Hydrogels with Magnetic Fluorescent Nanoparticles for Tissue Engineering

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Hydrogels with Magnetic Fluorescent Nanoparticles for Tissue Engineering

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Abstract

The goal of this project is to develop a vehicle for a therapy treatment that increases stem cell-mediated tissue regeneration in bone defects. Tissue engineering has been viewed as a better strategy to repair bone defects because the patient's own tissue can be used to complete the regeneration process. Current strategies look to increase blood vessel regeneration within defects to accelerate the repair process. The proposed mechanism can be achieved by increasing vascularization and successfully integrating a short term treatment that enhances biological repair in tissue and non-healing bone defects.

The vehicle is a poly-(ethylene glycol) (PEG)-based hydrogel with iron-oxide nanoparticles and incorporated adhesive peptide (RGD) to serve as a cellular matrix that responds to external magnetic forces. These forces promote mechanical signals that are translated into biological responses that will theoretically increase vascularization and cell growth. The 3D displacement of nanoparticles within the hydrogel matrix was measured. The effect of different magnetic field strengths, emitted by external permanent magnets, on the gel deformation was tested. Results showed a direct correlation between hydrogel deformation and the magnitude of external magnetic forces applied to the hydrogel. Human Umbilical Vein Endothelial Cells (HUVEC) have successfully been integrated within the hydrogel matrix and are predicted to sense mechanical forces and to increase the rate at which they make networks for vascularization.

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Introduction

The American Academy of Orthopedic Surgeons state that bone defects and musculoskeletal conditions affect 1 in every 2 two Americans. These conditions are due to problems caused by aging, trauma, arthritis, and injuries[1]. New technologies in tissue engineering are being developed to use patient's own tissue to aid in the regeneration process, a process better known as tissue engineering. These strategies include increasing blood vessel regeneration within bone defects.

Non-healing bone defects result in a hostile microenvironment surrounding the injured tissue causing extreme ischemia and a loss of circulating cells that contribute to regeneration. Current tissue engineered constructs for injured bone regeneration suffer from complications based on the slow progression of endogenous vascular repair and often fail at mending the bone defect. For these reasons, procedures are being developed to increase blood vessel regeneration within the tissue engineered constructs to improve the natural repair processes[2]. Therefore, developing a therapy that increases vascularization and successfully integrates the construct with the biological tissue is critical to clinical outcomes in non-healing bone defects.

Cells are able to respond to mechanical stimuli from their microenvironment by translating the mechanical information into biochemical signals, a process known as mechanotransduction. Adult human mesenchymal stem cells (hMSCs) respond to mechanical properties of the extracellular matrix (ECM). These cells are specifically useful for the study of bone regeneration because of their osteogenic differentiation and vasculogenic potential making them enticing cells to study for bone regeneration.

Furthermore, when hMSCs are combined with the growth hormone, vascular endothelial growth factor (VEGF), there is an increase in the mineralization and integration of the scaffold

with the native bone[1]. Therefore, we hypothesize that by combining both approaches, mechanotransduction and the co-delivery of VEGF with implanted hMSCs in injured bone tissue, we will be able to promote angiogenic behavior where new blood vessels form

To reach the injured bone area, a synthetic hydrogel can be used as the main delivery system of the hMSCs and VEGF. Hydrogels are cross-linked networks of water soluble polymers that create a 3D structure whose properties can be manipulated to mimic the natural ECM, while overcoming the lot-to-lot variabilities of naturally-derived matrices. Magnetic nanoparticles can be incorporated within the network to create ferrogels. Ferrogels are used for many biomedical applications, including targeted chemotherapeutic drug delivery to tumors[3], as they can be directed to specific sites by external magnetic forces. Therefore, this study focuses on investigating how changes in the mechanical properties of VEGF-containing ferrogels due to external magnetic forces can guide the extent of osteogenic differentiation and vasculogenic potential in hMSCs.

We **hypothesize** that this synthetic platform technology can be adapted for increased vascularization and osteogenesis within the engineered hydrogel and differentiation of hMSCs when external magnetic forces are applied to iron-oxide nanoparticles introduced into the hydrogel matrix. These poly (ethylene glycol) (PEG)-based hydrogels exhibit significant advantages by allowing incorporation of adhesive peptides (RGD), improved cross-linking efficiency, and reaction time scales appropriate for in situ gelation and in vivo applications. Moreover, these hydrogels demonstrate minimal toxicity and inflammation in vivo and the degradation products are rapidly excreted via the urine, which are important for the safety and translational potential of these hydrogels[6]. The fibronectin-derived oligopeptide, RGD, will be chemically incorporated as the prototypical adhesive ligand, a protein network foundation for most cells and organs. Therefore, these synthetic materials offer a highly significant opportunity to establish supportive

microenvironments for stem cells to repair tissue under the influence of a simple and inexpensive permanent magnet.

Literature Review

A. Hydrogels

Hydrogels, as in *Figure 1*, are cross-linked networks of water soluble polymers that create a tissue-like structure whose properties can be changed by altering its composition. Properties may include gel kinetics, homogeneity, and cell adherence and cell toxicity for cells. These properties are manipulated to aid in the development of new technologies for tissue engineering. Current uses of hydrogels include diagnostics, cellular immobilization, separation of biomolecules or cells, and barrier materials to regulate biological adhesion. Hydrogels are biocompatible and can integrate into body tissues due to their high water content. In addition, because of their high permeability, hydrogels permit for efficient transport of solutes[4].

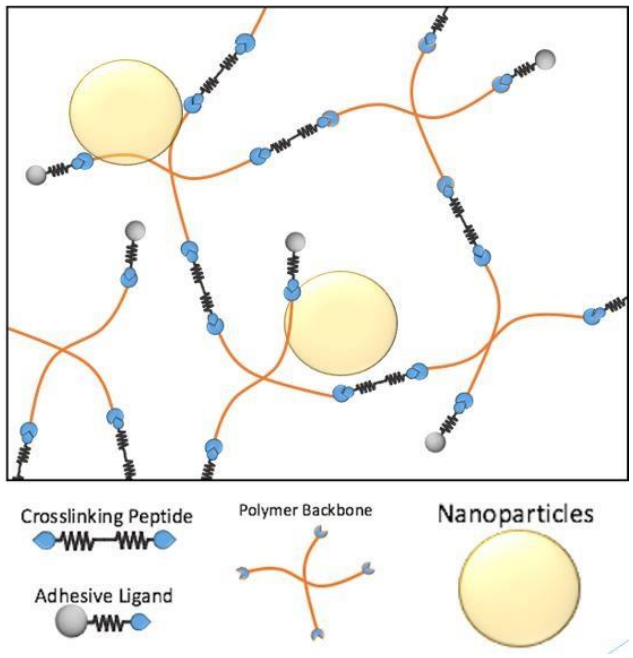


Fig 1: Hydrogel components and cross linking effect.

B. Magnetic nanoparticles

Magnetic nanoparticles have high in vitro and in vivo diagnostic applications because of their low interference with biological processes. They are small in size and do not retain magnetism after an external magnetic field is removed. Current applications in biomedicine include cell therapy, tissue repair, drug delivery, and magnetic resonance imaging (MRI), and other applications[5]. One of the main applications of magnetic hydrogels is for targeted chemotherapeutic drug delivery to tumors. This

application allows for tissue specific sites to be targeted by the drug coated particles. In turn, this reduces the potential toxic side effects on non-targeted tissue and increases the effectiveness of the drug on the patient. Another application is using hydrogels to target specific diseased tissue that can later be imaged with an MRI, enhancing the image contrast and increasing the overall accuracy of patient diagnostic[6].

C. Ferrogels

Hydrogel research incorporates magnetic nanoparticles within the network because of their specific properties and possible applications. Hydrogels that incorporate magnetic particles are known as ferrogels, because of the embedded iron oxide particles in the matrix[7]. The particles in the polymer network are attracted by the magnetic field that deforms the ferrogel and can control their shape and elasticity[6].

Studies measure the movement of these particles using computer simulations to test the deformation, elasticity, and magnetic response of ferrogels. Weeber and Holm used the software Extensible Simulation Package for Research on Soft matter systems, better known as ESPResSO, under two topographically different hydrogels, simple and diamond cubic structures. The software tracks the movement of the particles by calculating the position and orientation of other particles and tracing their movement. They proved that deformation response in a hydrogel is determined by a combination of factors, including the gel's degree of crosslinking and its elasticity[8].

D. Fast Iterative Digital Volume Correlation (FIDVC)[9]

The Franc Lab at Brown University developed a freely accessible MATLAB algorithm, known as the "Fast Iterative Digital Volume Correlation (FIDVC)" program [9]. It is capable of capturing large 3D non-linear deformation fields at a low computational cost. The program compares different images that correspond to the equal distances, but that have undergone deformation. It tracks these particles movement in the x, y, and z direction represented by displacement in u1, u2 and u3 as a graphical output from the program as seen in *Figure 2.* For example, in displacement component u3, greater deformation is exhibited by the blue pixels as they are moving towards the applied external force. No deformation is exhibited by the teal colored pixels. The yellow pixels are moving against the external influence. These pixel values are used for data analysis to determine the deformation exhibited in the z-stack images.

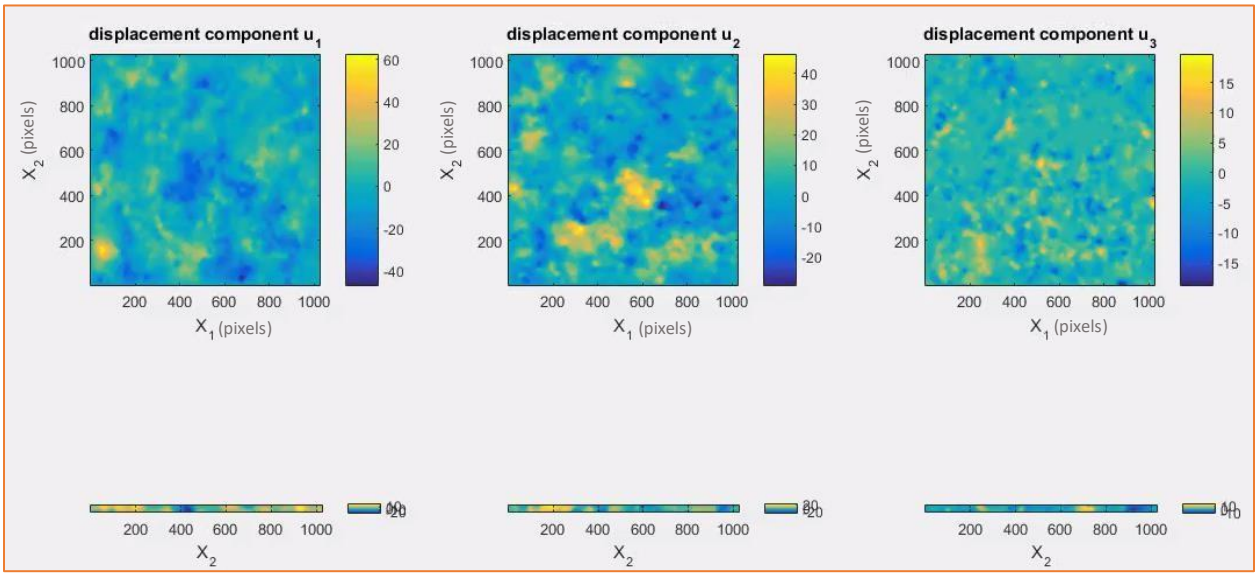


Fig 2: Fast Iterative Digital Volume Correlation (FIDVC) program output window that displays the deformation from an external permanent magnet as pixel movement according to an axis. Movement in the u1, u2, and u3 direction corresponding to the x, y, and z axis of the 3D stack images. The X_1 and X_2 axis are measured in units of pixels and correspond to the 2D side of the voxel imaged. These pixels contribute to a displacement of 12.068 µm/pixel.

E. Mechanotransduction

Mechanotransduction is the process in which cells are able to respond to mechanical stimuli from their M. Cells sense the stimuli and translate it into signals that regulate cell function and behavior. These forces are controlled by the cell and integrated in the tissue used to produce the final organism influencing the cell shape, proliferation, migration and apoptosis[10].

The purpose of this research is to engineer ferrogels and apply defined forces to them in order to study mechanotransduction events on cells. Applied forces will be determined from an applied magnetic field gradient that deforms the gel, which will be visualized by tracking the displacement of fluorescent particles in the gel. The research will establish the relationship among hydrogel properties, magnetic field strength, and gel deformation using microscopy and image analysis techniques. The ferrogels will work as a future vehicle to study cell mechanotransduction events.

Methods and Materials

The **objective** of the project is to engineer a synthetic PEG-based hydrogels with a fluorescently labeled iron-oxide component and incorporated adhesive ligand (RGD) that serves as a cellular matrix that responds to external magnetic forces as seen in *Figure 3*;

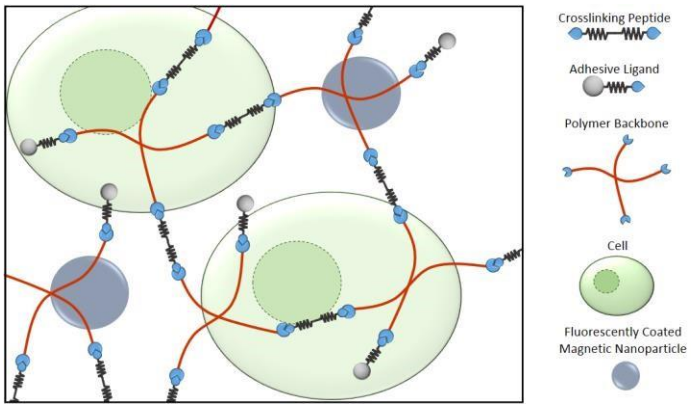
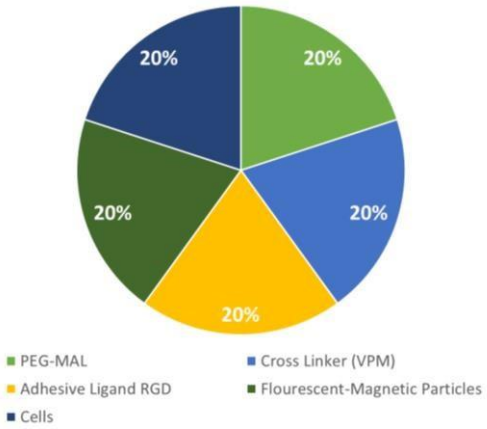


Fig 3: Hydrogel components and cross linking effect under the influence of fluorescent-magnetic nanoparticles.

Research Plan: To accomplish this, the following strategies are addressed to *Engineer a Poly (ethylene glycol) (PEG) hydrogel synthesis and fluorescent magnetic nanoparticle interaction. Hydrogel imaging for the determination of its deformation.*

a. PEG macromer (PEG-MAL, 20 kDa), GRGDSPC (RGD adhesive peptide, 1mM), and GCRDVPMSMRGGDRCG (VPM) cross-linker peptide, were used to create the hydrogels. PEG-MAL hydrogels (4.5% wt/v) were synthesized by reacting PEG-MAL with adhesive peptides, Human Umbilical Vein Cells (HUVEC), RGD, and with the magnetic nanoparticle solution. Followed by mixing with the VPM cross-linker at equal volume ratios, as seen in *Graph 1*, at the required concentrations



Graph 1: Graph distribution of total hydrogel components.

to obtain the desired final gel volume of 25 µL. All components were suspended in 10 mM HEPES buffer pH 7.3 to achieve desired concentrations.

b. Nano-screen MAG-UC/A iron-oxide (0.45 nM) blue fluorescent particles excited at 378 nm, with an emission of 413 nm and a 200 nm hydrodynamic diameter were used at different concentrations. A range of hydrogel weight/volume iron-oxide fluorescent particle concentrations were studied to determine the difference in deformation that they yield.

c. Model and 3D print using PLA Plastic, a non-magnetic stage for the confocal microscope in *Figure 4* to avoid interference between the external magnetic forces exerted by the permanent magnet and the ferrogels. Image hydrogels with a confocal microscope with a 60x objective.

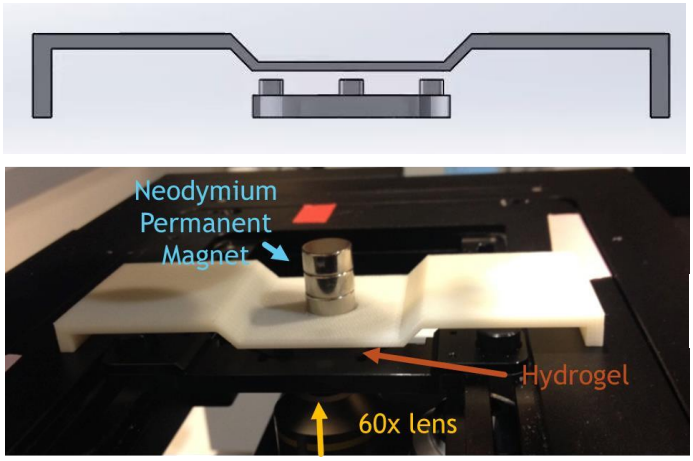


Fig 4: Non-magnetic Stage for confocal microscope **(A)** Solid Works design **(B)** 3D Printed design placed on confocal microscope.

d. Measure the 3D displacement exhibited by the hydrogel by running a Fast Iterative Digital Volume Correlation (FIDVC)[9] of a 24um thick set with 1um slices. FIDVC is a MATLAB based program created at Brown University that uses the images from two different displacements in the gels and measures their movement. The change in deformation was done by tracking the nanoparticle fluoresces at 378 nm excitation. Different field strengths with an increment in force from the number of Neudomyum permanent magnets (Sigma Aldrich) tested. This allowed us to determine the effect that magnetic field strength has on

gel deformation. For outcome measurements, the relationship between magnetic field strength, elastic modulus and gel deformation was determined.

e. Introduce HUVEC cells in the hydrogel matrix in order to determine the toxicity of the hydrogels with the fluorescent magnetic nanoparticles. Perform live-dead imaging on the hydrogels to determine their viability and how this changes over a period of time.

Results

A. FIDVC Particle Displacement Map

The Fast Iterative Digital Volume Correlation (FIDVC) program outputs a displacement map that tracks particle movements throughout the 3D stacked image or voxel. *Figure 5* shows the displacement map in the z-plane of the voxel. Greater hydrogel deformation is exhibited by the blue pixels as they move towards the applied external force. No deformation is exhibited by the teal colored pixels. The yellow pixels are moving against the external influence. These pixel values are used for data analysis to determine the deformation exhibited in the z-stack images. Each pixel corresponds to a specific size of 12.068 µm/pixel that was later used for the deformation analysis.

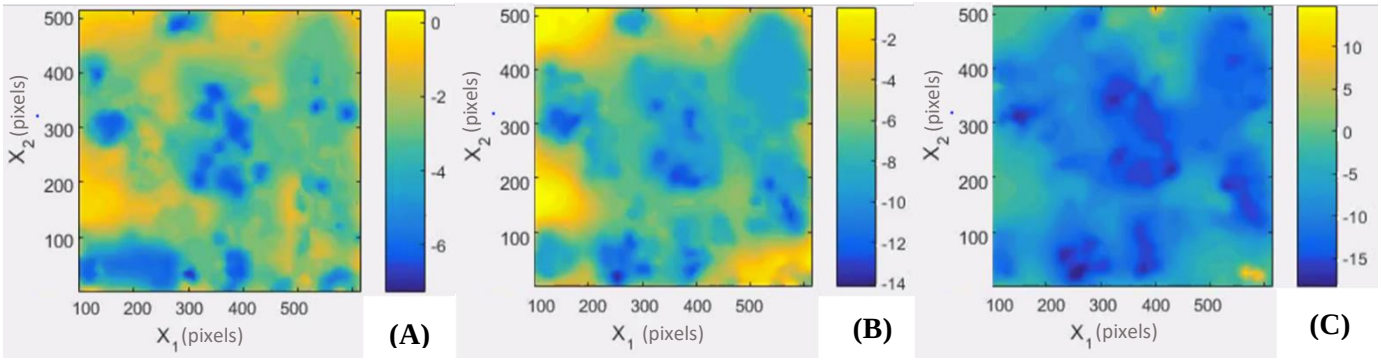
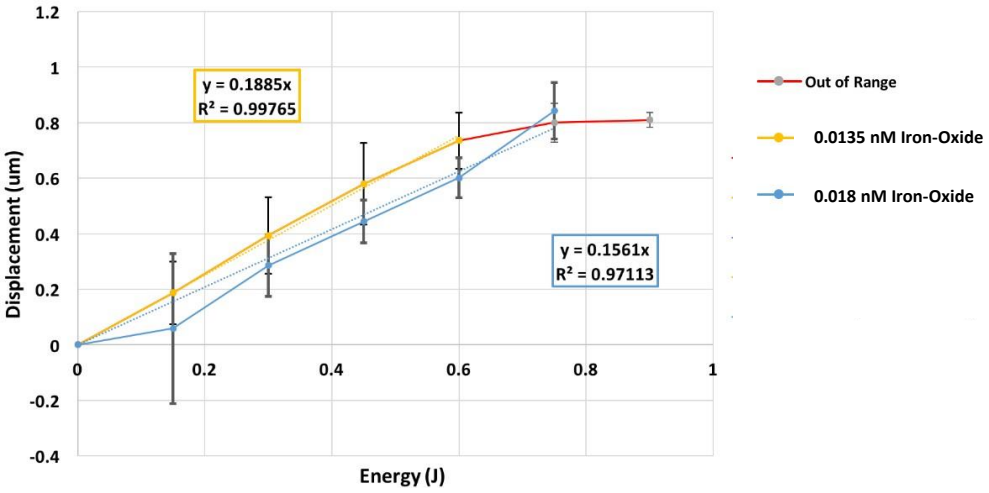


Fig 5: FIDVC program compares images at two different positions and plots its displacement. The X_1 and X_2 axis are measured in units of pixels and correspond to the 2D side of the voxel imaged with a displacement of 12.068 µm/pixel. An increase in the blue pixels indicates greater displacement towards the direction of the applied external force. As the number of magnets used increases, greater displacement exhibited, by the blue pixels, is represented in the map. **A)** Z-plane displacement with one magnet. **B)** Z-plane displacement with two magnets. **C)** Z-plane displacement with three magnets.

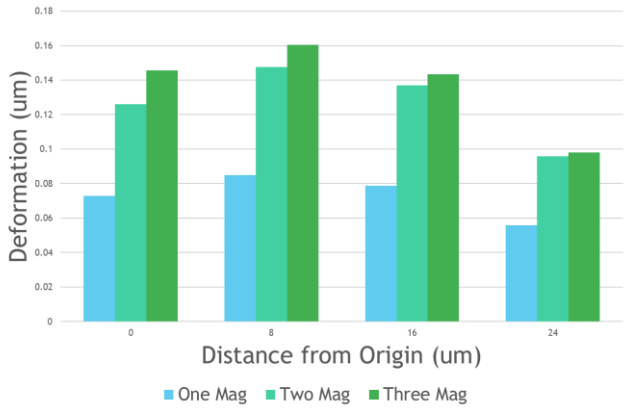
B. Iron-oxide fluorescent particle weight percentage.

Before the introduction of cells into the hydrogel, two magnetic particle molarities were tested, at a concentration of 0.0135 nM and 0.018 nM in the total gel volume to determine maximum deformation of the gel. An increase in the concentration of iron oxide in the hydrogel showed an increase in deformation of the gel of about 0.2 µm. These values were obtained from an analysis of the average displacements exhibited by the ferrogels. *Graph 2* compares the deformation exhibited by the 0.0135 nM and 0.018 nM iron oxide particle ferrogels. The permanents used are neudonyum permanent magnets with a maximum energy product of 247 kJ/m3. An increase in the amount of iron oxide in the particles and in the amount of magnets used (each contributing to about 0.15J of energy) increased the deformation of the hydrogel almost 0.2 µm. Both trend lines display goodness of fit with an R^2 of 0.99765 for the 4% of iron oxide.



Graph 2: Displacement vs. number of magnets. Displacement increases linearly with number of magnets for both 0.0135 nM and 0.018 nM iron-oxide particle concentration.

B. Effect of magnetic field strength.



Graph 3: Deformation vs. magnet deformation from origin. Displacement increases with an increase with number of magnets.

Magnetic field strength impacts gel deformation with an incremental correlation associated to the 3D displacement of the hydrogel. *Graph 3* represents the increase in deformation experienced by the hydrogel by increasing the force applied on the hydrogels by the external magnets. There is an increase of 0.08µm in deformation between the application of one magnet versus two magnets. This change in deformation decreases once the distance measured within the hydrogel surpasses 16 µm.

C. Effects of cells and magnetic particles.

The interaction between cells and magnetic particles will vary with different concentrations of magnetic particles introduced in the gel. The hypothesized scenario will use the smallest concentration of magnetic particles needed to cause deformation within the gel. This will ensure the deformation of a greater amount of cells without causing permanent damage to the cells. Live-Dead Stain Analysis will be performed on the hydrogels to determine if they can withstand the new conditions of their environment, before the addition of the external magnetic forces. The importance of this is to ensure that the cells are able to withstand their new microenvironment for vascularization and osteogenesis once implanted for in vivo applications.

Discussion:

Magnetic field strength modulated gel deformation with a differential effect to the 3D displacement of the hydrogel. The deformation values were quantified from the z-plane perspective because it measures the particle displacement in the plane where the external force was applied by the permanent magnet. Increasing the number of magnets, causes greater hydrogel deformation. As seen in *Figure 5*, the increase in blue pixels indicate greater particle displacement towards the direction of the applied external force. The pixel intensities from the displacement maps were used for the image analysis in determining the different effects of the magnets. After the use of 4 magnets, the displacement of the particles begins to plateau in *Graph 2*. This is most likely the result of the particles being out of range for the program to track. The use of three permanent magnets proved to cause the largest deformation in the hydrogel without being out of range of the 3D stack for the program to track as seen in *Figure 5*. There is an increase of 0.08μm in deformation between the application of one magnet versus two magnets.

The application of HUVEC cells into the matrix will also interfere with the deformation experienced within the gel. Once both processes have been mended together, we will be able to determine if the force of an external magnet causes an increase in deformation of the hydrogel. *Fig 5.* provides graphical representation of the displacements. Displacement is exhibited by the pixels represented in the graph that shifts towards the magnet. Increasing the amount of magnets applied to the hydrogels exhibit a visible change in the z-direction expressed by the increase in blue pixels on the graph.

Conclusion and Future Work:

To conclude, we were able to target the goal in the objective by building a nonmagnetic stage, iterating through different hydrogel engineering and designs and finally processing and analyzing the data obtained from the FIDVC program. The hypothesis was proven by confirming that magnetic field strength affects gel deformation with an incremental correlation associated to the 3D displacement of the hydrogel.

Future work involves continuing the set out experiments by commencing phase two of the investigation by introducing hMSCs and VEGF in a co-delivery introduced into the engineered hydrogels to promote vascularization in vivo. Other experimental variations that will be done during the next phase of the experiment include changing the polymer density of the PEG-MAL to promote a more rigid environment, changing the magnetic nanoparticle density to find the highest concentration of particles that are not toxic for the cells. The overarching goal will be to provide potential therapeutic options for stem cell-mediated tissue regeneration by increase the degree of vascularization for bone repair.

For the introduction of hMSCs and VEGF a trans-well will be used that allows for interaction between the hMSCs and the HUVEC cells, without physical contact. The HUVEC cells are endothelial cells and they can be used to form networks with other cells, in this case the hMSCs. These networks increase the angiogenic potential of the cells and thus their ability to vascularize and form blood vessels from previous vessels. If this experiment satisfies the hypothesis, *in vivo* studies will be performed by implanting these hydrogels on a critically sized bone defect on a mouse. Ultimately, this study will provide potential therapeutic options for stem cell-mediated tissue regeneration and increase the degree of vascularization for bone repair, classifying the

proposed plan as a highly innovative research project on the capabilities of mechanotransduction and vascularization.

References:

1. American Academy of Orthopedic Surgeons. (2016, March 1). One in two Americans have a musculoskeletal condition: New report outlines the prevalence, scope, cost and projected growth of musculoskeletal disorders in the U.S. . *ScienceDaily*. Retrieved November 15, 2016 from www.sciencedaily.com/releases/2016/03/160301114116.htm
2. García, J. R., & García, A. J. (2016). Biomaterial-mediated strategies targeting vascularization for bone repair. *Drug Delivery And Translational Research, 6*(2), 77-95. doi: 10.1007/s13346-015-0236-0
3. Abdeen, A. A., Lee, J., Bharadwaj, N. A., Ewoldt, R. H., & Kilian, K. A. (2016). Temporal Modulation of Stem Cell Activity Using Magnetoactive Hydrogels. *Advanced Healthcare Materials*. doi: 10.1002/adhm.201600349
4. Hoare, T. R., & Kohane, D. S. (2008). Hydrogels in drug delivery: Progress and challenges. *Polymer*, 49(8), 1993-2007. doi: http://dx.doi.org/10.1016/j.polymer.2008.01.027
5. Lu, W., Ling, M., Jia, M., Huang, P., Li, C., & Yan, B. (2014). Facile synthesis and characterization of polyethylenimine-coated Fe_3O_4 superparamagnetic nanoparticles for cancer cell separation. *Molecular Medicine Reports*, 9(3), 1080-1084. doi: 10.3892/mmr.2014.1906
6. Vatta Laura, L., Sanderson Ron, D., & Koch Klaus, R. (2006). Magnetic nanoparticles: Properties and potential applications *Pure and Applied Chemistry* (Vol. 78, pp. 1793).
7. Cezar, C. A., Kennedy, S. M., Mehta, M., Weaver, J. C., Gu, L., Vandenburgh, H., & Mooney, D. J. (2014). Biphasic ferrogels for triggered drug and cell delivery. Advanced Healthcare Materials, 3(11), 1869-1876. doi: 10.1002/adhm.201400095

8. Weeber, R., Kantorovich, S., & Holm, C. (2015). Ferrogels cross-linked by magnetic particles: Field-driven deformation and elasticity studied using computer simulations. *The Journal Of Chemical Physics*, 143(15), 154901-154901. doi: 10.1063/1.4932371

9. Bar-Kochba, E., Toyjanova, J., Andrews, E., Kim, K.S., Franck, C., "A Fast Iterative Digital Volume Correlation Technique for Large Deformation Measurements", Experimental Mechanics, 2014.

10. Iskratsch, T., Wolfenson, H., & Sheetz, M. P. (2014). Appreciating force and shape—the rise of mechanotransduction in cell biology. *Nature Reviews. Molecular Cell Biology*, 15(12), 825-833. doi:10.1038/nrm3903