

Effect of scaffold dilution on migration of mesenchymal stem cells from fibrin hydrogels

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Objective—To evaluate the effect of fibrin concentrations on mesenchymal stem cell (MSC) migration out of autologous and commercial fibrin hydrogels.

Sample—Blood and bone marrow from six 2- to 4-year-old horses.

Procedures—Autologous fibrinogen was precipitated from plasma and solubilized into a concentrated solution. Mesenchymal stem cells were resuspended in fibrinogen solutions containing 100%, 75%, 50%, and 25% of the fibrinogen precipitate solution. Fibrin hydrogels were created by mixing the fibrinogen solutions with MSCs and thrombin on tissue culture plates. After incubation for 24 hours in cell culture medium, the MSCs that had migrated onto the tissue culture surface and beyond the boundary of the hydrogels were counted. This procedure was repeated with a commercial fibrin sealant.

Results—Hydrogel-to-surface MSC migration was detected for all fibrin hydrogels. Migration from the 25% autologous hydrogels was 7.3-, 5.2-, and 4.6-fold higher than migration from 100%, 75%, and 50% autologous hydrogels, respectively. The number of migrating cells from 100%, 75%, and 50% autologous hydrogels did not differ significantly. With commercial fibrin sealant, the highest magnitude of migration was from the 25% hydrogels, and it was 26-fold higher than migration from 100% hydrogels. The 75% and 50% hydrogels resulted in migration that was 9.5- and 4.2-fold higher than migration from the 100% hydrogels, respectively.

Conclusions and Clinical Relevance—MSC migration from fibrin hydrogels increased with dilution of the fibrinogen component for both autologous and commercial sources. These data supported the feasibility of using diluted fibrin hydrogels for rapid delivery of MSCs to the surface of damaged tissues. (*Am J Vet Res* 2012;73:313–318)

Bone marrow–derived MSCs are considered to possess outstanding potential for use in healing tissues. Enthusiasm for use of MSCs as a therapeutic modality in orthopedic medicine has been motivated because MSCs can differentiate along numerous connective tissue lineages.¹ These results raise the possibility that MSC transplantation into damaged or diseased tissues during orthopedic repair may lead to neotissue formation and recovery of function. Additionally, MSCs are capable of producing bioactive factors that may stimulate native cells within the damaged tissue to participate in repair.² These data have stimulated both research and clinical evaluations to better understand the potential of MSCs to aid in healing tissues.

The preponderance of evidence that MSCs improve healing in veterinary medicine has involved clinically affected horses. Clinicians have injected autologous, undifferentiated, and culture-expanded MSCs into tendons, ligaments, and damaged joints and achieved suc-

ABBREVIATIONS	
FBS	Fetal bovine serum
FRP	Fibrinogen-rich precipitate
MSC	Mesenchymal stem cell

cessful outcomes.³ Treatment with MSCs has been most successful for tendon and ligament injuries, whereby MSC injections resulted in a return to work for a greater number of horses than would have been expected following conventional management.^{4–8} Similarly, the effect of intra-articular joint injections with MSCs has yielded promising results for return to athletic activity as determined at least 6 months following injection.⁴ The emergence of such data is likely to increase MSC use in equine patients.

Despite evidence supporting the use of MSCs to aid in the healing of orthopedically repaired tissues, the optimal method for administering cells for a given condition has not been determined. Implantation of MSCs in fibrin in horses with experimentally induced chondral defects resulted in increased chondrogenesis at 1 month but no difference at 8 months, compared with results for control horses.⁹ Direct intra-articular injection of MSCs in equine femorotibial joints with lesions of the menisci, meniscal ligaments, or cruciate ligaments has positive long-term results.⁴ In horses

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with experimentally induced arthritis,¹⁰ intra-articular injection of an adipose-derived stromal vascular fraction resulted in no beneficial changes but increased the concentration of the proinflammatory cytokine tumor necrosis factor- α in the synovial fluid of treated horses, compared with that of untreated control horses. In the same study,¹⁰ intra-articular injection of bone marrow-derived MSCs decreased the concentration of prostaglandin E₂ in the synovial fluid of treated horses relative to that in control horses, but this benefit is considered insufficient for clinical use of MSCs. Results of clinical and experimental studies are important to better define the potential of MSCs to aid in the healing of tissues. Concurrently, investigations into novel approaches for delivering MSCs to damaged tissues may lead to additional alternatives for administering MSCs to stimulate healing.

The early success reported for intra-articular injections of MSCs suggests that engraftment of cells on the surface of tissues can be sufficient to stimulate healing. In the study reported here, methods for highly localized delivery of MSCs were evaluated by exploring the potential of fibrin hydrogels to serve as a carrier scaffold. Fibrin-based materials generated from autologous blood or obtained from commercial sources¹¹ have been used extensively for medical applications. Originally used as an acellular tissue sealant,¹² fibrin hydrogel has also been considered for use in cartilage,^{9,13} bone,¹⁴ ligament,¹⁵ and tendon¹⁶ tissue engineering. Mesenchymal stem cells encapsulated in fibrin hydrogels maintain viability and proliferate.^{17,18} Fibrin hydrogel can support chondrogenesis of encapsulated MSCs *in vitro*¹⁹ and *in vivo*⁹ and has been used as an injectable component for seeding MSCs within porous scaffolds for cartilage²⁰ and bone²¹ tissue engineering, in addition to serving as a template for neotissue accumulation. It has also been found that MSCs possess the capability to migrate out of hydrogels into the pores of natural coral,²² the subcutaneous tissue of rats,²³ and the skin of humans²⁴ over a period of weeks. These data suggest fibrin hydrogels may be useful as a carrier scaffold for delivering MSCs to the surface of damaged tissue.

Dilution of fibrin hydrogels results in the production of a more open and homogenous fibrin network, compared with characteristics of undiluted constructs.¹⁸ On the basis of this observation, we hypothesized that a hydrogel with reduced fibrin content would be more conducive to MSC migration, thus modulating the ability of MSCs to migrate from the fibrin carrier to the surface of damaged tissue. To test this hypothesis, we used an *in vitro* method to evaluate MSC migration from various fibrin hydrogel formulations to a tissue culture surface over 24 hours.

Materials and Methods

Samples—Whole blood and bone marrow samples were collected from 6 horses (age 2 to 4 years) that were euthanized for reasons unrelated to the study. Before the horses were euthanized, 50 mL of whole blood was collected from each study horse in syringes containing 6.3 mL of citrate phosphate dextrose adenine-1^a; these samples were used for fibrinogen isolation (blood collection for these horses was approved

by the Institutional Animal Care and Use Committee of Colorado State University). After the horses were euthanized, bone marrow was aspirated from the iliac crest and used for MSC isolation (institutional animal and care and use committee approval was not necessary because the bone marrow aspirate was collected after horses were euthanized).

Autologous fibrinogen precipitation—Autologous fibrinogen was isolated from plasma by use of an ethanol precipitation technique.²⁵ Blood was allowed to separate overnight at 4°C, after which the plasma was centrifuged at 1,000 × g for 15 minutes to remove all remaining RBCs and WBCs. Plasma supernatant was collected and stored at -80°C. For each experiment, an FRP was collected by the addition of 0.88 mL of 100% ethanol to 5 mL of plasma, reaction of the mixture for 30 minutes on ice, and centrifugation at 1,500 × g for 15 minutes. The supernatant was aspirated, and the FRP pellet was warmed to 37°C and resuspended in 200 mL of fresh plasma.

MSC isolation and expansion—Mesenchymal stem cells were isolated in tissue culture by culturing the plastic-adherent fraction of nucleated bone marrow cells for 6 to 8 days in Dulbecco modified Eagle medium^b supplemented with 10% FBS^c until MSC colonies were evident. Colonies were then trypsinized and culture-expanded by seeding modified Eagle medium^b plus 10% FBS and 2 ng of fibroblast growth factor-2^d/mL at a density of 10⁴ MSCs/cm² until cells were grown to approximately 70% confluence. This culture technique has been used to obtain MSCs with strong chondrogenic potential.²⁶ Each MSC population was culture-expanded through 2 to 3 passages prior to seeding into fibrin hydrogels.

MSC encapsulation in fibrin hydrogels—Mesenchymal stem cells were encapsulated in autologous fibrinogen solutions containing 100%, 75%, 50%, and 25% of the resuspended FRP. The dilutions were created by adding PBS solution to the FRP samples. Mesenchymal stem cells were suspended in a solution of bovine thrombin^e (110 National Institutes of Health antithrombin U/mL reconstituted in 40mM CaCl₂) at a concentration of 15 × 10⁶ MSCs/mL. Hydrogels were created for each dilution by mixing 10 mL of the diluted FRP solution with 10 mL of the MSC-thrombin solution on the surface of a 12-well tissue culture plate, which resulted in a final cell concentration of 7.5 × 10⁶ MSCs/mL. Two hydrogels were cast in each well, with 3 wells/fibrin concentration. Fibrin hydrogels were covered with 2 mL of modified Eagle medium supplemented with 10% FBS and incubated at 37°C and 5% CO₂ for 24 hours.

Solutions with 100%, 75%, 50%, and 25% of a commercially available fibrin sealant^f (lyophilized human fibrinogen reconstituted in apoprotein fibrinolysis inhibitor; concentration, 75 to 115 mg/mL) were also created. Hydrogels were created for each dilution by mixing 10 mL of diluted commercial fibrin sealant with 10 mL of MSC-thrombin solution in the same manner and cell concentration as those used for the autologous hydrogels. The autologous

and commercial products were tested by use of cells from separate cultures.

For each experiment, a portion of MSCs that were not encapsulated in fibrin were returned to tissue culture flasks at a concentration of 10^4 cells/cm² and cultured overnight for purposes of obtaining a standard curve for the quantification assay.

Quantification of MSC migration—Migration of MSCs was quantified by collecting and counting the cells that migrated from the fibrin hydrogels and attached to the adjacent tissue culture surface. All processing was conducted by the same individual (BWH). Each well was washed with magnesium and calcium-free PBS solution. Seventy-five milliliters of 0.25% trypsin-EDTA^b was pipetted onto the surface of each well at a location adjacent to the fibrin hydrogels to minimize contact between the trypsin and the surface of the hydrogels. The trypsin was carefully pipetted to ensure that the area immediately surrounding each hydrogel was exposed to trypsin. After exposure to trypsin for 30 seconds, each well was inspected for detachment of the migrating cells by use of a light microscope. Trypsin was neutralized at the first sign of detachment by the addition of 1 mL of medium containing 10% FBS. The medium was collected and centrifuged for 5 minutes at $100 \times g$. The number of collected cells was quantified by use of a viable cell kit^s designed for the detection of low cell numbers. Centrifuged cells were resuspended in modified Eagle medium with 10% FBS (vol/vol) and 20% (vol/vol) of a solution contained in the viable cell kit and then seeded into wells of a 96-well plate. Each well was inspected to confirm that cell-seeded hydrogel fragments were not collected during trypsinization. Cells from monolayer cultures were trypsinized and seeded in parallel at 8 serial dilutions to generate data for a standard curve. After incubation for 12 to 16 hours, the wells were analyzed in a fluorescent plate reader at absorption and emission wavelengths of 570 and 600 nm, respectively. For each assay, a second-order fit to the standard curve resulted in a correlation coefficient of 0.999.

Protein analysis of the fibrinogen solution—Samples of the 100% autologous fibrinogen solution and plasma from each horse were saved for total protein analysis with a bicinchoninic acid protein assay.^h Volume of the FRP was calculated by use of the following equation:

$$V_{\text{FRP}} = V_{\text{T}} - V_{\text{P}}$$

where V_{FRP} is the volume of the FRP, V_{P} is the known plasma volume, and V_{T} is the measured total volume. The protein concentration of the FRP was calculated by use of the following equation:

$$C_{\text{FRP}} = ([C_{\text{T}} \cdot V_{\text{T}}] - [C_{\text{P}} \cdot V_{\text{P}}]) / V_{\text{FRP}}$$

where C_{FRP} is the protein concentration of the FRP, C_{T} is the protein concentration of the resuspended fibrinogen solution, and C_{P} is the protein concentration of the plasma alone.

Cell morphology and viability—Morphological characteristics of encapsulated and migrated MSCs were evaluated by use of differential interference microscopy at the start and end of each migration experiment. Viability of MSCs was subjectively evaluated at the end of each experiment by incubation of MSCs in a calcein-ethidium bromide solution^b for 1 hour, which was followed by evaluation with a fluorescent microscope.

Experimental design and statistical analysis—Autologous fibrinogen experiments were conducted by use of MSCs and plasma from all 6 horses. Commercial fibrin sealant experiments were conducted with MSCs from 4 of the 6 horses. Each data set was analyzed with the donor horse as a random effect. Post hoc comparisons between fibrin concentrations were performed by use of a mixed model on the logarithmically transformed data with the Kenwood-Roger method for estimating the denominator *df*. Because the experiments were conducted at different times with nonidentical cell populations, we did not perform comparisons between the autologous and commercial fibrin sources. Statistical softwareⁱ was used for analysis of the data. Values of $P < 0.05$ were considered significant.

Results

Cell morphology in fibrin hydrogels—In all autologous and commercial fibrin sealant hydrogels, viable MSCs were seeded evenly. Immediately after encapsulation, MSCs were round and easily identifiable as individual cells when viewed with differential interference contrast microscopy (Figure 1). After the 24-hour incubation period, most embedded MSCs were viable. In most cases, the cells assumed a more linear morphology and appeared to form an interconnected network of cells, as observed for 50% autologous and commer-

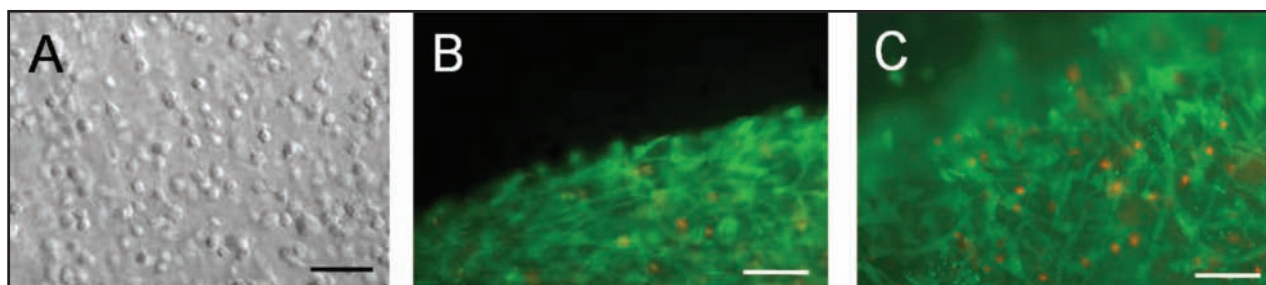


Figure 1—Photomicrographs depicting the morphology and viability of representative equine MSCs in fibrin hydrogels. Immediately after encapsulation (time 0), MSCs appeared round and evenly distributed throughout the hydrogels (unstained light microscope image from 50% autologous hydrogel; A). After culture for 24 hours, viable cells labeled with calcein (green) assumed a more linear morphology within autologous (B) and commercial fibrin sealant (C) hydrogels. Bar = 50 μm .

cial fibrin sealant hydrogels, respectively. The MSCs in 100% commercial fibrin sealant spread less than did MSCs in the diluted and autologous hydrogels, with round cells still evident after the 24-hour culture period (data not shown).

Autologous migration—Hydrogel-to-surface migration was detected for all autologous fibrin dilutions. In the 25% dilution, MSCs migrated around the entire perimeter of the hydrogel and were oriented in a

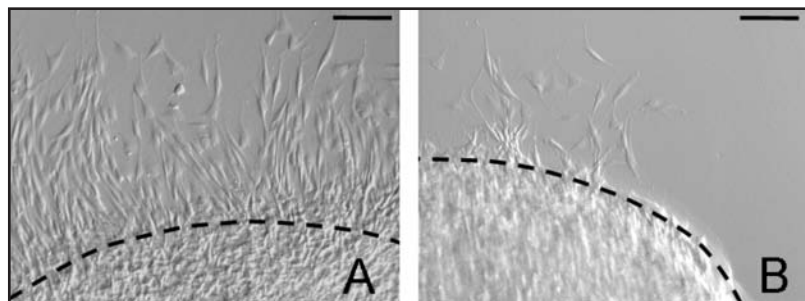


Figure 2—Unstained light photomicrographs depicting the morphology and patterns of MSC migration from autologous hydrogels. The MSCs migrating from a representative 25% hydrogel assumed a linear, spindle-shaped morphology; migration was uniform around the perimeter of the hydrogel (A). Migration from the 50%, 75%, and 100% hydrogels was less uniform, with areas of moderate and no migration (representative 50% hydrogel; B). In each panel, the black dotted line indicates the edge of the hydrogel. Bar = 100 μ m.

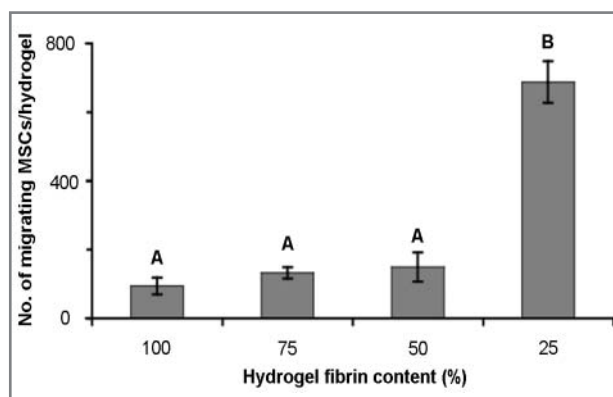


Figure 3—Quantification of MSC migration from autologous hydrogels. Values reported are mean \pm SE ($n = 6$ horses). ^{A,B}Values with different letters differ significantly ($P < 0.05$).

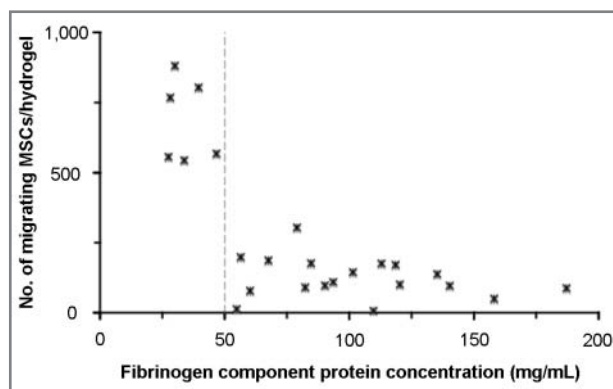


Figure 4—Mesenchymal stem cell migration after culture for 24 hours on the basis of hydrogel protein concentration. Each point represents migration for 1 horse. There is an apparent threshold to high-magnitude migration at approximately 50 mg/mL (vertical dashed line).

radial manner (Figure 2). In the 50%, 75%, and 100% hydrogels, migration was more sporadic, with areas of appreciable migration punctuated by areas of little or no migration (50% hydrogel). The mean number of MSCs migrating from the 25% hydrogels was 7.3-, 5.2-, and 4.6-fold higher than the number migrating from the 100%, 75%, and 50% hydrogels, respectively (Figure 3). The number of migrating cells did not differ significantly among the 100%, 75%, and 50% autologous hydrogels. The mean \pm SE FRP protein concentration of the 100% hydrogels was 137.3 \pm 30.2 mg/mL. Plotting mean migration per dilution for each horse against the continuum of FRP protein concentrations created by dilution revealed that there was high-magnitude migration when the FRP concentration was < 50 mg/mL (Figure 4).

MSC migration for the commercial fibrin sealant—Dilution of the commercial fibrin sealant yielded significant differences in the amounts of hydrogel-to-surface migration of MSCs across all dilutions (Figure 5). The greatest migration was from the 25% hydrogels and was 26-, 9.5-, and 4.2-fold higher than the migration in the 100%, 75%, and 50% hydrogels, respectively.

Discussion

In the study reported here, we evaluated the effect of fibrin concentration on MSC migration from autologous fibrin hydrogels and a commercial fibrin hydrogel in vitro by quantifying migration of encapsulated MSCs onto tissue culture plastic. In all dilutions tested for both fibrin sources, there was detectable hydrogel-to-surface migration of MSCs over the 24-hour culture period. Furthermore, it was possible to alter the degree of MSC migration out of fibrin hydrogels by diluting the fibrinogen component prior to cell encapsulation and polymerization with thrombin, with greater migration of MSCs from hydrogels with lower fibrin concentrations.

For autologous fibrin hydrogels, migration did not increase incrementally with successive dilution; instead, it was subject to a threshold-like response. Specifically, MSCs had a marked increase in the ability to migrate from the hydrogel and onto the plastic culture surface at an FRP protein concentration < 50 mg/mL. Although there were some differences in the protein concentration of the fibrinogen isolates from the 6 horses used for the study, the 25% dilution was sufficient to reduce the FRP protein concentration to < 50 mg/mL in all samples. Because fibrin content greater than this value did not result in significant increases in MSC migration, compared with the migration for undiluted hydrogel, these formulations would not be expected to rapidly deliver high numbers of MSCs to damaged tissues. This apparent threshold may be useful in determining the required dilution for fibrinogen isolated by means other than ethanol precipitation, which could result in a different FRP protein content.²⁷

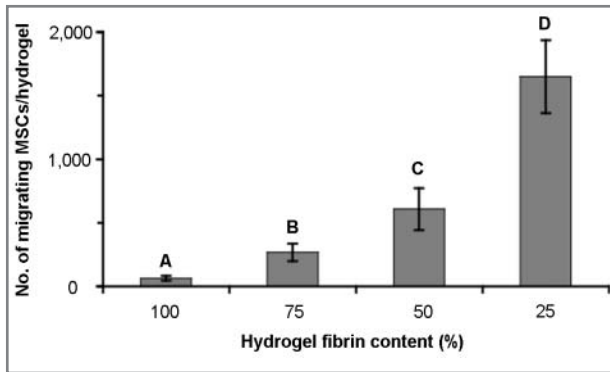


Figure 5—Quantification of MSC migration from commercial fibrin hydrogels after culture for 24 hours. Values reported are mean \pm SE (n = 4 horses). ^{A-D}Values with different letters differ significantly ($P < 0.05$).

Similar to results for the autologous hydrogels, the highest amount of MSC migration in the commercial fibrin sealant was from the 25% dilution. In contrast to results for the autologous product, significant increases in migration were detected for each successive dilution. This sensitive response of MSC migration to the commercial fibrin sealant indicated that cell delivery may be increased with minor dilutions of the commercial formulation. The trend of increasing migration with decreasing concentration of the commercial fibrin sealant resembled the behavior of MSCs in other studies,^{17,18,22} whereby dilution of the commercial fibrin formulation results in greater cell elongation and proliferation. It is important to mention that MSCs encapsulated in the undiluted commercial fibrin sealant had only a limited ability to spread within the hydrogel and migrate to the culture surface. This result is consistent with studies on the use of the undiluted commercial fibrin sealant in which little cell penetration was observed for cartilage repair in rabbits¹³ and minimal elongation of encapsulated MSCs was observed *in vitro*.²² These data suggest that a scaffold of the undiluted commercial fibrin sealant would not be an appropriate choice to achieve high amounts of MSC migration onto damaged tissues.

For both fibrin sources, migration from the 25% fibrin hydrogels was uniform and robust around the entirety of the perimeter, which indicated both formulations are capable of highly efficient delivery. This suggests other factors, including cost, convenience, accessibility, and safety, would likely have the greatest influence on a clinician's decision of which formulation to use. Migration from 50%, 75%, and undiluted hydrogels of both sources was less uniform with respect to location, which may have been attributable to the fact that heterogeneity of the fibrin network increases with fibrin concentration.¹⁸ Therefore, although a range of dilutions was capable of rapid delivery of MSCs, it is important to mention that hydrogels with a higher concentration may result in less uniform delivery than will 25% hydrogels.

The morphology of MSCs in monolayer culture correlates with proliferation and postexpansion differentiation.^{28,29} In general, MSCs that are relatively small and have a linear morphology are associated with high growth rate and differentiation, whereas larger cells that are less spindle-shaped suggest the onset of senes-

cence. In the present study, migrating MSCs retained a small and linear morphology that was similar to the morphology of those cells in expansion culture prior to hydrogel seeding. Although functional characterization of the cell population would be necessary to describe the phenotype of the migrating MSCs, this observation suggests that fibrin encapsulation and migration did not adversely affect the therapeutic potential of the cells.

The laboratory experiments in this study were designed to provide a highly controlled environment in which MSC migration could be quantified. These experiments were effective in resolving differences among hydrogel dilutions, but *in vivo* testing will be necessary to ensure the results of this study translate into the delivery of MSCs to damaged tissues. Several additional factors should be considered for *in vivo* applications. Although this study focused on the optimization of fibrin hydrogels to rapidly deliver MSCs to damaged tissue, it is important that the hydrogel remain in place for a sufficient time to allow for migration. Decreasing the fibrin concentration negatively affects the shear strength³⁰ and degradation rate²² of fibrin hydrogels, which makes durability a potential concern. Evidence of the high affinity of MSCs for damaged tissue has been determined for cartilage,³¹ with as little as 10 minutes of direct contact with a liquid MSC suspension with explanted cartilage being sufficient for high amounts of attachment. This example supports the feasibility of a short delivery period, which may obviate concerns about the adverse effects of dilution on hydrogel durability. Furthermore, a 10-fold dilution of the commercial fibrin sealant successfully delivered MSCs to an artificial nerve conduit,³² a result that indicates the potential of low-fibrinogen formulations in mechanically protected environments. For applications in which higher fibrinogen concentrations are needed, it is possible the migration detected for intermediate dilutions would increase with time beyond 24 hours, thereby potentially resulting in adequate seeding of damaged tissue. Migration at time points beyond 24 hours was not evaluated here because the potential artifact of cell proliferation of MSCs on exiting the gel would confound quantitative measures of migration, especially for the 25% hydrogels, for which extensive migration was observed around the entire perimeter of the hydrogels. Additional studies in which migration is quantified independent of postmigration proliferation are necessary to better define the ability of fibrin hydrogels to deliver MSCs to damaged tissues over a period of days.

- a. Terumo, Somerset, NJ.
- b. Invitrogen Corp, Carlsbad, Calif.
- c. Hyclone, Logan, Utah.
- d. R&D Systems, Minneapolis, Minn.
- e. MP Biomedicals, Solon, Ohio.
- f. Tisseel, Baxter Healthcare Corp, Deerfield, Ill.
- g. CellTiter-Blue, Promega, Madison, Wis.
- h. BCA protein assay, Pierce, Rockford, Ill.
- i. PROC MIXED, SAS, version 9.2, SAS Institute Inc, Cary, NC.

References

1. Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J Cell Physiol* 2007;213:341–347.

2. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. *J Cell Biochem* 2006;98:1076–1084.
3. Frisbie DD, Smith RK. Clinical update on the use of mesenchymal stem cells in equine orthopaedics. *Equine Vet J* 2010;42:86–89.
4. Ferris DJ, Frisbie DD, Kisiday JD, et al. Clinical follow-up of horses treated with bone marrow–derived mesenchymal stem cells for musculoskeletal lesions, in *Proceedings. 55th Annu Meet Am Assoc Equine Pract* 2009;59–60.
5. Fortier LA, Smith RK. Regenerative medicine for tendinous and ligamentous injuries of sport horses. *Vet Clin North Am Equine Pract* 2008;24:191–201.
6. Pacini S, Spinabella S, Trombi L, et al. Suspension of bone marrow–derived undifferentiated mesenchymal stromal cells for repair of superficial digital flexor tendon in race horses. *Tissue Eng* 2007;13:2949–2955.
7. Schnabel LV, Lynch ME, van der Meulen MC, et al. Mesenchymal stem cells and insulin-like growth factor-I gene-enhanced mesenchymal stem cells improve structural aspects of healing in equine flexor digitorum superficialis tendons. *J Orthop Res* 2009;27:1392–1398.
8. Smith RK. Mesenchymal stem cell therapy for equine tendinopathy. *Disabil Rehabil* 2008;30:1752–1758.
9. Wilke MM, Nydam DV, Nixon AJ. Enhanced early chondrogenesis in articular defects following arthroscopic mesenchymal stem cell implantation in an equine model. *J Orthop Res* 2007;25:913–925.
10. Frisbie DD, Kisiday JD, Kawcak CE, et al. Evaluation of adipose-derived stromal vascular fraction or bone marrow–derived mesenchymal stem cells for treatment of osteoarthritis. *J Orthop Res* 2009;27:1675–1680.
11. Silver FH, Wang MC, Pins GD. Preparation of fibrin glue: a study of chemical and physical methods. *J Appl Biomater* 1995;6:175–183.
12. Silver FH, Wang MC, Pins GD. Preparation and use of fibrin glue in surgery. *Biomaterials* 1995;16:891–903.
13. Brittberg M, Sjogren-Jansson E, Lindahl A, et al. Influence of fibrin sealant (Tisseel) on osteochondral defect repair in the rabbit knee. *Biomaterials* 1997;18:235–242.
14. Lechner S, Huss R. Bone engineering: combining smart biomaterials and the application of stem cells. *Artif Organs* 2006;30:770–774.
15. Soon MY, Hassan A, Hui JH, et al. An analysis of soft tissue allograft anterior cruciate ligament reconstruction in a rabbit model: a short-term study of the use of mesenchymal stem cells to enhance tendon osteointegration. *Am J Sports Med* 2007;35:962–971.
16. Chong AK, Ang AD, Goh JC, et al. Bone marrow–derived mesenchymal stem cells influence early tendon-healing in a rabbit achilles tendon model. *J Bone Joint Surg Am* 2007;89:74–81.
17. Catelas I, Sese N, Wu BM, et al. Human mesenchymal stem cell proliferation and osteogenic differentiation in fibrin gels in vitro. *Tissue Eng* 2006;12:2385–2396.
18. Ho W, Tawil B, Dunn JC, et al. The behavior of human mesenchymal stem cells in 3D fibrin clots: dependence on fibrinogen concentration and clot structure. *Tissue Eng* 2006;12:1587–1595.
19. Huang CY, Deitzer MA, Cheung HS. Effects of fibrinolytic inhibitors on chondrogenesis of bone-marrow derived mesenchymal stem cells in fibrin gels. *Biomech Model Mechanobiol* 2007;6:5–11.
20. Dickhut A, Gottwald E, Steck E, et al. Chondrogenesis of mesenchymal stem cells in gel-like biomaterials in vitro and in vivo. *Front Biosci* 2008;13:4517–4528.
21. Weinand C, Gupta R, Huang AY, et al. Comparison of hydrogels in the in vivo formation of tissue-engineered bone using mesenchymal stem cells and beta-tricalcium phosphate. *Tissue Eng* 2007;13:757–765.
22. Bensaid W, Triffitt JT, Blanchat C, et al. A biodegradable fibrin scaffold for mesenchymal stem cell transplantation. *Biomaterials* 2003;24:2497–2502.
23. Gregory CA, Reyes E, Whitney MJ, et al. Enhanced engraftment of mesenchymal stem cells in a cutaneous wound model by culture in allogenic species-specific serum and administration in fibrin constructs. *Stem Cells* 2006;24:2232–2243.
24. Falanga V, Iwamoto S, Chartier M, et al. Autologous bone marrow–derived cultured mesenchymal stem cells delivered in a fibrin spray accelerate healing in murine and human cutaneous wounds. *Tissue Eng* 2007;13:1299–1312.
25. Qiu LL, Levinson SS, Keeling KL, et al. Convenient and effective method for removing fibrinogen from serum specimens before protein electrophoresis. *Clin Chem* 2003;49:868–872.
26. Kisiday JD, Kopesky PW, Evans CH, et al. Evaluation of adult equine bone marrow– and adipose-derived progenitor cell chondrogenesis in hydrogel cultures. *J Orthop Res* 2008;26:322–331.
27. Yoshida H, Hirozane K, Kamiya A. Comparative study of autologous fibrin glues prepared by cryo-centrifugation, cryofiltration, and ethanol precipitation methods. *Biol Pharm Bull* 1999;22:1222–1225.
28. Siddappa R, Licht R, van Blitterswijk C, et al. Donor variation and loss of multipotency during in vitro expansion of human mesenchymal stem cells for bone tissue engineering. *J Orthop Res* 2007;25:1029–1041.
29. Solchaga LA, Penick K, Porter JD, et al. FGF-2 enhances the mitotic and chondrogenic potentials of human adult bone marrow–derived mesenchymal stem cells. *J Cell Physiol* 2005;203:398–409.
30. Glidden PF, Malaska C, Herring SW. Thromboelastograph assay for measuring the mechanical strength of fibrin sealant clots. *Clin Appl Thromb Hemost* 2000;6:226–233.
31. Koga H, Shimaya M, Muneta T, et al. Local adherent technique for transplanting mesenchymal stem cells as a potential treatment of cartilage defect. *Arthritis Res Ther* 2008;10:R84.
32. Kalbermatten DF, Kingham PJ, Mahay D, et al. Fibrin matrix for suspension of regenerative cells in an artificial nerve conduit. *J Plast Reconstr Aesthet Surg* 2008;61:669–675.