Supporting Information

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SI Materials and Methods

Human Mesenchymal Stem Cells. Fresh bone marrow aspirates were obtained from Cambrex and processed as in our previous studies (1). Bone-marrow–derived human mesenchymal stem cells (hMSCs) were isolated by their attachment to the plastic surface. Cells were expanded to the third passage in high glucose DMEM supplemented with 10% FBS, 1% penicillin–streptomycin, and 0.1 ng/mL bFGF (control medium), and used for the condensed mesenchymal cell body (CMB) formation.

Genetic Expression. RNA was purified from the samples according to the manufacturer's instructions using the TRIzol method (Life Technologies). Mesenchymal condensation transcriptional factors sex determining region Y (SRY)-box 9 (*SOX9*) and homeobox A2 (*HOXA2*), cell adhesion cadherin 2 (*CDH2*) gene, condensation extracellular matrix fibronectin (*FN1*), tenascin C (*TNC*), and syndecan 3 (*SDC3*) genes and housekeeping glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene were quantified by real-time PCR using TaqMan primers (Life Technologies) (Table S1).

Histology and Immunohistochemistry. After fixation, samples with decellularized scaffolds were decalcified with Immunocal solution, paraffin embedded, and cut into 5-μm sections that were stained with hematoxylin and eosin (H&E), Alcian blue for GAG, and trichrome. Samples were also immunohistochemically stained for collagens I, II, X, and lubricin (Abcam). *N*-cadherin and tenascin antibody (Millipore) were used for immunofluorescence staining.

Biochemical Analyses. DNA, GAG, and hydroxyproline contents were measured as previously described (2). In brief, the cartilage and subchondral regions were separated along the surface of the porous decellularized bone scaffold and the wet weights were determined. The samples ($n = 4$ per group) were digested in 0.5 mL proteinase K solution at 50 °C. DNA content was determined using the Picogreen assay (Molecular Probes). The sulfated GAG (s-GAG) content of the extract was determined using the 1,9-dimethylmethylene blue dye calorimetric assay with chondroitin-6-sulfate as a standard. Acid hydrolization was used to determine hydroxyproline content, which represents the amount of collagen.

Mechanical Testing. The compressive Young's modulus of the cartilage was measured in PBS using unconfined compression as previously described (3). The cartilage layer thickness was measured by subtracting the whole construct thickness from the thickness of the decellularized bone scaffold. The cylindrical constructs were compressed at 50 nm/s (~0.01% strain per second) up to 150 μm deformation, for up to 3,000 s, and the compressive load was measured. Young's modulus was calculated from the linear slope of the stress–strain curve.

Friction coefficient between the cartilage and glass was measured in PBS bath in a unconfined compression configuration as previously described (4). Continuous reciprocal sliding was used at a velocity of 1 mm/s and within a translational range of ±10 mm under 50 g constant load. The normal force, frictional force, and axial deformation of the cartilage were measured. All tests were terminated after 1,800 s. The time-dependent friction coefficient, μ_{eff}, was calculated from the ratio of the friction force to the normal force. The minimum friction coefficient, μ_{min}, and the equilibrium friction coefficient, μ_{eq}, represented the minimum value of μ_{eff} and the value achieved at the end of the run, respectively. Integration strength between the fused CMBs and native cartilage matrix was measured using a push-out test as in our previous studies (5). In brief, a cylindrical push-out rod (1.3 mm in diameter) was centered on the cartilage defect and lowered at 0.1% strain per second until the defect broke off. Peak force was measured and used to calculate peak shear stress.

Statistical Analysis. Pairwise comparisons of results were carried out using multiway analysis of variance (ANOVA) followed by Turkey's multiple comparison test (Prism Software) with $P < 0.05$ being considered as statistically significant.

1. Grayson WL, et al. (2010) Engineering anatomically shaped human bone grafts. *Proc Natl Acad Sci USA* 107(8):3299–3304.
2. Grayson WL, Bhumiratana S, Grace Chao PH, Hung CT, Vunjak-Novakovic G (2010) Spatial regulation of human mesenchymal stem cell differentiation in engineered osteochondral constructs: Effects of pre-differentiation, soluble factors and medium perfusion. *Osteoarthritis Cartilage* 18(5):714–723.
3. Mauck RL, et al. (2000) Functional tissue engineering of articular cartilage through dynamic loading of chondrocyte-seeded agarose gels. *J Biomech Eng* 122(3):252–260.
4. Krishnan R, Mariner EN, Ateshian GA (2005) Effect of dynamic loading on the frictional response of bovine articular cartilage. *J Biomech* 38(8):1665–1673.
5. Obradovic B, et al. (2001) Integration of engineered cartilage. *J Orthop Res* 19(6): 1089–1097.

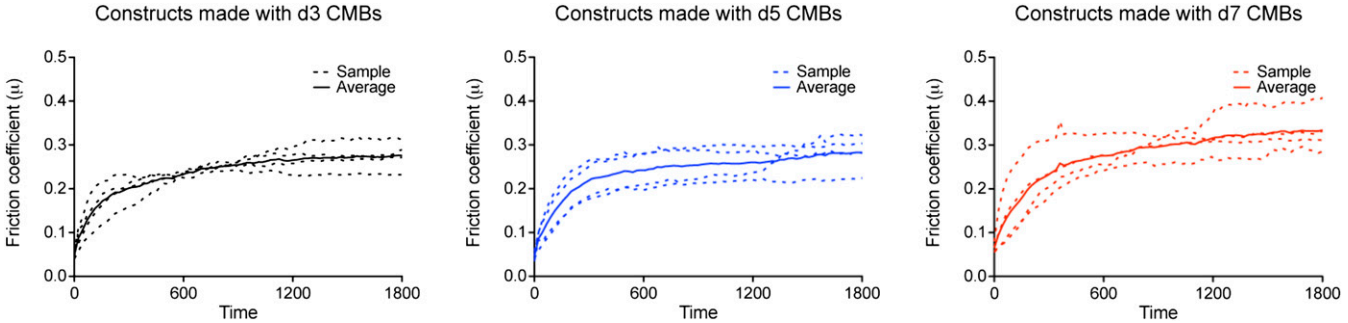
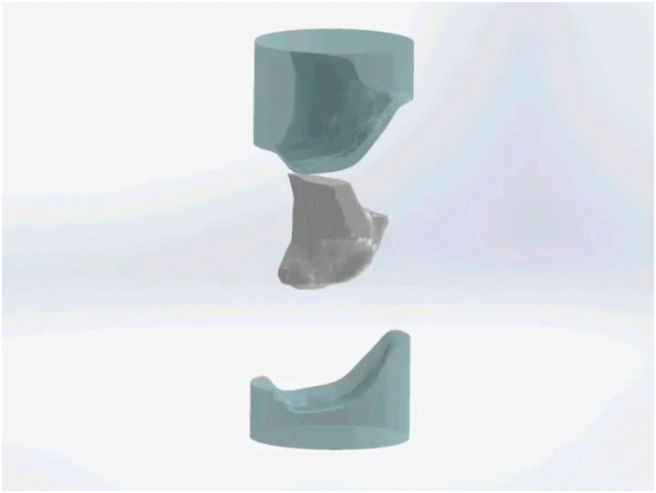


Fig. S1. Friction coefficient (μ) analysis of articular cartilage made with fusion of day 3, day 5, and day 7 CMBs. μ_{min} is the initial μ at the start of the test. μ_{eq} is the equilibrium μ value after 1,800 s of testing.

Table S1. List of TaqMan primers

Gene	TaqMan primer
GAPDH	Hs02758991_g1
SOX9	Hs01001343_g1
HOXA2	Hs00534579_m1
CDH2	Hs00983056_m1
FN1	Hs00365052_m1
TNC	Hs01115665_m1
SDC3	Hs01568665_m1



Movie S1. Animation of the steps involved in the formation of condylar cartilage by press-molding condensed mesenchymal bodies onto anatomically shaped porous bone scaffolds.

Movie S1