

Migration of Bone Marrow Derived Stem Cells in a Dilute Fibrin Matrix on Cartilage and Meniscus



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Abstract

Mesenchymal stem cells (MSCs) have the ability to differentiate into all mesenchymal cell types and the potential to self-renew, making them ideal for use therapeutically in tissues with limited innate healing capacity and high incidence of injury, such as cartilage and meniscus. The use of a fibrin gel carrier to deliver MSCs to the site of injury makes clinical use practical by allowing the appropriate amount of time for cells to bind to damaged tissue while keeping cells at the desired location. Fibrin gel must provide adequate attachment to tissue while allowing cell migration out of the carrier onto the tissue defect, making concentration an important factor in effective delivery of cells. Additionally, the suspension of fibrin gel in platelet-rich plasma (PRP) may enhance the migration of cells by incorporating platelet-derived cytokines, producing a chemotactic effect. In this study, varying concentrations of fibrin gels suspended in plasma or PRP were seeded with bone marrow derived equine MSCs genetically modified with green fluorescent protein (GFP) and placed on equine cartilage and meniscus tissue explants. Migration patterns of MSCs were studied using fluorescence microscopy. The ability of differing concentrations of fibrin gels to bind to tissue defects was compared macroscopically. Preliminary data shows fibrin concentrations as low as 25% bind strongly to tissue without compromising viability of MSCs. These results will allow further development of MSC-seeded fibrin gels as a clinical treatment.

Introduction

- MSCs show promise as a clinical treatment for joint defects due to their pluripotent character, allowing differentiation into damaged tissue types.
- It is hypothesized that use of a vehicle to deliver cells directly to joint defects would allow arthroscopic insertion of MSCs without need for more invasive surgery. Autologous fibrin gel has been used successfully in the clinical setting, making it an ideal vehicle for placement of stem cells in defects.
- Fibrin gels have previously been used at high concentrations. The ability of cells to migrate out of the carrier onto tissues may be jeopardized by the firmness of the fibrin gel once set.
- The following studies were performed to analyze the biomechanical ability of dilute fibrin matrix to both bond to tissues and allow migration of cells.
- The addition of platelet-rich plasma was analyzed to determine if further migration of cells would result from the presence of platelet-derived chemokines.

Materials and Methods

Cell Culture

- Bone marrow derived mesenchymal stem cells were harvested from the sternum and iliac crests of mature horses.
- Cell monolayers were cultured and expanded over 3-4 passages under aseptic conditions until 70% confluence was reached.

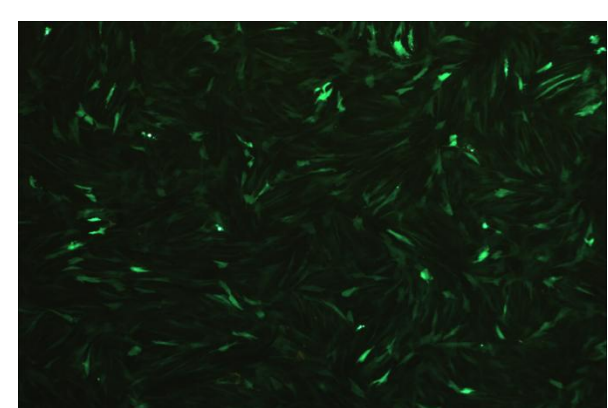


Figure 1. MSCs 3 days post-transduction with AAVGFP when visualized by fluorescence microscopy at 4x magnification.

- Transduction of cells with adeno-associated viral green fluorescent protein (AAVGFP) serotype 2 was performed for 4 hours in transduction media at a 4000 virus particle per cell concentration.
- Transduced cells were cultured in supplemented α -MEM for 3 days prior to seeding into fibrin gels and incubated at 37 °C.

Autologous Fibrinogen Precipitation

- Whole blood was collected from mature horses and centrifuged to remove red blood cells.
- Ice-cold absolute ethanol was added to the resulting plasma and allowed to precipitate for 30 minutes on ice.
- Precipitated fibrinogen was pelleted by centrifuging and the supernatant was aspirated.
- Fibrinogen pellets were resuspended in 200 μ l cell-seeded plasma after warming to 37 °C.
- From this stock solution, 50% and 25% concentrations were made by dilution in plasma or PRP.

Tissue Explant Preparation and MSC-Seeded Fibrin Gel Placement

- Cartilage and meniscus were harvested from the equine femoropatellar joint and defects were created with a 6 mm biopsy punch.
- Fibrinogen and bovine thrombin (0.14 KU/mL) were combined at a 1:1 ratio using a Duploject® syringe system, allowing direct placement of fibrin gel into defects. The resulting gels contained $\sim 10^6$ MSCs/ mL.
- Visualization of cells was performed with an Olympus IX70 fluorescent microscope equipped with an NB filter with an excitation of 470-490 nm and emission >515 nm at 24 hours, 48 hours, and 7 days post-seeding. Tissue explants were covered in supplemented α -MEM and incubated at 37 °C between time points.

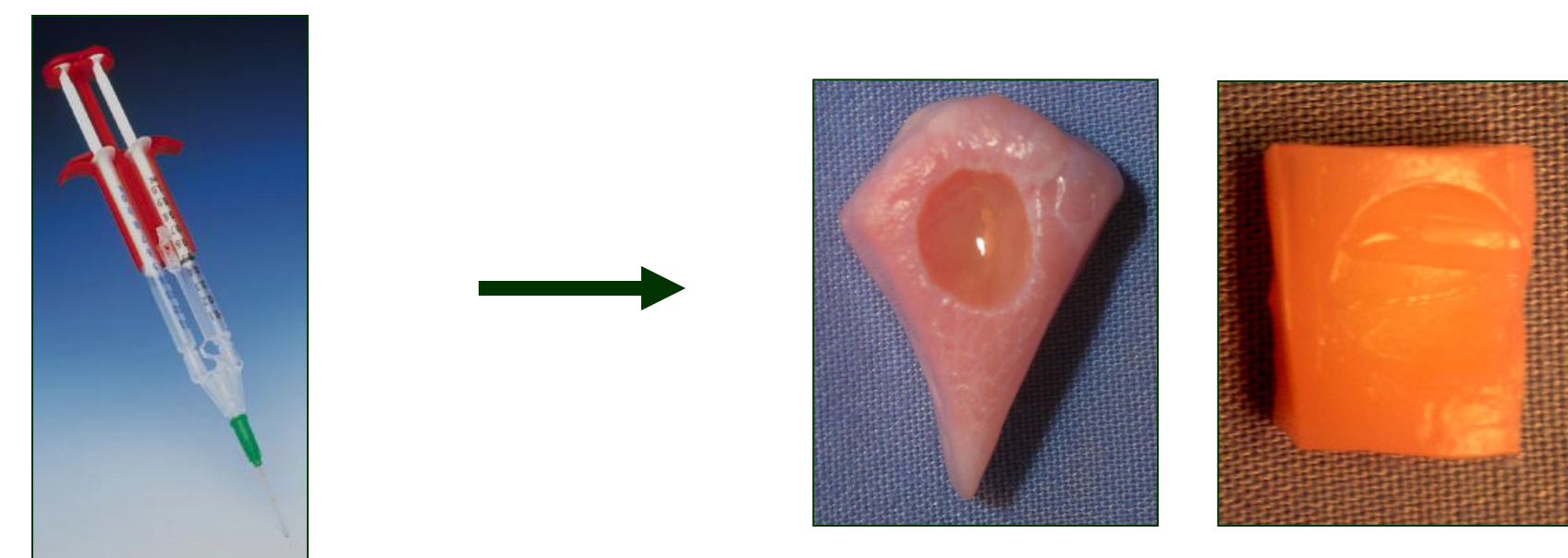


Figure 2. A Duploject® syringe system was utilized to simultaneously place and mix equal ratios of autologous MSC-seeded fibrinogen and bovine thrombin in meniscus (left) and cartilage (right) tissue defects.

Results

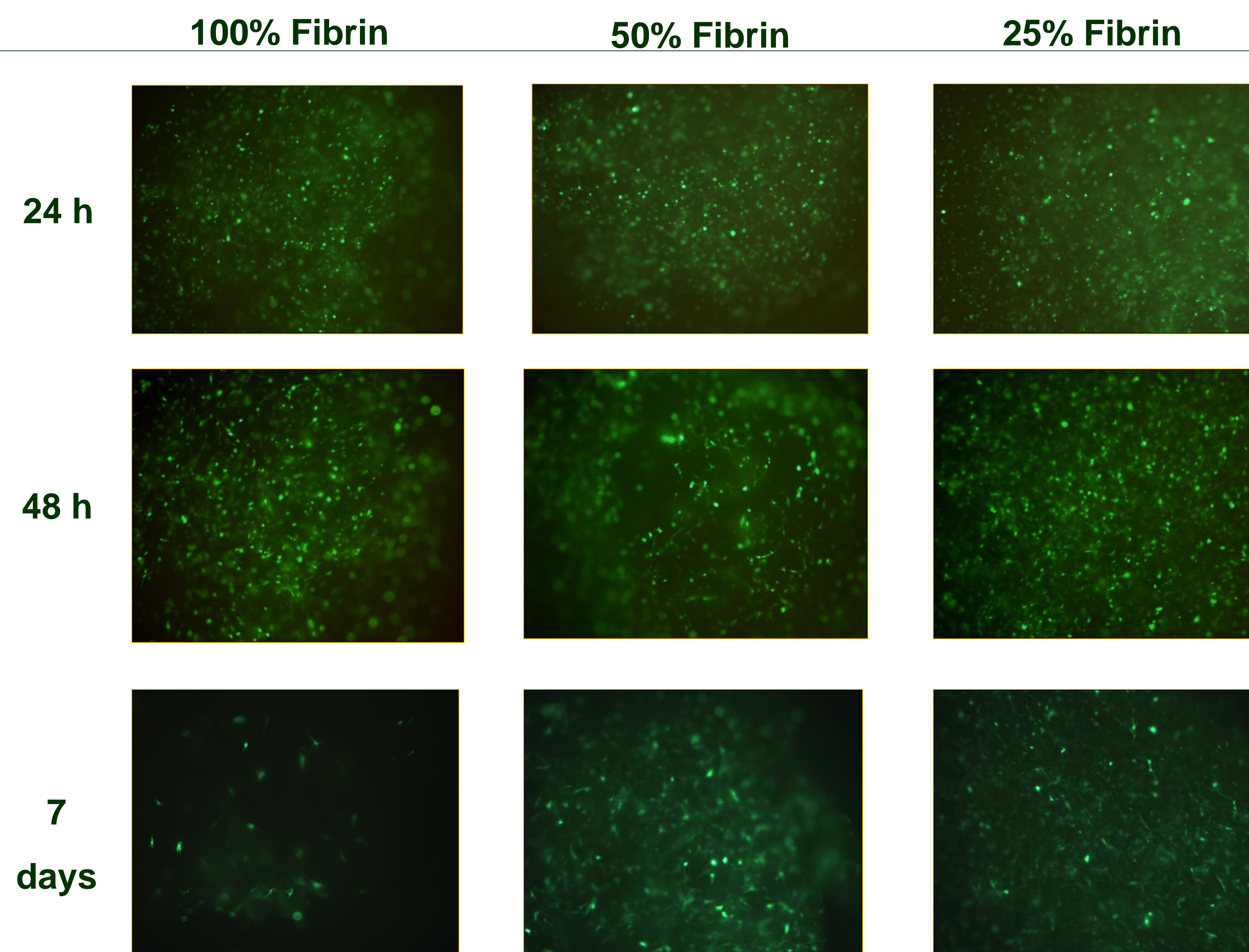


Figure 3. Fluorescence microscopy imaging at 4x magnification of AAVGFP genetically modified MSCs on cartilage defects demonstrated viability of cells at 24 h, 48 h, and 7 day time points. Though migration beyond borders of the tissue defects was not observed with any fibrin concentration, cells were able to live and change morphology within the fibrin gel environment regardless of concentration.

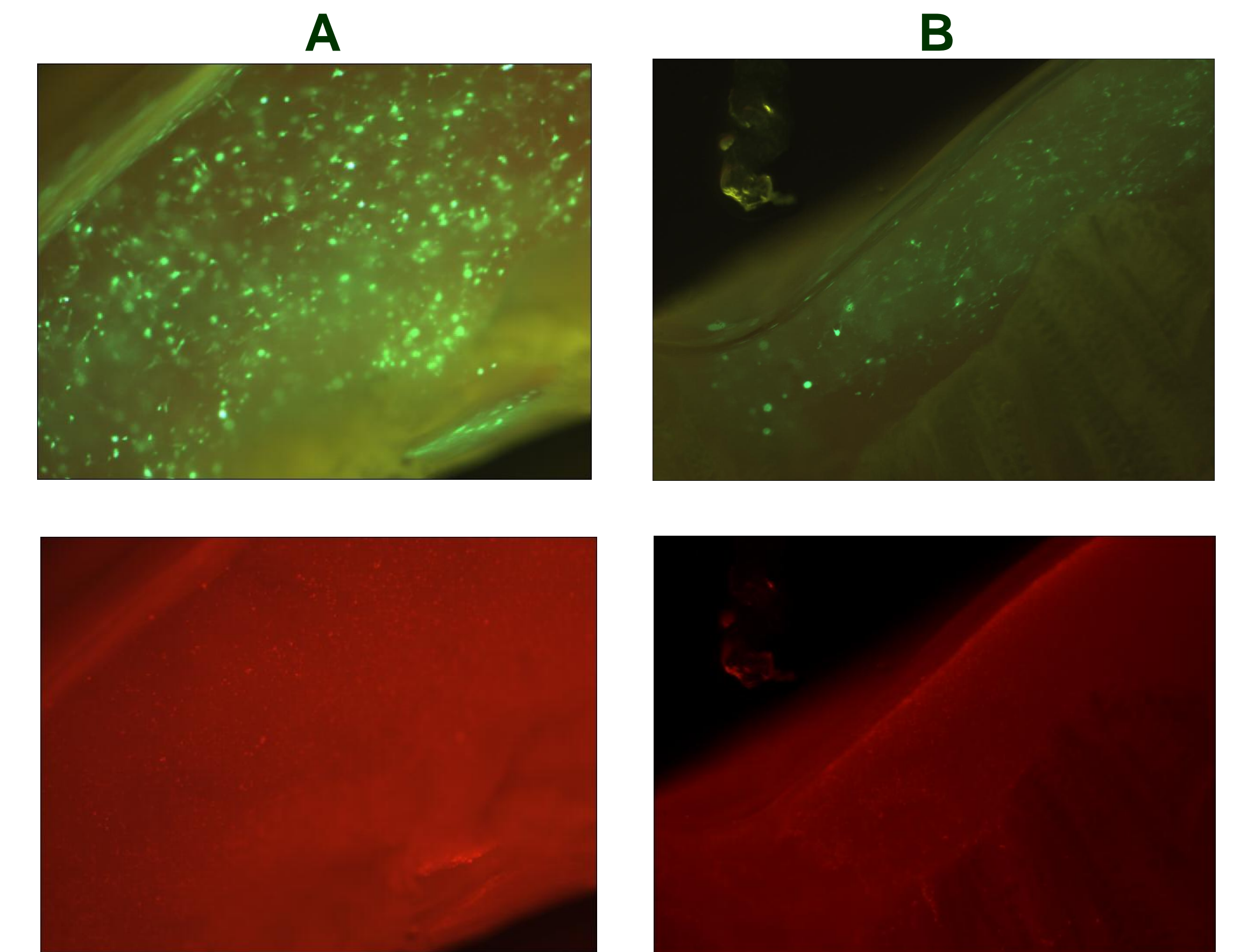


Figure 4. Cross-sections of meniscus tissue explants were cut and stained with ethidium bromide at 48 h (column A) and 7 day (column B) time points to visualize live/dead cells with fluorescence microscopy at 4x magnification. Though an observable number of cells were visualized as dead (red stain), many more appeared viable at both time points (green fluorescence).

Discussion and Conclusion

- 100%, 50%, and 25% fibrin gel concentrations remained bound to the tissue defect surface for 7 days despite repeated media changes. The 25% concentration was observed to set into gel form within seconds of application while allowing even coverage of the tissue defect, making it easier to handle than 100% concentrations, which hardened instantaneously and often became difficult to remove from the syringe needle.
- All fibrin concentrations appeared to be an acceptable environment for maintaining viability of cells over a 7 day period. However, migration of MSCs onto parent tissue was not demonstrated.
- It is hypothesized that 25% dilution of fibrin gel may still be too rigid to allow adequate migration of MSCs on to tissue surfaces. However, the biomechanical ability of the dilute gel to bind to tissues indicates further dilution without sacrifice of gel integrity may be possible.
- Though preliminary studies showed no observable effect of PRP on cell migration, further investigation is needed before reliable conclusions can be drawn.
- Future studies will test lower concentrations of fibrin gels, aiming to determine the best dilution to provide both gel integrity and cell migration. Biomechanical properties of gels prepared using alternative diluents such as phosphate buffered saline will also be examined.

Acknowledgements

- Major funding for this project was provided by the Merck-Merial Summer Research Program.
- The authors would like to thank the CSU Equine Orthopaedic Research Center barn staff for providing assistance in collection of research materials.
- Coordination of the Merck-Merial Summer Research Program at Colorado State University was graciously provided by Dr. Terry Nett and Dr. Colin Clay.