pISSN 1738-2696 · eISSN 2212-5469
http://dx.doi.org/10.1007/s13770-016-9120-3
ORIGINAL ARTICLE

TERM

Platelet-Derived Growth Factor-BB-Immobilized Asymmetrically Porous Membrane for Enhanced Rotator Cuff Tendon Healing

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Rotator cuff tear is a common musculoskeletal disease that often requires surgical repair. Despite of recent advances in surgical techniques, the re-tear rate of the rotator cuff tendon is very high. In this study, a platelet-derived growth factor-BB (PDGF-BB)-immobilized asymmetrically porous membrane was fabricated to investigate the feasibility for enhancing rotator cuff tendon regeneration through the membrane. PDGF-BB is recognized to promote tendon regeneration. The asymmetrically porous membrane was fabricated by polycaprolactone and Pluronic F127 using an immersion precipitation technique, which can allow selective permeability (preventing scar tissue invasion into defect region but allowing permeation of oxygen/nutrients) and incorporation of bioactive molecules (e.g., PDGF-BB) *via* heparin binding. The PDGF-BB immobilized on the membrane was released in a sustained manner over 42 days. In an animal study using Sprague-Dawley rats, the PDGF-BB-immobilized membrane group showed significantly greater regeneration of rotator cuff tendon in histological and biomechanical analyses compared with the groups of control (suturing) and membrane without PDGF-BB immobilization. The enhancing regeneration of rotator cuff tendon of the PDGF-BB-immobilized membrane may be caused from the synergistic effect of the asymmetrically porous membrane with unique properties (selective permeability and hydrophilicity) as a scaffold for guided tendon regeneration and PDGF-BB sustainedly released from the membrane. Tissue Eng Regen Med 2016;13(5):568-578

Key Words: Tendon; Rotator cuff; Membrane; Platelet-derived growth factor-BB; Tissue regeneration

INTRODUCTION

Rotator cuff tendon tear led to pain and functional disorder of shoulder is a common musculoskeletal disease that often requires surgical repair [1]. Surgical techniques are often used to reattach torn tendon to the bone. Despite of recent advances in surgical techniques, the rate of re-tear caused by suture breaking and suture slip from tendon still approaches up to 90% of cases [2-8]. This is generally attributed to insufficient restoration of native biological and mechanical properties at the injury site. A recent systematic review also showed that although arthroscopic transosseous equivalent repairs for large tears led to improved healing rates, failure rate still reached 25% [9]. For this reason, a number of efforts have focused on biological augmentation using small intestine submucosa, dermis, pericardium, etc. [1] to promote tendon healing as well as improve mechanical strength after repair. The use of various cells (e.g., mesenchymal stem cell and tenocyte) and growth factors [e.g., platelet-derived growth factor BB (PDGF-BB) and insulin-like growth factor-1] [10-13] have been also suggested as repair strategies. More recent focus is turning to synthetic extracellular matrix (ECM) augmentation to enhance rotator cuff tendon healing because tendon augmentation with biological tissues have not been widely accepted for clinical use due to the risk of evoking an inflammatory response and potential weakening of material properties after applying to host tissue [14]. Bioabsorbable polymeric matrices such as woven polylactic acid (PLA), poly(D,L-lactic-co-glycolic acid) nanofiber, chitin fiber, PLA/collagen hybrid nanofiber, and chitosan/hyaluronan hybrid fiber meshes have been investigated over the past decade [15-19].

In our previous studies [20,21], we reported that asymmetrically porous membrane fabricated by polycaprolactone (PCL)

Received: December 7, 2015
Revised: December 30, 2015
Accepted: January 7, 2016
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and Pluronic F127 using an immersion precipitation technique allows selective permeability (preventing scar tissue invasion into defect region but allowing permeation of oxygen/nutrients) and incorporation of bioactive molecules, which are essential criteria in a guided tissue regeneration (GTR) membrane used for effective target tissue regeneration. Based on this result, it was expected that if suitable growth factors to accelerate tendon healing were immobilized on the asymmetrically porous membrane, it may be an option for enhanced rotator cuff tendon healing and minimized retearing rate. In this study, PDGF-BB as a biological stimulus to promote rotator cuff tendon regeneration was incorporated onto the pore surfaces of the PCL/Pluronic F127 asymmetrically porous membrane *via* heparin binding. The regeneration of rotator cuff tendon through PDGF-BB-immobilized membrane was compared with those of membrane without PDGF-BB as well as a control (suturing without the membrane).

MATERIALS AND METHODS

Materials

PCL (Mw 80,000 Da; Aldrich, USA), tetraglycol (glycofurol; Sigma, USA), and Pluronic F127 (Mw 12,500; BASF, USA) were used to prepare an asymmetrically porous membrane. PDGF-BB, which is approved from US Food and Drug Administration (FDA) for human use [22], was selected as a growth factor to enhance the tendon regeneration and was purchased from R & D Systems (USA). All other chemicals were of analytical grade and were used as received. Water was purified (>18 mΩ) using a Milli-Q purification system (Millipore, USA).

Fabrication of PCL/Pluronic F127 membrane

Asymmetrically porous PCL/Pluronic F127 membranes were prepared by an immersion precipitation method [23]. Briefly, PCL pellets were dissolved in tetraglycol at 90°C (12 wt%) and Pluronic F127 powder was added in the PCL solution (5 wt%, PCL base). The PCL/Pluronic F127 mixture solution was poured in a mold (60×80×0.4 mm) and subsequently immersed into excess water for 1 hr at room temperature. The top surface of PCL/Pluronic F127 mixture solution was solidified by the contact of nonsolvent (water) and then the sublayer was gradually solidified by the diffusion of water into the solution (solvent-nonsolvent exchange). The asymmetrically porous PCL/Pluronic F127 membrane was obtained after washing in excess water to remove residual solvent and vacuum drying. The PCL membrane without Pluronic F127 was also prepared using the same procedure described above. Surface and cross-section morphologies of the prepared PCL/Pluronic F127 membrane were examined by a scanning electron microscope (SEM; Model S-3000N, Hitachi, Japan).

Growth factor immobilization and release test

PDGF-BB was incorporated onto the PCL and PCL/Pluronic F127 membrane (12×12×0.4 mm) via heparin binding. For this, the membrane was immersed in a heparin solution (3 mg/mL in 2 wt% NaCl solution) at 4°C for 3 hrs. The heparin-bound membrane was rinsed using a 2 wt% NaCl solution and water sequentially, and then freeze-dried. The amount of heparin incorporated on the membrane was determined using a Toluidine blue assay [24]. To immobilize the PDGF-BB onto the membrane, heparin-bound membrane was immersed in PDGF-BB solution (2 μg/mL) at room temperature for 9 hrs with gentle shaking. The PDGF-BB-immobilized membrane was washed three times using phosphate buffered saline (PBS; pH 7.4) and the loading amount of PDGF-BB immobilized on the membrane was quantified by a direct ELISA technique [25]. The PDGF-BB-adsorbed PCL and PCL/Pluronic F127 membranes without the heparin binding were also prepared using the same procedure described above to investigate the effect of heparin on the PDGF-BB immobilization. Figure 1 demonstrates schematic diagrams for the successive binding of the heparin and the growth factor onto the pore surface of the PCL/Pluronic F127 membrane. To investigate the release behavior of the

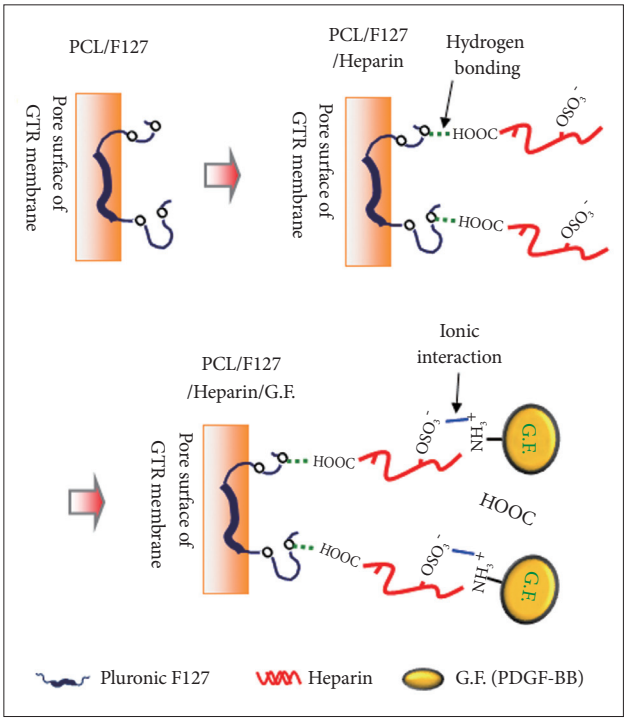

Figure 1. Schematic diagrams for the successive binding of the heparin and the growth factor onto the pore surface of the PCL/Pluronic F127 membrane. PCL: polycaprolactone, GTR: guided tissue regeneration, PDGF-BB: platelet-derived growth factor-BB.

PDGF-BB, the growth factor-immobilized (or adsorbed) membranes were incubated in 1 mL PBS containing 1% bovine serum albumin (Sigma) at 37°C for up to 42 days under mild shaking (~50 rpm). At pre-determined time periods, the whole media were harvested and replaced with fresh PBS. The amount of PDGF-BB released in the harvested medium was analyzed using the ELISA kit.

Surgical procedure

Sprague-Dawley rats (200−250 g) were chosen as an animal model to evaluate the effect of PDGF-BB-immobilized PCL/Pluronic F127 membrane as a GTR membrane on rotator cuff tendon regeneration. A total 36 rats were used for the analyses. The animals were divided into the following 3 groups (12 rats/group): suture repair group (Control), suture repair/GTR membrane-covered group (GTR), and suture repair/PDGF-BB-immobilized GTR membrane-covered group (PDGF-BB/GTR). The animal experiment was approved from the Institutional Animal Care and Use Committee of the Hannam University in Korea, and all procedures were performed according to the appropriate guidelines. Unilateral shoulder surgery using a sterile technique was conducted under anesthesia. The anesthesia was induced through an intramuscular injection of tiletamine/zolazepam (10 mg/kg; Zoletil 50®, Virbac Laboratories, France) and 2% xylazine hydrochloride (2 mg/kg; Rumpun®, Byely, Korea). A longitudinal anterolateral skin incision was made on left side, and the omovertebral and deltoid muscles were retracted. Osteotomy of the acromioclavicular arch just posterior to the acromioclavicular joint was performed, and the acromion was retracted to expose the infraspinatus tendon. The rotator cuff tendon was detached sharply at its insertion on the greater tuberosity, and a 2×2 mm defect involving the full-thickness of the infraspinatus tendon was created. The remaining distal fibrocartilaginous stump was resected from the greater tuberosity using a curette. In the control group, the defect was repaired by suturing using 5-0 nylon suture between tendon and greater tu-

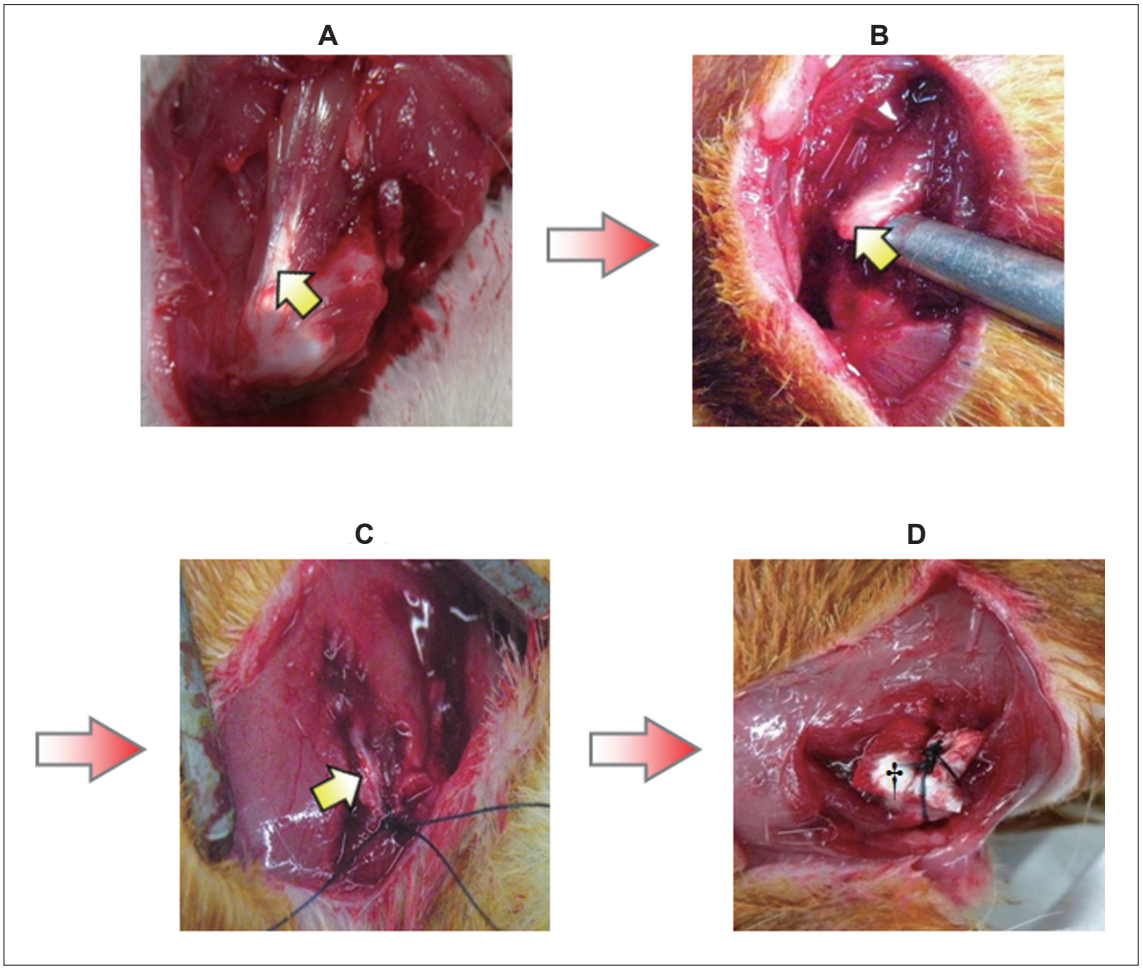

Figure 2. Photographs showing the surgical procedure; (A) the normal rotator cuff tendon, (B) injury tendon, (C) repaired tendon by suturing, and (D) GTR membrane (w/, w/o PDGF-BB)-applied tendon (arrow: tendon, †: GTR membrane). GTR: guided tissue regeneration, PDGF-BB: platelet-derived growth factor-BB.

berosity. In the membrane groups (GTR and PDGF-BB/GTR), the membranes (3×3×0.4 mm) were covered on the suture-repaired tendon defect with the micropore side of the membrane facing the defect, and were secured to the repaired tendon with a 5-0 nylon suture (Fig. 2). The deltoid and trapezius muscles were re-approximated and the incision was closed. The animals were returned to their cages and monitored until they recovered. They were allowed to perform normal cage activities without immobilization.

At 2 and 4 weeks after surgery, the animals were euthanized by overdose CO_2 inhalation. Each operated shoulder was evaluated macroscopically for signs of infection, wound dehiscence, adhesions, and rotator cuff integrity. All infraspinatus specimens were obtained by harvesting the scapula and humerus and removing all soft tissues except the infraspinatus muscle and tendon. For biomechanical testing, the scapula side of specimen was fixed at one custom-designed vise grip and the humerus side was held in another vise grip. The vise grips were attached to an universal testing machine (AG-X; Shimadzu, Japan) with a 10 kgf load cell. The tendon was aligned parallel to the direction of load application. The tendon was pulled with a crosshead speed of 10 mm/min and the ultimate stress to failure was recorded. For histological analysis, the specimens were fixed in 10% neutral buffered formalin and fully decalcified in 5% nitric acid, trimmed, and then embedded in paraffin. The paraffin-embedded specimens were cut into 5 μm transverse sections and stained with Hematoxyline & Eosin (H&E) and Safranin-O for observation by light microscopy (Model BX-51, Olympus, Japan). To evaluate the regenerated tendon qualitatively, we adopted a modified tendon-to-bone maturing score system [26] which grades histologic parameters including cellularity/vascularity (1=marked, 2=moderate, 3=mild, 4=minimal) and proportion of oriented fibers/large diameter fibers/continuity/bone ingrowth/fibrocartilage cells/tidemark (1: <25%, 2: 25−50%, 3: 50−75%, 4: >75%). The evaluation was conducted by an expert pathologist through a blinded experiment. A best score in this scoring system is 32.

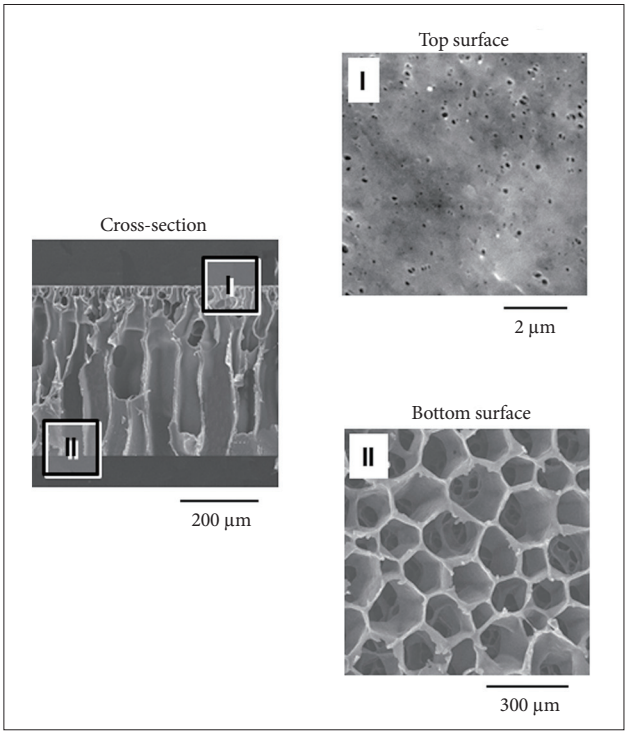

Figure 3. SEM photographs showing the morphologies of the cross-section, top, and bottom surfaces of the PCL/Pluronic F127 membrane fabricated by an immersion precipitation method. SEM: scanning electron microscope, PCL: polycaprolactone.

Statistical analysis

The data obtained from each group were averaged and expressed as mean±standard deviation. Student's t-test was adapted to determine the significance of differences between the groups. The differences were considered statistically significant at $p<0.05$.

RESULTS

Characterization of PCL/Pluronic F127 GTR membrane

Figure 3 shows the morphology of the PCL/Pluronic F127 membrane prepared by the immersion precipitation method [23]. Pluronic F127 was used as an intermediator to incorporate heparin and growth factor on the membrane as well as a hydrophilic additive to prepare hydrophilized PCL membrane. The top surface (water contact side) of the membrane had nano-size pores (~100 nm) which can effectively prevent scar tissue infiltration but permeate nutrients, while the bottom surface (mold contact side; tendon contact side in animal study) had micro-size pores (~200 μm) which can improve adhesion with tendon and guide tendon regeneration. Both sides of the membrane were connected by a channel-like pore structure. The formation of asymmetrically porous structure can be explained by gradual phase separation in the polymer (PCL/Pluronic F127 mixture) solution by exchange between solvent (tetraglycol) and non-solvent (water) [27]. The morphology of the PCL membrane without Pluronic F127 was similar to the PCL/Pluronic F127 membrane.

Growth factor loading amount and release profile

Heparin was incorporated on the PCL/Pluronic F127 membrane *via* hydrogen bonding between the ether group (-O-) of Pluronic F127 exposed on the pore surfaces of the membrane and the carboxylic acid group (-COOH) of heparin (Fig. 1). It

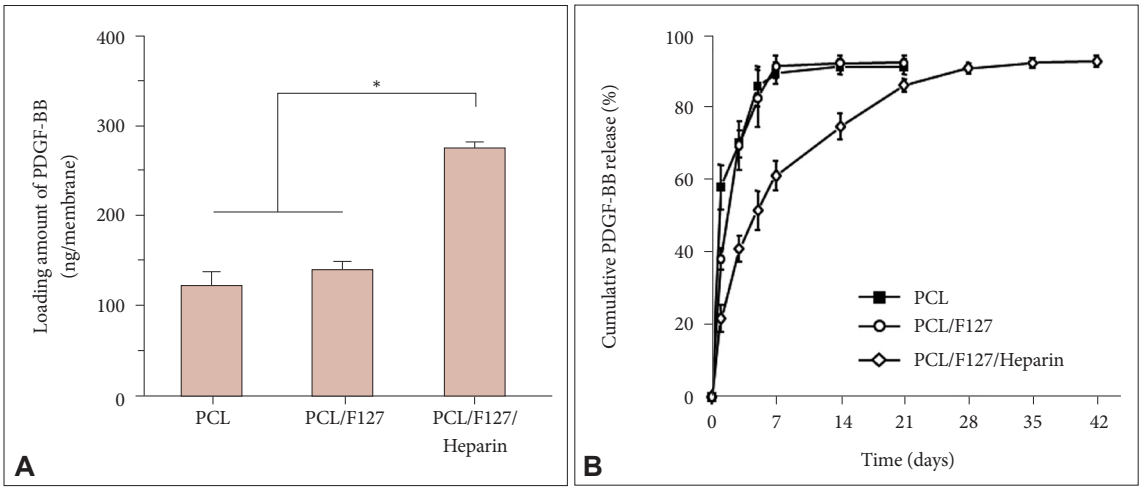

Figure 4. (A) Loading amount and (B) cumulative released amount of PDGF-BB from the PCL, PCL/Pluronic F127, and PCL/Pluronic F127/heparin membranes (n=3, *p<0.05). PDGF-BB: platelet-derived growth factor-BB, PCL: polycaprolactone.

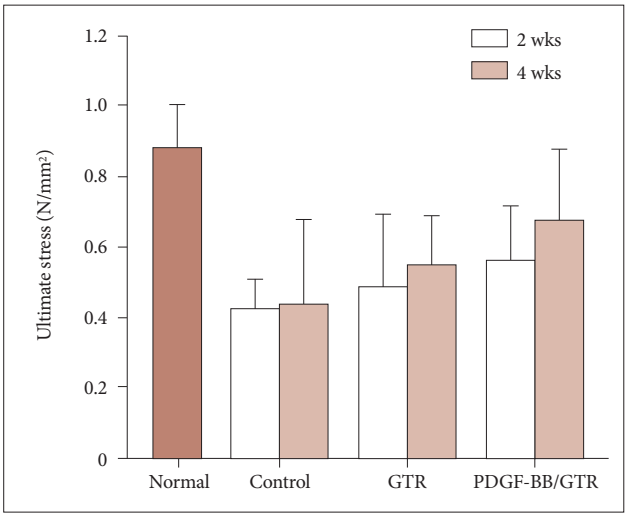

Figure 5. Ultimate stress at failure of the scapula and humerus specimens [Normal, Control (w/o GTR membrane), GTR membrane, and PDGF-BB/GTR membrane groups] harvested at 2 and 4 weeks after surgery (n=3). GTR: guided tissue regeneration, PDGF-BB: platelet-derived growth factor-BB.

was observed that the PCL/Pluronic F127 membrane allowed much larger heparin binding on the surfaces (9.16±1.28 μg/membrane) compared with the PCL membrane (2.25±0.44 μg/membrane) (not shown), indicating that the Pluronic F127 molecules are sufficiently exposed on the pore surfaces of the membrane and effectively bind with heparin. The heparin binding on the PCL membrane without Pluronic F127 may be caused by the physical adsorption of the heparin on the pore surfaces. PDGF-BB, which is recognized as an effective promoter for tendon regeneration [12], was incorporated onto the heparin-bound PCL/Pluronic F127 membrane through ionic interactions between the N-sulfate and O-sulfate groups of the heparin molecule and certain lysine and arginine residues in the growth factor, as well as high affinity between heparin and growth factor caused by the carboxyl-terminal extension (conformational change) of the dimeric PDGF-BB molecules [28,29]. The loading amount of PDGF-BB on the different membrane surfaces (PCL, PCL/Pluronic F127, and PCL/Pluronic F127/heparin) were estimated as 123.01±13.13 ng, 140.31±8.68 ng and 276.07±5.49 ng/membrane, respectively (Fig. 4A). The PDGF-BB detected on the membranes without heparin binding can be also understood by the physical adsorption of the PDGF-BB on the pore surfaces of the membrane. The heparin-bound PCL/Pluronic F127 membrane showed a moderate initial burst release of PDGF-BB, and then the PDGF-BB was released sustainedly up to ~90% of the initial loading over 42 days (Fig. 4B). On the other hand, the membranes without heparin binding showed a much greater initial burst for the initial 3 days. This high initial burst can be caused by the rapid desorption of physically bound PDGF-BB onto the membranes. Most of the PDGF-BB bound on the membranes without heparin was released within 7 days. We expected that the sustained release of PDGF-BB from the membrane would maximize its biological effectiveness for tendon regeneration by the prolonged bioactivity of the growth factor [30,31].

Tendon regeneration behavior

After 2 weeks of surgery, all rats were active and climbed up the cage without the limitation of shoulder motion. Grossly, the surgical site exhibited no signs of infection or wound dehiscence in any of the rats. Scar tissue was noted around the acromioclavicular joint, but there were no adhesions or contractures limiting the shoulder range of motion. There was no rupture or incomplete healing of repaired cuff at time of sacrifice. Figure 5 shows the ultimate stress at failure of the scapula and humerus specimens at 2 and 4 weeks after surgery. All specimens except normal group were failed at the repair (defect) site, while the

normal specimen was failed at the junctional region between tendon and scapula. The ultimate stress at failure of each group increased with time, indicating tendon regeneration in the rotator cuff defect. The degree of ultimate stress at failure among the groups was as follows: PDGF-BB/GTR>GTR>Control, even though the ultimate stresses were not comparable to normal group and the differences of the mechanical strength among them were not significant.

Figures 6 and 7 show the histological sections (with H&E and Safranin-O stainings) to compare tendon reconstruction in the rotator cuff defect among the control, GTR, and PDGF-BB/GTR groups at 2 and 4 weeks after surgery. In the control group, the defect tendon in rotator cuff was not re-connected with greater tuberosity until 2 weeks even suturing between tendon and greater tuberosity. The re-connected and maturated tissues were observed at 4 weeks. On the other hand, the membrane groups (GTR and PDGF-BB/GTR groups) allowed the reconnection of tendon to greater tuberosity at 2 weeks, and then the continuous maturation. There was capillary proliferation and the gap between the tendon and bone was filled in with fibrovascular granulation tissue in both membrane groups. In particular, the PDGF-BB/GTR group showed greater fibrocartilage-like tissues

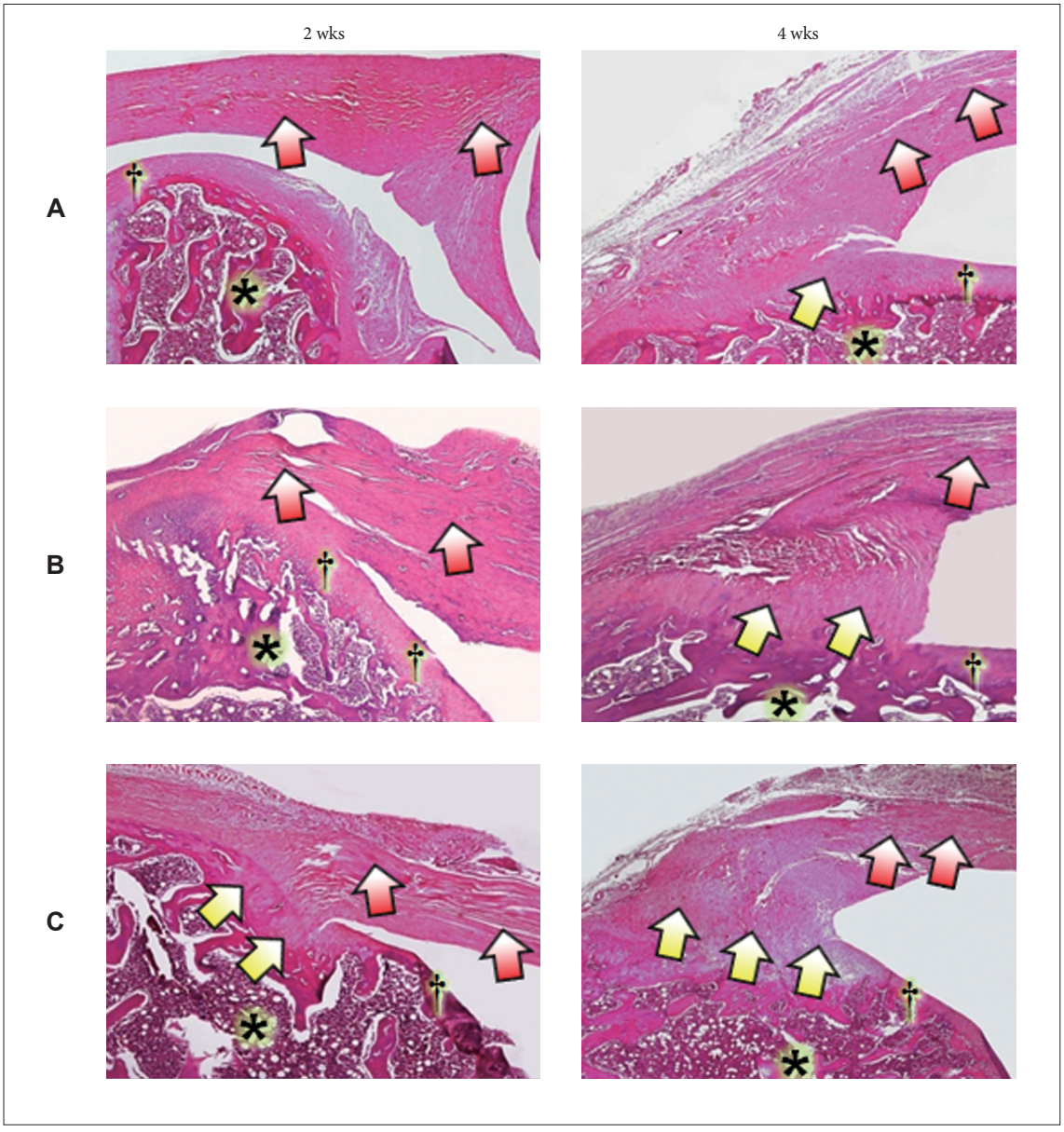

Figure 6. Histological sections of (A) Control (w/o GTR membrane), (B) GTR membrane, and (C) PDGF-BB/GTR membrane specimens at 2 and 4 weeks after surgery (H&E staining; ×40; *: bone, †: cartilage, yellow arrow: fibrocartilage, red arrow: tendon). GTR: guided tissue regeneration, PDGF-BB: platelet-derived growth factor-BB.

[spherical cartilage cells (lacuna structure)] and dense/oriented collagen fibers at the regenerated rotator cuff tendon (more similar to normal tendon-to-bone interface) compared with the other groups, indicating better environment for tendon regeneration than the control and GTR groups.

For qualitative analysis of the regenerated tendon at 2 and 4 weeks after surgery, the maturing of regenerated tendon was scored (Fig. 8) [26]. The GTR membrane groups showed higher maturing scores [GTR (9.3±0.3) and PDGF-BB/GTR (15.1±0.6) at 2 weeks; GTR (18.0±0.9) and PDGF-BB/GTR (23.4±0.8) at 4 weeks] compared to the control group (7.1±0.4 at 2 weeks and 11.0±0.9 at 4 weeks). Particularly, the PDGF-BB/GTR group had significantly greater maturing scores than the GTR group without the growth factor, suggesting the synergistic effect of the GTR membrane as a scaffold for tendon regeneration and PDGF-BB sustainedly released from the GTR membrane.

DISCUSSION

Torn rotator cuff tendon has a limited healing potential. Histologically, the rotator cuff tissue is mainly composed of an abundant and highly organized collagenous ECM with small

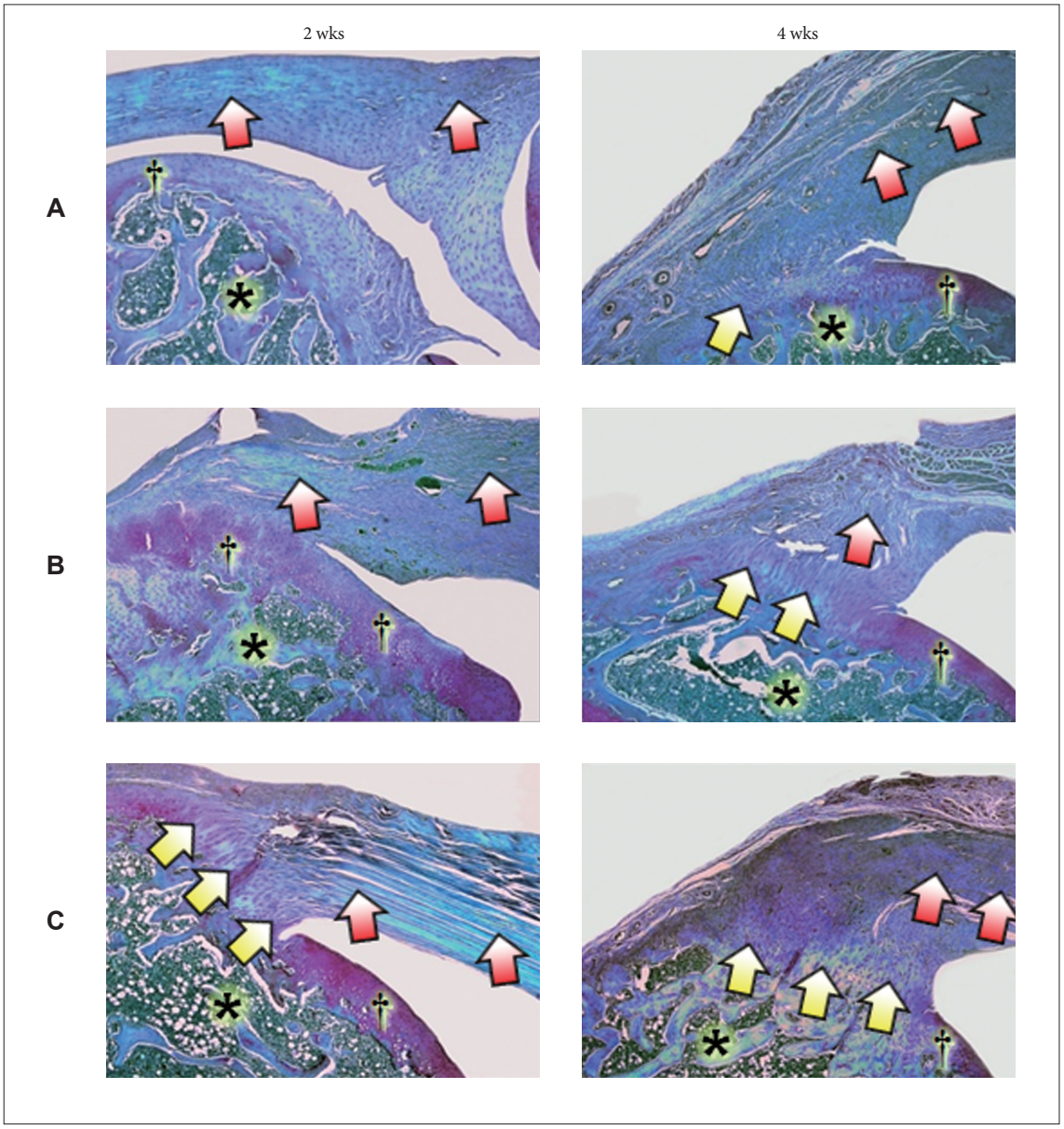

Figure 7. Histological sections of (A) Control (w/o GTR membrane), (B) GTR membrane, and (C) PDGF-BB/GTR membrane specimens at 2 and 4 weeks after surgery (Safranin-O staining: ×40, *: bone, †: cartilage, yellow arrow: fibrocartilage, red arrow: tendon). GTR: guided tissue regeneration, PDGF-BB: platelet-derived growth factor-BB.

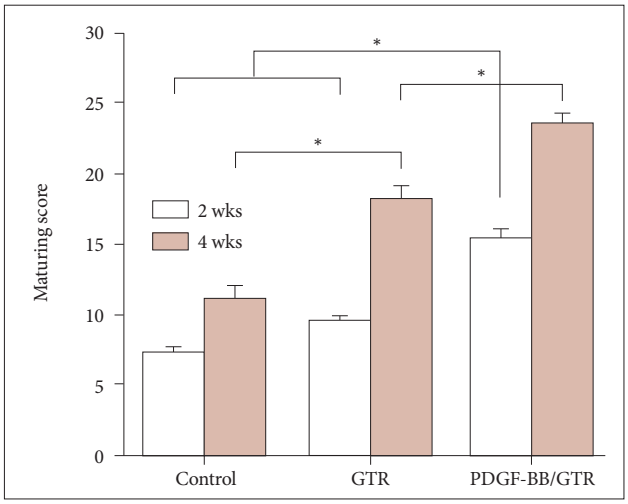

Figure 8. Tendon-to-bone maturing scores of Control (w/o GTR membrane), GTR membrane, and PDGF-BB/GTR membrane groups (after 2 and 4 weeks; n=3, *p<0.05). GTR: guided tissue regeneration, PDGF-BB: platelet-derived growth factor-BB.

number of cells supplied by poor vasculature [32]. Histological study of repaired rotator cuff demonstrates that the bone and tendon are joined by a layer of fibrovascular scar tissue consisting of mainly Type III collagen instead of four distinct zones, seen in the native rotator cuff insertion. This renders repaired tissue weaker than the original insertion site and may contribute to the substantial failures of repaired rotator cuff tendon [33,34]. Currently, the strategies to promote healing have been focused on native tendon tissue and ECM augmentations with the addition of growth factors. Tendon augmentation with the long head of biceps is a reasonable option and several advantages in that it is feasible during surgery, yet it sacrifices function of the biceps, a potential humeral head depressor [35]. ECM augmentation has been divided into xenograft, allograft, and synthetic materials. Tissue from animal and human donors has been commercially available and applied for tendon healing [36-42]. However, there are potential problems with allogenic ECMs. Inflammatory reaction to host tissues has been reported. These responses can cause degeneration of the rotator cuff tendon repair and decrease material properties as well, which may result in comparably increased re-tear rates due to the decreased tendon material properties [43-46]. These concerns have prompted the development of synthetic ECM grafts for surgical use. Novel synthetic materials have been introduced to overcome the weaknesses of biological materials. There are a variety of properties required for desirable synthetic ECMs. They include a proper biodegradation rate to match the rate of new tissue formation, mechanical strength to maintain the structure, biocompatibility, and positive interaction with surrounding cells to achieve well-organized tissues or organs [47]. To fulfill these requirements, poly(α-hydroxyl acid)s, including poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA) and their copolymers [poly(lactic-co-glycolic acid) (PLGA)], which are biocompatible, biodegradable and FDA-approved for human applications, have been commonly utilized [48]. However, their brittleness which limits the use for load-bearing tissue regeneration (e.g., cartilage, bone, tendon, ligament, etc.), and acidic by-products formed during degradation which can generate an inflammatory response, have been considered as practical limitations [49-52].

Recently, PCL has been gained increasing interest as a synthetic ECM for tissue regeneration, because of its flexibility, biocompatibility, and low toxicity of its degradation products *in vivo*. PCL is clinically used as biodegradable staple/suture for wound closure and is being investigated as meniscus and bone scaffolds for tissue engineering applications [49,51,53].

On this basis, PCL was considered as a matrix for rotator cuff tendon regeneration in the present study. Pluronic F127, which is an amphiphilic tri-block copolymer of polyethylene glycol and polypropylene glycol, was adapted as a hydrophilic additive and an intermediator to incorporate heparin and PDGF-BB on the membrane surface. Pluronic F127 is well known as the least toxic among Pluronic series (approved from FDA for human use) [54]. Tetraglycol, which has been widely adapted in parenteral products for intravenous, intramuscular, or intranasal injection [55-57], is used as a non-toxic co-solvent for both PCL and Pluronic F127. From the growth factor release profile, it was recognized that the heparin-bound membrane (PCL/Pluronic F127/heparin) has a significantly higher capacity and more sustained release of PDGF-BB than the other membranes without the heparin (PCL and PCL/Pluronic F127). This finding can be understood by ionic interactions between the N-sulfate and O-sulfate groups in the heparin and certain lysine and arginine residues in the growth factor [58]. The PDGF-BB detected on the membranes without heparin (PCL and PCL/Pluronic F127 membrane) can be also explained by the simple physical adsorption of PDGF-BB on the pore surfaces of the membrane, which may lead to a burst release at initial stage in the medium [59]. The sustained release of PDGF-BB from the heparin-bound membrane, which can prolong its biological effects, may be very helpful to regenerate tendon [60]. PDGF-BB was reported as one of the important growth factors involved in the healing process and correlated to increased levels of Type I collagen. It is present in low levels throughout normal cuff repair process [61,62]. Tendon healing occurs in three overlapping phases labeled as inflammatory, fibroblastic, and remodeling. Among three phases, PDGF-BB is involved in inflammatory and fibroblastic phases [63,64]. Some studies found the increased DNA and collagen synthesis in transduced fibroblasts as well as improved histology and biomechanics by PDGF-BB group in rotator cuff tendon repair model (rat). Furthermore, PDGF-BB/

Type I collagen matrix increased ultimate load to failure of cuff repairs in a sheep model [65,66]. There are limitations to the animal model used in this study. The rat model is a completely different milieu than found in humans. Tissue variations exist and surgically induced tears are not degenerative, as seen in humans. The remodeling process in healthy rats with great healing potential could not discriminate the effects of the addition of growth factors to the novel synthetic membrane on histology and material properties. It is interesting to note that none of the reconstructed group exhibited functional deficits, reflecting the promise of the PCL membrane in restoring function. Although we found both histological and mechanical benefits of the addition of a growth factor-immobilized polymer membrane, it is yet unclear how the polymer membrane and growth factor individually contribute to the entire remodeling process. In addition, the absence of inflammation and tissue rejection was encouraging. This study should be regarded as preliminary step toward the application of novel growth factor-immobilized polymer membrane to potentiate rotator cuff tendon healing and bridge cuff defects. Although rats are suitable for shoulder studies in that their anatomy is very similar to humans, it would be more desirable to perform the experiment in larger animal having chronic torn cuffs as seen in the human condition.

In conclusion, the PDGF-BB-immobilized GTR membrane seems to provide a suitable environment for healing of rotator cuff tendon in our system, probably due to the synergistic effect between the GTR membrane as a scaffold for tendon regeneration and PDGF-BB sustainedly released from the GTR membrane. Further studies using more clinically relevant models for rotator cuff tendon repair may accelerate the use of the PDGF-BB-immobilized GTR membrane in clinical fields.

Acknowledgements

This work was supported by grants of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute funded by the Ministry of Health & Welfare, Republic of Korea (HI14C0184 and HI13C1596).

Conflicts of Interest

The authors have no financial conflicts of interest.

Ethical Statement

All animal experiments were approved by the Institutional Animal Care and Use Committee of the Hannam University in Korea (HNU-IACUC 11-08).

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