INTERNATIONAL JOURNAL OF HEALTH & ANIMAL SCIENCE FOOD SAFETY

Keywords

Epigenetic, 5-aza-CR, 3D culture, Micro-bioreactor, Plasticity

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UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE VETERINARIE PER LA SALUTE, LA PRODUZIONE ANIMALE E LA SICUREZZA ALIMENTARE

Liquid Marble micro-bioreactor promotes 3D cell rearrangement and induces, maintains and stabilizes high plasticity in epigenetically erased fibroblasts

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Abstract

In the last years, many works demonstrated the possibility to directly interact with the epigenetic signature of an adult mature cell, through the use of epigenetic modifiers, (Pennarossa *et al.*, 2013; Brevini *et al.*, 2014, Chandrakantan *et al.*, 2016) and new mechanisms underlying this process have been recently described (Manzoni *et al.*, 2016). In particular, the small molecule 5-azacytidine (5-aza-CR) has been shown to induce a transient higher plasticity state in adult somatic cells, grown in standard 2D conditions. Recent evidence have also shown the possibility to regulate and maintain cell pluripotency through the use of 3D culture systems. In the experiments here presented, we combine the two approaches and investigate whether the simultaneous use of a 3D micro-bioreactor and 5-aza-CR is able to promote cell rearrangement, boost the induction of high plasticity and stably maintain it.

To this purpose, fibroblasts were either plated on plastic dishes (2D) or encapsulated in a Liquid Marble (LM) micro-bioreactor (polytetrafluoroethylene (PTFE)), which has been previously shown to support the growth of living microorganisms, tumor spheroids, fibroblasts, red blood cells, and embryonic stem cells (Ledda *et al.*, 2016). Cells were then erased with 5-aza-CR, for 18 hours and cultured in Embryonic Stem Cell (ESC) medium for up to 28 days. Morphological analysis and pluripotency related gene expression levels were monitored for the entire length of the experiments. 2D cells, kept a monolayer pattern and acquired a pluripotent state that was, however, transient and lost by day 6. In contrast the use of a 3D system maintained and stabilized the high plasticity state in LM cells until the end of the experiments (Fig. 1).

The data obtained demonstrate that cell rearrangement and interactions may modulate 5-aza-CR induced plasticity and suggest a correlation between 3D mechano-transduction-related pathways and epigenetic regulation of cell phenotype.

Acknowledgments:
Supported by Carraresi Foundation.
Authors are members of the COST Actions CA16119, BM1308 and CM1406.

ISSN: 2283-3927

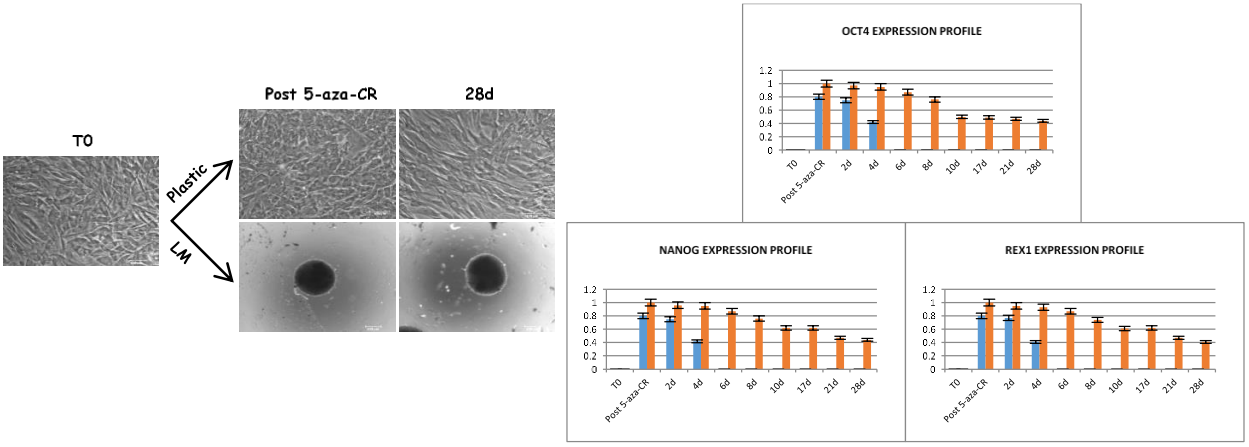

Fig.1: **A)** Morphological changes in human erased skin fibroblasts plated on standard plastic dishes (plastic) or encapsulated in a LM micro-bioreactor (LM) at different time points. **B)** OCT4, NANOG and REX1 expression changes in mammalian skin fibroblasts plated on plastic dishes (blue) or encapsulated in a LM micro-bioreactor (red), exposed to 5-aza-CR for 18 hours (Post 5-aza-CR) and subsequently cultured in ESC medium up to 28 days (28d). Gene expression levels are reported with the highest expression set to 1 and all other times relative to this.

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ISSN: 2283-3927