HiT-MACE: A High-Throughput Mechanical Activator for Cartilage Engineering to Study the Early Cartilage Response to Physiological and Supra-physiological Loads

Riccardo Gottardi[2,4], Elisa Capuana[1], Davide Marino[1], Vincenzo La Carrubba[1], Valerio Brucato[1], Rocky S. Tuan[2,3]

[1]University of Palermo, Palermo, Italy, [2]Center for Cellular and Molecular Engineering, University of Pittsburgh, PA, [3]The Chinese University of Hong Kong, Hong Kong, China, [4]Fondazione Ri.MED, Palermo, Italy
rig10@pitt.edu

Introduction

Articular cartilage is crucially influenced by mechanical loading in development, health, and disease. Regenerative medicine approaches need to incorporate mechanical loading as a key component to drive tissue repair as well as to generate engineered constructs that can substitute the damaged cartilage [1].
In this framework, we have developed a novel device for the high throughput compressive loading of native and engineered cartilage called **HiT-MACE** *(High Throughput Mechanical Activator for Cartilage Engineering)* to allow for the screening of up to 24 samples in one single run under different conditions (Fig.1).

The purpose of this work is the validation of this system by quantifying the device's accuracy to deliver mechanical stimulations, and the characterization of the mechanical and transcriptional response of chondrocytes within intact porcine articular cartilage under physiological and supra-physiological loading conditions.

Materials and methods

Device:

The HiT-MACE device is composed of two motors above a basis and of pistons attached to six compact actuators equipped with force sensors to provide force feedback during the loading of six samples at a time (Fig.1).

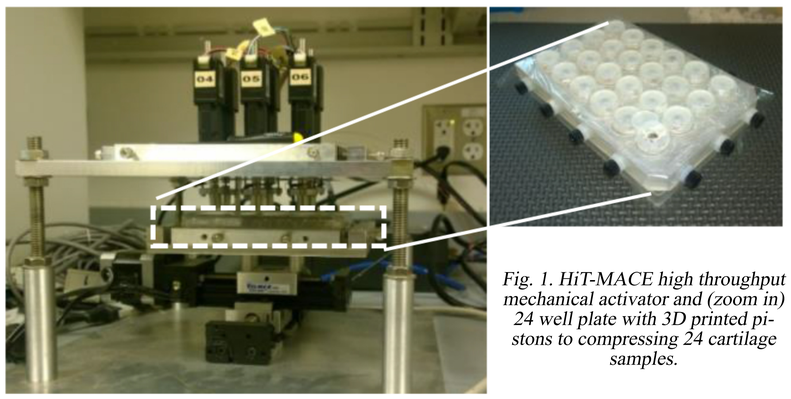

Fig. 1. HiT-MACE high throughput mechanical activator and (zoom in) 24 well plate with 3D printed pistons to compressing 24 cartilage samples.

A custom lid equipped with a flexible membrane as well as pistons and piston guides were manufactured by 3D printing in order to maintain sterile conditions during the compression.
A National Instrument DAQ board connects the system to a computer and a Labview user interface (Fig. 2).

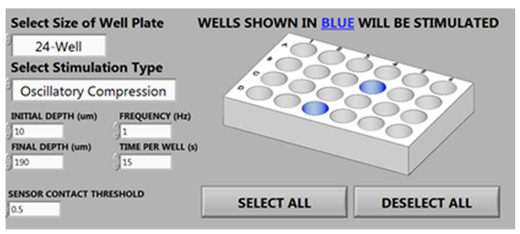

Fig. 2. Graphical user interface that displays in the screen of the computer in order to set the variables of the stimulation.

Device Validation:

We compared the Young's modulus of different compositions of PDMS and agarose cylinders (n=24), synthetic materials with mechanical properties in the range of cartilage, measured with HiT-MACE and with the Bose (now TAinstrument) ElectroForce 3230 mechanical tester.

Materials and methods

Cartilage early response to loads:

Articular cartilage cylinders of 4mm in diameter were harvested from freshly euthanized pigs from a local abattoir. After 48h equilibration in serum-free, TGF-β-free medium (DMEM, 2% penicillin/streptomycin, ITS, and ascorbate), samples were subject to physiologic cyclic loading (12% strain at 1Hz) for 30 minutes. A second set of samples was subject to supra-physiological loading (45% cyclic strain) for 30 minutes, and compared to the response to IL-1β exposure as an inflammation-based model of tissue damage.
All samples were then analyzed for gene expression by qRT-PCR of the extracted RNA (porcine genes: ACAN, BMP2, COL2, ALK5, PAI1, SMAD3, TGF-β1, COLX, ID1, MMP13) and by immunohistochemistry and histology.

Statistical analysis:

Results were compared by either t-Test or ANOVA following by a post hoc Tukey test with significance set at p=0.05.

Results

The developed stimulator is capable of accurate mechanical measurement, under compressive loading:

No significant difference was found between Young's moduli measured by the HiT-MACE and the ElectroForce mechanical tester, confirming the effectiveness of HiT-MACE.

Material	Measured Modulus_HiT-MACE (MPa)	Measured Modulus_Bose (MPa)
PDMS 5:1	1,45±0,13	1,46±0,07
PDMS 10:1	2,45±0,37	2,54±0,24
PDMS 20:1	0,90±0,17	0,89±0,13
2% Agarose	0,57±0,18	0,58±0,04
4% Agarose	1,60±0,38	1,77±0,08
6% Agarose	2,43±0,43	2,89±0,12

A physiological dynamic compression blocks hypertrophic differentiation of chondrocytes, restoring Tgf-β signaling:

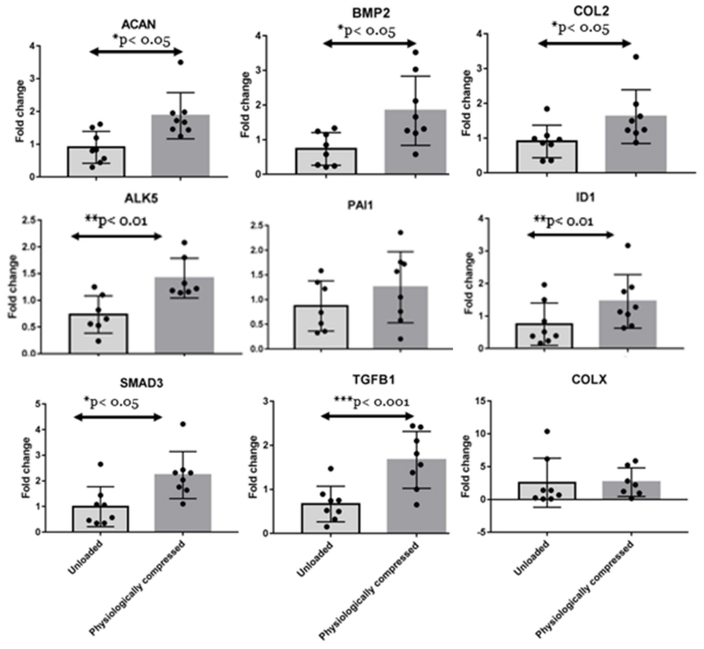

Fig. 3. Cartilage anabolic genes after physiological loading compared to the unloaded condition (n=8).

Results

Immunohistochemistry confirmed these gene expression in the cells within cartilage sections (Fig. 4).

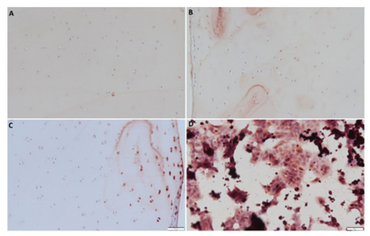

Fig. 4. Immunohistochemical staining for SMAD-3 of D0 (a), Unloaded (b), Loaded (c) and MRF-7 cells employed as positive control (d). Scale bar = 50 μm.

The upregulation of TGF-β1 as a compensatory response to promote tissue repair in supra-physiological/cytokines-treated conditions:

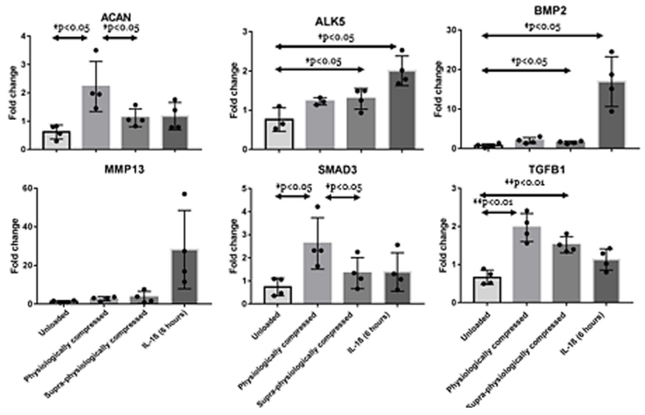

Fig. 5.Expression levels of mRNA in unloaded, physiologically loaded, supra-physiologically loaded and IL-1 treated groups (n=4).

Discussion

The use of a high throughput system such as our HiT-MACE allowed to rapidly generate a sizeable amount of data from multiple donors and to reach significance in a very short time. Cartilage subject to physiological loading rapidly responds with increased gene expression of anabolic markers along the TGF-β1 pathway (e.g., COL2, ACAN, SMAD3, etc.) (Fig. 3), confirming what previously reported by Madej et al. [2]
In supraphysiological loading, an Alk5, Bmp2 and Tgfb1 upregulation (Fig.5) could appear as a self-protective response in repairing of the tissue when it was subjected to an excessive load, whereas IL-1β clearly initiates a catabolic response as expected.

Significance / Clinical relevance

High throughput mechanical loading of cartilage and engineered constructs allows to generate large volumes of data and samples to effectively explore cartilage response to physiological and supraphysiological loading and to engineer more effective repair and rehabilitation strategies.

References

[1] Gottardi R. and Stoddart MJ, J Am Acad Orthop Surg (2018) 26(15):e321-e323.
[2] Madej W, et al. Osteoarthr. Cartil. (2016) 24(10):1807-1815.

Acknowledgements

Ri.MED Foundation, AR3T through NIH grant no. P2CHD086843, NIH (UG3TR002136)