

## From 2D Cell Phenotypes to 3D Live High-content Imaging: New Ways to Windows

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### Abstract

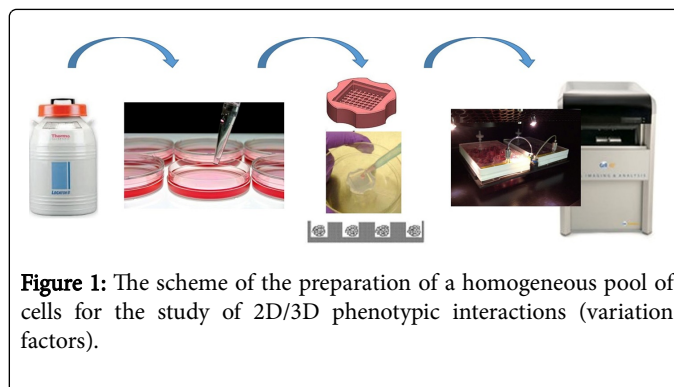
Researches in the field of cellular and molecular mechanisms of cell variability, based on phenotypic interactions, remain one of the most central and promising problems in modern biology. The current paper proposes a possible model for studying "variability windows" and cell phenotype reprogramming using different somatic cell types (epithelial and mesenchymal cells) as well as different cell culture systems - 2D monolayer, 3D single spheroids and microtissues accompanied with up-to-date live time-lapse microscopy analysis.

### Introduction

Conversion of genotype into 2D/3D - cell/tissue phenotypes remains the central challenge of current biology. The most important property of living systems is the non-linear signaling dialogue between gene regulatory networks and phenotype networks [1]. Later, variability on the basis of phenotypic interactions was added. The molecular/cellular mechanisms of variability are studied much worse than heredity. Waddington suggested that epigenetics should be designated as a branch of biology that studies the causal interactions between genes and their products, forming new pages and borders of phenotype (without mentioning interactions between 2D-/3D-phenotypes). Semantic contexts of phenotypic interactions remain poorly understood by means of the old genetics. However, convincing examples of «variability windows» are already discussed on a numerous cellular models, including phenotype -genotype linkers [2-4] or minimal somatic cell windows for direct reprogramming of cell phenotypes [5]. The concept of phenotypic induction (phenotypic programming) was studied in embryogenesis. In the case of «critical periods» of development, high sensitivity of forming signals to the embryonic signals and teratogens increases morphogenesis abnormalities [5,6]. With the help of automatic image analysis, phenotypic variability has been studied in 1,000 samples of yeast cells in 37 natural lines. Considerable background natural variability of the yeast lines has not been indicated [7-9]. Up to now direct interactions between the single phenotypes, 2D monolayer, 3D spheres, and neo-tissues remain largely unstudied. To find induced «variability windows», it is important to choose the direct interaction in micro-culture of single cells, monolayers, 3D spheres and new 3D lab tissues. Such a scheme of the experiment would allow determining the direct imaging of the target, depending on the status of the cells. It is necessary to completely eliminate or minimize the extracellular matrix signals (Figure 1).

Pre-prepared and characterized lines of epithelial (E) or mesenchymal (M) cells were thawed, cultured in monolayer and placed in 3D conditions in special non-adherent wells to form spheroids. In

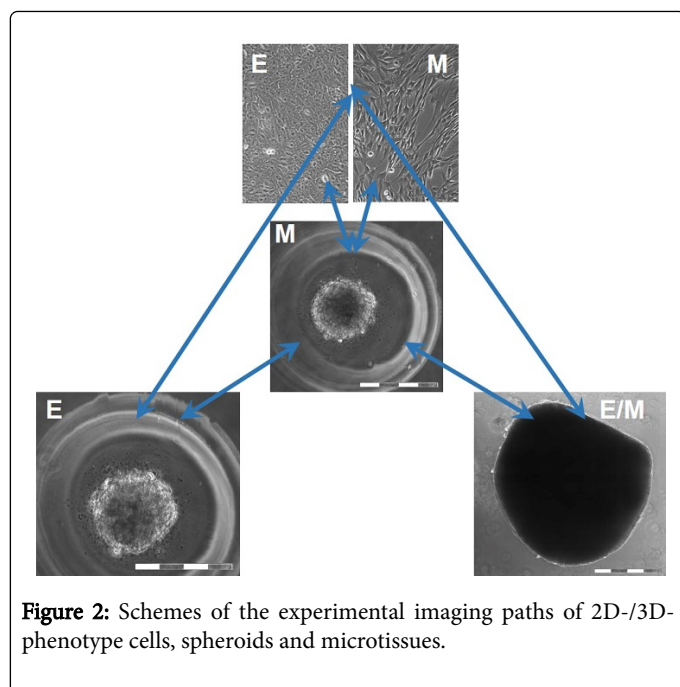
vivo observation of the spheroids formation was carried out in the time-lapse microscopy system Cell-IQ (CM Technologies). Fusion of spheroids with cells or with different spheroids or microtissues was also performed in non-adhesive wells. All 3D cultures were cultivated in the device camera for an extended in vivo microscopy. The cells of retinal pigment, buccal and amniotic epithelia were used as E-cultures. M-cultures were presented by multipotent mesenchymal stromal cells from bone marrow, subcutaneous adipose tissue, umbilical cord and eye limbus [7].



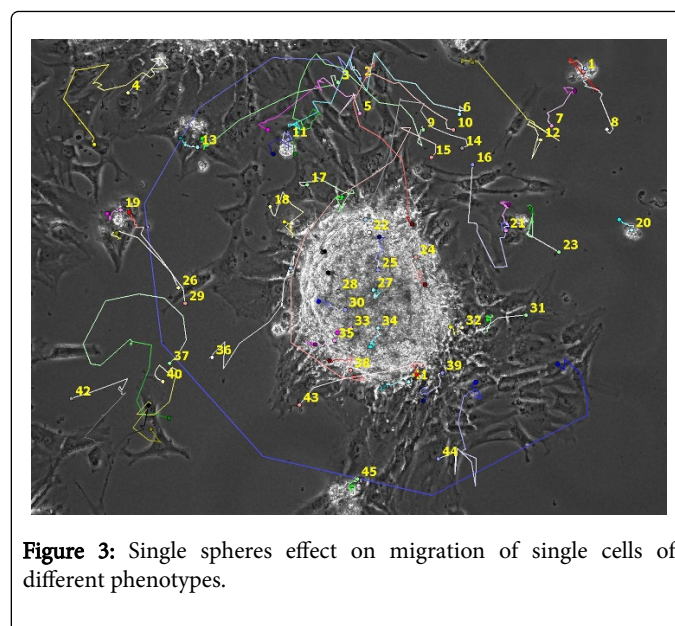
**Figure 1:** The scheme of the preparation of a homogeneous pool of cells for the study of 2D/3D phenotypic interactions (variation factors).

The working hypothesis of experiments: the cells' internal signaling system is able to induce new «phenotype windows» than more effectively the environment (Figure 2).

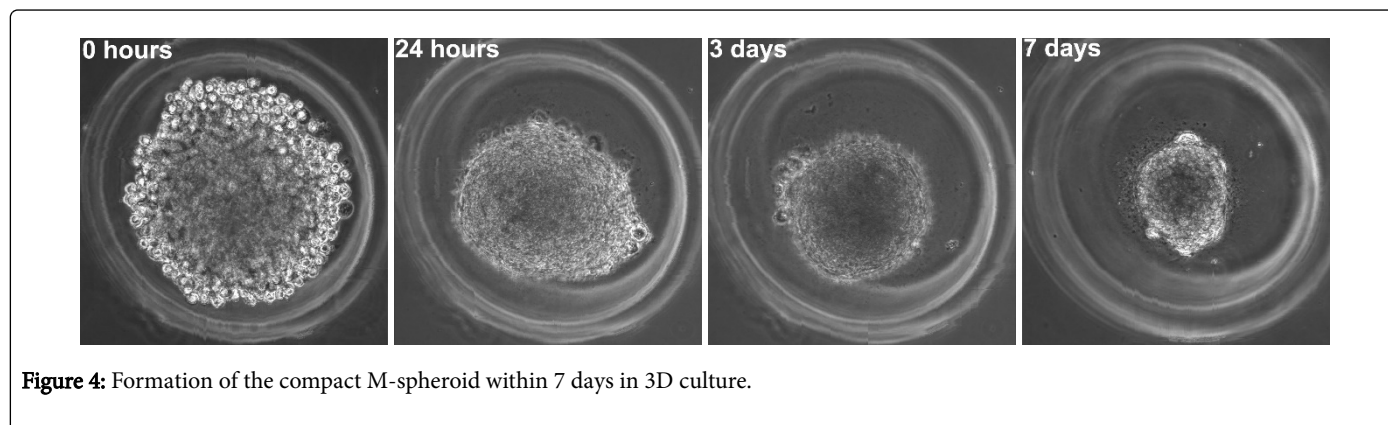
The spheroids were obtained from characterized epithelial (E) and mesenchymal (M) cell cultures under the 3D conditions. Then 7-14-day spheroids co-culturing resulted in the microtissue formation. In vivo changes were analyzed when adding the cell suspensions to spheroids and microtissues and besides, during spheroids interaction with each other and with the microtissues. The purpose of the experiments: the search for new phenotypes in the interaction of spheroids, new tissues and isolated cells under standard culture conditions (Figure 3).



Analysis of migration activity and motility of the cells was performed at transitions between 2D and 3D conditions. The trajectories of labeled single cells movement were numbered and marked with different colors. The subpopulations of cells with inhibition of migration activity were identified (Figure 4).



Aggregation of cells started within the first 5-7 hours, by the end of the first day a loose spheroid formed, and later compacted. The process of compaction gave rise to new phenotypes as part of spheroids. In addition, the compaction of the cells was labeled with local chromatin compaction [8] (Figure 5).



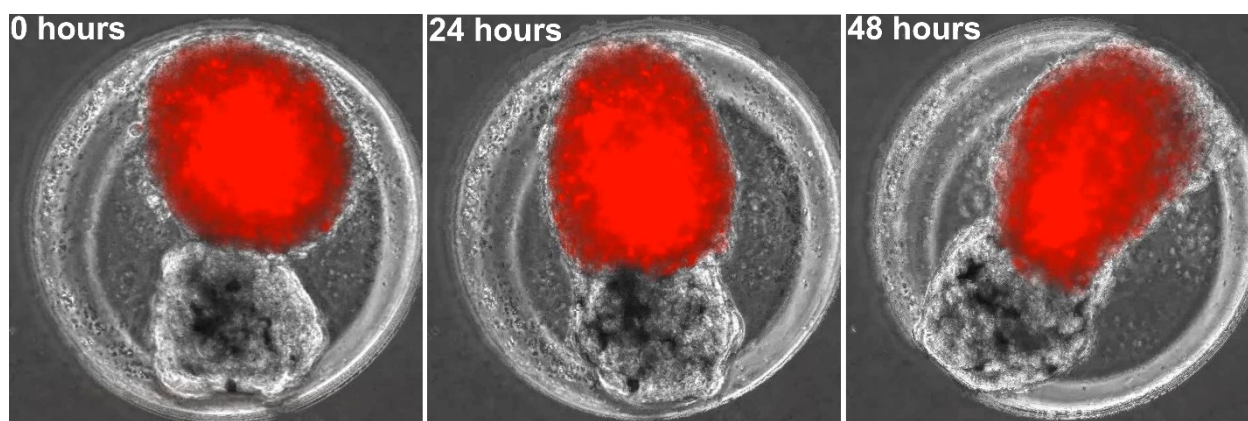
Epithelial cells of unstained E-spheroid migrate across the M-spheroid surface marked with red vital dye DiI. Interaction of E- and M spheroids proceeded without significant changes of the phenotype of mesenchymal and epithelial cells. The merger of the two spheroids has led to the stabilization of E- and M-phenotypes.

## Discussion and Summary

In recent years, molecular and cellular mechanisms of variability have been explored from different perspectives. Indeed, the last anniversary issue of PNAS US (2015. 112) contains a final review on this subject [9,10].

Heterogeneous interaction of endocrine cells of Langerhans islets is important for optimal gland function correction, especially for

coordinated hormone secretion [11]. Cell-cell interactions play an important role in spatial organization (pattern formation) in the development and adult organisms. Understanding of these crucial roles requires identification of cell phenotypes that are controlled by cell-cell interactions and spatial organizations of phenotype. However, assays for cell-cell interactions are mainly applicable at a population level. This level is incapable in elucidating of 3D status of tissue and with incomplete view of cell- cell profiles [12,13]. In another study, it was shown that the aggregates formed from the beta and alpha cells form ordered compact core of beta cells, whereas alpha - cells are packed in the outer layer. This arrangement improves the functional characteristics of the islets [14].



**Figure 5:** The process of hybrid E-M sphere forming where the layer of unstained epithelial cells migrates across the surface of the colored M-spheroid (red).

Heterogeneous interactions between hepatocytes and non-parenchymal cells are imperative for collective cell behavior and coordinated organ function [15]. Synergy of epithelial and mesenchymal windows are essential for cell plasticity and formation of 3D clusters of PP cells with hybrid E/M cell phenotypes [16]. Molecular imaging gives way to see fundamental biological processes in a new light [17]. Molecular imaging is a rapidly growing research branch that extends vision of living objects to a more meaningful dimension. For example, extended cell phenotypes phenomena, described by R.Dowkins, need new techno-imaging to describe in numbers multiple interactions of cells with eco-microsurroundings [18].

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