P.10 Study of cellular differentiation of embryonic carcinoma stem cells by AFM nanocytomechanics and Raman spectroscopy

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Studying the physics of biological systems is essential to deeply understand their behaviour. An in-depth investigation of the relationship between nanomechanical and biochemical signatures of biological systems is pivotal to improve our knowledge of the factors that influence their functions [1,2]. The biomechanical and biochemical markers of the undifferentiated stem cell status were obtained by combining Atomic Force Microscopy (AFM) nanoimaging and nanomechanics with Raman spectroscopy (RS). We used undifferentiated P19 embryonal carcinoma cells, a well-established model system, and exposed them to uniform differentiation instructive factors, such as retinoic acid (RA) and dimethyl sulfoxide (DMSO), to induce differentiation into neural and cardiomyocyte cells without initial formation of embryonic bodies (EBs). The main difference between undifferentiated and differentiated P19 cells was found to be in their shapes. Neurons are the largest and thickest cells compared to cardiomyocyte and undifferentiated P19 cells as indicated by their surface area and apparent volume (Fig. 1). Also the organisation of the cytoskeleton (Fig. 2) showed substantial differences. While undifferentiated P19 cells presented thin actin filaments running from the nuclear region towards the edge of the cell, the differentiated cells showed either thick actin filaments running parallel (neurons) or woven actin filaments (cardiomyocytes). As a consequence, differentiated cells displayed a higher surface roughness than undifferentiated P19 cells. Cell elasticity was found to be one of the main nanomechanical markers. Undifferentiated p19 cells were found to be more elastically deformable than differentiated cells. Interestingly, the adhesion energy of P19 cells was higher than that of cardiomyocytes but lower than that of neurons, indicating a difference in the cellular membrane fluidity. RS indicated clear differences in the biochemical makeup of cellular differentiation status of P19 stem cells. Differentiated neurons and cardyomyocites showed a decrease in DNA content, and therefore proliferation and cell growth, than undifferentiated cells. Moreover, neurons were characterised by higher protein content than P19 cells, whereas cardyomyctes displayed a decrease in the protein contents. These results demonstrate the great potential of the AFM/Raman combination as a tool for the profiling of differentiated and undifferentiated cells.

Fig 1

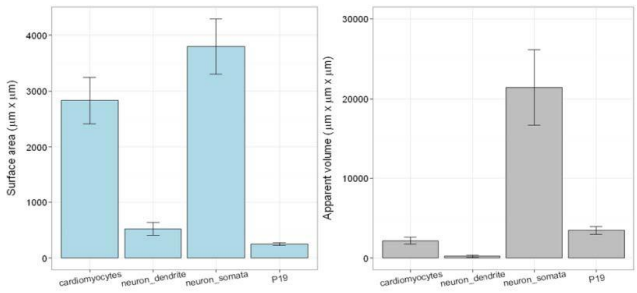
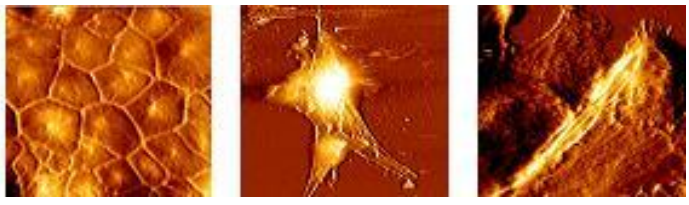


Fig 2



P19 **neurons** **cardiomyocytes**

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