RESEARCH ARTICLE

Platelet-activated serum might have a therapeutic effect on damaged articular cartilage

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Abstract

Platelet-activated serum (PAS) was collected from rabbits. This contains high concentrations of growth factors, including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF)-BB, and transforming growth factor-beta (TGF-β). PAS was injected into the knee joints of Japanese White rabbits subjected to anterior cruciate ligament transection (ACL-T) to investigate its therapeutic effects on articular cartilage. The effect of Avastin (an anti-VEGF monoclonal antibody) on VEGF expression was also investigated. The levels of VEGF, PDGF-BB, and TGF-β in PAS, platelet-rich plasma (PRP) and autologous serum from untreated rabbits were analysed by enzyme-linked immunosorbent assays. The samples ($n = 24$ rabbits) were divided into control (C), PAS (S), Avastin (A) and PAS + Avastin (S + A) treatment groups. Intra-articular injections were administered weekly for 7 weeks after ACL-T, during which the weight distribution ratios of the damaged limbs were evaluated. Histological evaluation was performed 12 weeks after ACL-T using Mankin score. The VEGF, PDGF-BB and TGF-β expression levels were significantly higher ($P < 0.05$) in the PAS than in the PRP or autologous serum samples. The weight distribution ratios of damaged limbs improved significantly after ACL-T in all treatment groups ($P < 0.05$). The proximal medial, distal medial and lateral aspects of joints in the treatment groups showed significant differences in Mankin scores compared with controls ($P < 0.05$). The damaged limb weight distribution ratios, Mankin scores and articular cartilage structure did not differ significantly among the three treatment groups, which all showed significant improvements in structure compared with controls. Copyright © 2017 John Wiley & Sons, Ltd.

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1. Introduction

Osteoarthritis (OA) is the most common joint disorder in the USA, and 10% of men and 13% of women aged 60 years and over have its symptoms (Zhang and Jordan, 2010). Articular cartilage is avascular tissue that is nourished solely by synovial fluid, so it has poor regenerative capacity (Paget, 1969). OA progresses along with trauma and the aging process, and complaints of problems in the knee joints are associated with increased physical activity in people of all ages. Many treatment methods have been used to control the progression of OA and improve both pain and functionality. Non-steroidal anti-inflammatory drugs, steroids, acetaminophen, opiates and other drugs have been used as analgesics, but these cause many adverse reactions (Bradley et al., 1991; Dieppe et al., 1993; Langman et al., 1994). Intra-articular hyaluronic acid (HA) injections and other minimally invasive treatments have been designed to alleviate the pain associated with degeneration of articular cartilage.

Although these are effective in the short term, they are insufficient for the treatment of chronic and serious forms of OA (Arrich et al., 2005; Petrella and Petrella, 2006). The 2013 American Academy of Orthopaedic Surgeons (AAOS) guidelines (http://www.aaos.org/Research/guidelines/OAKSummaryofRecommendations.pdf) strongly discourage the use of intra-articular injections of HA, glucosamine or chondroitin. Therefore, many surgical treatments including microfracture and mosaicplasty have been used to facilitate tissue regeneration. However, the widespread implementation of these methods has been hampered by observations of ossification and fibrosis at the transplant sites (Steadman et al., 1999; Szerb et al., 2005). Sporadic reports on treatment methods utilizing components taken from the patient's own blood indicate that this methodology can result in the effective repair of tissue damage (van Buul et al., 2011; Filardo et al., 2011; Kon et al., 2010; Patel et al., 2013; Sanchez et al., 2003; Wehling et al., 2007; Yoshioka et al., 2013). Of these, methods using platelet-rich plasma (PRP) have long been used in dentistry and oral surgery, and many basic research studies and clinical applications have been reported in the field of orthopaedics since the first study by Sanchez et al. (2003).

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Serum has been included as a component of culture media for promoting the proliferation of cells in regenerative medicine. Currently, sera of animal origins are used in such media. Believing that the use of autologous serum in culture media would be safer, the JMS Corporation (Hiroshima, Japan) has developed serum collection kits, CELLAID®. Here, rabbit serum obtained using CELLAID® kits was used to investigate its effect on the repair of damaged knee cartilage. These kits facilitate the collection of platelet-activated serum (PAS) from blood using a sterile and closed procedure. PAS includes high concentrations of growth factors, such as transforming growth factor-beta (TGF-β) and platelet-derived growth factor (PDGF)-BB. The kits also enable the long-term preservation of collected serum samples (Figure 1; Table 1). Here we investigated the effects of intra-articular injections of PAS on the histological repair of defects generated in knee joint articular cartilage in Japanese White rabbits.

Angiogenesis in synovial tissues is also a cause of inflammation, ectopic ossification and pain in cartilage tissues in patients with OA (Haywood *et al.*, 2003). Therefore, the suppression of angiogenesis can be therapeutic against OA. Nagai *et al.* (2010) reported that the intravenous injection of bevacizumab, a humanized anti-vascular endothelial growth factor (VEGF) monoclonal antibody (trade name, Avastin; Roche/Genentech, South San Francisco, CA, USA), facilitated the repair of articular

cartilage damage in the knee (Nagai *et al.*, 2010). Furthermore, the intra-articular injection of Avastin in models of OA produced by anterior cruciate ligament transection (ACL-T) was shown to induce articular cartilage repair (Nagai *et al.*, 2014). We hypothesized that the effect of Avastin on articular cartilage repair, combined with PAS obtained using CELLAID® kits might inhibit exogenous VEGF. Therefore, we also administered intra-articular injections of Avastin with and without PAS to test rabbits subjected to ACL-T. This work has already been published

Table 1. Comparison between PAS, autologous serum and PRP

Autologous serum
- Autologous serum conditioned
- Anti-inflammatory
- Inhibition of tissue degeneration
- Competitive blockade of IL-1 receptors

PRP
- Plasma with significantly elevated thrombocyte concentration from venous blood
- Activation of tissue regeneration by growth factors
- Growth factors are continuously released after the activation of thrombocytes

PAS
- Platelet is activated by platelet activation beads
- Including higher concentrated growth factors than autologous serum and PRP
- CELLAID® can be preserved at 4 ℃ or −80 ℃ to −20 ℃
- Platelet activation beads contained in CELLAID® complete all serum preparation steps within 1 h

PAS contained higher concentrations of cell growth factors (VEGF, TGF-β and PDGF-BB; released by shaking with platelet-activating beads) compared with autologous serum and PRP. PAS could be separated from the blood components using a closed and sterile procedure with CELLAID® kits, and could be cryopreserved at −80 ℃ for over 1 year without any loss in growth factor concentrations.
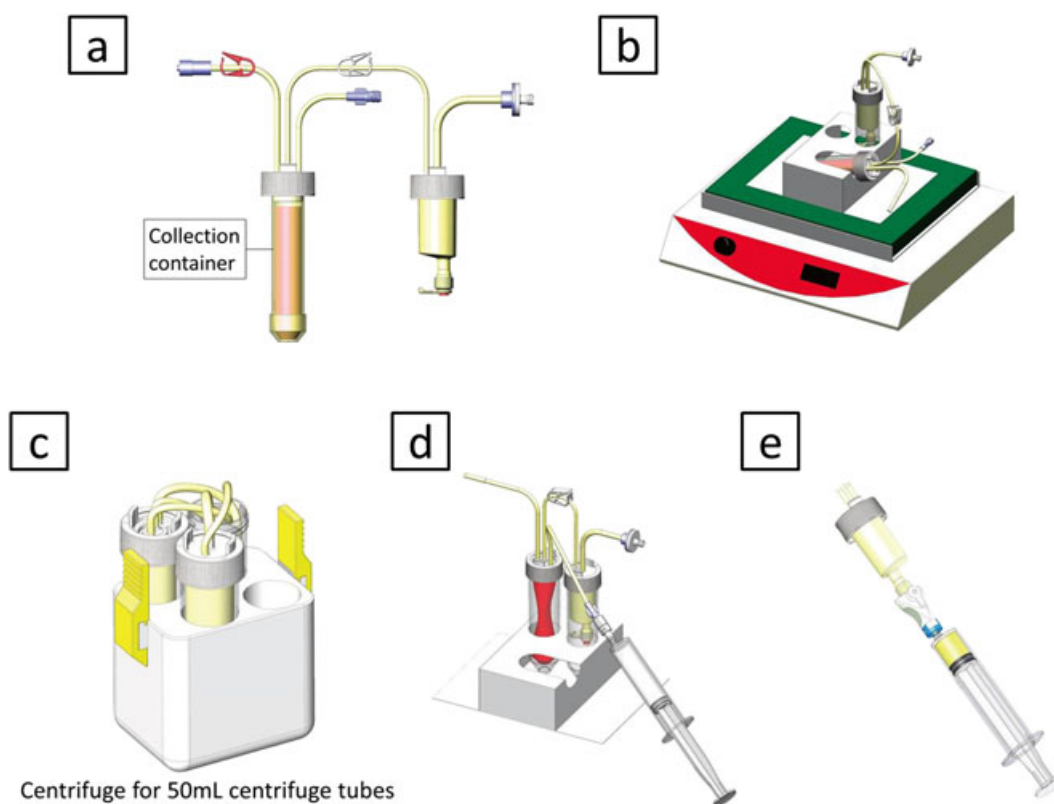IL, interleukin; PAS, platelet-activated serum; PRP, platelet-rich plasma.

Figure 1. Specifications of CELLAID® kits. These allow the separation of serum containing a high concentration of cell growth factors, such as transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF)-BB from blood components in a closed, sterile procedure. The growth factors are released by shaking with platelet-activating beads. It also allows for long-term cryostorage of the collected samples. (a) Fresh blood is introduced into the collection container, and (b) shaken for at least 30 min at room temperature. (c) The platelet-activating beads agglutinate rapidly. Centrifugation at 2000 *g* for 7 min is used to separate the serum from the beads. (d) The serum is transferred to storage container. (e) The storage container can be preserved at 4 ℃, −20 ℃ or −80 ℃. A plastic catheter is used to withdraw serum from the port [Colour figure can be viewed at wileyonlinelibrary.com]

as part of the conference proceedings for the 2014 Orthopaedic Research Society (ORS2014) in New Orleans (http://www.ors.org/Transactions/60/0355.pdf).

2. Materials and methods

2.1. Ethical approval

All animal experiments were approved by the Ethics Review Board of Tokai University, and were performed in accordance with the Tokai University Guidelines on Animal Use.

2.2. Quantitative analysis of growth factors

Platelet-activated serum was collected from five Japanese White rabbits aged 12 weeks (weight ~2.5 kg) using CELLAID® kits; the serum was cryopreserved at –80 °C. The levels of growth factors, including VEGF, PDGF-BB and TGF-β, in PAS, PRP and autologous serum samples obtained from two Japanese White rabbits (aged 12 weeks; weighing ~2.5 kg) were analysed using commercial enzyme-linked immunosorbent assay (ELISA) kits (Quantikine®; R&D Systems, Minneapolis, MN, USA). We collected PRP and autologous serum from two Japanese White rabbits (aged 12 weeks; weighing ~2.5 kg) using the following methods.

For PRP, venous blood samples were taken with anticoagulant-coated tubes to avoid platelet activation and degranulation. Samples were centrifuged at 1000 rpm for 5 min. At the bottom of the tube, the packed erythrocytes typically constitute 55% of the total volume. At the top of the tube, platelet-poor plasma (PPP) constitutes 40% of the volume. An intermediate layer of 5% is called the buffy coat. We aspirated the PPP and buffy coat layers using a sterile syringe. This was centrifuged at 1000 rpm for 5 min, and we aspirated the upper layer to collect the PRP.

For autologous serum, venous blood samples were taken with no anticoagulant. The tubes were shaken five times, kept for at least 30 min at room temperature, and then the upper layers were aspirated.

2.3. Histological evaluation of cartilage repair

We investigated the effect of PAS on articular cartilage repair in 24 Japanese White rabbits aged 12 weeks, subjected to ACL-T in the right knees with the animals under general anaesthesia. Based on the observations of Nagai et al. (2010, 2014), an Avastin intra-articular injection (25 mg/ml) group was included. Six rabbits (six knees) were each assigned to intra-articular injections of 1 ml phosphate-buffered saline (PBS) alone as a control (C) group, a PAS (S; 0.5 ml PAS + 0.5 ml PBS) group, an Avastin (A; 0.5 ml Avastin + 0.5 ml PBS) group and a PAS + Avastin (S + A; 0.5 ml PAS + 0.5 ml Avastin) group (n = 24 knees in all). The knees were injected with 1 ml of the treatment solutions in PBS weekly, from weeks 1–7 after ACL-T, and the weight distribution ratios of the damaged knees were measured (see below). The animals were killed 12 weeks after surgery by an intravenous overdose of anaesthetic. Medial and lateral tissues from the femoral and tibial ends of the right knees were collected and fixed and processed for histology, and subjected to haematoxylin and eosin and Safranin O staining. Immunohistochemistry was performed as described (Nagai et al., 2008). Briefly, sections were deparaffinized according to standard procedures. The sections were treated with 0.005% proteinase (type XXIV; Sigma-Aldrich, St Louis, MO, USA) for 30 min at 37 °C for antigen retrieval. For types I and II collagen, a primary mouse monoclonal antibody (Daiichi Fine Chemical, Toyama, Japan) diluted 1:200 in PBS + 1% bovine serum albumin (Sigma-Aldrich; final concentration 2.5 mg/ml) was placed on the section overnight at 4 °C. Blinded histological evaluations of the prepared tissue samples were conducted independently by three researchers using the histological score of Mankin et al. (1971; Mankin score).

2.4. Pain evaluation

The weight distribution ratios of the damaged limbs were measured using an incapacitance test meter (Linton Instrumentation, Norfolk, UK) during weeks 1–7 after surgery, and pain in the damaged limbs was evaluated using the following formula: damaged limb weight distribution ratio (%) = [weight exerted by the damaged limb/(that of the damaged limb + that of a healthy limb)] × 100. The incapacitance meter is a device that facilitates automatic and reproducible pain evaluation by measuring the weight distribution of both hind limbs using a dual channel weight-averaging technique. It is widely used to investigate pain-ameliorating effects (Ito et al., 2012).

2.5. Statistical analysis

Comparisons between groups were made using analysis of variance (ANOVA). Scheffe's post hoc test was applied, and $P < 0.05$ was assumed to be statistically significant.

3. Results

3.1. Quantitative analysis of growth factors

Analyses of the levels of VEGF, PDGF-BB and TGF-β expressed in PAS, PRP and autologous serum obtained from two rabbits measured by ELISA indicated significant increases in VEGF levels in PAS (117.5 pg/ml) and PRP (90.3 pg/ml) compared with autologous serum (72.5 pg/ml; Figure 2a). PDGF-BB and TGF-β levels were also significantly higher in PAS than in PRP and in the autologous serum (Figure 2b and c).

3.2. Pain evaluation

As shown in Figure 3a, the weight distribution ratios of damaged limbs showed a significant improvement in the
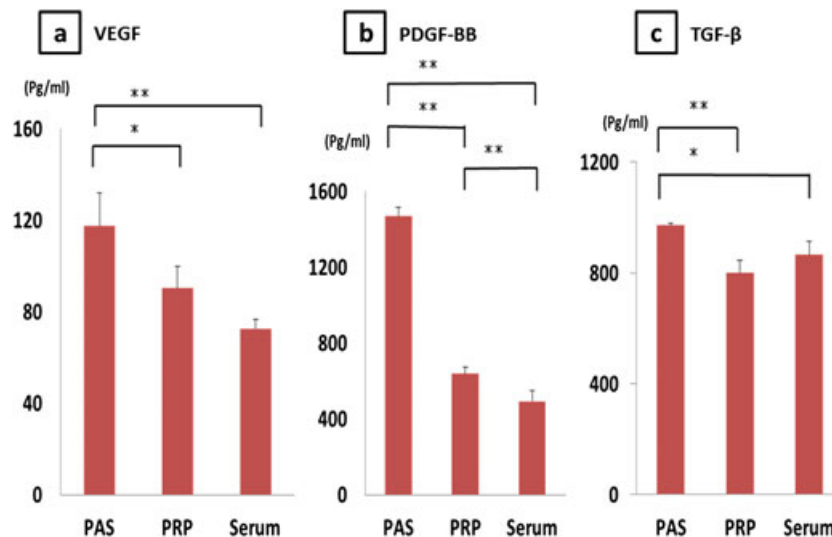
Figure 2. Quantitative analysis of growth factors. Platelet-activated serum (PAS), platelet-rich plasma (PRP) and autologous serum samples were collected from two Japanese White rabbits aged 12 weeks (weight ~2.5 kg). They were analysed for vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF)-BB and transforming growth factor-beta (TGF-β) expression levels using ELISA kits. (a) Significantly higher quantities of VEGF were observed in PAS and PRP than in the autologous serum samples. (b) PDGF-BB expression was significantly higher in PAS than in PRP and in the autologous serum samples, and the PDGF-BB level in PRP was significantly higher than that in autologous serum. (c) A higher TGF-β concentration was observed in PAS than in PRP and autologous serum samples. The vertical axis is in pg/ml; *$P < 0.05$ and **$P < 0.01$ [Colour figure can be viewed at wileyonlinelibrary.com]

three treatment groups from 5 weeks after ACL-T. A comparison of the weight distribution ratios of the damaged limbs in the treatment groups at 8 weeks after surgery showed significant improvements (46.3%, 49.5% and 48.0% in the S, A and S + A groups, respectively) compared with the C group (39.3%; $P < 0.05$). However, no significant differences were observed among the three treatment groups (Figure 3b).

3.3. Histological evaluation of repair tissue

Macroscopic examination of the tissue revealed redness on the surface of the articular cartilage on both the

proximal and distal aspects in the C group at 12 weeks after surgery. A gross examination of the damaged knee joints in the C group did not reveal any spur formation or cartilage loss, while there was a tendency for swelling and elevated temperature at the damaged sites. The articular cartilage histology findings (Safranin O, and Col-1 and Col-2 immunostaining) 12 weeks after surgery are shown in Figure 4a–d for the C, S, A and S + A groups, respectively. The upper row in Figure 4 shows the articular cartilage histology of damaged knees demonstrated by Safranin O staining 12 weeks after surgery. The surface layers of the joints in the C group showed irregularities, and the articular cartilage exhibited weak staining (Figure 4a). However, strong staining was observed in
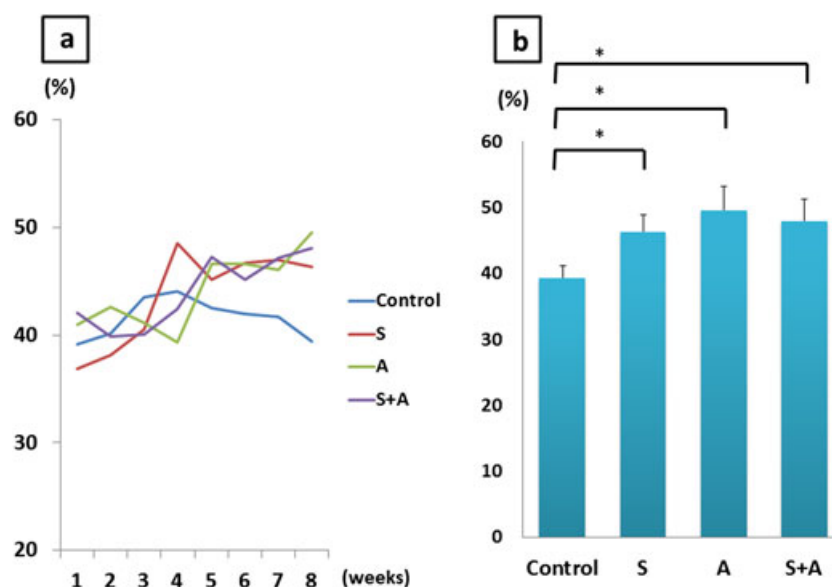

Figure 3. Pain evaluation using damaged limb weight distribution ratios (%). (a) The damaged limb weight distribution ratios improved 5 weeks after anterior cruciate ligament transection (ACL-T) surgery in the three treatment groups (S, A and S + A). (b) The damaged limb weight distribution ratios improved significantly in the treatment groups 8 weeks after surgery, compared with the control (C) group (*$P < 0.05$) [Colour figure can be viewed at wileyonlinelibrary.com]
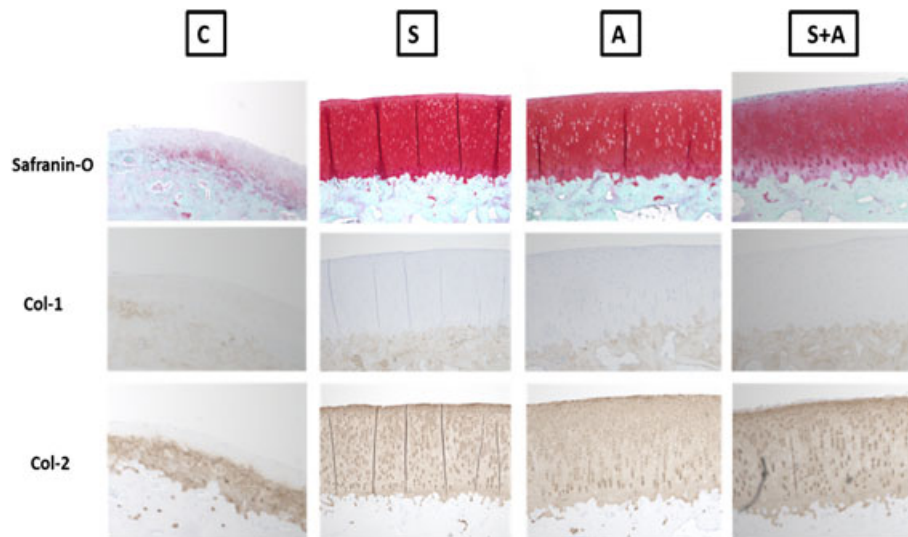
Figure 4. Histological findings of the developed repair tissue 12 weeks after surgery. C group: Safranin O staining, and Col-1 and Col-2 immunostaining of samples from the control group 12 weeks after surgery are shown. The C group samples exhibited articular surface layer irregularities and weak Safranin O staining of the articular cartilage. Both Col-1 and Col-2 expression levels were low at sites of weak Safranin O staining. S, A S + A groups: Safranin O staining, Col-1 and Col-2 immunostaining of samples from the S, A and S + A groups 12 weeks after surgery are shown. All three groups exhibited strong Safranin O staining of the articular cartilage. Col-2 was strongly expressed at sites of strong Safranin O staining, while Col-1 was only weakly expressed. The histological findings of the treatment groups did not differ significantly from those of the control group [Colour figure can be viewed at wileyonlinelibrary.com]

the treatment groups (Figure 4b–d). This suggested that the articular cartilage had degenerated in the C group.

3.4. Immunohistochemistry

The features of damaged knee tissues of the C, S, A and S + A groups, observed 12 weeks after surgery by immunohistochemical staining for Col-1 and Col-2 are shown in Figure 4a–d. Type II collagen (Col-2) expression suggests the repair of damaged articular cartilage by hyaline cartilage, while type I collagen expression (Col-1) suggests repair by subchondral bone fibrocartilage. We observed a strong type II collagen expression in the treatment groups 12 weeks after surgery. However, type I collagen was rarely observed in these sites, despite the strong Safranin O staining. Types I and II collagen were scarcely expressed in group C, accompanied by weak Safranin O staining. This suggests that the articular surface had been repaired effectively with hyaline cartilage in the treatment groups.

3.5. Histological scoring of repair tissue

The articular cartilage in four sites (proximal medial, proximal lateral, distal medial and distal lateral) was evaluated histologically 12 weeks after surgery using Mankin scores (Table 2). Mankin scores are shown as the average proximal medial, distal medial and distal lateral scores, respectively. These were 7.2, 6.2 and 8.0 in the C group; 4.0, 3.2 and 3.7 in the S group; 2.7 for each site in the A group; and 3.6, 2.2 and 3.2 in the S + A group (Figure 5 a, c and d). These scores indicated significant differences between group C and the treatment groups (*$P < 0.05$, **$P < 0.01$ in Figure 5). The scores did not differ significantly among the treatment groups. The average proximal lateral Mankin scores did not differ significantly among

Table 2. Mankin scores

I. Structur
    0: Normal
    1: Surface irregularities
    2: Pannus + irregularities
    3: Clefts to transitional
    4: Clefts to radial
    5: Clefts to calcified
    6: Disorganization

II. Cells
    0: Normal
    1: Diffuse hypercellularity
    2: Cloning
    3: Hypocellularity

III. Safranin-O staining
    0: Normal
    1: Slight reduction
    2: Moderate reduction
    3: Severe reduction
    4: No staining

IV. Tidemark
    0: Normal
    1: Vascularization

Mankin scores were used to evaluate cartilage repair histologically. The total potential scores ranged from 0 to 14, with 0 indicating normal, 1–3 indicating mild degeneration, 4–7 indicating moderate degeneration, and ≥ 7 indicating severe degeneration.

the groups: C, 4.5; S, 3.08; A, 3.2; and S + A, 2.8 (Figure 5b).

4. Discussion

The early stages of OA are characterized by destruction of hyaline cartilage and its extracellular matrix caused by release of the inflammatory cytokine interleukin (IL)-1 (Goldring and Goldring, 2004; Rutgers *et al.*, 2010; Smith *et al.*, 1997). The production of intracellular inflammatory substances promotes the breakdown of hyaline cartilage matrix (Fukui *et al.*, 2001; Goldring, 2000). There are
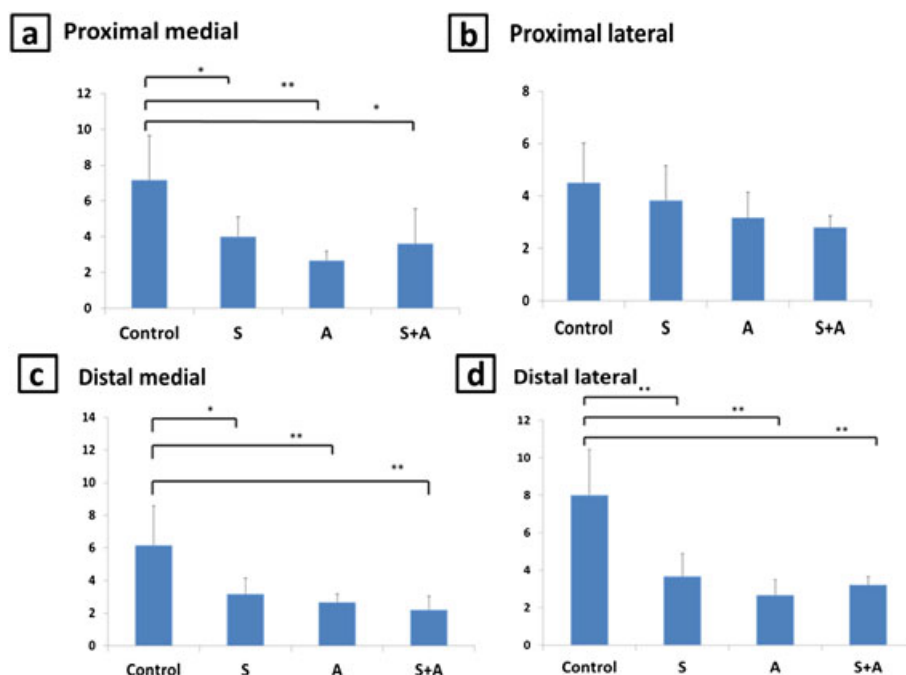
Figure 5. Mankin scores 12 weeks after surgery. The articular cartilage repair at each site was evaluated 12 weeks after surgery using the Mankin scoring system. (a) The proximal medial scores differed significantly between the C and the S, A and S + A groups. The treatment group scores did not differ significantly between each other. (b) The proximal lateral scores did not differ significantly between the C, S, A and S + A groups. (c) The distal medial scores differed significantly between group C and the S, A and S + A groups; however, there were no significant differences among the three treatment groups. (d) The distal lateral scores also differed significantly between group C and the treatment groups, but there were no significant differences among the latter. *$P < 0.05$ and **$P < 0.01$ [Colour figure can be viewed at wileyonlinelibrary.com]

new treatment options for patients with OA using components from their own blood, which facilitate histological repair (Arend *et al.*, 1994; Dinarello and Thompson, 1991; Granowitz *et al.*, 1991; Polisson, 2001). One of these is PRP, a plasma layer containing a large amount of platelets, obtained by the centrifugal separation of blood. The alpha granules within blood platelets contain growth factors, such as PDGF, TGF-β1, fibroblast growth factor and VEGF. High concentrations of these growth factors are present in PRP (Kon *et al.*, 2010). The injection of PRP into a joint results in the binding of a high concentration of growth factors to cell receptors that might help promote repair. Filardo *et al.* (2011) observed an early improvement in a group of patients with OA following the intra-articular injection of PRP.

Currently, serum (specifically animal-derived serum) is frequently included in the culture media used in regenerative medicine to promote stem cell growth. CELLAID® kits allow the collection of serum containing high concentrations of growth factors, such as TGF-β and PDGF-BB, to be separated from blood components via the shaking of platelet-activating beads, and collected in a closed and sterile environment. They also enable the collected samples to be cryopreserved at –80 °C for a long time without any loss in cell growth factor concentrations even after 1 year of storage (data not shown). The quantitative analysis of growth factors expressed in PAS collected using CELLAID® kits showed high concentrations of VEGF, PDGF-BB and TGF-β, compared with PRP or autologous serum (Figure 2). However, tissue degeneration, accompanied by conditions such as ectopic ossification, fibrotic degeneration and synovial hyperplasia, has been associated with angiogenic factors such as VEGF, which are

present in large quantities in platelets (Alini *et al.*, 1996; Aoyama *et al.*, 2004; Bluteau *et al.*, 2007; Ludin *et al.*, 2013). Moreover, high levels of VEGF have been reported in chondrocytes from articular cartilage in rabbits with OA (Jansen *et al.*, 2012). A previous report (Emami *et al.*, 2012) also suggested that VEGF might inhibit cartilage repair, thereby contributing to the exacerbation of OA. However, Nagai *et al.* (2010) have reported that an intravenous injection of Avastin induced effective repair of damaged articular cartilage. In another study comparing intravenous with intra-articular Avastin injections in rabbits subjected to ACL-T, Nagai *et al.* (2014) reported that intra-articular injections at approximately half the dosage used for intravenous injections suppressed ectopic ossification, fibrotic degeneration, synovial hyperplasia and pain. Emami *et al.* (2012) also reported that an intra-articular injection of Avastin in a rabbit model effectively reduced fibrotic degeneration.

We anticipated that there would be an increase in the VEGF levels in the PAS used here as a result of platelet activation, and an inhibition in cartilage histological repair caused by increased exogenous VEGF expression resulting from intra-articular PAS injections. Therefore, we added a group of rabbits including both Avastin and PAS treatments (S + A). Kon *et al.* (2010) and Wehling *et al.* (2007) have reported that PDGFs such as TGF-β and PDGF-BB serve as cell growth factors that can effectively induce articular cartilage repair. Our comparison of autologous serum and PRP with PAS revealed that the PAS contained high concentrations of TGF-β and PDGF-BB. This suggested that intra-articular injections of PAS might facilitate histological repair. Therefore, the S + A group was expected to exhibit significant repair compared with the control and other

treatment groups, because of the effect of high densities of growth factors and the anti-VEGF effect of Avastin. A comprehensive comparison of the pain scores, histological findings and histological scores of group C with the treatment groups 12 weeks after surgery indicated that there had been cartilage repair in the treatment groups. However, the S + A group did not exhibit significant repair compared with the other treatment groups. This was possibly because a single dose of Avastin exerts only a limited inhibitory effect on VEGF. This suggests that repeated Avastin doses administered over 1–2 weeks might provide optimal effects (Chung et al., 2011; Emami et al., 2012). We believe that the doses and numbers of Avastin injections might not have been sufficient to inhibit the increased levels of exogenous VEGF produced by platelet activation. Nagai et al. (2014) reported that elevated levels of matrix metalloproteinase (MMP)-3, MMP-13 and IL-1β detected 4 weeks after surgery were indicative of inflammation in synovial tissues of models of early OA; however, they did not observe a significant increase in VEGF levels.

In the present study, weekly intra-articular injections of PAS and/or Avastin were administered at 1–7 weeks after surgery, and the treatment outcome was assessed 12 weeks after surgery. VEGF levels in the synovial tissue begin to rise 4 weeks after surgery in conjunction with progression of OA, so a significant treatment outcome should have been exhibited in the S + A group by 12 weeks after surgery or by extending the period of PAS and Avastin administration. However, increasing the Avastin dosage and number of injections could lead to adverse drug reactions, such as haemorrhage, thrombosis, proteinuria, delayed wound healing and hypertension (Hurwitz et al., 2004). The use of an intra-articular injection, as in the present study, is likely to minimize the risk of such adverse drug reactions (Nagai et al., 2014). Our results suggest that the intra-articular injection of PAS at the 12-week point (i.e. short-term progression of OA) facilitated articular cartilage repair.

Platelet-rich plasma has also been the subject of hundreds of publications in recent years. Protocols for PRP preparation vary widely between authors and are often not well documented in the literature. A PRP classification system is needed to more accurately compare protocols. DeLong et al. (2012) suggested the 'PAW' classification system. The system is based on three components: (1) the absolute number of platelets; (2) the manner in which platelet activation occurs; and (3) the presence or absence of leucocytes. In this study, we collected PAS using CELLAID® kits. CELLAID® kits allow the separation of serum containing a high concentration of cell growth factors, but the transfer to storage container is performed with only eyeballing as a measuring tool. As a result, the CELLAID® system cannot exclude the buffy coat layer, which contains remained platelets and leucocytes. Because manual processing is not a clearly defined protocol, it might randomly lead to leucocyte-poor or pure PRP or leucocyte-rich plasma and PRP (Ehrenfest et al., 2009). The effect of highly concentrated leucocytes contained within PRP preparations has been debated.

Sundman et al. (2011) suggested that leucocytes contain and produce biologically active cytokines that are primarily catabolic or inflammatory and might influence the clinical outcome of PRP application. Delivery of concentrated leucocytes to a site of injury might not provide a favourable environment for tissue repair or healing.

Platelets contain factors leading to increased anabolic signalling; in contrast, leucocytes release catabolic signalling molecules. The optimal balance between anabolism and catabolism for tissue repair and the concentration of growth factors and cytokines required to maintain this balance remains unclear. Depending on the clinical application, preparations of PRP should be considered based on their ability to concentrate platelets and leucocytes with sensitivity to pathological conditions that will benefit most from increased platelets or reduced leucocyte concentrations. In addition, Sundman et al. (2014) suggested that PRP treatment also resulted in a significant reduction of MMP-13, an increase in HA synthase-2 expression in synoviocytes, and an increase in cartilage synthetic activity compared with HA. They also showed that PRP stimulated endogenous HA production and decreased cartilage catabolism; in addition, PRP showed similar effects to HA in the suppression of inflammatory mediator concentrations and expression of their genes in synoviocytes and cartilage. Boswell et al. (2013) suggested that increasing the platelet concentration within leucocyte-reduced PRP preparations would result in the delivery of more anabolic growth factors and less proinflammatory cytokines. Minimizing leucocyte numbers in PRP is more important than maximizing platelet numbers with respect to decreasing the expression of proinflammatory molecules and enhancing the expression of matrix molecules. As described above, it can be assumed that leucocytes contained in PAS might have inhibited cartilage repair and influenced our results. In the preparation of PAS using CELLAID® kits, it would be desirable to reduce the concentration of leucocytes.

In this study, the groups treated with PAS alone and Avastin alone both showed effective cartilage repair compared with the control group. Although some of the limitations of our study include deficiencies in selecting an optimal dosage, in the number of injections and in the duration of administration of Avastin, the combined administration of PAS and Avastin in the S + A group did not show a therapeutic effect on damaged articular cartilage by synergistic effects. In addition, the mechanism of action of PAS remains unknown. Although these findings must be verified comprehensively in further studies, the administration of PAS is expected to facilitate effective cartilage repair in human patients with OA.

5. Conclusion

Intra-articular injections of PAS showed therapeutic effects on cartilage histological repair and pain relief in a

Japanese White rabbit model of OA produced by ACL-T. However, no synergistic effects of Avastin with PAS were found.

Acknowledgements

Conflict of interest

We declare no conflicts of interest.

6. Author disclosure statement

MS received research funding from CellSeed Inc (Tokyo, Japan).

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