

In vivo and in vitro Cellular Ingrowth into a New Generation of Artificial Ligaments

K. Trieb H. Blahovec G. Brand M. Sabeti M. Dominkus R. Kotz

Department of Orthopedics, Center of Clinical and Experimental Oncology, Medical University of Vienna, Vienna, Austria

Key Words

Ligament advancement reinforcement system · Artificial ligament · Ingrowth · Histochemistry · Fibroblast

Abstract

Artificial ligaments are a useful tool in ligament reconstruction. Although the new generation of artificial ligaments shows encouraging clinical results, in contrast to earlier generations studies on the biological properties are lacking. Biopsies were taken from a ligament advancement reinforcement system (LARS) 6 months after implantation and investigated by histochemistry. An in vitro study seeding human fibroblasts or osteoblast-like cells (up to 10^6 cells for 21 days) on ligament pieces (5×5 mm) was conducted and analyzed by histochemistry. The biopsies showed complete cellular and connective tissue ingrowth in the LARS ligament. In vitro fibroblasts and osteoblast-like cells encapsulated the fibers by building a cellular net around them. To our knowledge, these findings demonstrate for the first time the cellular in-

growth into the LARS ligament. This mechanism might explain the strength and the inert behavior of the ligament without the synovialitis shown in clinical studies.

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Introduction

Artificial ligaments can be used for a number of indications. They are helpful tools in the reconstruction of ruptured ligaments (cruciate ligaments, rotator cuff, patellar tendon, collateral ligaments), the Achilles tendon, and extensor defects after wide tumor resection of the knee or humerus. The use of artificial ligaments has not been popular in the past because the ligaments introduced 20 years ago resulted in a high failure rate and inferior outcomes [1]. The improvement in new materials and the introduction of a new generation of artificial ligaments give promising results and satisfying outcomes, for instance for the ligament advanced reinforcement system (LARS) artificial ligament [2–5]. A prospective 24-month follow-up study suggests satisfactory results after reconstruction of the anterior cruciate ligament with a LARS ligament compared with bone-patellar-tendon-bone reconstruction, especially when early return to high levels of activity is

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Fax +41 61 306 12 34
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Univ. Prof. Dr. Klemens Trieb
Department of Orthopedics
University of Vienna, Währinger Gürtel 18–20
AT-1090 Vienna (Austria)
Tel. +43 1 40400 4070, Fax +43 1 40400 4077, E-Mail Klemens.trieb@akh-wien.ac.at

demanded [4]. Despite these promising results, reports about the biological properties of the LARS ligament and the reaction of cells and their possible ingrowth capacity are still lacking. It is the aim of this study to investigate the *in vivo* and *in vitro* behavior of fibroblasts and osteoblast-like cells to the LARS ligament.

Materials and Methods

Cells

Fibroblasts were isolated from tractus iliotibialis tissue obtained at elective hip surgery from 3 otherwise healthy patients (2 male, 1 female, mean age 58 ± 3 years). The tissue was processed by enzyme digestion with collagenase type IV (Sigma Chemicals, Deisenhofen, Germany) in a warm water bath (37°C) for 120 min as previously described [5]. Informed consent for using the cells and biopsies were given by the patients. Cells were then washed and incubated in RPMI 1640 medium (Gibco Laboratories, Paisley, UK) containing 10% fetal calf serum (FCS; Gibco) and 1% antibiotics (P/S, penicillin 10,000 U/ml and streptomycin 10,000 $\mu\text{g/ml}$; Gibco) in 75-cm² culture flasks (Falcon, Mountain View, Calif., USA). Cells were allowed to grow into continuous lines until they formed a monolayer.

The human osteoblast-like cell line MG-63 (CRL 1427, purchased from ATCC, Rockville, Md., USA) was incubated like the fibroblasts and allowed to grow until it had also formed a confluent monolayer.

After expansion, fibroblasts and osteoblast-like cells were treated in the same way and removed from their plastic support by 2-min exposure to trypsin EDTA (trypsin, 1:250; 2 g/l EDTA in modified Puck's saline; Gibco), washed three times, counted and used for further experiments.

Cell Ingrowth Experiments

The LARS ligament (polyethylenetetraphthalate, 6×40 cm; Surgical Implants and Devices, Arc-sur-Tille, France) was cut into 5×5 mm pieces and one piece was put into one well of a 24-well plate (1 piece/well; Falcon). Then 1 ml culture medium (RPMI, 10% FCS, 1% P/S) was added to each well containing different amounts of cells (0, 10^4 , 10^5 , 10^6 cells/well). One 24-well plate was chosen for each different time point of harvesting (0, 7, 14 and 21 days), resulting in 4 plates with 4 wells/ligament piece per well. The experiment was done parallel for fibroblasts and osteoblast-like cells (resulting in a total of 32 different ligament pieces). At the chosen time points, the pieces were harvested and embedded for further analysis.

Biopsies

In vivo biopsy was obtained from a 42-year-old patient at revision surgery for repeated effusions with synovectomy and a change in the polyethylene of the hinged axis. Six months before, reconstruction of the right knee extensor mechanism with the LARS ligament was done to improve function. More than 10 years earlier the patient had been treated for osteosarcoma by resection of the distal femur and replacement with a modular tumor endoprosthesis (HMRS; Howmedica) and neoadjuvant chemotherapy; the patient is now tumor free. At the latest revision, surgical biopsies were taken for routine histological examination.

Histochemistry

To optimize histological analysis, preliminary experiments had to be performed because embedding and cutting of the LARS ligament has not been described before. First, when the material was embedded in paraffin, it turned out that the ligament could not be cut because it was still intact and not destroyed. Then we changed to methylmethacrylate embedding which results in a harder block. Reproductive slides (5 μm) could then be obtained. The ligament pieces were divided before embedding and stained after cutting with hematoxylin eosin (HE) or azan blue, a staining method for connective tissue. Biopsies from the *in vivo* ligament were treated in the same way. Slides were analyzed under a light microscope and documented with a digital camera.

Results

Histochemistry of *in vivo* Biopsies

The biopsies taken at surgery showed complete cellular and connective tissue ingrowth of the LARS ligament after 6 months. Fibroblasts adhere to and surround the ligament fibers by building a capsule (fig. 1a, black arrow). Additionally, some endothelial cells can be found (fig. 1a, white arrow), suggesting the ingrowth of blood vessels. Under the azan blue staining, connective tissue is documented throughout the whole diameter of the ligament (fig. 1b).

In vitro Studies

The ingrowth capacity of fibroblasts and osteoblast-like cells was tested independently. Ingrowth could be found for both cell types. Interestingly, the ingrowth pattern was similar to the *in vivo* findings, fibroblasts and osteoblast-like cells adhere to the ligament fibers and build a capsule (fig. 2). The histological outcome does not differ between cell types, and complete encapsulation throughout the ligament pieces could be demonstrated after 14 days. The ligament was not destroyed or dispersed and remained histologically intact. Infiltration with leukocytes or a foreign body reaction were not observed.

Discussion

Due to theoretical advantages, artificial ligaments were introduced in the beginning of the 1980s and were popular to start with. Although different materials, for instance Dacron, Gore-Tex or Trevira, have been used, none of them had a satisfactory long-term outcome, and therefore they were not used further in clinical routine [6–10]. Some of the problems included synovitis, poor mechani-

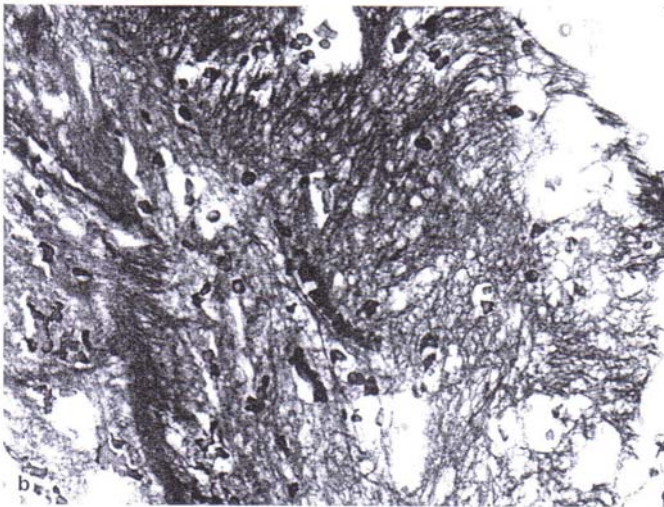
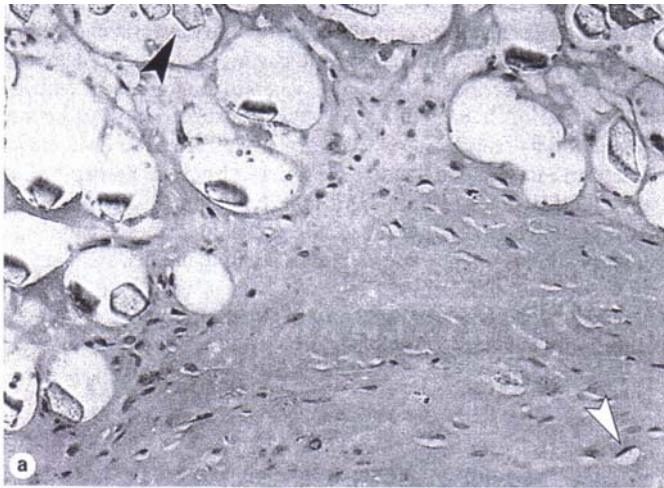


Fig. 1. Photomicrograph of a biopsy of the ligament. $\times 68$. **a** HE stain. Note the fibroblasts adhering to the fibers and building a 'second grid'. The fibers of the LARS ligament (black arrow) and endothelial cells (white arrow) can be seen. **b** Azan-blue stain showing connective tissue throughout the ligament.

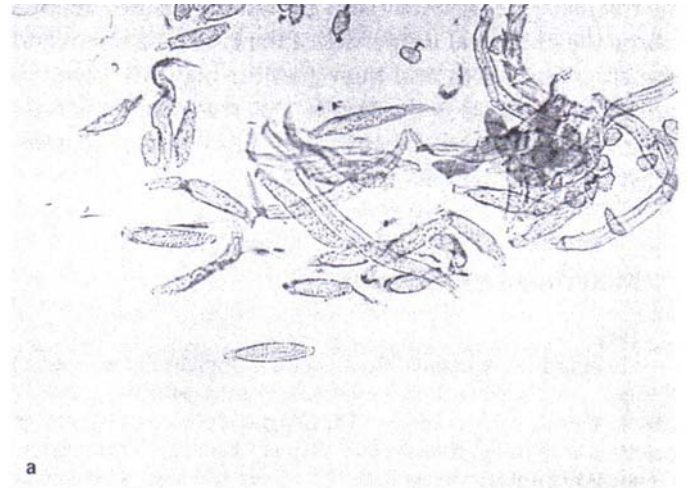


Fig. 2. Photomicrograph of the in vivo seeded fibroblasts (10^6 cells/well after 21 days). Note the adherence of the fibroblast to the fibers which results in a capsule. Magnification: **a** $\times 27$; **b** $\times 270$.

cal strength and rupture leading to a high failure rate. In the middle of the 1990s, a new generation of artificial ligaments was introduced, including the LARS artificial ligament (Surgical Implants and Devices, Arc-sur-Tille, France; polyethylenetetraphthalate) [2, 5]. The ligament is available in different designs for reconstruction of the cruciate ligament (left and right), and tendons and ligaments (for instance, patellar or Achilles tendon, rotator cuff, extensor mechanisms). A 24-month prospective randomized trial compared reconstruction of the anterior cruciate ligament with the LARS ligament and bone-

patellar-tendon-bone [4]. Results in both groups were comparable, especially with regard to complications. No adverse biological reactions could be observed in the LARS group including synovialitis, which suggests good in vivo compatibility. Despite these promising results, reports about the biological properties of the LARS ligament and the reaction of cells and their possible ingrowth capacity are still lacking. Additionally, no animal experiments using the LARS ligament are available so far.

This study shows the capacity of fibroblast to grow into the ligament in vivo and in vitro. Additionally osteoblast-

like cells also grow into the ligament in vitro. The cells adhere to the fibers and build a capsule around them. This might explain the biocompatibility of the ligament. In vivo the ligament was completely connected by the surrounding tissue. Due to this finding one might speculate about the mechanical properties of the LARS ligament. We think that the ligament gives a grid for the cells to build a new ligament. The next study will evaluate the mechanical properties of LARS ligaments loaded with fibroblasts. We will seed cells onto the ligament and then test the mechanical strength of the ligament with and

without cells. Further speculations might include a pre-load of the ligament in vitro with autologous fibroblasts propagated from a skin biopsy before implantation.

These findings suggest the high biocompatibility of the LARS ligament. Clinical investigations of this new generation of artificial ligaments have shown satisfactory results without causing synovialitis. The ingrowth of fibroblasts and osteoblast-like cells, which adhere to the fibers and build a capsule, might be an explanation. Further studies will have to evaluate the mechanical properties of the ligaments and tissue engineering possibilities.

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