** Proteins
 2 presented here were identified in the ECM of one cell-type and not detected in the others. *For Bm ECM*
 3 *proteins, only proteins present in at least 5 donors were considered (or in at least 3 donors for the*
 4 *secreted factors).*
 5
 6

Ad ECM		
<i>Collagens</i>	COL15A1	Collagen XV α 1
<i>Glycoproteins</i>	FGL2	Fibrinogen-like 2
	TNXB	Tenascin XB
<i>Regulators</i>	ITIH3	Inter-alpha (globulin) inhibitor H3
	SERPINA5	Serpin peptidase inhibitor, clade A, member 5
<i>Secreted factors</i>	SCUBE3	Signal peptide, CUB domain, EGF-like 3
	CTGF	Connective tissue growth factor
Der ECM		
<i>Glycoproteins</i>	LTBP4	Latent transforming growth factor beta binding protein 4
	MATN2	Matrillin 2
	THBS3	Thrombospondin 3
	THSD4	Thrombospondin, type 1 domain containing 4
<i>Regulators</i>	ADAMTS1	ADAM metallopeptidase with thrombospondin type 1 motif, 1
	ADAMTSL4	ADAMTS-like 4
	CD109	CD109 molecule
	P4HA1	Prolyl 4-hydroxylase, alpha polypeptide I
	PRSS12	Neurotrypsin
	SERPINC1	Serpin peptidase inhibitor, clade C, member 1
<i>Secreted factors</i>	MDK	Midkine
	SFRP1	Secreted frizzled-related protein 1
	WNT5A	Wingless-type MMTV integration site family, member 5A
Bm ECM		
<i>Glycoproteins</i>	AEBP1	AE-bindin prot. 1
	EDIL3	EGF-like repeats and discoidin I-like domains 3
	EFEMP2	Fibulin 3
	FGB	Fibrinogen β
	FGG	Fibrinogen γ
	MFGE8	Milk fat globule-EGF factor 8 protein
	VWA1	Von Willebrand factor A domain containing prot. 1
<i>Collagens</i>	COL14A1	Collagen XIV α 1
<i>Regulators</i>	CTSB	Cathepsin B
	LOXL1	Lysyl oxidase-like 1
	MMP2	Matrix metallopeptidase 2
<i>ECM-affiliated proteins</i>	ANXA5	Annexin A5
	LGALS3	Lectin galactoside-binding soluble 3
<i>Secreted factors</i>	S100A6/10	S100 calcium binding protein A6/10

3.4. Cell response to ECM substrates

7
 8
 9 Proteomic analyses revealed that ECM from Ad MSC, Bm MSC and NHDF shared a large set of
 10 common proteins, although significant variations in levels were detected for some proteins. Also,
 11 each matrix presents specific proteins that were not detected in others. We hypothesized that the
 12 differential ECM compositions might affect cell responses when cultured on each of the three cell-
 13 derived ECMs.
 14
 15
 16
 17
 18

3.4.1. Effect of the different cell-derived ECMs on cell survival

To investigate how cells respond to the different matrices, we evaluated and compared the cell number and cell morphology 72 h after seeding on each of the substrates, *i.e.*, Ad ECM, Bm ECM, Der ECM and TCPS (Fig 5).

All cell types plated on any of the ECM substrates showed a more aligned arrangement than on TCPS that can be explained by the fibrillar ECM architecture (Fig 1B), which likely guides cell morphology and migration [1]. Both types of MSC showed improved survival on cell-derived ECMs compared to TCPS, as indicated by the 1.5 increase in cell number (Fig 5B). In addition, MSC exhibited a different morphology on ECM compared to TCPS, with a more elongated shape and a smaller size, which may be related to the higher cell density (Fig 5A). On the other hand, NHDF survival was similar on TCPS as to the ECM-based substrates and NHDF do not exhibit significant morphologic changes beyond collective alignment.

These data indicate that ECM impacts cells differentially depending on the cell type. While the type of substrate does not affect NHDF survival, MSC number was significantly increased on ECM-based substrates compared to TCPS, regardless of the cellular origin of the ECM.

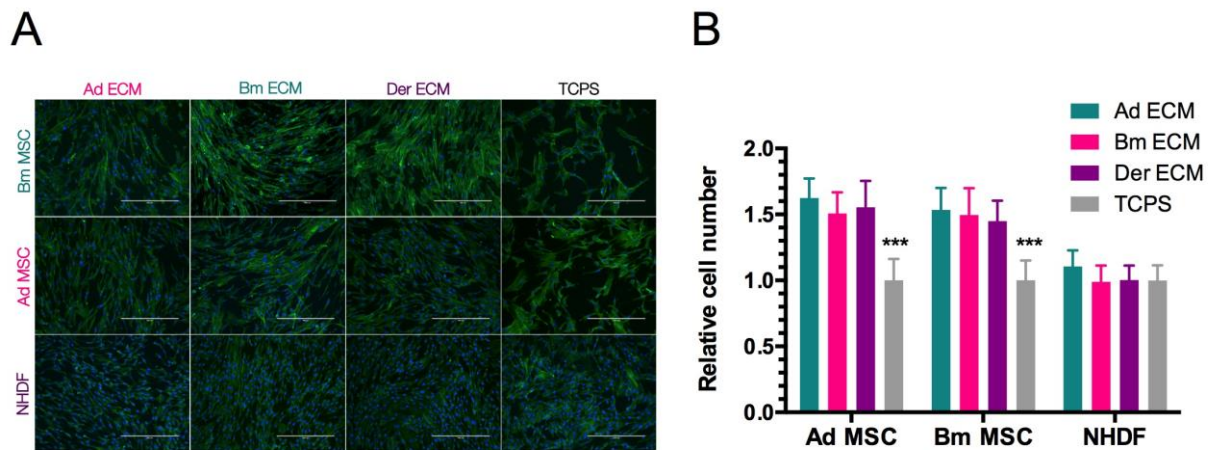


Figure 5: Cell survival on the different matrices. (A): Fluorescence microscopy pictures with actin cytoskeleton staining (Alexa Fluor® 488 Phalloidin) and cell nuclei (DAPI). Scale bar is 400 μ m. (B): Cell number relative to TCPS after 3 days in culture. Results show mean \pm SD of two independent experiments (n=6). Statistics were done using two-way ANOVA and Tukey's post test (***)p<0.001).

3.4.2. Cell-derived ECMs alter the cellular transcriptome

To investigate the effect of the substrates on the cell transcriptomes, RNA from the three cell types (*i.e.*, Ad MSC, Bm MSC and NHDF) was extracted after seeding the cells on each of the four substrates (*i.e.*, Ad ECM, Bm ECM, Der ECM, and TCPS) and the differential gene expression was evaluated across the different samples.

Few genes were differentially expressed when NHDF were seeded on TCPS as compared to cell-derived ECM, indicating a limited impact of the substrate on NHDF transcriptome (Fig 6A). This corroborates what was observed for cell growth and morphology. On the other hand, extensive significant differential gene expression was observed when MSC (both Ad MSC and Bm MSC) were plated on TCPS compared to ECM, suggesting that the signals from the substrate

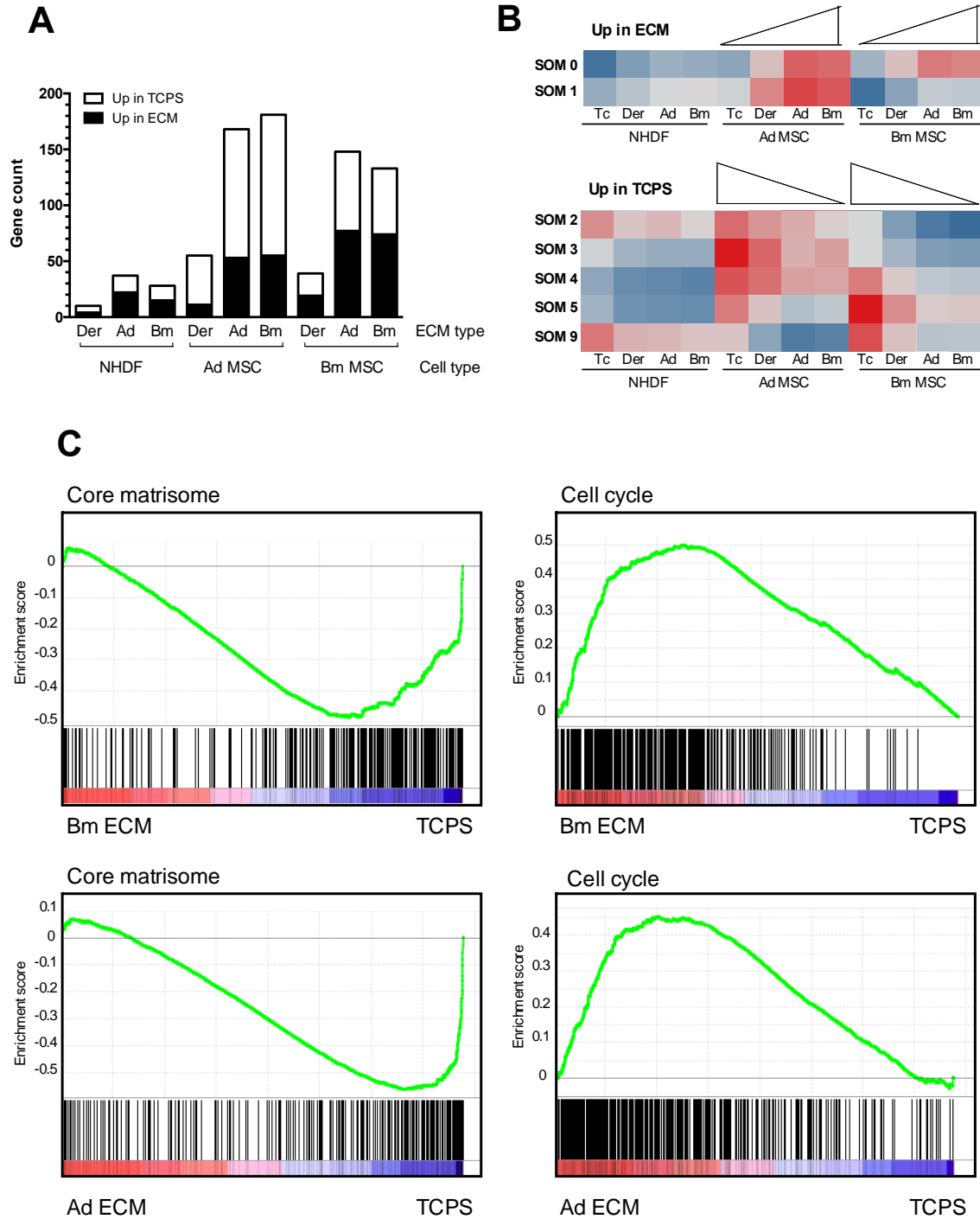
1 significantly alter the overall transcriptome of both types of MSC. The differentially expressed
2 genes are detailed in Supplementary Table IV.

3 310 genes with differential expression ($\text{abs log}_2\text{FC} \geq 1$, $\text{p-value} \leq 0.05$) in at least one of the three
4 comparisons (*Ad ECM versus TCPS*, *Bm ECM versus TCPS*, or *Der ECM versus TCPS*) for at
5 least one of the three cell types were selected for self-organizing map (SOM) clustering in order
6 to characterize the response of these genes in all comparisons. Clustering algorithms are
7 designed to elucidate general patterns in large data sets by grouping similar elements together
8 [40]. Here, the differentially expressed genes were grouped into 13 clusters that explained 81% of
9 the variance of the data. We focused our attention on the clusters in which differential responses
10 for Bm MSC on disparate matrices were observed. This corresponds to 7 different clusters that
11 displayed two main patterns: i) genes up-regulated in Bm MSC on MSC-derived ECM (Fig 6B
12 upper panel); and ii) genes expressed at a lower level in Bm MSC on MSC-derived ECM (Fig 6B
13 lower panel). The genes assigned to each of the cluster represented in Fig 6B are listed in
14 Supplementary Table V. For both Bm MSC and Ad MSC, the responses to MSC-derived ECM
15 (*i.e.*, Bm ECM or Ad ECM) were similar to one another, while the response to Der ECM was
16 intermediate between MSC-derived ECM and TCPS. As seen in Fig 6B, little variation across the
17 NHDF samples was observed using SOM clustering.

18

19 We then looked at the genes differentially expressed by MSC (both Ad MSC and Bm MSC) on
20 their own ECM compared to TCPS, and similar gene-set enrichments were observed for both cell
21 types (Fig 6C and Supplementary Table VI). The leading edge genes responsible for the
22 enrichment are listed in Supplementary Table VII. Of the genes more highly expressed on TCPS,
23 a large set encode ECM and ECM-associated proteins, as well as proteins involved in cell
24 adhesion (*e.g.*, ITGAV, ITGB8, CD36 or FGFR2). Increase in mRNA expression of ECM proteins
25 was validated by real time PCR for a subset of ECM proteins (Supplementary Figure 5A). Flow
26 cytometric analyses for cell surface receptors did not show differential expression, except for a
27 slight increase in ITGAV when Bm MSC were grown on TCPS compared to Bm ECM
28 (Supplementary Figure 5B). On the other hand, genes that regulate cell division were over-
29 expressed by MSC on cell-derived ECM as compared to TCPS (Fig 6C and Supplementary Table
30 V). In addition to genes involved in cell cycle, genes involved in MSC mobility and migration (*e.g.*,
31 genes coding for the CXCL chemokine family members, matrix proteases, PREX-1, GNG2) were
32 up-regulated when cells were on MSC-derived ECM *versus* TCPS.

33



1
2
3 **Figure 6: Influence of the substrate on the cell transcriptome.** (A): Differentially expressed genes
4 when Ad MSC, Bm MSC and NHDF were cultured on the different ECM compared to TCPS, with the
5 threshold of $\log_2 FC \geq 1$ and $p < 0.05$. (B): Gene response clustering with self-organizing maps (SOM). Each
6 row corresponds to a different gene cluster. Red represents up-regulation and blue represents down-
7 regulation (C): Enrichment plots for core matrisome gene set (left) and cell cycle KEGG pathway (right) in
8 MSC on their own ECM *versus* TCPS (Bm MSC top, Ad MSC bottom). Green curve indicates the enrichment
9 score. p -values < 0.002 .

4. Discussion

In the body, stem cells are in constant and intimate contact with the ECM. This matrix serves not only as a structural support, but as a reservoir of many biochemical and mechanical signals that are transduced via cell surface receptor-protein interactions and influence cell signaling pathways and dictate cell functions [1]. ECM components are at the center of this complex interplay and have a vital role in regulating physiological processes. During standard cell culture, stem cells are isolated from their native microenvironment (*i.e.*, the stem-cell niche), and need to adapt to a polymeric surface where ECM signals are limited to the serum proteins in the medium or adsorbed proteins on the plastic surface. This environment has been shown to affect cell biology; MSC cultured on ECM substrates compared to those on TCPS displayed different biologic features that impact cell morphology, motility and proliferation as well as, in other reports, differentiation [10,11]. In the context of stem cell biology, it is critical to decipher how individual ECM components regulate specific stem cell functions to better comprehend the natural role of the ECM. Understanding cellular processes requires a robust characterization of the ECM's molecular composition. In addition, this understanding will provide further insight for the rational design of ECM mimicking biomaterials for tissue engineering and regenerative medicine [18,41,42].

By employing a proteomic approach, we were able to provide a comprehensive characterization of the molecular composition of ECM produced *in vitro* by bone marrow-derived MSC (Bm ECM), adipose-derived MSC (Ad ECM) and human neonatal dermal fibroblasts (Der ECM). The analysis of Bm ECM of MSC from 6 different donors revealed a complex yet consistent protein composition. Specifically, the composition of these cell-derived ECMs (approximately 80 unique ECM and ECM-associated proteins) is rather complex as compared to protein-based substrates, which often comprise a surface coating of one or two proteins, and are sometimes employed for stem cell culture as an alternative to TCPS. The variance of protein expression across the Bm ECM formed by cells from the 6 different donors was relatively low (\log_2 fold change range of -0.6 to 0.65) indicative of a consistent matrix formed by Bm MSC from different donors. However, when the proteomic composition of Bm ECM was compared to Ad ECM and Der ECM, a distinct signature was observed for each of the three cell-derived matrices. Despite the sensitivity and precision of TMT labeling techniques, underestimation of protein abundance differences by a factor of 20 to 30% can occur due to ratio compression phenomena [43,44] and real effect sizes might be larger than what was measured. High-resolution techniques as well as correction algorithms have been developed to minimize the ratio compression and help detect subtle variations between samples.

The major proportion of Bm ECM proteins belongs to the core matrisome that includes collagens, glycoproteins and proteoglycans. A large variety of collagens were detected in Bm ECM, of which the fibrillar family was the most abundant (*i.e.*, collagens I, II, III, V and XI) that are known to contribute to the molecular architecture and mechanical properties of many tissues [45–47]. In addition, Fibril Associated Collagens with Interrupted Triple helices (FACIT) collagens that associate to the surface of fibrillar collagens were identified (collagens XII and XIV that associate with fibrillar collagen I to facilitate fibril thickening), along with network-forming collagens (collagen IV) and beaded-filament forming collagens (collagen VI) [45]. The presence of collagens in the ECM was associated with proteinases that specifically degrade collagens (MMP-2 that lyses collagens I, IV, V) as well as collagen cross-linkers such as the LOX-like family members [48]. While Bm ECM and Ad ECM present a similar collagen composition, the expression level of collagens in Der ECM was overall lower. Dermal tissues are elastic, while collagens confer stiffness and strength to tissues, as the collagen fibers have a high capacity for energy storage but minimal elasticity as a consequence of their semi-crystalline packing [45,49,50]. In addition, overall glycoprotein levels were found to be increased in Der ECM compared to Ad ECM and Bm

1 ECM, although differences in the levels of specific glycoproteins (e.g., fibulin-2) were observed
2 between both MSC-derived ECMs. These glycoproteins are implicated in the ECM structural
3 organization and have somewhat restricted tissue distribution [51]. For example, a major function
4 of fibulin-2 is the formation of large proteoglycan networks and its overexpression in Ad ECM and
5 Der ECM might be responsible for different ECM macromolecular architectures, compared to Bm
6 ECM. Similarly, fibrillins form microfibrils that provide tissue with long-range extensibility and
7 associate with elastic fibers [52,53]. The different ratios of core matrisome proteins lead to different
8 supramolecular assemblies that might ultimately provide the matrices with different mechanical
9 properties [49,50,54].

10 In addition to the core matrisome ECM proteins, numerous other proteins that contribute
11 to ECM function and dynamics were detected. Regulators maintain a balance between ECM
12 stability and dynamic remodeling by degrading proteins (e.g., cathepsin B, Adamalysin ADAMTS
13 that degrade fibronectin and collagen IV, or sulfatase SULF1 that cleaves proteoglycans and
14 removes GAG chains) or by modifying ECM topography through protein crosslinking (e.g., TGM2
15 and LOX). ECM remodeling promotes cell movement and migration, and the released proteolytic
16 fragments may also play a role in cell signaling [32]. These proteases are tightly regulated at the
17 transcriptome level, and some of them are secreted as pro-enzymes that require extracellular
18 activation. This is the case for the matrix metalloproteinase MMP-2 that needs to be activated by
19 other proteinases to be functional, such as the transmembrane type MMP-14, also identified in
20 Bm ECM [33]. Proteinases can be inactivated by protease inhibitors such as TIMP3 and A2M
21 found in Bm ECM. While several proteases were identified in Bm ECM, Ad ECM and Der ECM, it
22 is not clear from these data what their relative activity is and on-going work is characterizing their
23 role in ECM remodeling during stem cell culture.

24 Interestingly, ECM impacts different cells in different ways. Human neonatal dermal
25 fibroblasts survive as well on TCPS as on cell-derived ECM, and minimal changes at the RNA
26 levels were observed, suggesting that this cell type is negligibly impacted by the substrate over
27 72 h. In contrast, MSC number was significantly increased on ECM compared to TCPS as well as
28 to a fibronectin-coated substrate (unpublished data), indicating that a synergy of multiple proteins
29 is advantageous. Interestingly, the differences among the compositions of ECMs from different
30 tissue types do not seem to affect MSC survival, as the cell number was similarly increased on
31 each ECM. This suggests that MSC survival depends more on the common core of proteins than
32 on the unique proteins expressed in each of the cell-derived ECM. However, the different ECMs
33 might affect other cell functions that were not investigated in this study. **Future work will focus on
34 the secretome, another major regulator of MSC fate.**

35 Transcriptomic analysis revealed that the culture of MSC on TCPS induces an up-
36 regulation of genes that code for ECM and ECM-associated proteins as well as proteins implicated
37 in cell adhesion as compared to MSC on cell-derived ECM. On the other hand, NHDF seem less
38 affected by the type of substrate they are seeded on. One hypothesis is that MSC on TCPS are
39 more sensitive to certain ECM signals than other cell types, such as NHDF. In addition, MSC
40 population was increased on ECM and the cells over-express several genes implicated in cell
41 cycle regulation and migration. **Interactions with ECM and its components impact MSC function
42 compared to TCPS where MSC receive restricted molecular cues.** Perhaps, the lack of pertinent
43 ECM signals at early time points during TCPS culture or the relative cost of construction of a new
44 ECM is responsible for the lower number of MSC on TCPS as compared to cell-derived ECM.

45 46 **5. Conclusions**

47
48 In conclusion, we characterized the molecular composition of ECM deposited *in vitro* by bone
49 marrow-derived MSC as well as adipose-derived MSC and neonatal dermal fibroblasts. While the
50 three matrices share a set of common proteins, each ECM displayed distinct features specific to
51 the tissue of origin that might be related to specialized tissue functions. MSC derived from bone

1 marrow or adipose tissue both showed increased cell number on the cell-derived ECMs *versus*
2 standard TCPS; however, the unique ECM signatures had a minimal impact on MSC growth as
3 cell number was similarly increased across the different cell-derived ECMs. In addition, significant
4 changes in MSC transcriptome profiles were observed between both culture conditions; MSC on
5 TCPS over-expressed genes coding for matrisome proteins and MSC on cell-derived ECM over-
6 expressed genes implicated in cell cycle regulation and migration. On the other hand, the type of
7 substrate had a limited effect on NHDF behavior. These data corroborate the importance of ECM
8 signals for the *in vitro* culture of MSC. Utilizing these methods to further characterize ECM protein
9 composition as well as understanding ECM-stem cell interactions can provide insight into stem
10 cell biology and inform the design of next-generation ECM-mimicking substrates for *in vitro* stem
11 cell culture.

12 **Acknowledgements**

13 The authors gratefully acknowledge J. Wyckoff for expert advice with confocal imaging of the cell-
14 derived ECM as well as M.W. Tibbitt and O.S. Fenton for helpful feedback on the manuscript. This
15 work was supported in part by StemBioSys, the Helmsley foundation, grants from the National
16 Cancer Institute – Tumor Microenvironment Network (U54 CA126515/CA163109), the Howard
17 Hughes Medical Institute, of which ROH is an Investigator, and in part by Support Grant to the
18 Koch Institute (P30-CA14051 from the National Cancer Institute).

19 **Author contribution**

20 H.R. designed and performed the experiments, analyzed the data and wrote the manuscript; B.L.
21 designed, performed and analyzed cell culture experiments together with H.R.; A.N. designed and
22 analyzed the proteomic experiments together with H.R. and Ad.R.; B.L. and A.N. critically revised
23 the manuscript; F.Z. and B.L. designed and performed the SEM imaging; M.P. performed cell
24 culture experiments together with H.R. and B.L.; C.A.W. performed and analyzed the
25 transcriptome data together with H.R.; Ad.R. performed and analyzed the proteomic data together
26 with H.R. and A.N.; R.L., R.O.H. and D.G.A. supervised the design and execution of the study and
27 critically revised the manuscript.

28 **References**

- 29 [1] R.O. Hynes, The extracellular matrix: not just pretty fibrils., *Science*. 326 (2009) 1216–9.
- 30 [2] D.E. Discher, P. Janmey, Y.-L. Wang, Tissue cells feel and respond to the stiffness of their
31 substrate., *Science*. 310 (2005) 1139–1143.
- 32 [3] D.E. Discher, D.J. Mooney, P.W. Zandstra, Growth factors, matrices, and forces combine and
33 control stem cells., *Science*. 324 (2009) 1673–1677.
- 34 [4] S.W. Lane, D.A. Williams, F.M. Watt, Modulating the stem cell niche for tissue regeneration., *Nat.*
35 *Biotechnol.* 32 (2014) 795–803.
- 36 [5] A.J. Engler, S. Sen, H.L. Sweeney, D.E. Discher, Matrix elasticity directs stem cell lineage
37 specification., *Cell*. 126 (2006) 677–689.
- 38 [6] F.M. Watt, W.T.S. Huck, Role of the extracellular matrix in regulating stem cell fate., *Nat. Rev.*
39 *Mol. Cell Biol.* 14 (2013) 467–473.
- 40 [7] A. Banfi, A. Muraglia, B. Dozin, M. Mastrogiacomo, R. Cancedda, R. Quarto, Proliferation kinetics
41 and differentiation potential of ex vivo expanded human bone marrow stromal cells: Implications for
42 their use in cell therapy., *Exp. Hematol.* 28 (2000) 707–715.
- 43 [8] S.P. Bruder, N. Jaiswal, S.E. Haynesworth, Growth kinetics, self-renewal, and the osteogenic
44 potential of purified human mesenchymal stem cells during extensive subcultivation and following
45 cryopreservation., *J. Cell. Biochem.* 64 (1997) 278–294.
- 46 [9] K. Stenderup, J. Justesen, C. Clausen, M. Kassem, Aging is associated with decreased maximal
47 life span and accelerated senescence of bone marrow stromal cells., *Bone*. 33 (2003) 919–926.

- 1 [10] C.P. Ng, A.R.M. Sharif, D.E. Heath, J.W. Chow, C.B.Y. Zhang, M.B. Chan-Park, et al., Enhanced
2 ex vivo expansion of adult mesenchymal stem cells by fetal mesenchymal stem cell ECM.,
3 *Biomaterials*. 35 (2014) 4046–4057.
- 4 [11] M.C. Prewitz, F.P. Seib, M. von Bonin, J. Friedrichs, A. Stissel, C. Niehage, et al., Tightly
5 anchored tissue-mimetic matrices as instructive stem cell microenvironments., *Nat. Methods*. 10
6 (2013) 788–794.
- 7 [12] S. Abraham, M.J. Riggs, K. Nelson, V. Lee, R.R. Rao, Characterization of human fibroblast-
8 derived extracellular matrix components for human pluripotent stem cell propagation., *Acta*
9 *Biomater.* 6 (2010) 4622–4633.
- 10 [13] X.-D. Chen, Extracellular matrix provides an optimal niche for the maintenance and propagation of
11 mesenchymal stem cells., *Birth Defects Res. C. Embryo Today*. 90 (2010) 45–54.
- 12 [14] J. Li, K.C. Hansen, Y. Zhang, C. Dong, C.Z. Dinu, M. Dzieciatkowska, et al., Rejuvenation of
13 chondrogenic potential in a young stem cell microenvironment., *Biomaterials*. 35 (2014) 642–653.
- 14 [15] B. Antebi, Z. Zhang, Y. Wang, Z. Lu, X.-D. Chen, J. Ling, Stromal-cell-derived extracellular matrix
15 promotes the proliferation and retains the osteogenic differentiation capacity of mesenchymal
16 stem cells on three-dimensional scaffolds., *Tissue Eng. Part C. Methods*. 21 (2015) 171–181.
- 17 [16] Y. Lai, Y. Sun, C.M. Skinner, E.L. Son, Z. Lu, R.S. Tuan, et al., Reconstitution of marrow-derived
18 extracellular matrix ex vivo: a robust culture system for expanding large-scale highly functional
19 human mesenchymal stem cells., *Stem Cells Dev.* 19 (2010) 1095–1107.
- 20 [17] S. Gobaa, S. Hoehnel, M. Roccio, A. Negro, S. Kobel, M.P. Lutolf, Artificial niche microarrays for
21 probing single stem cell fate in high throughput., *Nat. Methods*. 8 (2011) 949–955.
- 22 [18] F. Gattazzo, A. Urciuolo, P. Bonaldo, Extracellular matrix: A dynamic microenvironment for stem
23 cell niche, *Biochim. Biophys. Acta - Gen. Subj.* 1840 (2014) 2506–2519.
- 24 [19] A. Byron, J.D. Humphries, M.J. Humphries, Defining the extracellular matrix using proteomics., *Int.*
25 *J. Exp. Pathol.* 94 (2013) 75–92.
- 26 [20] A. Naba, K.R. Clauser, S. Hoersch, H. Liu, S.A. Carr, R.O. Hynes, The matrisome: in silico
27 definition and in vivo characterization by proteomics of normal and tumor extracellular matrices.,
28 *Mol. Cell. Proteomics*. 11 (2012) M111.014647.
- 29 [21] A. Naba, K.R. Clauser, R.O. Hynes, Enrichment of Extracellular Matrix Proteins from Tissues and
30 Digestion into Peptides for Mass Spectrometry Analysis., *J. Vis. Exp.* (2015) e53057.
- 31 [22] A. Naba, K.R. Clauser, H. Ding, C.A. Whittaker, S.A. Carr, R.O. Hynes, The extracellular matrix:
32 Tools and insights for the “omics” era., *Matrix Biol.* 49 (2016) 10–24.
- 33 [23] A. Naba, S. Hoersch, R.O. Hynes, Towards definition of an ECM parts list: an advance on GO
34 categories., *Matrix Biol.* 31 (2012) 371–372.
- 35 [24] H. Johnson, A.M. Del Rosario, B.D. Bryson, M.A. Schroeder, J.N. Sarkaria, F.M. White, Molecular
36 characterization of EGFR and EGFRvIII signaling networks in human glioblastoma tumor
37 xenografts., *Mol. Cell. Proteomics*. 11 (2012) 1724–1740.
- 38 [25] T. Ternent, A. Csordas, D. Qi, G. Gomez-Baena, R.J. Beynon, A.R. Jones, et al., How to submit
39 MS proteomics data to ProteomeXchange via the PRIDE database., *Proteomics*. 14 (2014) 2233–
40 2241.
- 41 [26] J.A. Vizcaino, A. Csordas, N. del-Toro, J.A. Dienes, J. Griss, I. Lavidas, et al., 2016 update of the
42 PRIDE database and its related tools., *Nucleic Acids Res.* 44 (2016) D447–56.
- 43 [27] M. Reich, T. Liefeld, J. Gould, J. Lerner, P. Tamayo, J.P. Mesirov, GenePattern 2.0., *Nat. Genet.*
44 38 (2006) 500–501.
- 45 [28] V.K. Mootha, C.M. Lindgren, K.-F. Eriksson, A. Subramanian, S. Sihag, J. Lehar, et al., PGC-
46 1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in
47 human diabetes., *Nat. Genet.* 34 (2003) 267–273.
- 48 [29] A. Subramanian, P. Tamayo, V.K. Mootha, S. Mukherjee, B.L. Ebert, M.A. Gillette, et al., Gene set
49 enrichment analysis: a knowledge-based approach for interpreting genome-wide expression
50 profiles., *Proc. Natl. Acad. Sci. U. S. A.* 102 (2005) 15545–15550.
- 51 [30] <http://matrisomeproject.mit.edu>, The Matrisome Project, (n.d.).
- 52 [31] R.O. Hynes, A. Naba, Overview of the matrisome-An inventory of extracellular matrix constituents
53 and functions, *Cold Spring Harb. Perspect. Biol.* 4 (2012).
- 54 [32] P. Lu, K. Takai, V.M. Weaver, Z. Werb, Extracellular matrix degradation and remodeling in
55 development and disease., *Cold Spring Harb. Perspect. Biol.* 3 (2011).
- 56 [33] S. Loffek, O. Schilling, C.-W. Franzke, Series “matrix metalloproteinases in lung health and

- 1 disease": Biological role of matrix metalloproteinases: a critical balance., *Eur. Respir. J.* 38 (2011)
2 191–208.
- 3 [34] R. Chiquet-Ehrismann, R.P. Tucker, Tenascins and the importance of adhesion modulation., *Cold*
4 *Spring Harb. Perspect. Biol.* 3 (2011).
- 5 [35] N. Karin, The multiple faces of CXCL12 (SDF-1alpha) in the regulation of immunity during health
6 and disease., *J. Leukoc. Biol.* 88 (2010) 463–473.
- 7 [36] R. Donato, Functional roles of S100 proteins, calcium-binding proteins of the EF-hand type.,
8 *Biochim. Biophys. Acta.* 1450 (1999) 191–231.
- 9 [37] H. Steen, M. Mann, The ABC's (and XYZ's) of peptide sequencing., *Nat. Rev. Mol. Cell Biol.* 5
10 (2004) 699–711.
- 11 [38] S.-E. Ong, M. Mann, Mass spectrometry-based proteomics turns quantitative., *Nat. Chem. Biol.* 1
12 (2005) 252–262.
- 13 [39] P.L. Ross, Y.N. Huang, J.N. Marchese, B. Williamson, K. Parker, S. Hattan, et al., Multiplexed
14 protein quantitation in *Saccharomyces cerevisiae* using amine-reactive isobaric tagging reagents.,
15 *Mol. Cell. Proteomics.* 3 (2004) 1154–1169.
- 16 [40] G.E. Garrigues, D.R. Cho, H.E. Rubash, S.R. Goldring, J.H. Herndon, A.S. Shanbhag, Gene
17 expression clustering using self-organizing maps: analysis of the macrophage response to
18 particulate biomaterials., *Biomaterials.* 26 (2005) 2933–2945.
- 19 [41] W.L. Murphy, T.C. McDevitt, A.J. Engler, Materials as stem cell regulators., *Nat. Mater.* 13 (2014)
20 547–557.
- 21 [42] R. Peerani, P.W. Zandstra, Enabling stem cell therapies through synthetic stem cell-niche
22 engineering., *J. Clin. Invest.* 120 (2010) 60–70.
- 23 [43] S.Y. Ow, M. Salim, J. Noirel, C. Evans, I. Rehman, P.C. Wright, iTRAQ underestimation in simple
24 and complex mixtures: "the good, the bad and the ugly", *J. Proteome Res.* 8 (2009) 5347–5355.
- 25 [44] S.Y. Ow, M. Salim, J. Noirel, C. Evans, P.C. Wright, Minimising iTRAQ ratio compression through
26 understanding LC-MS elution dependence and high-resolution HILIC fractionation., *Proteomics.* 11
27 (2011) 2341–2346.
- 28 [45] S. Ricard-Blum, The collagen family., *Cold Spring Harb. Perspect. Biol.* 3 (2011) a004978.
- 29 [46] E.G. Canty, K.E. Kadler, Procollagen trafficking, processing and fibrillogenesis., *J. Cell Sci.* 118
30 (2005) 1341–1353.
- 31 [47] K.E. Kadler, C. Baldock, J. Bella, R.P. Boot-Handford, Collagens at a glance., *J. Cell Sci.* 120
32 (2007) 1955–1958.
- 33 [48] C. Bonnans, J. Chou, Z. Werb, Remodelling the extracellular matrix in development and disease.,
34 *Nat. Rev. Mol. Cell Biol.* 15 (2014) 786–801.
- 35 [49] J.K. Mouw, G. Ou, V.M. Weaver, Extracellular matrix assembly: a multiscale deconstruction., *Nat.*
36 *Rev. Mol. Cell Biol.* 15 (2014) 771–785.
- 37 [50] L.D. Muiznieks, F.W. Keeley, Molecular assembly and mechanical properties of the extracellular
38 matrix: A fibrous protein perspective., *Biochim. Biophys. Acta.* 1832 (2013) 866–875.
- 39 [51] R. Timpl, T. Sasaki, G. Kostka, M.-L. Chu, Fibulins: a versatile family of extracellular matrix
40 proteins., *Nat. Rev. Mol. Cell Biol.* 4 (2003) 479–489.
- 41 [52] C.M. Kielty, C. Baldock, D. Lee, M.J. Rock, J.L. Ashworth, C.A. Shuttleworth, Fibrillin: from
42 microfibril assembly to biomechanical function., *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 357
43 (2002) 207–217.
- 44 [53] C.M. Kielty, T.J. Wess, L. Haston, J.L. Ashworth, M.J. Sherratt, C.A. Shuttleworth, Fibrillin-rich
45 microfibrils: elastic biopolymers of the extracellular matrix., *J. Muscle Res. Cell Motil.* 23 (2002)
46 581–596.
- 47 [54] W.P. Daley, S.B. Peters, M. Larsen, Extracellular matrix dynamics in development and
48 regenerative medicine., *J. Cell Sci.* 121 (2008) 255–264.
- 49