Deploying iPSC-derived M2 Macrophages to Mitigate PTOA in 2D, 3D and in vivo Models

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INTRODUCTION: Post-traumatic osteoarthritis (PTOA), a degenerative disease caused by traumatic injury, is a major cause of joint pain and disability. An imbalance between pro-inflammatory M1 macrophages and anti-inflammatory M2 macrophages has been proposed as a central driver of the disease, leading to tissue damage and accelerated disease progression characterized in several local cell types including articular chondrocytes and fibroblast like synoviocytes (FLS). iPSC-derived M2 macrophages (iMac-M2) offer a promising therapeutic approach to slow or even halt the progression of PTOA. To evaluate their treatment efficacy 2D, 3D and in vivo models can be employed and provide complementary insights: 2D models allow dissection of specific cellular mechanisms on individual cell types in the joint, 3D models capture multicellular interactions and better mimic the native joint environment, and in vivo models validate physiological relevance in a more complex system. We hypothesized that iMac-M2 therapy would decrease inflammatory markers and limit degeneration.

METHODS: To evaluate iMac-M2 treatment efficacy, several models were employed: 2D and 3D co-culture models as well as in vivo rat model to assess translational impact. Human iPSCs were differentiated into iMonocytes and subsequently into iMacs over 5 days using a previously established protocol. The iMacs were then further polarized into iMac-M2s over two additional days. Expression of M1 and M2-associated genes were measured under varying inflammatory media compositions and timepoints to evaluate the phenotypic stability of the iMac-M2s. The anti-inflammatory effects of the cells were subsequently tested using an in vitro 2D model: iMac-M2s were co-cultured separately with chondrocytes and FLS that had been inflamed with IL-1β and LPS for 24 hours. Pro-inflammatory markers in chondrocytes and FLS were assessed after 24 hours using gene expression. Gene expression of catabolic markers was also assessed after 14 days in the FLS cells and 28 days in the chondrocyte culture. To validate the translational efficacy of iMac-M2 treatment, an in-vivo rat model was performed: cells were injected into a DMM injured rat knee 1-week post injury. Disease progression and pain was assessed through gait analysis and weight bearing tests. Subchondral bone changes were monitored using µCT. For additional validation of iMac-M2 treatment, an additional 3D model will be assessed: Chondrocytes and FLS will be printed onto a petri dish surface using bioink (keeping cell populations separate), however, sharing media, mimicking a shared joint environment. The iMAC-M2s will then be co-cultured in the plate and gene expression on anabolic and catabolic markers will be performed to assess triple co-culture effects.

RESULTS: The phenotypic stability of the iMac-M2 cells was assessed to determine if they maintain their anti-inflammatory phenotype under stressful conditions. Gene expression of M2-associated genes (Stat3, cMyc, and Stat6) and M1-associated genes (Stat1, HIF1α, and Stat5) showed a dynamic regulation that was dependent on inflammatory media type and duration (**Fig. 1**). In the 2D in vitro model, iMac-M2 treatment in chondrocyte and FLS co-culture (**Fig. 2**) led to a reduction in gene expression levels of pro-inflammatory markers as well as a decrease in catabolic markers. In the in vivo rat model, iMac-M2 treated rats exhibited a more normal gait pattern and rear paw weight distribution, validated by gait analysis and weight bearing testing (**Fig. 3**). µCT analysis showed that iMac-M2 treated rats maintain subchondral bone structure over time when compared with the saline-treated DMM group (**Fig. 4**). For the projected 3D co-culture model with chondrocytes, FLS, and iMac-M2s, we hypothesize that treatment will reduce inflammatory and catabolic markers in both cell populations (**Fig. 5**).

DISCUSSION: Our findings show that iMac-M2s retain plasticity under varying inflammatory conditions and timepoints but preserve an anti-inflammatory phenotype even under some inflammatory stress. In a 2D in vitro culture, iMac-M2 treatment reduced catabolic and pro-inflammatory gene expression in chondrocyte co-culture as well as FLS co-culture, suggesting they have a direct influence on inflammatory signaling and matrix degradation mechanisms. In vivo, treated rats exhibited improved gait and weight distribution, as well as preservation of subchondral bone structure, supporting a disease-modifying trajectory in PTOA. Integration of 2D and in vivo models complement treatment validation efforts, while the projected 3D-co-culture system will offer a more physiologically relevant model to evaluate multi-cellular interactions. Future work in 3D joint modelling may allow for more cell type integration and can more closely mimic the joint structure and environment.

SIGNIFICANCE/CLINICAL RELEVANCE: Developing iMac-M2s as a cell therapy offers a minimally invasive option for both symptom alleviation and disease course alteration for PTOA by targeting inflammatory processes that contribute to the disease. Using a complementary 2D, 3D and in vivo model approach allows for more thorough treatment validation across the translational spectrum.

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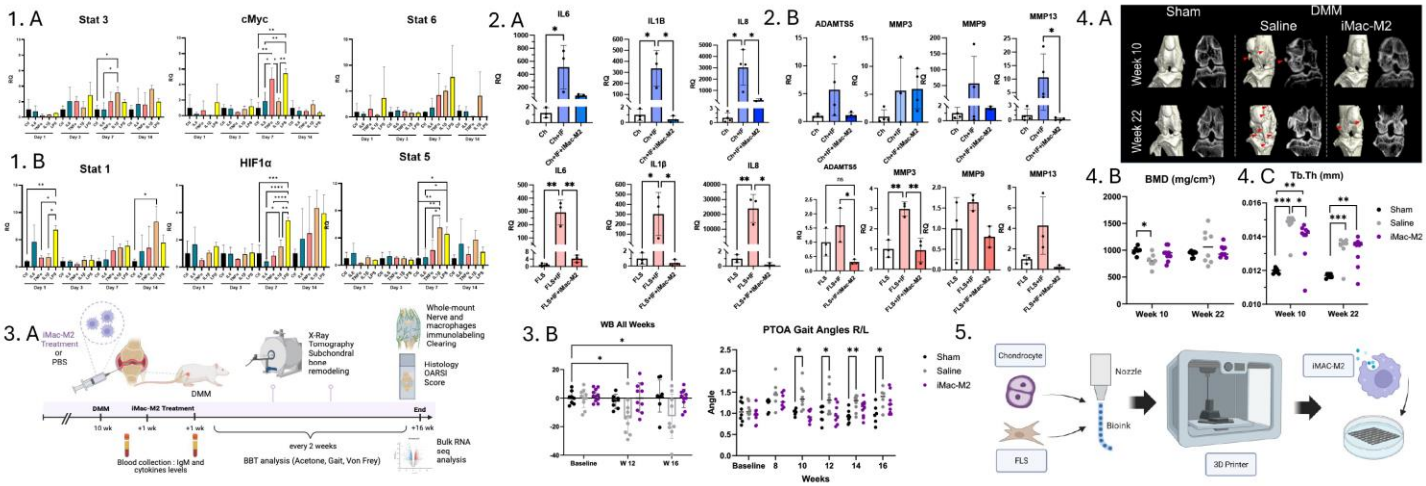

Fig. 1. Phenotypic stability of iMac-M2 macrophages under inflammatory conditions: **(A)** Gene expression of M2-associated genes (Stat3, cMyc, and Stat6) measured by RT-qPCR across the same inflammatory conditions and timepoints. (**B**) Gene expression of M1-associated genes (Stat1, HIF1α, and Stat5) showing a dynamic regulation depending on stimulus and duration. Data are presented as relative quantification (RQ) compared to control (non-inflamed) iMac-M2. **Fig. 2.** *In vitro* anti-inflammatory effects of iMac-M2 on Chondrocytes and FLS from Osteoarthritic Patients. **(A)** Gene expression of pro-inflammatory cytokines 24 hours after inducing inflammation in chondrocytes (blue) and FLS (peach) (**B**) Gene expression of catabolic enzymes 14 days post-inflammation in chondrocytes (blue) and FLS (peach). **Fig. 3. (A)** Schematic representation of the experimental design. **(B)** Weight bearing and Gait analyses indicating improved pain measures in iMAC-M2 treated group. **Fig. 4. (A)** µCT imaging of subchondral bone structure in Sham, DMM-Saline, and DMM-iMac-M2 groups at weeks 10 and 22. 3D reconstructions and coronal cross-sections highlight bone degeneration (red arrows) in the saline-treated DMM group, whereas iMac-M2 treatment maintains bone structure over time. **(B)** Quantification of bone volume fraction (BV/TV) at weeks 10 and 22. **(C)** Quantification of trabecular thickness (Tb.Th) at weeks 10 and 22. **Fig. 5.** Projected schematic for 3D model of chondrocyte, FLS and iMac-M2 co-culture.