High-throughput screening using a novel joint organoid for discovery of disease modifying drugs for osteoarthritis

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INTRODUCTION: Osteoarthritis (OA) is characterized by chronic inflammation and erosion of cartilage, leading to pain, stiffness, and poor function. OA significantly impacts patient ambulation and limits a person's ability to engage in physical activities, including walking, running, and other sporting pursuits. Given that OA affects approximately 15% of the U.S. population, and increases with advancing age, effective methods for managing OA are critical for the nation's health and welfare, and financial viability. However currently, there are no diseases modifying drugs for OA (DMOADs). Organoids derived from cells obtained from patients with different medical conditions have the potential to revolutionize personalized medicine, drug development, and disease modeling. In this respect, we have extended the use of using human joint organoids to screen drugs for OA using a high-throughput approach. Here, we propose a unique engineered 3D osteochondral organoid that is physiologically analogous to the native joint and capable of disease modeling at an individual patient level. We collect MSCs from OA patient's bone marrow and collect macrophages from blood samples. We hypothesize that the joint organoid will facilitate high-throughput screening and identification of drugs on a personalized level to regulate macrophage function and modulate the inflammatory state, for the treatment of OA.

METHODS: We collected MSCs from the bone marrow of surgical waste in OA patients undergoing joint replacement. Monocytes were isolated from blood using venipuncture. Then, monocytes were differentiated to macrophages by the induction medium (RPMI+10%FBS+macrophage colony-stimulating factor). To establish a personalized platform for precision medicine, we cultured MSCs in a U-bottom 384 well plate. MSCs self-aggregated and formed a 3D structure after 1 day. One bone sphere and one cartilage sphere was self-assembled together to form osteochondral organoids in 2 days by coculture. Macrophages were added in the 3D osteochondral organoids. Cells from 2 OA patients generated the corresponding osteochondral organoids (IRB 51386).

We added 10 ng/mL IL-1β to each well at day 1 to induce OA-like degeneration. An FDA-approved drug library (Cayman Chemical or ApexBio Technology) were applied to the osteochondral organoid. These drugs include anti-inflammation drugs, corticosteroids, and other small molecules. After 5 days of treatment, Gelatinase/collagenase and MMP13 from the culture supernatants were evaluated.

RESULTS: We established patient-derived osteochondral organoids incorporating MSCs, chondrocytes, and macrophages, and exposed them to an FDA-approved drug library to assess modulation of MMP activity, a key marker of OA-related cartilage degradation. Organoids generated from two OA patients were successfully screened, and five candidate drugs were identified. Four drugs—mitoxantrone, verteporfin, vitamin B12, and doxycycline—were consistently effective in both patients, suggesting potential broad therapeutic relevance. Notably, rifapentine was uniquely identified in Patient 1, whereas actinomycin emerged only in Patient 2, reflecting inter-individual variation in drug responses. These results demonstrate the feasibility of using osteochondral organoids for high-throughput screening and highlight their utility in capturing both common and patient-specific drug effects that could guide precision OA therapy.

DISCUSSION: Our osteochondral organoid platform enabled identification of candidate drugs that modulate MMP activity in patient-derived samples. Common hits (mitoxantrone, verteporfin, vitamin B12, doxycycline) suggest shared mechanisms, while patient-specific responses (rifapentine in Patient 1, actinomycin in Patient 2) highlight inter-individual variability. These findings demonstrate the feasibility of personalized drug screening for OA and support further validation of patient-tailored therapeutic strategies.

SIGNIFICANCE/CLINICAL RELEVANCE: OA management plays a critical role in restoring patient quality of life by reducing pain, improving joint function, enhancing physical activity levels, and improving long-term health outcomes. Joint organoids developed directly from cells harvested from individual patients with various medical conditions provide a sustainable platform for comprehensive drug screening without the use of laboratory animals. Processing of additional samples from patients with/without OA using the osteochondral organoid platform will facilitate high-throughput screening of potential DMOADs compounds for personalized treatment of OA.

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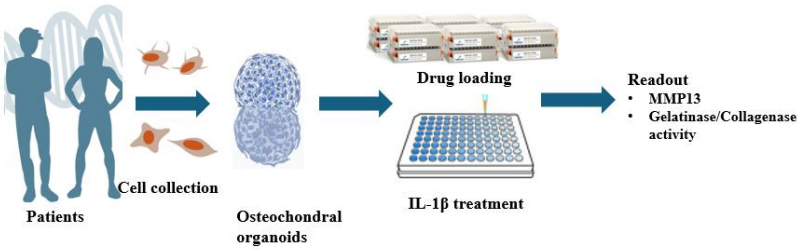

Figure 1. Workflow of a personalized high-throughput drug screening platform. Patient-derived MSCs, chondrocytes, and macrophages are used to generate osteochondral organoids, treated with IL-1β to model OA, and screened with FDA-approved drugs.

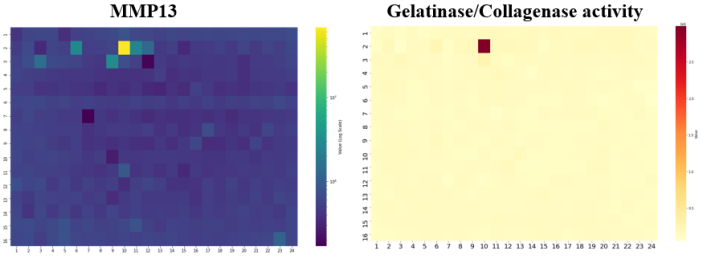

Figure 2. Drug screening heatmaps. (Left) MMP13 inhibition profile across 384 compounds. (Right) Gelatinase/Collagenase inhibition pattern for identical drug library. Each cell represents one compound's effect, with color intensity corresponding to activity.

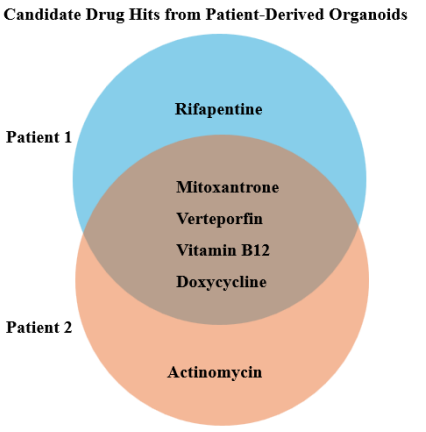

Figure 3. Venn diagram of candidate drug hits from patient-derived organoids. Four drugs were shared between patients, while rifapentine and actinomycin were patient-specific.