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Muscle-derived stem cell seeded fibrin gel interposition produces greater tendon strength and stiffness than collagen gel in vitro

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Dear Sir

We have reported that muscle-derived stem cells (MDSCs) enhance flexor tendon healing better than bone marrow stromal cells in an in vitro tendon healing model (Ozasa et al., 2014). While many hydrogel-based cell delivery vehicles have been investigated, the ideal carrier remains to be established due to a lack of comparative studies of one carrier versus another. A disadvantage of using a collagen carrier is that it cannot be harvested from the host as an autologous substance for clinical use. Another concern in using collagen gel for mesenchymal stem cells (MSC) transplantation is the potential for heterotopic tissue differentiation. Fibrin is widely used clinically for a variety of applications and is a logical matrix for clinically directed cell delivery.

In this study we compared fibrin gel and collagen gel supplemented with growth and differentiation factor-5 (GDF-5 as carriers of MDSCs) in an in vitro tendon healing model.

A total of 64 flexor digitorum profundus tendons were harvested from eight dogs. Zone D segments were used for mechanical testing, and zone A segments were used for histological and biochemical assessment.

The tendons were divided into four groups: (1) repaired tendon without any gel patch interposition (no cell group); (2) repaired tendon with fibrin gel patch interposition (FG group); (3) repaired tendon with MDSC-seeded collagen gel patch supplemented with GDF-5 interposition (CG-MG group); and (4) repaired tendon with MDSC-seeded fibrin gel patch supplemented with GDF-5 interposition (FG-MG group).

MDSCs were isolated by a modified preplate technique, as described previously (Ozasa et al., 2014). Collagen gel preparation followed a previously published procedure (Zhao et al., 2009). A 100- μ L aliquot of the GDF-5 supplemented cell suspension was added onto the preincubated gel, for a total dose per gel of 10 ng of rhGDF-5 and 2×10^5 cells.

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Conflict of interests

None declared.

For the fibrin gel with cell seeding group, a 10- μ L aliquot of bovine fibrinogen (5 mg/mL) (Sigma-Aldrich, St. Louis, MO, USA) was mixed with the pelleted MDSCs supplemented with 1 μ L of rhGDF-5 (10 ng/ μ L). A 3- μ L bovine thrombin solution (25 U/mL) (Sigma) was mixed with the fibrinogen solution to convert the fibrinogen to fibrin. This mixture was incubated at 37 °C for 0.5 h until gelation. The fibrin gel-only patch was fabricated as above, with no MDSCs added. As with the collagen gel, the total dose per fibrin gel was 10 ng of rhGDF-5 and 2×10^5 cells. To evaluate stem cell residency, MDSCs were labelled with Vybrant^R DiI cell labelling solution (Molecular Probes, Eugene, Oregon, USA), according to the manufacturer's instruction before seeding into gel patches.

Tendon segments were repaired with two single loop sutures. Two gel patches were implanted at the repair site before tightening the suture loop. The repaired tendons were incubated for 2 or 4 weeks. Repaired zone D tendon segments were assessed mechanically (eight per group) and repaired zone A segments were assessed histologically (three per group).

Mechanical testing was performed as previously described (Ozasa et al., 2014). Before testing, the tendon repair sutures were cut bilaterally, with care taken not to disrupt the repair site, thus permitting assessment of the strength of the healing tissue rather than the composite strength including the suture. Pullout strength at failure and stiffness were analysed with one-way factorial analysis of variance. The Tukey-Kramer post hoc test for each pair comparison was performed if a significant difference was found. All results were reported as means (standard deviation). The significance level was set to $p < 0.05$ in all cases.

Failure strength and stiffness were higher in the FG-MG ($P < 0.05$) than in the other groups at both time points. Confocal microscopy showed DiI labelled MDSCs in the repair site in cell-seeded groups at both 2 and 4 weeks. DiI-labelled cells had migrated into the cut tendon ends at 4 weeks (Figure 1).

Our study showed that GDF-5-treated MDSCs in a fibrin gel scaffold increased failure strength and stiffness of flexor tendon in this tissue culture model. Fibrin gel was a significantly better cell carrier than collagen gel in terms of the resulting stiffness and strength after healing. As previously reported, use of fibrin gel alone did not affect failure strength and stiffness (Lusardi and Cain, 1994). Further in vivo studies are necessary to confirm the clinical validity and applicability of these results.

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References

- Lusardi DA, Cain JE Jr. The effect of fibrin sealant on the strength of tendon repair of full thickness tendon lacerations in the rabbit Achilles tendon. *J Foot Ankle Surg.* 1994; 33:443–7. [PubMed: 7849667]

- 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; 39:1706–13. [PubMed: 24909566]
- Zhao C, Chieh HF, Bakri K, et al. The effects of bone marrow stromal cell transplants on tendon healing in vitro. *Med Eng Phys.* 2009; 31:1271–5. [PubMed: 19736035]

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Figure 1.

Cells labelled with Vibrant DiI cell labelling solution were observed under confocal microscopy with red fluorescence at 4 weeks. Blue fluorescence indicates nuclei. (Original magnification $\times 100$. Scale bar represents 100 μm). (A) No cell group, (B) FG group, (C) CG-MG group and (D) FG-MG group.