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Epigenetic conversion;  
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# Matrix stiffness and oxygen tension modulate epigenetic conversion of mouse dermal fibroblasts into insulin producing cells

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## Abstract

*In vivo*, cells are surrounded by a three-dimensional (3-D) organization of supporting matrix, neighboring cells and a gradient of chemical and mechanical signals (Antoni, *et al.*, 2015). However, the present understanding of many biological processes is mainly based on two-dimensional (2-D) systems that typically provides a static environment. In the present study, we tested two different 3-D culture systems and apply them to the epigenetic conversion of mouse dermal fibroblasts into insulin producing-cells (Pennarossa, *et al.*, 2013; Brevini, *et al.*, 2015), combining also the use of two oxygen tensions. In particular, cells were differentiated using the Polytetrafluoroethylene micro-bioreactor (PTFE) and the Polyacrylamide (PAA) gels with different stiffness (1 kPa; 4 kPa), maintained either in the standard 20% or in the more physiological 5% oxygen tensions. Standard differentiation performed on plastic substrates was assessed as a control. Cell morphology (Fig.1A), insulin expression and release were analyzed to evaluate the role of both stiffness and oxygen tension in the process. The results obtained showed that 1 kPa PAA gel and PTFE system induced a significantly higher insulin expression and release than plastic and 4 kPa PAA gel, especially in low oxygen condition (Fig.1B). Furthermore, comparing the efficiency of the two systems tested, 1 kPa PAA gel ensured a higher insulin transcription than PTFE (Fig.1C). Recent studies show the direct influence of substrates on lineage commitment and cell differentiation (Engler, *et al.*, 2006; Evans, *et al.*, 2009). The evidence here presented confirm that the use of an appropriate stiffness (similar to the pancreatic tissue), combined with a physiological oxygen tension, promote  $\beta$ -cell differentiation, with beneficial effects on cell functional activity and insulin release. The present results highlight the importance of 3-D cell rearrangement and oxygen tension to promote *in vitro* epigenetic conversion of mouse fibroblasts into insulin-producing cells.

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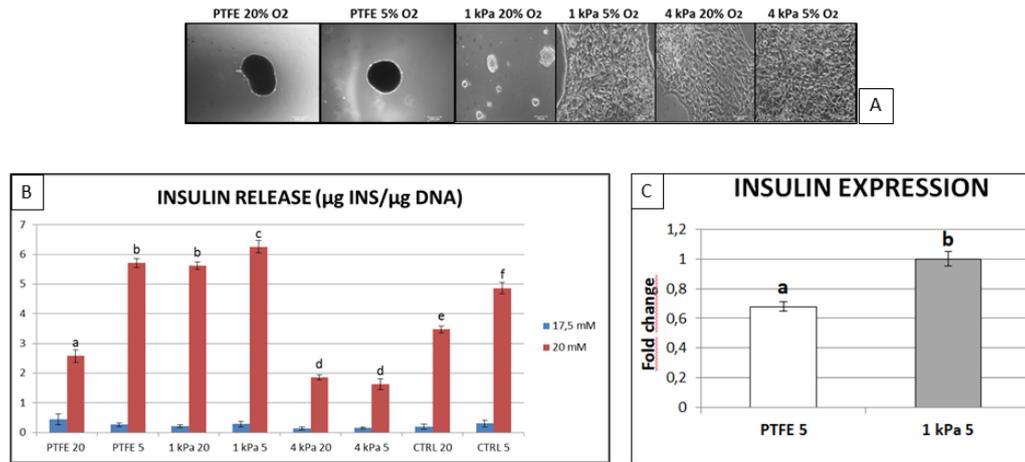


Fig.1: **A**) representative image of cells differentiated in PTFE (20% and 5% oxygen) and on PAA gels (20% and 5% oxygen); **B**) insulin release in all samples after hyperglycemic stimulation with 20 mM of glucose. Different superscripts indicate statistical differences among the samples (SPSS software,  $p \leq 0,05$ ). Insulin release is expressed as mean value  $\pm$  SD; **C**) insulin expression in the more efficient experimental groups (PTFE 5% O<sub>2</sub>; 1 kPa 5% O<sub>2</sub>). Different superscripts indicate statistical differences between the samples (SPSS software,  $p \leq 0,05$ ). Gene expression levels are reported with the highest expression set to 1 and the other relative to this.

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