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Comparison of different harvesting methods from the flat and long bones of rats

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Abstract

Different harvesting methods have been developed for bony augmentation before implantation. The aim of the present study was to assess the viability of endochondral (femoral) and membranous (mandibular) bone cells harvested by different methods under standard conditions in an animal model, and to investigate the surface of the bone in the harvested area. Samples of mandibular and femoral bone were harvested using a drilling burr, a piezoelectrical device, or a Safescraper®. Blocks of bone that had been harvested with cutting forceps were used as controls. The size of the samples was measured and they were examined by conventional microscopy and immunohistochemical analysis; osteoblast-like cells were also cultured. The surface of the harvested area was analysed with scanning and conventional microscopy. There was no significant difference between mandibular and femoral bone in the size of particles harvested, but bone chips were significantly smaller when a drilling device had been used in both harvesting areas. Viability of cells in these smaller particles was significantly less than among cells harvested with a piezoelectrical device or Safescraper®. Scanning microscopy showed a smooth bony surface where a drilling burr or piezoelectrical device had been used, whereas small disruptions were observed after the Safescraper® had been used. Harvesting of particulate bone is feasible using a drilling burr, piezoelectrical device, or Safescraper® from mandibular and femoral bone. The piezoelectrical device and the Safescraper® gave comparable results concerning the viability of osteoblast-like cells, and so are preferred to a drilling burr.

Keywords: Bone; Piezosurgery; Safescraper; Augmentation; Harvesting; Surgery

Introduction

Autogenous bone grafting is widely used for augmentation before insertion of a dental implant. It is most frequently commonly used for raising the sinus floor and augmentation of the alveolar ridge. Autogenous bone, unlike artificial bone substitutes, does not cause any immunological reaction and has optimal biocompatible remodelling patterns,[1] so it outclasses all artificial bone substitutes in its osteoinductive and osteogenic properties.[2] The integration of autogenous bone is, however, influenced by several factors such as the origin of the graft,[3] local growth factors,[4] structural composition,[5] and the method of harvesting.[6]

Autogenous bone grafts can be harvested in different ways, but the reports are contradictory about questions of the size of bone chips and the viability of cells.[7,8] However, bone chips can be harvested using dental drilling burrs, a new piezoelectrical device, and in combination with a bone trap or a Safescraper®.

The different methods vary not only in the time required for harvesting, the incidence of complications, and the size of the graft, but also in the viability of the cells.[9–11] The success of bony augmentation, however, is primarily dependent on osteogenic potency and the viability of osteoblastic cells, which induce angiogenesis and facilitate long-term incorporation.

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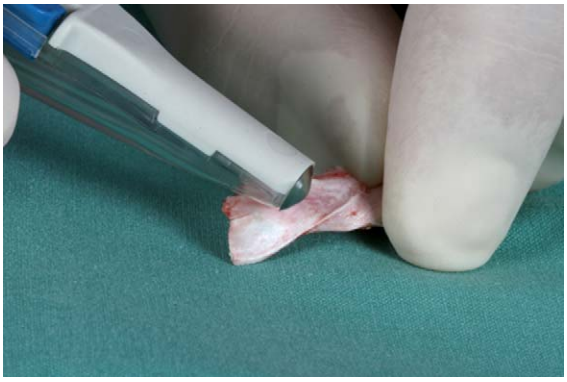

Fig. 1. Harvesting of bone chips using a Safescraper® on the mandible of a Lewis rat after resection of the surrounding soft tissues.

Several areas of origin are possible, but bone grafts for use in maxillofacial surgery are usually taken from the tibia,[12] iliac crest,[13] chin,[14] zygomaticoalveolar crest,[15] and mandibular angle.[16] Harvesting bone from different regions results in various resorption rates and long-term success, and whether membranous or endochondral bone is the material of choice for bone augmentation is still controversial.

The aim of the present study, therefore, was to analyse systematically the harvesting area and the influences of new, different, harvesting methods on the viability and morphology of membranous and endochondral bone in animals.

Materials and methods

Animals

We used a total of 40 male Lewis rats, body weight 300–330 g, which were distributed randomly into four groups ($n = 10$ in each group). In the first group the bone was harvested from the mandible and the femur using a burr; in the second group we used a piezoelectrical device (Mectron, Piezosurgery, Carasco, Italy); in the third group a Safescraper® (Meta, Reggio Emilia, Italy) (Fig. 1); and in the fourth group (control) blocks of bone from the mandible and femur that were harvested using bone-cutting forceps. The animals were kept and the experiments organised in accordance with the German Animal Protection Act.

Harvesting of bone

The rats were killed with an injection of T61 (Intervet, Unterschleissheim, Germany). Femur and mandible were removed and any adherent tissue sharply dissected.

A conventional steel rotating burr with eight blades (ISO 021, Komet, Lemgo, Germany) was used for drilling at a speed of 1000 rpm in saline solution. Harvesting with the piezoelectrical device (Piezosurgery, Mectron Medical Technology, Carasco, Italy) took 30 s at 29 kHz and an amplitude of 200 μm on the bone under permanent cooling with saline solution. The Safescraper® was repeatedly drawn over the bone surface (Fig. 1). The bone chips harvested were collected in the chamber of the Safescraper®. Bone blocks 2 mm × 2 mm × 1 mm were cut directly off the bone using bone-cutting forceps.

Size of chips of bone

Samples were measured under 10× magnification. Linear measurements of the largest and smallest diameter of each bone chip were recorded (Analysis, Muenster, Germany). Fifteen chips from each sample of bone in each group were chosen for measurement. All examinations were made by one examiner who was unaware of the design and purpose of the study.

Bony surface

Light and scanning microscopy were used, and the specimens were placed in 3.5% formaldehyde (pH 7.4) for 24 h before being rinsed in water. After the specimens had been decalcified for 14 days in 10% ethylene diaminetetraacetic acid (EDTA) (in 0.3 M Tris-hydrochloric acid pH 7.4) and embedded in paraffin, sections 5 μm thick were cut and stained with haematoxylin and eosin according to our standard protocol before analysis.

Isolation and cultivation of osteoblast-like cells

Primary osteoblast-like cells were isolated according to a protocol described by Frosch et al.[17] The cleaned bony fragments were kept on ice in Hanks' buffered salt solution (HBSS PAA, Coelbe, Gemany) until they were processed.

The fragments were placed on 100-mm cell culture dishes (Greiner, Frickenhausen, Germany), covered with culture medium 5 ml, and incubated at 37 °C in a humidified incubator containing an atmosphere of 91.5% air and 8.5% carbon dioxide. The culture medium was Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, Hepes buffer 20 mmol, penicillin 1000 IU/ml, and streptomycin 0.1 mg/ml (all PAA, Coelbe, Gemany). After one week another 5 ml of culture medium was added.

After an incubation period of two weeks, cell layers were gently rinsed with HBSS and then incubated with trypsin/EDTA (PAA, Coelbe, Gemany). Cells released from the culture surface were washed with two changes of culture medium and resuspended in a limited amount of culture medium. The total cell count was measured using a Neubauer haemocytometer.

Immunocytochemistry

The cells were fixed in 4% paraformaldehyde (Sigma Aldrich, Taufkirchen, Germany) for 10 min. After they had been blocked for 20 min with PBS containing 5% fetal