

Chiara Rinoldi, Afsoon Fallahi, Iman K. Yazdi, Jessica Campos Paras, Ewa Kijeńska-Gawrońska, Grissel Trujillo de Santiago, Abuduwaili Tuoheti, Danilo Demarchi, Nasim Annabi, Ali Khademhosseini, Wojciech Swieszkowski*, Ali Tamayol**

Mechanical and Biochemical Stimulation of 3D Multi-layered Scaffolds for Tendon Tissue Engineering

Number of pages: 5

Number of figures: 5

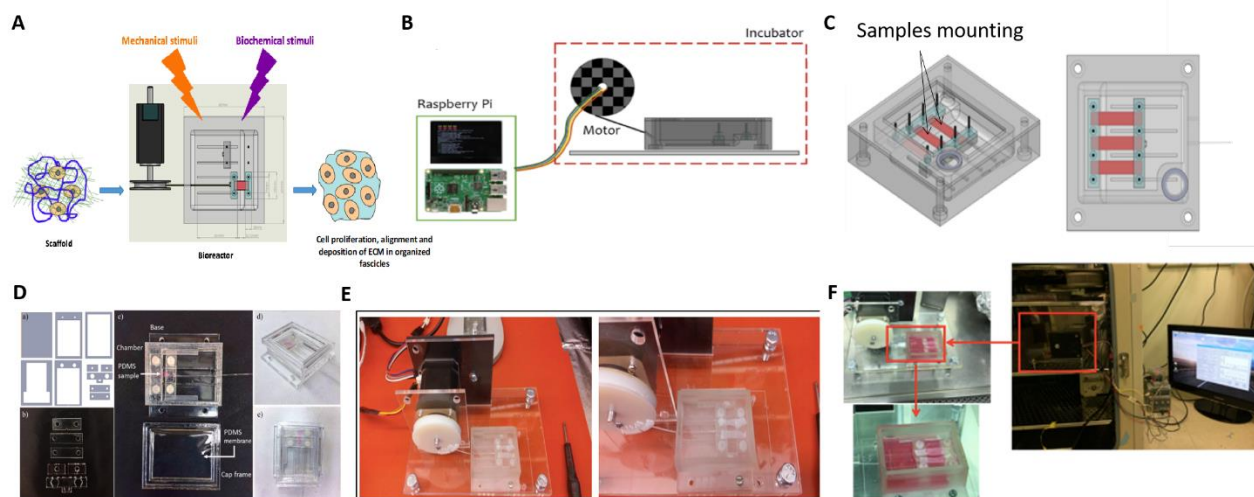


Figure S1. Bioreactor model. A) Schematic demonstration of bioreactor function and purpose. The samples are mounted into the bioreactor system and stimulated under tensile cycle load to perform the dynamic culture of the cell and improve cell proliferation alignment and ECM deposition. B) The bioreactor model provides a raspberry electronic circuit which is connected to a motor that is linked to the bioreactor chamber through a wire to stimulate the scaffolds. C) The chamber design in SolidWorks. D) A PMMA model was designed, built and assembled in order to minimize the working volumes and maintain the sterility. E) Samples were loaded in the chamber and mechanically stimulated 4 hours/day for 7 days. F) The ML scaffolds were mounted into the bioreactor system, incubated and cultured under dynamic condition.

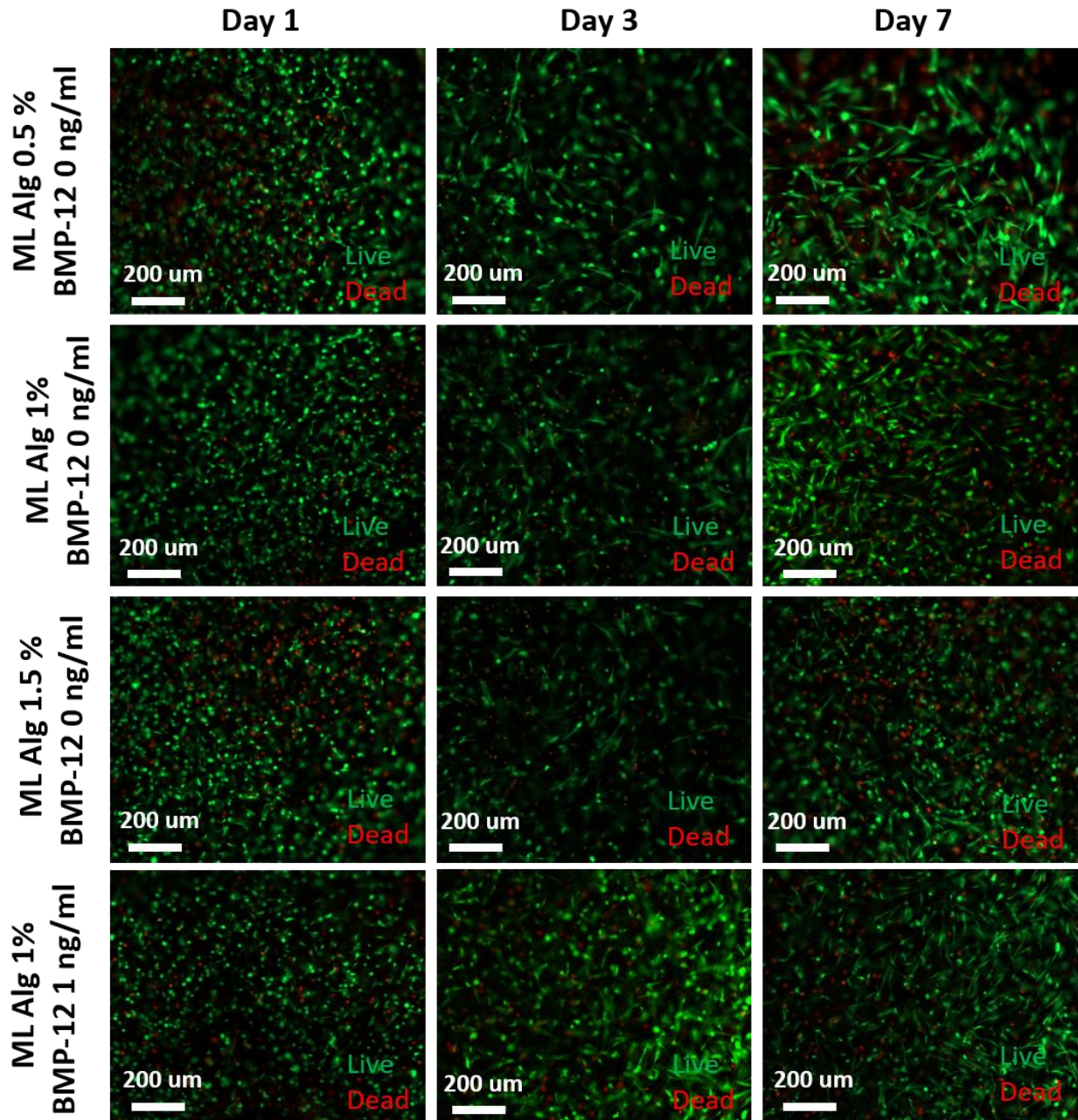


Figure S2. Fluorescence images of alive (green) and dead (red) cells cultured for 7 days into multi-layered scaffolds: MSCs encapsulated into 10%-0.5%, 10%-1%, 10%-1.5% (w/v) GelMA-Alg hydrogel layers in absence of BMP-12; MSCs encapsulated into 10%-1% (w/v) GelMA-Alg hydrogel and treated with 1 ng/mL BMP-12. MSCs cultured into the multi-layered scaffolds show high viability (> 94%), spreading and elongation in every condition tested ($n=3$).

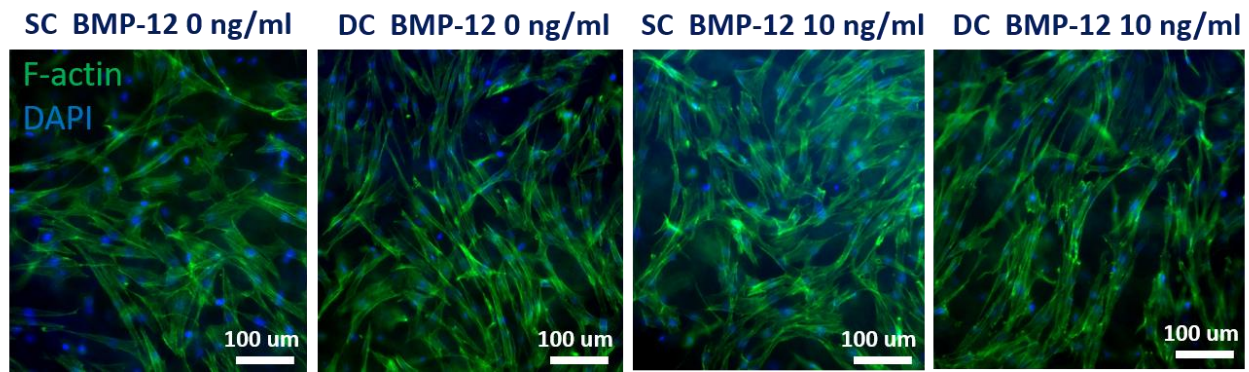


Figure S3. Mechanical and biochemical stimulation on cell cytoskeleton of MSCs encapsulated into multilayered scaffolds. Fluorescence images of the cell actin (green) and DAPI (blue) after 7 days of culture ($n=3$).

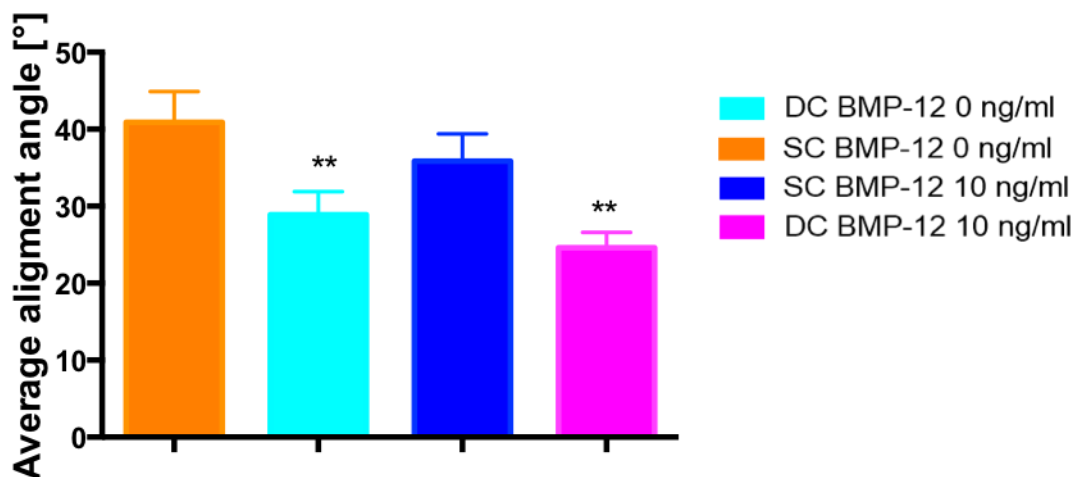


Figure S4. Quantification of the average alignment angle of MSCs within the multi-layered scaffolds, reporting that the mechanical stretching significantly improves the average cell alignment compared to static condition. Direction of the stretching is considered as 0°. ($n=3$, significant differences are determined compared to SC BMP 0 ng/ml condition; $**p < 0.01$).

