Effect of Fibrin Formulation on Initial Strength of Tendon Repair and Migration of Bone Marrow Stromal Cells in Vitro

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Background: Cell-based tissue engineering techniques have been introduced to improve tendon repair outcomes. The purpose of this study was to determine optimal concentrations of fibrinogen and thrombin for use as a scaffold to deliver stromal cells to the tendon repair site.

Methods: Lacerated flexor digitorum profundus tendons from forty canine forepaws underwent simulated repair with fibrin gel interposition. The tendons were divided into five groups with different ratios of fibrinogen (mg/mL) to thrombin (NIH units/mL) used to form the gels. These ratios, which ranged from those found in normal hemostasis to those used clinically as adhesives, were 5:25 (the physiological ratio, used as a control), 40:250 (a low adhesive concentration of fibrinogen and a low adhesive concentration of thrombin [low-low group]), 80:250 (high-low group), 40:500 (low-high group), and 80:500 (high-high group). The failure load and tensile stiffness at time zero, compressive stiffness of the fibrin gel, and cell viability and migration were evaluated.

Results: The failure loads of the high-low and high-high groups were significantly higher than that of the control group. The tensile stiffness of the high-high group was significantly higher than that of the control group. The high-low and high-high groups had significantly higher compressive stiffness than the other groups. While there was no significant difference among the groups regarding cell viability, the cells in the control, low-low, and low-high gels were spindle-shaped whereas those in the high-low and high-high groups were rounded. Cells migrated across scratch gaps within twenty-four hours in the control, low-low, and low-high groups, but not in the high-low and high-high groups.

Conclusions: Higher concentrations of fibrinogen resulted in stronger and stiffer gels, but the strength was far less than that of a tendon suture and these gels were associated with a more rounded cell morphology and reduced cell migration. Therefore, lower concentrations of fibrinogen should be used if a fibrin gel is employed to deliver cells for tendon repair.

Clinical Relevance: Concentrations of fibrinogen lower than those used in fibrin glue may be more appropriate if fibrin is employed to create a cell delivery matrix for tendon repair.

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Although new suture materials¹⁻³, suture techniques^{4,5}, and postoperative rehabilitation protocols^{6,7} have improved clinical outcomes, functional restoration fol-

lowing flexor tendon injury and repair in zone II remains a substantial concern for hand surgeons because of the high rate of complications, such as rupture at the repair site and

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adhesion formation^{8,9}. To overcome this problem, tissue engineering techniques have been introduced to deliver cells to the repair site at the time of surgery, with encouraging preliminary results in both in vitro and in vivo models¹⁰⁻¹³. With these techniques, cells are delivered by a vehicle such as a suture¹⁴, collagen gel¹⁵, platelet-rich-plasma clot¹⁶, or fibrin gel¹⁷.

A number of studies have been performed to investigate the effect of fibrin scaffolds seeded with stem cells with and without growth factor augmentation^{16,18-20}. The results of these reports have not been consistent, with some studies demonstrating that fibrin scaffolds increased cell viability, proliferation, or differentiation^{18,20} and others showing that fibrin may adversely affect these parameters^{16,18,20}. An in vitro tendon repair model showed that fibrin gel, acting as a carrier of growth differentiation factor-5 (GDF-5)-treated muscle-derived stem cells, enhanced tendon healing, as evaluated both mechanically and histologically, compared with a collagen gel at both two weeks and four weeks²¹. However, the concentrations of fibrinogen and thrombin in that study were based on levels necessary for hemostasis, 5 mg/mL of fibrinogen and 25 NIH units/mL of thrombin. Fibrin is also used as a tissue repair glue, and the degree of adhesion of fibrin depends on the concentrations of fibrinogen and thrombin, which are much higher in fibrin glue than they are in normal hemostasis²²⁻²⁵.

The aim of this study was to determine, from both mechanical and cell biology perspectives, optimal concentrations of fibrinogen and thrombin for a fibrin gel used for tendon repair. We hypothesized that the ratio of fibrinogen to thrombin in a fibrin gel may have different effects on adhesive strength as compared with its effects on cell survival and mobility.

Materials and Methods

Mechanical Testing Methods

Forty forepaw flexor digitorum profundus tendons were harvested from mixed-breed two-year-old male dogs that had been killed for other Institutional Animal Care and Use Committee-approved studies. The tendons were randomly divided into five groups, after which they underwent transection and simulated repair with fibrin gel interposition with one of five different ratios of fibrin formulations based on previously published studies^{22,23}: 5 mg/mL of fibrinogen and 25 NIH units/mL of thrombin (physiological concentration; control group), low adhesive concentration of fibrinogen (40 mg/mL) and low adhesive concentration of thrombin (250 NIH units/mL) (low-low group), high adhesive concentration of fibrinogen (80 mg/mL) and low adhesive concentration of thrombin (250 NIH units/mL) (high-low group), low adhesive concentration of fibrinogen (40 mg/mL) and high adhesive concentration of thrombin (500 NIH units/mL) (low-high group), and high adhesive concentration of fibrinogen (80 mg/mL) and high adhesive concentration of thrombin (500 NIH units/mL) (high-high group).

Each fibrin gel was fabricated from 6 μ L of bovine fibrinogen (Sigma-Aldrich) and 2 μ L of bovine thrombin solution (Sigma-Aldrich), at the concentrations noted above.

The adhesive strength of each specimen was evaluated with use of a previously described in vitro tendon repair model²⁶. Each specimen was cut to a standard length of 30 mm and transected at the midpoint. The fibrin gel was interposed between the transected tendon ends by delivering fibrinogen and thrombin simultaneously with use of two pipettes and then longitudinally compressing the lacerated site with use of two forceps for five seconds. The fibrin gel was allowed to cure for three minutes before biomechanical testing.

A single loop suture of 6-0 polypropylene (Prolene; Ethicon), 5 mm in length, was placed at each end of the test specimen in advance to connect the tendon to a custom-designed microtester for mechanical evaluation. The device included a load transducer (GSO-50; Transducer Techniques), a linear potentiometer (TR-50; Novotechnik), and a stepper-motor-driven stage with hooks to capture the suture loops of the repaired tendon when mounted on a platform. This platform had a channel cut beneath the repair site to prevent adhesion of the fibrin gel to the platform (Fig. 1).

The specimens were distracted at a rate of 0.1 mm/sec until the repair site completely separated. Force and displacement data were collected at a sample rate of 100 Hz. Failure load was normalized by the tendon cross-sectional area. Failure load was identified, and tensile stiffness was defined by the slope of the linear region of the force-displacement curve. The cross-sectional area of the tendon at the repair site was evaluated after testing by scanning a 1.5-mm-thick slice from one of the transected tendon ends and using ImageJ software (National Institutes of Health) to calculate the area. We evaluated the compressive stiffness of each fibrin gel using indentation testing with an electromechanical test system (ElectroForce 3200; Bose) configured with a 3-mm-diameter planar indenter tip. Fibrin gels with each of the five described fibrinogen-thrombin ratios were poured into wells (five wells per gel composition) of a ninety-six-well dish (Costar 3595; Corning), filling each well to a depth of approximately 5 mm. Each well of the same gel composition was mixed separately. The gels were compressed under displacement control at a rate of 5 mm/min up to a penetration into the gel of 2 mm. Force and displacement data were collected at a sample rate of 20 Hz. Compressive stiffness was calculated from the slope of the linear region of the force-displacement curve.

Cell Biology Methods

Bone marrow was harvested from one mixed-breed dog that had been killed for other Institutional Animal Care and Use Committee-approved studies. Bone marrow was collected, under sterile conditions, from each humerus. Cells were pelleted, and supernatant fluid was removed by centrifugation at 1500 rpm for five minutes at room temperature. The bone marrow pellet was resuspended in cell culture minimum essential medium (MEM) with Earle salts (Gibco), 10% fetal bovine serum (Gibco), and 1% antibiotics (Antibiotic-Antimycotic; Gibco) and then plated onto two 100-mm culture dishes in 10 mL of MEM. Bone marrow stromal cells were incubated at 37°C with 5% CO₂ and 95% air

Fig. 1
Repaired canine tendon mounted on the experimental platform. The loops sewn into the tendon ends were set on the hooks.

at 100% humidity. The culture medium was replaced every three days. Bone marrow stromal cells were harvested at 70% to 80% confluence with 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA) (Gibco) and expanded. The fibrin gels (as previously described) were seeded with 1.3×10^5 cells from second-passage bone marrow stromal cells.

Cell viability and migration were assessed. Three gels from each group were fabricated from a mixture of a 40- μ L aliquot of bovine fibrinogen with the pelleted bone marrow stromal cells added to 12 μ L of bovine thrombin solution, with each constituent having the respective concentrations described above. The gels were incubated at 37°C for 0.5 hours to complete gelation. The fibrin gels were then dispensed onto a twenty-four-well plate with MEM and Earle salts, 10% fetal bovine serum, and 1% antibiotics, and incubated at 37°C in a 5% CO₂ humidified incubator for one, three, or seven days. The culture medium was changed every other day.

After one, three, or seven days in culture, cells were incubated with calcein AM (acetomethoxy) and ethidium homodimer-1 fluorescent dye (EthD) solution (LIVE/DEAD Viability/Cytotoxicity Kit, for mammalian cells; Life Technologies) to stain for living and dead cells, according to the manufacturer's protocol²⁷. Stained specimens were examined with a laser scanning confocal microscope (LSM780; Zeiss) for living and dead-cell counting and to examine cell morphology. Living and dead cells on a 10 $\times$ field (one 10 $\times$ field equals 0.73 mm²; three fields were picked randomly for each group) were counted with use of ImageJ software, and the area of each fibrin gel was obtained with use of ImageJ software as well. The individuals performing the counts were not blinded to the treatment group. Counterstaining was performed with DAPI (4',6-diamidino-2-phenylindole) with use of VECTASHIELD Mounting Medium with DAPI (Vector Laboratories). We calculated the living-cell count contained in each fibrin gel by multiplying the living-cell count per 1 mm² by the area of each fibrin gel (mm²). The results were reported as the living-cell count

contained in each fibrin gel—i.e., the mean density of living cells per 10 $\times$ field and the ratio of dead cells to total cells (living and dead) counted at days one, three, and seven.

Three dishes from each group were also prepared for a scratch assay to assess cell migration. In this test, a 200- μ L pipette tip (700 to 900 μ m in width) was used to score the fibrin gel in each dish²⁸. Cell migration was then observed under the microscope at twelve and twenty-four hours. The individuals performing the migration assay were not blinded to the treatment group.

Statistical Analysis

Failure load, failure stress, and tensile stiffness were analyzed with one-way factorial analysis of variance (ANOVA). The significance level was set to $p < 0.05$ in all cases. For the cell biology studies, the differences among the fibrin compositions with respect to the mean living-cell count of each fibrin gel and the mean ratio of dead cells to total cells were analyzed with the Kruskal-Wallis test. A Bonferroni-corrected Mann-Whitney U test was performed for each pairwise comparison that showed a significant difference.

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Results

All specimens failed at the repair site. There were significantly higher failure loads in the high-low ($p = 0.041$) and high-high ($p = 0.018$) groups than in the physiological control group (Table I). Since tendon cross-sectional area did not differ

Fig. 2

Representative immunohistochemical images of cell growth at three and seven days after repairs performed with fibrin gels with different fibrinogen and thrombin concentrations. Note that cells grown in the low-fibrin conditions are spindle-like, whereas the cells in the high-fibrinogen groups are rounded. Control = physiological fibrinogen and thrombin concentrations, LL = low-fibrinogen and low-thrombin concentrations, LH = low-fibrinogen and high-thrombin concentrations, HL = high-fibrinogen and low-thrombin concentrations, and HH = high-fibrinogen and high-thrombin concentrations.

X10⁵ cells / one fibrin patch

Fig. 3-A

Fig. 3-A Mean count of living cells (per one fibrin gel patch). Kruskal-Wallis test results at each time point showed no significant difference among groups. Control = physiological fibrinogen and thrombin concentrations, LL = low-fibrinogen and low-thrombin concentrations, LH = low-fibrinogen and high-thrombin concentrations, HL = high-fibrinogen and low-thrombin concentrations, and HH = high-fibrinogen and high-thrombin concentrations. **Fig. 3-B** Ratio of dead cells to total cells. Kruskal-Wallis test results at each time point showed no significant difference among groups. Control = physiological fibrinogen and thrombin concentrations, LL = low-fibrinogen and low-thrombin concentrations, LH = low-fibrinogen and high-thrombin concentrations, HL = high-fibrinogen and low-thrombin concentrations, and HH = high-fibrinogen and high-thrombin concentrations.

Dead cell / total cell ratio

Fig. 3-B

significantly among groups (average, 7.8 mm²), normalizing the failure load did not substantially change the respective differences between groups (Table I).

Tensile stiffness in the high-high group was significantly higher than that in the control group ($p = 0.017$) (Table I). Indentation testing showed that the compressive stiffness of the high-low and high-high groups was significantly higher than that of the other groups ($p < 0.01$), and that of the low-low group was significantly higher than that of the control and low-high groups ($p < 0.01$) (Table I).

Two types of cell morphology were observed: round and spindle-shaped (Fig. 2). Round cells were noted in the high-

high and high-low groups, while spindle-shaped cells were noted in the other groups.

The mean count of living cells contained in each gel did not differ significantly among the groups at any time point (Fig. 3-A). The ratio of dead to total cells was $<30\%$ in each group and also did not differ significantly among the groups at any time point (Fig. 3-B).

The migration assay showed cells migrating across the gap at twelve hours in the control and low-low groups, and at twenty-four hours in the low-high group. There was no cell migration in the high-low or high-high groups at twenty-four hours (Fig. 4).

Fig. 4

Scratch wound assay. Bone marrow stromal cells migrated across scratch gaps within twenty-four hours in the control, low-low, and low-high groups. There was no cell migration at twenty-four hours in the high-low and high-high groups. The white bars represent 200 μm . Control = physiological fibrinogen and thrombin concentrations, LL = low-fibrinogen and low-thrombin concentrations, LH = low-fibrinogen and high-thrombin concentrations, HL = high-fibrinogen and low-thrombin concentrations, and HH = high-fibrinogen and high-thrombin concentrations.

TABLE I Results of Mechanical Tests

	Tendon*			Gel*
	Failure Load (mN)	Failure Stress (mN/mm ²)	Tensile Stiffness (mN/mm)	Compressive Stiffness† (mN/mm)
Control	36.1 (29.9)	6.8 (7.1)	34.7 (19.4)	0.003 (0.001)
Low-low	122.1 (119.7)	12.4 (8.7)	97.9 (94.4)	0.107‡ (0.057)
Low-high	75.7 (54.9)	9.4 (6.7)	50.4 (36.5)	0.047 (0.018)
High-low	164.3§ (112.7)	20.7# (13.5)	143.6 (96.1)	0.280‡ (0.080)
High-high	178.8** (76.1)	20.7# (7.0)	170.7†† (112.9)	0.193‡ (0.082)
ANOVA	P = 0.008	P = 0.008	P = 0.009	P < 0.001

*The values are given as the mean with the standard deviation in parentheses. †Regarding mean compressive stiffness, there were significant differences between the low-low group and the low-high group, the low-low group and the high-low group, the low-low group and the high-high group, the low-high group and the high-low group, and the low-high group and the high-high group ($p < 0.05$ for each). ‡ $P < 0.05$ (compared with control). § $P = 0.041$ (compared with control). # $P = 0.030$ (compared with control). ** $P = 0.018$ (compared with control). †† $P = 0.017$ (compared with control).

Discussion

Previous work has shown that a fibrin gel with physiological concentrations of fibrinogen and thrombin used as a cell-seeded scaffold resulted in better tendon healing than that achieved with a collagen gel in an in vitro tendon repair model²¹. These findings suggest that a fibrin gel scaffold may play an important role in the success of cell-seeded tissue engineering in tendon repair. In the present study, we looked at both mechanical properties and the cell biology of different fibrinogen and thrombin concentrations, similar to those used clinically for fibrin glue^{22,23}, and made two clinically relevant observations.

First, while the failure loads in the high-low and high-high groups were significantly higher than the failure loads in the control group, the strength of those gels may not be clinically relevant in the context of tendon repair. Our observation that fibrin formulations with a high fibrinogen concentration have higher adhesive strength is similar to that in a study of fibrin glue in a rat skin model, which showed that the failure load was highest with use of 39 mg/mL of fibrin and 200 NIH units/mL of thrombin²³. However, even the strongest of these fibrin gels—i.e., those with a strength of roughly 150 mN—fails to approach the strength of a tendon suture repair, which is typically 20 to 30 N or more²⁹. Thus, we concluded that the adhesive strength of the fibrin gel is not a clinically important factor in determining the composition of a gel for cell delivery.

Second, the concentration of fibrinogen in the gel affected cell viability, shape, and motility, which may, in contrast to gel strength, have important implications for the use of fibrin gels as cell carriers for the purpose of tissue engineering. Stromal and stem cell therapeutics require cell migration for tissue healing³⁰. In this study, we found that the low-fibrinogen gels allowed for cell migration, whereas the high-fibrinogen gels did not. Additionally, morphological findings showed that, in the low-fibrinogen groups, bone marrow stromal cells assumed a spindle shape, as would be expected in tendon, whereas bone marrow stromal cells in the high-low and high-

high groups maintained a rounded shape³¹. We believe that both of these findings relate to the greater stiffness of these gels, as noted on the indentation testing. Matrix stiffness can drive the stromal cell lineage and differentiation^{32,33}. Kim et al. similarly reported that mesenchymal stem cells maintained a rounded shape in fibrin gel with a higher, nonphysiological concentration of fibrinogen (127 to 256 mg/mL)³⁴. In contrast, our physiological controls, as well as our low-low and low-high groups, showed a spindle-shape cell morphology, which reflects the morphology of tenocytes^{35,36}.

There was no significant difference among groups, at any time point, regarding the mean count of living cells in this study. Zurita et al. studied four combinations of fibrinogen and thrombin with the apoptotic marker TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) and the proliferation marker Ki-67 after using fibrin gel as a scaffold for bone marrow stromal cells for seven days¹⁶. They concluded that the most favorable combination for survival of bone marrow stromal cells was 20 mg/mL of fibrinogen and 87 NIH units/mL of thrombin, values intermediate between those of our control and low-low groups. The ratio of dead to total cells in our study was <30%, a finding consistent with previous reports^{18,27}. Gugerell et al. reported that thrombin concentrations of >400 NIH units/mL induced apoptosis of human keratinocytes²⁷. Given our findings, we agree with Gugerell et al. that much lower thrombin concentrations are appropriate for fibrin gels used for cell delivery. However, a nonphysiological gel composition may be easier to handle than a physiological one. The lower viscosity and stiffness of our control gel made it difficult to handle, as was previously reported¹⁶.

On the basis of the findings of this study, we concluded that, when used for tendon repair, gels with a fibrinogen concentration in the 40 mg/mL range may be preferable to the fibrin in a physiological clot because they are stronger and easier to handle. They are also preferable to the higher-fibrinogen-concentration gels used in commercially available fibrin glues as they are better able to support cell migration, which should

improve the ability of the delivered cells to improve healing, and the small difference in adhesive strength is not clinically important. On the basis of the reports cited above, we also suggest that lower thrombin concentrations are preferable since they seem to be associated with better cell viability, although this was not observed in our study.

This study has several limitations. First, it was performed with use of canine tendons. The results could differ in human tendons. Second, since this is an in vitro study the results in terms of cell migration may not model in vivo results. It is likely that growth factors and cytokines surrounding the tendon affect cell viability, migration, morphology, and mechanical properties in vivo. Third, strictly speaking, we did not measure the stiffness of the fibrin gel, but rather the stiffness of the tendon-gel composite. However, the difference between this and the actual repair site stiffness was small as the tendon is much stiffer than the gel. Finally, the assessments were not blinded in this study.

In conclusion, in view of the effects on both mechanical properties and cell activity, the results of this study support the

notion that concentrations of fibrinogen higher than those associated with physiological hemostasis but lower than those used in fibrin glue should be used to compose a scaffold if the goal is to provide some adhesive strength and deliver cells for tendon repair. ■

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References

- McDonald E, Gordon JA, Buckley JM, Gordon L. Comparison of a new multifilament stainless steel suture with frequently used sutures for flexor tendon repair. *J Hand Surg Am.* 2011 Jun;36(6):1028-34. Epub 2011 Jun 4.
- Miller B, Dodds SD, deMars A, Zagoreas N, Waitayawinyu T, Trumble TE. Flexor tendon repairs: the impact of FiberWire on grasping and locking core sutures. *J Hand Surg Am.* 2007 May-Jun;32(5):591-6. Epub 2007 May 8.
- Su BW, Protopsaltis TS, Koff MF, Chang KP, Strauch RJ, Crow SA, Rosenwasser MP. The biomechanical analysis of a tendon fixation device for flexor tendon repair. *J Hand Surg Am.* 2005 Mar;30(2):237-45. Epub 2005 Mar 23.
- Al-Qattan MM, Al-Rakan MA, Al-Hassan TS. A biomechanical study of flexor tendon repair in zone II: comparing a combined grasping and locking core suture technique to its grasping and locking components. *Injury.* 2011 Nov;42(11):1300-2. Epub 2011 Mar 5.
- Peltz TS, Haddad R, Scougall PJ, Nicklin S, Gianoutsos MP, Walsh WR. Influence of locking stitch size in a four-strand cross-locked cruciate flexor tendon repair. *J Hand Surg Am.* 2011 Mar;36(3):450-5. Epub 2011 Feb 17.
- Chesney A, Chauhan A, Kattan A, Farrokhfar F, Thoma A. Systematic review of flexor tendon rehabilitation protocols in zone II of the hand. *Plast Reconstr Surg.* 2011 Apr;127(4):1583-92. Epub 2010 Dec 29.
- Trumble TE, Vedder NB, Seiler JG 3rd, Hanel DP, Diao E, Pettrone S. Zone-II flexor tendon repair: a randomized prospective trial of active place-and-hold therapy compared with passive motion therapy. *J Bone Joint Surg Am.* 2010 Jun;92(6):1381-9. Epub 2010 Jun 3.
- Dy CJ, Hernandez-Soria A, Ma Y, Roberts TR, Daluiski A. Complications after flexor tendon repair: a systematic review and meta-analysis. *J Hand Surg Am.* 2012 Mar;37(3):543-551.e1. Epub 2012 Feb 6.
- Tang JB. Clinical outcomes associated with flexor tendon repair. *Hand Clin.* 2005 May;21(2):199-210. Epub 2005 May 11.
- Juncosa-Melvin N, Boivin GP, Gooch C, Galloway MT, West JR, Dunn MG, Butler DL. The effect of autologous mesenchymal stem cells on the biomechanics and histology of gel-collagen sponge constructs used for rabbit patellar tendon repair. *Tissue Eng.* 2006 Feb;12(2):369-79. Epub 2006 Mar 22.
- Martinello T, Bronzini I, Perazzi A, Testoni S, De Benedictis GM, Negro A, Caporale G, Mascarello F, Iacopetti I, Patrino M. Effects of in vivo applications of peripheral blood-derived mesenchymal stromal cells (PB-MSCs) and platelet-rich plasma (PRP) on experimentally injured deep digital flexor tendons of sheep. *J Orthop Res.* 2013 Feb;31(2):306-14. Epub 2012 Aug 14.
- Morizaki Y, Zhao C, An KN, Amadio PC. The effects of platelet-rich plasma on bone marrow stromal cell transplants for tendon healing in vitro. *J Hand Surg Am.* 2010 Nov;35(11):1833-41. Epub 2010 Oct 15.
- Zhao C, Chieh HF, Bakri K, Ikeda J, Sun YL, Moran SL, An KN, Amadio PC. The effects of bone marrow stromal cell transplants on tendon healing in vitro. *Med Eng Phys.* 2009 Dec;31(10):1271-5. Epub 2009 Sep 6.
- Guyette JP, Fakharzadeh M, Burford EJ, Tao ZW, Pins GD, Rolle MW, Gaudette GR. A novel suture-based method for efficient transplantation of stem cells. *J Biomed Mater Res A.* 2013 Mar;101(3):809-18. Epub 2012 Sep 8.
- Young RG, Butler DL, Weber W, Caplan AL, Gordon SL, Fink DJ. Use of mesenchymal stem cells in a collagen matrix for Achilles tendon repair. *J Orthop Res.* 1998 Jul;16(4):406-13. Epub 1998 Sep 25.
- Zurita M, Otero L, Aguayo C, Bonilla C, Ferreira E, Parajón A, Vaquero J. Cell therapy for spinal cord repair: optimization of biologic scaffolds for survival and neural differentiation of human bone marrow stromal cells. *Cytotherapy.* 2010 Jul;12(4):522-37. Epub 2010 May 15.
- Breen A, O'Brien T, Pandit A. Fibrin as a delivery system for therapeutic drugs and biomolecules. *Tissue Eng Part B Rev.* 2009 Jun;15(2):201-14. Epub 2009 Mar 3.
- Aoyagi Y, Kuroda M, Asada S, Bujo H, Tanaka S, Konno S, Tanio M, Ishii I, Aso M, Saito Y. Fibrin glue increases the cell survival and the transduced gene product secretion of the ceiling culture-derived adipocytes transplanted in mice. *Exp Mol Med.* 2011 Mar 31;43(3):161-7. Epub 2011 Feb 23.
- de la Puente P, Ludeña D. Cell culture in autologous fibrin scaffolds for applications in tissue engineering. *Exp Cell Res.* 2014 Mar 10;322(1):1-11. Epub 2013 Dec 28.
- Hankemeier S, van Griensven M, Ezechieli M, Barkhausen T, Austin M, Jagodzinski M, Meller R, Bosch U, Krettek C, Zeichen J. Tissue engineering of tendons and ligaments by human bone marrow stromal cells in a liquid fibrin matrix in immunodeficient rats: results of a histologic study. *Arch Orthop Trauma Surg.* 2007 Nov;127(9):815-21. Epub 2007 Jun 14.
- Ozasa Y, Gingery A, Thoreson AR, An KN, Zhao C, Amadio PC. A comparative study of the effects of growth and differentiation factor 5 on muscle-derived stem cells and bone marrow stromal cells in an in vitro tendon healing model. *J Hand Surg Am.* 2014 September; 39(9): 1706–1713. Published online 2014 June 6
- Portilla-de Buen E, Orozco-Mosqueda A, Leal-Cortés C, Vázquez-Camacho G, Fuentes-Orozco C, Alvarez-Villaseñor AS, Macías-Amezcuca MD, González-Ojeda A. Fibrinogen and thrombin concentrations are critical for fibrin glue adherence in rat high-risk colon anastomoses. *Clinics (Sao Paulo).* 2014;69(4):259-64. Epub 2014 Apr 10.
- Byrne DJ, Hardy J, Wood RA, McIntosh R, Cuschieri A. Effect of fibrin glues on the mechanical properties of healing wounds. *Br J Surg.* 1991 Jul;78(7):841-3. Epub 1991 Jul 1.
- Murakami M, Tono T, Okada K, Yano H, Monden T. Fibrin glue injection method with diluted thrombin for refractory postoperative digestive fistula. *Am J Surg.* 2009 Nov;198(5):715-9. Epub 2009 May 1.
- Yoshida H, Hirozane K, Kamiya A. Adhesive strength of autologous fibrin glue. *Biol Pharm Bull.* 2000 Mar;23(3):313-7. Epub 2000 Mar 22.
- Ikeda J, Zhao C, Moran SL, An KN, Amadio PC. Effects of synovial interposition on healing in a canine tendon explant culture model. *J Hand Surg Am.* 2010 Jul;35(7):1153-9. Epub 2010 Jun 11.
- Gugerell A, Schossleitner K, Wolbank S, Nürnberger S, Redl H, Gulle H, Goppelt A, Bittner M, Pastener W. High thrombin concentrations in fibrin sealants induce apoptosis in human keratinocytes. *J Biomed Mater Res A.* 2012 May;100(5):1239-47. Epub 2012 Feb 23.

- 28.** Huleihel L, Ben-Yehudah A, Milosevic J, Yu G, Pandit K, Sakamoto K, Yousef H, LeJeune M, Coon TA, Redinger CJ, Chensny L, Manor E, Schatten G, Kaminski N. Let-7d microRNA affects mesenchymal phenotypic properties of lung fibroblasts. *Am J Physiol Lung Cell Mol Physiol*. 2014 Mar 15;306(6):L534-42. Epub 2014 Jan 17.
- 29.** Schuind F, Garcia-Elias M, Cooney WP 3rd, An KN. Flexor tendon forces: in vivo measurements. *J Hand Surg Am*. 1992 Mar;17(2):291-8. Epub 1992 Mar 1.
- 30.** Cox S, Cole M, Tawil B. Behavior of human dermal fibroblasts in three-dimensional fibrin clots: dependence on fibrinogen and thrombin concentration. *Tissue Eng*. 2004 May-Jun;10(5-6):942-54. Epub 2004 Jul 22.
- 31.** Deans RJ, Moseley AB. Mesenchymal stem cells: biology and potential clinical uses. *Exp Hematol*. 2000 Aug;28(8):875-84. Epub 2000 Sep 16.
- 32.** Dingal PC, Discher DE. Combining insoluble and soluble factors to steer stem cell fate. *Nat Mater*. 2014 Jun;13(6):532-7. Epub 2014 May 23.
- 33.** Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. *Cell*. 2006 Aug 25;126(4):677-89. Epub 2006 Aug 23.
- 34.** Kim I, Lee SK, Yoon JI, Kim E, Kim M, Ha H. Fibrin glue improves the therapeutic effect of MSCs by sustaining survival and paracrine function. *Tissue Eng Part A*. 2013 Nov;19(21-22):2373-81. Epub 2013 Jul 16.
- 35.** Clegg PD, Strassburg S, Smith RK. Cell phenotypic variation in normal and damaged tendons. *Int J Exp Pathol*. 2007 Aug;88(4):227-35. Epub 2007 Aug 19.
- 36.** Lavagnino M, Wall ME, Little D, Banes AJ, Guilak F, Arnoczky SP. Tendon mechanobiology: Current knowledge and future research opportunities. *J Orthop Res*. 2015 Jun;33(6):813-22. Epub 2015 Apr 27.