J Clin Periodontol 2005; 32: 966–972 doi: 10.1111/j.1600-051X.2005.00772.x

Journal of Clinical Periodontology

Analysis of rat calvaria defects implanted with a platelet-rich plasma preparation: histologic and histometric observations

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Pryor ME, Polimeni G, Koo K-T, Hartman MJ, Gross H, April M, Safadi FF, Wikesjö U. Analysis of rat calvaria defects implanted with a platelet-rich plasma preparation: histologic and histometric observations. J Clin Periodontol 2005; 32: 966–972. doi: 10.1111/j.1600-051X.2005.00772.x. © Blackwell Munksgaard, 2005.

Abstract

Objectives: It has been suggested that degranulating platelet α-granules release growth factors having a potential to modulate bone formation. The objective of this study was to evaluate the osteoconductive potential of a platelet-rich plasma (PRP) preparation.

Methods: Thirty adult male Sprague–Dawley rats were used. The PRP preparation was obtained from 10 ml of whole blood drawn from one age-matched donor rat. The preparation was processed by gradient density centrifugation and stored at $-80^{\circ}C$ until use. Using aseptic techniques, the PRP preparation soak loaded onto an absorbable collagen sponge (ACS) or ACS alone was surgically implanted into contralateral critical size 6-mm calvaria osteotomies in 18 animals. Twelve animals received ACS *versus* sham surgery in contralateral defects. Animals were sacrificed at 4 and 8 weeks when biopsies were collected for histologic and histometric analysis.

Results: The animals were maintained without adverse events. Bone formation was highly variable in sites receiving PRP and control treatments. Defect bone fill at 4 weeks averaged (± SD) 28.8 ± 27.4% (PRP/ACS) *versus* 39.1 ± 24.4% (ACS; $p = 0.2626$) and 62.0 ± 20.0% (ACS) *versus* 71.6 ± 32.2% (sham surgery; $p = 0.1088$), and at 8 weeks 81.0 ± 12.9% (PRP/ACS) *versus* 64.5 ± 28.1% (ACS; $p = 0.2626$) and 75.6 ± 34.1% (ACS) *versus* 74.1 ± 24.2% (sham surgery; $p = 0.7353$). Remnants of the ACS biomaterial were observed at both 4 and 8 weeks in sites implanted with PRP/ACS or ACS.

Conclusions: The results suggest that the PRP preparation has a limited potential to promote local bone formation.

Key words: bone; growth factors; osteogenesis; platelet-rich plasma; tissue engineering

Accepted for publication 9 February 2005

A number of growth factors are sequestered in platelets including platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), and insulin-like growth factor-1 (IGF-1) (Schliephake 2002). PDGF may affect wound healing including bone as it is synthesized and secreted by platelets, macrophages, and endothelial cells. While PDGF secreted from platelets plays an important role in initial wound healing, its subsequent secretion from macrophages continues the events of wound healing through up-regulation of other growth factors and cells that ultimately promote fibroblastic and osteoblastic functions (Ross et al. 1986, Heldin & Westermark 1999). Nevertheless, a role for PDGF in local bone formation is not uncontroversial. PDGF has been suggested to antagonize osteoinductive proteins such as osteogenin, and osteoconductive surfaces such as hydroxyapatite (Goodkin & Pierce 1993). TGF-β is found in high concentrations in platelets and bone (Sporn & Roberts 1989, Slater et al. 1995). When released from degranulating platelets or secreted by macrophages, TGF-β exerts chemotactic and mitogenic effects on fibroblasts, marrow stem cells, endothelial cells, epithelial cells, and pre-osteoblastic cells (Rudkin & Miller 1996). TGF-β participates in the inhibition of osteoclastic activity by stimulating chemotactic migration of

osteoblasts to the site of injury (Sporn & Roberts 1989). The release of endogenous TGF-β_1 is not critical, however, for initiation and progression of tissue repair. In fact, endogenous TGF-β_1 may increase inflammation and retard wound closure (Koch et al. 2000). Finally, IGF-1 secreted from platelets stimulates proliferation of osteoprogenitor cells to increase the number of cells capable of synthesizing bone matrix (Lind 1996). Although IGF-1 demonstrates mitogenic activity for osteoprogenitor cells, its mitogenic effects appear to be less pronounced than that of TGF-β and PDGF (Canalis et al. 1989).

Since physiologic concentrations of growth factors may not be sufficient to stimulate local bone formation, the use of exogenous growth factors to supplement endogenous biological mediators has been explored. Platelet-rich plasma (PRP) is a volume of autologous plasma that contains a platelet concentration above baseline values (Marx 2001). The preparation of PRP involves the sequestration of platelets in plasma by gradient density centrifugation with the intent to enhance the concentration of growth factors (Whitman et al. 1997, Marx et al. 1998, Anitua 1999). It has been reported that PRP preparations may increase the concentrations of platelets up to 338% (Marx et al. 1998). The genuine effect of PRP on osteogenesis is, however, controversial. Several studies using histologic techniques suggest that PRP preparations may enhance local bone formation (Marx et al. 1998, Kim et al. 2002, Fennis et al. 2004), while others have not been able to confirm these findings (Aghaloo et al. 2002, Fürst et al. 2003b, 2004, Jakse et al. 2003). Generally, PRP preparations have been combined with osteoconductive biomaterials potentially obscuring a genuine osteoconductive effect of PRP. In contrast, type I collagen biomaterials are considered appropriate carriers for the evaluation of growth factors because of rheologic properties, biocompatibility, and their resorbable nature (McPherson 1992). The utility of collagen-based vehicles for the delivery of growth factors found in a platelet releasate preparation in wound-healing sites has been shown to provide a sustained biologic effect when compared with a carrier-free platelet releasate preparation (McPherson 1992). The objective of this study was to evaluate the osteoconductive potential of a PRP preparation using an absorbable collagen sponge carrier without osteogenic/conductive or inductive effects using a critical-size rat calvarial defect model.

Materials and Methods

Animals

Thirty 22-week-old male Sprague–Dawley rats, weight approximately 525 g, were used following a protocol approved by the Institutional Animal Care and Use Committee, Temple University, Philadelphia, PA, USA. The animals were individually housed in plastic cages in a monitored environment (21°C; 12:12 light cycle). They had ad libitum access to drinking water and a standard laboratory rat food pellet diet. The animals were monitored for signs of infection and discomfort pre- and post-surgery until their date of euthanasia.

PRP preparation

The PRP preparation was obtained from one age-matched Sprague–Dawley rat. The animal was anaesthetized using isoflurane inhalation anaesthesia (E-Z Anesthesia, Euthanex Corp., Palmer, PA, USA; 4–5% induction; 2–3% maintenance). An aorta heart puncture with complete exsanguination was performed using 5-ml vacutainer tubes containing 0.84 ml acid-citrate-dextrose (ACD) and 10 mM EDTA. A total of 10 ml of whole blood was obtained. The blood was transferred to a centrifuge tube and spun at 450 × *g* for 30 min. The PRP was removed and placed into a new tube. An equal volume of Tyrode's buffer (137 mM NaCl, 2.7 mM KCl, 2 mM MgCl, 0.5 mM NaH_2PO_4, 5 mM glucose, 10 mM HEPES, 0.2% BSA; pH 7.4) was added to the PRP. The diluted PRP was centrifuged at 1400 × *g* for 15 min. to pellet the platelets. The platelet pellet was resuspended in 2-ml Tyrode's buffer. Platelet counts were determined from this preparation using a Coulter counter. Ten microlitres of 20% Triton X-100 (F.C. 0.1%) was added to lyse the cells. As soon as the solution cleared, the lysate (PRP preparation) was placed into 1.5-ml microcentrifuge tubes and spun at 16,000 × *g* for 15 min. to pellet cell debris. All procedures were performed at room temperature. The cell lysate was aliquoted and stored at −80°C.

Experimental surgery

Anaesthesia and pain control followed recommended routines for the species. The animals were anaesthetized using isoflurane inhalation anaesthesia (E-Z Anesthesia, Euthanex Corp.; 4–5% induction; 2–3% maintenance). Buprenorphine HCl, 0.02–0.03 mg/kg, was administered pre-surgically. Prior to surgery, the animal's head was shaved, washed with a disinfectant, and stabilized by a nose cone apparatus (Euthanex Corp.). A midline incision was made from the nasofrontal area to the external occipital protuberance along the mid-sagittal suture. The skin and underlying tissues including the temporal muscle were reflected bilaterally to expose the full extent of the calvaria (Fig. 1).

In each animal, one calvarial through-and-through osteotomy, 6.0 mm in diameter, was trephined into the dorsal portion of the parietal bone on each side of the mid-sagittal suture using a dental hand piece and a trephine bur (#11-31-0050; Ace Surgical Supply Co. Inc., Brockton, MA, USA) under constant irrigation of sterile saline. The trephined bone was removed from the surgical field. Using aseptic techniques, 42 µl of the PRP preparation was used to soak load a precut (∅ 6 × 1.5 mm) absorbable collagen sponge (ACS; CollaCote®, Sulzer Dental Inc., Carlsbad,

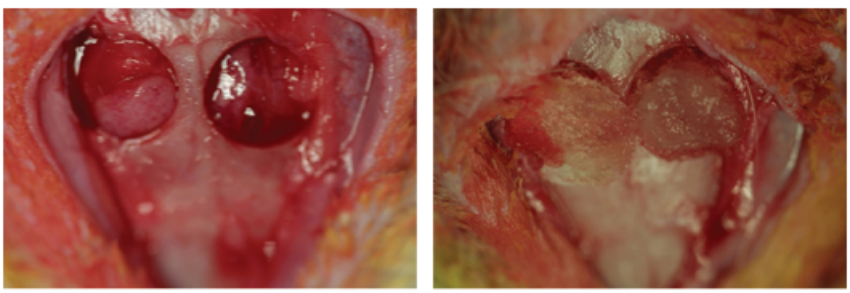

Fig. 1. Bilateral craniotomy defects before (left) and after implantation of platelet-rich plasma/absorbable collagen sponge (ACS) and ACS.

CA, USA). The PRP/ACS construct or ACS alone was implanted into contralateral calvaria osteotomy defects in 18 animals. Twelve animals received ACS without the PRP preparation *versus* sham surgery in contralateral calvaria osteotomy defects. The margins of the wound were closed using autoclips (Autoclip® Wound Closing System, Stoelting Co., Wood Dale, IL, USA). A bacitracin–neomycin–polymyxin ointment (Vetropolycin® Ophthalmic Ointment, Pharmaderm, Melville, NY, USA) was applied to the eyes and the animal was monitored until anaesthesia recovery. Animals were sacrificed at 4 and 8 weeks post-surgery by CO_2 inhalation. The cranial bone including the bilateral defects was removed in total, rinsed in water, and placed into 10% buffered formalin.

The animals were randomized to receive PRP/ACS *versus* ACS (10 animals; observation interval 8 weeks); PRP/ACS *versus* ACS (eight animals; observation interval 4 weeks); ACS *versus* sham surgery (seven animals; observation 8 weeks); and ACS *versus* sham surgery (five animals; observation 4 weeks).

Histotechnical preparation

Calvarial specimens were fixed in 10% buffered formalin for 3–5 days, and decalcified in formic acid and sodium citrate for 24 h. The specimens were washed with tap water, dehydrated with ascending concentrations of ethyl alcohol, cleared in xylene, and infiltrated with paraffin. Serial sections (7 μm) parallel to the mid-sagittal suture were cut from the centre of each defect using a microtome (RM2155, Leica Microsystems GmbH, Nussloch, Germany) and stained with haematoxylin and eosin.

Histologic and histometric evaluation

The most central portion of each defect was identified and subject to histologic and histometric analysis. On the average three central sections were used for the histologic evaluation, representing approximately 80 μm of the central aspect of the osteotomy defect. The sections were viewed and analysed for new bone formation, residual ACS, residual clot elements, soft tissue elements, and inflammatory reactions by one calibrated examiner (MEP) using incandescent and polarized light microscopy (BX 60, Olympus, America Inc., Melville, NY, USA). The following linear measurements were recorded for the most central section for each defect using a microscope digital camera system (DP10, Olympus, America Inc.) and a PC-based image analysis system (Image-Pro Plus™, Media Cybernetic, Silver Springs, MD, USA):

- Defect width: the distance between the margins of the original bone defect.
- Bone fill: the length of newly formed bone along an axis bridging the gap between the defect margins.

Percentage bone fill was calculated based on defect width and bone fill measurements. Examiner reproducibility for the histometric analysis was evaluated by calculating the correlation coefficient for repeated measurements ($r = 1.0$; $p < 0.0001$). A defect was considered exhibiting *limited bone fill* when $\leqslant 25\%$ of the defect width was filled with bone; *partial bone fill* when $> 25\%$, $\leqslant 90\%$ of the defect width was filled with bone; and *complete bone fill* when $> 90\%$ of the defect width was filled with bone.

Statistical analysis

Summary statistics (means $\pm$ SD) were calculated based on individual animal measurements for the various groups. The Wilcoxon sign-rank test was used to evaluate differences between protocols within the experimental groups. The level of significance was set at 5%.

Results

The animals were maintained without adverse events. A platelet count of 1.76×10^9 cells/ml was obtained from the PRP preparation. Two specimens were damaged in the histological processing for the PRP/ACS group, and two ACS and one sham-surgery specimens for animals receiving ACS *versus* sham surgery, and were thus not included in the analysis.

Histologic observations

The histologic observations for sites implanted with PRP/ACS *versus* ACS and ACS *versus* sham surgery at 4 weeks post-surgery are described in Figs 2–5. The defect sites exhibited variable healing within and between treatments, ranging from limited bone formation to complete defect closure

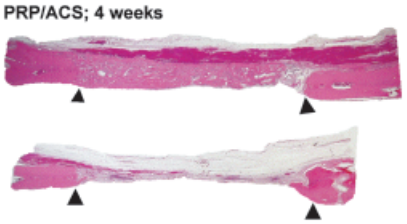

Fig. 2. Photomicrographs of critical size 6-mm rat craniotomy defects following implantation of platelet-rich plasma (PRP)/absorbable collagen sponge (ACS) in animals receiving PRP/ACS *versus* ACS in contralateral defects, 4 weeks post-surgery. The top photomicrograph shows complete bone fill. The lower photomicrograph shows limited bone fill. Residual elements of the maturing fibrin clot including RBCs and granulation tissue residing in fibrous connective tissue bridge the defect. Arrowheads outline the borders of the original defect (haematoxylin and eosin).

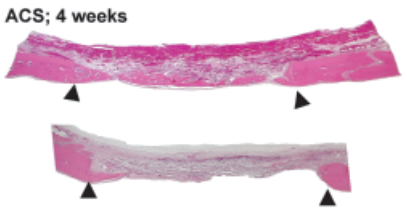

Fig. 3. Photomicrographs of critical size 6-mm rat craniotomy defects following implantation of absorbable collagen sponge (ACS) in animals receiving platelet-rich plasma/ACS *versus* ACS in contralateral defects, 4 weeks post-surgery. The top photomicrograph shows complete bone fill. ACS residing in fibrous connective tissue bridges the defect. The lower photomicrograph shows limited bone fill. Loose granulation tissue, residual clot elements, and ACS residing in fibrous connective tissue bridge the defect. Arrowheads outline the borders of the original defect (haematoxylin and eosin).

(Tables 1 and 2). Remnants of the ACS carrier were observed in defects receiving this treatment. There were no remarkable differences between experimental and control sites at 8 weeks, although bone formation was more advanced at this observation interval.

Histometric analysis

The results of the histometric analysis for animals receiving PRP/ACS *versus* ACS are shown in Tables 3 and 4. At 4 weeks post-surgery, the mean defect width, bone fill, and percentage bone fill for sites receiving PRP/ACS amounted to 4.4 ± 0.2 and 1.3 ± 1.3 mm, and

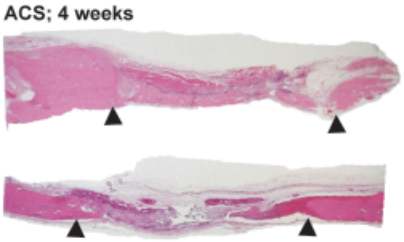

Fig. 4. Photomicrographs of critical size 6-mm rat craniotomy defects following implantation of absorbable collagen sponge (ACS) in animals receiving ACS *versus* sham surgery in contralateral defects, 4 weeks post-surgery. The top photomicrograph shows partial bone fill. Islands of bone are present at the right defect border. An inflammatory infiltrate is present in fibrous connective tissue bridging the defect. The lower photomicrograph also shows partial bone fill. Residual fibrin clot elements including RBCs and ACS in fibrous connective tissue bridge the defect. Arrowheads outline the borders of the original defect (haematoxylin and eosin).

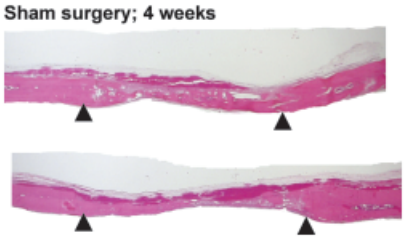

Fig. 5. Photomicrographs of critical size 6-mm rat craniotomy defects following sham surgery in animals receiving absorbable collagen sponge (ACS) *versus* sham surgery in contralateral defects, 4 weeks post-surgery. The top photomicrograph shows complete bone fill. The lower photomicrograph shows partial bone fill. Residual elements of a maturing fibrin clot including RBCs and ACS residing in fibrous connective tissue bridging the defect. Islands of bone formation are present at the right defect border. Arrowheads outline the borders of the original defect (haematoxylin and eosin).

28.8 ± 27.4%, respectively. The corresponding values for sites receiving ACS were 4.4 ± 0.7 and 1.7 ± 1.1 mm, and 39.1 ± 24.4%. At 8 weeks post-surgery, the mean defect width, bone fill, and percentage bone fill for sites receiving PRP/ACS amounted to 4.3 ± 0.4 and 3.5 ± 0.8 mm, and 81.0 ± 12.9%, respectively. The corresponding values for sites receiving ACS were 4.3 ± 0.4 and 2.8 ± 1.3 mm, and 64.5 ± 28.1%. There were no statistically significant differences in defect bone fill at 4 or 8 weeks post-surgery.

Table 1. Histologic observations of bone fill in contralateral craniotomy defects receiving PRP/ACS *versus* ACS. Healing at 4 and 8 weeks

	PRP/ACS (weeks)		ACS (weeks)	
	4	8	4	8
Limited bone fill	5	0	3	1
Partial bone fill	2	5	4	7
Complete bone fill	1	3	1	2
N	8	8	8	10

PRP, platelet-rich plasma; ACS, absorbable collagen sponge.

Table 2. Histologic observations of bone fill in contralateral craniotomy defects receiving ACS *versus* sham surgery. Healing at 4 and 8 weeks

	ACS (weeks)		Sham surgery (weeks)	
	4	8	4	8
Limited bone fill	0	1	0	0
Partial bone fill	3	2	2	4
Complete bone fill	0	4	2	3
N	3	7	4	7

ACS, absorbable collagen sponge.

Table 3. Histometric evaluation of bone fill in contralateral craniotomy defects receiving PRP/ACS *versus* ACS. Healing at 4 weeks (means ± SD)

	Defect width (mm)	Bone fill (mm)	Bone fill (%)
PRP/ACS	4.4 ± 0.2	1.3 ± 1.3	28.8 ± 27.4
ACS	4.4 ± 0.7	1.7 ± 1.1	39.1 ± 24.4
p-value	0.7794	0.4008	0.2626

PRP, platelet-rich plasma; ACS, absorbable collagen sponge.

Table 4. Histometric evaluation of bone fill in contralateral craniotomy defects receiving PRP/ACS *versus* ACS. Healing at 8 weeks (means ± SD)

	Defect width (mm)	Bone fill (mm)	Bone fill (%)
PRP/ACS	4.3 ± 0.4	3.5 ± 0.8	81.0 ± 12.9
ACS	4.3 ± 0.4	2.8 ± 1.3	64.5 ± 28.1
p-value	0.8336	0.4008	0.2626

PRP, platelet-rich plasma; ACS, absorbable collagen sponge.

Table 5. Histometric evaluation of bone fill in contralateral craniotomy defects receiving ACS *versus* sham surgery. Healing at 4 weeks (means ± SD)

	Defect width (mm)	Bone fill (mm)	Bone fill (%)
ACS	4.3 ± 0.8	2.7 ± 1.0	62.0 ± 20.0
Sham surgery	3.8 ± 0.8	2.6 ± 1.0	71.6 ± 32.2
p-value	0.6547	0.5930	0.1088

ACS, absorbable collagen sponge.

The results of the histometric analysis for animals receiving ACS *versus* sham surgery are shown in Tables 5 and 6. At 4 weeks post-surgery, the mean defect width, bone fill, and percentage bone fill for sites receiving ACS amounted to 4.3 ± 0.8 and 2.7 ± 1.0 mm, and 62.0 ± 20.0%, respectively. The corresponding values for sham surgery were 3.8 ± 0.8 and 2.6 ± 1.0 mm, and 71.6 ± 32.2%. At 8 weeks post-surgery, the mean defect width, bone fill, and percentage bone fill for sites receiving ACS amounted to 4.6 ± 0.4 and 3.6 ± 1.7 mm, and

Table 6. Histometric evaluation of bone fill in contralateral craniotomy defects receiving ACS *versus* sham surgery. Healing at 8 weeks (means ± SD)

	Defect width (mm)	Bone fill (mm)	Bone fill (%)
ACS	4.6 ± 0.4	3.6 ± 1.7	75.6 ± 34.1
Sham surgery	4.1 ± 0.5	2.9 ± 1.1	74.1 ± 24.2
p-value	0.1232	0.1763	0.7353

ACS, absorbable collagen sponge.

75.6 ± 34.1%, respectively. The corresponding values for sham surgery were 4.1 ± 0.5 and 2.9 ± 1.1 mm, and 74.1 ± 24.2%. There were no statistically significant differences in defect bone fill at 4 or 8 weeks post-surgery.

Discussion

The objective of this study was to evaluate the potential of a PRP preparation to promote local bone formation. PRP in an ACS carrier and control treatments, ACS alone and sham surgery, were applied to critical-size rat calvaria defects. Eighteen Sprague–Dawley rats received PRP/ACS and ACS, and 12 rats received ACS and sham surgery in contralateral defects. The histological and histometric analysis following a 4- and 8-week healing interval suggests that the PRP preparation had a limited potential to enhance osteogenesis. There were no appreciable differences in local bone formation among the PRP and control treatments at any observation interval.

Rat calvaria defects have been used to evaluate the biologic potential of various devices, as well as osteoinductive and/or osteoconductive biomaterials and biologics to promote bone regeneration. For example, barrier membranes for guided bone regeneration (Dahlin et al. 1991, Bosch et al. 1995), autograft bone (Turnbull & Freeman 1974, Dahlin et al. 1991), demineralized bone powder (Mulliken & Glowacki 1980), coral porous calcium carbonate (Naaman & Ouhayoun 1998), growth and differentiation factors (Takagi & Urist 1982, Thaller et al. 1993, 1998, Marden et al. 1993, 1994, Bosch et al. 1996, Winn et al. 1999, Blom et al. 2001, Ahn et al. 2003), or combinations thereof have been evaluated. The craniotomy defects used in the aforementioned studies have varied in size ranging from 2 to 8 mm in diameter. Schmitz & Hollinger (1986) have suggested that an 8-mm defect is suitable to evaluate candidate biomaterials for bone regeneration and constitutes a critical-size defect, i.e., a defect that will not resolve within the lifetime of the experimental animal unless implanted with an osteoconductive or osteoinductive technology. Others have defined and successfully used smaller (5-mm) critical-size defects (Bosch et al. 1995, 1996, 1998, Blom et al. 2001). Based on information from previous studies and anatomical restrictions preventing creation of larger-size defects, we used bilateral 6-mm trephine osteotomies in 22-week-old Sprague–Dawley rats. Since this protocol used adult rats, the potential for bone regeneration was reduced and only limited spontaneous healing was expected.

Bone formation among animals receiving ACS and sham surgery was highly variable. No significant differences were noted among these conditions for the 4- and 8-week observations. Many of the defects demonstrated osseous non-union including fibrous connective tissue with only minor bone apposition emanating from the defect margins. This was a consistent finding irrespective of treatment at 4 and 8 weeks post-surgery. These observations corroborate previous reports that demonstrated limited bone healing in sham-surgery controls (Turnbull & Freeman 1974, Mulliken & Glowacki 1980, Takagi & Urist 1982, Dahlin et al. 1991, Thaller et al. 1993, Bosch et al. 1995, 1996, Naaman & Ouhayoun 1998, 1998, Ahn et al. 2003). Interestingly, this study demonstrated complete defect closure at 4 and 8 weeks post-surgery for some sites receiving ACS alone or sham surgery, which has not been reported previously for cranial critical-size defects in the rat.

The histologic observations verified fragments of the ACS carrier at both 4 and 8 weeks post-surgery for sites implanted with PRP/ACS and ACS alone. There were no noteworthy differences between these sites. This finding is consistent with previous reports observing residual ACS when used as a carrier for recombinant human bone morphogenetic protein-2 in canine models (Sykaras et al. 2001, Selvig et al. 2002). Both Selvig et al. (2002) and Sykaras et al. (2001) observed remnants of ACS carrier at 8 or 12 weeks post-surgery using transmission electron or incandescent light microscopy. Other collagen matrices have been observed up to 3 months post-implantation in a subcutaneous rat model (Anselme et al. 1990).

PRP in combination with various bone biomaterials has been evaluated for a number of clinical indications including mandibular resection defects (Marx et al. 1998), extraction sites (Anitua 1999), sinus augmentation (Danesh-Meyer et al. 2001), alveolar ridge augmentation (Shanaman et al. 2001), atrophic mandibles (Robiony et al. 2002), and intra-bony defects (Camargo et al. 2002). These studies indicate that PRP used in conjunction with various bone biomaterials may or may not enhance bone formation and maturation. Animal models have also been used to evaluate the regenerative potential of PRP for peri-implant bone augmentation (Kim et al. 2002, Fürst et al. 2003a, Schlegel et al. 2003, Zechner et al. 2003), sinus floor elevation combined with bovine hydroxyapatite (Fürst et al. 2003b) or autogenous bone (Jakse et al. 2003), mandibular resection defects combined with autogenous bone (Fennis et al. 2002, 2004, Choi et al. 2004), and cranial defects combined with autogenous (Aghaloo et al. 2002) or anorganic bovine bone (Kim et al. 2001, Aghaloo et al. 2004). These studies also offer conflicting evidence concerning the efficacy of PRP when used in conjunction with bone biomaterials. Whereas previous studies have commonly combined PRP with osteoconductive/inductive biomaterials, the present study used ACS shown not to have osteoconductive/inductive effects. This protocol may therefore more effectively discern the genuine osteoconductive potential of a PRP preparation. We could not find any remarkable or statistically significant differences between PRP/ACS and the ACS control at any observation interval, suggesting a limited osteoconductive potential for this PRP preparation. This and experimental observations by others provide an overall impression that PRP has limited, if any, stimulating effect on local bone formation.

In a parallel study, we determined the concentrations of some growth and

extracellular matrix factors in the PRP preparation (manuscript in preparation). The concentrations of PDGF-AB, TGF-β_1, and thrombospondin-1 (TSP-1) obtained were 94.4 , 5.99 pg/ml, and 250 mg/ml, respectively. Still others have evaluated purified or recombinant growth factors also included in PRP as candidate therapies in rodent calvaria critical-size defects. A histologic evaluation of IGF-1 implanted into 8-mm critical-size rat calvaria defects showed complete closure indistinguishable from the surrounding bone at 8 weeks post-implantation *versus* a virtually unchanged bone gap in the controls (Thaller et al. 1993). Bosch et al. (1996) reported a histologic evaluation of bone closure in 5-mm rat calvaria critical-size defects following surgical application of rhTGF-β_1 in a gelatin sponge carrier. None of the rhTGF-β_1 or carrier control sites exhibited complete bone closure. In all, differences in the healing potential among candidate therapies may relate to the presentation of the biologic agent. The carrier system may significantly influence the efficacy of the agent, as might dosages influence the outcome of therapy. The presentation and amount of growth and extracellular matrix factors in the present study may have been incompatible with significant osteogenic stimulation. Nevertheless, the results in the present study and those reported in the literature may also suggest that PRP and some growth factors may limitedly promote local bone formation.

In summary, the PRP preparation in the present study did not enhance bone regeneration in the critical size 6-mm rat calvaria defects over that observed in the control. Bone formation was highly variable in sites receiving the PRP preparation, as well as in controls. Remnants of the ACS biomaterial were observed at both 4 and 8 weeks in sites implanted with PRP/ACS or ACS. The results suggest that the PRP preparation has a limited potential to promote local bone formation.

Acknowledgements

The authors thank Alexis Agelan, DVM, and Lewis T. Bright, Temple University, for excellent veterinary and animal technical care; Thelma E. Montara for skilful histotechnical processing; and Joanne M. Manns, PhD, for processing the PRP preparation.

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