3D PHOTOPOLYMERIZED SCAFFOLD PORE SIZE REGULATES MESENCHYMAL STEM CELL PHENOTYPE

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Introduction

Mechanical stimuli from the environment affect mesenchymal stem cells (MSCs) morphology and functionality [1]. We observed an increased stemness maintenance for MSCs grown into a 3D custom-made scaffold [2]. We are now investigating the role of scaffold architecture in maximizing the cell stemness maintenance over the time, and the identification of molecular pathways most significantly involved in this process.

Methods

Custom made organic-inorganic polymeric scaffolds were produced by the two-photon polymerization technique with different dimension of cubic pores (15x15x15μm^3, 20x20x20 μm^3) or graded pore (Named Nichoid), with a range between 10 and 30 μm transversely and 15 μm in the vertical direction.

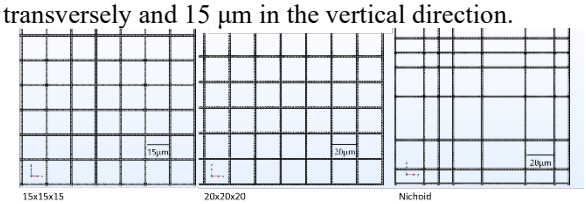


Fig.1: Representative portion of the 3D investigated scaffolds (top view).

Finite element analyses (COMSOL Multiphysics®) were performed to evaluate the displacement (d_i) and bending and longitudinal stiffnesses ($K_i=F_i/d_i$) of each pores type. We built the model in stationary condition, and linear elastic and isotropic material properties (E=3.03GPa, υ=0.49, ρ=1200kg/m^3) were imposed. We tested three load cases (|F|=70nN), one for each direction, placed at half-length of bars for representative pores (Fig.1).

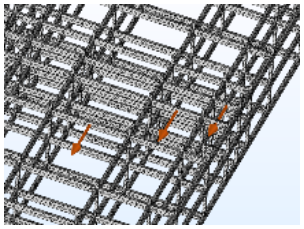


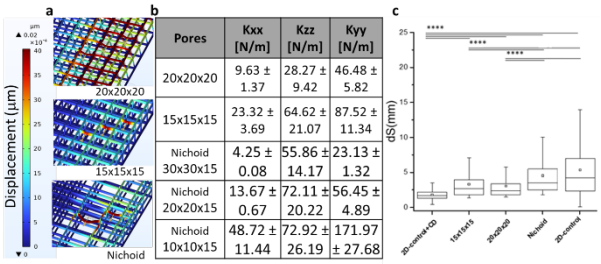
Fig.2: Example of a meshed scaffold composed by graded pores, meshed by COMSOL Multiphysics®.

10^{4}MSCs were cultured for 24h and 7days, in 3D and 2D samples, using standard culture conditions. As pharmacological control, 1μm Cytochalasin-D for 1h was used to reduce internal cellular tension.

Fluorescence imaging was performed to investigate cell migration capability in situ and ex situ, nuclear morphology and nucleoskeletal organization. RNA-Seq and Bioinformatic Data Analysis were used to investigate the gene expression deregulation induced by the different scaffolds.

Results

RNA-Seq analyses indicate that culture conditions significantly affect gene expression. Among all the 3D conditions tested, cell stemness significantly increased only in Nichoid scaffolds. Computational analysis demonstrates that this condition induces increased gradients in the displacement and stiffness of the microscopic trusses that form the pore microgrid (Fig.2a,b). Fluorescence imaging shows that the 3D scaffold geometry does not affect the organization of several proteins primarily involved in the mechanotransduction pathway; on the contrary, it influences significantly the cell capability to migrate (Fig.2c).



Pores	Kxx [N/m]	Kzz [N/m]	Kyy [N/m]
20x20x20	9.63 ± 1.37	28.27 ± 9.42	46.48 ± 5.82
15x15x15	23.32 ± 3.69	64.62 ± 21.07	87.52 ± 11.34
Nichoid 30x30x15	4.25 ± 0.08	55.86 ± 14.17	23.13 ± 1.32
Nichoid 20x20x15	13.67 ± 0.67	72.11 ± 20.22	56.45 ± 4.89
Nichoid 10x10x15	48.72 ± 11.44	72.92 ± 26.19	171.97 ± 27.68

Fig.2: a) Representation of truss displacement under the point load F_x=70nN. b) Pore stiffness along the three investigated directions; c) Analysis of MSCs capability to migrate.

Discussion

MSCs cultured in our 3D scaffolds do not show a significant cell reshaping and remodelling of the main structural proteins; however, it is appreciable a significative gene deregulation. Our hypothesis is that this phenomenon is guided by a modulation of the cell confinement and migration properties.

Our findings reveal novel aspects of the MSCs culture in 3D, representing a step forward in the control of stem cells *via* purely mechanical conditioning, thus paving the way to new strategies for MSCs translation to clinical applications.

References

1.Steward AJ et al. J Anat. 2015;227(6):717-31.
2.Remuzzi A. et al. Cells 2020, 9, 1873

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