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Mandibular reconstruction: A clinical and radiographic animal study on the use of autogenous scaffolds and platelet-rich plasma

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Abstract. This paper reports on an experimental animal study evaluating a method of mandibular reconstruction. After a successful pilot study, 28 goats underwent a continuity resection of the mandibular angle. Primary reconstruction was carried out using specially designed osteosynthesis plates and screws. The defect was bridged by the original cortical scaffold, filled with an autogenous bone graft from the iliac crest. To accelerate bone healing, platelet-rich plasma (PRP) was mixed with the particulate bone graft in 14 goats. All goats had uneventful healing. The osteosynthesis plates and screws withstood immediate loading for periods varying from 3 weeks to 3 months. The use of PRP appeared to enhance bone healing considerably.

Key words: mandibular reconstruction; platelet-rich plasma (PRP); experimental animals.

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The mandible, because of its symphyseal curvature and gonial angle, poses a challenge to the reconstructive surgeon. Its gentle curves and the otherwise intricate contour require a reconstructive technique that takes into account the complex anatomy in all three dimensions. Numerous techniques have been suggested to bridge defects or to reconstruct a lost part of the mandible as closely as possible. Methods proposed and for a large part still applied in clinical conditions include the use of bridging plates, free non-vascularized and vascularized bone grafts, either pedicled or based on microvascular anastomosis[3,4,6,9,18,21,23].

All these techniques have inherent advantages and disadvantages and can be used in selected patients[10]. Despite advances made, the main shortcoming of most techniques is the inability to exactly copy the volume and contour of the lost bone, let alone to restore the proper three-dimensional relationship. As a result, it is often difficult to provide these patients with adequately functioning dentures. Contour abnormalities are also usually painfully visible, giving rise to the stigmata that characterize the oral cancer patient[15,17].

In an attempt to improve on mandibular reconstruction, it was thought to be mandatory to create an animal model that would allow for the testing of various methods making use of tissue engineering techniques. Mandibles of primates, of course, would come close to the human model but are not readily available for obvious reasons. Ruminating animals, like sheep and goats, also have mandibles that come close to the human anatomy as far as the gonial region is concerned. These animals, however, exert tremendous forces on their mandibles when ruminating. Current plating systems are not adequate to withstand this excessive loading, which makes them less suitable[16].

The aim of the present study was to test a model in which a defect was made in the mandibular angle of a goat. Subsequently, primary reconstruction was carried out, making use of two specially designed plates with monocortical screws and cortical scaffolds filled with an autogenous bone graft, with and without the addition of platelet-rich plasma (PRP). If successful, this model can be used for further experiments testing various modes of reconstruction[5,14].

Pilot study

One healthy, mature (2.5 years of age) female Saanen goat, weighing about 60 kg was used for the pilot study. Before surgery, a blood sample was taken from the goat to ensure that the animal was CAE/CL arthritis free. The animal was housed in a stable. National guidelines for the care and use of laboratory animals were observed. The operation was performed under general anaesthesia. The anaesthesia was induced by an i.v. injection of pentobarbital and maintained by isoflurane 2–3% through a constant volume ventilator, administered through an endotracheal tube. The goat was connected to a heart monitor.

To reduce the risk of perioperative infection, the goat was treated with three gifts of ampicillin, the second and third gifts were depots:

- During the operation: Albipen 15%, 3 ml/50 kg s.c.
- Postoperative day 1: Albipen LA, 7.5 ml/50 kg s.c.
- Postoperative day 3: Albipen LA, 7.5 ml/50 kg s.c.

Before surgery, the skin at the operation site was shaved and disinfected using povidine iodine solution.

A continuity resection on the right side of the mandible, measuring 4 cm at the lower border and 1.5 cm in the retromolar area, was performed including the angle of the mandible through a submandibular approach (Fig. 1). Before resection, two pre-shaped osteosynthesis-plates were adapted, using proprietary bending tools, in order to maintain the correct position of the mandible. Computer-aided design (CAD) was used to make the osteosynthesis plates fit the contour of a goat mandible and they were automatically transformed into the computer-aided manufacturing (CAM) process (CAD-CAM). This guaranteed that the shape of the plates would fit an average goat mandible, which obviated the need for major bending of the plates. The plates had a thickness of 1.5 mm and a width of 5.4 mm. They were manufactured out of titanium alloy (DIN 17851:11.90 titanium alloys chemical composition: ASTM F136-96). The centre-drive® screws had a diameter of 2.3 mm and the pitch is 1.25 mm. The screws were manufactured out of alloyed titanium (DIN 17851:11.90 titanium alloys chemical composition: ASTM F136-96).

Fig. 1. Magnitude of defect in the mandible. CAD/CAM titanium plates maintain three dimensional position and stabilize the fragments.

The neurovascular bundle entering the mandible was ligated in order to control bleeding after resection. The segment was then resected, using a fissure burr nr. 2 and subsequently placed back in its original location and fixed with the pre-shaped plates. The bone gaps on either side of the reimplanted segment were approximately 3 mm wide. The soft tissues were closed in layers using resorbable Vicryl 3*0 sutures. Immediate postoperative radiographs were made. Flunixine 1.5 mg/kg (Finadyne®) was given for 3 days to alleviate postoperative pain. The procedure was performed unilaterally in order to avoid malocclusion and subsequent jaw function disturbance.

The animal had an uneventful recovery and from the first day was eating and ruminating. After 6 weeks, the animal was sacrificed and the mandible retrieved for radiographic and histological evaluation. It appeared that the plates had not broken, during this 6-week period, no displacement of the mandible had occurred. Superficial bone formation was visible mainly on the attachment site of the masseter muscle and had completely covered the bone gaps.

Materials and methods

The success on this preliminary experiment caused the Animal Ethical Committee of the University of Nijmegen to grant permission for further studies. A total of 28 goats were used for the first part of a larger study to test various surgical techniques. Surgery was carried out according to the above-mentioned description, except for the treatment of the resected segment.

The 28 goats were divided in two groups of 14 animals each. In the first group of 14 animals, the bone marrow of the resected segment was removed. The cortical tray was then filled with an autogenous particulate bone graft taken from the anterior iliac crest. For this reason the skin in this area was shaved and treated with povidine-iodine solution. An incision of 5 cm was made on top of the iliac crest. Subsequently subperiosteal elevation was carried out on the lateral aspect of the crest.

Approximately 5 cc of cortico-cancellous bone was harvested by means of a trepan[12]. The wound was closed in layers using resorbable sutures (Vicryl®, Ethicon). The graft was ground in a bone mill (Leibinger®). Consequently the particulate bone graft consisted of cancellous and cortical bone. Perforations, located approximately 5 mm apart

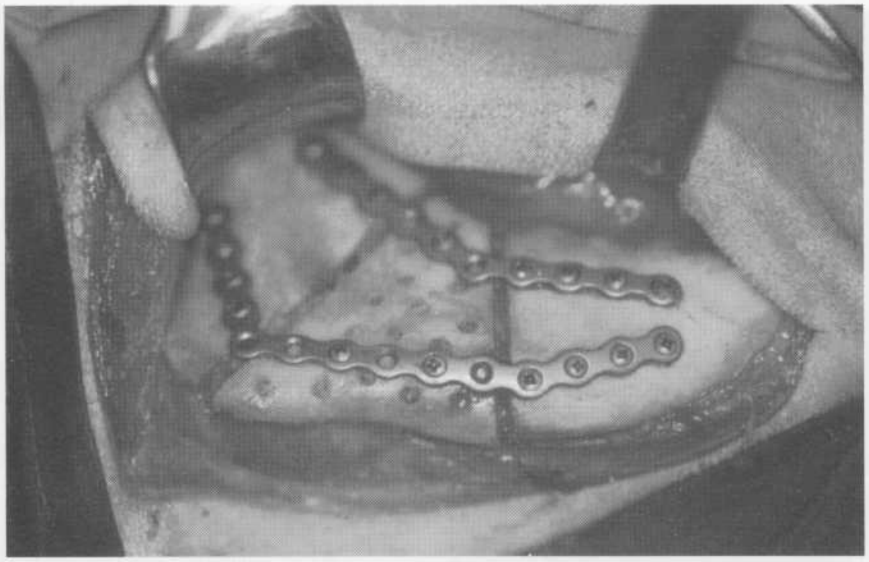

Fig. 2. Cortical tray filled with corticocancellous bone chips placed back in the original position and fixed to plates and screws. Burr holes with round burr nr. 2 have been made in the cortex to facilitate capillary ingrowth.

were made in the cortical tray with a round burr no. 2 to facilitate vascular ingrowth. The trays were placed back in their original position and fixed with two plates (Figs 1 and 2).

The 14 goats in the second group underwent the same treatment, except that the bone graft was mixed with PRP. For this reason, 250 cc of blood was drawn 2 days before surgery. This blood was treated by centrifugation at the transfusion laboratory in various cycles[2]. In this way, a PRP suspension was obtained (platelet count exceeding 800×10^9). This was activated at the time of surgery with a 10% calcium chloride solution and 300 i.u. of bovine thrombin (Fibriquick®, Organon Teknika) to form a gel[19,22], whilst the erythrocytes were given back[7,11]. Surgery was carried out unilaterally, randomly divided over both sides of the jaw and iliac crests.

Radiographs of the reconstructed sites were made directly after surgery. After 3, 6 and 12 weeks respectively, the animals were sacrificed by an overdose of pentobarbital. The mandibles were retrieved for radiographic and histologic evaluation. Radiographic evaluation took place in a standardized way after resection of the hemi-mandible. Radiographs were made with a mobile X-ray machine (Siemens Mobilett B) using a long-cone technique from a distance of 70 cm (40 kV, 28 mA). Two independent examiners, who were not informed about which animal was treated with PRP, assessed the radiographs. They were asked to score on a checklist as depicted in Table 1. Parameters involved included bridging of both osteotomy sites by new bone, fading of the perforations made in the cortical bone, callus formation at the lower borders of the osteotomies and resorption both at the lower border as well as centrally in the graft. The details of the scoring system are presented in Table 1.

Results

All goats had an uneventful recovery and were walking around within 3 hours. Within 36 hours, all 28 goats were eating and ruminating again. Their weight remained stable, although one goat had lost 5 kg. This appeared to be caused by an infection with intraoral exposure around the cranial plate.

On sacrifice, no plate fractures were noted; however three loose screws were found in the one goat with plate exposure, yet the plate was intact and appeared to be fixed to the bone with the remaining screws.

All specimens of both the first and second groups showed the same overall picture in that abundant bone formation was seen partially overgrowing the apparently intact plates. This was most prominent in the muscle attachment zones and virtually not present on the lingual side (Fig. 3). In all cases the bone gaps appeared to be bridged by newly formed bone.

The radiographs taken at sacrifice were comparable to the ones taken immediately postoperatively in that no loose screws were seen and the plates showed no fractures. The results of the scoring of the radiographic parameters are presented in Table 2. Typical examples of specimens from each group at the 6-week interval are presented in Figures 4 and 5.

The mean scores of the two groups at the 3-week interval hardly showed any differences (Table 2). At the 6- and 12-week intervals, however, the obliteration of bone gaps and cortical perforations appeared to indicate bone healing. In the PRP group, however, this was more markedly present. Bone healing, as manifested by the obliteration of the bone gaps at the osteotomy sites, was particularly present in the PRP group at 6 weeks. This appeared to be statistically significant (Student *t*-test, one-tailed, two-sample equal variance, $P<0.05$). This is well illustrated by the presence of radio-opaque areas at the osteotomy gaps exclusively seen in the PRP group at 6 and 12 weeks (Fig. 5). Angular and central resorption was less visible in the PRP group at all time intervals. This, however, was not statistically significant.

Table 1. Parameters and scoring system used for radiographic evaluation

Variable	Score
Bone gaps (anterior and posterior osteotomy sites)	0=completely visible 1=partial obliteration 2=>90% obliteration 3=radio-opacity around obliterated gap
Perforations (made by burr holes)	0=completely visible 1=<50% obliteration 2=>50% obliteration 3=no visible perforations
Callus formation (at anterior and posterior osteotomy sites)	0=none 1=present
Central resorption (internal resorption)	0=non 1=present
Angular resorption (external resorption at osteotomy site at gonial angle)	0=none 1=present

Fig. 3. Lateral surface of reconstructed mandible at sacrifice after 6 weeks showing overgrowth of bone. Plates are partially covered by bone (6 weeks postoperatively. Non-PRP).

Table 2. Mean results from scoring. Forms completed by two independent referees

	Obliteration of bone gaps	Obliteration of perforations	Callus formation	Angular resorption	Central resorption
Group 1 (non-PRP)					
3 wks $n=6$					
Mean	0.92	1.17	0.92	0.33	0.17
SD	0.67	0.72	0.29	0.49	0.39
6 wks $n=6$					
Mean	1.58*	1.58	0.67*	0.17	0.33
SD	0.90*	0.90	0.49*	0.39	0.49
12 wks $n=2$					
Mean	1.5*	1.75	0	0.25	0.75*
SD	1.29*	1.26	0	0.5	0.5*
Group 2 (PRP)					
3 wks $n=6$					
Mean	1.17	1.17	0.92	0.33	0.25
SD	1.11	0.94	0.29	0.49	0.45
6 wks $n=6$					
Mean	2.33*	1.67	1*	0.08	0.33
SD	0.78*	0.65	0*	0.29	0.49
12 wks $n=2$					
Mean	3*	2.5	0.5	0.25	0*
SD	0*	0.58	0.58	0.5	0*

*Statistically significant differences between groups 1 and 2 ($p<0.05$). SD: standard deviation.

The results from the histologic examinations and the histomorphometry will subsequently be reported.

Discussion

Current research on mandibular reconstruction making use of bioengineering techniques focuses on the prefabrication of bone grafts at distant sites and the restoration of lost parts of the jaw at the defect site[1,16,20]. The latter technique usually makes use of scaffolds, alloplastic, homogenous or even autogenous. It remains to be seen which of these pathways will prevail, although each may have its unique advantages.

This experiment shows for the first time that mandibles with continuity defects can be stabilized even in ruminating animals, provided adequate fixation is used, using a specially designed titanium mandibular osteosynthesis system (Martin) with monocortical screws as described before.

The technique used gave rise to satisfactory results, whereby the mandible remained stable, allowing the animals to eat and ruminate. Radiographs and clinical examination revealed no fractures of the plates. This is different from the results reported by LINDQUIST et al.[8], who used four types of single reconstruction plates for similar but smaller defects in sheep. The results with three out of four plating systems were similar in that apparently in approximately 50%, one large plate could not withstand the ongoing forces caused by ruminating, resulting in fracture of the plates or screws over a 2-month period. There is, however, a major difference in study design in that LINDQUIST et al. left the defect open, whereas in the present study the defect was grafted. The Animal Ethical Committee of the University of Nijmegen did not grant permission to test the plates on non-grafted defects.

In the present study in only one goat, three screws had become loose. This was likely to be due to the somewhat high position of the cranial plate, which had eroded through the mucosa and became exposed to the oral environment.

The buccal bone formation as seen in almost all animals is somewhat puzzling. It is unlikely that this should be considered periosteal bone because in goats periosteal osteoblasts remain attached to the cortical bone after periosteal elevation. The bone-forming capacity of the periosteum is, therefore, rather limited, which is different from the human situation[13]. Furthermore, before the perforations in the trays were made, the surface of the cortical bone was cleansed to remove attached soft tissues by means of an acrylic burr. In addition, when stripping subperiosteally in the muscle attachment zones, the periosteum is usually severely damaged and subsequently tends to retract. For these reasons we consider this bone formation to be reactive.

The results of the radiographic examinations including the assessment of the callus formation unequivocally point towards rapid bone healing in the PRP group. These results are in keeping with the results on the clinical application of PRP, as reported by MARX et al[11]. The use of autogenous cortical scaffolds is, of course, somewhat unusual, because it may seldomly be used in the clinical

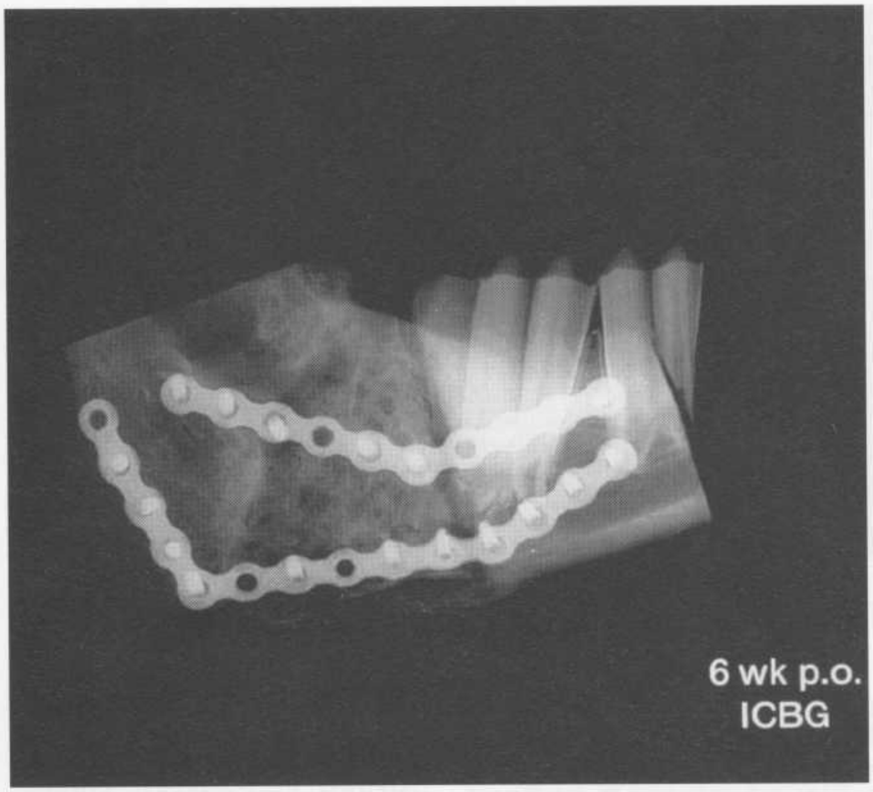

Fig. 4. Radiograph taken after 6 weeks at sacrifice from a goat in which no PRP was used. Note that the osteotomy lines and burr holes are still visible, while clear radiolucent areas are seen within the grafted site. Periosteal bone formation is visible on the anterior osteotomy site, whereas angular resorption is also seen (arrow).

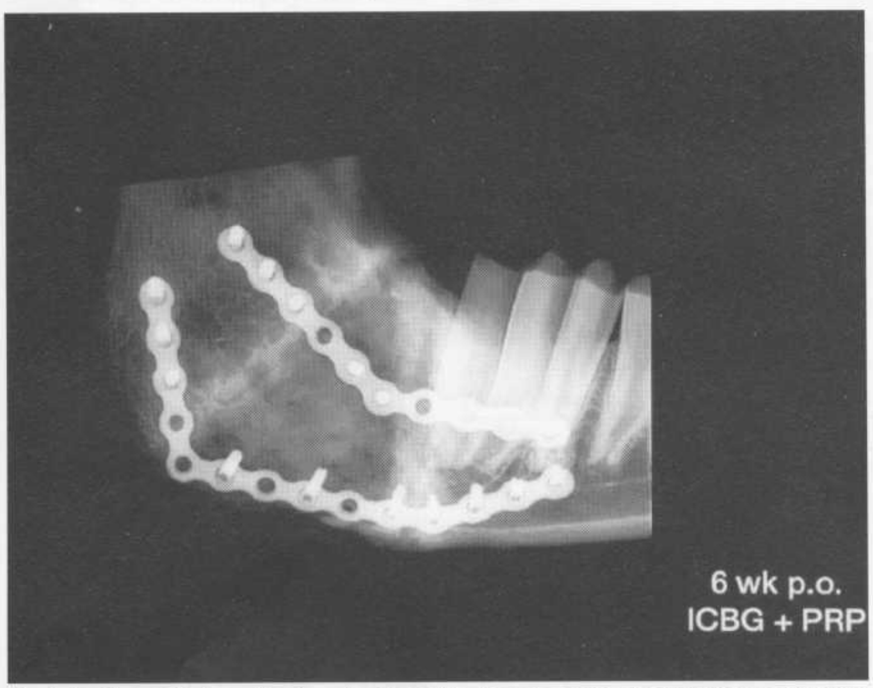

Fig. 5. Radiograph taken after 6 weeks at sacrifice from a goat in which PRP was used. Note radio-opaque areas at the original osteotomy sites, while the burr holes have almost disappeared. There are no signs of radiolucent areas within the grafted bone. The contour of the lower border is clearly defined without resorption. Callus formation can be seen on the anterior osteotomy.

situation. Yet, it was thought to be necessary to begin this set of experiments with this mode of treatment in order to have a baseline when testing alloplastic scaffolds. It is recognized that in the autogenous cortical scaffolds bone-inductive factors may be present that may influence the results. In fact, this may be part of the reason why the plates and the osteotomy gaps were partially covered with a superficial layer of bone, especially at the muscle attachment zones.

Studies are underway to test various scaffolds including autogenous as well as alloplastic materials making use of autogenous cortico-cancellous bone grafts, PRP and histomorphometric analysis of the newly formed bone.

The results of this study clearly demonstrate that the animal model chosen and the plating system used fulfil the requirements for testing various mandibular reconstruction methods. The specially designed plates, which hardly needed any adaptation, and the monocortical screws provided enough stability to allow the animals to eat and ruminate and thus, to survive the experimental period.

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