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EXPERIMENTAL INVESTIGATION

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Photodynamic laser therapy for rheumatoid arthritis

Cell culture studies and animal experiments

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Abstract The introduction of arthroscopic techniques has improved the surgical therapy of rheumatoid arthritis. The additional application of the holmium:yttrium aluminum garnet (Ho:YAG) laser likewise holds great promise by providing complete hemorrhagic control. Unfortunately, a minimally invasive solution for use in smaller joints has not yet emerged. The present study describes the possible treatment of these joints by means of photodynamic laser therapy. Cell culture studies with human synovial fibroblasts obtained from patients with rheumatoid arthritis have demonstrated a cytotoxic effect after administration of Photosan-3 as a photosensitizer and subsequent laser irradiation at 630 nm. For the in vivo studies, IgG-induced arthritis in rabbits, which is histologically consistent with the proliferative phase of rheumatoid arthritis, was used as the animal model. The histologic picture following photodynamic laser therapy with Photosan-3 revealed complete synovial destruction which also extended to the border of the subjacent joint capsule. In contrast, bradytrophic structures, e.g. cartilage, menisci, and ligaments, remained unchanged at both the macroscopic and microscopic levels. Therefore, photodynamic laser therapy can be considered a new method in the surgical treatment of inflammatory disease of the synovial membrane. It has the advantage of being minimally invasive, while offering a high degree of efficacy and selectivity.

Key words Synovectomy · Rheumatoid arthritis · Photodynamic laser therapy

Introduction

During recent years, arthroscopic synovectomy has increasingly displaced the open procedures conventionally used in the treatment of rheumatoid arthritis (RA) [11]. The arthroscopic technique has the merit of being less traumatic and more precise [5]. Recent studies suggested that further improvements may be achieved by use of the holmium:yttrium aluminum garnet (Ho:YAG laser) [12]. Besides ensuring complete hemorrhagic control in animal models with only minimal postoperative articular effusion and less postoperative pain [8], application of this laser is also associated with a decrease in the formation of fibrous tissue [4]. Despite these advances, there is still a lack of minimally invasive methods which might be suitable for the small joints of the hands and feet usually involved in RA. The concept of using photodynamic laser therapy similar to its application in oncology was suggested by our group as early as 1989 [9]. The principle of photodynamic therapy provides for the administration of a drug (photosensitizer) which after exposure to light quanta of a suitable wavelength produces a cytotoxic effect that can be utilized for a specific therapeutic application [3, 13]. This study describes relevant cell culture studies on human synovial fibroblasts and the successful transposition of the findings obtained to an animal (arthritis) model using photodynamic therapy.

Materials and methods

Nine patients with a diagnosis of RA satisfying the modified American Rheumatism Association (ARA) criteria [1] underwent arthroscopic synovectomy after having given informed consent. The synovial tissue obtained was scissored under sterile conditions. Following enzymatic digestion with a collagenase-DNAse solution with constant stirring for 4 h at 37°C, the samples were run through a Ficoll density gradient, and the nonadherent cells were discarded after 24-h incubation. Further culturing was done in RPMI 1640 medium with the addition of 10% fetal calf serum, 1% sodium pyruvate, 1% L-glutamine, and 1% penicillin-streptomycin solution at 37°C and 5% CO_2. The culture medium was changed every 3 days. Fibroblast morphology was detectable after 2 weeks in 99% of the cells. Trypan blue staining yielded a vitality > 95%.

A dye laser (Spectra-Physics 375 B, Spectra-Physics, Mt. View, Ca.) pumped by an argon ion laser (Spectra-Physics 2035) and operating at a wavelength of 630 nm was used for the experiments. A dichloromethane-ethylene glycol solution served as the chromophore. The beam was focused onto the target by a plano-convex lens (focal length f = 40 mm) and was then transmitted through a 400 nm glass-clad quartz fiber. The irradiance at the fiber tip was measured before and after each irradiation using a Scientec Powermeter Mentor MA 10 (Scientec, Boulder, Co.).

As directed by the manufacturer, Photosan-3 (provided under the BMFT Collaborative Study "Photodynamic Laser Therapy") was stored protected from light at 4°C until use. Initially, cell growth was determined after incubation for 2 h using different concentrations of Photosan-3. Three cultures were grown for each experiment. Serial dilutions of Photosan-3 were prepared with concentrations varying from 0.1 to 20 mg/ml. To determine the effect of the laser radiation alone, a radiant exposure of 2 J/cm^2 was delivered, no change in cell growth being detectable below this level. For the photodynamic therapy tests, the cell cultures were incubated with the respective Photosan-3 concentrations at a density of 1×10^4 cells/cm^2. This was followed 2 h later by a radiant exposure of 2 J/cm^2 and measurement of the cell count vs the nonirradiated controls after 24 h.

For the animal experiments, which were carried out on ten male cross-bred rabbits (body weight 2500–3500 g), approval had been obtained from the regional state board in Hannover (no. 504-42502-92/577). The animals were immunized by intracutaneous injection of a mixture (2 ml) of heat-aggregated 2% human IgG (HAGG) and complete Freund's adjuvant (Sigma, Deisenhoven). Intradermal testing with 0.1 ml of 2% HAGG was done after 3 weeks. Animals showing a positive skin reaction (nine of ten) were boostered with an intraarticular injection of 0.2 ml of 2% HAGG into the right knee joint [7]. By the end of the 1st week, inflammation of the synovial membrane with swelling and hematoma formation was evident in all rabbits. The distribution of animals in the various treatment groups is depicted in Table 1. Two animals received intravenous injections of 5 mg Photosan-3/kg body weight 24 h before starting the photodynamic therapy. Intraarticular injections were given to another two animals 1 h before starting the therapy. After administration of the photosensitizer, the animals were kept in the dark. After intramuscular anesthesia with 1 ml of 10% ketamine and 0.05 ml of 2% Rompun, the joints were punctured with an 18-gauge needle in the upper recess. Photodynamic therapy was initiated using a 400 nm quartz fiber whose tip was prepared for homogenous radial radiation at a length of 20 mm. The light dose was between 25 and 50 J/cm length. After the treatment, the animals did not exhibit any clinical complaints and regained unrestricted activity after 24 h. After 1 week the animals were killed, and the joints were removed; a lateral access was used, and photographic documentation was obtained simultaneously. Samples for histologic study were taken from specific sites (Table 2). Staining was carried out with hematoxylin-eosin and azan solution.

Table 1 Distribution of experimental animals (n = 9)

No.	Therapy
1 + 2	Untreated negative controls
3	Photosan-3 (i.v.)
4	Photosan-3 (i.a.)
5	Laser irradiation
6 + 7	Photosan-3 (i.v.) plus laser irradiation
8 + 9	Photosan-3 (i.a.) plus laser irradiation

Table 2 Sampling sites for histologic study

Medial aspect of upper recess
Lateral aspect of upper recess
Anterior horn of inner meniscus
Anterior horn of outer meniscus
Cartilage covering posterior patellar surface
Cartilage covering medial femoral condyle
Medial collateral ligament, bottom layer
Anterior cruciate ligament

Results

In the cell culture studies, radiant exposures of 2 J/cm^2 did not produce a change in cellular growth. After incubation with Photosan-3 alone, the cell counts were found to be lowered after 24 h. However, the decrease never exceeded 10%, even at concentrations of 20 mg/ml. In contrast, incubation with Photosan-3 and subsequent irradiation produced complete cytotoxicity at concentrations of 10 mg/ml and above (Fig. 1).

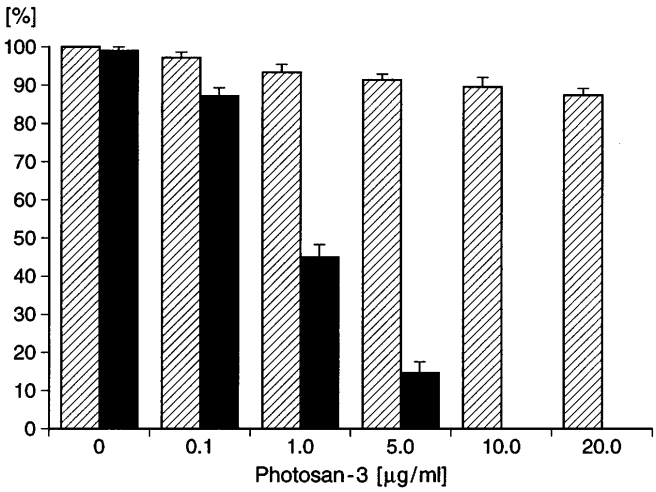
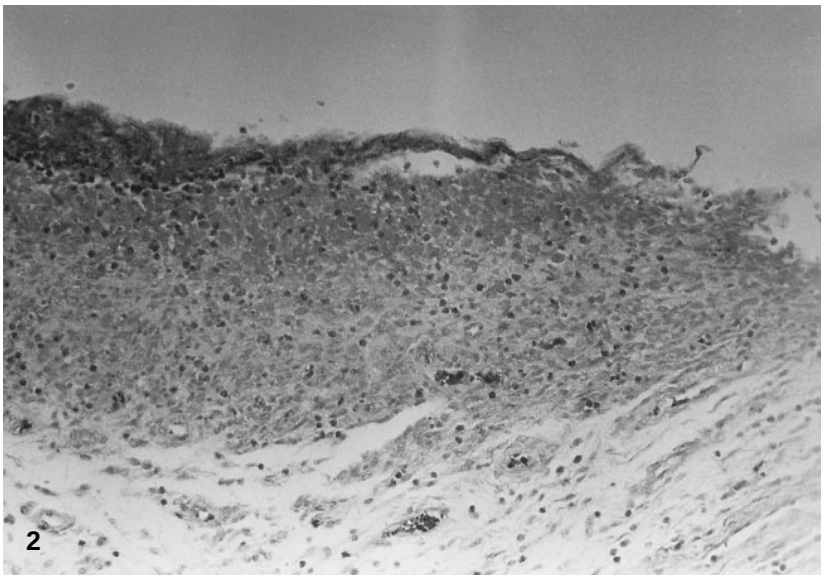


Fig. 1 Cytotoxic effect of different Photosan-3 concentrations. Cell counts are plotted on the *ordinate* and Photosan-3 concentrations, on the *abscissa. Hatched bars* indicate the effect of incubation with Photosan-3 without laser irradiation; *black bars* show the effect of Photosan-3 concentration and subsequent laser radiation at 630 nm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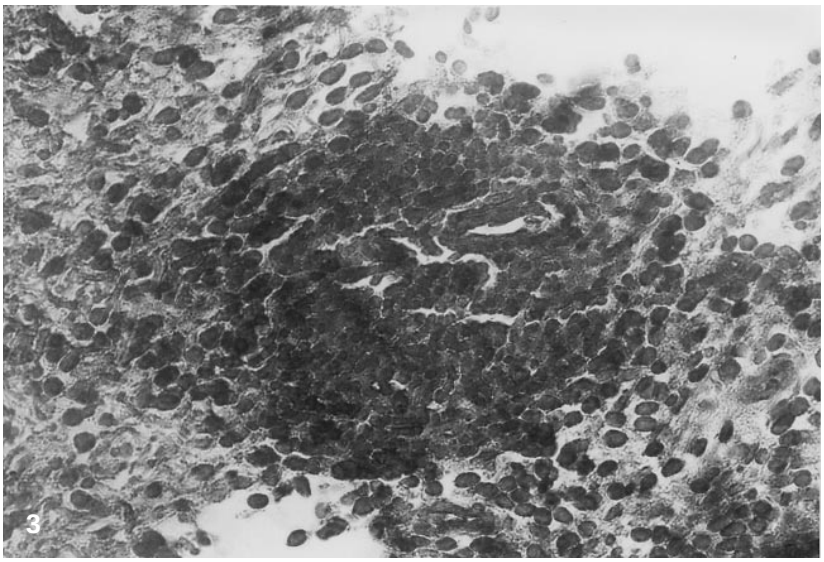
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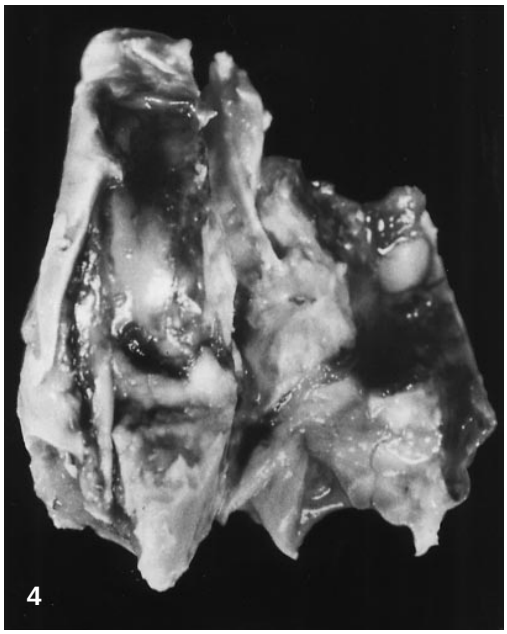
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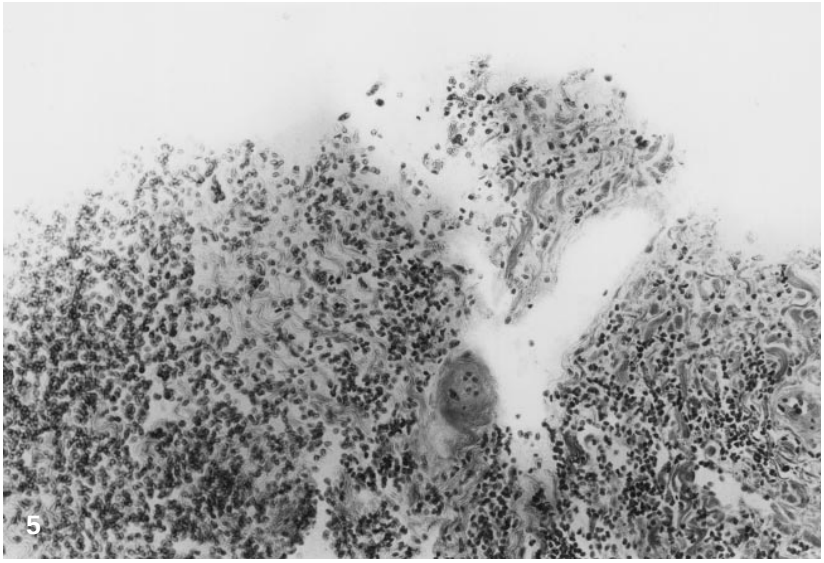
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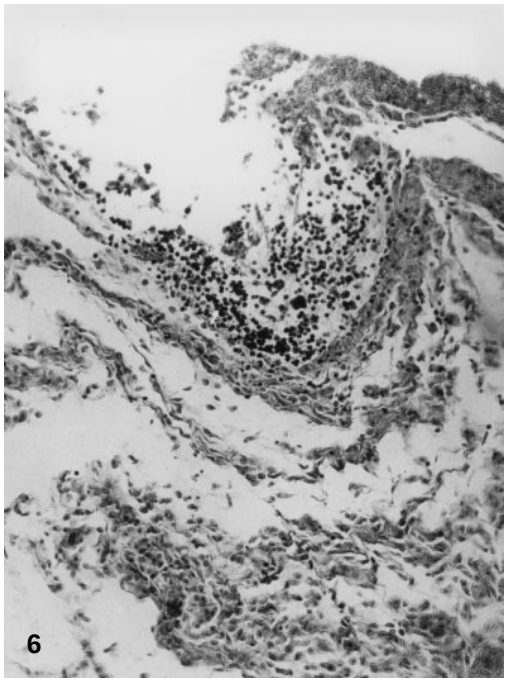
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◀ **Fig. 2** Photomicrograph showing the synovial membrane of upper recess in IgG-induced arthritis (azan stain, × 100). Lining layer of synovial cells has widened, comprising up to 30 layers of syncytial cells. The widened villous stroma shows substantial infiltration with lymphoplasmic cells. The optical sections of numerous vessels can be recognized

Fig. 3 Photomicrograph showing the synovial membrane of upper recess in IgG-induced arthritis (azan stain, × 200). Follicle-type structures are detectable in the lymphoplasmic cell infiltrate

Fig. 4 Photograph showing rabbit knee joint following photodynamic therapy (intraarticular administration of Photosan-3) and laser irradiation at 630 nm. The joint was opened via a lateral access, followed by medial dislocation of the patella. The entire synovia has developed a reddish-brown coloration; gray regions are also observed. In contrast, no macroscopic changes are seen affecting cartilaginous, meniscal, and ligamentary structures

Fig. 5 Photomicrograph showing the synovial membrane of upper recess after photodynamic therapy with Photosan-3 (azan stain, × 100). Abundant erythrocytes are seen throughout the villous stroma. The hemorrhage extends from the synovial lining cells to the boundary between loose fibrous tissue and joint capsule

Fig. 6 Photomicrograph showing synovial membrane of upper recess following photodynamic therapy with Photosan-3 (azan stain, × 100). Above the hemorrhagic exudate, the lining layer of synovial cells is lifted, together with adjacent portions of villous stroma

A positive skin reaction was noted in nine of ten immunized animals. After intraarticular booster doses, these animals developed swelling and effusions. Histologically, there was substantial stromal proliferation and extension of the lining layer of synovial cells to as many as 30 cell layers (Fig. 2). Besides hypervascularization, infiltration with mononuclear cells was seen throughout the stroma; in some regions, follicular structures were detectable (Fig. 3). The histologic picture thus resembled that seen in the proliferative phase of RA.

Intravenous or intraarticular administration of Photosan-3 without laser irradiation did not affect the articular morphology, nor did exclusively laser radiation have a noticeable effect. In contrast, a distinct reddish-brown coloration of the synovial membrane was noted on gross observation after intraarticular or intravenous doses of Photosan-3 with subsequent laser irradiation. Cartilage surfaces, menisci and ligamentary structures, however, did not show any changes (Fig. 4). Histologically, substantial hemorrhage throughout the stroma was seen to extend to the border of the tough collagenous fibrous tissue of the joint capsule (Fig. 5). Starting from this boundary, the synovial membrane was found to be lifted from the subjacent tissue. In some areas, complete desquamation of the entire synovial membrane was demonstrable (Fig. 6). The residual histologic features were stems of villi of dense connective tissue with fewer cell infiltrates; hemosiderin inclusions were present in places (Fig. 7). In the preparations obtained after intravenous doses of Photosan-3 and subsequent laser irradiation, the same changes could be seen but were less extensive. In contrast to the substantial synovial membrane changes, the histologic appearance of the cartilaginous, meniscal, and ligamentary structures had remained unchanged (Figs. 8, 9).

Discussion

The present study was designed to develop a photodynamic therapeutic concept for use in RA. Besides the histologic features of stromal proliferation, enlargement of the lining layer of synovial cells, and hypervascularization, a pronounced cellular infiltration is also seen in RA. Type II and III cells with fibroblast morphology account for some 70%–80% of the cellular elements of pannus tissue [2]. Therefore, human synovial fibroblast cultures obtained from RA patients were employed as a cell model. Following incubation with Photosan-3 and subsequent laser radiation at 630 nm, a complete cytotoxic effect was triggered in these cells. It thus became evident that Photosan-3 may be used successfully for the photodynamic therapy of RA. Attempts to substantiate these results in vivo led to the establishment of the animal (rabbit) model of IgG-induced arthritis, which constitutes an adjuvant type of arthritis histologically satisfying the criteria of the early proliferative phase of RA [7]. It is characterized by a distinct proliferation of the synovial stroma with increased vascularization and a substantial broadening of the lining layer of synovial cells. There is a coexistent infiltration with lymphoplasma cells exhibiting a partially follicular-type structure. After the successful establishment of the arthritis model, photodynamic therapy with the intravenous or intraarticular administration of Photosan-3 and subsequent radiant exposure at 630 nm was started. At both the macroscopic and microscopic levels, there was distinct hemorrhage involving the entire synovial membrane, followed by desquamation which extended to the innermost capsular layer. On the other hand, examination of the cartilage, menisci, and ligamentary structures did not reveal any changes in morphology. Photodynamic therapy with Photosan-3 thus leads to the total destruction of the inflamed synovial membrane. By preserving the other articular structures, especially the cartilage, photodynamic laser therapy offers a high degree of selectivity. Laser radiation also reaches articular spaces which are inaccessible to mechanical instrumentation [9]. The ablation achieved in the course of photodynamic laser therapy will clearly be more precise and comprehensive [6]. Application of the laser in the rabbit joint through an 18-gauge needle illustrates the minimally invasive nature of the procedure. The present study has described, for the first time, the basic surgical applicability of photodynamic therapy in the treatment of RA under in vivo conditions. The results further document its potential application in smaller joints, e.g. the finger joints, which are practically always affected in RA. With the development of applicators permitting uniform radiation of even small joints, further miniaturization of the procedure will be possible

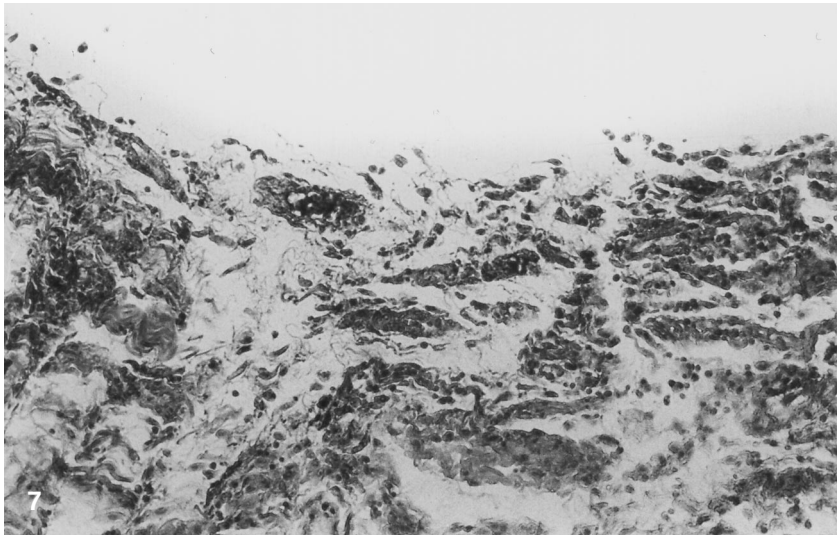

Fig. 7 Photomicrograph showing synovial membrane of upper recess following photodynamic therapy with Photosan-3 (azan stain, × 100). After desquamation of the uppermost synovial layers, villous stems denuded of synovial lining cells project into the joint space. Cellular infiltration is less marked. Hemosiderin inclusions are seen in places

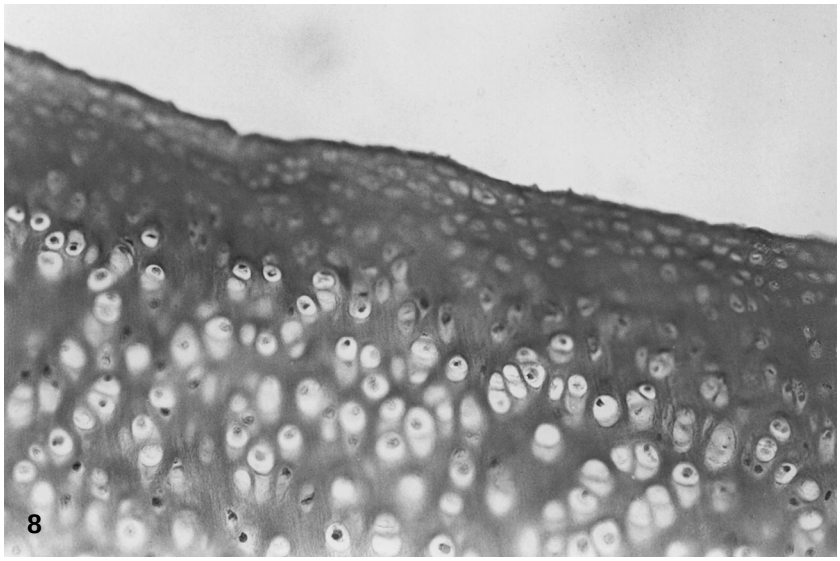

Fig. 8 Photomicrograph showing cartilage of patellar surface following photodynamic therapy with Photosan-3 (azan stain, × 100). In contrast to an untreated joint of a control animal, microscopic changes are not detectable

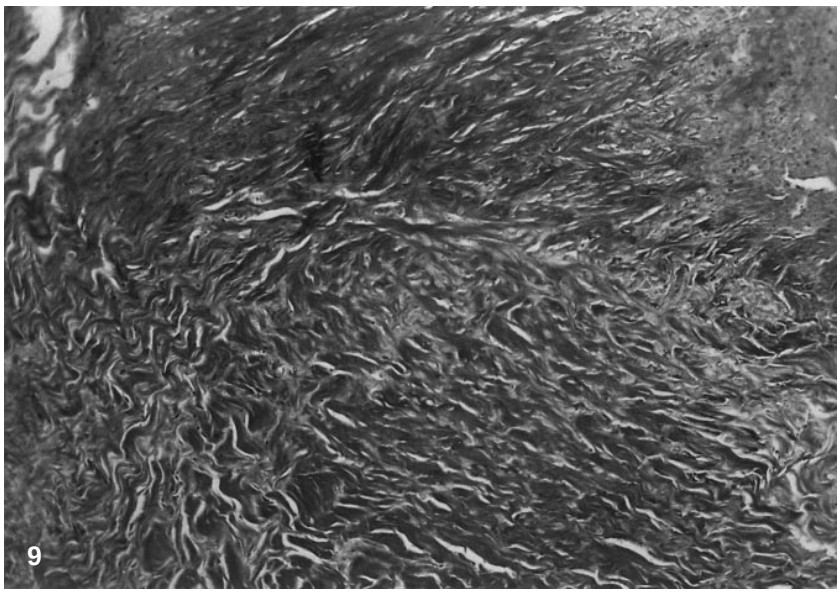

Fig. 9 Photomicrograph showing inner rim of meniscus following photodynamic therapy with Photosan-3 (azan stain, × 100). In contrast to an untreated control, the meniscus does not reveal any changes, except for some slight desquamation of the synovial lining in the basal region

[10]. The novel technique of photodynamic laser therapy will permit an early surgical intervention in RA; it is also minimally invasive. Its intermediate-term efficacy and practicability in man will, therefore, be the subject of further studies.

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