Maintenance of the gliding surface of tendon autografts in dogs

Sven-Olof Abrahamsson and Richard Gelberman

To determine whether there are differences in the extent to which the superficial cellular layers remain viable and contribute to the healing process, extra- and intrasynovial hindlimb donor tendons of 12 dogs (24 tendons) were labeled with 3H-thymidine in vitro and transferred to the medial and lateral toes of the forepaw. After transplantation, the distribution and amounts of remaining labeled cells within the healing tendon grafts were determined at 1 and 3 weeks after surgery by scintillation countings and autoradiographic examinations.

The two types of tendon grafts showed different turnover rates for the elimination of labeled DNA. At 1 week, 25 percent of labeled DNA remained in the extrasynovial grafts and 65 percent in the intrasynovial grafts. The two types of tendon grafts also showed different healing responses histologically. Autradiographic examinations showed that the majority of labeled cells were located in the periphery of the control tendons and that the numbers of labeled cells within the newly proliferated superficial areas of both types of tendon grafts decreased over time. These findings indicate that intrasynovial flexor tendons may possess improved prerequisites for superficial cellular survival and tendon gliding following autogenous tendon grafting.

Massachusetts General Hospital, Department of Orthopedics, 15 Parkman Street - Suite 527, Boston, MA 02114-2600, USA. Correspondence: Dr. S-O Abrahamsson, Department of Hand Surgery, Malmö General Hospital, S-214 01 Malmö, Sweden. Tel 46 40-331725. Fax -928855
Submitted 93-07-10. Accepted 94-06-11

The restitution of function of the tendon following autogenous grafting depends on the extent to which the gliding surface is maintained or restored (Braithwaite and Brockis 1957, Lindsay and McDougal 1961, Boyes and Stark. 1971, Urbaniak et al. 1974). Using extrasynovial donor tendons, previous authors have observed that the tendon surface is consistently obliterated after grafting by circumferential adhesions to the digital sheath (Peacock 1959, Flynn et al. 1969). Through a process of surface remodeling, tendon gliding is variably restored, depending on the effectiveness of elongation of the intervening scar.

Recent studies have suggested that intrasynovial donor tendons may survive the grafting process without undergoing widespread cellular necrosis (Gelberman et al. 1992a,b, Seiler et al. 1993). We determined the extent to which the superficial layers of healing intra- and extrasynovial donor tendons remain viable and contribute to the healing process following tendon grafting to the digital sheath.

Animals and methods

12 adult mongrel dogs, weighing 25–35 kg, were used. All procedures were performed under sterile conditions in an animal operating facility. The dogs were anesthetized with an intravenous dose of sodium Thiamylal (0.5 mL/kg of body weight) and Acepromazine (0.02 mL/kg of body weight). The animals were orotracheally intubated and maintained on 1 percent Halothane anesthetic. 1 peroneal tendon graft (extrasynovial) of each of the 2 hindlimbs and 2 deep flexor tendon grafts (intrasynovial) of 1 of the hindpaws were harvested. The tendons were placed in sterile tubes containing MCDB 105 medium (Sigma Chemical Co; Abrahamsson et al. 1989), supplemented with gentamicin (50 μg/mL), ascorbic acid (50 μg/mL) and bovine serum albumin (1 mg/mL) and transferred to the laboratory facilities for radioactive labeling.

Culture and labeling protocols for tendon grafts

Before culture, all intra- and extrasynovial tendon grafts were rinsed in DMEM medium (Gibco Laboratories, Gaithersburg, MD, supplemented with HEPES buffer (3.57 mg/mL), ascorbic acid (50 μg/mL), $NaHC0_3$ (3.7 mg/mL) and streptomycin/penicillin (Gibco Laboratories, Gaithersburg, MD), placed in 6-multidish plates, one tendon per well (Fisher, Atlanta, GA). 6 mL of fresh supplemented DMEM medium was added to each well.

In order to have the 3H-thymidine uptake opti-

Copyright © Scandinavian University Press *1994. ISSN 0001-6470. Printed in Sweden – all rights reserved.*

mized by dog tendons cultured in DMEM, different labeling procedures were tested in separate experiments. The optimal procedure was found to be comparable to that of rabbit tendons cultured in MCDB 105 medium (Ahrahamsson 1991). The tendons were preincubated for 24 h at 37 °C in a water-saturated atmosphere of 5 percent CO_2. On the second day, the supplemented medium was replaced with fresh medium containing 10 percent fetal calf serum. On the third day, the procedure was repeated and tendon segments were labeled for 24 h with 3H-thymidine (7 µCi/mL; Radiochemical Center, Amersham, England). On the fourth day, the tendons were rinsed once and chase-incubated 4 times for 30 min in supplemented medium. In separate experiments we found that this procedure completely removed the abundance of 3H-thymidine from the medium. 1 flexor tendon and 1 peroneal tendon of each animal were placed in a sterile tube containing supplemented medium MCDB 105 and transferred to the operating facility for completion of the tendon grafting procedure. The remaining 2 tendon grafts, serving as control tendons, were divided and either collected in a freezer at –20 °C for later synchronous determination of the incorporation of 3H-thymidine, or placed in formalin for autoradiographic examination.

Grafting procedure, postoperative treatment and harvest of tendon grafts

After culture, deep flexor tendons of the second and fifth left forepaw toes of the previously operated dogs were excised from the digital sheath area. The labeled intrasynovial deep flexor tendon graft was then transplanted through a midlateral incision to the digital sheath of the second toe and the extrasynovial peroneal tendon graft to the digital sheath of the fifth toe. The flexor tendon grafts were oriented according to their original in situ relationship to the phalanges and pulleys. Distally, the tendon grafts were passed through longitudinal incisions in the recipient tendon stumps. They were secured distally with Kessler 3–0 nylon sutures over padded dorsally placed buttons at the distal phalanges and with 4–0 braided dacron mattress sutures between the grafts and the recipient tendon stumps. The tendon sheath was closed with interruptured 4–0 braided dacron. Proximally, at the level of the distal forearm, the tendon graft was woven into the distal end of the remaining tendon and secured with two horizontal mattress sutures of 4–0 braided dacron. Hemostasis was obtained and the wounds were closed.

Postoperatively, the animals were placed in polyurethane shoulder spica casts with their elbows at 90° and their wrists at 45° of flexion. Early controlled passive mobilization was initiated on the day after surgery. The wrists and digits were passively flexed and extended to the limits of a dorsal extension bloc for ten minutes daily.

At 1 and 3 weeks, respectively, 6 dogs were killed and the labeled extra- and intrasynovial tendon grafts were harvested, divided into proximal and intermediate segments and either collected in a freezer at –20 °C for later synchronous quantification of the remaining 3H-thymidine-labeled DNA or placed in formalin for autoradiographic examination (Abrahamsson et al. 1989). In addition, segments of the unoperated superficial flexor tendons surrounding the deep flexor tendons at the level of the first metacarpal bone were collected for determination of indirectly incorporated 3H-thymidine.

Determination of incorporation rates

After lyophylization and weighing, tendon segments labeled with 3H-thymidine were dissolved in 1 M potassium hydroxide at 37 °C for 4 h, neutralized and cooled on ice. Nucleic acids were precipitated by adding trichloroacetic acid (TCA). After centrifugation, the supernatant was discarded and the procedure repeated. Since no radioactivity was found in the supernatant, there was no possibility that free 3H-thymidine significantly affected the labeled tendons. The final washed pellet was dissolved in sodium hydroxide, neutralized and mixed with liquid scintillant (Opti-Fluor; Packard Instr. Co.) and counted in a scintillation counter (Beckman Inc., Fullerton, CA). The content of 3H-thymidine, expressed as disintegrations per min per mg dry weight tendon, was used as a measure of the amount of synthesized DNA in vitro (cell proliferation). The amount of remaining 3H-thymidine in extrasynovial and intrasynovial tendon grafts was expressed as percent of simultaneously labeled, but not grafted, control tendons in each dog. The amount of indirect radioactive uptake in surrounding superficial flexor tendons was expressed as disintegrations per min per mg dry weight tissue (dpm/mg dwt).

Autoradiography

As tendon segments were collected, they were fixed in formalin, dehydrated, embedded in paraffin and cut into longitudinal 10 µm sections by standard techniques. The sections were deparaffinized, rehydrated in tromethamine buffered solution (TRIS) and dipped into nuclear track emulsion (NTB 3, Kodak Co, Rochester, NY) diluted 1:2 in distilled water. The slides were dried, exposed in light-tight boxes for 2 weeks and developed in Kodak developer D-19

Table 1. The amount of 3H-thymidine remaining in extrasynovial peroneal and intrasynovial flexor tendon grafts. Values are presented as percentage of nontransplanted control tendons, median and range (n 6)

	1 week	*P*-value	3 weeks
Extrasynovial peroneal grafts	28 (2–50)	0.02	0.4 (0.1–3.1)
P-value	0.04		0.7
Intrasynovial flexor grafts	63 (111–12)	0.009	1.0 (0.3–3.2)

Table 2. The amount of 3H-thymidine in normal superficial flexor tendons surrounding tendon grafts. Values are presented as disintegrations per min per mg dry weight tissue (dpm/mg dwt), median and range (n 6).

	1 week	*P*-value	3 weeks
Tendons around extrasynovial grafts	63 (20–115)	0.06	26 (17–36)
P-value	0.3		0.2
Tendons around intrasynovial grafts	93 (31–182)	0.02	18 (17–25)

The values at 3 weeks were comparable to that of background

(Kodak Co, Rochester, NY). Slides were further stained with hematoxylin and eosin, examined under light microscopy and replication-scored by counting cells covered by dark granules.

Statistics

The results are presented as median and range. As the distribution of values within groups was unknown, the non-parametric Wilcoxon rank test was used when comparing 2 groups with each other. Paired testing gave no advantages. A value of $P < 0.05$ was considered significant.

Results

No ruptures or infections were noted among the grafts. At both intervals of healing, the tendon grafts of extrasynovial origin showed a slightly greater quantity of adhesions, decreased turgor and increased numbers of vessels compared to the intrasynovial tendon grafts.

Determination of 3H-thymidine

The rates of cellular turnover in the extrasynovial peroneal tendon grafts and intrasynovial flexor tendon grafts, represented by the median amount of 3H-thymidine remaining in the proximal and intermediate segments, are expressed as percentages of control values (Table 1). In both extrasynovial peroneal tendon grafts and intrasynovial flexor tendon grafts, the percentage of 3H-thymidine remaining following transplantation was reduced by time. Higher values were noted in the intrasynovial peroneal tendon grafts at 1 week.

We also determined the rates of indirect uptake by surrounding tissues, represented by the amounts of 3H-thymidine (dpm/mg dwt) in the unoperated superficial flexor tendons surrounding the intrasynovial tendon grafts at 1 and 3 weeks (Table 2).

Autoradiography

In control extrasynovial peroneal extensor tendons, the uptake of 3H-thymidine was concentrated heavily in the cells within the surrounding loose connective tissue and in the cells of the most superficial regions of the tendons (Figure 1). Few labeled cells were observed within the tendon's endotenon. In control intrasynovial flexor tendons, up to 50 percent of the cells within the epitenon and a few cells within the most superficial regions of the tendon stained heavily with 3H-thymidine (Figure 2). Similar findings were made in the different segments of the intrasynovial tendons (Abrahamsson et al. 1989).

Extrasynovial tendon grafts at 1 week were surrounded by a several layer thick hyperproliferative zone of fibroblast-like cells (Figure 3). Cells originating from the surface appeared to extend into the central portion of some of the tendon grafts, forming a partial and patchy reorganization of the endotenon. Segments of longitudinally-arranged vessels were seen in most of the grafts. Cells with persistent heavy uptake of 3H- thymidine and a few cells with a reduced amount of grains were observed within the surrounding, newly formed cellular layer, as well as in the most superficial layers of the endotenon. In contrast, intrasynovial tendon grafts at 1 week had a slightly thickened epitenon which was composed primarily of 2–3 layers of spindle-shaped cells (Figure 4) and a morphologically preserved endotenon. Compared to control tendons, there were fewer superficial cells demonstrating a heavy uptake of 3H-thymidine. Few cells with reduced amounts of grains were found.

Extrasynovial tendon grafts at 3 weeks had increased thickening of the surrounding zone of fibroblast-like cells compared to those examined at 1 week. The patchy reorganization of endotenon was observed in 50 percent of specimens and vessels were observed within all grafts. Intrasynovial tendon grafts had a slightly thickened epitenon and longitudinally-oriented vessels were seen occasionally with-

Figure 1. Autoradiograph of control extrasynovial peroneal tendon demonstrating a heavy 3H-thymidine uptake concentrated superficially and in the paratenon (arrow). HE, × 200.

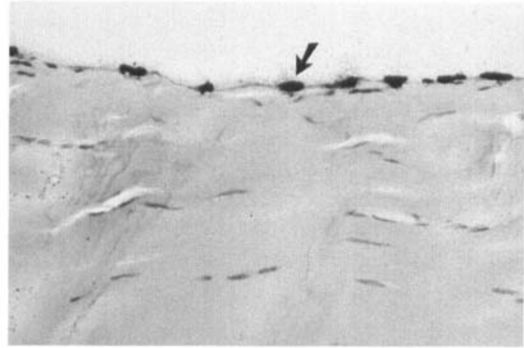

Figure 2. Autoradiograph of control intrasynovial flexor tendon demonstrating a moderate uptake in the epitenon layer of fibroblasts (arrow). HE, × 300.

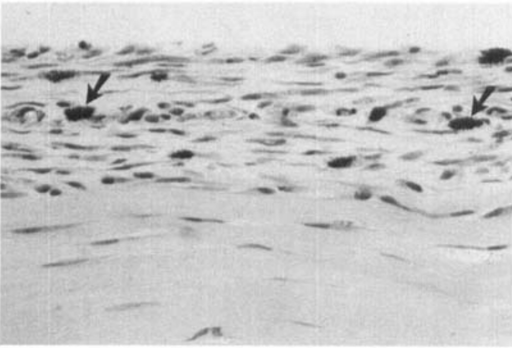

Figure 3. Autoradiograph of extrasynovial tendon graft 1 week after labeling and transplantation. The hypercellular superficial zone containing fibroblast-like cells demonstrates persistent 3H-thymidine uptake (arrows). HE, × 300.

Figure 4. An intrasynovial flexor tendon graft at 1 week with a 2–3 cell layer epitenon containing cells with persistent labeling (arrows). HE, × 300.

in the predominantly preserved endotenon. There were no cells with a persistent heavy uptake of 3H-thymidine in either of the 2 types of grafts.

Discussion

While previous studies have implied differences in surface cellular viability between extrasynovial peroneal and intrasynovial flexor tendon grafts (Gelberman et al. 1992a, b, Seiler et al. 1993), none has focused on the survival characteristics of superficial fibroblasts, those cells that affect the gliding function of the tendon grafts directly. We found areas of hypercellularity, neovascularization and surface reorganization in the extrasynovial grafts. Intrasynovial grafts, in contrast, showed little evidence of adhesion formation, less striking surface reorganization and cellular response in superficial cellular layers.

Autoradiographic examinations demonstrated decreasing numbers of labeled cells within the hyperproliferative superficial areas of both types of tendon grafts over time. Few of the labeled cells in both tendon types showed evidence of dividing cells with reduced amounts of grains. These data indicate a relatively high turnover rate for labeled superficial tendon cells, irrespective of the site of origin. These findings differ from those of Lindsay and Birch (1964), who noted that the numbers of labeled cells increased within tendon grafts themselves, but decreased in surrounding tissues. Their animal tendons, however, were labeled indirectly by intravenous injections of 3H-thymidine.

Autoradiographic findings correlated well with the values obtained with scintillation counting of the remaining radioactive uptake in the extrasynovial and intrasynovial tendon grafts. The high turnover rates for labeled cells in both types of tendons indicate that the grafting procedure results in a progressive degradation of surface cells over time.

Little information has been provided regarding normal turnover rates of surface cells in dense regular connective tissue. Cleaver (1967), reporting that the turnover of thymidine varied depending on the tissue studied, found that the initial half-times varied

between 1 and 60 days. Our findings that the measurable uptake by superficial flexor tendons at 1 week was lost within the following 2 weeks indicates a relatively high turnover rate for the surface layers of normal unoperated intrasynovial tendons. Applied to the labeled tendon grafts, this observation further underlines the importance of the measured differences in remaining activity between extra- and intrasynovial tendon grafts at 1 week and may explain the lack of activity in both types of grafts at 3 weeks.

There are limitations to the methods utilized in this study. The amounts of degradation, measured as remaining radioactivity, may be reduced by reutilization of thymidine (Cleaver 1967). While most of the incorporated thymidine is reduced to carbon dioxide and water as cells degrade, minor amounts of thymidine can be reused by other tendon cells or by cells in the surrounding tissues. In our study, this phenomenon may have been reflected by the minor uptake noted in cells of the superficial flexor tendons at 1 week. Relatively little data are available regarding the metabolic and proliferative activity of the tendon's internal (endotenon) fibroblasts. The lack of activity among endotenon fibroblasts may indicate that the cells have not been exposed to labeled thymidine. The observation, however, that some of the mesenchymal cells found along blood vessels within the core of the tendon had evidence of uptake indicates that the central parts of the tendon grafts probably were exposed to the 3H-thymidine. The failure of endotenon fibroblasts to demonstrate labeling may therefore reflect a lack of proliferative activity among these cells. For our goals, however, the use of direct tendon labeling with 3H-thymidine provided a unique method of determinating the fate of fibroblasts within the gliding surface layers of intrasynovial and extrasynovial tendon grafts.

Although we observed high turnover rates for labeled surface cells in both extra- and intrasynovial tendons, perhaps the most relevant observation clinically relates to the significant differences in cellular turnover at 1 week noted between the 2 types of tendon grafts. Histologically, the proliferation of superficial cells and the reorganization of the superficial regions of the tendon grafts was far more pronounced in the extrasynovial than in the intrasynovial donor tendons. These differences may indicate improved survival characteristics for intrasynovial tendons and may correlate well with previous histological and biomechanical observations and support the premise that intrasynovial deep flexor tendons may be especially suited for cellular survival and tendon gliding (Gelberman et al. 1992a, b, Seiler et al. 1993).

Acknowledgements

This study was supported by NIH grant AR 33097 and by grants from the Swedish Medical Research Council K92-17F-10029-01, the Swedish Society of Medicine, the Österlund Foundation, the Tore Nilsson Foundation, the Medical Faculty, University of Lund and the Royal Physiographic Society. The authors thank Carol Trahan, Orthopaedic Research, Massachusetts General Hospital for her invaluable technical assistance and Docent Claus Rerup, Department of Pharmacology, Lund University, for statistical advice.

References

Abrahamsson S O. Matrix metabolism and healing in the flexor tendon. Experimental studies on rabbit tendon. Scand J Plast Reconstr Surg Hand Surg (Suppl 23) 1991: 1–51.

Abrahamsson S O, Lundborg G, Lohmander L S. Segmental variation in microstructure, matrix synthesis and cell proliferation in rabbit flexor tendon. Scand J Plast Reconstr Surg Hand Surg 1989; 23 (3): 191–8.

Boyes J H, Stark H H. Flexor-tendon grafts in the fingers and thumb. A study of factors influencing results in 1000 cases. J Bone Joint Surg (Am) 1971; 53 (7): 1332–42.

Braithwaite F, Brockis J G. The vascularization of a tendon graft. Br J Plast Surg 1957; 4: 130–5.

Cleaver J E. Thymidine metabolism and cell kinetics. J Wiley & Sons Inc, New York 1967.

Flynn J E, Wilson J T, Child C G, Graham J H. Heterogenous and autogenous-tendon transplants in dogs. J Bone Joint Surg (Am) 1969; 42: 91.

Gelberman R H, Seiler J G, Rosenberg A E, Heyman P, Amiel D. Intercalary flexor tendon grafts. A morphological study of intrasynovial and extrasynovial donor tendons. Scand J Plast Reconstr Surg Hand Surg 1992a; 26 (3): 257–64.

Gelberman R H, Chu C R, Williams C S, Seiler J G, Amiel D. Angiogenesis in healing autogenous flexor-tendon grafts. J Bone Joint Surg (Am) 1992b; 74 (8): 1207–16.

Lindsay W K, Birch J R. The fibroblast in flexor tendon healing. Plast Reconstr Surg 1964; 34 (3): 223–32.

Lindsay W K, McDougall E P. Digital flexor tendons. An experimental study-III. The fate of autogenous digital flexor tendon grafts. Br J Plast Surg 1961; 13: 293–304.

Peacock E E. A study of the circulation in normal tendons and healing grafts. Ann Surg 1959; 149: 415–28.

Seiler J G, Gelberman R H, Williams C S, Woo S L, Dickersin G R, Sofranko R, Chu C R, Rosenberg A E. Autogenous flexor-tendon grafts. A biomechanical and morphological study in dogs. J Bone Joint Surg (Am) 1993; 75 (7): 1004–14.

Urbaniak J R, Bright D S, Gill L H, Goldner J L. Vascularization and the gliding mechanism of free flexor-tendon grafts inserted by the silicone-rod method. J Bone Joint Surg (Am) 1974; 56 (3): 473–82.