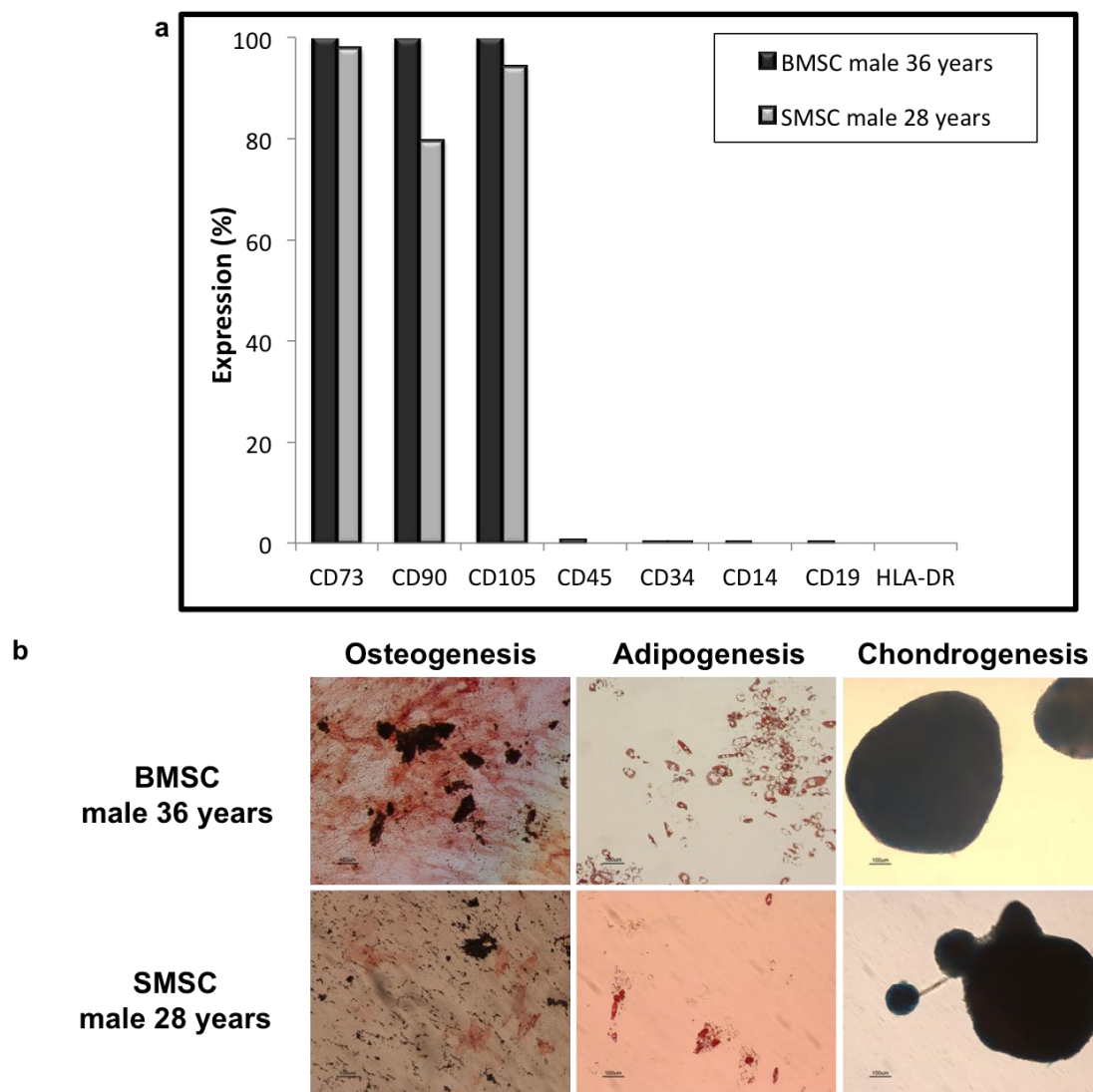


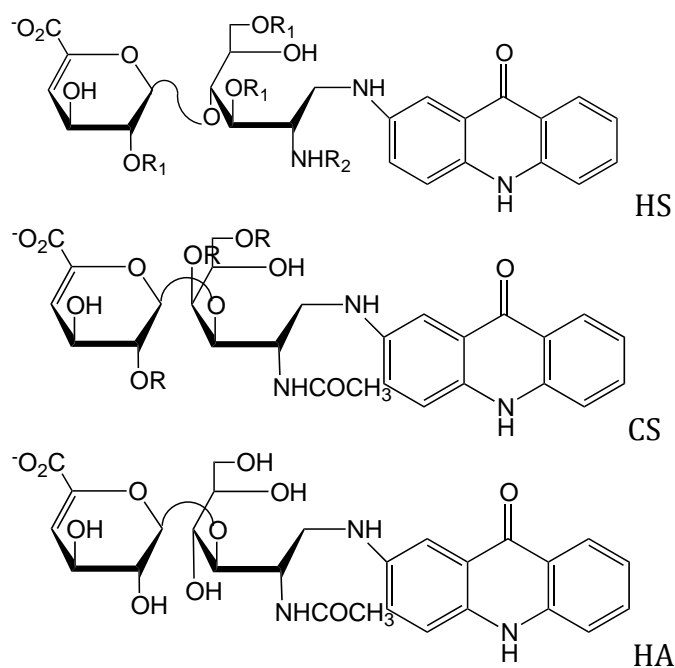
Compositional and structural analysis of glycosaminoglycans in cell-derived extracellular matrices

João C. Silva^{1,2,4}, Marta S. Carvalho^{1,3,4}, Xiaorui Han², Ke Xia², Paiyz E. Mikael², Joaquim M. S. Cabral^{1,4}, Frederico Castelo Ferreira^{1,4} and Robert J. Linhardt^{2,3*}

Supplementary information



Supplementary Figure 1 Characterization of MSC isolated from bone marrow aspirates (BMSC) collected from a male donor (36 years) and of MSC isolated from synovium aspirates obtained from a male donor (28 years). (a) Immunophenotypic characterization of BMSC and SMSC assessed by flow cytometry. (b) Multi-lineage differentiation potential of BMSC and SMSC. Osteogenic, adipogenic and chondrogenic differentiation was confirmed after 14 days induction by ALP/von Kossa, Oil Red-O and Alcian Blue staining, respectively.



Supplementary Figure 2 Structures of AMAC derivatized disaccharides used in LC-MS/MS analysis.

Supplementary Table 1. Total GAG amounts obtained for the different cell-derived ECM produced from chondrocytes, BMSC and SMSC presented as ng of GAG/mg of dry ECM. Results are presented as mean \pm SD of three independent samples (n=3).

	Total GAG (ng/mg)			
	HS	CS	HA	Total
<i>Chondrocyte-ECM</i>	15.7 \pm 3.5	86.4 \pm 35.5	10.3 \pm 5.6	112.4 \pm 43.6
<i>BMSC-ECM</i>	39.4 \pm 16.4	79.4 \pm 22.6	88.0 \pm 19.6	206.8 \pm 44.0
<i>SMSC-ECM</i>	7.7 \pm 0.8	34.6 \pm 2.3	31.5 \pm 8.4	73.8 \pm 9.5

Supplementary Table 2. HS and CS disaccharides amounts obtained for the different cell-derived ECM produced from chondrocytes, BMSC and SMSC presented as ng of GAG/mg of dry ECM. Results are presented as mean \pm SD of three independent samples (n=3).

HS disaccharide (ng/mg)								
	TriS	NS6S	NS2S	NS	2S6S	6S	2S	0S
<i>Chondrocyte-ECM</i>	0.0 \pm 0.0	0.3 \pm 0.3	3.1 \pm 0.3	3.2 \pm 0.6	0.0 \pm 0.0	0.4 \pm 0.1	0.2 \pm 0.2	8.4 \pm 2.5
<i>BMSC-ECM</i>	0.0 \pm 0.0	0.2 \pm 0.0	1.5 \pm 0.9	11.1 \pm 5.1	0.0 \pm 0.0	1.1 \pm 0.5	0.2 \pm 0.2	25.3 \pm 9.9
<i>SMSC-ECM</i>	0.0 \pm 0.0	0.1 \pm 0.0	1.0 \pm 0.1	1.1 \pm 0.4	0.0 \pm 0.0	0.3 \pm 0.2	0.0 \pm 0.0	5.2 \pm 0.3
CS disaccharide (ng/mg)								
	TriS	2S4S	2S6S	4S6S	4S	6S	2S	0S
<i>Chondrocyte-ECM</i>	0.0 \pm 0.0	0.1 \pm 0.0	2.2 \pm 1.1	0.3 \pm 0.2	22.8 \pm 8.0	54.8 \pm 23.1	0.2 \pm 0.2	6.0 \pm 3.0
<i>BMSC-ECM</i>	0.0 \pm 0.0	0.2 \pm 0.2	0.7 \pm 0.6	0.3 \pm 0.1	43.3 \pm 10.7	32.9 \pm 10.7	0.2 \pm 0.2	1.9 \pm 0.6
<i>SMSC-ECM</i>	0.0 \pm 0.0	0.0 \pm 0.0	0.3 \pm 0.1	0.9 \pm 0.1	19.2 \pm 1.5	13.3 \pm 1.1	0.0 \pm 0.0	0.9 \pm 0.2

Supplementary Table 3. Total GAG amounts presented as ng/dish for the different cell-derived ECM and respective cell culture monolayers prior to decellularization treatment. Results are presented as mean \pm SD of three independent samples (n=3).

	Total GAG (ng/dish)			
	HS	CS	HA	Total
<i>Chondrocyte</i>	209.5 \pm 24.6	338.2 \pm 42.2	125.0 \pm 11.8	672.7 \pm 70.9
<i>Chondrocyte-ECM</i>	27.9 \pm 6.3	153.6 \pm 63.1	18.3 \pm 10.0	199.8 \pm 77.5
<i>BMSC</i>	105.7 \pm 11.5	113.9 \pm 14.3	873.8 \pm 160.3	1093.4 \pm 184.4
<i>BMSC-ECM</i>	42.4 \pm 17.7	85.6 \pm 24.3	94.9 \pm 21.2	222.9 \pm 47.5
<i>SMSC</i>	57.8 \pm 3.5	334.8 \pm 178.7	86.5 \pm 17.1	479.1 \pm 199.3
<i>SMSC-ECM</i>	14.0 \pm 1.4	63.0 \pm 4.2	57.5 \pm 15.4	134.4 \pm 17.3

Supplementary Table 4. HS and CS disaccharide amounts presented as ng/dish for the different cell-derived ECM and respective cell culture monolayers prior to decellularization treatment. Results are presented as mean \pm SD of three independent samples (n=3).

HS disaccharide (ng/dish)								
	TriS	NS6S	NS2S	NS	2S6S	6S	2S	0S
<i>Chondrocyte</i>	0.0 \pm 0.0	1.5 \pm 0.0	23.8 \pm 2.3	38.8 \pm 5.4	0.1 \pm 0.1	4.6 \pm 1.0	0.3 \pm 0.0	140.4 \pm 16.9
<i>Chondrocyte-ECM</i>	0.0 \pm 0.0	0.5 \pm 0.5	5.4 \pm 0.5	5.7 \pm 1.1	0.0 \pm 0.0	0.8 \pm 0.3	0.4 \pm 0.3	14.9 \pm 4.5
<i>BMSC</i>	0.0 \pm 0.0	0.4 \pm 0.0	16.6 \pm 2.1	28.8 \pm 3.2	0.1 \pm 0.1	0.0 \pm 0.0	0.0 \pm 0.0	59.8 \pm 6.1
<i>BMSC-ECM</i>	0.0 \pm 0.0	0.2 \pm 0.0	1.6 \pm 1.0	11.9 \pm 5.5	0.0 \pm 0.0	1.2 \pm 0.5	0.2 \pm 0.2	27.2 \pm 10.7
<i>SMSC</i>	0.1 \pm 0.0	0.2 \pm 0.2	4.3 \pm 0.4	7.5 \pm 1.3	0.0 \pm 0.0	0.8 \pm 0.5	3.4 \pm 0.6	41.5 \pm 0.9
<i>SMSC-ECM</i>	0.0 \pm 0.0	0.1 \pm 0.1	1.7 \pm 0.2	2.1 \pm 0.8	0.0 \pm 0.0	0.5 \pm 0.4	0.1 \pm 0.0	9.4 \pm 0.5
CS disaccharide (ng/dish)								
	TriS	2S4S	2S6S	4S6S	4S	6S	2S	0S
<i>Chondrocyte</i>	0.0 \pm 0.0	4.8 \pm 0.3	9.0 \pm 1.0	4.5 \pm 0.2	257.0 \pm 40.0	58.0 \pm 1.8	0.3 \pm 0.0	4.5 \pm 0.5
<i>Chondrocyte-ECM</i>	0.0 \pm 0.0	0.2 \pm 0.1	3.9 \pm 2.0	0.6 \pm 0.4	40.5 \pm 14.1	97.4 \pm 41.1	0.4 \pm 0.3	10.6 \pm 5.4
<i>BMSC</i>	0.0 \pm 0.0	1.1 \pm 0.3	1.0 \pm 0.1	1.3 \pm 0.4	85.5 \pm 11.7	20.9 \pm 3.4	0.0 \pm 0.0	4.1 \pm 0.5
<i>BMSC-ECM</i>	0.0 \pm 0.0	0.2 \pm 0.2	0.7 \pm 0.6	0.3 \pm 0.1	46.7 \pm 11.5	35.5 \pm 11.5	0.2 \pm 0.2	2.0 \pm 0.7
<i>SMSC</i>	0.1 \pm 0.0	0.2 \pm 0.0	2.0 \pm 0.3	4.6 \pm 0.7	233.3 \pm 114.4	74.9 \pm 55.8	3.4 \pm 0.6	16.3 \pm 7.0
<i>SMSC-ECM</i>	0.0 \pm 0.0	0.1 \pm 0.0	0.5 \pm 0.2	1.6 \pm 0.1	35.0 \pm 2.7	24.1 \pm 2.0	0.1 \pm 0.0	1.6 \pm 0.4

Supplementary Table 5. Average percentage GAG composition for the different cell-derived ECM and respective cell culture monolayers prior to decellularization treatment. Results are presented as mean \pm SD of three independent samples (n=3).

	Total GAG relative %		
	HS	CS	HA
<i>Chondrocyte</i>	31 \pm 2	50 \pm 1	19 \pm 2
<i>Chondrocyte-ECM</i>	15 \pm 3	77 \pm 2	9 \pm 4
<i>BMSC</i>	10 \pm 1	10 \pm 1	80 \pm 2
<i>BMSC-ECM</i>	18 \pm 5	38 \pm 6	43 \pm 9
<i>SMSC</i>	13 \pm 5	68 \pm 9	19 \pm 4
<i>SMSC-ECM</i>	10 \pm 1	47 \pm 6	42 \pm 7

Supplementary Table 6. Average percentage HS and CS disaccharide composition for the different cell-derived ECM and respective cell culture monolayers prior to decellularization treatment. Results are presented as mean \pm SD of three independent samples (n=3).

HS disaccharide relative %								
	TriS	NS6S	NS2S	NS	2S6S	6S	2S	0S
<i>Chondrocyte</i>	0 \pm 0	1 \pm 0	11 \pm 0	18 \pm 1	0 \pm 0	2 \pm 0	0 \pm 0	67 \pm 1
<i>Chondrocyte-ECM</i>	0 \pm 0	2 \pm 2	20 \pm 3	21 \pm 1	0 \pm 0	3 \pm 1	1 \pm 1	53 \pm 4
<i>BMSC</i>	0 \pm 0	0 \pm 0	16 \pm 0	27 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	57 \pm 0
<i>BMSC-ECM</i>	0 \pm 0	1 \pm 0	4 \pm 1	28 \pm 2	0 \pm 0	3 \pm 0	0 \pm 0	65 \pm 3
<i>SMSC</i>	0 \pm 0	0 \pm 0	7 \pm 0	13 \pm 1	0 \pm 0	1 \pm 1	6 \pm 1	72 \pm 3
<i>SMSC-ECM</i>	0 \pm 0	0 \pm 0	12 \pm 1	15 \pm 4	0 \pm 0	4 \pm 3	0 \pm 0	68 \pm 3
CS disaccharide relative %								
	TriS	2S4S	2S6S	4S6S	4S	6S	2S	0S
<i>Chondrocyte</i>	0 \pm 0	1 \pm 0	3 \pm 0	1 \pm 0	76 \pm 2	17 \pm 2	0 \pm 0	1 \pm 0
<i>Chondrocyte-ECM</i>	0 \pm 0	0 \pm 0	2 \pm 0	0 \pm 0	27 \pm 2	63 \pm 1	0 \pm 0	7 \pm 1
<i>BMSC</i>	0 \pm 0	1 \pm 0	1 \pm 0	1 \pm 0	75 \pm 1	18 \pm 1	0 \pm 0	4 \pm 1
<i>BMSC-ECM</i>	0 \pm 0	0 \pm 0	1 \pm 0	0 \pm 0	55 \pm 3	41 \pm 2	0 \pm 0	2 \pm 0
<i>SMSC</i>	0 \pm 0	0 \pm 0	1 \pm 0	2 \pm 1	71 \pm 4	21 \pm 6	1 \pm 0	5 \pm 1
<i>SMSC-ECM</i>	0 \pm 0	0 \pm 0	1 \pm 0	3 \pm 0	56 \pm 1	38 \pm 1	0 \pm 0	3 \pm 1