Bhattacharjee et al.:
A Human Osteoarthritis Mimicking Goat Cartilage
Explant-Based Disease Model for Drug Screening

Supplementary Data

Tab. S1: Age, gender, and grade of OA of all the patients included in the study

| Serial no. | Gender | Age | OA grade |
|------------|--------|-----|----------|
| 1          | M      | 62  | 4        |
| 2          | F      | 55  | 3        |
| 3          | M      | 65  | 4        |

Tab. S2: Details of the antibody used in the study

| Antibody            | Source                  | Catalog no. | Concentration/dilution | Clone/isotype      |
|---------------------|-------------------------|-------------|------------------------|--------------------|
| Anti-Collagen II    | DSHB                    | II-II6B3    | 6.00 µg/mL             | Monoclonal/IgG1    |
| Anti-Chondroitin sulfate | DSHB                  | 9BA12      | 6.20 µg/mL             | Monoclonal/IgM     |
| Anti-Aggreccan      | DSHB                    | 12/21/1-C-6| 6.40 µg/mL             | Monoclonal/IgG1    |
| Anti-Collagen X     | Sigma Aldrich           | C7974       | 4.25 µg/mL             | Monoclonal/IgM     |
| Anti-MMP13          | Sino Biologicals        | 102015-T10  | 1:250                  | Polyclonal/IgG     |
| Anti-ADAMTS4        | Sino Biologicals        | 106767-108  | 1:250                  | Polyclonal/IgG     |
| Anti-INOS           | Sino Biologicals        | 102279-T02  | 1:250                  | Polyclonal/IgG     |
| Anti-p38            | Sino Biologicals        | 10081-MM07  | 1:100                  | Monoclonal/IgG/IgM |
| Anti-rabbit IgG     | Jackson Immuno Research | 111-165-003| 2.00 µg/mL             | Polyclonal/IgG     |
| Anti-mouse IgG/M    | Jackson Immuno Research | 115-545-044| 2.00 µg/mL             | Polyclonal/IgG+IgM|

Tab. S3: List of primer sequences for qPCR

| Name      | Sequence                              |
|-----------|---------------------------------------|
| MMP13     | F: 5'-GAGCACTCTGTTCCCATCTA-3' R: 5'-GAGACTGATCCCTGGACATC-3' |
| ADAMTS4   | F: 5'-CTGACCTCTTCAAGAACTTCCC 3' R: 5'-GGGGTGACCAGCTGTAAGTAG 3' |
| COL10A1   | F: 5'-TGCTTTCACTGTTATCTTCTCC 3' R: 5'-TCCGCTTCTGGTGTAGAATG 3' |
| RUNX2     | F: 5'-CTTCACAAATCTCCTCCCAAGTAGCTACC 3' R: 5'-GGTATTAGATCATCAAGCTTCTGGT 3' |
Fig. S1: Effect of IL1β treatment on cartilage matrix markers
Immunofluorescence micrographs of goat cartilage explant sections stained for aggregan (green, top), decorin (green, bottom), and nuclei (blue) (scale bar – 100 μm).
Fig. S2: Live/dead staining and metabolic activity of chondrocytes in goat cartilage explants

(A) Staining for live and dead chondrocytes with fluorescein diacetate (green) and propidium iodide (red) in goat cartilage explants after 7 and 14 days of treatment with IL1β respectively (scale bar – 50 µm). 
(B) Resazurin assay for metabolic activity of chondrocytes in goat cartilage explants after 7 and 14 days of IL1β treatment. ns, not significant (one-way ANOVA with Tukey’s correction for multiple comparisons).
Fig. S3: Effect of IL1β treatment on chondrocyte hypertrophic markers
(A) Immunofluorescence micrographs of goat cartilage explant sections stained for collagen type X (Coll X) (green), osteopontin (OPN) (red), and nuclei (blue) (scale bar – 100 μm). (B) Fluorescence intensity quantification using image-based analysis. *, p < 0.05 with respect to control cartilage explants, n = 4 (one-way ANOVA with Tukey’s correction for multiple comparisons).
Fig. S4: Comparison of inflammatory marker expression in goat \textit{ex vivo} OA model with human OA cartilage
(A) Immunofluorescence micrographs of goat and human cartilage sections stained for NFκB (green) and nuclei (blue); and iNOS (red) and nuclei (blue) (scale bar – 100 µm). (B) Image analysis-based quantification of % NFκB and iNOS positive cells. ns, no significant difference between the groups (one-way ANOVA with Tukey’s correction for multiple comparisons).
Fig. S5: Comparison of goat ex vivo OA model with human OA cartilage for collagen II and aggrecan expression
Immunofluorescence micrographs of goat cartilage explant sections stained for collagen type II (Coll II) (green), aggrecan (Aggn) (green), and nuclei (blue) (scale bar – 100 μm).
Fig. S6: Live/dead staining of chondrocytes in goat cartilage explants after treatment with cytokines (IL1β or TNFα or IL1β+TNFα)
Staining for live and dead chondrocytes with fluorescein diacetate (green) and propidium iodide (red) in goat cartilage explants after 7 and 14 days of treatment with different cytokines (scale bar – 50 µm).
Fig. S7: Live/dead staining of chondrocytes in goat ex vivo OA model after treatment with celecoxib, BMP-7 or rapamycin

Staining for live and dead chondrocytes with fluorescein diacetate (green) and propidium iodide (red) in goat cartilage explants after (A) 7 days and (B) 14 days of treatment with different concentrations of celecoxib, BMP7 or rapamycin: CB1, celecoxib 0.1 µM; CB2, celecoxib 1 µM; B1, BMP7 50 ng/mL; B2, BMP7 100 ng/mL; R1, rapamycin 0.1 µM; R2, 1 µM. (scale bar – 100 µm).
Fig. S8: Gene expression analysis for OA markers in goat ex vivo OA model
Quantification of mRNA expression for (A) MMP13, (B) ADAMTS4, (C) collagen type X (COL10A1) and (D) runt-related transcription factor 2 (RUNX2) normalized to GAPDH expression in goat cartilage explants after treatment with IL1β. *, p < 0.05 vs control cartilage explants; ns, not significant, n = 6 (one-way ANOVA with Tukey’s correction for multiple comparisons).

Fig. S9: Gene expression analysis of ex vivo OA model after treatment with celecoxib, BMP7 and rapamycin
Quantification of mRNA expression for (A) MMP13 and (B) ADAMTS4 (normalized to GAPDH expression) in goat cartilage explants after treatment with different concentrations of celecoxib, BMP7 and rapamycin. CB1, celecoxib 0.1 µM; CB2, celecoxib 1 µM; B1, BMP7 50 ng/mL; B2, BMP7 100 ng/mL; R1, rapamycin 0.1 µM; R2, 1 µM. *, p < 0.05 vs IL1β-treated cartilage explants, n = 6 (one-way ANOVA with Tukey’s correction for multiple comparisons).