Mechanobiology-informed Tissue Engineering: Developing scaffold-based therapies for adult bone repair by harnessing age-altered JNK3 activation in stem cells

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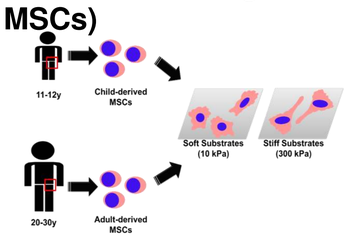
INTRODUCTION

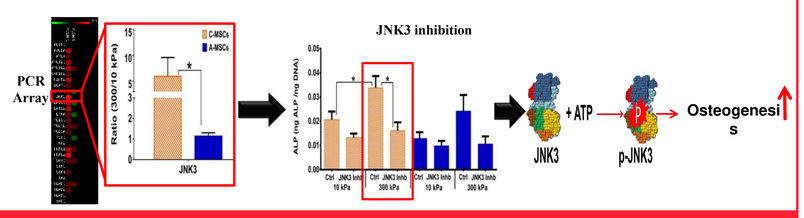
Regenerative capacity of bone decreases with age

* Children have a renowned capacity to repair fractured bones quickly.

Children-MSCs (C-MSCs) have greater mechanically-activated osteogenic potential than adult-MSCs (A-MSCs)

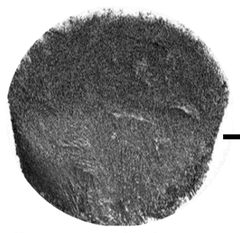


Child-MSCs have greater stiffness-activated osteogenic and angiogenic response than adult-MSCs



JNK3 identified as potential therapeutic target for recapitulating children's bone repair in adults (González-Vázquez, *et al.* Acta Biomaterialia, 2017; González-Vázquez, *et al.* ORS 2018).

3D collagen-based scaffold as therapeutic delivery platform for bone healing



Collagen-nanohydroxyapatite (Coll-nHA = JNK3*Free)

Cunniffe GM *et al.*, J. Mater. Sci.: Mater. Med. 2010;
Cunniffe GM *et al.*, ACS Appl. Mater. Interfaces, 2016.

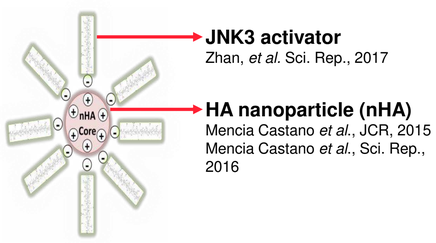
Proven to successfully promote bone healing *in vivo* by delivering plasmid DNA into rat calvarial critical size defects (Curtin et al., Adv. Healthcare Mater., 2016).

Proven to successfully promote osteogenesis by delivering miRNAs into MSCs (Mencia Castano *et. al.*, Scientific Reports, 2016).

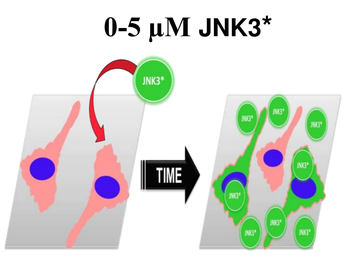
AIMS

To design a mechanobiology-enhanced therapeutic delivery platform that recapitulates children's bone healing capacity in adults.

Aim 1: To formulate and validate a therapeutic molecule able to facilitate JNK3 activation intracellularly.

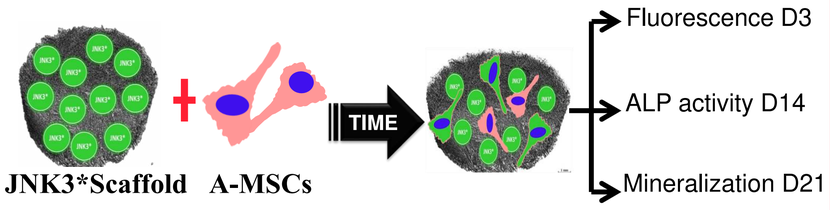


JNK3 activator-FITC-nHA (JNK3*)

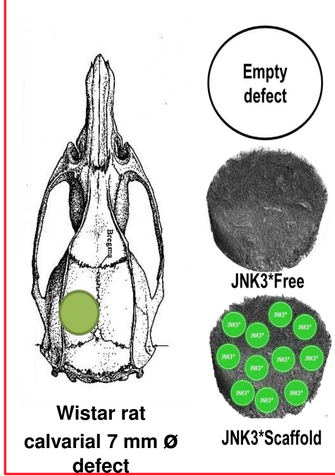


- Zetasizer Nano Z: particles size and zeta potential.
- Flow cytometry and fluorescent microscopy: intracellular uptake.
- Alamar Blue: cell viability.
- Alkaline phosphatase (ALP) activity: JNK3* effects on MSC osteogenesis.

Aim 2: To fabricate an off-the-shelf JNK3 activated collagen nanohydroxyapatite 3D scaffold (**JNK3*scaffold**) and evaluate its capacity to enhance the osteogenic capacity of adult-MSCs.

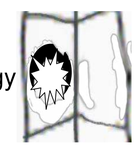


Aim 3: To assess the JNK3*scaffold capacity to accelerate bone healing in a rat critical size calvarial bone defect model.



A: After **14 days** assess
1. New bone formation: µCT
2. Blood vessel infiltration: histology
3. PCR array: customised 96 genes array containing specific cell markers, osteogenic and angiogenic pathways.

B: After **28 days** assess
1. New bone formation: µCT
2. Neovascularisation: histology



RESULTS

JNK3 activator (JNK3*) nanoparticle was successfully taken up by child- and adult-MSCs

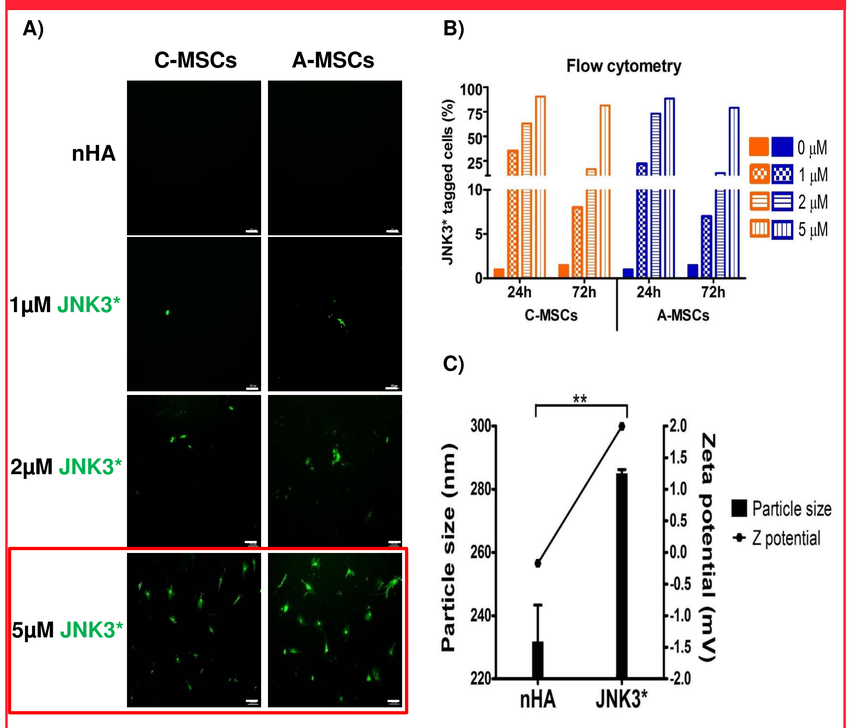


Figure 1. JNK3 activator uptake was only positive when complexed with nHA (JNK3*). Adults- and children-MSC JNK3* uptake is concentration dependent (A), with 5 µM JNK3* showing up to 90.3% C-MSCs and 88.4% A-MSCs uptake at 24h with no significant change after 72h (B). Z-potential confirmed that JNK3* presented optimal features for cellular uptake (285 nm and positively charged) (C). ** = p < 0.01.

JNK3 activation in adult-MSCs enhances their osteogenic potential

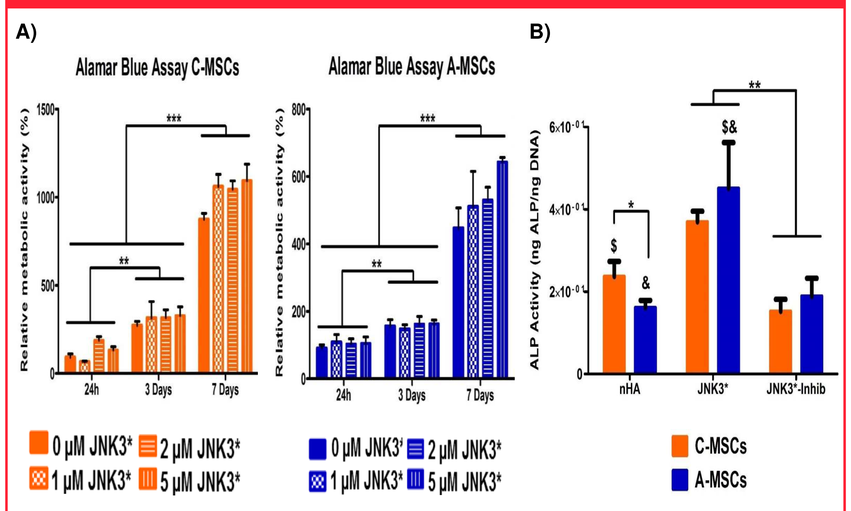


Figure 2. None of the treatments caused negative effects in cell viability after 7 days in culture (A). The ALP activity of C-MSCs was higher than A-MSCs when treated with the carrier alone (nHA). Moreover, the A-MSC ALP activity increased twice after the treatment with 5 µM JNK3*, reaching similar levels than C-MSCs. Interestingly, when JNK3* was utilized in combination with the JNK3 specific inhibitor the ALP activity increase was abolished, demonstrating that JNK3 activation is a key modulator for enhancing osteogenesis (B). * = p<0.05; ** = p < 0.01; *** = p<0.001; $,& = p<0.05.

3D JNK3* scaffold enhances MSC mediated osteogenesis

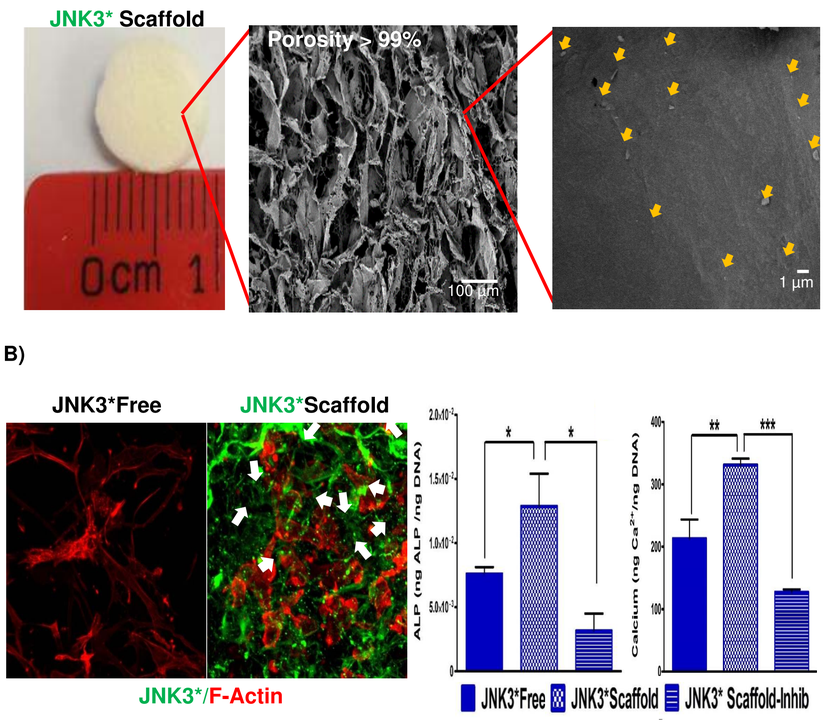


Figure 3. Incorporation of JNK3* particles into the type I collagen scaffolds did not affect the architecture of the scaffold. JNK3*scaffolds are highly porous (99.39 ± 0.35%). Yellow arrows point to nanoparticles of JNK3* homogeneously distributed on the collagen (A). White arrows point to A-MSCs (dyed in red) seeded on JNK3* scaffolds (JNK3* dyed in green) with positive JNK3* uptake after three days in culture (B). More importantly, ALP activity and mineralization assays demonstrated that JNK3* scaffold further enhanced the osteogenic potential of A-MSCs by facilitating JNK3 activation (C). *p < 0.05, **p < 0.01.

RESULTS

JNK3*scaffold promotes vessel infiltration and upregulates osteogenic and angiogenic pathways in mesenchymal cells

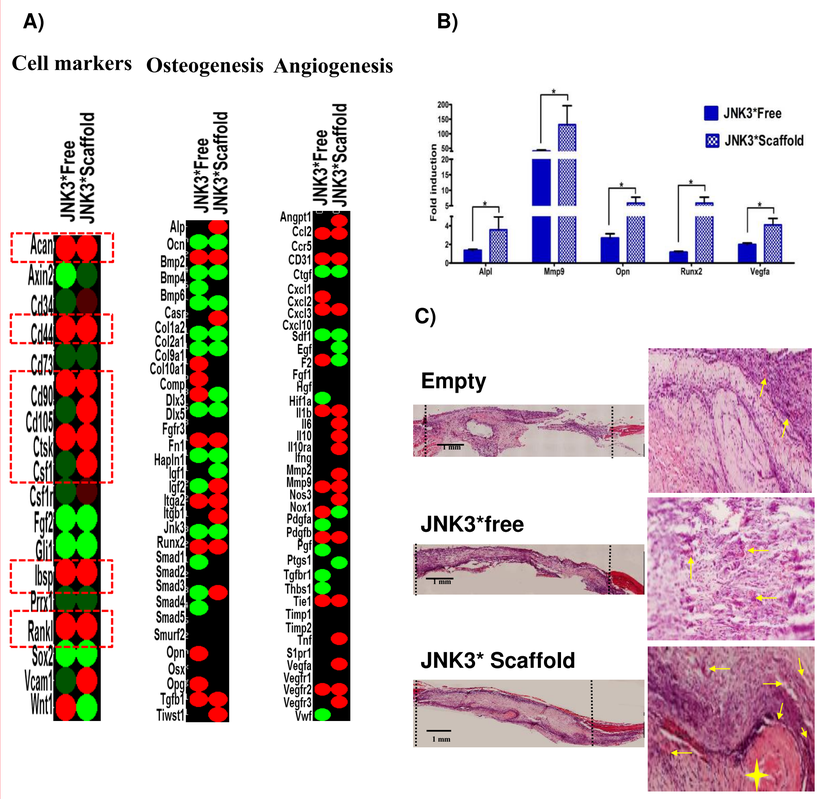


Figure 4. host cells that infiltrated JNK3*free and JNK3*scaffolds –after only two weeks of implantation- were positive for mesenchymal (CD90, CD105, CD44, Csf), osteoblasts (Ibps) and osteoclast (Rankl, Ctsk) markers, no significant differences were observed between the two scaffolds. Interestingly, only JNK3*scaffolds induced upregulation of genes associated to the osteogenic and angiogenic pathways (A). It is to highlight the statistically significant upregulation of Alp, Runx2, Opn, Vegf and Mmp9 only observed in JNK3*scaffold (B). Finally, JNK3*scaffolds exhibited the highest blood vessel infiltration (yellow arrows) and new bone formation when compared with JNK3*free and empty (C). H/E staining. * = p<0.05, n=8.

JNK3* scaffold accelerates bone healing *in vivo*

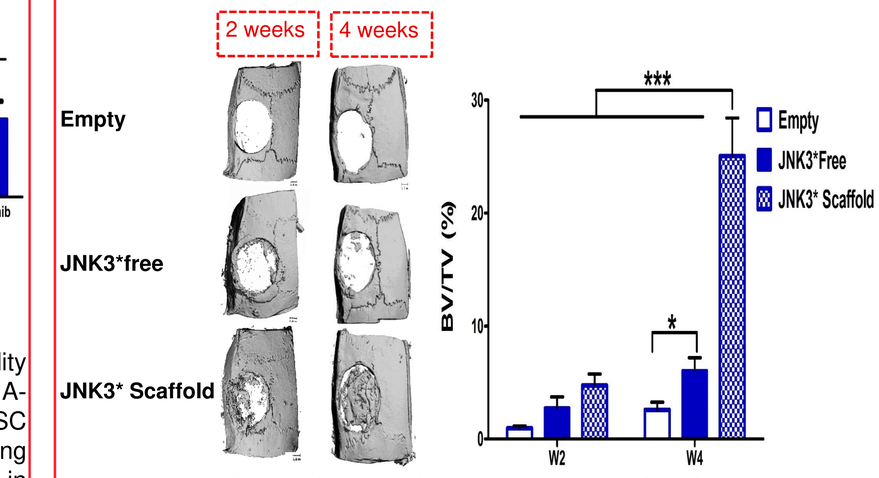


Figure 4. JNK3*scaffold promoted faster bone healing than JNK3*free scaffolds or defects left empty, reaching almost full defect closure as early as 4 weeks. µCT analysis showed that JNK3*scaffold treated defects have the highest bone volume fraction of all. Mean ± SEM (n=8). *** = p<0.001.

DISCUSSION

- This study describes the development of a JNK3-activated scaffold which accelerate bone healing and facilitates blood vessel infiltration by upregulating osteogenic and angiogenic pathways in mesenchymal cells.
- Calcium production and ALP activity by MSCs seeded on the JNK3*scaffolds *in vitro* is significantly higher than JNK3*free scaffolds and was blocked when JNK3 activation was inhibited.
- JNK3* nanoparticles recapitulated child-MSCs osteogenic capacity in adult-MSCs.

SIGNIFICANCE

This cell-free, off-the-shelf, mechanobiology-informed therapeutic delivery platform recapitulated children's bone healing capacity in adults by facilitating JNK3 activation.

ACKNOWLEDGEMENTS

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