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Applying macromolecular crowding to enhance extracellular matrix deposition and its remodeling *in vitro* for tissue engineering and cell-based therapies ☆

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ABSTRACT

With the advent of multicellular organisms, the exterior of the cells evolved dramatically from highly aqueous surroundings into an extracellular matrix and space crowded with macromolecules. Cell-based therapies require removal of cells from their crowded physiological context and propagating them in dilute culture medium to attain therapeutically relevant numbers whilst preserving their phenotype. However, bereft of their microenvironment, cells under perform and lose functionality. Major efforts currently aim to modify cell culture surfaces and build three dimensional scaffolds to improve this situation. We discuss here alternative strategies that enable cells to re-create their own microenvironment *in vitro*, using carbohydrate-based macromolecules as culture media additives that create an excluded volume effect at defined fraction volume occupancies. This biophysical approach dramatically enhances extracellular matrix deposition by differentiated cells and stem cells, and boosts progenitor cell differentiation and proliferation. We begin to understand how well cells really can perform *ex vivo* if given the chance.

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1. Introduction

The human body is comprised of approximately 411 different cell types [1], and they reside in or migrate through microenvironments comprising a variety of extracellular matrix (ECM) [2]. Cell-based therapies hinge on one particular platform technology: *ex vivo* cell culture. This technology is based on the sudden separation of cells from their tissue microenvironment (harvest, mechanical and enzymatic destruction) and placing them on plastic in the presence of huge amounts of salt water, additives, and fetal calf serum, still an ill-defined compound but difficult to replace.

Cell culture has come a long way from glass flasks to modern cell culture polystyrene that has been treated with gas plasma to make it more cell-friendly. Huge efforts in industry have been made to ensure quality and reproducibility of the hardware, as well as cell culture sera and media compositions. These efforts are reflected in textbooks that specifically deal with establishing cell cultures [3]. So far, this technology has served us well, but it has become forgotten over time that what we have in cell culture flasks and in many bioreactors is pathological: the disproportion of aqueous medium to cell mass, the substitution of an organic support by plastic, and the lack of macromolecular crowdedness as such, a hallmark of the microenvironment of cells in metazoans. In fact, if such a disproportion of fluid to cell would occur in the human body this would incur immediate medical attention, yet biologists all over the world are complacent to grow cells under conditions that in the clinical world would be addressed as oedema or effusion.

It is therefore small wonder that cell-based therapy has now reached a glass ceiling in its attempts to create larger three dimensional structures. One reason is the limited diffusion of oxygen and nutrients into the tissue in structures thicker than 1–2 mm [4–8], which is a mass transfer problem *in vitro*, and a microvascularisation issue after implantation. The other limitation, which we propose to remove by applying macromolecular crowding (MMC), is set by the ability to create structures coherent enough to be manipulatable. Scaffold-free approaches involving cell printing and cell sheet technology are both dependent on the presence of sufficient ECM to stabilise the structures [9,10], but unfortunately the deposition of ECM *in vitro* with uncrowded conditions is an inefficient process. Stem cell based therapies are currently hitting a roadblock because *ex vivo* propagation of stem and progenitor cells on tissue culture polystyrene results in decaying proliferation and differentiation capacities [11,12]. A recent technology feature summarised the worldwide efforts by materials scientists and chemical engineers to produce microenvironments for stem cells [13]. On one hand it is intriguing to witness efforts to supercede some 700 million years of evolution and materials testing, on the other hand this testifies the glaring disregard for the *capacity of cells themselves to manufacture their ECM*. In this review, we share possible ways to control and augment this cellular capacity by re-introducing macromolecular crowding into culture systems, in order to create a well-developed ECM that can provide cellular cohesion and tissue strength to aid in tissue engineering, and building microenvironments for stem cell work both for basic research and for therapeutic applications.

2. What is macromolecular crowding (MMC) and how does it work?

2.1. MMC is an ancient biological principle

This review does not intend to give a comprehensive overview of crowding theory, but rather aims at explaining the principle of macromolecular crowding (MMC) to the practitioner. Our focus is the effect of MMC on the formation of ECM, and we intend to highlight the basics of this ancient principle of life to scientists in regenerative medicine. Possible applications include improving cell performance in culture and preserving the phenotype of precious progenitor cells to facilitate cell-based therapy. With this disclaimer in mind and the intention to bridge the current gap between theory and application, we would like to begin with the statement that *all living systems are highly crowded* [14].

This is true of the interiors of cells, whether bacterial, animal, or plant, and the exterior of most cells of multicellular organisms. The crowding element is derived from macromolecules such as proteins, carbohydrates, lipids and nucleic acids, that form macromolecular complexes and supramolecular assemblies such as cellular organelles and membranes [15]. Although up to 40% of the cytoplasmic volume can be occupied by macromolecules [16,17], the usual range lies between 20 and 30% [18]. Notably, the volume-occupying macromolecules also tend to have a net fixed electrical surface charge. The question arises whether crowding is just an incidental occurrence during evolution or was necessary for the origin of life. Interestingly, the earliest life forms (viruses, archaea and prokaryotes) have been found to have crowded structural and functional units. The total concentration of protein and RNA inside bacteria like E. coli is in the range of 300–400 g/L. As we go up the tree of evolution, crowding persists as a highly conserved property of higher organisms [19].

2.2. MMC and the excluded volume effect

Macromolecular crowding functions by way of the excluded-volume effect (EVE) and is often referred to as the "volume of a solution that is excluded to the center of mass of a probe particle by the presence of one or more background particles in the medium" [19]. *Fractional volume occupancy* Ψ *(FVO)* denotes the fraction of the total volume occupied by macromolecules (Fig. 1). Thermodynamically, volume exclusion lowers the configurational and conformational freedom (entropy) leading to elevated basal free energy of the reactant macromolecules and a number of downstream effects [20]. These may be identified as (1) folding of biopolymers (e.g. proteins and nucleic acids) into native states optimal for function [21], (2)

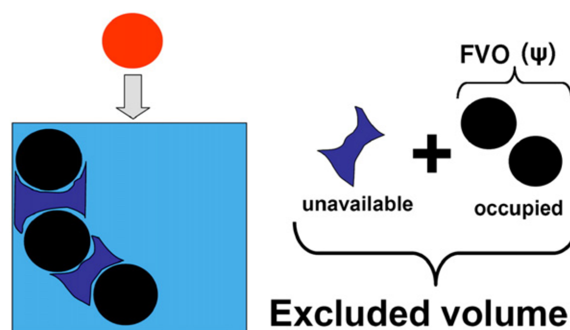

Fig. 1. A simplified representation of the generation of EVE through the presence of macromolecules. The schematic reflects the situation that a test molecule (red) encounters in a given volume element (box). The crowders (black) occupy about 30% of this volume, the fractional volume occupancy (FVO, ψ) therefore is 30% (v/v). While FVO can be calculated, the additional unavailable volumes represent a challenge to compute as several factors such as electrostatic repulsion and hydration shell need to be considered.

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