

MAXILLARY SINUS AUGMENTATION WITH AUTOLOGOUS BONE ALONE OR IN COMBINATION TO EQUINE BONE: A COMPARATIVE HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY IN MAN



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ABSTRACT

Aim: Rehabilitation of the edentulous posterior maxilla with dental implants may be a problem due to insufficient bone volume. The purpose of the present study was to perform a comparative histological and immunohistochemical evaluation of microvessel density (MVD), vascular endothelial growth factor (VEGF) and nitric oxide synthase (NOS) expression in maxillary sinus augmentations using autologous bone alone and in combination to equine bone.

Materials and Methods: A total of 16 patients participated in this study. All patients underwent maxillary sinus augmentation with autologous bone (n= 3 patients) and a 50:50 mixture of autogenous bone and equine bone (n= 7 patients). Control cores were harvested from pre-existing non augmented bone under the sinus floor (n = 6 patients). A total of 16 specimens were retrieved and evaluated.

Results: In all the groups the greatest values of MVD were always found in the sites grafted with autologous bone. When comparing the different graft materials statistically significant differences in the MVD values were found between control group vs autologous (P < 0.01) and control group vs autologous + equine (P < 0.01). The higher and lower intensities of VEGF and NOS3 expression were prevalent in the sites grafted with autologous bone with statistically significant differences between control group vs autologous (P < 0.05). No statistically significant differences (P > 0.05) were found when comparing high and low intensities of NOS1 expression among the groups.

Discussion and Conclusions: The results obtained showed that the mixture of autologous and equine bone used was biocompatible, and its use was associated with new blood vessels ingrowth during healing, which has been found to be extremely important for bone formation.

INTRODUCTION

Rehabilitation of the edentulous posterior maxilla with dental implants may be a problem due to insufficient bone volume following edentulism and pneumatization of the maxillary sinus. In this anatomical situation, it can be very difficult to obtain primary stability; therefore, a sinus augmentation procedure, with the use of bone grafts, may then be necessary. The aim of the present study was to histologically and immunohistochemically evaluate the performance of autologous bone used alone and in combination to equine bone in maxillary sinus augmentation procedures in man.

MATERIALS AND METHODS

A total of 16 patients participated in this study. All patients underwent maxillary sinus augmentation with autologous bone (n= 3 patients) and a 50:50 mixture of autogenous bone and equine bone (n= 7 patients). Control cores were harvested from pre-existing non augmented bone under the sinus floor (n = 6 patients). A total of 16 specimens were retrieved and evaluated.

All the specimens were fixed in formalin (10% neutral buffered formalin). The specimens were then put in 70% alcohol solution, dehydrated and embedded in paraffin. Three micron sections were subsequently obtained with a Leitz 1512 microtome and all the sections stained with Hematoxylin-Eosin. The immunohistochemical staining of CD31, VEGF, NOS1 and NOS3 was performed using the strep-ABC (Streptavidine-Biotine-Peroxidase) method. In order to unmask the antigens, a microwave oven and a 2.1% content of citric acid was used related to the antibodies, CD31 (1:50), VEGF (1:100), NOS1 (1:150), NOS3 (1:100) (Novocastra, Newcastle Upon Tyne, UK).

The CD31, VEGF, NOS1 and NOS3 were evaluated using a quantitative method.

A light microscope (Leica DMR, Leica Microsystems, Milano, Italy) was used to perform the evaluations. This optical system was associated with a digitizing pad and a histometry software package with image-capturing capabilities (Leica, Qwin V3, Leica Microsystems, Milano, Italy). Regarding VEGF, NOS1 and NOS3 the evaluation was conducted after having distinguished two different intensities of the expression of low and high; the intensities were recognized by the PC software as green and red, respectively.

Results were statistically analyzed using Dunn's Multiple Comparisons Test, for independent samples, where statistically significant differences were accepted as p < 0.05. All the measurements were expressed as a mean ± standard deviation.

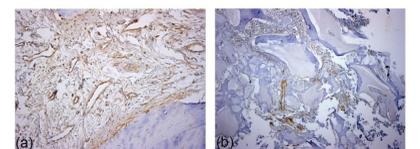
RESULTS

Immunohistochemical data

MVD

In all the groups examined, greater values of MVD were always found in the sites grafted with autologous bone. The MVD count was performed in the areas surrounding the newly formed bone

	Mean ± SD
Autologous bone	34.66±2.78
Autologous+equine bone	32.28±3.10
Control	24.16±2.45



Autologous vs. Autol + equine ns P>0.05

Autologous vs. Control*** P<0.001

Autol. + Equine vs. Control*** P<0.001

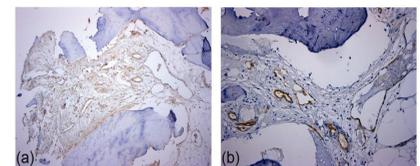
*** significance

ns not significant

VEGF

Vascular endothelial growth factor was expressed in all groups examined with different intensities of expression among the groups. The evaluation was performed at the level of the endothelial cells lining the vessels

	High (Mean ± SD)	Low (Mean ± SD)
Autologous bone	71.67±7.64	28.33±7.64
Autologous+equine bone	59.29±17.66	40.71±17.66
Control	36.67±15.38	63.33±15.38



HIGH INTENSITY:

Autologous vs. Autol + equine ns P>0.05; Autologous vs. Control* P<0.05; Autol. + Equine vs. Control ns P>0.05

LOW INTENSITY:

Autologous vs. Autol + equine ns P>0.05; Autologous vs. Control* P<0.05; Autol. + Equine vs. Control ns P>0.05

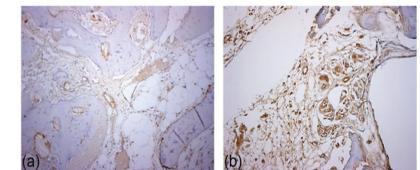
* significance

ns not significant

NOS3

NOS3 was expressed in all groups examined with different intensities of expression among the groups. The evaluation was performed at the level of the endothelial cells lining the vessels

	High (Mean ± SD)	Low (Mean ± SD)
Autologous bone	77.33±5.77	26.66±5.77
Autologous+equine bone	60.71±16.69	39.28±16.69
Control	33.33±17.22	66.66±17.22



HIGH INTENSITY:

Autologous vs. Autol + equine ns P>0.05; Autologous vs. Control* P<0.05; Autol. + Equine vs. Control ns P>0.05

LOW INTENSITY:

Autologous vs. Autol + equine ns P>0.05; Autologous vs. Control* P<0.05; Autol. + Equine vs. Control ns P>0.05

*** significance

ns not significant

NOS1

NOS1 was expressed in all groups examined. The evaluation was performed at the level of the endothelial cells lining the vessels. No statistically significant difference (P> 0.05) were found when comparing high and low intensities of NOS1 expression among the groups.

DISCUSSION AND CONCLUSIONS

The sinuses augmented with autologous bone in combination to equine bone showed similar immunohistochemical results when compared with the sinuses augmented with autologous bone alone. Therefore, the present data point out that the use of autologous bone alone and the related discomfort for patients could be avoided as the equine bone substitute demonstrated to be a good alternative. Indeed, the results obtained showed that the equine graft material used was biocompatible, and its use was associated with new blood vessels ingrowth during healing, which has been found to be extremely important in bone formation.