Controlled Dielectrophoretic Assembly of Bio-inorganic Composite Materials using Live Cells and Functionalized Particles

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Introduction

Large research efforts have been directed in the last few years toward the fabrication of materials with electronic and photonic functionality by assembly of micro- and nanoparticles. One exciting new concept that we extend here is that live biological cells can be used as "particles" in similar types of assembly. This will yield new classes of biodevices with potentially rich areas of application, such as in biosensors with direct electrical detection. The assembly of live cells and synthetic nanoparticles also yields new biomaterials, in which the functionality of the cells is coupled to the functionality of the organized structure. In this manner, the functionality of the entire structure can be altered. Yeast and fibroblast cells in suspension can be readily organized in chains and arrays by the use of dielectrophoresis (DEP). Presently we are developing techniques for binding the arrays of cells into new biomaterials and sensor elements. We perform this binding by using functionalized nano- and microparticles as a biocolloidal "glue" between the cells (Fig. 1). These particles have on their surfaces lectins that bind to polysaccharides on the outer cell membrane. This nanoparticle-cell assembly process can be conveniently initiated and controlled by the strength and frequency of the AC electric field. When the suspension contains microparticles or nanoparticles the characteristics of the dipoles induced in the cells and particles vary with frequency, voltage and electrolyte concentration. This leads to the assembly of new types of cell-particle structures such as chains where the cells and particles alternate, arrays from cells or combined cells and particles. This process raises a variety of important fundamental questions such as how the frequency-dependent dipolar polarization of the cells leads to the formation of different types of structures and how the nanoparticles are attracted to the areas of higher field intensity between the cells. The role of the operating parameters and the structure of the phases formed by our method have been characterized both experimentally and theoretically.

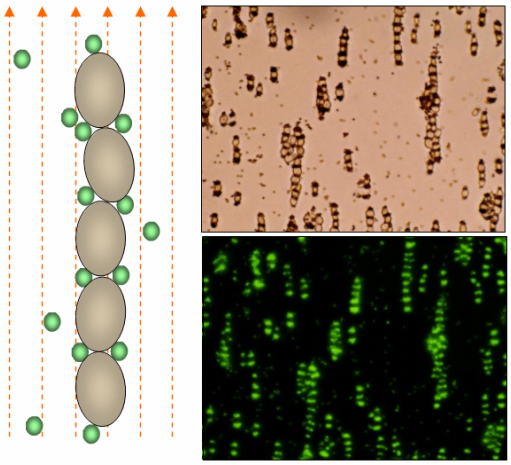

Figure 1. Assemblies of yeast cells and 1 μm fluorescent microparticles made by AC electric field. The specific organization of the particles in the junction between the cells (compare the regular and fluorescence images) occurs because of the induced dipolar interactions.

Experimental Section

Substrate Fabrication. Glass microscope slides were cleaned by immersing in Nochromix solution overnight. They were washed thoroughly with deionized Millipore water and dried in an oven at 60^{o}C. For coplanar electrodes, the dried slides were covered with TEFLON Thread seal tapes in areas where no electrodes were desired. The tape acts as a mask during vapor deposition. A 10 nm layer of chromium was deposited on the glass first, to provide better gold adhesion to the substrate. A 100 nm gold layer was then deposited on top of the thin chromium layer. The distance between the two planar electrodes formed by this procedure can be controlled by varying the width of the masking tape. The gap for different electrodes was varied from 4 to 8 mm. For four-point electrodes, four needles were orthogonally injected into the chamber. The electrode setups are shown in Fig. 2.

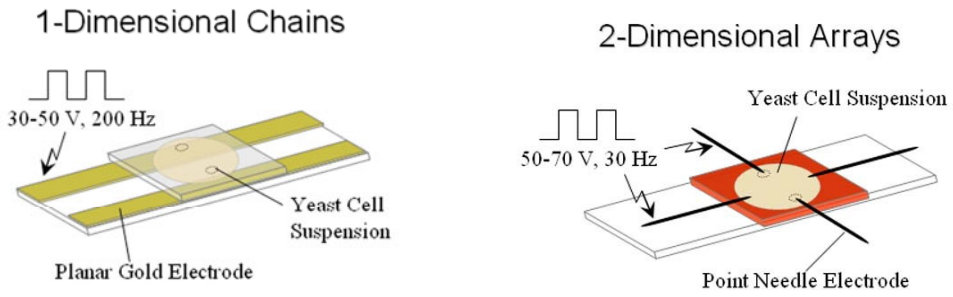

Figure 2. The electrode setup: coplanar (a) and 4-point orthogonal (b) for cell organization in chains and 2-D arrays, respectively.

Cell Suspensions. Baker's dried active yeast, *Saccharomyces cerevisiae* (5 μm) was purchased from MP Biomedicals, Inc. NIH/3T3 mouse fibroblast cells (20 μm) were borrowed from Dr. Haugh's lab. Yeast cell suspensions of concentration 0.1 wt % and NIH/3T3 mouse fibroblast cell suspensions of concentration 0.08 wt % were freshly prepared in a 10 μM phosphate buffered saline aqueous solution purchased from Sigma. Cell viability was tested both before and after the experiments.

Experimental Setup. Approximately 20 μL of cell suspension (for chains) and 550 μL of cell suspension (for 2-D arrays) were loaded in perfusion chambers purchased from Grace Bio Labs (HBW13 and PC1R-2.0, respectively). The chamber was securely sealed on top of a microscope slide and attached to coplanar electrodes (for chains) and four orthogonal electrodes (for 2-D arrays). The electrodes were connected to a function generator that produced *square waves* in the frequency range 10-5000 Hz. An amplifier increased the generated signal to the desired working voltage of 20-80 V. A 1 μF capacitor was included in the circuit to filter any direct component of the signal. Digital multimeters connected in the circuit read the voltage applied and the current passing through the suspension. A master switch allowed starting and stopping the process whenever needed. The assembly process was continuously monitored using an Olympus

BX61 confocal microscope and the images were taken using an Olympus DP70 camera attached to the microscope.

Results and Discussion

Dielectrophoresis was used as a precise, controllable and scalable tool for assembling organized structures from millions of cells simultaneously. The assembly was easily controlled by varying the strength and frequency of the electric field between coplanar electrodes. A range of experiments were conducted for frequencies and voltages between 10-5000 Hz and 20-60 V, respectively. The results were characterized for obtaining optimum conditions required for cell chains of a particular length. For example, low frequencies (<100 Hz) and high voltages (>50V) gave us longer cell chains than vice versa. Once, the conditions were set, long, permanent and rigid cell chains were fabricated by alternately trapping protein functionalized particles in the junctions between the cells (Fig. 3). We used many different types of particles, mainly magnetic particles coated with protein so that the final assembly could be manipulated with an external magnetic field.

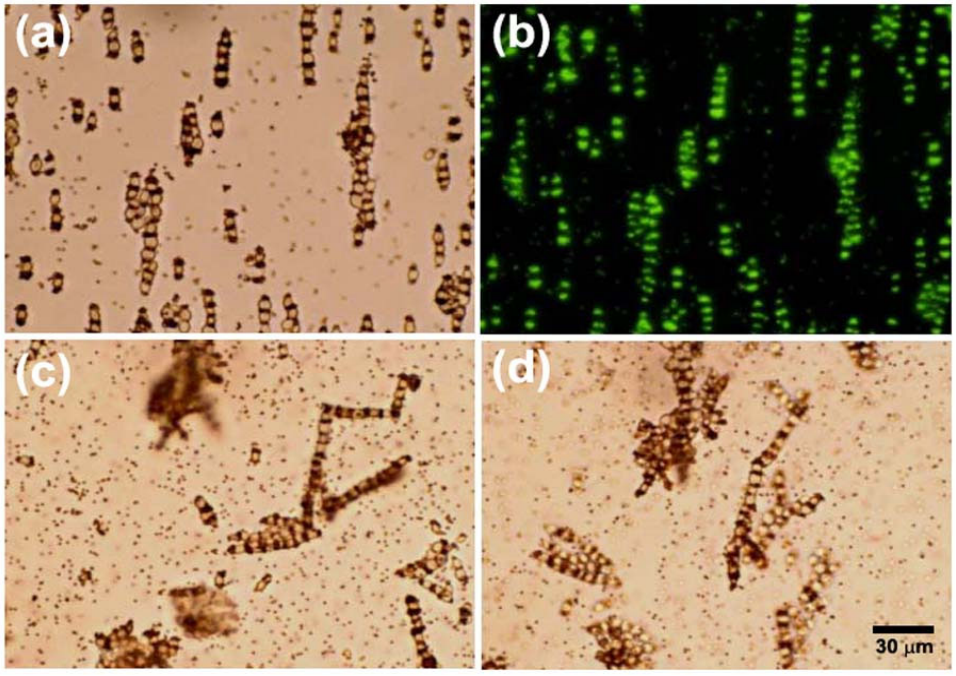

Figure 3. Cell chains obtained using fluorescent lectin conjugated flouraldehyde latex. (a) & (b) complementary images of striped "tiger-like" chains formed from 0.2 wt% cells and 0.1 wt% particles in AC electric fields of 30 V, 200 Hz. The final protein concentration obtained in the solution was 0.05 mg lectin/mg latex. (c) & (d) AC field 'off'. Chains develop hinges after perturbation. The images indicate rigidity of the chains.

The concept of cell chaining in AC electric fields was extended to 2-D array formation from live cells, an equivalent of a new biomaterial. When a high concentration of cells (sufficient to form at least a monolayer) was subjected to an AC electric field for a long time (~45 mins), the cells first organized themselves into chains.

The chains after reaching an optimum length interacted laterally with each other giving rise to an intermediate 2D foam-like structure. The packing of cells was improved by using a custom designed 4-point orthogonal electrode unit. Cells formed chains by aligning with the closest neighbor in the direction of the electric field. By switching different pairs of electrodes "on-&-off", we were able to align cells in all directions until they formed a highly dense and uniform hexagonally closed packed (hcp) cell crystal. This experiment was repeated with magnetic particles in the same way as before and we obtained some very easily manipulative magnetic membranes shown in Fig. 4. The membranes were also of monolayer thickness and highly elastic (no quantitative results). Similar data was obtained with mammalian cells. These membranes have future as biomedical dressings, artificial tissues, coatings, biofilters etc.

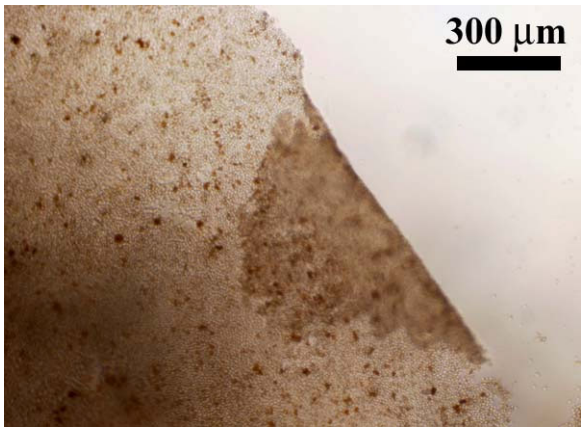

Fig. 4. A folded magnetic membrane of yeast cells. The membrane is a matrix of cells and lectin-coated magnetic nanoparticles.

In summary, we have extended the concept of particle dielectrophoresis to live cells and particles in order to yield new kinds of biomaterials and sensor elements. We have conducted exploratory research to illustrate novel techniques of synthesizing permanent cell chains & arrays, co-assembled irreversible cell-particle microstructures and 2D membranes of cells using functionalized nano- and microparticles as biocolloidal binders. These particles attach to cells via biospecific lectin-polysaccharide interactions. The presence of synthetic particles between cells adds a new dimension to the class of biocomposite materials wherein the functionality of the particles gets combined with the functionality of the organized structure. For example, irreversibly bound 2D matrix of cells and magnetic particles has been manipulated en masse (as a membrane) by external magnetic fields. Operating system parameters like frequency and voltage, proved to be vital in defining the length, morphology and functionality of the organized structures, have been optimized. These assemblies have potential applications as biosensors, bioreactors, biomedical dressings etc.