Surface Modification Combined Tissue Engineering Patch for Flexor Tendon Repair: A Short-Term In Vivo

INTRODUCTION:
Surface modification with hyaluronic acid plus lubricin (cd-HA-Lub) after flexor tendon repair effectively increases the tendon gliding ability, decreases adhesions, and improves digit function in a canine model both in vitro and in vivo\textsuperscript{1,2}. However, these benefits were compromised by delayed tendon healing\textsuperscript{2}, probably due to lubricin’s anti-adhesive properties\textsuperscript{3}. The purpose of this current study was to use a cell and growth factor based tissue engineering technique to enhance tendon healing. We hypothesized that a gel patch seeded with bone marrow stromal cells (BMSCs) stimulated by growth differentiation factor 5 (GDF-5) would counteract the adverse effect of cd-HA-Lub on tendon healing.

METHODS:
Fabrication of GDF-5-BMSC Patch: Three weeks before scheduled surgery, bone marrow was harvested from the host animal and incubated for patch making. 24 hours before surgery, 0.5% PureCol bovine dermal collagen (Inamed Corporation, Fremont, CA) was prepared with 200μl mixed solution into each well of 48-well dish. The 2nd or 3rd passage of BMSC was trypsinized, concentrated to 2.0X10\textsuperscript{6} cells/ml and mixed with 100ng/ml of GDF-5 (MBL, Woburn, MA). Then, 10μl of cell and GDF-5 suspension was pipetted onto a prepared gel well and incubated for 24 hours. Thus, each gel patch contained about 0.2X10\textsuperscript{6} BMSCs.

Tendon Repair and Treatment: Twelve mixed-breed adult dogs weighing 20 to 25 kg were used with the approval of our Institutional Animal Care and Use Committee. Under general anesthesia, the flexor digitorum profundus (FDP) tendons of the 2nd and 5th digits were exposed and lacerated 5 mm distal to the proximal pulley and repaired with a 2-strand modified Pennington technique using 4/0 FiberWire (Ethicon Inc., Somerville, NJ) to secure to a custom-made soft jacket was used to close and reinforce the repair. Following tendon repair, the surface modification with cd-HA-Lub was done (2). Briefly, a solution of 1% sodium hyaluronate (HA) (95%), 1.5x10\textsuperscript{3}MW, Acros), 10% gelatin (Sigma), 1% 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (Sigma) and 1% N-hydroxysuccinimide (NHS) (Pierce), 0.1 M Mes (pH 6.0) was prepared. Then, the repaired tendon was coated with this compound and maintained for 5 minutes, and then the lubricin with a volume of 0.2 ml of 260 μg/ml was applied to the surface. Either the 2nd or 5th FDP tendon was randomly selected for the patch and surface coating treatment. The other digit served as a control undergoing tendon repair only. Following flexor tendon repair, a high radial neurectomy was performed to eliminate the elbow and wrist extensor function and thereby prevent weight bearing. A custom-made soft jacket was used to maintain the surgical paw under the chest. Therapy was started on postoperative day 5 with a synergistic motion protocol\textsuperscript{2} once per day until sacrifice. The dogs were sacrificed at postoperative day 10.

Evaluation: The surgical digits and contralateral normal paws were dissected for work of flexion (WOF) evaluation. The digit was mounted onto a custom jig with a wire that fixed the MCP joint and allowed free PIP and DIP motion. A Motion Monitor System (Motion Analysis Corporation, Santa Rosa, CA) was used to measure the joint motion. The WOF was then normalized (nWOF) by the DIP and PIP motion based on previously reported protocols\textsuperscript{4}.

After measuring WOF, the repaired tendons were further dissected, keeping the proximal pulley intact. The gliding resistance between the tendon repair site and proximal pulley was then measured using a custom tendon-pulley frictional testing device, as previously described\textsuperscript{5}. Finally, the repaired tendons were secured to a servohydraulic testing machine (MTS Systems, Eden Prairie, MN) and distracted to failure at a rate of 20 mm/min. 10 FDP tendons normal paws were also lacerated and repaired with the same procedures of in vivo model served as in vitro (0 time) control group. Maximum breaking force and stiffness were recorded.

Two tendons in each group from two dogs were harvested for cell viability examination. BMSCs were labeled with PKH26 red fluorescent cell linker (Sigma, St. Louis, MO) before seeding in the gel patch. Ten days after tendon repair, the tendon samples were observed with a confocal microscope (LSM310 Zeiss, Germany).

A one way ANOVA was used to analyze the differences on nWOF, gliding resistance, repair strength, and stiffness between repaired FDP tendons. Any p-value < 0.05 was reported as statistically significant.

RESULTS:
The nWOF of the normal digit was significantly lower than the repaired tendons in both treated and control groups (p < 0.05). The nWOF of the treated tendons was significantly lower than the control tendons (p < 0.05) (Figure 2). The gliding resistance of control tendons was significantly higher than the treated tendons (p < 0.05) (Figure 3). There was no significant different in maximum failure strength among repaired tendons at 0 time, either treated or non-treated tendons (Figure 4). The stiffness of both treated and non treated repaired tendons at 10 days was significantly higher than the repaired tendon at 0 time (Figure 5). The seeded BMSCs were viable after 10 day transplantation (Figure 6).

SIGNIFICANCE:
The surface treatment of a repaired flexor tendon with cd-HA-Lub improves digit function, but at the cost of repair strength. In this short term study, the repair strength and stiffness were not affected by this surface modification when a BMSC seeded gel patch is added to the repair. Compared to previous report\textsuperscript{2}, the cell-based patch increased repair stiffness after 10 days of surgery. The GDF-5 stimulated BMSCs are viable after 10 days. This study demonstrated that this cell based therapy may potentially enhance tendon healing, or at least counter the adverse effects of the surface treatment while maintaining its beneficial anti-adhesive effects. Long-term investigation is needed to explore this cell based therapy through the completion of tendon healing.

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REFERENCES: