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Organ printing: Fiction or science

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Abstract. Aggregates of living cells (i.e. model tissue fragments) under appropriate conditions fuse like liquid drops. According to Steinberg's differential adhesion hypothesis (DAH), this may be understood by assuming that cells are motile and tissues made of such cells possess an effective surface tension. Here we show that based on these properties three-dimensional cellular structures of prescribed shape can be constructed by a novel method: cell aggregate printing. Spherical aggregates of similar size made of cells with known adhesive properties were prepared. Aggregates were embedded into biocompatible gels. When the cellular and gel properties, as well as the symmetry of the initial configuration were appropriately adjusted the contiguous aggregates fused into ring-like organ structures. To elucidate the driving force and optimal conditions for this pattern formation, Monte Carlo simulations based on a DAH motivated model were performed. The simulations reproduced the experimentally observed cellular arrangements and revealed that the control parameter of pattern evolution is the gel-tissue interfacial tension, an experimentally accessible parameter.

Keywords: Tissue engineering, differential adhesion, fusion, spherical aggregate, tissue liquidity

1. Introduction

It has long been suggested that under appropriate conditions morphogenetic tissue motions can be interpreted by assuming that tissues possess liquid-like properties [10]. Aggregates made of motile and adhesive cells round up when incubated in gyratory shakers. When aggregates are prepared from cells of different types randomly intermixed, the morphogenetic phenomenon of sorting takes place and the final equilibrium pattern of the system corresponds to a configuration in which cells of one type form a sphere surrounded by the cells of the other type. Such behaviors are characteristic for liquids. A liquid drop in the absence of external forces assumes a spherical shape to minimize its surface energy. Two immiscible liquids (e.g., water and oil) when intermixed, phase separate and the more cohesive liquid (water) becomes engulfed (surrounded) by the less cohesive one (oil). Such configuration again minimizes the total surface or interfacial energy of the system.

The molecular basis of tissue liquidity has been established by Steinberg's Differential Adhesion Hypothesis (DAH) [10]. DAH stipulates that tissues possess measurable surface or interfacial tension whose origin is in the adhesive properties of their constituent cells. Different tissues have different surface tensions due to the differential adhesion between the cells that compose them. Foty and coworkers have measured the tensions of a number of embryonic tissues [2]. Their values where consistent

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with the mutual engulfment behavior of these tissues, that is the more cohesive tissue with the higher surface tension always sorted out of the less cohesive tissue with lower surface tension [2]. Computer simulations [3] and *in vivo* experiments have provided further support for DAH [4,5].

The analogy between tissues and liquids makes it possible to apply quantitative modeling methods to morphogenetic phenomena. However, it is important to keep in mind that despite analogies, fundamental differences remain. Liquids equilibrate by molecular motions driven by thermal fluctuations, whereas the motion of cells is ameboid and is fueled by energy producing chemical reactions (i.e. ATP hydrolysis).

In the present work we demonstrate that tissue liquidity can be used to construct organ structures in a novel way: by layer-by-layer cell aggregate printing. To print one needs ink, paper and printer. Here we describe some details of the bioink and biopaper and present results obtained by manual printing. Details of the true printer have been described elsewhere [8].

2. Bioink and biopaper

Traditional organ or tissue engineering uses biocompatible gels as scaffolds to accommodate cells to be grown in number until they can be harvested for specific applications [1,6]. This is a slow process, which has also limitations as far as the shape of the tissue to be engineered is concerned. We propose instead, to use spherical cell aggregates consisting of many thousands of cells. These can be delivered (e.g., printed) into gels in specified geometry. Due to the liquid properties of these tissue fragments, provided the gel properties are appropriately adjusted, they will fuse into organ structures (see below). Thus our bioink consists of spherical cellular aggregates and the biopaper is a biocompatible gel. Figure 1 shows unicolor and multicolor bioinks. In the former case the aggregate contains cells of one type, whereas in the latter case several cell types may be included, depending on the specific requirements. For example if blood vessels are to be constructed, the multicolor aggregate will contain both endothelial and muscle cells. In this case, the self-organizing properties of cells and tissues will guarantee the "right" (physiological) arrangement of cells in the final structure. In Fig. 1 this is illustrated by the sorted cellular pattern.

Fig. 1. Left panel: spherical aggregate (of 500 micron diameter) of chicken embryonic heart cells containing approximately 40,000 living cells. Such aggregates made of cells of one type constitute the unicolor bioink. Right panel: an initially random mixture of two cell types when equilibrated (i.e. sorted) assumes a configuration with one tissue surrounded by the other. Such mixed aggregates comprise the multicolor bioink. The aggregates shown in the figure contain two types of Chinese hamster ovary cells (CHO) transfected with E-cadherin, one containing 25% more of the adhesion molecule than the other. The diameter of these aggregates is 450 microns. (Image prepared and kindly provided by R. Foty.)

3. Printing tubular organ structures

To demonstrate the feasibility of organ printing, we have manually printed a concentric circle of aggregates using unicolor bioink (Fig. 2) into gels of varying composition. Here we report results with collagen gels of two different concentrations. Once the aggregates have been placed into the gel, we let the system evolve in time. Depending on the gel properties the system reached various configurations. As can be seen in Fig. 2, for a permissive gel, with collagen concentration of 1.7 mg/ml, the system evolves towards the minimum energy configuration, corresponding to a single sphere. Permissive in the present context means a gel, which favors cell movement. It is well known that cells are able to crawl along collagen fibers [9] and the more concentrated the gel the more intensive is this movement. Thus a gel with 1.0 mg/ml is much less permissive. At the end of the observation (168 h after preparation) in this case the aggregates fuse into a ring or thick disc. Printing the circular pattern layer-by-layer and using an appropriate biopaper thus can lead to a tubular organ structure with lumen. When the bioink has the right composition such a process may result in a blood vessel or gut fragment.

Figure 2 demonstrates the importance of the biopaper; its properties are crucial for structure formation. (Note also the shrinking of the gel, especially in the 1.7 mg/ml case.) In order to gain further insight into controlling the biopaper properties, we have carried out Monte Carlo simulations of the described experimental process. We have placed cells and gel particles into a three-dimensional discrete lattice and assumed specific adhesive molecular interactions between them (gel–gel, cell–cell and gel–cell). We then prepared the experimental initial arangeemnt (Fig. 2) and let this system evolve towards its lowest energy configuration according to the standard Metropolis Monte Carlo algorithm [7]. A more detailed analysis of such a model reveals that the only control parameter of this pattern evolution is the interfacial tension γ_{cg} between the gel and the cellular material, which is the combination of the three molecular interaction parameters. Thus, in the simulations it is this parameter that determines how permissive is the biopaper. As Fig. 2 demonstrates, a relatively low value of γ_{cg} is analogous to the

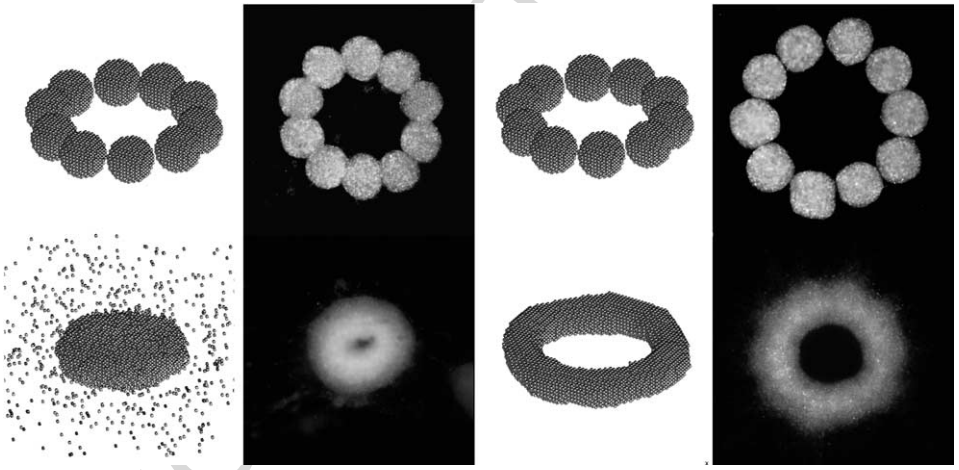

Fig. 2. Experimental and computational realization of a simple organ structure. Upper panels show initial configurations, lower panels show final configurations. First panel from left: simulation of the fusion of 10 spherical aggregates (each containing 123 cells) for a permissive gel with small gel–tissue interfacial tension $\gamma_{cg} = 0.25$. Second panel: manual printing of 10 spherical aggregates of CHO cells with diameter of 500 microns into a permissive collagen gel of 1.7 mg/ml concentration. The final configuration shown has been reached in 168 h. Third panel: model simulations for a non-permissive gel with $\gamma_{cg} = 2.0$. Fourth panel: manual printing of 10 spherical aggregates of CHO cells with diameter of 500 microns into a non-permissive collagen gel of 1.0 mg/ml concentration. The final configuration shown has been reached in 168 h.

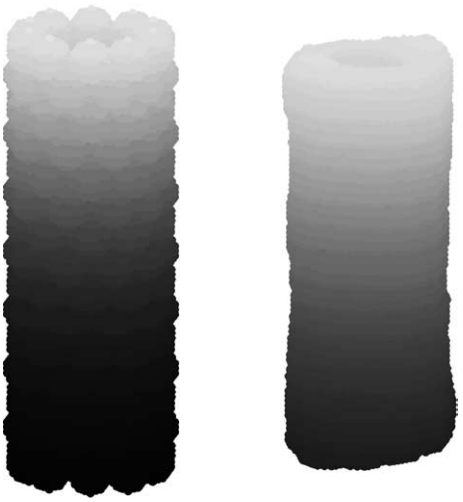

Fig. 3. Initial (*left panel*) and final (*right panel*) configuration of 15 rings, 10 aggregate each, each aggregate containing 123 cells in the simulations.

concentrated collagen solution, whereas a larger interfacial tension corresponds to a less permissive gel. The almost perfect match between experiments and simulations suggests that the parameter γ_{cg} largely determines the final geometry after aggregate fusion takes place. Clearly γ_{cg} depends in a complicated way on the chemical composition of the gel, but once this dependence is established, the outcome of pattern evolution can be predicted.

In Fig. 3 we present simulation results for the case when a large number (15) of layers (of 10 aggregates each) are printed. As the figure indicates, for the right interfacial tension the desired tubular structure can be achieved.

It has to be emphasized that rings and tubes are not final equilibrium states of the cellular systems and thus do not represent the most stable structures. These would correspond to spheres, as discussed earlier. However, as the simulations showed, rings and tubes can be made deeply metastable, meaning they could persist for a long time, long enough to eliminate the gel and thus conserve these structures. Elimination of the gel can, for example, be accomplished by a slight change in temperature (in some cases a one degree change is sufficient to induce the sol–gel transition).

4. Conclusion

The results presented in this work provide strong evidence that liquid-like properties of tissues, established many years ago can be exploited in a novel way to construct three-dimensional organ structures of specified shape. Moreover, the same liquid properties allow the introduction of the concept of bioink and the delivery by printing of cells in large numbers into scaffold gels, the biopaper. We have demonstrated the feasibility of such an ambitious program with printing very simple albeit nontrivial organ structures using unicolor bioink. We have shown how computer simulations can aid in the optimization of this technology. Although many technical difficulties will have to be overcome before noble organs, such as liver will be possible to construct, we hope the results of this work are strongly suggestive of organ printing being much more than fiction.

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