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# Low, but not too low, oxygen tension and macromolecular crowding accelerate extracellular matrix deposition in human dermal fibroblast culture

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ABSTRACT

A key challenge of *in vitro* organogenesis is the development in timely manner tissue equivalents. Herein, we assessed the simultaneous effect of oxygen tension (0.5%, 2% and 20%), foetal bovine serum concentration (0.5% and 10%) and macromolecular crowding (75 μg/ml carrageenan) in human dermal fibroblast culture. Our data demonstrate that cells cultured at 2% oxygen tension, in the presence of carrageenan and at 0.5% serum concentration deposited within 3 days in culture more extracellular matrix than cells grown for 14 days, at 20% oxygen tension, 10% serum concentration and in the absence of carrageenan. These data suggest that optimal oxygen tension coupled with macromolecular crowding are important *in vitro* microenvironment modulators for accelerated development of tissue-like modules *in vitro*.

**Statement of Significance**

To enable clinical translation and commercialisation of *in vitro* organogenesis therapies, we cultured human dermal fibroblast at 2% oxygen tension, under macromolecular crowding conditions (75 μg/ml carrageenan) and at low foetal bovine serum concentration (0.5%). Within 3 days in culture, more extracellular matrix was deposited under these conditions than cells grown for 14 days, at 20% oxygen tension, 10% FBS concentration and in the absence of crowding agents. These data bring us closer to the development of more clinically relevant tissue-like modules.



## 1. Introduction

The extracellular matrix (ECM) is a complex structure that provides mechanical support to the tissues and influences fundamental cellular processes, such as proliferation, differentiation, migration and apoptosis [1–3]. Bereft of this optimal milieu, cells lose their phenotype, during *in vitro* expansion [4–6]. Further, direct cell injections, lacking this native ECM, demonstrate poor cell localisation at the injury site upon implantation [7]. For these reasons, tissue engineering has depended, among others, on artificial scaffolds, natural or synthetic in origin, to maintain the native cell function *in vitro* and to enhance cell localisation [8–13]. However, drawbacks associated with artificial scaffolds (e.g. acute rejection, foreign body response, risk of disease transmission, non-ideal degradation profile, mismatched mechanical properties) have triggered investigation into scaffold-free therapies, which exploit the inherent ability of cells to produce their own ECM, thus forming a completely biological and tissue-specific implantable device [14–17]. Despite the promising preclinical and clinical data, the main limitation of this approach is the very long culturing time required to create an implantable device (e.g. 5 weeks to produce a corneal equivalent [18], 28 weeks to produce a blood vessel equivalent [19]), resulting not only in an expensive manufacturing process, but also in phenotypic drift of the cultured cells. To this end, current research is focused in various *in vitro* microenvironment modulators to both maintain cell phenotype *in vitro* and to enhance ECM deposition [20].

Molecular oxygen is an essential signal that regulates developmental processes, cell fate and tissue function [21,22]. Extracellular oxygen concentration can fluctuate from 0.5% to 14% within

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an organ, depending on how far cells are located from capillaries [23]. The cell response to decreases in oxygen tension is mediated by the hypoxia-inducible factor 1 (HIF-1), which regulates fundamental cell processes, including differentiation, proliferation, migration, glucose metabolism, angiogenesis and ECM synthesis [24–26]. As tissues and their cell types need specific oxygen levels, considerable research effort has been directed towards optimisation of oxygen supplementation in cell cultures and engineered tissues [27].

An alternative approach to modulate the *in vitro* microenvironment and to subsequently enhance matrix deposition is based on macromolecular crowding (MMC). *In vivo*, both the extracellular space and the intracellular environment are tightly packed with various biological macromolecules, which make the milieu extremely crowded. Volume exclusion by solutes leads to the decrease of the entropy of the crowded environment and produces an increase in the thermodynamic activity of solutes [28]. MMC has shown to affect protein structure, folding, shape, conformational stability, enzymatic activity and protein-protein interaction [29]. *In vitro*, cells are cultured in large volumes of dilute aqueous media that do not provide a sufficiently crowded physiological environment. In fact, typical cell culture media supplemented with 5–20% serum have solute concentration of 4–16 mg/ml, which fails to imitate even the localised density of body fluids (e.g. blood has solute concentration of 80 mg/ml). Macromolecular crowding, the addition of inert macromolecules in the culture media, has been shown to positively affect biological processes and to increase many-fold ECM deposition in various permanently differentiated and stem cell culture systems [30–33].

In the present study, we ventured to assess the simultaneous effect of oxygen tension and MMC in human dermal fibroblast culture, as means to enhance ECM synthesis and subsequent deposition, respectively.

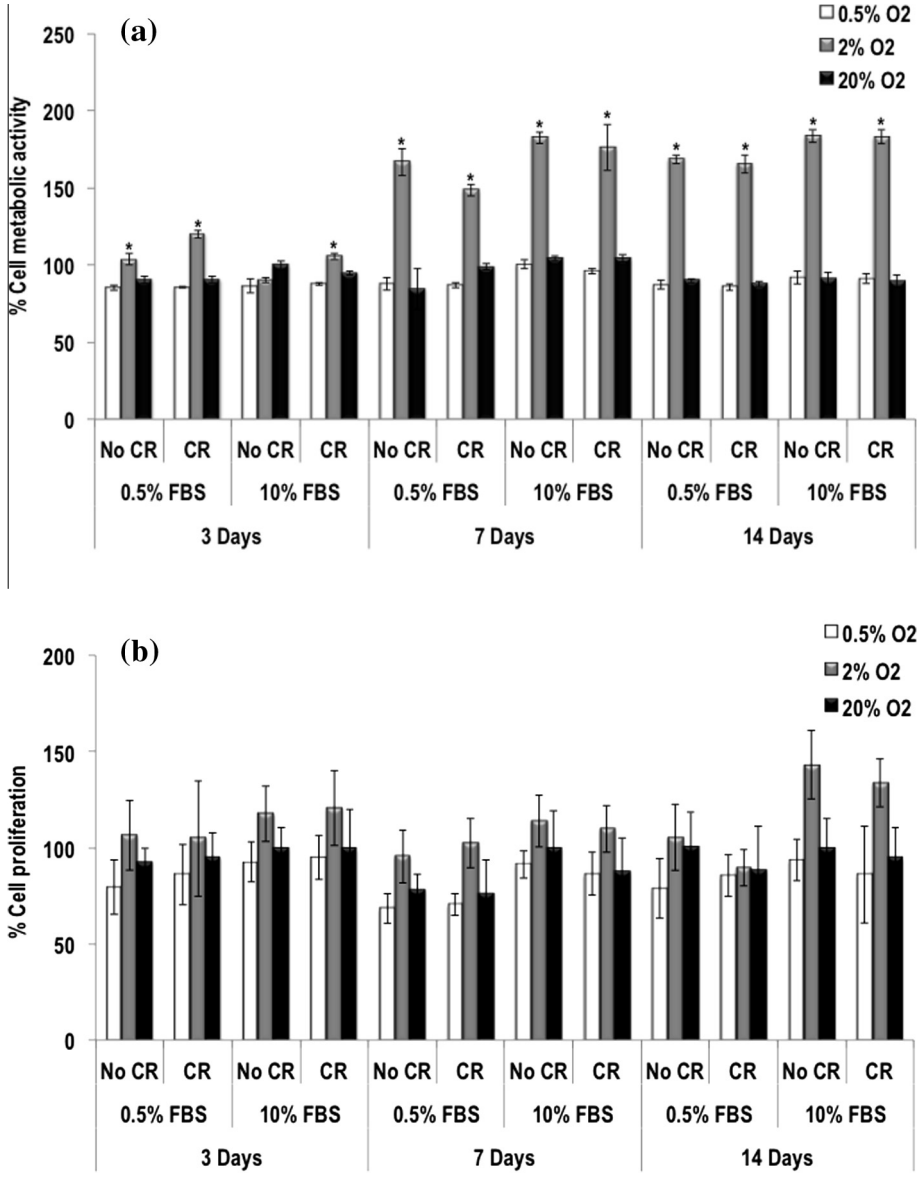


**Fig. 1.** Cell metabolic activity analysis (alamarBlue®) revealed that at all time points (3, 7 and 14 days), human dermal fibroblasts cultured at 2% oxygen tension exhibited significantly higher ($p < 0.0001$) metabolic activity than cells cultured at 0.5% and 20% oxygen tensions, independently of the FBS concentrations (0.5% and 10%) and presence (75 μg/ml CR) or absence of crowder. Cell metabolic activity (%) was normalised to day 3, 10% FBS, 20% $O_2$ and no CR (a). Cell proliferation (nuclei DAPI staining and subsequent counting) was not statistically affected ($p > 0.05$) as a function of time in culture (3, 7 and 14 days), FBS concentrations (0.5% and 10%), oxygen tensions (0.5%, 2%, 20%) and presence (75 μg/ml CR) or absence of crowder (b). Note: ∗ indicates statistically significant difference.