Cartilage Explant-Based High-Throughput Screening Identifies Clinically Promising Candidates for Osteoarthritis

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INTRODUCTION: Osteoarthritis (OA) is a leading cause of pain and disability, yet no therapies prevent cartilage degeneration or modify disease progression. Current treatments are limited to symptom relief, leaving an unmet need for disease-modifying strategies [1]. Glycosaminoglycan (GAG) loss is an early marker of cartilage breakdown and a reliable measure for evaluating protective therapies. Most existing OA drug screening efforts rely on simplified 2D monolayer or 3D hydrogel culture systems [2], which lack the structural and biochemical complexity of native cartilage, limiting translational relevance. To address this gap, we developed a tissue-based high-throughput screening (HTS) platform that preserves the native cartilage matrix and uses click chemistry for longitudinal detection of GAG loss. Here, we apply this platform to evaluate FDA-approved drugs. Our goal is to identify repurposing candidates with potential for clinical translation in OA.

METHODS: Experimental conditions were adapted from our prior GAG quantification workflow [3] to a 96-well format. Juvenile bovine cartilage explants (1-2 months old, 3 mm diameter, 1 mm thickness, ~7 mg) were isolated from middle-zone tissue using a custom cutting tool for uniformity (Fig 1). Explants were cultured in chondrogenic medium and assayed for GAG loss using a copper-free, click-chemistry method. Samples were labeled with GalNAz and clicked with AZ488 fluorophore to enable longitudinal GAG quantification over 10 days in the presence of IL-1β (2 ng/mL) and 10 μM of an FDA-approved drug. A subset of 235 compounds from a 2,906-compound drug library (MedChemExpress, HY-L022) was screened across three plates: DP3, DP8, and DP11. Each plate included vehicle (0.3% DMSO), IL-1β (0.3% DMSO), and IL-1β + resveratrol (100 μM) (n=4/group) as negative, disease, and positive controls. GAG release into media was measured every other day. On day 10, explants were papain-digested to quantify remaining labeled GAG, and longitudinal GAG loss was normalized to total labeled GAG per explant. Control replicates were checked for outliers (Table 1 Eq. 1; excluded if $|z|>3.5$). Assay robustness was assessed using Z'-factor between vehicle and IL-1β (Eq. 2; acceptable if Z'>0) and control coefficients of variation (Eq. 3; acceptable if CV<25%) [4]. Drug effects were expressed as percent protection (%Protection) relative to controls (Eq. 4) and summarized by robust z-score vs IL-1β distribution (Eq. 5) [5]. Each drug plate was tested in duplicate; if both passed quality control (QC), positive controls agreed within 10%, and inter-plate CV<25%, results were averaged. Otherwise, only the passing plate was used. Hits were defined as compounds with ≥50% protection and robust z-score<–3.

RESULTS: Both DP3 plates passed QC (Plate 1: Z'=0.75, CVs=2-21%; Plate 2: Z'=0.62, CVs=5-17%). One plate each from DP8 (Z'=0.61, CVs=5-24%) and DP11 (Z'=0.04, CVs=1-12%) met QC standards, so only those were analyzed. IL-1β consistently induced greater GAG loss than vehicle, while resveratrol, an anti-oxidative and anti-inflammatory polyphenol, reproducibly reduced IL-1β-induced GAG loss (Fig 2). Since DP3 positive controls agreed within 10%, results from both plates were averaged. Of the 235 drugs screened, most clustered near the IL-1β response with little or no protection (Fig 3A-B). Applying predefined thresholds (≥50% protection and robust z-score<–3) identified 52 hits (Fig. 3C) across multiple pharmacological classes (Fig 4).

DISCUSSION: This pilot study establishes the feasibility of a cartilage explant-based HTS for OA drug repurposing. Consistent GAG loss responses to IL-1β and resveratrol confirmed assay robustness. Screening identified 52 candidate compounds, including modulators of immune, inflammatory, metabolic, and oxidative stress pathways, which align with known OA pathology [6-7]. Additional categories, such as DNA repair and anti-infectives, suggest novel or underexplored chondroprotective mechanisms. Drugs targeting immune, inflammatory, metabolic, and oxidative stress pathways provided the strongest protective effects, supporting their therapeutic potential. A limitation is the reliance on GAG loss to define efficacy, without evaluating collagen degradation or cell viability [8-9]. Validated hits will undergo further *in vitro* and *in vivo* assays, and text mining will help prioritize safe, affordable, and clinically translatable candidates. Next steps include expanding screening to the full 2,906-compound library and testing bovine hits in healthy and OA human explants. **SIGNIFICANCE:** This study establishes a proof-of-concept for a cartilage explant-based HTS platform to rapidly identify drug repurposing candidates for OA. Leveraging FDA-approved compounds offers the potential to fast-track therapeutic development and improve patient outcomes.

REFERENCES: [1] Cho+ 2021. [2] Foster+ 2021. [3] Porter+ 2022. [4] Iversen+ 2012. [5] Brideau+ 2003. [6] Ansari+ 2020. [7] Wei+ 2023. [8] Tschaikowsky+ 2022. [9] Charlier+ 2016.

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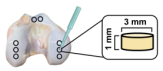

Fig 1 Assay Design. Cartilage was harvested from juvenile bovine knee joints to screen 2,906 FDA-approved drugs for their ability to reduce IL-1β-induced glycosaminoglycan (GAG) loss in a native tissue microenvironment.

Table 1 Equations used for assay assessment and hit identification

Equation	Name	Formula
Eq. 1	Robust z (control outlier)	$z_{outlier} = 0.6745 \times \frac{x_i - median_{ctrl}}{MAD_{ctrl}}$
Eq. 2	Z'-factor	$Z' = 1 - \frac{3(\sigma_{Veh} + \sigma_{IL1\beta})}{\lvert\mu_{IL1\beta} - \mu_{Veh}\rvert}$
Eq. 3	Coefficient of variation (CV)	$CV = \frac{\sigma/\sqrt{n}}{\mu} \times 100\%$
Eq. 4	Percent protection	$\%Protection = \frac{\mu_{IL1\beta} - x_{drug}}{\mu_{IL1\beta} - \mu_{Veh}} \times 100\%$
Eq. 5	Robust z (drug vs IL-1β)	$z_{drug} = 0.6745 \times \frac{x_{drug} - median_{IL1\beta}}{MAD_{IL1\beta}}$

Key: x_i = observed value for single control sample; ctrl = control condition (vehicle, disease, or positive); Veh = vehicle control; IL1β = IL-1β control; σ = standard deviation; μ = mean; n = number of samples; MAD = median absolute deviation. Unless noted, all statistics are computed on normalized GAG loss values within plate.

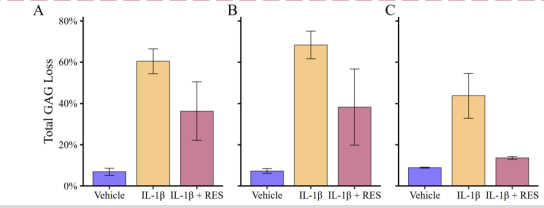

Fig 2 Protective Effects of Positive Controls. Total GAG loss from cartilage treated with vehicle, IL-1β, or IL-1β + resveratrol (RES) across three drug plates: DP3 (A), DP8 (B), and DP11 (C). IL-1β consistently induced greater GAG loss compared with vehicle, while RES reproducibly reduced induced loss by a similar magnitude across all plates. Sample sizes were n ≥ 7 (A), n = 4 (B,), and n ≥ 3 (C) per condition.

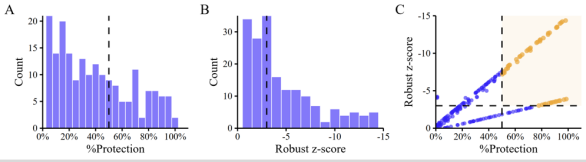

Fig 3 Assay Result Distributions for Hit Selection. (A) Counts of all compounds by %Protection. (B) Counts of compounds by robust z-score. (C) Scatter plot where the shaded region indicates hit-selection cutoff (≥ 50% protection and z < –3). Dashed lines show thresholds. Axes were scaled to show potentially protective drugs, and compounds outside these ranges were not shown. 52 hits were identified.

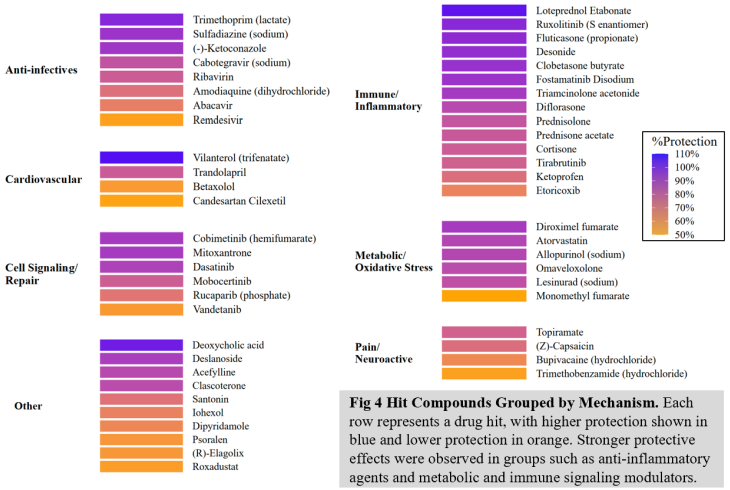

Fig 4 Hit Compounds Grouped by Mechanism. Each row represents a drug hit, with higher protection shown in blue and lower protection in orange. Stronger protective effects were observed in groups such as anti-inflammatory agents and metabolic and immune signaling modulators.