

Supplementary File 1

Evaluation of the safety and regenerative potential of human mesenchymal stem cells and their extracellular vesicles in a transgenic pig model of cartilage-bone injury *in vivo* – preclinical study

Anna Łabędź-Masłowska^{*,1}, Jarosław Wieczorek^{*,2}, Maciej Mierzwiński³, Małgorzata Sekuła-Stryjewska^{1,4}, Sylwia Noga^{1,4}, Jolanta Rajca^{3,5}, Piotr Duda⁶, Katarzyna Milian-Ciesielska⁷, Elżbieta Karnas¹, Katarzyna Kmiotek-Caller¹, Agnieszka Szkaradek¹, Zbigniew Madeja¹, Krzysztof Ficek³, Jacek Jura^{#,8} Ewa Zuba-Surma^{#,1}

¹Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland.

²University Center of Veterinary Medicine UJ-UR, University of Agriculture in Krakow, Krakow, Poland.

³Department of Science, Innovation and Development, Galen-Orthopaedics, Bierun, Poland.

⁴Malopolska Centre of Biotechnology, Jagiellonian University, Krakow, Poland.

⁵Spin-Lab Centre for Microscopic Research on Matter, University of Silesia in Katowice, Katowice, Poland.

⁶Institute of Biomedical Engineering, Faculty of Science and Technology, University of Silesia in Katowice, Katowice, Poland.

⁷Department of Pathomorphology, Jagiellonian University Medical College, University Hospital, Krakow, Poland.

⁸Department of Reproductive Biotechnology and Cryoconservation, National Research Institute of Animal Production, Balice, Poland.

*First coauthors.

#Corresponding coauthors.

Correspondence: Ewa Zuba-Surma e-mail: ewa.zuba-surma@uj.edu.pl

Jacek Jura e-mail: jacek.jura@iz.edu.pl

Figure S1

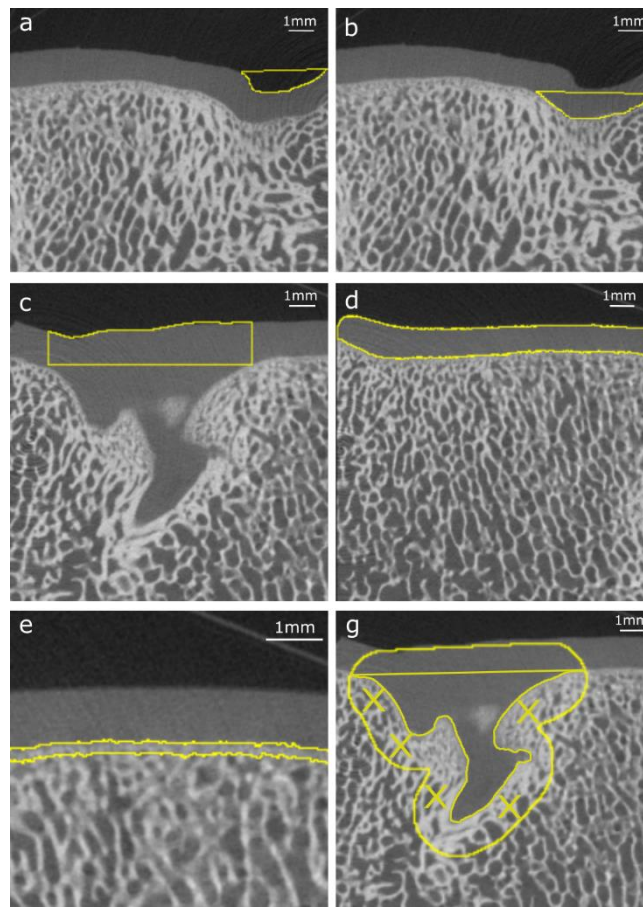


Fig. S1. ImageJ analysis of cartilage-bone samples. The volume of interest (VOI) marked for: a) cartilage defect, b) bone defect, c) cartilage thickness above bone defect, d) cartilage thickness above healthy bone (in unaffected region), e) subchondral bone plate thickness, f) the trabecular bone around the defect (X-marked VOI).

Figure S2

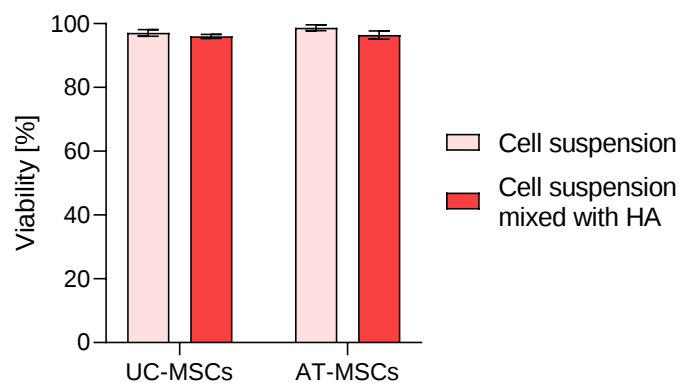


Fig. S2. Viability of AT-MSCs and UC-MSCs before and after mixing with high molecular weight hyaluronic acid (HA) by trypan blue dye exclusion method.

Table S1. Summary data for analysis of volume of cartilage defect. *ns*- non-significant.

Unit no.	Volume of cartilage defect [mm ³]					
	Group of animals					
	Control	HA	AT-MSCs	UC-MSCs	AT-MSC-EVs	UC-MSC-EVs
1	8.09	0.00	1.17	14.71	24.18	20.32
2	9.02	0.00	0.00	0.00	3.67	2.15
3	7.83	26.89	0.18	0.00	0.00	0.00
4	16.11	11.26	0.00	0.00	34.76	0.00
5			0.00	0.00	0.00	
6			0.00	0.00		
N	4	4	6	6	5	4
Mean	10.26	9.54	0.23	2.45	12.52	5.62
SD	3.93	12.73	0.47	6.01	15.99	9.85
<i>p</i> vs. Control	-	<i>ns</i>	<0.01	<0.05	<i>ns</i>	<i>ns</i>
<i>p</i> vs. HA	<i>ns</i>	-	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

Table S2. Summary data for analysis of volume of bone defect. *ns*- non-significant.

Unit no.	Volume of bone defect [mm ³]					
	Group of animals					
	Control	HA	AT-MSCs	UC-MSCs	AT-MSC-EVs	UC-MSC-EVs
1	49.60	13.76	4.05	20.01	74.11	22.35
2	84.06	0.50	0.00	0.65	77.00	85.08
3	19.67	70.14	0.83	35.38	0.00	0.00
4	22.36	10.46	0.00	0.00	6.70	0.00
5			0.00	0.00	52.05	
6			0.00	4.56		
N	4	4	6	6	5	4
Mean	43.92	23.72	0.81	10.10	41.97	26.86
SD	29.98	31.46	1.62	14.57	36.63	40.22
<i>p</i> vs. Control	-	<i>ns</i>	<0.01	<i>ns</i>	<i>ns</i>	<i>ns</i>
<i>p</i> vs. HA	<i>ns</i>	-	<0.05	<i>ns</i>	<i>ns</i>	<i>ns</i>

Table S3. Summary data for analysis of cartilage thickness above healthy bone. *ns- non-significant.*

Unit no.	Cartilage thickness <u>above healthy bone</u> [mm]					
	Group of animals					
	Control	HA	AT-MSCs	UC-MSCs	AT-MSC-EVs	UC-MSC-EVs
1	1.12	1.26	1.22	1.35	0.95	1.64
2	1.14	1.05	1.43	1.14	1.45	1.29
3	1.08	1.17	1.04	1.36	1.00	1.30
4	1.05	1.10	0.83	1.13	1.41	1.19
5			1.47	0.70	1.17	
6			0.94	1.05		
N	4	4	6	6	5	4
Mean	1.10	1.15	1.16	1.12	1.20	1.36
SD	0.04	0.09	0.26	0.24	0.23	0.20
<i>p</i> vs. Control	-	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
<i>p</i> vs. HA	<i>ns</i>	-	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

Table S4. Summary data for analysis of cartilage thickness above bone defect. *ns- non-significant.*

Unit no.	Cartilage thickness <u>above bone defect</u> [mm]					
	Group of animals					
	Control	HA	AT-MSCs	UC-MSCs	AT-MSC-EVs	UC-MSC-EVs
1	0.00	1.26	1.08	0.00	0.00	0.00
2	0.90	0.88	1.43	1.14	0.90	0.91
3	0.00	0.00	0.46	1.36	1.00	1.30
4	0.46	0.00	0.83	1.13	1.26	1.19
5			1.47	0.70	1.36	
6			0.94	1.05		
N	4	4	6	6	5	4
Mean	0.34	0.54	1.04	0.90	0.90	0.85
SD	0.43	0.64	0.38	0.49	0.54	0.59
<i>p</i> vs. Control	-	<i>ns</i>	<i><0.01</i>	<i><0.01</i>	<i><0.01</i>	<i><0.01</i>
<i>p</i> vs. HA	<i>ns</i>	-	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

Table S5. Summary data for analysis of subchondral bone plate (SBP) – bone mineral density (BMD). *ns- non-significant.*

Unit no.	Subchondral bone plate - BMD [g Hap/cm ³]						
	Group of animals						
	Healthy limb	Control	HA	AT-MSCs	UC-MSCs	AT-MSC-EVs	UC-MSC-EVs
1	0.65	0.67	0.68	0.6	0.52	0.69	0.61
2	0.76	0.72	0.64	0.62	0.6	0.63	0.65
3	0.69	0.59	0.63	0.70	0.61	0.61	0.64
4	0.69	0.69	0.63	0.60	0.6	0.64	0.68
5	0.65			0.50	0.65	0.60	
6	0.58			0.68	0.6		
N	6	4	4	6	6	5	4
Mean	0.67	0.67	0.65	0.62	0.60	0.63	0.65
SD	0.06	0.06	0.02	0.07	0.04	0.04	0.03
<i>p</i> vs. Healthy limb	-	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
<i>p</i> vs. Control	<i>ns</i>	-	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
<i>p</i> vs. HA	<i>ns</i>	<i>ns</i>	-	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

Table S6. Summary data for analysis of subchondral bone plate (SBP) - thickness. *ns- non-significant.*

Unit no.	Subchondral bone plate - thickness [mm]						
	Group of animals						
	Healthy limb	Control	HA	AT-MSCs	UC-MSCs	AT-MSC-EVs	UC-MSC-EVs
1	0.19	0.36	0.36	0.22	0.18	0.23	0.20
2	0.13	0.36	0.36	0.21	0.23	0.16	0.14
3	0.19	0.29	0.31	0.25	0.20	0.18	0.19
4	0.15	0.37	0.32	0.18	0.18	0.23	0.19
5	0.14			0.24	0.11	0.19	
6	0.16			0.17	0.21		
N	6	4	4	6	6	5	4
Mean	0.16	0.35	0.34	0.21	0.19	0.20	0.18
SD	0.03	0.04	0.03	0.03	0.04	0.03	0.03
<i>p</i> vs. Healthy limb	-	<i>ns</i>	<i>ns</i>	<0.05	<i>ns</i>	<i>ns</i>	<i>ns</i>
<i>p</i> vs. Control	<0.01	-	<i>ns</i>	<0.01	<0.01	<0.01	<0.05
<i>p</i> vs. HA	<0.01	<i>ns</i>	-	<0.01	<0.01	<0.01	<0.05

Table S7. Summary data for analysis of trabecular bone – bone mineral density (BMD).
ns- non-significant.

Unit no.	Trabecular bone - BMD [g Hap/cm ³]						
	Group of animals						
	Healthy limb	Control	HA	AT-MSCs	UC-MSCs	AT-MSC-EVs	UC-MSC-EVs
1	0.77	0.76	0.82	0.62	0.65	0.83	0.83
2	0.76	0.70	0.69	0.72	0.68	0.74	0.88
3	0.81	0.76	0.76	0.74	0.79	0.73	0.74
4	0.76	0.91	0.73	0.78	0.63	0.90	0.71
5	0.79			0.51	0.71	0.79	
6	0.70			0.75	0.60		
N	6	4	4	6	6	5	4
Mean	0.76	0.78	0.75	0.69	0.68	0.80	0.79
SD	0.04	0.09	0.05	0.10	0.07	0.07	0.08
<i>p</i> vs. Healthy limb	-	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
<i>p</i> vs. Control	<i>ns</i>	-	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
<i>p</i> vs. HA	<i>ns</i>	<i>ns</i>	-	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

Table S8. Summary data for analysis of trabecular bone – bone to total volume ratio (BV/TV). *ns- non-significant.*

Unit no.	Trabecular bone – BV/TV [%]						
	Group of animals						
	Healthy limb	Control	HA	AT-MSCs	UC-MSCs	AT-MSC-EVs	UC-MSC-EVs
1	59.39	59.71	69.99	63.10	68.57	57.19	75.76
2	56.18	73.02	70.13	56.89	70.25	57.98	68.92
3	56.39	69.07	57.32	87.24	80.94	57.67	56.61
4	60.63	76.13	74.75	62.83	53.52	69.98	60.41
5	49.52			50.99	37.00	71.37	
6	54.04			59.42	70.38		
N	6	4	4	6	6	5	4
Mean	56.03	69.48	68.05	63.41	63.44	62.84	65.43
SD	3.97	7.13	7.49	12.50	15.65	7.18	8.60
<i>p</i> vs. Healthy limb	-	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
<i>p</i> vs. Control	<0.05	-	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
<i>p</i> vs. HA	<0.05	<i>ns</i>	-	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>