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Synthesis, Development, Characterization and Effectiveness of Bovine Pure Platelet Gel-Collagen-Polydioxanone Bioactive Graft on Tendon Healing

Article by Moshiri A, Oryan A and Meimandi-Parizi A. J Cell Mol Med. 2015 Jun;19(6):1308-1332.

Commentary by **Lawrence A. DiDomenico, DPM and Emlyn K. Forsung, DPM**

The authors certify that they have no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted article.

CONDENSATION

Purpose of Study

To investigate whether bovine platelet gel (BPG) can accelerate tendon repair

Approach

Extraction of the collagen type I from bovine tendon and its purity was confirmed by SDS-PAGE, followed by electrospinning. The electrospun collagen fibers were obtained by electrospinning the collagen molecules onto a dual-plate device. In order to produce the tridimensional collagen gel, the electrospun bovine tendon type I collagen molecules were mixed with electrospun collagen fibers and polymerized in an incubator at 4 degrees Celsius. During the polymerization, the collagens were then aligned under 12 Tesla magnetic fields. The composite materials were cut into several pieces according to the size and shape of the rabbit's Achilles apparatus followed by crosslinking using irradiation to increase the mechanical properties of the scaffold, so that it could hold the surface tensions.

The bioimplant (CI-PDS) was prepared by wrapping a polydioxanone sheet around each collagen piece. It was left for drying at room temperature to avoid any in vivo tissue reaction with the chemical solvents. The final product was repeatedly washed to maintain its sterility until surgery. The peripheral blood samples were collected from healthy bovines which were free of any

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infective diseases, and centrifuged which resulted in the formation of three layers: the red blood cells, white blood cells or platelets and plasma. The plasma and the buffy coat layers were centrifuged again, which also resulted in the production of three layers: white blood cells at the bottom, platelet-rich plasma (PRP) at the middle and platelet-poor plasma (PPP) at the top. The PRP and PPP were studied under stereomicroscope to confirm that the samples were free from bovine WBCs and RBCs.

Finally, the PRP and PPP preparations were lyophilized and pulverised into a sterile powder. The powder was added to 0.9% NaCl to achieve a concentration of 2,000,000 per each µl. The fully-dehydrated CI-PDS were placed in a plate containing a solution of protein rich platelets which led to the absorption of the solution to the scaffold. The attachment of the platelets to the collagen fibers was confirmed by SEM, TEM and light microscopy. Based on the morphology of the scaffolds, three groups were observed: CI, CI-PDS and CI-PDS-BPG. The coagulation profiles, activated partial thromboplastin time, prothrombin time, degradation rate and levels of platelet growth factor were determined. The functional activity of the platelets was determined by platelet aggregation testing, live and dead cell assay and immunofluorescence microscopy.

The in vivo study was conducted on 160 male New Zealand rabbits which were randomly assigned to experimental and control groups (CI, CI-PDS and CI-PDS-BPG). Tendon injury was inflicted by making a 2-cm excision of the Achilles and the paratenon. Surgical reconstruction involved producing a 2-cm gap between tendons and insertion of implants into the gaps. The animals in each group were evaluated at 60 and 120 days after tendon injury and surgical reconstruction. There were a pilot group which consisted of 40 rabbits, they were also randomly assigned to four groups and were evaluated at 20 and 40 days after injury to study the host-implant interactions. The pre-euthanasia assessments were conducted on animals before anaesthetizing and finally euthanizing them by using intra-cardiac injection of 1 mg/kg gallamine triethiodide. The features of the healing tendons of the animals after euthanasia were assessed for the following characteristics: dry matter content, water uptake and delivery characteristics, gross morphological, histopathological and scanning electron microscopic features.

What Investigators Accomplished

- The biconvex discoid shape of the non-activated platelets produced after first step centrifugation (PFSC), platelets produced after second step centrifugation (PSSC) and platelets produced after lyophilisation and saline solving procedure (PLSSP) were observed having 2-3 µm diameter. The activated platelets in the platelet gel had pseudopodia emission which was confirmed by TEM and SEM. The number of platelets observed in the Cl-PDS-BPG was 2 x 10. The implant was made to absorb the platelets and the structure of the platelets which was confirmed by SEM, TEM and light microscopy. The amount of activated platelets to total platelets was 89.61 ± 6.54% which indicated that the activation method

was effective. No significant difference concerning partial thromboplastin time was observed between NBC and PFSCs and also between PFSC and PSSCs, whereas the prothrombin and clotting time increased significantly when compared to controls. The degradation rate of the PRP significantly decreased after the centrifugation when compared with controls. Similarly, the degradation rate of BPG decreased because of LSSP on comparing with controls.

- There was no significant difference between the LTA of PFSCs and PSSCs and between the LTA of the PSSCs and those of PLSSPs in light transmission aggregometry. The growth factors platelet-derived growth factor (PDGF)-AA, -AB, -BB and insulin-like growth factor 1 (IGF-1) were higher than that of PFSCs. The levels of growth factors of the PLSSPs were reduced when compared to PSSCs, the activation of the PLSSPs to the platelet gel increased the levels of growth factors when compared to the controls. The BPG increased the number of viable cells in the CI-PDS-BPG compared to the CI and CI-PDS, improved the cellular proliferation, cell maturation and matrix production.

- The BPG lead to a superior distribution of the cultured fibroblasts inside the scaffold and the cells. The immunofluorescence microscopy, SEM and light microscopy confirmed there were no significant differences observed between numbers of the cultured fibroblasts in different parts of the CL-PDS-BPG scaffolds. The clinical examination revealed that the treatment with BPG improved the tarsal flexion degree, weight distribution, heel and toe position, pain on palpation and swelling of the injured area compared with the animals which were untreated or treated with Cl or Cl-PDS. No significant differences were observed between the transverse diameter of the injured and contralateral tendons after the operation. Although after the implantation of the prosthetic implant, the transverse diameter of the injured tendons increased on days seven and 14, however it gradually decreased until day 120 after injury. Even positive results were observed in regards of surface temperature in the animals after the implantation when compared to control.

- The treatment with BPG improved the echogenicity, homogeneity, peritendinous adhesion, regeneration volume, intensity of the peritendinous adhesion and regenerative proportion compared to the control, collagen and collagen-PDS treated groups. There were no significant changes observed in the number of RBCs between multiple groups but after the implantation of prosthetic implants in tendon defects the number of leucocytes decreased in all treated groups compared to the control group.

- The treatment with BPG increased the scoring values for the development of peritendinous adhesion, hyperaemia, general appearance of the neotenon, muscle atrophy and muscle fibrosis compared to the control, CI and CI-PDS treated groups. The treatment with BPG significantly decreased total cellularity, total fibroblast, immature fibroblast and lymphocyte and even increased number of mature fibroblast and fibrocyte, macrophages, blood vessels and collagen density compared to control tendons, CI

tendons and CI-PDS observed at 60 days after injury. The cellularity, total fibroblasts, number of immature fibroblast, inflamma tory cells, blood vessels and cell density decreased in all the groups when compared to 60-day levels after injury. At 60 and 120 days after injury, the treatment with BPG significantly increased transverse diameter density of the collagen fibrils, fibers and fiber bundles compared to controls, and it improved the scored values of the tissue alignment, maturity of the collagen fibrils and crimp pattern compared to the control, CI and CI-PDS treated groups. It was also observed that after the implantation, the inflammation and the transverse diameter of the injured area increased which accelerated fibroplastic response. The treatment with BPG significantly increased the dry matter and hydroxyproline contents of the injured area compared the other groups. The animals which were treated with BPG had a close behaviour of water uptake and water delivery to normal tendons, CI and CI-PDS treated tendons score.

Investigators' Observations

This study suggests that BPG is an "accessible, reliable, cost-effective and powerful healing promotive source of the platelets and growth factors" and can be used as an alternative option to autogenous and allogenous forms of platelets, and that CI-PDS-BPG is a valuable graft option over classic grafts for managing tendon injuries.

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COMMENTARY

This article can have a big influence on the future of acute tendon repair. The idea of having the availability of a scaffold for repair of the Achilles tendon is needed in particular cases with great ruptures/tears and significant tissue loss. This study showed the effectiveness of bovine pure platelet gel-collagen-polydiaxone bioactive graft in acute tendon injury in animal studies.

Treatment with BPG significantly enhanced the scoring values for the development of peri-tendinous adhesions. It also significantly increased the transverse diameter and density of the collagen fibrils, fibers and fiber bundles, and sooner differentiated the collagen fibrils to fibers and fibers to fiber bundles compared to the controls. The results of the study showed that the

BPGs, when embedded within collagen implant with polydioxanone (artificial tendon), significantly enhances cytocompatibility of the implant at in vitro level but also improves scaffold biocompatibility and biodegradability in vivo. BPG was proven to be successful.

The reluctance in using an off-the-shelf product in acute tendon injury will be due to the availability of PRP from the patient, the reaction that it may cause and the expense. Compared to off-the-shelf BPG, PRP is readily available and is free. PRP has also been proven to be effective in facilitating tendon healing. As it comes from the patient, PRP has no chance of host reaction. Even though this study shows that BPG has a great potential, it will need to be tested in humans in a clinical trial setting (most foot and ankle surgeons will be reluctant to try a product which has not been tested and approved by the FDA).

The results of this study may have an impact in the in the treatment of acute tendon injuries in the future. As of now, the readily available PRP from patients will remain the standard, compared to an off-the-shelf product such as BPG.