

Repair of Tendon Defect with Dermal Fibroblast Engineered Tendon in a Porcine Model

WEI LIU, M.D., Ph.D.,* BIN CHEN, M.D.,* DAN DENG, M.D., FENG XU, M.D.,
LEI CUI, M.D., Ph.D., and YILIN CAO, M.D., Ph.D.

ABSTRACT

Harvesting autologous tenocytes for tendon engineering may cause secondary tendon defect at the donor site. Dermal fibroblasts are an easily accessible cell source and do not cause major donor site defect. This study aims to explore the possibility of tendon engineering using dermal fibroblasts. A total of 45 hybrid pigs were randomly divided into three groups: experimental group ($n = 15$)—repair of tendon defect with a dermal fibroblast engineered tendon; control group 1 ($n = 15$)—repair of defect with a tenocyte engineered tendon; and control group 2 ($n = 15$)—repair of defect with a scaffold alone. Both autologous dermal fibroblasts and tenocytes were seeded on polyglycolic acid (PGA) unwoven fibers to form a cell-scaffold construct and cultured *in vitro* for 7 days before *in vivo* implantation to repair a defect of flexor digital superficial tendon. Specimens were harvested at weeks 6, 14, and 26 for gross, histological, and mechanical analyses. Microscopy revealed good attachment of both dermal fibroblasts and tenocytes on PGA fibers and matrix production. *In vivo* results showed that fibroblast and tenocyte engineered tendons were similar to each other in their gross view, histology, and tensile strength. At 6 weeks, parallel collagen alignment was observed at both ends, but not in the middle in histology, with more cellular components than natural tendons. At weeks 14 and 26, both engineered tendons exhibited histology similar to that of natural tendon. Collagens became parallel throughout the tendon structure, and PGA fibers were completely degraded. Interestingly, dermal fibroblast and tenocyte engineered tendons did not express type III collagen at 26 weeks, which remained observable in normal pig skin and control group 2 tissue using polarized microscopy, suggesting a possible phenotype change of implanted dermal fibroblasts. Furthermore, both fibroblast and tenocyte engineered tendons shared similar tensile strength, about 75% of natural tendon strength. At 6 weeks in control group 2, neo-tissue was formed only at the peripheral area by host cells. A cord-like tissue was formed at weeks 14 and 26. However, the formed tissue was histologically disorganized and mechanically weaker than both cell-engineered tendons ($p < 0.05$). These results suggest that dermal fibroblasts may have the potential as seed cells for tendon engineering.

Department of Plastic and Reconstructive Surgery, Shanghai Ninth People's Hospital; Shanghai Tissue Engineering Center, Shanghai Second Medical University, Shanghai, P. R. China.

*These two authors contributed equally to this work.

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INTRODUCTION

DEVELOPMENT OF TISSUE-ENGINEERING TECHNIQUE offers a promising approach for regeneration and repair of musculoskeletal tissues, such as bone, cartilage, and tendon.¹ Previous studies showed that tissue-engineering technique was feasible to generate tendon tissue in small animal models.² A study in our group demonstrated that tendon tissue could be generated in a hen model using polyglycolic acids and tenocytes and could be used to repair a defect of the flexor digitorum profundus tendon. Importantly, the engineered tendon exhibited histological features and biomechanical properties similar to those of natural tendon.³

However, use of tenocytes as seed cells to engineer tendon tissue certainly has its limitations in the practical application of an engineered tendon, because isolation of autologous tenocytes itself can cause a tendon defect. In recent years, researchers have also searched for an alternative cell source, such as bone marrow stromal cells, for *in vivo* tendon engineering in small animal models.⁴⁻⁶ Considering the fact that both tenocytes and dermal fibroblasts are derived from mesoderm and have similar characteristics in cell morphology and extracellular matrix components, we hypothesized that dermal fibroblasts may replace tenocytes as seed cells for *in vivo* tendon engineering. In this study, repair of a porcine flexor digital superficial tendon defect was used as an *in vivo* model to demonstrate that dermal fibroblasts could serve as seed cells to engineer tendon tissue *in vivo*. In addition, both tenocytes and dermal fibroblasts were used as seed cells to demonstrate that they could form tendons with histological, biomechanical, and biochemical characteristics similar to each other.

MATERIALS AND METHODS

Animals

Four-week-old hybrid pigs were used in this study. Animals, either male or female and weighting ~10 kg, were purchased from Shanghai Chuansha Breeding Factory. An institutional review committee of Shanghai Second Medical University approved all animal study protocols.

Isolation and *in vitro* culture of dermal fibroblasts and tenocytes

The pigs were anesthetized by intramuscular injection of ketamine (2 mg/kg) and 2.5% sodium pentobarbital (0.2 mL/kg). The skin tissue was obtained from an abdominal area (an ellipse area, $7 \times 4 \text{ cm}^2$) and tendon tissue was obtained from digital flexor superficial tendons. Both autologous dermal fibroblasts and tenocytes were isolated and cultured using a previously described method.³ Briefly, epidermis was removed first from the skin by dis-

treatment (0.24 U/mL in medium; Roche, Mannheim, Germany) overnight at 4°C and mechanical separation, and then both harvested dermis and tendon were minced into small pieces ($2 \times 2 \times 2 \text{ mm}^3$). The tissue fragments were rinsed with phosphate-buffered saline (PBS) three times followed by the digestion with 1.5 mg/mL type II collagenase (Worthington, Freehold, NJ) in serum-free Dulbecco's modified Eagle's medium (DMEM, Gibco, Grand Island, NY) at 37°C on a rotator. The resulting cell suspension harvested at 3 and 6 h post-digestion was filtered through a sterile nylon mesh (Tetko, Elmsford, NY) to remove tissue residues. The filtrate was further centrifuged, and cell pellets were washed with PBS twice and then re-suspended in DMEM culture medium containing 10% fetal bovine serum (FBS, Gibco), L-glutamine (292 µg/mL), penicillin (100 U/mL), streptomycin (100 U/mL), and ascorbic acid (50 µg/mL). The extracted cells were plated on 100 mm culture dishes (1×10^6 cell/dish) and incubated at 37°C in a humidified atmosphere containing 95% air and 5% carbon dioxide. When cultured primary cells reached 90% confluence, they were detached with 0.25% trypsin and 0.1% ethylenediaminetetraacetic acid (EDTA, Gibco) and subcultured at the density of 1×10^6 cell/dish.

Cell-scaffold construct

Polyglycolic acid (PGA) unwoven fibers (Albany International Research, Albany, NY) were used as a scaffold material. Briefly, 20 mg PGA fibers (15 µm in diameter) were arranged into a cord with a length of 40 mm and a diameter of 0.5 cm, similar to that of porcine flexor digital superficial tendon. This construct was then wrapped with a biomembrane of acellular intestinal submucosa membrane (Shanghai Jing Huang Medical Appliance, Shanghai, China). Each construct was soaked in 70% ethanol for 1 h and washed with PBS three times followed by three washes with DMEM plus 10% FBS. The medium was removed and the construct was subsequently air dried for 30 min under ultra-violet light. The cultured dermal fibroblasts or tenocytes were respectively collected and re-suspended in culture medium at a density of 5×10^7 cell/mL. In a culture dish, 3.5×10^7 cells (for either cell type) were seeded evenly onto the PGA fibers, and the cell-scaffold construct was kept in an incubator for 4 h, allowing complete adhesion of the cells to the PGA fibers. Culture medium was then added, and the cell-scaffold construct was incubated for 7 days prior to *in vivo* transplantation. During this incubation period, the culture medium was changed every other day. As a control for both dermal fibroblast and tenocyte engineered tendons, the construct was similarly prepared without cell seeding.

Scanning electron microscopic (SEM) examination

After 7 days of *in vitro* culture, part of the cell-scaffold construct was prepared for SEM examination. Briefly, samples were prefixed with 2% glutaraldehyde

for 2 h at 4°C and washed twice with PBS at 4°C followed by post-fixation with 1% osmic acid for 2 h at 4°C. After two washes in PBS, the samples were dehydrated with gradient ethanol and dried to a critical point (HCP-2, Hitachi, Tokyo, Japan). The samples were then mounted, sputter-coated with gold (BAL-TEC, Philips, Eindhoven, The Netherlands), and examined with a scanning electron microscope (Philips-XL-30) to observe cell attachment and matrix production on the PGA scaffold.

Experimental design

The 45 hybrid pigs included in this study were randomly divided into three groups, including 1) the experimental group ($n = 15$)—a tendon defect was repaired by a dermal fibroblast engineered tendon; 2) control group 1 ($n = 15$)—a tendon defect was repaired by a tenocyte engineered tendon; and 3) control group 2 ($n = 15$)—a tendon defect was repaired by the PGA scaffold alone. Only the left posterior leg of each pig was involved in the study; the right leg served as a natural tendon control or as a donor for autologous tenocytes.

Surgical procedure

The pigs were anesthetized by intramuscular injection of ketamine (2 mg/kg) and 2.5% sodium pentobarbital (0.2 mL/kg) with intubation. An incision was made at the dorsal aspect of the left posterior leg above the ankle joint. After exposure of the flexor digital superficial tendon, a 2-cm-long tendon fragment was resected to create a tendon defect. Both ends of the severed tendon were allowed to retreat by themselves, resulting in a tendon defect of 3 cm in length. The cultured cell-scaffold construct was then implanted to bridge the tendon defect in the experimental group ($n = 15$) and in control group 1 ($n = 15$) using Kessler's method of tendon repair. The wrapped bio-membrane was also extended to host tendon ends to cover the interface area. The construct without cells was used to bridge the defect in control group 2 ($n = 15$). Specimens were harvested post-repair at weeks 6 ($n = 5$ /each group), 14 ($n = 5$ /each group), and 26 ($n = 5$ /each group), respectively, for gross, histological, and transmission electronic microscope (TEM) examination and biomechanical analysis. Of the five harvested tendons, one sample was used for histological and TEM examinations, the remaining used for biomechanical test.

Macrographic examination

Specimens harvested from different groups were compared to each other and to natural tendons regarding their tissue color and appearance, and the interface healing between engineered and host tendons was also examined.

Hematoxylin and eosin and trichrome staining

Harvested specimens were fixed in 4% paraformaldehyde overnight. After washes in PBS, the tissues were

paraffin-embedded and sectioned at 5 μ m for H&E and trichrome staining. Histologic sections were examined to observe PGA degradation, cellular density, collagen production and alignment, and interface healing between engineered and host tendons.

Polarizing microscope examination

To determine the contribution of collagens I and III in the formed tissues of different groups, a polarizing microscope was used to examine H&E-stained tissue samples of 26 weeks, as previously described.⁷

Transmission electron microscopic (TEM) examination

Specimens harvested at different time points were prepared as described in SEM examination. After fixation and dried to a critical point, the samples were mounted and sputter-coated with gold for viewing in a transmission electron microscope (Quanta 200 FEI) to examine the distribution and diameter of collagen fibrils in the engineered and natural tendons. KS400 Image analysis software (version 3.0, Zeiss) was used to analyze the cross-section image of the collagen fibrils, and about 100 collagen fibrils were randomly selected from each image to measure and obtain an average diameter of the collagen fibrils.

Biomechanical analysis

Fresh specimens harvested at various time points were submitted to tensile strength measurement using a biomechanical analyzer (Instron, Canton, MA), similar to a previously study.³

Statistical analysis

An ANOVA test was applied to analyze group difference in collagen fibril diameter and tissue tensile strength. Two-sample t test was also used to analyze the tensile strength difference between the experimental group and control group 1 and the difference between the experimental group and control group 2. A p value of <0.05 was considered statistically significant. SAS software (version 6.12) was applied in statistical analysis.

RESULTS

Phase-contrast microscopic and SEM examination of cell-scaffold construct

After *in vitro* culture of the cell-scaffold construct for 7 days, both fibroblasts and tenocytes were found to produce matrices on the PGA scaffold when observed under a phase contrast microscope (Fig. 1). SEM examination further demonstrated that both cell types adhered to and spread on the PGA fibers very well and produced extracellular matrices around the polymer fibers (Fig. 2).

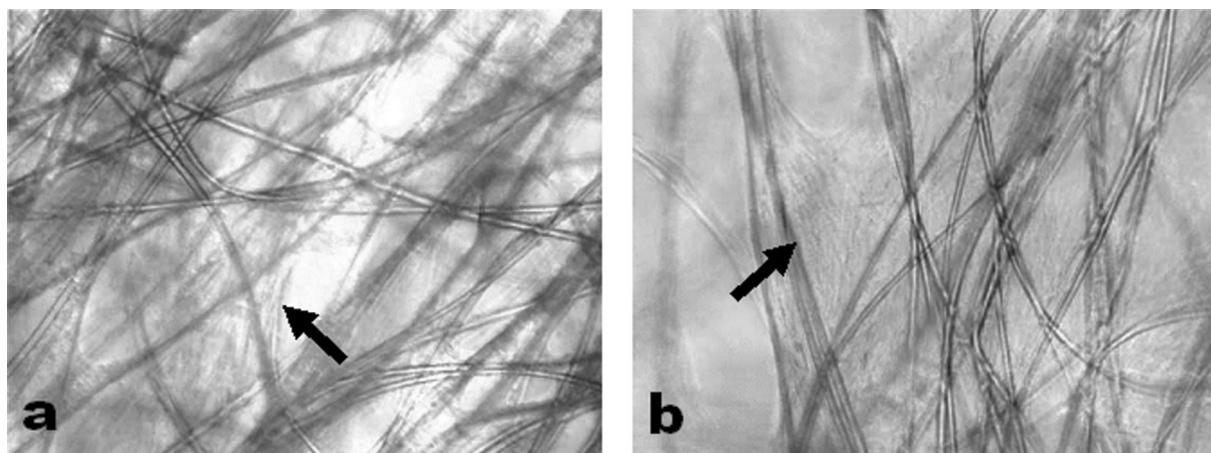


FIG. 1. Phase contrast microscopic view of an *in vitro* cultured cell-scaffold construct. Both dermal fibroblasts (A) and tenocytes (B) produce matrices on PGA fibers, indicating good biocompatibility between the cells and the scaffold material. Arrow indicates cell attachment and matrix production.

Macrographic examination

Experimental group. At 6 weeks post-repair, a relatively dark color, cord-like neo-tendon was formed at the site of tendon defect. No polymer fibers were grossly visible within the newly formed tissue, and good interface healing was observed. In addition, a moderate level of adhesion was noted between the neo-tendon and its surrounding tissue. Furthermore, the neo-tendon was thicker than a natural tendon at its corresponding area (Fig. 3A).

At 14 weeks post-repair, engineered tendons exhibited a cord-like shape with no material residue visible. The engineered tendons healed well, with host tendons at the interface. The tendons remained thicker than their normal counterparts (Fig. 3D).

At 26 weeks, the engineered tendons appeared as white-

colored, cord-shaped tissue, comparable to its natural tendon. Host and engineered tendons healed very well at the interface and became difficult to distinguish from one another. In addition, the engineered tendon remained a little thicker than its corresponding natural tendon. A moderate level of tissue adhesion was observed between the engineered tendon and its surrounding tissue. Although the color of the engineered tendon approached that of natural tendon, the engineered tendon did not exhibit a shiny-white, smooth surface as that observed in natural tendon, suggesting that a longer time period may be needed for tissue remodeling (Fig. 4A and D).

Control group 1. The macrographic view of tendons engineered with tenocytes exhibited similarly to that of

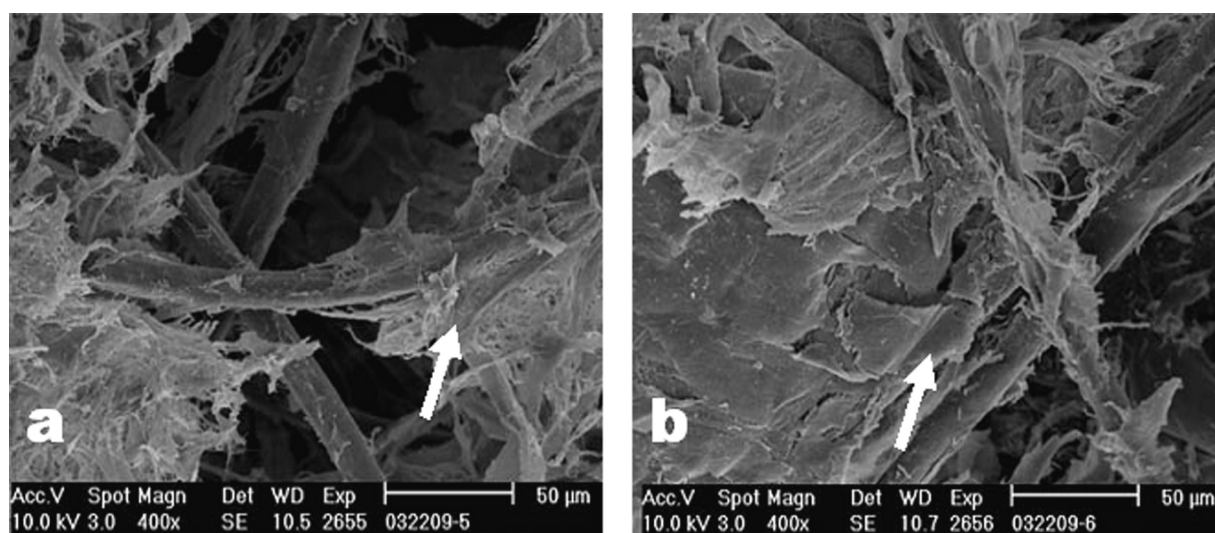


FIG. 2. Scanning electron microscopic view of an *in vitro* cultured cell-scaffold construct, which shows adhesion and matrix production of dermal fibroblasts (A) and tenocytes (B) on PGA fibers. Arrow indicates cell attachment and matrix production.

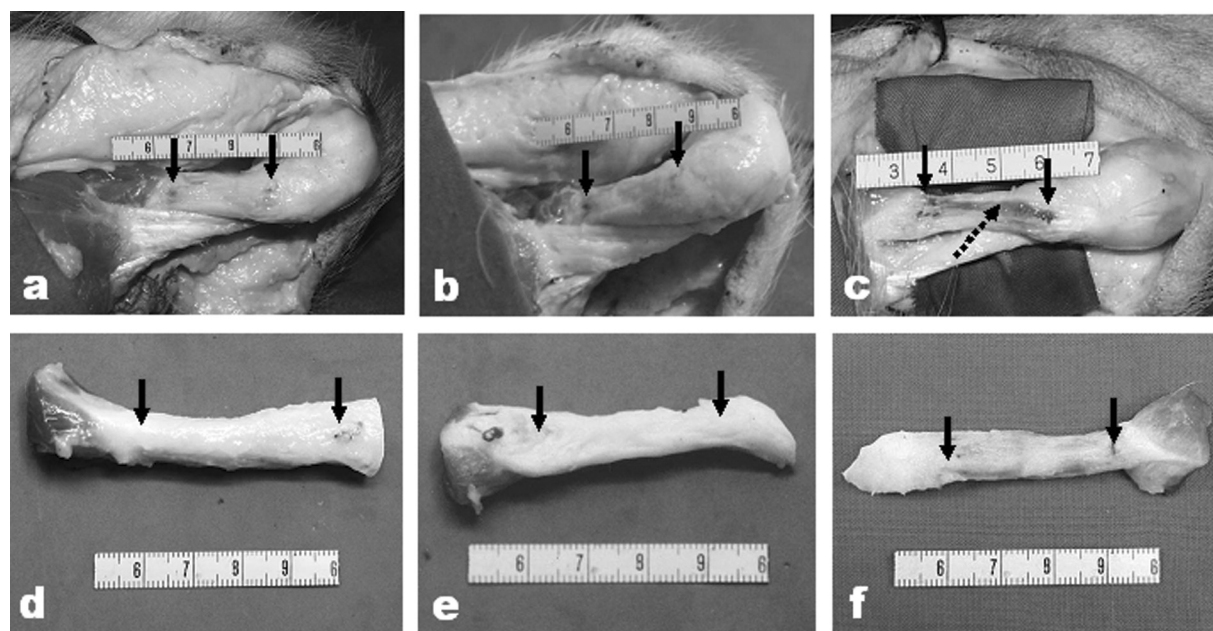


FIG. 3. Repair of tendon defects at 6 weeks (A–C) and gross view of harvested tissues (D–F) at 14 weeks. Tissues formed between arrows are a dermal fibroblast engineered tendon (A and D), a tenocyte engineered tendon (B and E), and a formed tissue in control group 2 (C and F), respectively. (C) Dotted arrow indicates scaffold biomaterial where no neo-tissue is formed. (Color images available online at www.liebertpub.com/ten.)

the experimental group at all three time points (Figs. 3B and E, 4B and E), indicating that tendons engineered either by dermal fibroblasts or by tenocytes can achieve a similar tendon structure.

Control group 2. Interestingly, a neo-tissue with a cord-like shape also formed at 6 weeks post-repair in control group 2 where a PGA scaffold alone was implanted. In contrast to the other two groups, the neo-tissue was observed only at the peripheral area of the cord-like structure, and yellow-colored scaffold biomaterial remained visible at the central area (Fig. 3C). This phenomenon strongly suggests that host cells migrated from the surrounding tissue participated in the neo-tissue formation. However, these cells that invaded into the scaffold were apparently insufficient to form a homogenous tissue at the time point of 6 weeks. In addition, a much more severe tissue adhesion was observed between the neo-tissue and its surrounding tissue. At the time points of 14 (Fig. 3F) and 26 (Fig. 4C and F) weeks post-repair, the formed tissue exhibited a dark-red color with a diameter thicker and surface rougher than its counterparts in the experimental group and control group 1. In addition, the formed tissue revealed severe fibrotic adhesion to its surrounding tissue and a stiff tissue texture, suggesting that the formed tissue resembles a fibrotic tissue more than a tendon tissue.

Hematoxylin and eosin and trichrome staining

6 weeks post-repair. At this time point, fibroblasts and tenocytes produced similar structures in their respective

neo-tendons under histological examination. H&E and trichrome staining showed that the structures of neo-tendon tissues contained abundant fibroblasts or tenocytes and extracellular matrices in their respective groups. Non-degraded PGA fibers remained visible. In addition, the neo-tendons contained more cellular components than their corresponding natural tendon tissue (Fig. 5A, B, and F). Histology also showed that collagen fibers were arranged in a relatively parallel form at both ends of the neo-tendons (Fig. 5A and B), whereas a random pattern of collagen arrangement was observed in the middle part of the tendons (Fig. 5D and E). Furthermore, good interface healing was observed in both groups, although different collagen patterns were observed at the sides of the neo-tendons and their host tendons, respectively (Fig. 5G and H). Moreover, trichrome staining revealed substantial matrix production in both types of engineered tendons at 6 weeks post-repair (data not shown).

In contrast, a neo-tissue that formed in control group 2 at the peripheral area of the PGA scaffold exhibited an irregular tissue structure with non-degraded PGA fibers. Although the scaffold was implanted without cell seeding, both H&E and trichrome staining demonstrated the presence of fibroblasts migrated from the surround tissue and the presence of extracellular matrices produced by these invaded cells. Overall, cellular components were relatively less than those of the other two groups. Additionally, collagen alignment was disorganized at both ends of the neo-tissue compared to that of the other two groups (Fig. 5C). Nevertheless, good interface healing

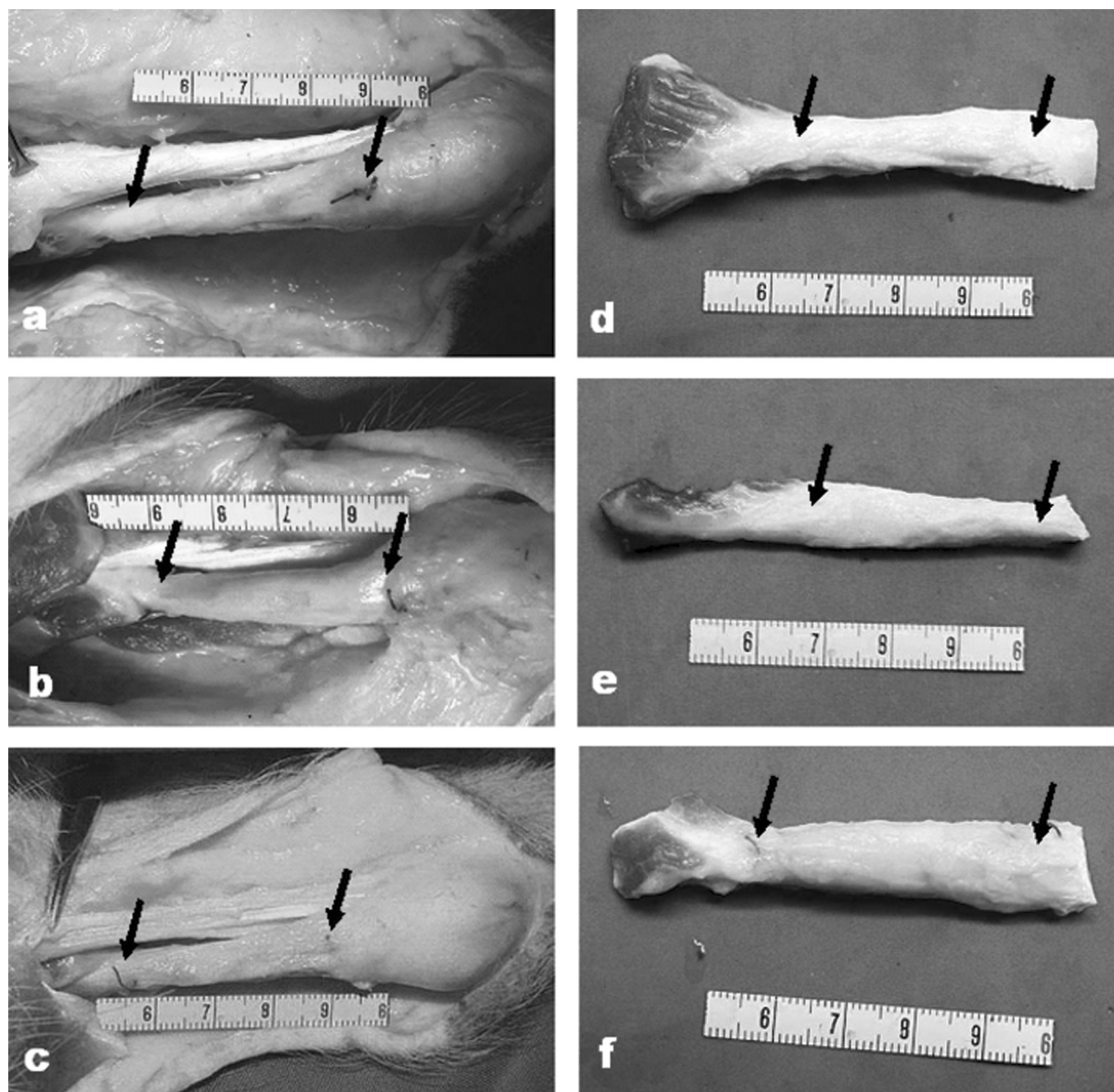


FIG. 4. Repair of tendon defects by gross view of a fibroblast engineered tendon (**A and D**, between arrows), a tenocyte engineered tendon (**B and E**, between arrows) and a formed tissue in control group 2 (**C and F**, between arrows) at 26 weeks. Note that both engineered tendons have a relatively smooth surface, whereas the control tissue has a rough surface. (**A–C**) Before harvesting; (**D–F**) after harvesting. (Color images available online at www.liebertpub.com/ten.)

was also observed in this group, although more infiltrated inflammatory cells were observed at the side of the neo-tissue (Fig. 5I).

14 weeks post-repair. At this time point, both H&E and trichrome staining demonstrated organized collagen fibers that aligned longitudinally along the axis of the loading force in both the experimental group and control group 1. This organized collagen pattern was observed not only at both ends (Fig. 6A and B), but also in the middle part (Fig.

6D and E). In addition, the PGA fibers were completely degraded at this time point, and matrix production in these two groups (Fig. 6A and B) was comparable to that of the natural tendon (Fig. 6C). Interestingly, cellular components decreased when compared to the neo-tendons of 6 weeks. These phenomena indicate that mechanical loading and tissue remodeling are important for the maturation of the engineered tendons. Moreover, histology also showed good interface healing between the engineered and natural tendons (Fig. 6G and H).

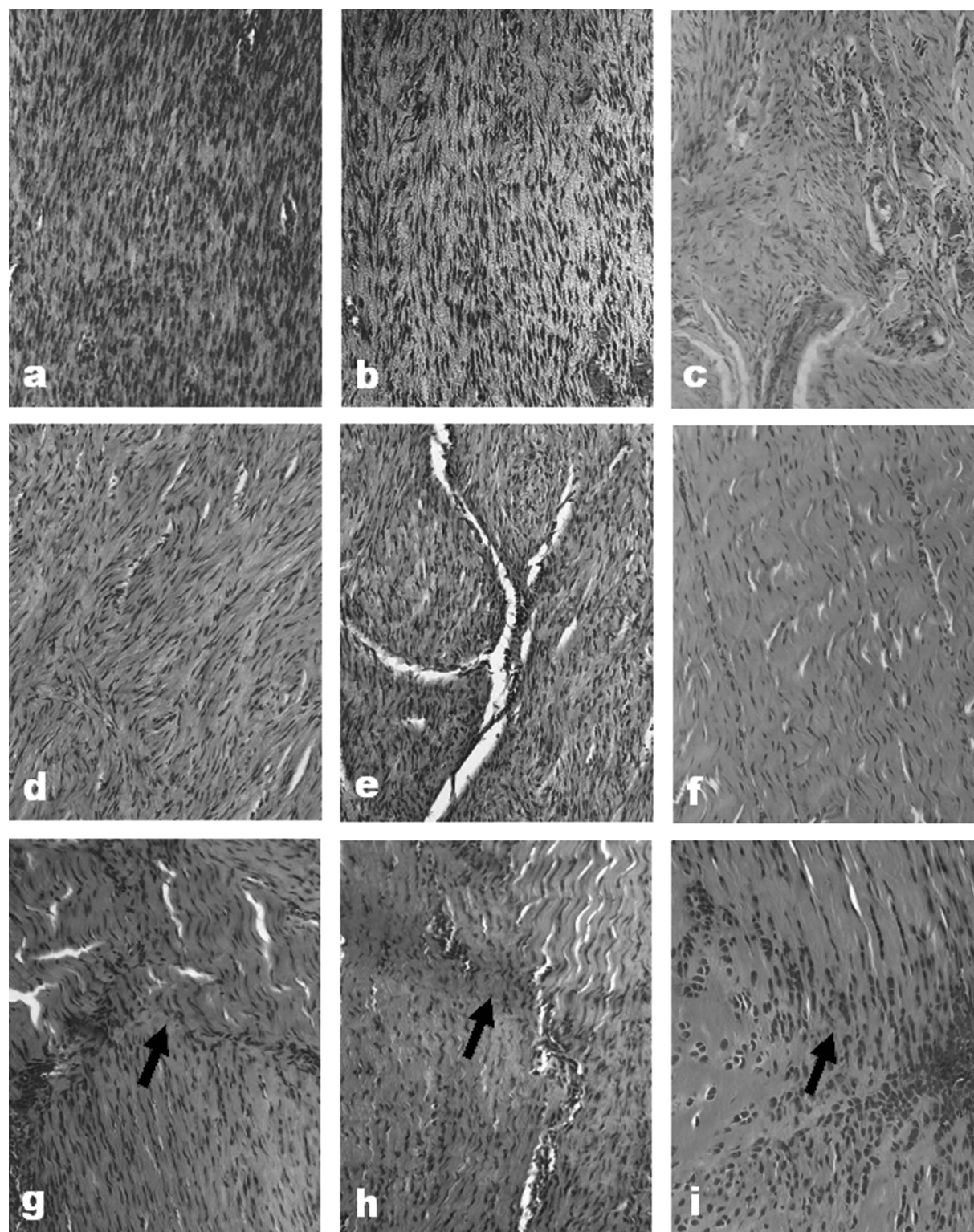


FIG. 5. Histological finding of formed tissues at 6 weeks. H&E staining shows the structures of a fibroblast engineered tendon (**A, D, and G**), a tenocyte engineered tendon (**B, E, and H**), a control tissue of control group 2 (**C and I**), and a natural tendon (**F**). Note that relatively parallel collagen alignment is observed at the end part (**A and B**), but not at the middle part of engineered tendons (**D and E**) and not at the end part of the tissue in control group 2 (**C**). Arrows (**G–I**) indicate an interface healing between a host tendon (upper portion) and engineered tendons or a formed control tissue (lower portion). Original magnification $\times 100$. (Color images available online at www.liebertpub.com/ten.)

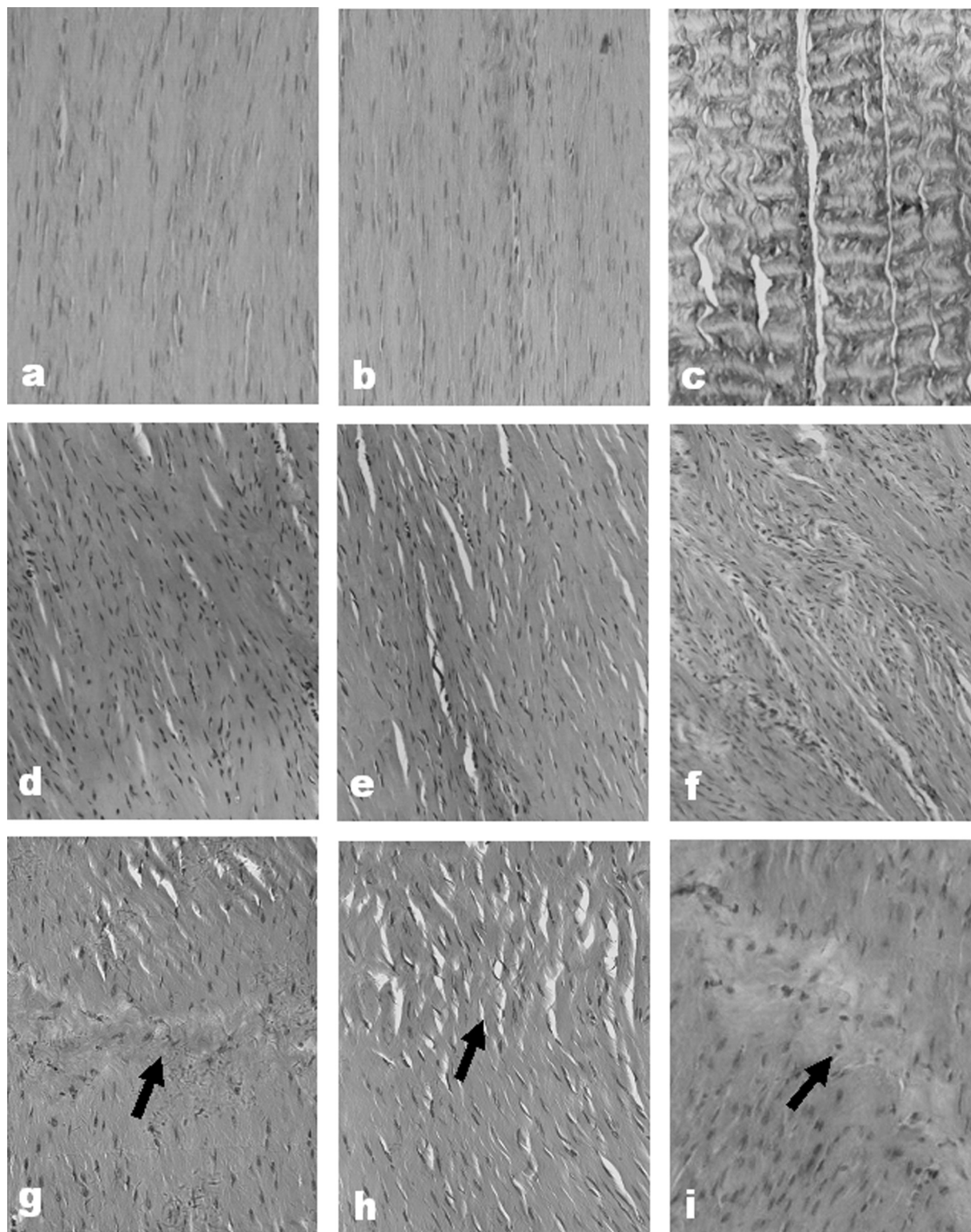


FIG. 6. Histological finding of formed tissue at 14 weeks. Histology shows that collagen fibers become longitudinally aligned at both end part (A) and middle part (D) of a fibroblast engineered tendon, and at both end (B) and middle (E) parts of a tenocyte engineered tendon. The collagen fiber alignment remains relatively disorganized at the end part of the tissue in control group 2 (F). Arrows indicate the interface healing between a host tendon (upper portion) and fibroblast (G) as well as tenocyte (H) engineered tendons or the tissue of control group 2 (I) (lower portion). Matrix production in engineered tendons (A and B) is comparable to that of normal tendon (C). H&E staining (D–H); trichrome staining (A–C and I). Original magnification $\times 200$ (G–I); $\times 100$, all others. (Color images available online at www.liebertpub.com/ten.)

In contrast, H&E staining showed a relatively disorganized pattern of collagen fibers in the formed tissue of control group 2, even at the location of both ends. Additionally, the PGA fibers were completely degraded at this time point (Fig. 6F). Trichrome staining also showed good interface healing and a high level of matrix production (Fig. 6I).

26 weeks post-repair. At 26 weeks, the fibroblast engineered tendon revealed a histological structure similar to that of the normal tendon to a certain level in both H&E and trichrome staining. In addition, cellular components further decreased and approached a level comparable to those of the natural tendon (Fig. 7A). As expected, tenocyte engineered tendon (Fig. 7B) shared a similar histologic structure with fibroblast engineered tendon. In addition, organized collagen alignment in the middle part and good interface healing were also observed in these two groups (data not shown). In contrast, overall collagen alignment remained disorganized in the formed tissue of control group 2 (Fig. 7C) although relatively parallel collagen alignment could be found in some localized area (data not shown).

Polarizing microscope examination

Natural tendon tissue usually does not contain collagen III, whereas this type of collagen is a component of skin tissue. To examine the collagen components in engineered tendons at 26 weeks, polarizing microscope was applied. As previously described,⁷ type III collagen should reveal an image of weak light reflection with a green color under polarized light, while type I collagen should exhibit a strong light reflection with a golden color.

Interestingly, type III collagen was easily detected in the tissue sections of control group 2 and normal pig skin (Fig. 7G, H, and K), but not in either fibroblast engineered tendon (Fig. 7E and I) or tenocyte engineered tendon (Fig. 7F and J), and not in natural tendon as well (Fig. 7L). This finding indicates that dermal fibroblasts may undergo a phenotype change during *in vivo* tendon formation under a mechanical loading. Besides, collagen I was also expressed in these tissues. As expected, type I collagen was the predominant collagen type in both fibroblast and tenocyte engineered tendons (Fig. 7E, F, I, and J), as well as in natural tendon tissue (Fig. 7L). Nevertheless, the collagen bundles were apparently thicker in the natural tendon than in both engineered tendons, suggesting that there remains difference in the collagen ultrastructure between the engineered and the natural tendons, at least at 26 weeks post-repair.

Distribution and diameter of collagen fibrils

TEM examination demonstrated evenly distributed collagen fibrils in the formed tissues of all three groups, similar to that of natural tendon. Additionally, a bimodal

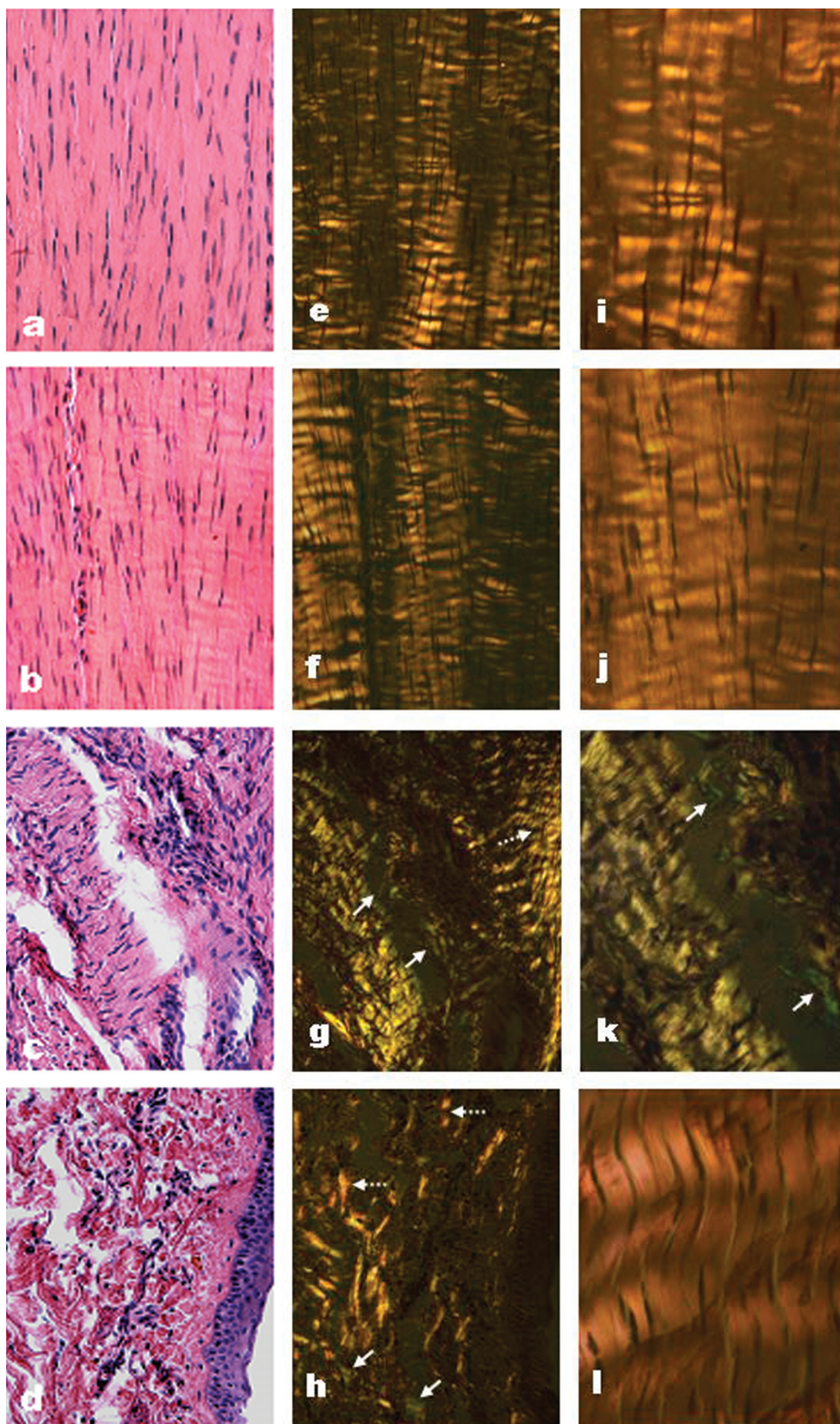
distribution of fibrils was observed in natural tendon due to the presence of both thick and thin fibrils, although most of them were thick fibrils. Interestingly, an average fibril diameter of the natural tendon (145.61 ± 18.76 nm) was much bigger than that of all three group tissues (~ 50 nm, Fig. 8). However, no apparent difference in collagen fibril diameters was found among the specimens harvested at different time points. To quantitatively analyze collagen fibril diameters, a cross-section image of collagen fibrils was analyzed with a KS400 Image analysis software (version 3.0, Zeiss) to obtain an average diameter. An ANOVA test demonstrated a statistically significant difference in fibril diameters among natural tendon and formed tissues of three groups at 6 weeks ($p < 0.05$, Fig. 8). Nevertheless, neither a significant difference was found among the specimens of three groups at each of three time points (6, 14, and 26 weeks) nor a significant difference among specimens of the same group harvested at different time points ($p > 0.05$, Table 1), although the histologic structure changed significantly with time.

Biomechanical analysis of engineered tendon

To analyze the mechanical property of the engineered tendons, tensile strength was chosen as a parameter. As shown in Figure 9 and Table 2, the tensile strength significantly increased with time in the formed tissues of three groups ($p < 0.05$) when analyzed by an ANOVA test. No significant difference was observed in the natural tendon group ($p > 0.05$). In addition, a statistically significant difference in tensile strength was detected among the tissues of different groups at each of three time points ($p < 0.05$, Table 2). A two-sample *t* test comparison with each other's strength showed that there was no significant difference in tensile strength between fibroblast and tenocyte engineered tendons in all three time points ($p > 0.05$). However, the difference between the engineered tendon (experimental group) and the formed tissue (control group 2) was significant at 6 weeks ($p < 0.05$), but not at 14 weeks ($p > 0.05$). The difference became significant again at 26 weeks ($p < 0.05$). Furthermore, at 26 weeks, the tensile strength of engineered tendons in the experimental group and control group 1 had reached 31.0 ± 2.2 and 32.4 ± 3.6 Mpa respectively, which were accounted for 74 and 76% of normal tendon's strength. This result also suggests that both fibroblast and tenocyte engineered tendons can achieve similar tensile strength that might be strong enough to perform normal tendon functions, at least from the point view of mechanical property.

DISCUSSION

Functional repair of a tendon defect remains a challenge in reconstructive surgery, mainly because of lack-



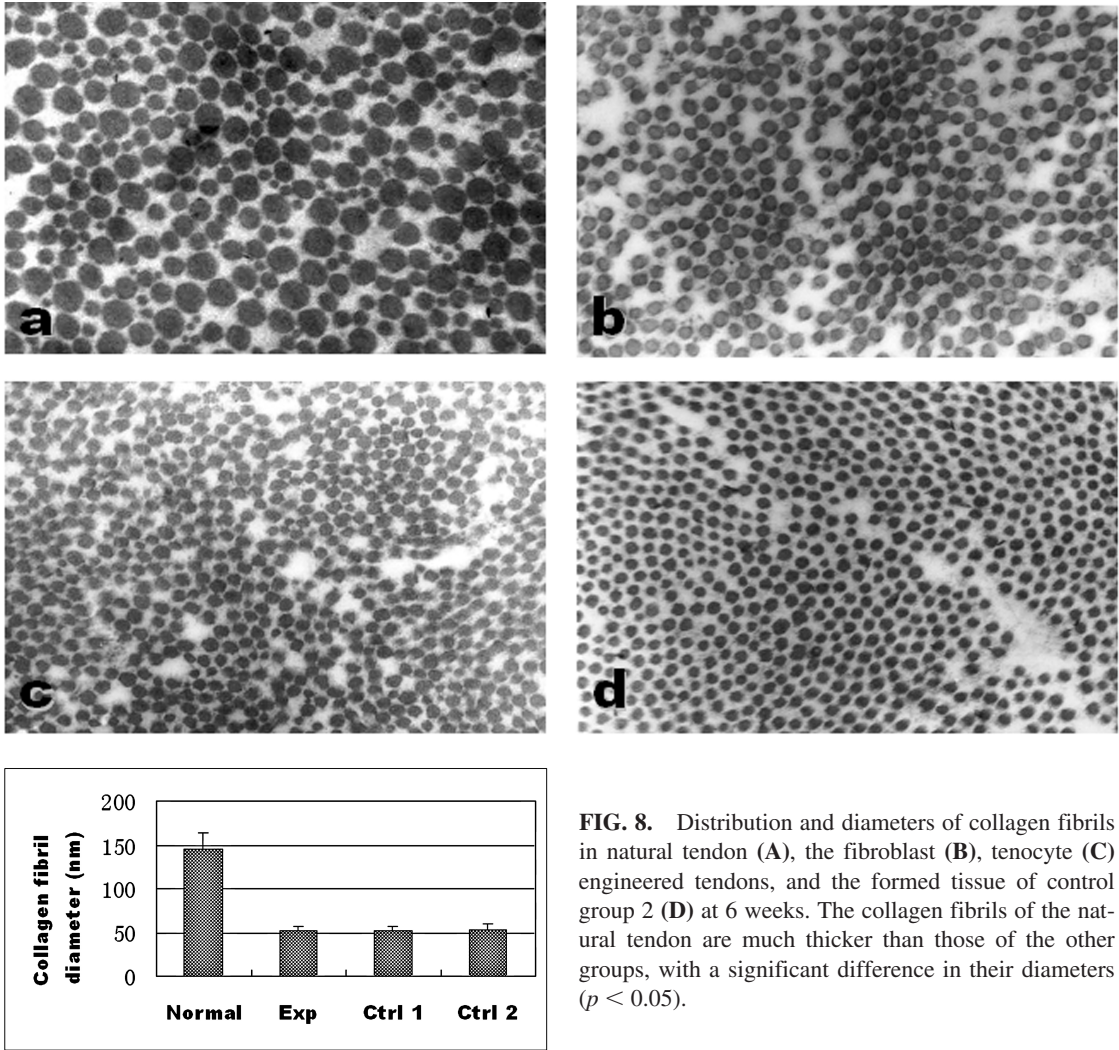


FIG. 8. Distribution and diameters of collagen fibrils in natural tendon (A), the fibroblast (B), tenocyte (C) engineered tendons, and the formed tissue of control group 2 (D) at 6 weeks. The collagen fibrils of the natural tendon are much thicker than those of the other groups, with a significant difference in their diameters ($p < 0.05$).

ing ideal tissue grafts rather than surgical techniques. Prosthetic replacements are not ideal substitutes for tendon repair because they heal poorly with tendon tissues and do not integrate into host tissue^{8–10}; nor will a xenograft or an allograft be an ideal substitute until immune rejection can be overcome.¹¹ In the era of regenerative medicine, tissue regeneration and repair should become a preferred approach for tendon repair.

In a pioneering experiment, Cao *et al.* demonstrated that a neo-tendon tissue could be generated in the subcutaneous tissue of nude mice using degradable polymer and tenocytes,¹² indicating that tissue engineering might

be a promising approach for tendon repair. Cao *et al.* recently also reported that a tissue engineered tendon could be generated in a hen model with gross view, histology, and mechanical property similar to those of a natural tendon, and the engineered tendon could be used to repair a tendon defect.³ This finding further indicates that tissue-engineered tendon may provide a source of autologous tendon graft for functional repair of a tendon defect.

However, a major concern for engineered tendon repair is the cell source rather than the technique itself. Harvesting autologous tendon to isolate tenocytes for tendon

FIG. 7. Histological finding of formed tissue at 26 weeks. H&E staining shows histological structures of fibroblast (A), tenocyte (B) engineered tendons, a control tissue in control group 2 (C) and normal pig skin (D). Collagen III (delicate collagen fibers with a light-green color) is detected only in the polarized images of control tissues (G and K) and in normal pig skin (H), as indicated by white arrows. In addition, collagen I (golden color, indicated by white dotted arrows) is also detected in these tissues. In the polarized images of fibroblast (E and I) and tenocyte (F and J) engineered tendons and natural tendons (L), collagen I (golden color) is the predominant collagen type. original magnification $\times 400$ (I–K); $\times 200$, all others.

TABLE 1. COLLAGEN FIBRIL DIAMETERS IN (nm) DIFFERENT GROUPS AT DIFFERENT TIME POINTS

	6 weeks	14 weeks	26 weeks	p value
Exp (<i>n</i> = 4)	50.58 ± 5.57	53.88 ± 3.08	52.54 ± 4.53	>0.05
Ctrl 1 (<i>n</i> = 4)	51.26 ± 3.74	53.97 ± 3.55	51.38 ± 5.71	>0.05
Ctrl 2 (<i>n</i> = 4)	50.75 ± 2.95	52.70 ± 3.67	53.18 ± 6.31	>0.05
p value	>0.05	>0.05	>0.05	

ANOVA analysis of weeks 6, 14, and 26 specimens finds no significant difference in fibril diameters in each of three groups (horizontal), and ANOVA analysis of three group specimens finds no significant difference at each of three time points (vertical).

engineering is obviously impractical for clinical tendon repair; thus, the search for an alternative cell source has become a key issue for current tendon tissue-engineering research.

Awad *et al.*⁴ and Young *et al.*⁵ initially used bone marrow stromal cells (BMSC) as seed cells for tendon engineering. They mixed BMSCs with a collagen gel to repair the defects of patellar and Achilles tendons respectively in a rabbit model. Although the biomechanical properties were improved when compared with the control tissue formed by the scaffold that was implanted without cell seeding, the engineered tissues failed to display a histological structure similar to that of a normal tendon. Ouyang *et al.*⁶ recently reported successful engineering of a rabbit Achilles' tendon by using allogeneic BMSCs. The experiment showed that knitted poly-lactide-co-glycolide (PLGA) scaffolds seeded with BMSCs could generate a tendon tissue with a histological structure similar to that of a normal tendon. What has caused the difference of the results between these studies remains to be determined.

Caplan proposed that BMSCs contain mesenchymal stem cells that can be induced to differentiate into various cell types, including tenocytes.¹³ Different from osteogenic and chondrogenic differentiation, it remains relatively unclear which chemical compound or growth factor will definitely induce BMSCs into tenocytes. Unlike the collagen scaffold used by Awad's and Young's studies, Ouyang's study used knitted PLGA as a scaffold material that could bear a strong mechanical loading after *in vivo* implantation with seeded BMSCs, and the mechanical stretch might be an important factor for tenogenic differentiation of implanted BMSCs. One concern for using BMSCs to engineer a tendon is the lack of specific markers to identify differentiated tenocytes derived from BMSCs. Additionally, committed tenogenic differentiation of implanted BMSCs is certainly an important issue. Harris *et al.* recently reported the formation of ectopic bone at the site of a tendon defect where a BMSC-collagen construct was implanted.¹⁴

Compared to BMSCs, dermal fibroblasts share more common characteristics with tenocytes. Both cell types are terminal differentiated cells and originate from mesoderm. During *in vitro* culture, both exhibit similar cell morphology. Biochemically, both cells produce major extracellular matrix type I collagen, although dermal fibroblasts also produce type III collagen. Functionally, both cells form connective tissues to carry out tissue-supporting functions. In addition, dermal fibroblasts are an easily accessible cell source. Harvesting of a small piece of skin tissue will not cause major donor site defect. Furthermore, dermal fibroblasts are easily expanded during *in vitro* culture.

The result of this study demonstrated that tendons engineered by two different cell types exhibited similar features in their gross view, histology, and biomechanical property, indicating that dermal fibroblasts may replace tenocytes for tendon engineering. Importantly, the dermal fibroblast engineered tendon also resembled a natural tendon in its histological structure. In addition, at 26 weeks, its tensile strength reached 74% of natural tendon strength, similar to that of tenocyte engineered tendon

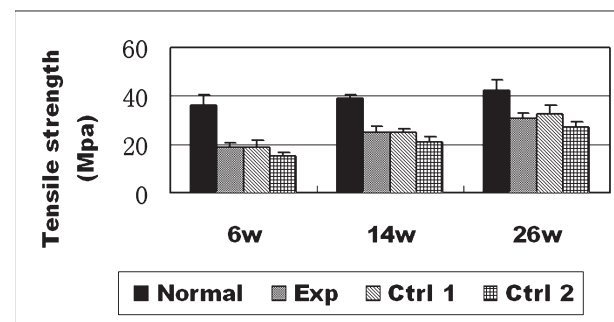


FIG. 9. Mechanical property of the natural tendon, the engineered tendons, and the formed tissue of control group 2 at different time points. Tensile strength in all formed tissues increases with time, and the fibroblast and tenocyte engineered tendons at 26 weeks respectively reach 74 and 76% of normal tensile strength.

TABLE 2. TENSILE STRENGTH (MPa) OF ENGINEERED TENDONS AT DIFFERENT TIME POINTS

	6 weeks	14 weeks	26 weeks	p value
Normal (n = 4)	36.4 ± 3.9	38.8 ± 1.7	42.4 ± 3.9	>0.05
Exp (n = 4)	18.9 ± 1.9	24.8 ± 2.6	31.0 ± 2.2	<0.05
Ctrl 1 (n = 4)	19.0 ± 2.7	24.7 ± 1.8	32.4 ± 3.6	<0.05
Ctrl 2(n = 4)	15.3 ± 1.7	21.1 ± 2.4	26.8 ± 2.5	<0.05
p value	<0.05	<0.05	<0.05	

ANOVA analysis of weeks 6, 14, and 26 specimens finds no significant difference in tensile strength in normal group, but significant difference in other three groups (horizontal), and ANOVA analysis of four group specimens finds significant difference at each of four time points (vertical). In addition, two sample *t* test shows no significant difference between Experimental (Exp) and control (Ctrl) 1 group at all three time points, but a significant difference between Exp and Ctrl 2 at either 6 or 26 weeks.

(76%), indicating that dermal fibroblasts can also generate a functional tendon, at least from the point view of biomechanical property.

One concern in this study is the difficulty to exclude the role played by endogenous fibroblasts in the formation of engineered tendons. As shown in Figure 3, a neo-tissue also formed in control group 2 when a scaffold alone was implanted. This result clearly demonstrates that host cells were involved in the neo-tissue formation by invading into the scaffold. However, the tissue histology of tendons engineered by either fibroblasts or tenocytes showed a regular structure similar to that of normal tendon, whereas the formed tissue in control group 2 was structurally disorganized, resembling a fibrotic tissue. Although the cellular components in the formed tissue of control group 2 were not characterized, these invaded cells should be fibroblasts as they generally exist in many fibrotic tissues. We thus believe that the difference between the experimental group and control group 2 is not due to the cell type, but rather to the cell distribution within the implanted scaffold material. To form an organized tendon tissue structure, cells should be evenly seeded onto a scaffold. After implantation, these cells can form a homogenous tissue structure that can undergo timely tissue remodeling with mechanical loading, resulting in a regular tissue structure. In contrast, cells migrated from host tissue cannot be evenly distributed within the scaffold, leading to a disorganized structure.

The phenomenon of tissue formation by invaded host cells was also observed in Ouyang’s study of Achilles’ tendon repair in a rabbit model,⁶ which showed that a neo-tissue also formed in the implanted scaffold without cell seeding at 4 weeks. In contrast to our study, they demonstrated that at 8, 12, and 24 weeks post-repair, both engineered tendons with or without allogeneic BMSC seeding exhibited a tissue structure similar to each other with no obvious difference from the natural tendon. This

result might be attributed to the replacement of allogeneic BMSCs with invaded fibroblasts. It may also be due to the small size of the defect (1 cm long), which is relatively easily repaired by invaded host fibroblasts; the formed tissue, in turn, is relatively easily remodeled into an organized structure within a short period of time. Regarding this study, it will be interesting to observe whether the collagen alignment pattern of control group 2 will become more organized if given a much longer time period for tissue remodeling.

As shown in Figures 5 and 6, the mechanical loading certainly played a role in forming an organized collagen pattern that arranged along the axis of the loading force. In addition, the mechanical loading also seemed to contribute to a possible phenotype change of implanted dermal fibroblasts. As shown in Figure 7, type III collagen was detected in the pig skin and in the disorganized structure of control group 2, but not in dermal fibroblast or tenocyte engineered tendons, and not in the natural tendon as well. Further studies may reveal more detailed mechanism behind this phenomenon.

Most reported studies have usually used small animal models for tendon engineering.² This study used a large mammal pig as a model in order to mimic the human tendon forming process as best as possible. Unlike this study, our previous study of tendon engineering in a hen model demonstrated that no tissue formed if a scaffold was implanted alone within an intact tendon sheath. Such a model might better fit the goal of tendon engineering and repair using dermal fibroblasts to exclude the effect of host fibroblasts. Despite the drawback of the animal model in this study, the result clearly demonstrated that dermal fibroblasts could generate a tendon tissue similar to a natural tendon, although the partial contribution of host fibroblasts cannot be excluded yet. Additionally, the result of this study also suggests that both seed cells and biomaterial are essential for generating a relatively

large sized tendon tissue that has an organized tissue structure.

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Address reprint requests to:
Yilin Cao, M.D., Ph.D.

Department of Plastic and Reconstructive Surgery
Shanghai Ninth People's Hospital
Shanghai Tissue Engineering Center
Shanghai Second Medical University
639 Zhi Zao Ju Road
Shanghai
P. R. China, 200011

E-mail: yilincan@yahoo.com