INTRODUCTION:

Reconstruction of injured ligaments is still a problem. The success of the reconstruction is highly dependent on the quality of healing of the transplanted tendon in the bone tunnel. This in turn depends on a number of factors, including the type of graft fixation. Inadequate fixation often results in tunnel widening and, in consequence, a reduction in the quality of the contact established between the outer surface of the tendon and the surrounding bone. Insufficient contact between the tendon and bone often results in an unsatisfactory outcome of the ligament reconstruction. Although various materials are being used experimentally to promote tendon graft-to-bone healing, their effectiveness has not been clinically proven. In this study, microporous poly lactide beads of controlled pore size impregnated with peripheral blood were implanted in bone tunnels in rabbits to assess their potential to enhance tendon graft osteointegration in ligament reconstruction surgery.

METHODS:

Polylactide beads. The beads have a pore size in the range of 200 - 400 μm were produced from poly(DL-lactide) - PURAC Biocem, Gorinchem, The Netherlands. Animals, Twenty four male New Zealand rabbits 8-12 months old and 3-3.5 kg body weight were used in the study (Ethic Committee approval 668/09, July 7, 2009). The animals were divided into 4 groups of 6 animals each: Group 1 (control), no PLA; implantation time 6 weeks; Group 2, PLA beads, implantation time 6 weeks; Group 3 (control), no PLA beads, implantation time 12 weeks; Group 4, PLA beads, implantation time 12 weeks. The animals were kept in individual cages for a few days before surgery to adapt to the housing environment. No food was given during the day preceding surgery. Surgery. Anesthesia: atropinum sulfuricum 0.06 mg/kg, intramuscularly (Polfa, Poland), ketamine hydrochloride 40 mg/kg (Biowet, Poland), Xylazine HCL 10 mg/kg (Biowet, Poland), administered intramuscularly and antibiotic protection Linco-Spectin SS, 1 ml/kg subcutaneously per 7 days (Pfizer Animal Health, UK). Under general anesthesia the bone tunnel with diameter of 2.6 - 3.5 mm and length 8 - 12 mm was drilled in the proximal tibial metaphysis using a drill equipped with adequate drill bits. Next, the long digital extensor tendon of the right hind limb was detached from its lateral femoral condyle and implanted in the bone tunnel of the proximal tibial metaphysis applying appropriate tension. At this time, the PLA beads were mixed with blood from the bleeding part of the bone wall to form a homogenous paste and packed clockwise into the bone tunnel around the tendon starting from its lateral entrance. After a tight lateral application, the procedure was repeated at the medial outlet. (Fig. 1). The average amount of PLA beads needed to fill the tunnel was 60 mg. The free end of the graft was fixed to the tibial periostium at the opposite tunnel entrance with a 4-0 monofilament Prolene suture (Ethicon®). Subcutaneous wounds were closed in layers using 3/0 absorbable Safi Braun® braided polyglycolic acid sutures and the skin was closed with 4-0 monofilament Prolene suture (Ethicon®). The rabbits were placed in cages and allowed free movements. The animals were euthanized humanely 6 and 12 weeks after implantation using barbiturate overdose. The control and PLA implanted bones were harvested for evaluation. Evaluation. The material collected was evaluated histologically using 100 μm thick slices. The stains used were Masson-Goldner trichrome, hematoxylin-eosin and safranin O. The variables analyzed were: the presence of new bone, the type and density of cells, organization of collagen fibers, vascularization, cartilage orientation and healing of the grafted tendon along the longitudinal and the lateral cross-sections of the tunnels. In the latter case the slices were collected from the tissues harvested at the entrance, the midpoint, and the exit of the tunnels. In addition, the specimens were examined for macrophage-like cells, lymphocytes, and foreign body cells.

RESULTS:

At 6 and 12 weeks postimplantation histological sections of harvested specimens showed significant tendon-to-bone healing in all animals with bone tunnels filled with PLA-blood paste. Abundant vascularized new bone was however formed almost exclusively in those areas where PLA-blood paste has been successfully delivered, whereas in other areas along the longitudinal tendon axis not reached by PLA-blood paste there was only a minute amount of bone or none at all. There was very little new bone formation in the control specimens for a comparable implantation time.

DISCUSSION:

Healing of grafted tendon in a bone tunnel is a problem in ligament reconstructive surgery. The differences between the cross-sectional geometry of the graft and the bone tunnel are the reasons that after graft transplantation there are zones formed within the tunnel where there is no direct contact between tendon and bone. These zones filled with blood adversely affect graft osteointegration and may lead to graft failure. Various approaches have been tried experimentally to promote graft-to-bone healing with varying degrees of success. These include growth factors, hydroxypaptite and/or magnesium cements, and mesenchymal stem cells, to mention but a few. In the present study biodegradable, microporous osteoconductive polymer carrier was used as a filler to pack the spaces between the outer surface of the tendon graft and bone in the tunnel. We hypothesised that the porous structure of the carrier that allows for the flux of nutrients and proliferation of cells would promote graft osteointegration. Impregnation of the carrier with blood to form a paste facilitates its introduction into the tunnel and importantly, adds an osteoinductive component to the carrier. All these factors should improve the outcome of ligament reconstruction surgery.

CONCLUSIONS:

Adaptable, pliable paste consisting of microporous PLA beads and peripheral blood used to fill vacant areas between the tendon and bone in the bone tunnels promoted tendon-to-bone healing in rabbits subjected to surgery. This healing effect can be intensified by mixing PLA beads with autogenous marrow blood, platelet-rich plasma and/or growth factors. Uneven distribution of the PLA paste in the bone tunnel around the grafted tendon results in a patchy bone formation. The new bone is almost exclusively formed in those areas where PLA paste is in direct contact with both the tunnel bone and the outer surface of the tendon, thus creating conditions that facilitate bone regeneration. Further study is required to define optimal handling procedure to ensure uniform distribution of PLA paste throughout the bone tunnels.