

CONTROL OF SPATIALLY PERIODIC PATTERNS OF CELL AGGREGATION ON SOFT ECM SUBSTRATA: EXPERIMENT VERIFICATION OF MECHANOCHEMICAL MODEL

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INTRODUCTION: Development of spatial pattern and form is one of the central issues in embryology and is included under the general name of morphogenesis. Turing showed mathematically how certain combinations of chemical reaction kinetics and diffusion rate should lead to chemical instabilities, capable at least in principle of producing the desired type of spontaneous morphogenesis [1]. Subsequently, some actual combinations of chemicals have been found which spontaneously generate patterns in approximately the desired way, and the general principles underlying all such 'reaction-diffusion system' have become the major concept of morphogenesis.

Rather contrast to this view point, Harris et al have found the generation of spatial patterns created by mechanical instabilities [2]. When fibroblasts were cultured on the collagen gel, and the contraction of the gel as a whole is physically restrained by attachment of its margin to a plastic substratum, then the effect of the fibroblasts' traction is to break up the cell-matrix mixture into a series of aggregations of cells and compressed matrix. These aggregations are interconnected by linear tracts of collagen fibres aligned under the tensile stress exerted by fibroblast traction. Oster and Murray showed mathematically how mechanical force between fibroblast and collagen gel germinated cell aggregation, and they predicted distribution of fibroblasts on the collagen gel become periodic pattern [3]. Although their theory was formulated in terms of measurable quantities such as cell densities, forces, and so on, there seems to be no established experiment fact to prove their theory. In this study, we have observed varieties of geometric patterns of fibroblast (3T3 Swiss albino) distribution, when they were cultured on collagen gel attached on plastic substrata.

METHODS: To clarify periodic structure of the distribution pattern of the cell, we have observed the wide area of collagen gel. 9cm diameter of collagen gels were prepared on polystyrene Petri dishes with kept attached to the dish. Then the measurements were taken at 2cm x 2cm square center of the gel using CCD camera to obtain

images of cell distributions. Images were analyzed by using Fast Fourier Transformation (FFT) method to examine periodic structure of the geometric patterns.

RESULTS:

The patterns generated spontaneously on the collagen gels vary depending on initial cell population densities and concentration of collagen gels. Geometric shapes of patterns were depended on concentration of collagen (*Fig1*).

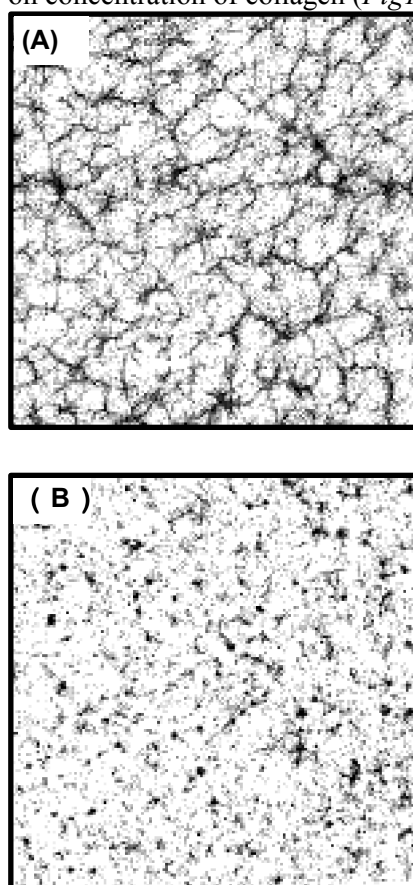


Fig1: Cell aggregation pattern generated on collagen gel. Each figure shows fourth day after cell seeded. Concentration of collagen gels are A) 0.3mg/ml, B) 3.0 mg/ml.

When the concentration of collagen was low (0.3 to 1.0(mg/ml)), cell aggregation formed 'Network like' shape. As increasing concentration of collagen (3.0(mg/ml)), cell aggregation formed 'island like' shape. FFT method revealed all the patterns

generated on collagen gels have periodic structure. The wavelength of patterns was only depended on initial cell population density, and the initial cell populated density were increased; the wavelength of patterns was decreased. Size of wavelength was approximately seven times larger than average distance between the cells initially populated.

DISCUSSION & CONCLUSIONS: From these results and analysis, range of cell-cell mechanical interaction through the collagen fibre only depends on initial cell populated density and this can be explained by Murray's theory qualitatively. Also, we suggested the cause of different geometric shapes of pattern formed on different concentration of the gels was asymmetry of the cell-cell interaction was remarkable when concentration of gel decrease.

REFERENCES: ¹ Turing, A.M.(1952) Phil. Trans. Roy. Soc. B237:37. ²Harris, A.K., Stopak, D., and Wild, P. (1984) J. Embryol. exp. Morphol. 80:1. ³Murray, J.D., Oster, G.F, and Harris, A.K. (1983) J. Math Biol. 17:125.