Supplementary Information (ESI): Fig. S1, Table 1, Fig. S2.

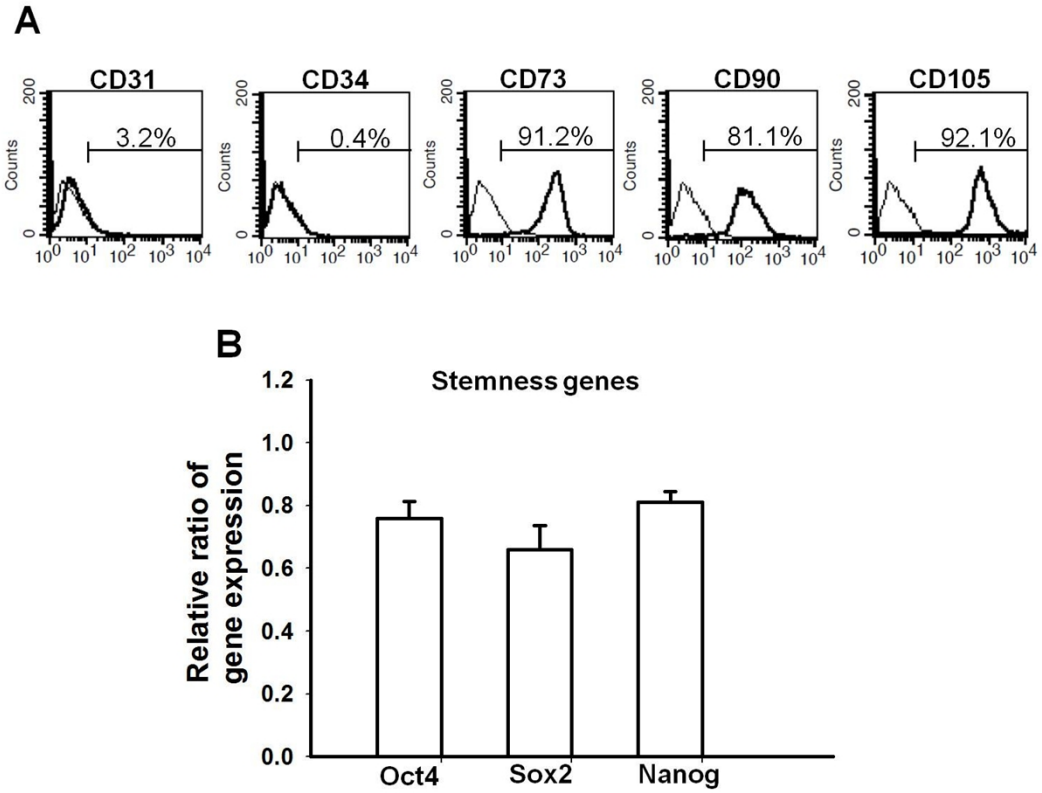

Fig. S1. Characterization of ADSCs. The expressions of (A) surface markers (CD31, CD34, CD73, CD90, and CD105) and (B) stemness genes (Oct4, Sox2, and Nanog) were analyzed each by flow cytometry and qPCR. The expression was normalized to GAPDH (the housekeeping gene). These data confirmed that ADSCs used in this study were genuine MSCs.

Table S1. The characteristics of the spheroids derived on various biomaterial substrates after 72 h.

	Petri dish	PVA	CS	CS-HA
Spheroid size (μm)	43 ± 5	73 ± 4	78 ± 5	91 ± 12
Cell number in a spheroid	814 ± 29	1049 ± 19	1034 ± 46	1515 ± 32
Total cell number used for implantation	1×10^6	1×10^6	1×10^6	1×10^6
Estimated number of spheroids for implantation	1028 ± 21	953 ± 18	967 ± 35	660 ± 26

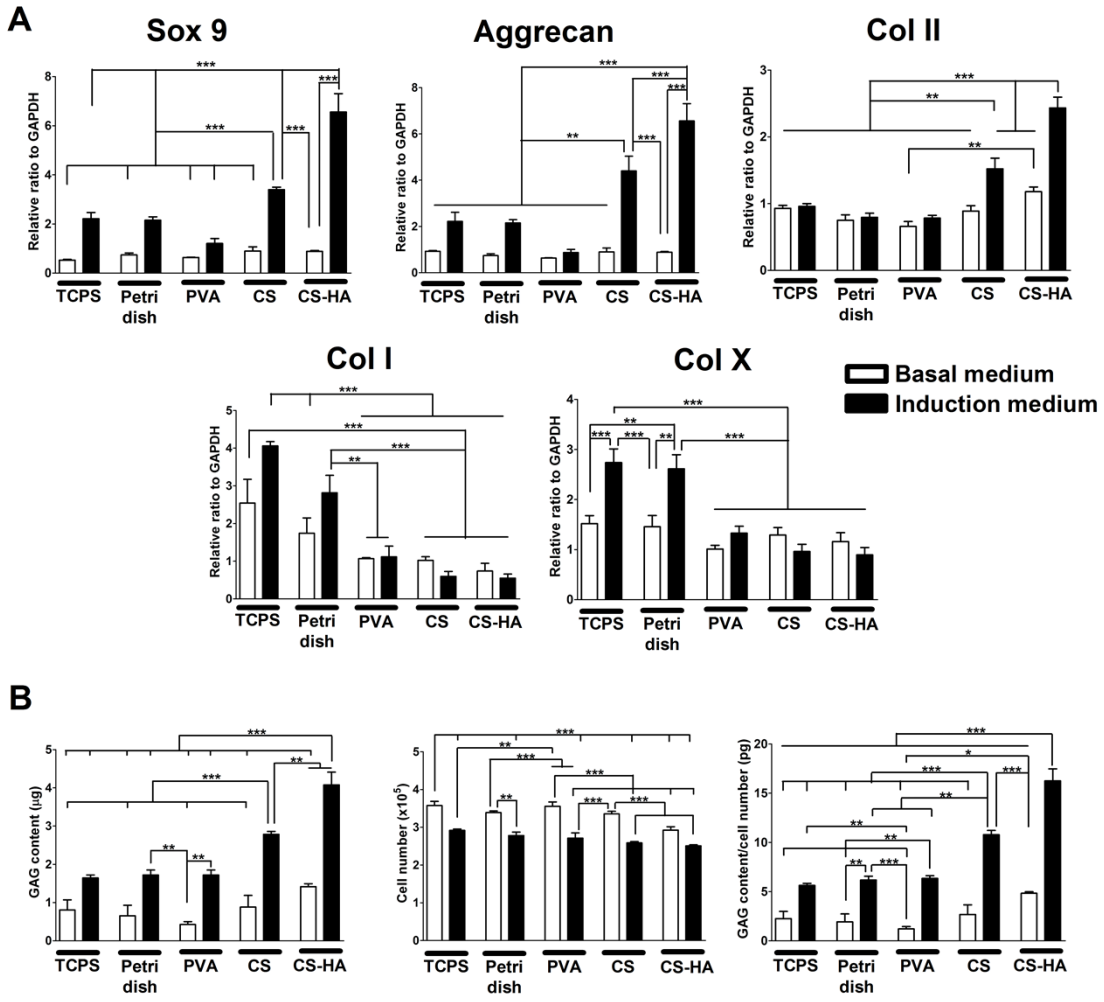

Fig. S2. The chondrogenic potential of various spheroids. (A) Gene expression was analyzed by qRT-PCR (Table S2). The expression was normalized to GAPDH (housekeeping gene). ADSCs were seeded on various substrates for 72 h in basal medium and cultured in the chondrogenic induction medium for another 7 days before the analysis of gene expression. The chondrogenic induction medium was DMEM-LG/ /F12 supplemented by 10 ng/ml TGF-β3, 0.1 μM dexamethasome, 1% insulin-transferring-selenium (ITS) premix 100×, 50 μg/ml L-ascorbate-2-phosphate, 40 μg/ml L-proline, and 1% penicillin–streptomycin. (B) The GAG/DNA analysis by the biochemical assay. ADSCs were seeded on various substrates for 72 h in basal medium and cultured in the chondrogenic induction medium for another 14 days before the biochemical analysis. The GAG content was analyzed by dimethylmethylene blue (DMMB) assay with the standard curve generated from chondroitin sulfate C. The concentration was measured by a UV/Vis spectrophotometer with a wavelength 525 nm. The cell number was evaluated by the DNA Hoechst 33528 dye assay, where the fluorescence intensity was measured by a

fluorescence spectrophotometer with excitation at 365 nm and emission at 458 nm. The cell number was obtained based on a standard curve made from known numbers of cells. *, $p< 0.05$; **, $p< 0.01$; ***, $p< 0.001$.

Table S2. The primer sequences used for qRT-PCR analysis (annealed at 62°C).

Genes	Primer sequences
Sox 9	5’-GGCTCCGACACCGAGAATAC
	3’-GTCGTAGCCCTTGAGCACCT
Aggrecan	5’-CTCTGGGTCCCCTGATTCTG
	3’-AGAGGCAGGCCTGATGTCTC
Col II	5’-GTGTGGTTTGGGGAGACCAT
	3’-GTGGCTTCATCCAGGTAGGC
Col I	5’-GGGCGAAAGAGGAGAAGGAT
	3’-GCCACAAGTGGTGCAAATGT
Col X	5’-TAAAAGGCCCACAACCCAAC
	3’-GGGGTTCCATAGCCTGGTTT
GAPDH	5’-CTGAGAACGGGAAGCTGGTC
	3’-GAGGGGGCTGAGATGATGAC