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Effect of hyaluronic acid on the osseointegration of dental implants☆

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Abstract

The mechanism of osseointegration is related to many factors, including the quality of the bone, the biocompatibility and surface characteristics of the implant material, the surgical technique, and functional loading. The purpose of this study was to investigate the effects of hyaluronic acid gel on the osseointegration of implants placed in defined areas of the mandible in rabbits. Hyaluronic acid is known to have an osteoinductive effect during regeneration of bony defects, and we thought that it might also have a favourable effect on osseointegration, a specialised mechanism to heal bone. Ten New Zealand rabbits aged 10 weeks and weighing 2.5–3.0 kg were used, and sites for implants that were far enough from the apices of the teeth in the mandibular molar area were chosen. Two cavities were prepared in each rabbit, one (anterior) for the control implant, and one (posterior) for the implant with hyaluronic acid gel (Medical Instinct GmbH, Bovenden). New bone and the osteoid matrix content around the dental implants were evaluated histologically and histomorphometrically two months after the operation, and no significant difference was found between the two groups.

Keywords: Hyaluronic acid; Dental implant; Osseointegration; Rabbit Mandible; Histopathology; Bone Repairing

Introduction

Branemark et al were to our knowledge the first to describe "osseointegration": the mechanism by which titanium implants and living bony tissue heal without inflammation.[1] It was defined by Albrektsson et al[2] as direct microscopic contact between living bone and implants, and Zarb et al added to the description of osseointegration as a connection between a bone and a functionally-loaded implant that was asymptomatic.[3]

Osseointegration of dental implants is important, and includes the circumferential tissue response that includes inflammation, neoangiogenesis, osteoinduction, and osteoconduction, followed by a remodelling phase. First, blood fills the bony cavity after osteotomy, and then the cellular components of blood such as erythrocytes, thrombocytes and white cells migrate to the bone:implant contact area and a dense fibrin clot develops. The provisional matrix forms on the fibrin network, to finish the first step of osseointegration.

Research into advanced modifications of the surface of the implant has included microtechnological and nanotechnological procedures. New and complex modifications have been made to the surfaces to provide alternative approaches with different micrometre and nanometre scales. Some of these surfaces ınclude loading osteoinductive and osteoconductive agents such as collagen, bone morphogenetic protein 2, and

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chondroitin sulphate to the surface of the implant, and "carrier" agents such as hyaluronic acid are used to load these materials on to the surface of the implant.

Hyaluronic acid (or hyaluronan) is a glycosaminoglycan that contains linear polysaccharides that are formed by consecutive chains of D-glucuronide acid and N-acetyl glycosamine monosaccharides. It is one of the prevalent components of the extracellular matrix, is found in all living organisms, and plays an important part in the morphogenesis of healing tissue.[4] It has been reported to stimulate cell migration, adhesion, proliferation, and differentiation, leading to formation of bone.[5–8]

We assumed that hyaluronic acid, which is known to have favourable effects on morphogenesis and tissue healing, may also affect the first stages of osseointegration of dental implants. The aim of this study, therefore, was to evaluate its effect on osseointegration of dental implants in the mandibles of rabbits.

Material and methods

The study was supported by the Scientific Research Project Unity of Kirikkale University, and consent was given by the local Animal Studies Ethics Committee on 16 December 2013. The study took place at the Huseyin Aytemiz Animal Research Laboratory of Kirikkale University. Ten New Zealand rabbits, 10 weeks old and weighing 2.5–3.0 kg were studied.

Operative technique

For general anaesthesia we used xylazine (Rompun) 5 mg/kg and ketamine hydrochloride (Ketalar) 50 mg/ kg injected intramuscularly. The sites of operation were disinfected with povidone iodine (Betadine), and articaine hydrochloride (Ultracain D-S Forte) was injected locally into the operating sites. A skin incision 2.5 cm long was made parallel to the inferior border of the mandible, and a mucoperiosteal flap was raised with subperiosteal dissection (Fig. 1).

Dental implants 2 mm in diameter and 4 mm long were specially designed and produced by Nucleoss Implants Ltd (Izmir) (Fig. 2). Two implant cavities were prepared in the molar region of the mandible. One implant was inserted into the anterior cavity and acted as the control, and the posterior cavity had the gel-formed hyaluronic acid (Medical Instinct GmbH, Bovenden) applied to the cavity after the implant had been inserted (experimental group), giving 10 in each group (Fig. 3). The animals were killed after two months.

Preparation of samples and histomorphometric analysis

The mandibles were removed and dissected, and stored in 10% formaldehyde solution. All samples containing the dental implants and the surrounding hard tissue were fixed in 4% buffered formaldehyde. They were then dehydrated in a graded series of alcohol and xylene, and embedded in light-curing resin (Technovit). Undecalcified ground sections were prepared using sawing and grinding. The blocks of bone were cut into sections 200 μm thick mid-axially in a buccolingual plane. The central section was harvested and then polished to a final thickness of 40 μm (Exakt ®, EXAKT Advanced Technologies). Sections were stained with Goldner trichrome stain.

Digital images were then taken, and histomorphometric samples were measured using a computer-based analysis program (AnalySIS LS Research, Versiyon 5.0, Olympus Soft Imaging Solutions). Bone-implant contact and bone-osteoid

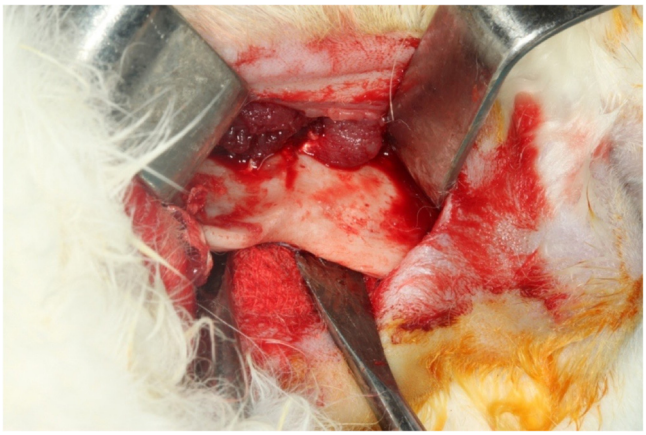

Fig. 1. Raising the mucoperiosteal flap.

Fig. 2. The specially-designed implants.

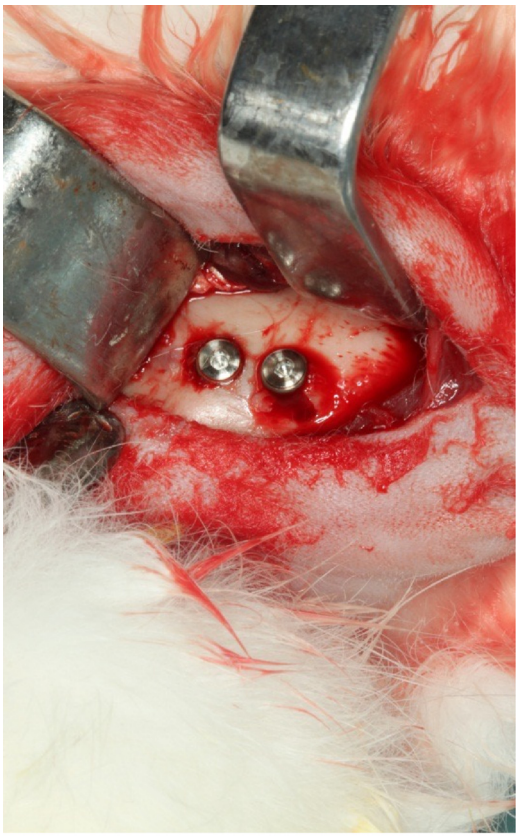

Fig. 3. Implantation and application of hyaluronic acid gel.

contact in each histological section were calculated by measuring the length of the implant that was in contact with bone, mineralised bone, and osteoid. Evaluation of the contact between bone and implant was calculated as the amount of direct contact between mineralised bone and the titanium surface.

Statistical analysis

Normal distribution of the data was confirmed with the Shapiro–Wilk test. To compare the significance of the differences between groups, we used a *t* test for independent samples. Analysis was by the R 3.0.1 software (www.r-project.org) and probabilities of less than 0.05 were accepted as significant.

Results

The operation was well-tolerated by the animals, and there was no conspicuous reduction in body weight. After the animals had been killed we found that three of the 10 control implants had failed and these were therefore excluded. Osseointegration was successful for all other implants, and we found no signs of infection in either group. The newly-formed osteoid matrix and bony tissue were evaluated histologically in both groups (Figs. 4 and 5). There was more extensive osteoid tissue and new bony tissue seen in the experimental group but there were no significant differences between the groups (Fig. 5). The measurements are shown in Table 1.

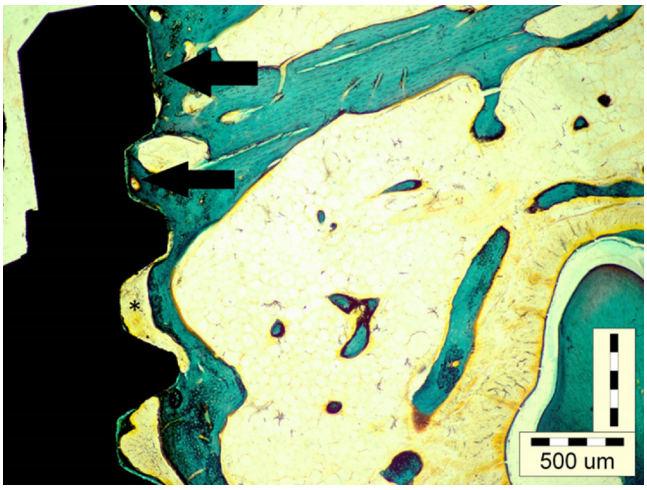

Fig. 4. Histological section from an animal in the control group (Goldner trichrome stain, bar = 500 μm).

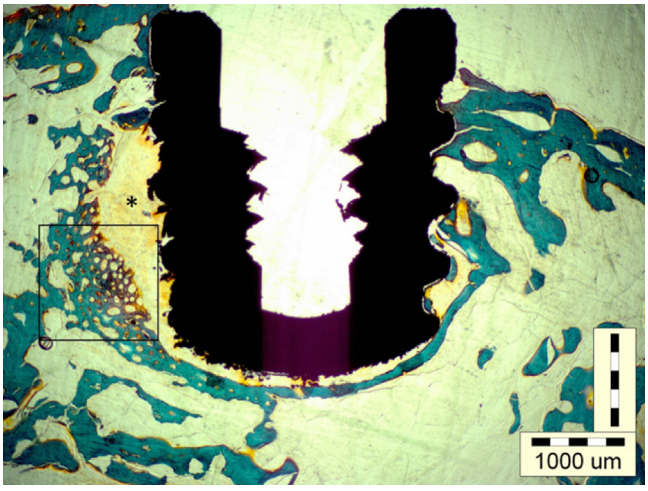

Fig. 5. Histological section from an animal in the experimental group (Goldner trichrome stain, bar = 1000 μm).

Table 1
Histomorphometric analysis. Data are given as mean (SD) μm.

Area	Control group (n = 7)	Experimental group (n = 10)
Bone	2697.7 (1605.8)	3252.3 (1675.7)
Bone plus osteoid	4704.1 (2935.9)	5887.3 (2504.6)

There is no significant difference between the groups.

Discussion

Hyaluronic acid is one of the important glycosaminoglycans in the extracellular matrix, and is synthesised by synoviocytes, fibroblasts, and chondrocytes. It is involved

in cell proliferation and migration, and is a mechanism for tissue repair and for the progression of some malignant tumours. Its main functions are the reduction of inflammation during wound healing, supporting cell proliferation and re-epithelialisation, and the reduction of scarring. It is used in ophthalmology, dermatology, orthopaedics, and rheumatology, and in dentistry it is used for temporomandibular joint disorders, the treatment of oral ulcers, and in surgical operations.

We aimed to investigate its effects in gel form on the osseointegration of dental implants. During osseointegration, the woven bone that formed during the first stage of healing adapts to the loading forces and is converted to lamella bone, which consists of parallel fibres. There are similar stages during the healing of bony defects and osseointegration of dental implants. Many factors that stimulate bone healing therefore have favourable effects on the osseointegration of implants. However, we know of no published study that clearly examines the effects of hyaluronic acid on the osseointegration of dental implants.

A number of publications have examined its effects on bone healing. One of the earliest showed that high-molecular-weight hyaluronic acid functions by effectively protecting osteoinductive growth factors in the local environment because of their physicochemical properties.[9] Through the induction of differentiation of osteogenic cells, bone can accelerate the process of formation of new bone during wound healing.[9] Engstrom et al investigated its effects on the height of alveolar bone and bony healing, and found that there was only 1 mm difference between those treated with hyaluronic acid and controls after 12 months.[10] Other workers have reported that its use in sinonasal cavities and defects in calvarial bone in mice may have osteogenic effects.[11] Aslan et al reported that a bone cavity that was filled with hyaluronic acid and bone grafts healed better than one containing bone grafts alone.[4]

Because we knew that it had favourable effects on the healing of bone, we investigated its effect on the osseointegration of dental implants. Our hypothesis was that hyaluronic acid, known to accelerate osteogenic cell differentiation, would have a favourable effect on the osseointegration of dental implants. Our results showed that, although there was no significant difference between the groups, the experimental group had more extensive osteoid tissue and new bone around the bone-implant interface.

Osseointegration is in direct relation to both the implant surface and the bone, with no collagen tissue or fibroblast matrix. Histological sections from the experimental group showed more osseointegrated areas than those from the control group (Table 1). We think that the effects of hyaluronic acid on osseointegration will be supported by the results of further studies with larger study and control groups.

Peri-implant tissue reactions may be assessed in non-decalcified tissue sections using conventional histological techniques. Embedding specimens of bone in methacrylate offers an important advance in terms of avoiding decalcification, as it maintains bony integrity in the bone:implant contact area. Schwarz et al concluded that using conventional histological staining such as toluidine blue and Masson Goldner trichrome in non-decalcified tissue sections is a valuable way to evaluate the initial and early stages of wound healing around endosseous titanium implants.[12] Gruber reported that Goldner staining had the advantage that it provided superior staining for discriminating between osteoid and mature bone.[13] In our study histological sections were stained with Goldner's trichrome for light microscopy, which resulted in orange/red for osteoid and green/blue for mineralised tissue. The active bone remodelling was apparent from a thick layer of osteoid and an active border of osteoblasts.

The study has some limitations, one of which is the lack of early and mid-term histological examination. The reason for the restriction is that the Animal Ethics Committee would not consent to increasing the number of animals that we could study. Another limitation is the lack of functional loading of osseointegrated implants. This, which is inherent in an animal model, can be overcome by making special prosthetic restorations.

In conclusion, we have shown that the hyaluronic acid may have had a favourable impact on healing of soft tissue and bone. There is a need for further work to be done.

Conflict of interest

We have no conflicts of interest.

Ethics statement/confirmation of patients' permission

Ethics consent was given by the Local Animal Studies Ethic Committee of the University. No patients were studied.

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