Biofabrication of the osteochondral unit through biphasic layering of wild-type and RUNX2 KO primary human MSCs

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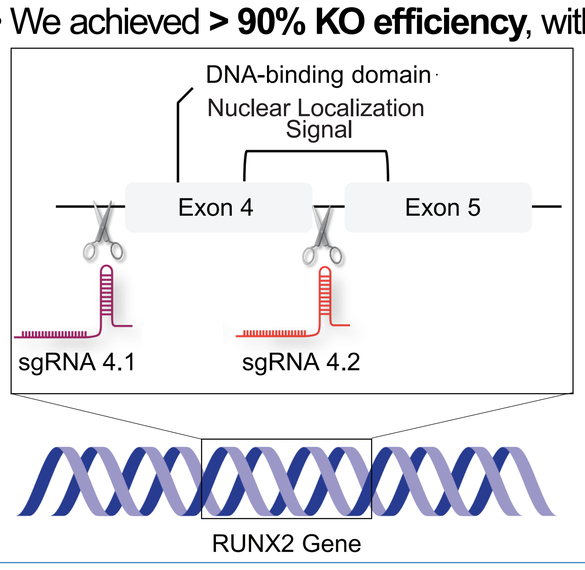
Introduction

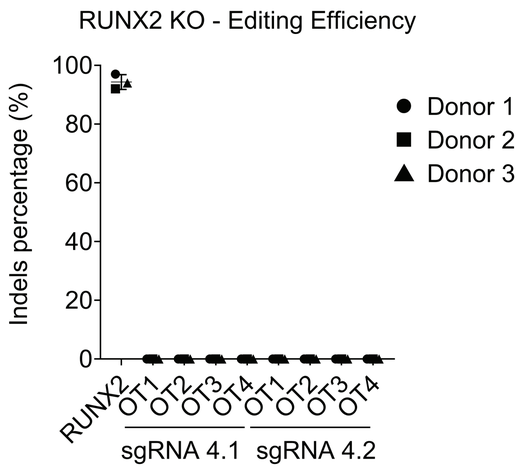
The osteochondral (OC) unit, composed of hyaline, calcified cartilage and subchondral bone, provides structural and load-bearing support[1]. OC defects cause joint instability and lead to osteoarthritic degenerative changes, yet current surgical treatments cannot fully restore the native tissue[2]. Tissue engineering of the OC unit using mesenchymal stem cells (MSCs) represents a promising tool for regenerative approaches, however, MSCs committed to the cartilaginous phenotype undergo instead endochondral ossification, hindering the formation of distinct cartilage and bone layers[3]. Furthermore, the *in vitro* maturation of tissue engineered OC constructs is achieved using transwell systems and multiple culturing media, separate maturation processes for the different layers, or complex multi-material systems[4]. Here we propose a novel strategy for the engineering the OC unit by using wild-type (WT) and runt-related transcription factor 2 (RUNX2) knockout (KO) primary human MSCs to create multi-layered, zonally organized constructs that can be fully cultured under the same conditions and ultimately lead to functional OC tissue maturation.

Methods and Results

• RUNX2 KO in primary human MSCs

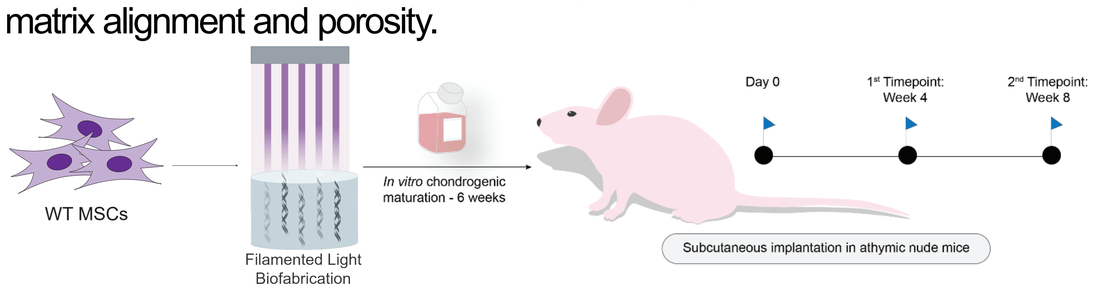
- We used a **nonviral CRISPR-Cas9** ribonucleoprotein complex (RNP) to KO RUNX2 in three MSCs donors and characterized on-target and off-target (OT) editing events with Sanger Sequencing.
- We achieved **> 90% KO efficiency**, with no off-target effects.



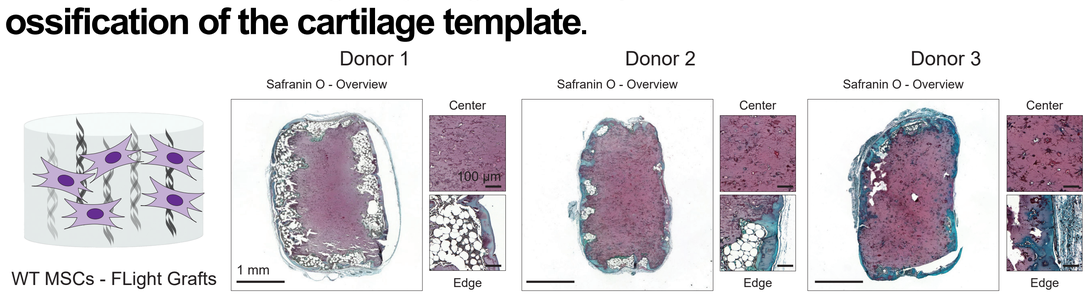


• FLight Biofabrication supports WT MSCs endochondral ossification and KO MSCs hyaline cartilage maturation

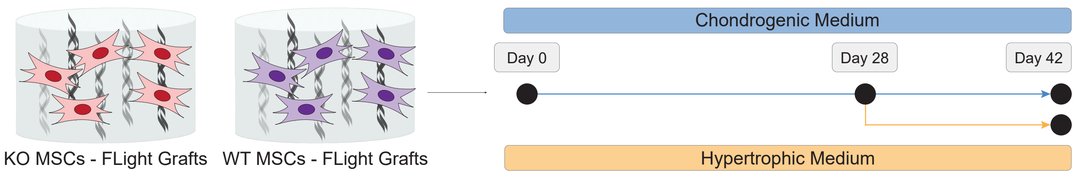
- WT MSCs were encapsulated in bovine cartilage decellularized extracellular matrix (**dECM**) hydrogels, matured *in vitro* for 6 weeks and implanted subcutaneously *in vivo* to trigger **endochondral ossification**.
- Grafts were printed using Filamented Light (**FLight**) biofabrication, to induce matrix alignment and porosity.

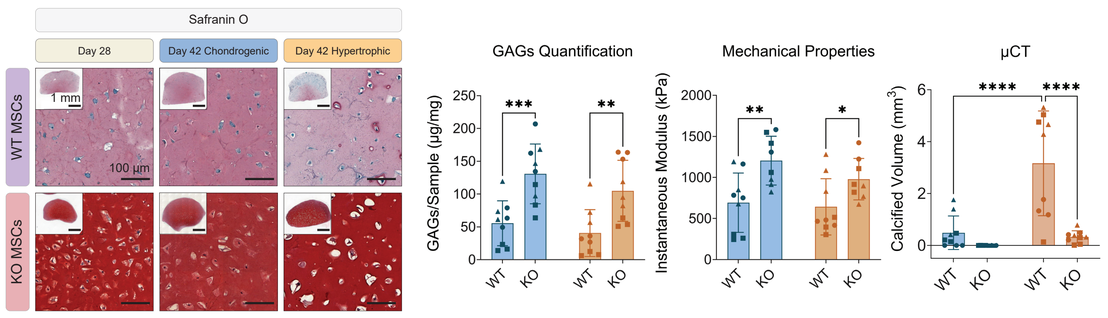


- *In vivo*, **WT MSCs FLight** hydrogels led to pronounced **cell infiltration** and **ossification of the cartilage template**.



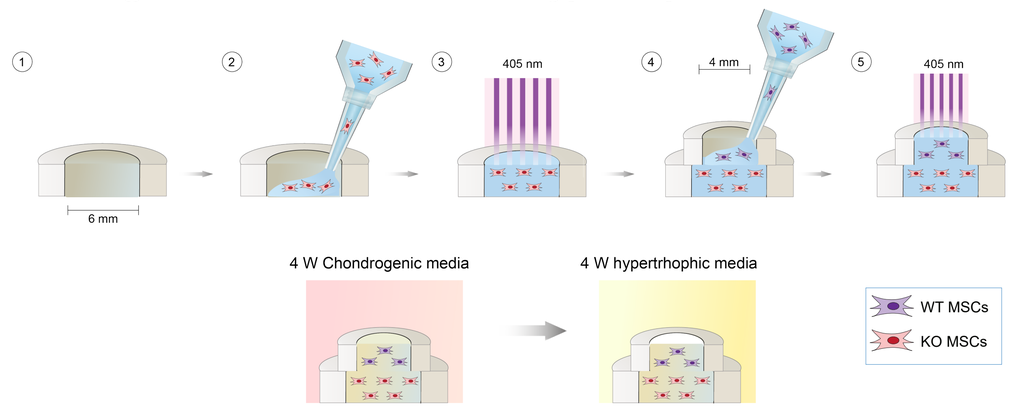
- *In vitro*, **KO MSCs FLight** grafts displayed **enhanced cartilage formation** in chondrogenic and hypertrophic media compared to WT.



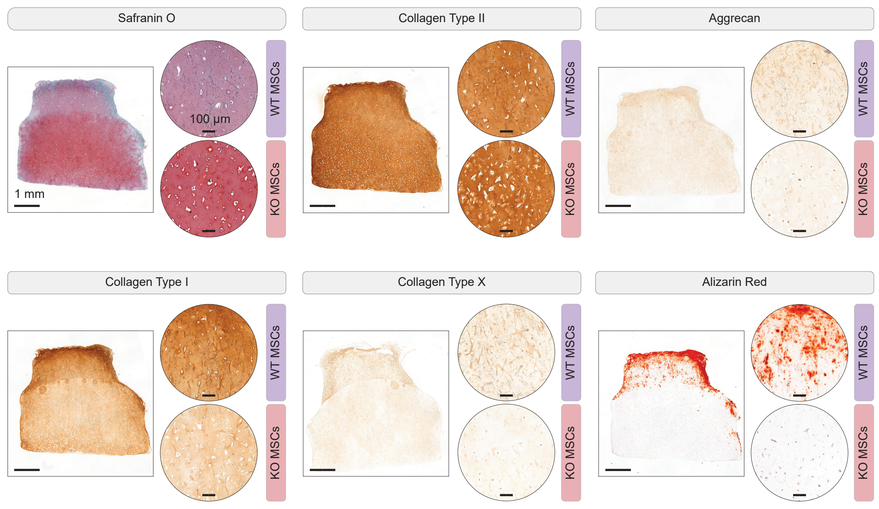


• OC tissue-engineered bilayer constructs retain specific zonal organization *in vitro*

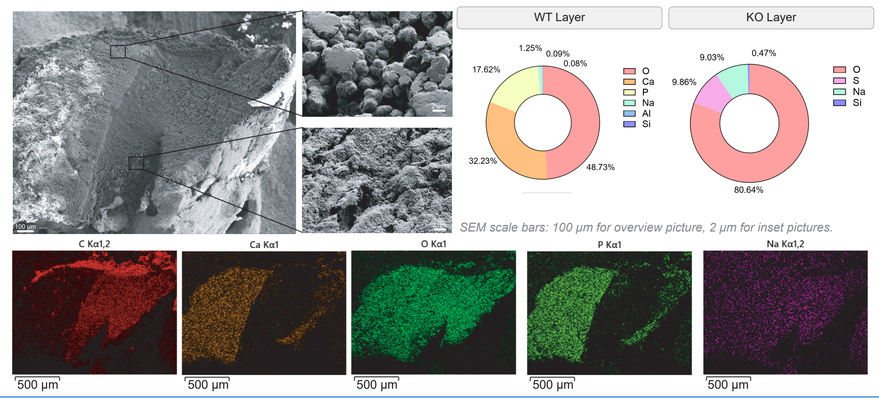
- WT and KO MSCs in dECM hydrogels were sequentially casted, crosslinked with FLight Biofabrication, and cultured for **4 weeks in chondrogenic media + 4 weeks in hypertrophic media**.



- Histologically, **KO** MSCs led to **stable cartilage** maturation, with optimal GAGs retention; the **WT** layer displayed a **hypertrophic** phenotype, with GAGs loss, matrix calcification and collagen type I deposition.



- Scanning electron microscopy (SEM) confirmed a bilayered organization, with **calcium** and **phosphate** localizing to the **WT** layer.



Conclusions

In this study, we use a single biomaterial platform and uniform culturing condition in combination with WT and RUNX2-KO MSCs to create a tissue-engineered OC unit with zonal, native tissue organization. We envision our constructs to be suitable for future clinical treatment of OC defects and for the creation of a novel *in vitro* model of the OC unit.

References

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