Published in final edited form as:

Langmuir. 2017 June 20; 33(24): 6167–6181. doi:10.1021/acs.langmuir.7b01493.

Polyurethane Microgel based Microtissue: Interface-guided Assembly and Spreading

Michael J. Hill¹, Debanjan Sarkar^{1,2,*}

¹Department of Biomedical Engineering, University at Buffalo, The State University of New York, Buffalo, NY 14260, USA

²Department of Chemical and Biological Engineering, University at Buffalo, The State University of New York, Buffalo, NY 14260, USA

Abstract

Colloidal gels are 3-dimensional networks of microgel particles and can be utilized to design microtissues where the differential adhesive interactions between the particles and cells, guided by their surface energetics, are engineered to spatially assemble the cellular and colloidal components into 3-dimensional microtissues. In this work we utilized a colloidal interaction approach to design cell-polyurethane (PU) microgel bimodal microtissues using endothelial cells (ECs) as a normal cell model and a non-malignant breast cancer cell line (MCF-7) as a cancer cell model. PU microgels were developed from a library of segmental polyurethanes with poly (ethylene glycol) soft segment and aliphatic diisocyanate/L-tyrosine based chain extender as hard segment to modulate the interactions between PU colloidal particles and cells. Surface energy of the microgel particles and cells were estimated using Zisman's critical surface tension and van Oss-Good-Chaudhury theory (vOGCT) from liquid contact angle analysis. Binary interaction potentials between colloidal PU particles and cells and the ternary interaction between colloidal PU particle, cell, and collagen I/Matrigel® were calculated to explain the formation of microtissues and their spreading in extraneous biomatrix respectively, by using classical and extended DLVO theory (XDLVO). Furthermore, rheological analysis and in silico simulations were used to analyze the assembly and spreading of the PU microgel based microtissues. In-vitro experiments showed that ECs and MCF-7 displayed more differentiated (EC spreading/MCF-7 lumen formation) character when mixed with microgel particles that were stable in aqueous medium and more undifferentiated character (EC non-spreading/MCF-7 spreading) when mixed with microgel particles unstable in aqueous medium.

Graphical abstract

*Corresponding author: D. Sarkar, Ph: 716-645-8497, Fax: 716-645-2207, debanjan@buffalo.edu.

Associated Content

Supporting information. Compucell3D Simulations methods and additional figures and tables related to experimental results.

The authors declare no competing financial interest

Keywords

polyurethane; microtissue; colloidal; microgel; DLVO

Introduction:

Microtissues are a physiologically relevant 3-dimensional model of homotypic or heterotypic cellular aggregates which are used to mimic and predict biological responses of tissues. The formation and stability of microtissues is guided by the intermolecular force characteristics of cell surfaces. Steinberg's differential adhesion hypothesis (DAH) and different variations of the original DAH have been used to explain how tissues physically develop and behave through interfacial interactions.[1–3] The DAH postulates that all cellular sorting or compartmentalization phenomena are due to the differential work of adhesion between different cell types and their extracellular matrix (ECM) components.[4] This theory has major implications for developmental biology, tissue engineering, and the understanding of tissue pathologies such as cancer where major morphological changes in tissues can be understood as a differential adhesion phenomenon.[5, 6]

However, in general, microtissues are cell-only aggregates without extracellular matrix components within the 3-dimensional structures, and therefore, can only partially reconstitute the diverse contact relations of the cellular microenvironment. Designing biomaterial based microtissues, where the biomaterial provides a matrix component with tailored surface chemistry, can synchronize cell-matrix and cell-cell interactions to reestablish the contacts between cells and the surrounding matrix for proper organization and maintenance of cellular niches. Cell sorting phenomena between unlike cells can be understood in the context of heterocoagulation where the asymmetric force fields between interacting materials can lead to attraction or repulsion as a function of distance.[7, 8] Therefore, controlling interfacial interactions between the cells and biomaterial based matrix components can assemble these two into a physiologically relevant microtissue structure. Introduction of colloidal nano or microscale particles as a matrix along with cells is predicted to regulate the adhesive balance between these components and ultimately define the formation and stability of the microtissue. Similar microgel particle scaffolds have been

used to support cells in the past, however, surface chemical linking is typically utilized to form the particle based gel, rather than the balance of colloidal forces, and the role of natural differential adhesion in subsequent cell behavior is obscured.[9, 10] Since microgel particles can assemble into self-spanning 3-dimensional networks to form colloidal gels through inter-particle colloidal interactions, these materials represent a viable matrix component for the microtissue where interfacial adhesive interactions between cells and microgel particles can be modulated to differentially assemble the cellular and matrix components.[11, 12] For instance, oppositely charged poly (N-isopropylacrylamide) based microgels, displayed sorting behavior similar to living cells which was dependent on ionic concentration and temperature as a modulator of the interaction potential.[13]

We envisioned developing biomaterial based microtissues where the matrix component is derived from segmental polyurethane (PUs) based microgel particles. PU microgel particles can assemble into colloidal gel-like structures through inter-particle interactions which are regulated by the molecular structure of PU segments.[14–16] Segmental variation of PU composition allows tuning of the adhesive interactions of PU microgel particles and, thus, as matrix components they are ideal to engineer the adhesive interactions between cells in microtissues. The DAH can be used as a guiding principle to predict the assembly and spreading behavior of bimodal microtissues consisting of both cellular (large) and microgel particle derived matrix (small) elements based on their differential contact relations. Both the binary interactions between cells and particles as well as the ternary interactions between each microtissue component and the extraneous biomatrix phases can be correlated to assess the formation, stability, and spreading behavior of microtissues. To develop PU based microtissues, endothelial cell (EC) and epithelial cell types were chosen as these cells are widely used for microtissue development where differential adhesive interactions have been acknowledged as a design principle.

In particular, EC microtissues are used for tissue engineering, understanding of vasculogenesis, and for injection into ischemic tissues.[17, 18] The DAH has been acknowledged as a design principle in matrix free microtissues with multiple cell types, e.g. uniluminal EC and smooth muscle cell (SMC) spheroids where SMCs spontaneously form the outermost layer of the microtissue.[19] When embedded in a collagen gel, these spheroids elongated into an ellipsoid shape reminiscent of vascular formation during development. Similarly, for epithelial cells, when mixed aggregates of luminal (LEM) and myoepithelial (MEP) cells were plated on nonadhesive agarose gels LEM segregated to the outer surface whereas when they were plated on basement membrane matrix (Matrigel®), MEP were outer most (the physiologically correct orientation). [20] In parallel, malignant epithelial cell line MCF-7 (breast epithelial cancer cell line) microtissues have also been utilized as a model of cancer transformation due to their lack of normal differentiated luminal morphology when plated on Matrigel®. [21] When $\beta 1$ integrins were blocked, MCF-7 regained their differentiated morphology, demonstrating that the enhanced interaction between the surfaces of the cancer cells and their ECM components was disrupting their normal morphological features.[22]

These examples revealed that alterations in contact relations between cells with one another and their matrices is a fundamental aspect of normal versus abnormal tissue morphology and

behavior. However, lack of matrix components within the cellular aggregates of these microtissues excludes the effect of cell-matrix versus cell-cell interactions in a 3D context, which can be addressed by designing microgel based microtissues, essentially with PU colloids as the matrix component. Utilizing, a DAH based physical approach can underline the exact nature of the contact relations between cellular and matrix components which leads to tissue formation and their responses.

Thus, in this work, we developed and analyzed PU microgel based microtissues with normal ECs and pathological cancer cell MCF-7 to understand the interfacial interaction guided assembly of cellular and matrix components into microtissue like structures. Using microgels from segmental PUs with poly(ethylene glycol) (PEG) as “hydrophilic” polyether soft-segment with L-tyrosine based chain extender (desaminotyrosyl tyrosine hexyl ester, DTH) and aliphatic hexamethylene diisocyanate as “hydrophobic” hard segment can modulate interfacial adhesion between the particles and cells to form microtissues with differential spreading or morphological characteristics.[14] The molecular design of PU segments is altered to achieve different levels of adhesive interaction by varying the aqueous stability (i.e. their ability to remain dispersed in aqueous medium) of particles either through altering the chain length (i.e. molecular weight (M.W.)) of PEG soft segment or by introducing ionic character in the hard segment (where either negatively charged 2,2-Bis(hydroxymethyl)propionic acid (DMPA) or positively charged N-methyl diethanolamine (NMDA) as ionic chain extender was used in equimolar ratio with DTH). Importantly, these PU microgels have been used for chondrogenesis of mesenchymal stem cell (MSC) microtissues where incorporation of microgels as matrix promoted chondrogenic differentiation compared to cell-only microtissues.[23] Thus, this group of segmental PUs, as shown in figure 1, is expected to induce different levels of adhesive interactions which can be understood by exploring a colloidal interaction design approach to develop and control the EC and MCF-7 microtissues behavior. Using two cell types can induce different adhesive interactions and spreading characteristics under differential colloidal interaction pressures as cancer cell surface properties are significantly different compared to normal cells. [24] These microtissues are formed by aggregating the cells with PU microgels, and their assembly and stability are modulated by the differential adhesive interactions between these cells and colloidal PU particles (figure 1). Subsequently their spreading response in extraneous biomatrix of Collagen I or Matrigel® (to model stromal and basement membrane of ECM) is characterized. Both the DLVO theory of colloidal stability and the extended DLVO (XDLVO) theory is used to analyze the aqueous stability of cells and particles as well as their tendency to aggregate and form the microtissue.[25] To complement this approach, oscillatory rheological analysis and in silico simulations are used to understand the microtissue assembly and their spreading response which is further verified from in vitro cell experiments. Understanding how differentiated normal cells and cancer cells respond uniquely to the presence of colloidal particles of various surface characteristics is important to predict microtissue formation. Additionally, information concerning the effect of the surface characteristics of colloidal particles on the stability of tissues has wide reaching implications for the fields of nanomedicine, understanding the effects of particulate debris from implants, as well as solid-state carcinogenesis (the Oppenheimer effect).[26–28]

Experimental Section:

Materials:

PEG with 600, 1000, and 2000 M.W. were purchased from Sigma Aldrich. Hexam ethylene diisocyanate (HDI), dimethyl formamide, and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) were purchased from TCI and dimethyl sulfoxide (DMSO), L-tyrosine, and 3-(4-hydroxyphenyl)propionic acid (DAT) were purchased from Alfa Aesar. The 15 mm coverslips were purchased from Warner Instruments, while all other chemicals and solvents were purchased at Sigma Aldrich and used as received unless otherwise noted. Cryopreserved human umbilical vein endothelial cells (HUVEC) and Endothelial Cell Growth Medium containing 2% serum with growth factor supplement (EC medium) were purchased from Promo Cell. MCF-7 cells were donated from Dr. Yun Wu's laboratory in the department of biomedical engineering at University at Buffalo. HUVECs were cultured in EC medium and MCF-7 were cultured in cell culture medium (CCM) consisting of RPMI with 10% FBS and 1% Pen-Strep. Roswell Park Memorial Institute medium (RPMI) and fetal bovine serum (FBS) were purchased from Invitrogen CA and Atlanta Biologicals GA. Cells were typically used between passage 4-7. Matrigel® and rat tail Collagen I in .2 N acetic acid were purchased from Corning at a concentration of 10 mg/ml and 3.3 mg/ml respectively, 4',6-diamidino-2-phenylindole (DAPI) and rhodamine-phalloidin were purchased from Invitrogen CA.

Polyurethane Synthesis and Colloidal Microgel Preparation

DTH was synthesized according to established protocols.[29, 30] PUs were synthesized according to a previously published protocol via a two-step polycondensation reaction between different dried PEG (M.W.: 600, 1000, 2000) and HDI as aliphatic diisocyanate, followed by DTH as chain extender.[31] Since the polyurethanes were synthesized with 1:1 molar ratio of soft and hard segment, increasing PEG molecular weight resulted in decreased hard segment fraction in the polymer. Additionally, ionic PU were synthesized by replacing half the molar concentration of DTH with DMPA or NMDA, added simultaneously with DTH in the second step of the reaction. For PEG1000-DMPA-HDI-DTH triethylamine was added at ~ 1:1 molar ratio with PEG while for PEG1000-NMDA-HDI-DTH glacial acetic acid at ~ 0.505 molar ratio to PEG was added and both allowed to react for 2 hours at 50° C, in order to confer ionic properties to these PU. The reaction mixture was precipitated in cold ether, washed thoroughly with ether, and dried at room temperature overnight. PU molecular weight measured by gel permeation chromatography (GPC) results with DMF solutions at 3 mg/ml are shown in table 1. PU colloids were prepared by precipitating 5-10% (w/v) DMF solutions of PU in 4M aqueous solution of sodium chloride followed by homogenization at 4000 RPM. Colloidal PU suspensions were dialyzed into excess PBS overnight before use to remove small molecule impurities and DMF. All experiments utilizing PU colloids were performed in PBS (or in cell culture medium) unless otherwise indicated.

Contact Angle Measurements

Samples for contact angle measurement were prepared by washing the dialyzed suspensions with DI water, drying, and solvent casting Piranha (3:1, H₂SO₄: H₂O₂) washed glass coverslips from 1% weight DMSO solutions of the dissolved colloids. Solvent cast films

were dried at ~50° C overnight. For cell contact angle measurements, monolayers of both cell types were formed on similarly prepared coverslips by culturing for 48 hours with their respective media. One group was then fixed with 2.5% glutaraldehyde for 30 minutes at room temperature and 2 hours at 5°C. The second group was allowed to air dry without fixation.[32] Both glutaraldehyde fixed and non-fixed monolayers were examined to determine whether cell surface components are washed away in the preparation process.[32] Samples were then rinsed with DI water and allowed to dry. Contact angles were measured using a Rame-Hart Inc. Model 100-00 115 NRL C.A. goniometer. The “slowly advancing contact angle method” was used.[33] Both sides of the droplet profile were measured as a second and third drop were placed on top of the first. An average of six values was taken to represent the contact angle of the drop. A flamed platinum wire (heated to a dull red glow) was used to apply liquid droplets of several diagnostic liquids, each to a different area of the material surface. The diagnostic liquids used and their theoretical surface energy components or “gamma values” are as shown in Table S1. Zisman plots were used to obtain the critical surface tension, γ_c (CST) in order to estimate the van der Waals character of microtissue components while vOGCT is used to estimate Lewis acid (γ^+) or Lewis base character (γ^-) as required by XDLVO using water/glycerol as polar liquids and n-bromo naphthalene as the apolar liquid. Collagen 1 and Matrigel® were measured on films of similar concentration and thickness as used in the cell experiments on tissue culture polystyrene (TCP).

Colloid Size, ζ -potential, Concentration, and Volume Fraction

Size and ζ -potential were obtained using a Malvern Zeta-sizer with a 640 nm laser. Dialyzed PU suspensions were diluted 100-200X by PBS. Averages were taken of 5 runs of 10 measurements each. For concentration measurements a Malvern NanoSight LM10 was used with similar dilutions as DLS measurements. Camera level, gain, and particle threshold detection were kept similar for each suspension and an average of 3 measurements from 90 s videos was taken. The volume fraction (ϕ) of the colloidal gels was then calculated according to EQ. 1 where a is the particle radius, N is the particle number concentration of the suspension, and V is the volume of the final centrifuged gel, obtained by estimating an equal volume of water into a separate 15 ml centrifuge tube. [34]

$$\phi = \frac{4}{3}\pi a^3 \frac{N}{V} \quad \text{EQ. 1}$$

This calculation assumed that all of the particles in the suspension were present in the centrifuged gel, a reasonable assumption as the supernatant was clear when looked at in a 40x bright field microscope. ζ -potential was obtained at similar concentrations to DLS measurements with 5 runs of 10 measurements and Smoluchowski theory for thin double layers was used for the calculations. Matrigel® ζ -potential was measured in 10 mM PBS (−20 mV) and collagen I was taken from the literature (−7 mV).[35] Values for HUVEC (−13 mV) and MCF-7 (−20 mV) were also taken from literature values.[36, 37]

Oscillatory Rheological Characterization

PU microgel suspensions were centrifuged at 3000 RPM for 5 minutes to form the colloidal microgels. Samples were loaded into a Malvern Bohlin C-VOR Rheometer using a 10 mm parallel plate geometry with a metal spatula. After sample loading, temperature was equilibrated to 37° C prior to performing a logarithmic amplitude sweep using stress control at 1 Hz. Frequency sweeps were performed using stress control with the lowest possible value from the linear region determined via the amplitude sweep. Low viscosity silicone oil was used to prevent evaporation of water from the hydrated samples. Elastic shear modulus (G') and loss modulus (G'') were plotted. For collagen I and Matrigel® rheology, samples were diluted to the concentrations used for cell experiments (0.33 mg/ml and 1.5 mg/ml respectively) and 50 μ l sample were allowed to gel between the rheometer plates for 30 minutes at 37° C, prior to testing.

Compucell3D Simulations

Microtissue spreading was modelled using the open source Compucell3D platform, a “cellular Potts” model which uses modified Metropolis dynamics.[38] This allowed the theoretical prediction of microtissue morphological parameters as measured contact relations, particle size, and cell type were varied according to EQ. S1 where each term can be accounted for in the Hamiltonian (H). Briefly, simulations consist of cells (green objects) and microgel particles (blue objects) which occupy multiple sites on a square lattice. This model accounts for stochastic, metabolically driven cell membrane fluctuations with amplitude governed by the temperature (T) through “index-copy” attempts to sample adhesive contacts with energy (J) through a Monte Carlo type process with Boltzmann acceptance probability, analogous to models of ferromagnetism. [39] The fractal dimension (D_f value) of simulated microtissue spreading was calculated using ImageJ (NIH) plugin FracLac. Additional details and images of zero hour time points (figure S1) of the simulations are included in the supplementary information.

Microtissue Preparation, Viability, Spreading, and Immunofluorescent Staining

The experimental process for microtissue assembly and spreading is shown schematically in figure 1. Colloidal suspensions were centrifuged and re-suspended in EC medium for HUVEC microtissues or CCM for MCF-7. Cell suspensions containing 50,000 cells were mixed with polymer suspensions giving a final concentration of 0.3 mg/ml wet weight of microgel. After mixing with a pipette, suspensions were centrifuged at 3,000 RPM for 5 minutes in a 1.5 ml centrifuge tube. Microtissues were allowed to mature in centrifuge tubes for 48 hours prior to seeding in either Collagen I or Matrigel®. Collagen I gels were prepared according to manufacturers’ instructions at a concentration of 0.33 mg/ml, while Matrigel® was prepared to a final concentration of 1.5 mg/ml by diluting with EC medium or CCM. Microtissues were then pipetted into the bulk of 100 μ l of the gel which had been allowed to gel at 37° C for 40 minutes and covered with EC medium or CCM. Microtissue spreading was observed using a bright field microscope for 72 hours. Circularity index (C.I.), migration distance, and D_f were calculated manually using imageJ and imageJ plugin FracLac. C.I. value of one denotes perfectly circular and C.I. value of less than one denotes less circular morphologies. Migration distance was defined as the distance between the

center and the furthest spread leading edge of the asymmetrically spreading microtissue. The skeletonized border of aggregates was measured using the sliding box technique. After the 72 hour time point microtissues were fixed using 4% formalin for one hour and after washing stained with DAPI and phalloidin to visualize the cell nucleus and actin cytoskeleton. Stained microtissues were examined using a Nikon fluorescent microscope and images were processed using the Nikon software. Rheological characterizations of tissues were performed by scaling up the amount of polymer and cells while maintaining their relative ratios. Characterizations were performed on the same day tissues were constructed to ensure cell viability was maintained in the larger, macroscopic constructs. Viability studies were performed on microtissues after 48 hours to ensure that PU microgels did not have a cytotoxic effect on either cell type. Table S2 shows the cell viability measurements at 48 hours from a 4 hour Alamar blue study, according to manufacturers' protocol. Viability across all groups was similar without significant difference. Therefore differences in spreading behavior of the microtissues could not be attributed to differences in cell viability.

Statistical Analysis

Data was presented as an average of microtissues from 5 different wells for each PU microgel group with standard deviations (figures 5, 6, and 7). Significance testing was performed using analysis of variance (ANOVA) with $p < 0.05$ and $p < 0.1$ post-hoc two-sided Fisher's test. Bars connecting groups which are significantly different are marked with a * $p < 0.05$ and # $p < 0.1$. Two p-values were used to analyze the trends for cellular response patterns.

Theoretical

Microgel/Cell Symmetrical Interactions

According to DLVO theory, colloidal stability between monodisperse particles of the same material is based on the balance between long-range attractive van der Waals forces and short-ranged electric double-layer repulsion.[40] The XDLVO theory accounts for the effects of hydrogen bonding and interaction with the solvent which is not considered by DLVO. [25] According to the XDLVO theory, the short-range contributions from Lewis acid-base interactions can outweigh the entropic repulsion of double-layers with a potential that decreases exponentially with separation of surfaces and which can be either attractive or repulsive depending on the relative H-bonding character of particles and solvent. Double-layer interactions between similar particles were modeled by EQ. 2 where, ϵ is the dielectric permittivity of free space, l is the separation distance between particle surfaces, R is particle radius, surface potential (Ψ) is equated with the ζ -potential, and the Debye length (k) of 10 Å was used for physiological conditions. [41] For Lifshitz-van der Waals forces γ_c was substituted for γ_i^{LW} according to EQ. 3. This was justified on the observed basis that apolar liquids with different surface tensions can give similar contact angle values on the same polymer surface (table S3 and S4). Hamaker constants were found according to EQ. 4 between symmetrical surfaces (1) in a medium (3) and the potential curves were found by EQ. 5. [42] Lewis acid-base components of PU (γ_s) were found by using EQ. 6 with contact angles and "gamma values" of two polar (water/glycerol) and one apolar (n-bromo naphthalene) liquid (γ_d) to solve three simultaneous linear equations.[43] Lewis acid/Lewis

base potentials are calculated according to EQ. 7 and EQ. 8. In EQ. 8 λ is the experimentally determined decay length of H-bonding interactions of ~ 0.6 nm while l_0 is the minimum equilibrium distance of the interaction (~ 1.6 Å).

$$\Delta G_I^{EL} = .5\epsilon R\psi_0^2 \ln[1 + \exp(-kl)] \quad \text{EQ. 2}$$

$$\gamma_i^{LW} = \gamma_c \quad \text{EQ. 3}$$

$$A_{11} = 1.8585 E^{-14} \gamma_i^{LW} \text{ and } A_{131} = (\sqrt{A_{11}} - \sqrt{A_{33}})^2 \quad \text{EQ. 4}$$

$$\Delta G_I^{LW} = \frac{-AR}{12l} \quad \text{EQ. 5}$$

$$\gamma_l(1 + \cos \theta_l) = 2(\sqrt{\gamma_s^{LW} \gamma_l^{LW}} + \sqrt{\gamma_s^+ \gamma_l^-} + \sqrt{\gamma_s^- \gamma_l^+}) \quad \text{EQ. 6}$$

$$\Delta G_{131}^{AB''} = -4(\sqrt{\gamma_1^+ \gamma_1^-} + \sqrt{\gamma_3^+ \gamma_3^-} - \sqrt{\gamma_1^+ \gamma_3^-} - \sqrt{\gamma_1^- \gamma_3^+}) \quad \text{EQ. 7}$$

$$\Delta G_I^{AB} = \pi R \lambda \Delta G_{l_0}^{AB''} \exp\left[-\frac{l_0 - l}{\lambda}\right] \quad \text{EQ. 8}$$

Cell-Particle/Extracellular matrix Interactions

When a colloidal system is asymmetrical, the equations from the field of “heterocoagulation” must be used. Generally double-layer forces as well as van der Waals forces can be attractive or repulsive in this case. [44] This theory holds in the case of cell-particle interactions across water and bulk protein phases, or in the case of mutually dehydrated interactions between cells and particles. We used EQ. 9 (particle-cell) and EQ. 10 (particle-ECM i.e. extraneous protein phase of either Collagen I or Matrigel®) the Hogg-Healy-Ferstenau equation for double layers between dissimilar particles and the equation for interaction between planar and spherical geometries, respectively.[7, 45] We also used the XDLVO equation, EQ. 11, for dissimilar solutes in a medium. Hamaker constants were found according to EQ. 12 and the van der Waals interaction for asymmetrical spheres according to EQ. 13. [46] Van der Waals interaction between a sphere and plate is found by multiplying EQ. 5 by 2.[42]

$$V(l) = \pi \epsilon_r \epsilon_0 \frac{R_1 R_2}{R_1 + R_2} \{ (\psi_{01} + \psi_{02})^2 \ln(1 + \exp[-kl]) + (\psi_{01} - \psi_{02})^2 \ln(1 - \exp[-kl]) \} \quad \text{EQ. 9}$$

$$V(l) = \pi \epsilon_r \epsilon_0 R \left\{ (2\psi_{01}\psi_{02}) \ln \left(\frac{1 + \exp[-kl]}{1 - \exp[-kl]} \right) + (\psi_{01} + \psi_{02})^2 \ln(1 - \exp[-2kl]) \right\} \quad \text{EQ. 10}$$

$$\Delta G_{132}^{AB''} = 2(\sqrt{\gamma_1^+ \gamma_3^-} + \sqrt{\gamma_2^+ \gamma_3^-} + \sqrt{\gamma_1^- \gamma_3^+} + \sqrt{\gamma_2^- \gamma_3^+} - 2\sqrt{\gamma_3^+ \gamma_3^-} - \sqrt{\gamma_1^+ \gamma_2^-} - \sqrt{\gamma_1^- \gamma_2^+}) \quad \text{EQ. 11}$$

$$A_{132} = A_{12} + A_{33} - A_{13} - A_{23} \quad \text{EQ. 12}$$

$$V(l) = -\frac{A}{6} \left(\frac{2R_1 R_2}{l^2 - (R_1 + R_2)^2} + \frac{2R_1 R_2}{l^2 - (R_1 - R_2)^2} + \ln \frac{l^2 - (R_1 + R_2)^2}{l^2 - (R_1 - R_2)^2} \right) \quad \text{EQ. 13}$$

In general, microtissue spreading can be interpreted by the total change in energy (G or V) of each phase where both bulk and surface changes are taken into account.

Results and Discussion

Polyurethane Microgel Surface Energy

As the soft or hard segment content is varied, the surface polyether (PEG) and ionic content of the PU microgels is changed resulting in increasing or decreasing surface van der Waals force and Lewis acid/Lewis base character which alters the aqueous stability. The contact angles of PU microgels and cells (monolayer) are shown in tables S3 and S4 while calculated surface energies are shown in table S5 according to EQ. 6. Zisman plots of all surfaces are also shown in Figure S2. According to the Zisman plots, PEG600-HDI-DTH, PEG1000-HDI-DTH, and PEG1000-NMDA-HDI-DTH were the most apolar with highest CST in the 30-40 dynes/cm range. PEG2000-HDI-DTH and PEG1000-DMPA-HDI-DTH had γ_c in the bioadhesive minimum (20-30 dynes/cm) of the Baier curve at 26 and 24.8 dynes/cm, respectively.[47] Measured CST likely correlated with quantity of surface PEG. Therefore lower CST is due to more surface exposed PEG and a higher quantity of bound water (water γ_c = 21.8 dynes/cm). Layers of bound water act as kinetically stable low energy barriers to colloidal pair-interactions. [48] We calculated Lewis acid/Lewis base character, according to vOGCT, using both water/glycerol and water/formamide polar liquid combinations (table S5 and S6) and found that with the former liquid pair, all PU were monopolar basic and with the latter pair all had a small Lewis acid component which was highest for PEG1000-NMDA-HDI-DTH and lowest for PEG1000-DMPA-HDI-DTH. Previous work has correlated electron donating character to a more negative ζ -potential. [49] Both calculations, combined, would suggest that the positively charged PEG1000-NMDA-HDI-DTH likely has highest ζ -potential and PEG1000-DMPA-HDI-DTH has the most negative ζ -potential.

For cell monolayers, Zisman plots indicated that HUVECs had lower CST of ~ 22 dynes/cm than MCF-7 (~26 dynes/cm) and were more polar as indicated by the more positive Zisman slope. [50] According to vOGCT, the water/glycerol combination showed that HUVEC had high Lewis acid character whereas MCF-7 were monopolar basic. However, the water/formamide combination showed higher Lewis acidity for the MCF-7. Since water/formamide combination gives higher Lewis acid character to protein surfaces while according to table S5, glycerol possibly predicts higher Lewis acidity for polysaccharides (agar control), it is possible that the cancer cell line has more surface protein and fewer surface polysaccharides which may be responsible for these differential interactions with diagnostic liquids. [51] Previous refractive index (RI) mapping studies have demonstrated the increased RI of cancer cells due to a hypothesized increased protein content. [52]

Size, ζ -potential, and Volume Fraction

Table 1 shows that the size of PU microgel particles varies from 450 nm (PEG2000-HDI-DTH) to 2 μ m (PEG600-HDI-DTH). Size of particles is a fundamental aspect of Brownian effects as well as stability in the equations of DLVO theory and XDLVO. Additionally GPC results showed that PEG600-HDI-DTH had the highest M.W. followed by PEG2000-HDI-DTH. Therefore particle size must have been a function of inherent polymer chain aqueous stability rather than polymer chain size alone, ζ -potential of all PU microgels was low (< 30 mV) likely due to the phenomenon of PEG shielding.[53] In support of this, previous work with DMPA chain-extendors with relatively hydrophobic soft-segment led to a much more negative ζ -potential.[54] It was noteworthy that PEG1000-NMDA-HDI-DTH had the highest potential, as predicted, and PEG1000-DMPA-HDI-DTH had the most negative potential. Volume fraction measurements revealed that, in general, colloidal gels which were formed from smaller particles ($D < 1.2 \mu$ m) led to lower volume fraction gels. However, this was not a linear correlation as there are numerous competing contributing factors to colloidal gel volume fraction.[55] PEG600-HDI-DTH and PEG1000-HDI-DTH had highest volume fraction (0.69 and 0.73 respectively) and may be randomly but closely packed due to the higher Peclet number (Eq. A.1) during shear-induced gel formation. This information was used to calculate interaction potentials and support rheological data.

Symmetric Interactions between Microgels

Analysis of symmetrical particle-particle interactions revealed that according to XDLVO theory, PEG600-HDI-DTH, PEG1000-HDI-DTH, and PEG1000-NMDA-HDI-DTH produced unstable aqueous dispersions with energy minimums of approximately minus 4, 3, and 1 kT respectively (figure 2.a). Whereas, PEG2000-HDI-DTH and PEG1000-DMPA-HDI-DTH both produced stable dispersions with long-range repulsive interactions of ~ 1 kT. For DLVO (figure 2.b), similar relative relations were obtained except that all unstable particles had deep energy minimums only which should lead to uncontrolled and irreversible coagulation. Since this was not the case (nor was it expected) it was evident that XDLVO predictions were more accurate in the case of PEG PU microgels. These results showed that a decreasing M.W. of PEG promoted aqueous instability while introduction of ionic character promoted aqueous stability (regardless of measured ζ -potential). In the case of the cells, HUVECs were predicted to be stable by 1 kT and MCF-7 unstable by minus 3 kT according to XDLVO theory. However, MCF-7 also had greater short ranged repulsion due

to monopolar basic-basic interactions (not shown). This prediction agrees with the higher ζ -potential of MCF-7 as well as the common finding of increased expression of negatively charged groups on cancer cell surfaces. [49, 56] DLVO theory gave similar predictions except that MCF-7 were much more unstable (-10 kT minimum). Greater long-range (van der Waals) attraction with increased short-range repulsion is consistent with a number of past observations of cancer cells. These observations include the faster attachment to glass than normal (aqueously stable) cells, the ability to migrate on apolar surfaces (long-range apolar-apolar interactions), and generally remaining more rounded on high energy surfaces than normal counterparts (greater short-ranged repulsion). [24, 57, 58]

Asymmetric Interactions between Cells/Microgels/ECM Phases

The asymmetric particle-cell interactions across water revealed that due to their stability HUVECs should repel all PU microgel particles (figure 2.c and d). However, in figure 2.d and e, MCF-7 had strong attractions to the unstable particles and repulsive interactions with stable particles. In particular, the strength of attraction was highest with PEGIOOO-HDI-DTH, followed by PEG600-HDI-DTH, and PEG1000-NMDA-HDI-DTH. In order to further examine possible interactions we also calculated the dehydrated interaction between cell and particle across vacuum (figure S3). These interactions represent the situation as vicinal water between cells and particles is displaced over time due to the entropic favorability.[59] This is important due to the fact that microtissues were allowed to mature for 48 hours prior to the experiments. It was determined that in the dehydrated interaction HUVECs (figure S3.a and b) had an attractive potential for unstable particles which was considerably less (~ 5 kT) than that of MCF-7 (figure S3.c and d) and a repulsive potential for stable particles. These asymmetric cell-particle interactions along with symmetric particle-particle and cell-cell interactions determine the sign of the internal microscopic energies which will enhance or resist microtissue spreading.

The ternary interactions between particles/cells and ECM phases were also calculated for both collagen I and Matrigel® (figure 3) using the equations for a plate and sphere. In addition to the interaction of PU microgel with ECM across water, the interaction between cells and particles with the ECM as solvent phase was also calculated. This would be the situation as cells and particles begin to spread into the semi-solid ECM phase. Figure 3.a and b shows the interaction potentials between cells/particles across water with collagen I while figure 3.g and h shows the interactions with Matrigel®. According to XDLVO theory PEG2000-HDI-DTH and PEG1000-DMPA-HDI-DTH (the microgels stable in aqueous medium) both had a repulsive interaction with the collagen I. The unstable particles were all attracted to the collagen I phase with PEG600-HDI-DTH having the highest attraction. This order of interactions was similar for the DLVO theory, except that PEG1000-DMPA-HDI-DTH was slightly attracted (~ 1 kT). For XDLVO particle interactions with Matrigel®, the relations were similar but quantitatively less, due to the lower y_c of Matrigel® compared to collagen I. DLVO theory gave different predictions due to the high measured ζ -potential of Matrigel®. Therefore PEG1000-NMDA-HDI-DTH was most attracted followed by PEG600-HDI-DTH. MCF-7 attraction to collagen I was much greater than that for Matrigel®. This is consistent with the DAH which states that not only should cancer cells be more cohesive than normal counterparts, they should also have greater attraction to stromal

tissue than basement membranes, causing them to migrate toward capillaries. In contrast to this, HUVEC had low attraction with collagen I and a small repulsion with Matrigel® that went to zero at about 10 nm separation.

In figure 3.c and d, collagen I represented the intervening medium between HUVECs and particles (figure 3.i and j for Matrigel®). The unstable particles all had repulsive maximums due to the repulsive van der Waals force between particles and cells in the protein phase which has γ_c intermediate between the two. This effect would be important if particles diffuse into the ECM phase first, producing a positive disjoining pressure across the intervening protein film, which tends to increase its thickness. Such a pressure could represent a back pressure against cell migration into the ECM phases. Contrary to this, the stable particles had attractive minimums between 10 and 20 nm, which would produce the opposite effect. DLVO theory had similar predictions (figure 3.j) except that the repulsive van der Waals forces were even stronger. In figure 3.e and f it was shown that MCF-7 microtissues displayed similar behavior except that PEG2000-HDI-DTH was closer to being repulsive, due to the matching γ_c with MCF-7. The effect of substituting Matrigel® (figure 3.k and l) for collagen I would be to reduce the attractions of the stable particles and increase the repulsive forces for the unstable particles, due to the lower γ_c of Matrigel®.

Rheological Characteristics of Colloidal Gels and Microtissues

Rheological data can support the particle size data theoretically through calculation of the Peclet number (Pe). The Pe of the particles at 1 Hz is shown in table 1 as calculated via EQ. A.1.[60] Higher Pe implies that hydrodynamics control the interactions and lower Pe number implies thermodynamic forces control the interactions.[61] Figure 4.a and figure 4.b shows the amplitude sweeps and frequency sweeps of the PU microgel colloidal gels. According to the amplitude sweeps, PEG600-HDI-DTH had the highest G' and G'' followed by PEG1000-HDI-DTH and PEG1000-NMDA-HDI-DTH, due to their larger size and aqueous instability. Whereas, the other two stable microgel particles had much lower G' and G'' with more liquid-like character. Additionally, frequency sweeps showed a lack of frequency dependence for the unstable particles and a higher dependence for the stable particles. This could be due to Brownian effects dominating at low frequency and hydrodynamics dominating at higher Pe number flow, whereas the unstable particles were too large for such a Brownian effect to dominate in the frequency range used. These observations, taken together, confirm both volume fraction data (higher ϕ = more pair interactions) and calculated interaction potentials of PU microgels.

In figure 4.c and figure 4.e amplitude sweeps as well as frequency sweeps (figure 4.d and f) of HUVEC and MCF-7 microtissues are shown. In addition, table S7 shows the G' and G'' as well as the ratio of G' to G'' in tabular form. Control, cell only MCF-7 microtissues had higher G' than G'' and G'' was higher than that of HUVEC control microtissues. The frequency sweeps showed that MCF-7 had solid-like character at low frequencies and more liquid-like character at higher frequency. The higher G' and G'' of MCF-7 could be linked with the calculated negative energy minimum which tends to induce aggregation of the cells and energy loss during flow. The low G' relative to G'' is linked to the higher short range repulsion of MCF-7 as they experience pair interactions under the organizing forces of shear.

For the bimodal microtissues, the PU microgel particles tended to generally decrease the bulk properties of MCF-7 microtissues while increasing those of HUVECs. For both cell types, however, PEG600-HDI-DTH produced a very strong tissue due to the irreversible coagulation of PEG600-HDI-DTH under the applied shear.

For MCF-7 microtissues, unstable particles caused microtissues to become exceedingly liquid-like in character with G'' dominating G' . This effect was much less for the HUVEC microtissues. For both cell types, mixing with stable particles resulted in a tissue of increased solidity compared to the unstable particles as seen in table S7. We interpreted these findings in light of recent work on depletion forces where the interaction potential between big (larger colloidal) and small (depletion force mediating) particles was predicted to modulate the depletion interaction.[62] For big-big (cell-cell) attraction combined with a big-small (cell-particle) attraction, a repulsion through attraction effect is obtained, which could be responsible for the liquidity of MCF-7 microtissues mixed with unstable particles. Additionally, big-big repulsions combined with small-small repulsions can enhance the classical attractive depletion force, which would be responsible for the solid-like tissue obtained with HUVEC mixed with stable particles. The probable bulk free energy changes of the microtissues were used in conjunction with measured surface properties to interpret microtissue spreading.

CompuCell3D Simulations of Microtissue Contact Relations

Simulations of microtissue spreading as well as calculated D_f values are shown in figure 5. A higher D_f value for a cancerous cell line may indicate increased invasive behavior while for HUVEC it may indicate sprouting or capillary forming behavior.[63] The J values used for contact relations for each binary (cell-particle) as well as ternary interaction (particle ECM) are shown in tables S8 and S9, as taken from the dehydrated interaction potentials between cells/particles and that across water for particle/cell/ECM interactions. The particle volumes and Lagrange multipliers were chosen relative to the measured particle sizes via DLS and the observed rheological properties of the colloidal gels. By reducing the strength of some of the plugins which have been previously used we were able to isolate the effect of the differential measured contact relations.

Figure 5.a and c shows that HUVEC microtissues with stable particles (PEG2000-HDI-DTH, PEG1000-DMPA-HDI-DTH) had higher fractal dimension borders ($D_f \sim 1.35$) of spreading than unstable particle microtissues ($D_f < 1.30$). Therefore, microgel particles with repulsive interactions increased cell-cell repulsion and the interaction of HUVEC with ECM tissues. Simplistically, particles with negative energy minimums segregated to the outside due to the greater attraction with ECM phases relative to HUVEC while stable particles which repel ECM phases segregated to the inside. This latter case led to increased sprouting and branching of HUVEC into the ECM phase due to the lower repulsion between HUVEC and ECM phases than the stable particles. PEG1000-NMDA-HDI-DTH microtissue simulations showed slightly increased D_f value above the control, however this difference was not statistically significant. This indicated that an instability more than minus 1 kT was required to produce a lower D_f value from the cell-only microtissues.

For MCF-7 (figure 5.b and d), opposite trends were observed and the unstable PU microgel particles caused an increased fractal dimension of the spreading border of microtissues. For instance, PEG1000-HDI-DTH and PEG600-HDI-DTH exhibited the greatest mean D_f value, which for the latter was statistically significant. The exception was PEG1000-NMDA-HDI-DTH which had no interaction (using hydrated potential) with MCF-7 and thus the tissue spread uniformly. Therefore MCF-7 microtissues with unstable particles tend to cause MCF-7 to segregate to the outside, due to the cohesive competition between cell-cell and particle-particle interactions. When mixed with stable particles, MCF-7 segregated to the inside due to their inherent tendency to flocculate and the absence of competitive $J_{\text{particle-particle}}$ interactions. These simplistic predictions were used as a guide to interpreting microtissue spreading in-vitro.

Microtissue Spreading in ECM Phases

Figure 6.a and b and figure 7.a and b show the spreading characteristics of HUVEC and MCF-7 microtissues respectively. For HUVECs in collagen I, stable particle microtissues had lower circularity (figure 6.c) than the unstable particle microtissues. D_f values (figure 6.e) were higher for stable particle microtissues which had rougher borders and more visible sprouting as demonstrated by the fluorescent images. This difference in means was statistically significant for PEG2000-HDI-DTH but not for PEG1000-DMPA-HDI-DTH microtissues. However, we also examined migration distance (figure 6.g) which showed that migration distance was significantly greater for PEG1000-DMPA-HDI-DTH than for PEG600-HDI-DTH and PEG1000-NMDA-HDI-DTH. We characterized the time dependent changes in morphological features in both protein phases (figure S4 and S5). The time dependent changes further highlight the stability with time of the morphological features of unstable-particle HUVEC microtissues and the increasing D_f values of stable-particle microtissues. The lack of extensive spreading which was observed for HUVECs mixed with unstable particles could be due to ECs segregating inside or the repulsive van der Waals forces which would exist between HUVECs and the unstable particles with collagen I as the solvent phase, as discussed. Combined with differential contact relations, according to the rheological data, the microgel particles stable in aqueous medium caused HUVEC microtissues to become more solid-like in character giving an unfavorable bulk G of spreading. However, the surface and colloidal interaction predictions clearly gave negative G of spreading and overcame this bulk factor as demonstrated by the in silico simulations, especially with increased time of culture.

For MCF-7 in collagen I, the unstable particle tissues had lower circularity (figure 7.c) and higher D_f values (figure 7.e) in opposition to the behavior observed for HUVEC microtissues. Migration distance (figure 7.g) was also significantly greater for unstable particles such as PEG600-HDI-DTH and PEG1000-NMDA-HDI-DTH than for stable particles such as PEG1000-DMPA-HDI-DTH. This outcome was predicted by the simulations. Exceptions to this trend (such as similar D_f value of PEG600-HDI-DTH and PEG1000-DMPA-HDI-DTH) could be explained by the different starting morphological features of the microtissues. For instance, it was observed that for the PEG1000-DMPA-HDI-DTH microtissues a luminal shape was present reminiscent of the differentiated acinar forming epithelial cells. Since this microgel was the most stable in water, it is possible that

the added stability as well as increased solid-like character (table S7) gave an overall interaction potential more similar to a differentiated cell type. Such reversion in transformed characteristics of MCF-7 can occur due to multiple factors that were discussed in the introduction. In keeping with this hypothesis, PEG1000-NMDA-HDI-DTH microtissues displayed elongated shapes reminiscent of a broken toroidal or “luminal” microtissue shape. It is possible that the increased interaction between MCF-7 and NMDA based microgels across water or in the dehydrated state caused flocculation and a lower ability of the cells to rearrange. The unstable particles, in general, gave an overall more disorganized appearance to the spreading microtissues as evidenced by the increased D_f values. The changing morphological features with time are shown in figures S6 and S7. The changing circularity and D_f values with time as well as bright field images confirm that unstable particle microtissues spread further with higher D_f values and in general had a higher initial D_f value of the microtissues. The negative bulk G contribution due to the increased liquidity of MCF-7 unstable particle microtissues would also contribute to increased spreading and rough-borders while the opposite would hold for stable particle microtissues.

In general, the trends in collagen I and Matrigel® were similar for both HUVEC (figure 6.b, d, f, h) and MCF-7 (figure 7.b, d, f, h), but the differences were more significant in collagen I. A possible reason for this is the increased interaction potential of cells and particles for collagen I, along with differential bulk AG of spreading in the ECM phases. Figure S8 shows that the Matrigel® had a much higher ratio of G' to G'' than collagen I at the concentrations used. The higher solidity of the Matrigel® phase along with its lower interaction potential could have acted as a barrier to microtissue spreading.

The Balance of Colloidal Forces Controls Microtissue Behavior

The use of microgels as a matrix component for microtissue development has significant relevance to reconstitute the physiological correct orientation of cell-matrix and cell-cell interactions. Our analysis showed that interface guided interactions between cells and a microgel derived matrix component is a guiding principle to define the assembly and subsequent stability of microtissues. Thus, this physical approach based on the DAH can be used to understand how cellular and matrix components of microtissues interact. The DAH has been proven using different experimental techniques for many different model systems. [5, 6, 64–67] However the thermodynamic aspect of the surface and colloidal energies involved is still obscure in terms of current competing theories of surface energy. For instance, a recent study used the DAH concepts to interpret the morphological characteristics of mixed EC-MSC microtissues plated on various polymer substrata. [68] However the intermolecular force characteristics of these interactions was not measured. In other studies, using simulations, empirically chosen potentials between cells have also been assumed. [69] We sought a combinatorial colloidal force approach to utilize a PU microgel family with tunable surface adhesive interactions to modulate microtissue assembly and behavioral characteristics. Through systematic variation of PU segmental composition, it was possible to extract the different components of interfacial interactions and to identify their roles in the assembly of cellular and matrix components of microtissues. Thus, this approach represents a possible frame work for controlling cell-self-assembly via modulating interaction potentials and depletion forces.

Our results showed that a differentiated cell type such as HUVEC has higher colloidal stability than cancer cell types such as MCF-7 which tends to aggregate. This result is in keeping with the well-known abnormal behavior of cancer cells. Therefore, interaction with stable versus unstable microgel particles gave opposite behaviors for both microtissue types. While there was some correlation with double-layer effects alone, the ζ -potentials were too low to explain particle stability overall and the DLVO and XDLVO H-bonding model provided greater insights. We used volume fraction measurements as well as rheological characterization and in silico simulations to support our contact angle studies of the PU and cells. This information qualitatively predicted the spreading characteristics of microtissues based on each cell type. Figure 8 demonstrates the trend of these experiments schematically. When mixed with particles stable in aqueous medium (higher surface PEG) cells displayed more differentiated characteristics (MCF-7 lumen/HUVEC capillaries) whereas unstable particles (more surface hard-segment) led to undifferentiated characteristics (MCF-7 spreading/HUVEC non-spreading).

The finding that MCF-7 regained a morphology reminiscent of differentiated epithelial cells when mixed with stable particles and undifferentiated characteristics such as increased spreading when mixed with unstable particles indicated that colloidal forces of nano or microparticles are important characteristics in nanomedicine and TE applications that are currently underappreciated. For instance a recent study showed that microvesicles released from tumors have been shown to disrupt normal epithelial morphology. [70] Whether this is due to payload molecules delivered, an alteration in the balance of colloidal forces, or some combination of both is still unknown. Furthermore, evidence from the well-known field of solid-state carcinogenesis has suggested that in rodent models bulk polymers produce tumors more often than bulk metals, however, the opposite relation holds for particulate matter of each substance.[26, 27, 71] The higher γ_c of metals relative to polymers could be related to the findings of this study where higher γ_c microgel particles induced undifferentiated characteristics of microtissues.

In the case of HUVECs, it was shown that a more differentiated morphology (sprouting/capillary networks) was also observed when mixed with stable particles as opposed to unstable particles where spreading is minimal. A simplistic view is that stable particles impart enhanced stability to the tissue as a whole that allow cells to rearrange into a differentiated morphology. However, we did not investigate whether specific biological changes were also a part of this process, which is likely the case. For instance, the influence of differential cell secreted ECM molecules within different PU microtissues was not examined and likely leads to complex effects. Furthermore, in-vivo cells typically interact with a thin basal lamina via specific integrin mediated interactions. The extent to which predicted XDLVO H-bonding interactions between microgels and cells can model this behavior is uncertain. Future studies utilizing contact angle analysis of integrin proteins and ECM proteins could determine whether XDLVO can predict specific (integrin-ECM) biological interactions as well (which depend on “specific” arrangements of polar groups). Therefore further studies are necessary to focus on both biological and colloidal force aspects of particle-tissue interactions. Such work can enhance understanding of the adverse effects of accumulation of fine particulate matter in organisms as well as guide possible future therapies for cancer and other pathological conditions.[28, 72] Overall, this study

provides significant understanding in establishing the role of interfacial adhesion in microtissue assembly where microgel based matrix components can differentially regulate the assembly and stability of a microtissue.

Summary and Conclusions

We demonstrated the use of biocompatible PEG based PU microgels as a matrix component of microtissues to regulate the assembly and spreading characteristics of both a differentiated (HUVEC) and cancer (MCF-7) cell type. Bimodal microtissues which incorporated stable colloidal particles imparted more differentiated morphological and behavioral characteristics while unstable particles had the opposite effect. The DLVO and XDLVO theories complimented one another in these studies. It was shown that XDLVO and the incorporation of H-bonding characteristics into the theory fit the PEG based PU microgel stabilities better than DLVO, due to the low measured ζ -potentials. This approach provides new insight into the design principles for microtissue formation from a physical understanding of interfacial interactions. Future work should involve characterization of a variety of different cell types with both conventional and novel nanoparticle or microparticles systems which have potential for regenerative medicine therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Research reported in this publication was supported by the National Institute Of Biomedical Imaging And Bioengineering of the National Institutes of Health under Award Number R03EB022201. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. This work was partly supported by SUNY MAM Network of Excellence funds. We thank Dr. Robert E. Baier for numerous technical discussions and access to contact angle goniometer, Calvin Cheah for synthesis of some polymers, Yuan Yuan for GPC results, Dr. Mark Swihart for access to Nanosight, Dr. Chong Chen for access to DLS and GPC, as well as Dr. Yun Wu for donation of MCF-7 cells.

Appendix

The Peclet number is calculated according to EQ. A.1.

$$Pe = \frac{6\pi\eta_m\dot{\gamma}a^3}{kT} \quad \text{EQ. A.1}$$

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Figure 1: Schematic representation of experimental design and approach:

PEG based PU structures with DTH chain extender and aliphatic HDI diisocyanate. Surface chemistry of microgels is varied by altering M.W. of PEG or using a binary chain-extender system with a 1:1 ratio of either positively charged NMDA or negatively charged DMPA molecules. Mixed cell-PU microgel microtissues are allowed to mature for 48 hours before embedding in ECM phase with Collagen I representing a stromal tissue model while Matrigel® representing a basement membrane protein mixture model.

Figure 2: Symmetric and Asymmetrical Spherical Interactions:

A) Symmetrical interactions between microgel particles calculated according to XDLVO and B.) DLVO theory C.) HUVEC asymmetrical sphere-sphere interactions across water calculated according to XDLVO and D.) DLVO theory E.) MCF-7 asymmetrical sphere-sphere interactions across water calculated according to XDLVO and F.) DLVO theory

Figure 3: Interaction of Cells with ECM Phases to Predict Ternary Interaction of Microtissue Spreading:

A.) Asymmetrical interactions between microgel/cell spherical particles and flat collagen I phase according to XDLVO theory and B.) DLVO Theory C.) Interaction between HUVEC and microgel particles with collagen I as solvent phase according to XDLVO theory and D.) DLVO theory E.) Interaction between MCF-7 and microgel particles with collagen I as solvent phase according to XDLVO theory and F.) DLVO theory G.) Asymmetrical interactions between microgel/cell spherical particles and flat Matrigel® phase according to XDLVO theory and H.) DLVO Theory I.) Interaction between HUVEC and microgel particles with Matrigel® as solvent phase according to XDLVO theory and J.) DLVO theory K.) Interaction between MCF-7 and microgel particles with Matrigel® as solvent phase according to XDLVO theory and L.) DLVO theory.

Figure 4: Rheological Characterization of Colloidal Gels and Microtissues:

A.) Colloidal gels formed from PU microgel suspensions amplitude sweeps B.) Colloidal gels formed from PU microgel suspensions frequency sweeps C.) HUVEC-PU microgel scaled up microtissues amplitude sweeps D.) HUVEC-PU microgel scaled up microtissues frequency sweeps E.) MCF-7 PU microgel scaled up microtissues amplitude sweeps F.) MCF-7 PU microgel scaled up microtissues frequency sweeps. Amplitude sweeps are performed at 1 Hz frequency and frequency sweeps at < 5 Pa stress

Figure 5: CompuCell3D Simulations of Microtissue Spreading:

A.) ECM phase is represented by the black background, smaller blue (or yellowish) objects represent PU microgel particles, and larger green objects represent cells. Initial spherical random mixtures of cells and particles randomly sample adhesive contact relations in a Monte Carlo (MC) type process for ~72 hours (time calculated from number of MC steps). Simulations snapshots are taken at 72 hour time point. Energetically favorable MC steps are accepted while energetically unfavorable steps are accepted with Boltzmann acceptance probability. B.) D_f value of HUVEC simulations at 72 hours C.) D_f value of MCF-7 simulations at 72 hours. Statistical comparison used ANOVA with Fisher's post-hoc test. Bars connecting groups which are significantly different are marked with a * $p < 0.05$ and # $p < 0.1$. Bars connecting groups with no symbol indicates no statistical difference.

Figure 8: Schematic Representation of Microtissue Assembly and Spreading:

Stable particles (PEG2000-HDI-DTH and PEG1000-DMPA-HDI-DTH) likely have more surface PEG as judged by contact angle measurements compared to unstable particles (PEG600-HDI-DTH, PEG1000-HDI-DTH, and PEG1000-NMDA-HDI-DTH) which likely have more surface hard segment. Mixing cells with stable particles led to microtissues that displayed overall more morphologically differentiated characteristics such as a luminal morphology and non-spreading behavior for MCF-7 and capillary network formation and spreading character for ECs. Unstable particles led to opposite behavior where MCF-7 microtissues displayed more spreading and disorganized appearance and EC microtissues did not spread. The opposite spreading and non-spreading behavior between ECs and MCF-7 when mixed with similar particles was due to the higher critical surface tension and different H-bonding character of MCF-7 compared to ECs. (example microtissue morphologies manually traced from bright field images)

Table 1:

Physical Properties of PU Microgel Colloidal Particles and Colloidal Gels

PU	PU Microgel Colloidal Dispersion					PU Microgel Colloidal Gel			
	M.W. (kDa)	Size (nm)	Concentration (#/ml)	Zeta potential, ξ (mV)	Volume Fraction (ϕ)	Elastic Modulus G' (Pa)	Loss Modulus G'' (Pa)	Peclet # @ 1 Hz	
PEG2000-HDI-DTH	60.8	470 $\pm$ 80	2.51E ¹¹	-5.3 $\pm$ 1.7	0.27	7.50	4.00	0.057	
PEG1000-HDI-DTH	57.0	1200 $\pm$ 250	2.16E ¹⁰	-9.0 $\pm$ 0.6	0.73	1500	530	0.950	
PEG600-HDI-DTH	90.2	2010 $\pm$ 300	1.61E ¹⁰	-7.0 $\pm$ 1.0	0.69	2525	1820	4.500	
PEG1000-DMPA-HDI-DTH	36.6	985 $\pm$ 290	8.73E ⁹	-11.0 $\pm$ 1.3	0.20	12.0	2.50	0.530	
PEG1000-NMDA-HDI-DTH	39.4	1100 $\pm$ 500	2.68E ¹⁰	-1.6 $\pm$ 1.0	0.25	562	72.0	0.730	

Concentration and size data are used to calculate volume fractions. Rheological data is taken from figure 4.