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Supporting Information

Investigating the Potential of Human Placental Derived Extracellular Matrix Sponges Coupled with Amniotic Membrane-Derived Stem Cells for Osteochondral Tissue Engineering

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Experimental Section

Flow cytometric/immunofluorescence analysis/gene expression studies (section 2.11)

The isolated cells were incubated with 1 µg/10^6 cells FITC/PE-conjugated antibodies (1. FITC anti-CD105; 2. FITC anti-CD44; 3 PE anti-CD166; 4. PE anti-CD45; all purchased from BioLegend, USA) for 40 min at 4 °C in the dark. The cells were analysed on a flow cytometer (FACSCalibur, Becton Dickinson, San Jose, CA, USA) after washing the cells with PBS to remove unspecific binding of the antibodies. 1000 events were collected and the data analysed by Cell Quest Software (Becton Dickinson, San Jose, CA, USA).

The isolated cells were characterized by immunofluorescence. Briefly, the cells were cultured till confluence on lysine coated coverslips and fixed with 4% formaldehyde. The fixed cells were permeabilized with 0.1% Triton X-100 and 1% bovine serum albumin (BSA, Sigma, USA) was used to block unspecific sites and later incubated with antibodies. The antibodies used were FITC/PE conjugated CD44, CD105 and CD166 (Biolegend, USA).

Further gene expression studies of OCT4, NANOG and SOX2 were further carried out in the isolated cells to confirm their pluripotency.

Results and discussion

As shown in Figure S1, the immunophenotypical profile of the isolated cells revealed that they were positive for human mesenchymal stem cell marker such as CD105, CD44, CD166 and negative for the hematopoietic marker CD45. Also from Figure S2, as evidenced by immunofluorescence, the isolated cells expressed characteristic stem cell markers such as CD44, CD166 and CD105. RT-PCR further revealed (Figure S3), that the

isolated cells expressed octamer-binding transcription factor (OCT4), the homeobox transcription factor NANOG, and sex-determining region Y-box 2 (SOX2), the genes known to be required for self-renewal and pluripotency.

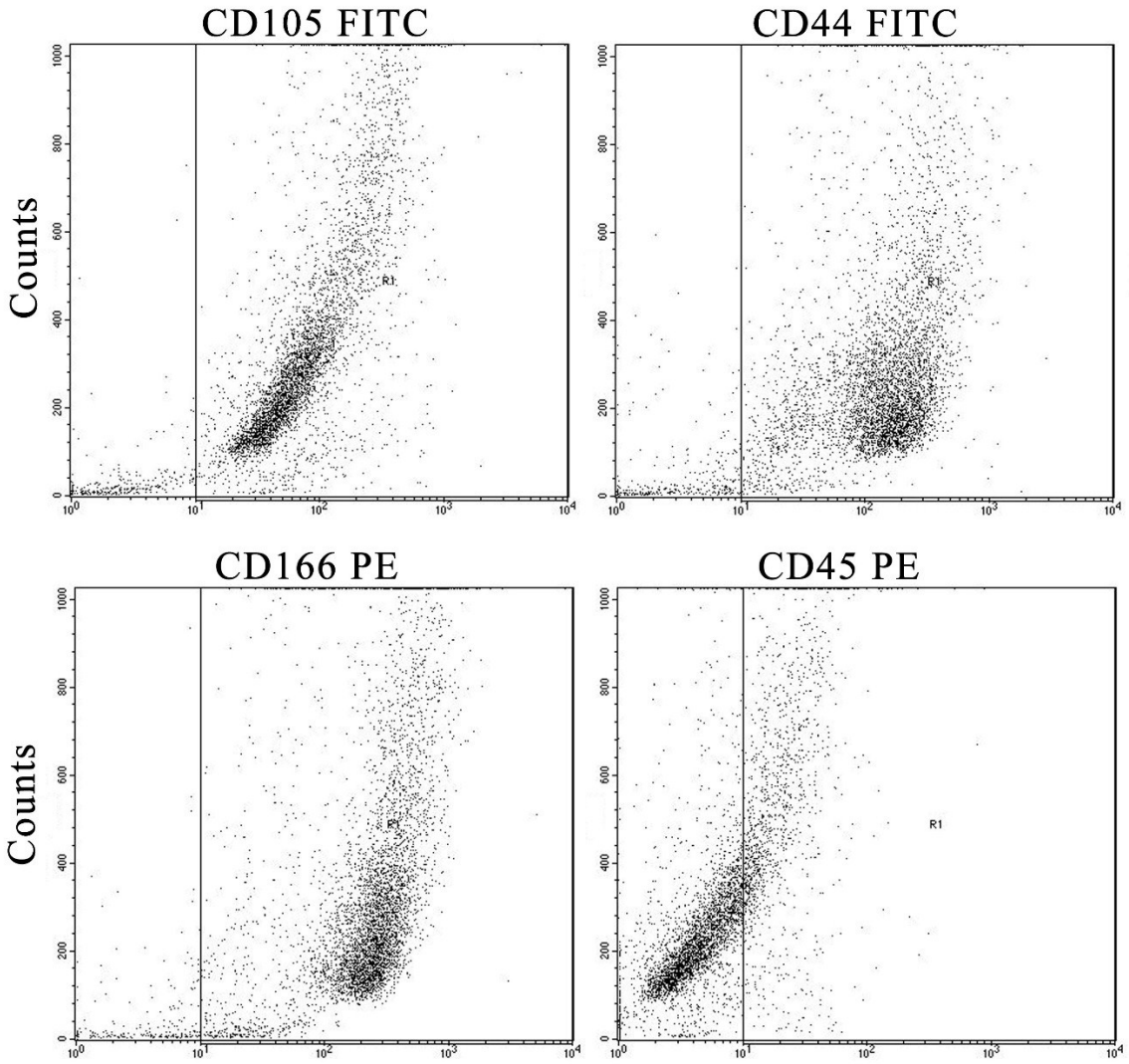

Figure S1: Immunophenotypical characterization of the isolated cells analyzed by flow cytometry. The isolated cells (HAMSCs) expressed CD105, CD44, CD166 and negative for CD45.

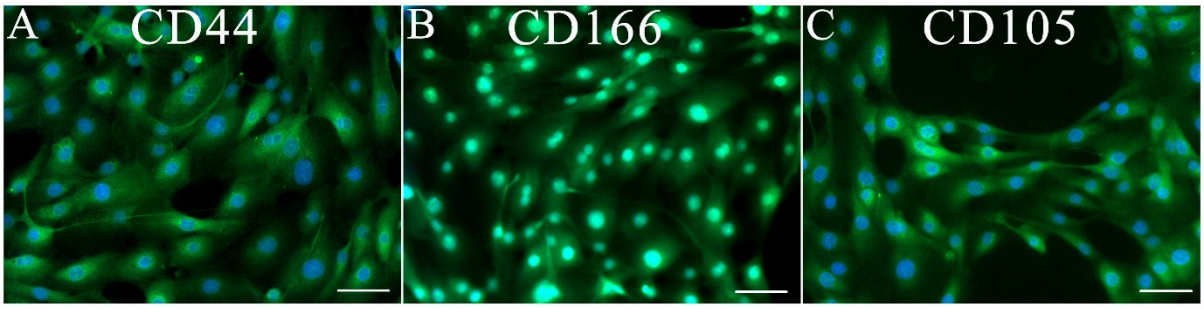

Figure S2: Immunofluorescence based detection of characteristic stem cell markers namely CD44, CD166 and CD105 (Scale bar = 100μm).

Figure S3: RT-PCR based analysis for RNA expression of pluripotency genes (OCT4, NANOG and SOX2) in the isolated cells.

Table S1. Primers for RT-PCR used in the study

	Genes	Primer Sequences		Amplicon Size (bp)
		Forward 5'-3'	**Reverse 5'-3'**	
	GAPDH	CCATGGAGAAGGCTGGGG	CAAAGTTGTCATGGATGACC	195
Pluripotent	OCT4	CCTCACTTCACTGCACTG	CAGGTTTTCTTTCCCTAG	100
	SOX2	CCCAGCAGACTTCACAT	CCTCCCATTTCCCTCGTT	95
	NANOG	GCTTGCCTTGCTTTGAAG	TTCTTGACTGGGACCTTG	200
Osteogenic	COL I	CAACCTCAAGAAGGCCCT	TTACAGGAAGCAGACAGGGC	250
	OPN	CCAGAGTGCTGAAACCCA	TTAATTGACCTCAGAAGATGCACT	250
	OCN	ATGAGAGCCCTCACACTCCTC	GCCGTAGAAGCGCCGATAGGC	294
Chondrogenic	ACAN	TGCATTCCACGAAGCTAACCTT	GACGCCTCGCCTTCTTGAA	84
	COL II	GGCAATAGCAGGTTCACGTACA	CGATAACAGTCTTGCCCCACTT	600
	SOX9	AGCGAACGCACATCAAGAC	GCTGTAGTGTGGGAGGTTGAA	110

Histomorphological evaluation (Section 2.18)

Semi-quantitative histomorphological analysis was done to evaluate the repair tissue according to ICRS Visual Histological Assessment grading scale (Table S2). A representative score for each parameter was determined by averaging the scores of the three observers.

Table S2: ICRS Visual Histological Assessment Scale

Features		**Score**
I. Surface	Smooth/continuous Discontinuities/irregularities	3 0
II. Matrix	Hyaline Mixture: hyaline/fibrocartilage Fibrocartilage Fibrous tissue	3 2 1 0
III. Cell distribution	Columnar Mixed/columnar-clusters Clusters Individual cells/disorganized	3 2 1 0
IV. Cell population viability	Predominantly viable Partially viable <10% viable	3 1 0
V. Subchondral bone	Normal Increased remodeling Bone necrosis/granulation tissue Detached/fracture/callus at base	3 2 1 0
VI. Cartilage mineralization (calcified cartilage)	Normal Abnormal/inappropriate location	3 0