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Mechanical evaluation of mandibular defects reconstructed using osteogenic protein-1 (rhOP-1) in a sheep model: a critical analysis

M. Abu-Serriah[1], A. Kontaxis[2], A. Ayoub[1], J. Harrison[3], E. Odell[4], J. Barbenel[2]

[1]Oral and Maxillofacial Surgery, Glasgow Dental Hospital and School, Glasgow, UK; [2]Bioengineering Unit, University of Strathclyde, Glasgow, UK; [3]The Bone Metabolism Unit, Western Infirmary, Glasgow, UK; [4]Department of Oral Pathology, King's College London, London, UK

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Abstract. Osteoinductive bone morphogenetic proteins (BMPs) have been used extensively in experimental and clinical orthopaedic research. It is a natural progression for these growth regulators to be tested in the craniofacial region. The aim of this investigation was to analyse the mechanical properties of the sheep mandibles reconstructed using recombinant human osteogenic protein type 1 (rhOP-1). A unilateral 35 mm osteoperiosteal continuity defect was created at the parasymphyseal region of the mandible in six adult sheep. The animals were sacrificed 3 months after surgery and mechanical properties of the regenerated bone at the operated sides (OS) were compared to the corresponding bone at the non-operated side (NOS). The regenerated tissue at the OS were then submitted for histological and histomorphometric analysis. Although all the animals achieved complete bony union, a wide range of mechanical properties was found. The rhOP-1-induced bone achieved a mean of 36% of the strength of the bone at the NOS ($P < 0.05$). The mean value of the stiffness of the OS was 24% of the NOS ($P < 0.05$). While half of the samples of the OS had 'weak' mechanical properties (9–25% strength compared to NOS) and a low stiffness (6–18%), the rest showed relatively higher strength (47–63%) and were stiffer (35–47%). Unlike the NOS, the operated sides failed under tensile stresses and cracks initiated at the superior border of the mandible. The wide mechanical variations suggest that further basic bone biology research is needed to provide better understanding of the cellular and molecular events which take place during the process of osteoinduction.

Key words: rhOP-1; BMP; mandible; sheep; mechanical.

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The mandible is an important bone of the facial skeleton and performs vital mechanical and cosmetic functions. Large continuity mandibular defects can be severely disabling, disfiguring, and are difficult to reconstruct[13]. Although various autogenic, allogenic and alloplastic methods of reconstructing these defects have been reported in the literature, all have serious drawbacks. Reconstruction of skeletal defects by osteoinduction using bone morphogenetic proteins (BMPs) may become

a realistic treatment alternative. Such an approach would eliminate or reduce the disadvantages associated with conventional reconstructive procedures.

Several studies on the use of BMP for reconstructing continuity mandibular defects in large mammals have been published[4,5,16,22–24], but little is known about the mechanical characteristics of the newly regenerated bone.

The mandible is designed to withstand masticatory forces, and so mechanical characterisation of the newly formed bone is of paramount importance. The aim of this study was to describe the mechanical properties of osteoperiosteal continuity defects in sheep mandible, which had been reconstructed using recombinant human osteogenic protein type 1 (rhOP-1 or rhBMP-7) and bovine collagen type-I as a carrier.

Material and methods

The investigation was conducted on six adult female Scottish Grey Face sheep and was approved by the Ethics Committee of the University of Glasgow and the Home Office (Scientific Procedures Act, 1986).

Operative technique

The surgical procedure was performed as described before[1]. Briefly, inhalational general anaesthesia was induced using Diazepam (0.2 mg/kg, Phoenix Pharma Limited, Gloucester, UK) and Saffan™ (9 mg/kg intravenously; Schering-Plough Animal Health, Middlesex, UK). Prophylactic antibiotic (7.0 mg amoxicillin, 1.75 mg clavulanic acid, Pfizer, Kent, UK) in a dose of 2.5 ml/50 kg was given intramuscularly at the time of induction. Five millilitre of local anaesthetic (Xylocaine 2% with adrenaline 1:800,000) was infiltrated, and unilateral exposure of the mandible was achieved via the submandibular approach using a cutting diathermy (Electrosurg®, Kruuse, North Yorkshire, UK). Supra-periosteal dissection was then carried out buccally and lingually and the diastema between the fourth lower incisor and the first premolar tooth was identified (Fig. 1). The mental neurovascular bundle was identified, ligated and cut. A 35 mm osteoperiosteal continuity defect was created and the integrity of the mandible was maintained using a custom-made stainless steel bone plate (Fig. 1).

Implant material was prepared following the manufacturer's instructions. The defect was filled with rhOP-1 and bovine collagen type-I. The volume of the bone defect (approximately 7 cm^3) determined the amount of rhOP-1 used. The defect was packed passively with the implant material giving, an equivalent dose of 1.0 mg of rhOP-1/cm^3 of the defect, and closed in layers.

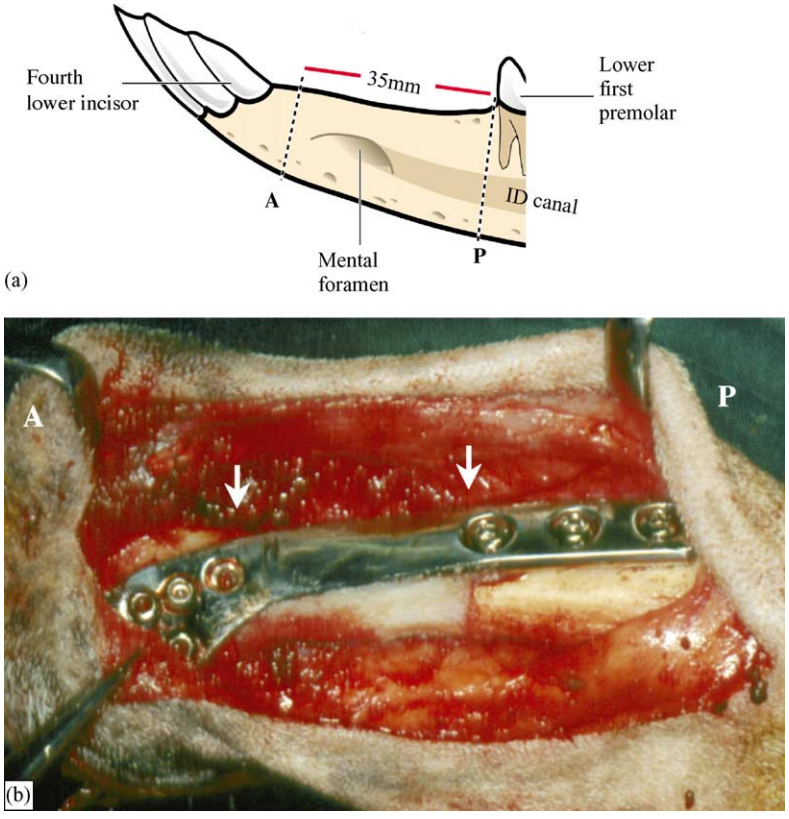

Fig. 1. (a) A diagrammatic view illustrating the site of the surgical defects and the anatomical structures involved. **A** and **P** represent the anterior and posterior ends of the defect. Inferior dental (**ID**). (b) The custom-made bone plate and implant material in place. The surgical defect (*between the arrows*). **A**: anterior; **P**: posterior.

Experimental protocol

Radiographic and ultrasonographic assessments were carried out at 0, 2, 4, 8, and 12 weeks of the follow-up period. Three months post-operatively, the animals were sacrificed with an overdose of pentobarbitone sodium BP 20% (w/v) (Fort Doge Animal Health, Southampton, UK). The mandibles were then removed and all the attached soft tissues were removed to prevent slippage of the test samples during mechanical loading. The bone plate was carefully removed and manual manipulation of the operated side was carried out to determine the nature of union. The mandibles were split into hemimandibles and were kept initially in the refrigerator for 1 h prior to storage at −80 °C. The samples were left to thaw in the refrigerator overnight before the day of testing.

A cantilever (single-point bending) test was chosen for mechanical testing of the mandibles, and a custom-made holding device (a jig) was used to hold the samples firmly during the testing procedure (Fig. 2). During the preparation and testing process, the bone samples were wrapped in wet tissue paper in order to minimise dehydration. The lower border of the mandible was kept in contact with the metallic base of the jig with the lingual surface oriented vertical to the floor.

The test was performed using an Instron mechanical testing machine (Instron, High Wycombe, UK). After placing the hemimandible in the jig, the assembly (i.e., the bone specimen and the jig) was fixed into the movable lower cell of the Instron machine with holding screws. The lower cell was then moved upwards at 5 mm/min, bringing the bone sample into contact with the contact component of the mandible-loading tool. The point of force application was 35 mm anterior to the posterior interface between the newly formed and native bone. Cantilever bend testing was continued until bone failure was observed. Failure was defined as an instantaneous drop in the recorded loading force.