A tool for evaluating novel osteoarthritis therapies using multivariate analyses of human cartilage-synovium explant co-culture

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SUMMARY

Objective: There is a need to incorporate multiple tissues into in vitro OA models to evaluate novel therapeutics. This approach is limited by inherent donor variability. We present an optimized research tool: a human OA cartilage-synovium explant co-culture model (OA-EXM) that employs donor-matched lower and upper limit response controls combined with statistical approaches to address variability. Multiple rapid read-outs allow for evaluation of therapeutics while cataloguing cartilage-synovium interactions.

Design: 48-h human explant cultures were sourced from OA knee arthroplasties. An OA-like cartilage-synovium co-culture baseline was established relative to donor-matched upper limit supraphysiological pro-inflammatory cytokine and lower limit OA cartilage or synovium alone controls. 100 nM dexamethasone treatment validated possible “rescue effects” within the OA-EXM dual tissue environment. Gene expression, proteoglycan loss, MMP activity, and soluble protein concentrations were analyzed using blocking and clustering methods.

Results: The OA-EXM demonstrates the value of the co-culture approach as the addition of OA synovium increases OA cartilage proteoglycan loss and expression of MMP1, MMP3, MMP13, CXCL8, CCL2, IL6, and PTGS2, but not to the extent of supraphysiological stimulation. Conversely, OA cartilage does not affect gene expression or MMP activity of OA synovium. Dexamethasone shows dual treatment effects on synovium (pro-resolving macrophage upregulation, protease downregulation) and cartilage (pro-inflammatory, catabolic, and anabolic downregulation), and decreases soluble CCL2 levels in co-culture, thereby validating OA-EXM utility.

Conclusions: The OA-EXM is representative of late-stage OA pathology, captures dual interactions between cartilage and synovium, and combined with statistical strategies provides a rapid, sensitive research tool for evaluating OA therapeutics.

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Introduction

Osteoarthritis (OA) is a complex disease with a multitude of factors contributing to its progression including low-grade synovial inflammation. Significant changes to innate immunity, including infiltrating and synovial resident monocytes and macrophages, are key contributors to OA progression. However, tools to evaluate new therapeutics that integrate the role of innate immune cells in OA pathogenesis are limited. There is a need for ex vivo assessment tools of OA therapeutics that can dually account for human cartilage-synovium tissue interactions.

Single chondrocyte or synoviocyte cultures have been mainstays of in vitro OA research; cells are typically expanded from primary tissue or a progenitor cell source. Alternatively, cartilage or synovium are kept intact as tissue explants to retain endogenous matrix topology as a trade-off for increased heterogeneity. Single tissue cultures are often treated with pro-inflammatory cytokine stimulation or mechanical stressors to mimic disease that are in far
excess of physiological levels\textsuperscript{14}. Although single tissue investigations allow greater control over experimental conditions, they silo outcomes and do not reflect key crosstalk in OA pathology\textsuperscript{14,15}.

Use of human cartilage-synovium explant co-cultures were first reported for rheumatoid arthritis\textsuperscript{16}, given its predominance of synovial hyperplasia and inflammation. Recently, cartilage-synovium co-culture explant models of healthy animal tissue treated with compression injury or exogenous cytokines have been used to induce an OA-like state\textsuperscript{17–20}. Beekhuizen \textit{et al.} developed a human OA cartilage-synovium explant co-culture model\textsuperscript{11} that demonstrated proteoglycan synthesis inhibition by OA synovium without ened by anti-inflammatory corticosteroid rescue. This study importantly suggests that a human cartilage-synovium explant co-culture is capable of capturing known pathophysiological effects of synovium on cartilage and OA clinical outcomes\textsuperscript{22,23}. However, this study used an extended timeline and did not address human donor heterogeneity, which is a limiting factor in widespread use of co-culture explant models as a research tool.

Building on existing contributions, we present a “human OA cartilage-synovium explant co-culture research model” (referred to as OA-EXM). The OA-EXM encompasses an entire suite of culture conditions, methods, and statistical strategies to establish a useful late-stage OA cartilage-synovium explant baseline to evaluate therapeutics, with significant features including: (1) optimized readouts at a rapid 48-h timepoint sensitive to significant changes in multiple variables; (2) statistical analyses and clustering strategies to account for donor-derived variability; (3) an expanded toolkit of \textit{simultaneous}, quantitative readouts of cartilage and synovium gene expression, glycosaminoglycan (GAG) loss, soluble matrix metalloproteinase (MMP) activity and soluble protein concentrations. The OA-EXM provides a 48-h “snapshot” of multivariate outcomes in an intact, human disease setting accounting for dual cartilage-synovium tissue interactions and is thus a useful research tool for evaluating OA therapeutics.

\textbf{Method}

\textit{Total knee arthroplasty processing and explant tissue culture}

Cartilage and synovium from late-stage OA (Kellgren–Lawrence grade 3–4) total knee arthroplasties (Table I) were acquired with patient consent and institutional ethics approval (UHN REB 14-7483-AE). Full-depth cartilage is cut from subchondral bone and sectioned with a biopsy punch (1.5 mm diameter) and pooled before random distribution per 20 or 40 mg wet weight into 24-well plates (800 μL). Synovium is minced with scissors after removing fat tissue, then distributed per 40 mg wet weight into 0.4 μm transwell inserts (100 μL).

Explant medium is a 1:1 ratio of Roswell Park Memorial Institute (RPMI) 1640 and Dulbecco’s modified Eagle medium (DMEM), low glucose, supplemented with 1% fetal bovine serum (FBS), 1% gentamicin, 1 mM sodium pyruvate, 50 μg/mL L-proline, 50 μg/mL L-ascorbic acid 2-phosphate, and 1X insulin-transferrin-selenium (ITS-G). Explant tissue is incubated separately for a 2-day acclimatization period\textsuperscript{14–18} before start of experiments.

Explant tissue and conditioned medium are harvested at days 2 and 7 of culture by snap freezing and stored at –80°C for analysis.

\textit{Explant tissue co-culture experimental design for statistical analyses}

The OA-EXM [Fig. 1(A)] is constructed with experimentally controlled fixed factors of multiple levels for compatibility with mixed linear modeling. Recommended options for analysis are detailed in \textit{Statistics}.

Two fixed factors are used: “Culture” and “Treatment”. “Culture” is a 2-level factor: (1) single tissue alone (SOLO; cartilage-alone or synovium-alone) or (2) cartilage-synovium co-culture (CCUL). “Treatment” is a 3-level factor: (1) baseline (BASE; medium alone); (2) positive cytokine control (POS; 5 ng/mL oncostatin M (OSM) and 5 ng/mL interleukin 1β (IL1β) for cartilage, or 100 ng/mL interferon γ (IFNγ) for synovium; PeproTech); or (3) corticosteroid (DEXA; 100 nM dexamethasone\textsuperscript{27,28}; BioReagent). “Treatment” is nested under “Culture” for a total of four experimental “Culture-Treatment” groups per tissue: SOLO-BASE, SOLO-POS, CCUL-BASE, CCUL-DEXA. SOLO-BASE and supraphysiological pro-inflammatory positive control (SOLO-POS) represent the lower and upper limit responses specific to each donor. SOLO-POS uses supraphysiological cytokines to elicit known pro-inflammatory and pro-catabolic effects tailored specifically for cartilage\textsuperscript{29–32} or to promote pro-inflammatory macrophage polarization in synovium\textsuperscript{10,33,34}. The OA-EXM therefore comprises of the OA co-culture environment testing baseline (CCUL-BASE) with established upper (SOLO-POS) and lower (SOLO-BASE) limits. The OA-EXM also includes test therapeutic “Treatment” groups on top of its co-culture platform; in this study, we validate the current version of the OA-EXM with known therapeutic, dexamethasone, generating an additional CCUL-DEXA subset.

\textit{Gene expression by quantitative polymerase chain reaction (qPCR)}

Frozen explant tissue is fragmented using a liquid nitrogen pre-chilled BioPulverizer (BioSpec) for RNA isolation using a Plant Total RNA Mini Kit (Geneaid) for cartilage or RNeasy Plus Universal Mini Kit (QIAGEN) for synovium. cDNA is generated with SuperScript\textsuperscript{TM}

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<table>
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SD, standard deviation.

\textbf{Table I}

Tissue donor demographics for total knee replacement explants
IV VILO™ Master Mix (Invitrogen). qPCR is run on cartilage- and synovium-specific gene panels (Supplementary Table 1) using custom primers (Supplementary Table 2, Invitrogen) and FastStart Universal SYBR Green Master Mix (Roche) on a QuantStudio™ 5 (ThermoFisher) or 7900HT (Applied Biosystems). Results are normalized (ΔΔC₇), represented as log₂(fold-change) against reference genes B2M, TBP, and RPL13A (geometric mean) and to the donor-matched single tissue baseline (SOLO-BASE) median. Undetected C₇ values of samples are imputed as 40 for fold-change calculations.

Fig. 1

Human OA joint explant model (OA-EXM) consisting of cartilage-synovium co-culture is viable for 2–7 days. (A) Schematic of model. Cartilage and synovium from late-stage OA knee replacements are processed for a 48-h acclimatization period. Cartilage controls are established alone (baseline; SOLO-BASE) or with OSM and IL1β as a positive pro-inflammatory control (SOLO-POS). Synovium controls are alone (SOLO-BASE) or pro-inflammatory IFNγ-treated control (SOLO-POS). Cartilage and synovium in co-culture (CCUL-BASE) is representative of an OA baseline state. Treatment within the cartilage-synovium co-culture is validated in this iteration using 100 nM dexamethasone (CCUL-DEXA). (B) Representative (N = 1) DAPI stain on cartilage and synovium show distribution of cells in day 7 explant co-cultures. Bars represents 400 μm. (C) Representative (N = 1) hematoxylin & eosin staining of synovium show structure before and after day 7 explant co-culture. Bars represent 100 μm. (D) Representative (N = 1) cell populations within synovium digest on day of knee replacement. A CD14⁺ population is identified within a CD45⁻/CD90⁻ lymphocyte gate. (E) Representative (N = 3) Safranin-O/Fast Green staining of cartilage show proteoglycan content in day 7 explant culture between cartilage SOLO-BASE and pro-inflammatory SOLO-POS.

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Proteoglycan loss, soluble proteins, and soluble protease activity

Proteoglycan loss represented by sulfated GAG concentration in medium is detected by dimethylmethylen blue (DDMB) assay against a chondroitin sulfate (sodium salt from shark cartilage, Sigma–Aldrich) standard A525 curve. Soluble protein concentrations in medium are detected using LEGENDPlex™ immunoassay (BioLegend) on a FACS® Canto (BD Biosciences). Values below or above the limit of detection were imputed as half or twice the lower and upper limits if ≥ 67% of samples were within range. Soluble total MMP activity is measured using the MMP Activity Assay kit (abcam). All kits are used according to manufacturer’s protocol.

Statistics

JMP Pro 14 (SAS) is used. Each randomized well is assigned into an experimental “Culture-Treatment” group with 3 technical replicates per group from a single arthroplasty. “Donor” is used as a random effect, blocking factor, or normalization factor in statistical analyses to account for donor variability; each donor is considered a biological replicate for each experimental group. The number of replicates and “Culture-Treatment” groups differ between donors due to yield variability in surgical tissue acquisition. Table II details statistical methods used to account for donor variability. Supplementary Fig. 1 illustrates the study datasets, sample sizes, and selected analyses as a practical guideline to adopt the OA-EXM.

Linear regression (Fit Model, Mixed Model) was used for GAG loss. Regression assumptions are fulfilled by test of normal residuals (Supplementary Fig. 2). Effect size of each factor is represented by mixed model parameter estimates.

Unbiased hierarchical clustering (Ward method) is used for multivariate gene and soluble protein profiles. “Donor” labels are appended post hoc. Discriminant analysis (Linear, common covariance method; grouped by “Culture-Treatment”) is used for dimensionality reduction. The percent misclassified and entropy R² value indicate the fit of “Culture-Treatment” groups into distinct clusters. Gene expression fold-change outcomes are summarized by mean and 95% confidence interval (CI) using un-pooled estimates of standard error. Statistical testing of individual genes was omitted in favour of cluster-based classification representative of overall effects to expression profile.

Results

Co-culture environment of the OA-EXM is conducive to structural maintenance and viability

The OA-EXM was optimized from existing protocols. Media supplementation ensures explant viability for up to 7 days (Supplementary Fig. 3), with mass per well of cartilage and synovium based on feasible harvest per donor and minimum-volume requirements for downstream multivariate analyses. Low serum-supplemented conditions allow investigation of multiple tissues without serum growth factor interference and is conducive to future investigations of cellular therapies.

Cartilage and synovium morphology are maintained for 7 days as seen by 4',6-diamidino-2-phenylindole (DAPI) nuclear staining (Fig. 1(B)) and hematoxylin & eosin staining (Fig. 1(C)). Both cluster of differentiation (CD) 90⁺ fibroblasts and CD45⁻/CD14⁻ monocytes/macrophages are present within explant synovium (Fig. 1(D)), corroborating with reports of elevated percentages of macrophage populations in the OA joint. Proteoglycan retention in cartilage explants at baseline contrasts with visible loss when treated with supraphysiologically of pro-inflammatory cytokines (Fig. 1(E)), demonstrating the upper limit response of cartilage degradation within the OA-EXM.

Cartilage-synovium co-culture conditions in the OA-EXM set an OA-like baseline demonstrating significant effects of synovium on cartilage matrix loss, anabolic and inflammatory gene expression

OA cartilage alone (SOLO-BASE) has a lower limit of steady GAG loss (µg/mL) at baseline at 48-h while supraphysiologically cytokine stimulation (SOLO-POS) defines the upper limit (Fig. 2(A)). Addition of OA synovium in co-culture (CCUL-BASE) significantly increases GAG loss (P = 0.0479) compared to the lower limit, but not as much

### Table II

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<td>MMP activity</td>
<td>Block-centered t-test or ANOVA</td>
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<td>Yes, as Block factor (equal n replicates within blocks)</td>
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A – Statistical method used in JMP 14 software. B – If the method uses “Culture-Treatment” experimental grouping or if the analysis is unbiased. C – How the OA tissue donor is accounted for in the statistical method.

ANOVA, analysis of variance; GAG, glycosaminoglycan; MMP, matrix metalloproteinase.
as the upper limit ($P < 0.09001$). Using donor-specific limits, least squares regression (Fig. 2(B), Table III) accounts for “Donor” contribution to total variance and reattributes effect estimates of “Culture” and “Treatment”. This mixed linear analysis predicts GAG loss of SOLO-BASE, CCUL-BASE, and SOLO-POS as: 0.838 (0.595, 1.07), 1.14 (0.908, 1.38), and 3.03 (2.79, 3.27). The OA-EXM thus captures additional GAG loss in cartilage-synovium co-culture compared to cartilage alone, with further capacity to statistically account for donor variability using upper and lower limits.

The OA-EXM captures 48-h cartilage expression changes within a curated OA-relevant 28-gene panel representing extracellular matrix (ECM) metabolism, chemotaxis and adhesion, and inflammation (Supplementary Table 1). The OA-EXM detects the effects of synovium on cartilage in the CCUL-BASE group (Fig. 3(A), Supplementary Table 3): the top three downregulated genes are anabolic (ACAN, COL2A1, SPP1), while the top three upregulated genes are pro-inflammatory and chemotactic (OXCL1, IL6, NOS2). Matrix proteases (MMP1, MMP3, MMP13, ADAMTS4, ADAMTS5) and other pro-inflammatory (PTGS2, IL1B) genes are also upregulated. Addition of synovium affects 48-h cartilage gene expression in the same direction as SOLO-POS, albeit at a diminished magnitude. Individual donor effects are accounted for as fold-change in gene expression is relative to each donor’s cartilage-alone expression (SOLO-BASE).

The OA-EXM concurrently captures 48-h synovium gene expression changes within a curated 9-gene panel focused on macrophage polarization and fibrosis (Supplementary Table 4). Supraphysiological IFNγ (SOLO-POS) upregulates fold-change of NOS2 and pro-inflammatory macrophage surface marker HLA-DR. In contrast to cartilage gene expression, the OA-EXM shows non-significant change in synovium gene expression with the addition of OA cartilage (CCUL-BASE) within the 48-h co-culture period. Again, donor-matched normalization to synovium-alone (SOLO-BASE) accounts for donor variability.

An alternative configuration of the OA-EXM explored increasing the cartilage: synovium ratio from 1:2 (20:40 mg) to 1:1 (40:40 mg). The effects of synovium on cartilage are maintained despite reduced synovium per cartilage (Supplementary Fig. 4). Meanwhile, increasing the cartilage: synovium ratio does not change synovium gene expression or produce significant effects of cartilage on synovium (Supplementary Fig. 5). Given the lack of...
changes to synovium even in the presence of additional OA cartilage, the suggested cartilage: synovium ratio for the OA-EXM is more practically limited to the lower 1:2 ratio due to limited availability of cartilage from end-stage OA arthroplasty.

The OA-EXM captures donor-specific soluble protein profiles

The OA-EXM allows for analysis of soluble factors from both cartilage and synovium. Soluble OA-relevant cytokines (interleukin (IL) IL6, IFNγ, IL10, IL1β, tumor necrosis factor (TNF) α), chemokines (chemokine (C–C motif) ligand (CCL) 2, chemokine (C–X–C motif) ligand (CXL) 8, CXL10), and adipokines (adiponectin, resistin, leptin, adipin) were measured in a donor subset (N = 4). All proteins, except IL10, IL1β, and TNFα, were present in ≥67% of samples (Supplementary Table 5).

Both OA cartilage and synovium alone produce detectable levels of the remaining 9 proteins. IL6, IFNγ, CCL2, CXL8, adiponectin, resistin, leptin, and adipin were found in higher concentrations in the OA synovium-alone, whereas CXL10 was primarily secreted by OA cartilage. Further, the OA-EXM captures a differential co-culture baseline soluble factor profile compared to OA cartilage or synovium alone. All soluble factors except CXL10 are higher in coculture than cartilage-alone [Fig. 3(B)]. Comparing co-culture with synovium-alone with donor blocking (P < 0.005 for all proteins), CCL2 and IL6 have the greatest differences [Fig. 3(C)]. Both CCL2 and IL6 levels are higher in cartilage-synovium co-culture compared to synovium alone (P = 0.097; P = 0.0081, respectively). All means and comparisons are found in Supplementary Tables 6 and 7. The cartilage-synovium baseline in the OA-EXM is a distinctive soluble factor environment of OA-relevant factors that is comparable to OA cartilage-synovium baseline in the OA-EXM is a distinctive soluble factor environment of OA-relevant factors that is comparable to OA cartilage-synovium baseline (SOLO-POS). Fig. 4(A) represents the log2(fold-change) values from the cartilage gene expression panel of 28 outputs reduced to three canonical dimensions. This differentiates the four canonical groups of SOLO-BASE (cartilage), SOLO-POS (cartilage), CCL-BASE, and CCL-DEXA with only 5% sample misclassification (Supplementary Table 8). Adding dexamethasone (CCL-DEXA) not only “rescues” the profile from CCL-BASE to SOLO-BASE (downwards on Canonical3), but further shifts on both the Canonical1 and 2 axes. Unbiased two-way hierarchical clustering [Fig. 4(B)] similarly shows the four “Culture-Treatment” groups as separate clusters.

In parallel, dexamethasone alters the synovium gene expression profile away from cartilage-synovium co-culture baseline (CCL-BASE; Fig. 4(A)). Dexamethasone added in co-culture down-regulates expression of MMP1 and MMP3 combined with upregulation of pro-resolving macrophage scavenger receptors (CD163 and MRC1) normalized to synovium-alone (SOLO-BASE; Supplementary Table 8). An additional subset demonstrates similar MMP1, MMP3 downregulation and CD163,
MRC1 upregulation in synovium-alone treated with dexamethasone (Supplementary Fig. 9).

Discriminant analysis (Fig. 4(A), Supplementary Fig. 10) and unbiased hierarchical clustering [Fig. 4(C)] confirms an overlap between the synovium SOLO-BASE and CCUL-BASE groups, which is the source of a 19.6% misclassification for synovium gene expression (Supplementary Table 8). Addition of pro-inflammatory IFNγ to synovium (SOLO-POS) shifts the cluster away from both SOLO-BASE and CCUL-BASE synovium profiles. Regardless of the overlap between SOLO-BASE and CCUL-DEXA produces a separate expression profile shifted from these two groups (downwards on Canonical1).

OA-EXM cartilage-synovium co-culture baseline downregulates anabolic matrix and upregulates pro-inflammatory cartilage gene expression, which correspond to pro-inflammatory changes to the soluble factor profile. (A) Day 2 cartilage gene expression as log2(fold change) relative to reference genes and cartilage baseline (SOLO-BASE). Diamonds represent mean and 95% confidence interval. All experimental groups represented are significantly different from SOLO-BASE by post-hoc Dunnett comparison ($p < 0.05$), $N = 21$ donors * 3 replicates. (B) Soluble factor profile represented by hierarchical clustering and (C) select block-centered scatterplots comparing cartilage baseline (SOLO-BASE, cartilage), synovium baseline (SOLO-BASE, synovium) and cartilage-synovium baseline (CCUL-BASE). Heatmap represents high-to-low protein concentration with maximum–minimum relative to each secreted protein (where minimum ≥ 0 pg/mL). Diamonds represent mean and 95% confidence interval, block factor = donor, * = $p < 0.05$ by post-hoc Tukey–Kramer comparison, $N = 4$ donors * 3 replicates.

Fig. 3

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Treatment with dexamethasone (CCUL-DEXA) significantly reduces MMP activity [Fig. 5(A)], but not GAG loss [Fig. 5(B)] compared to co-culture baseline (CCUL-BASE). Soluble MMP activity was significantly reduced with CCUL-DEXA compared to CCUL-BASE ($P = 0.0397$) when accounting for donor blocking (donor effect $P < 0.0001$). In contrast, there is no difference ($P = 0.181$) in GAG loss between CCUL-BASE and CCUL-DEXA, even after accounting for significant donor effect ($P = 0.0177$). This is consistent with downregulation of MMP1 and MMP3 expression in the CCUL-DEXA group relative to SOLO-BASE and CCUL-BASE for both cartilage (Fig. 4(B), Supplementary Fig. 6) and synovium (Fig. 4(C), Supplementary Fig. 8).

The OA-EXM detects changes to soluble CCL2 and CXCL8 with dexamethasone treatment while others were not significantly altered (Fig. 5(C), Supplementary Table 9). Addition of dexamethasone decreases CCL2 compared to co-culture baseline ($P = 0.0072$, Fig. 4).
CXCL8 concentrations unexpectedly increased ($P = 0.0116$) with dexamethasone treatment. Unbiased hierarchical clustering [Fig. 5(C)] of soluble protein concentrations shows no distinct clustering of dexamethasone (CCUL-DEXA) and co-culture baseline (CCUL-BASE) groups. Only one of three donors shows separation between CCUL-DEXA and CCUL-BASE soluble protein profiles within its donor cluster.

Taken together, the OA-EXM is a sensitive tool that captures the nuanced effects of dexamethasone in terms of reduced pro-inflammatory and catabolic cartilage and synovium gene expression, reduced MMP activity, and reduced soluble CCL2 levels without concomitant rescues in GAG loss or soluble CXCL8 levels at a 48-h “snapshot”.

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Discussion

We present a novel human OA joint explant cartilage-synovium co-culture model (OA-EXM) that includes a full suite of explant culture protocols, experimental designs to account for an OA co-culture baseline with donor-specific upper and lower limit controls, treatment groups, as well as appropriate statistical methods. The OA-EXM rapidly captures dual cartilage and synovium gene expression, soluble protein concentrations, GAG loss, and MMP activity at a rapid 48-h timepoint “snapshot”. Human late-stage OA explants allow for non-culture expanded cells with baseline human disease phenotypes. The OA-EXM queries an end-stage OA-like baseline using cartilage-synovium co-culture; supraphysiologic factors are only used to capture donor-matched upper limits of multiple readouts. Tissue donor heterogeneity, often a deterrent to the use of human explant tissue is directly addressed by use of single tissue and supraphysiologic cytokine controls to capture donor-specific response ranges combined with various statistical tools such as clustering and incorporation of donor as a block factor in sample sizes ranging from $N = 3$ to $N = 21$. The OA-EXM incorporates OA synovium effects and confirmed increased GAG loss, pro-inflammatory and catabolic cartilage gene expression relative to OA and age-mo, and reduced secretion of CXCL1. Conversely, the OA-EXM shows minimal effects of OA cartilage on OA synovium regardless of cartilage: synovium co-culture ratios in terms of synovium gene expression at 48-h.

Dexamethasone was used to validate the utility of the OA-EXM. Clinically, dexamethasone has shown acute pain reduction in OA49,50 and reduces detrimental effects induced by mechanical injury and pro-inflammatory cytokines in human cartilage explant in vitro51. The OA-EXM captured dual effects of dexamethasone on cartilage and synovium, inhibiting cartilage catabolic, chemokacic, and pro-inflammatory gene expression as previously reported27,51 while upregulating pro-resolving macrophage marker expression and downregulating both synovium MMP gene expression in synovium and concomitant MMP activity. Dexamethasone has been observed to have strong pro-resolving macrophage polarization effect52,53 and an anti-inflammatory effect on OA synovium explants by targeting pro-inflammatory synovial macrophage polarization10.

Unexpectedly, the OA-EXM captured non-significant effects of dexamethasone treatment on GAG loss; the model captures downregulated cartilage expression of anabolic matrix genes to magnitudes greater than anabolic downregulation in the OA cartilage-synovium co-culture baseline. This aligns with controversial clinical reports of greater cartilage loss and radiographic OA progression54,55 with repeat corticosteroid treatment. Despite a consensus on dexamethasone’s effects in MMP downregulation, mixed effects on apoptosis and GAG loss have been reported in cell and cartilage explant cultures56. Inhibition of MMP expression and activity concurrently with anabolic matrix gene downregulation has not been previously reported in a human arthritic co-culture setting. The OA-EXM simultaneously captures mixed effects of dexamethasone and can thus be a useful research tool for investigating dosage, duration, and novel formulation or delivery strategies for treatments including dexamethasone conjugates57,58 that may recapitulate beneficial effects without activating anti-anabolic pathways.

The OA-EXM has a number of important limitations. Our data shows that it can be vaunted for its rapid 48-h “snapshot” readout utility and 7-day viability, but it is not optimized for prolonged culture durations. Longer culture durations could capture more extensive matrix remodeling or long-term treatment effects with additional histological and matrix protein quantification. Long-term explant cultures have been previously used21,26 and are valuable in modeling extended changes in OA. Further, the OA-EXM does not use healthy or early-stage joint tissue and thus is not currently configured for capturing longitudinal disease evolution.

The OA-EXM configuration is optimized with considerations to ease-of-use and to primarily establish a controlled human OA-like cartilage-synovium baseline environment using donor-matched tissues and control conditions. The use of donor-matched single tissue controls allows the capture of a range of response to test therapeutics, but only in relevant to late-stage OA. Incorporation of additional joint components including fat pad, synovial fluid, infiltrating monocytes/macrophages, and subchondral bone would be considered in future iterations of the OA-EXM research tool. Additionally, the use of a transwell system recapitulates soluble factor interactions between cartilage and synovium that are more reflective of physiological conditions4,5 but does not account for possible direct tissue interactions.

The OA-EXM did not capture significant effects of cartilage on synovium. The synovium gene panel was curated toward macrophage-mediated response and was thus less comprehensive than the cartilage panel. Further, the synovium is a heterogeneous tissue with additional variability arising from its mixed cellular composition that could obliterate cell population-specific changes. Despite this, incorporation of OA synovium is essential to the OA-EXM as a contributor of active soluble proteins and its multiple effects on OA cartilage. Single cell RNA sequencing, cell sorting, or flow cytometry could be integrated into a future OA-EXM toolset to parse meaningful synovial changes in face of population heterogeneity.

Although the OA-EXM accounts for donor variability through upper and lower limit control groups and statistical strategies, this utility is limited to determining the significance of treatment or culture effects as a whole. In its current iteration, the OA-EXM effectively screens for novel therapies with significant effects but cannot predict which patients could be responders to test treatments. Within our sample datasets, the effects of OA synovium on cartilage were of greater magnitude in a subset of donors, suggesting the existence of donor subtypes that could be segregated within the OA-EXM. Annotation of these donors with existing clinical data yielded no correlations (data not shown). More sensitive clinical measurements and potential biomarkers5,23,59,60 could be used in future correlations to patient heterogeneity.

The OA-EXM allows for rapid 48-h multivariate readouts that provide holistic simultaneous “snapshots” of the OA joint cartilage and synovium tissue’s inflammatory, anabolic, and catabolic status. The model is sufficiently sensitive to capture potentially favourable and less favourable gene, protein, enzyme activity and proteoglycan losses upon challenge with a well-known anti-inflammatory treatment, and thus represents a useful tool to investigate novel OA therapeutics. Importantly, the OA-EXM as presented incorporates statistical tools and controls to capture donor heterogeneity characteristic to OA. The OA-EXM represents a significant advance in in vitro tools available to OA researchers to evaluate therapies and their mechanisms.

Author contributions

All authors have made substantial contributions to all three requirements for authorship. MWYC, AGA, SV contributed to the design and conception of the study. MWYC and AGA contributed to conducting experiments. MWYC, AGA, NM, and RG contributed to acquiring reagents, patient tissue, and data. MWYC, AGA, SV contributed to analyzing and interpreting data. All authors contributed to the writing and final review of the manuscript. MWYC (mable.chan@uhnresearch.ca) and SV (sowmya.vishwanathan@uhnresearch.ca) take responsibility for the integrity of this work.

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All authors declare no conflicts of interest within the scope of this manuscript. MWYC and RG declare no conflicts of interest exist outside of this scope. AGA receives income from BlueRock Therapeutics and holds co-inventorship on the patent WO2007128115A1 on immune privileged and modulatory progenitor cells. NM declares equity in biotechnology company Arthritis Innovation Corp, and equity and funds from Arthur Health as its Chief Executive Officer. SV declares 60% ownership in Regulatory Cell Therapy Consultants, Inc., a private regulatory consulting company.

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Appendix A Supplementary data
Supplementary data to this article can be found at https://doi.org/10.1016/j.joca.2021.09.007.

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