Physical Analysis of Cellular Chain Formation via Label-Free Negative Magnetophoresis

Randall M. Erb,[1] Melissa D. Krebs,[2] Bappaditya Samanta,[3] Vincent M. Rotello,[3] Eben Alsberg,[2] Benjamin B. Yellen[1]
[1]Duke University, Durham, NC, [2]Case Western Reserve University, Cleveland, OH, [3]Univ of Massachusetts, Amherst, MA

Statement of Purpose: Recently, we have demonstrated the formation of viable linear cellular structures via label-free negative magnetophoresis,[1] (Fig 1). This magnetic assembly method allows for cellular manipulation without attachment or endocytotic internalization of magnetic particles that can adversely affect normal biological functioning. Instead, negative magnetophoresis relies upon magnetizing the extracellular fluid surrounding the particles, such as cells, which then behave as controllable magnetic cavities which interact with each other leading to chaining formation under applied field. Previous work has demonstrated that particles size-commensurate with cells (~10μm) may exhibit different chaining kinetics than smaller Brownian-affected particles (<4μm) that have been well-characterized by diffusion-limited cluster aggregation (DLCA) models.[2] We have discovered the dependence of variables affecting these kinetics, including applied field strength, fluid magnetization, and, most surprisingly, particle concentrations – a variable previously believed to be independent in DLCA models. Accurate characterization of the dependencies of the kinetics will allow inverse magnetophoretic techniques to be employed to build highly controlled cellular structures for tissue engineering applications.

Methods: Suspensions of bovine serum albumin- (BSA-) passivated 12-nm Fe_3O_4 nanoparticles, (ferrofluid), were synthesized and used at concentrations ranging from 0-30mg/mL. These BSA-ferrofluids were used to magnetize extracellular fluids leading to the formation of viable cellular chains. To characterize the chaining kinetics of cell-sized particles, 10-μm polystyrene colloids were used for their monodispersity. The number of particle chains, n, of length S were counted over time, t, through the analysis of fluorescent micrographs and found to be consistent with the general features of DLCA models, namely, that $n_S \sim t^{-w}$ and $S \sim t^{z}$, where z and w were previously considered to be constant exponents.

Results: A time sequence for polystyrene particles in 100 Gauss field is presented in Fig 2. As time progresses, the effective chain size increases dramatically, producing long chains of nonmagnetic particles in ferrofluid. The temporal decrease in fluorescence intensity indicates the presence of magnetic image forces pushing the particles away from the glass-fluid interface. This image force allows the kinetics to be studied independent of local surface interactions that might otherwise affect particle mobility.

In this work, we systematically tested the dependence of the scaling exponents, z and w, on both the particle and ferrofluid concentrations and found that they are not constant over all experimental conditions, contrary to predictions by DLCA models. We attribute this deviation to the strong particle-particle interactions that dominate Brownian diffusion in this experimental system. In fact, a major discovery of this work is that there appears to be a linear relationship between the dynamic exponents and the concentration of the particles and ferrofluid (Fig 3).

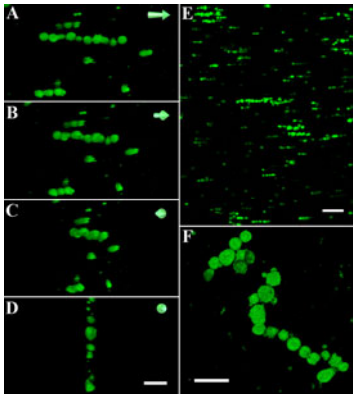

Figure 1. *Confocal micrographs of HUVECs chained in BSA-ferrofluid. (a-d) rotated images of oriented linear cell chains. The arrow indicates the direction of the magnetic field. Scale bar = 50-μm. (e) Low mag view of cells under magnetic field. Scale bar = 200-μm. (f) View of cell chains 1 hr after removal of magnetic field. Scale bar = 50-μm.*

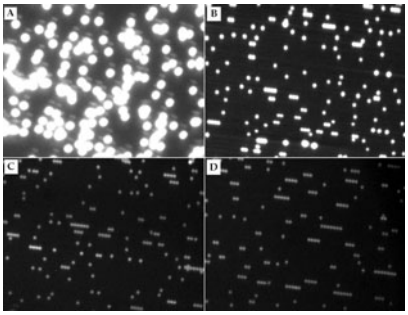

Figure 2. *Sequence of photomicrographs obtained for particle concentrations of 2.4% and ferrofluid concentration of 30 mg/mL showing the growth of particle chains at various times: [A] 0 s, [B] 300 s, [C] 600 s, and [D] 1600 s. Particle intensity initially decreases due to the image forces present in the system.*

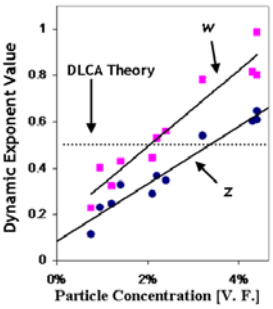

Figure 3. *Dependence of dynamic exponents* z and *w on the concentration of 10-μm polystyrene particles within the ferrofluid. Linear dependence between exponents and particle concentration exists that is not predicted by current DLCA theory.*

Conclusions: In this work, we present our investigations of the growth of cell-sized nonmagnetic particles within ferrofluid to analyze the experimental variables that are critical to the assembly of cellular chains for applications in tissue engineering. We discovered that the chain growth for cell-sized particles exhibit different kinetics than well-characterized Brownian-affected particles. Also, these growth kinetics are greatly improved by using larger fields and particle concentrations; however the type of ferrofluid and its concentration are also important variables to be considered for utility in tissue engineering.

References: [1]Krebs et al. *In Submission.*
[2]Cernak et al. Phys Rev E 2004:70:031504.