

Sinus lift with autologous bone only or in conjunction with equine bone: comparative histological and immuno-histochemical study on patient

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SCIENTIFIC REPORT

Introduction

The rehabilitation of an edentulous posterior maxilla and subsequent implant placement is a very often problematic procedure due to insufficient bone volume resulting from edentulism and pneumatization of the maxillary sinus. Under these anatomical conditions, it is truly difficult to obtain primary implant stability. It might therefore be necessary to intervene with a surgical procedure for sinus lift and bone material graft. The purpose of this study was to evaluate with histological and immunohistochemical tests the efficacy of grafting autologous bone alone and in conjunction with equine bone in sinus lift *in vivo*.

Materials and methods

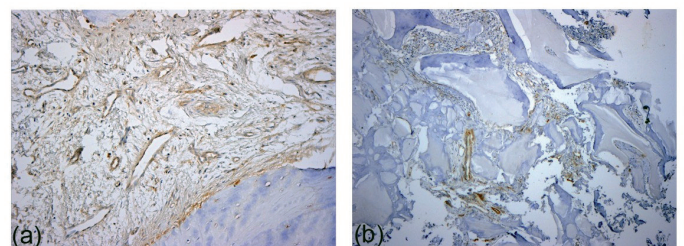
16 patients underwent a surgical sinus lift procedure. Three of them were grafted with autologous bone only, 7 a mixture (50:50) of collagenated equine bone (OSP-OX34) and autologous bone. Control-biopsies were taken from the remaining 6 patients, near the site to be regenerated. Sixteen biopsy samples were collected in total. The biopsies were preserved in formalin (10%) and subsequently dehydrated in a 70% ethanol solution and fixed in paraffin. A microtome (Leitz 1512) was used to obtain 3 μ m sections, subsequently fitted on slides and stained with H&E. Immunohistochemical staining of CD31, VEGF, NOS1 and NOS3 was obtained by the strep-ABC method (*Streptavidine-Biotine-Peroxidase*). To highlight the antigens, antibodies for CD31, VEGF, NOS1, NOS3 were used (Novocastra, Newcastle Upon Tyne, UK). The results were evaluated by optical microscope (Leica DMR, Leica Microsystems, Milan, Italy). This optical system was associated to a specific software for histomorphometric analysis that measured the intensity of the color (Leica, Qwin V3, Leica Microsystems, Milan, Italy). For VEGF, NOS1 and NOS3 the tests were conducted after distinguishing two different densities of high and low expression. Microvascular density (MVD) was then measured. The results were then statistically analyzed with *Dunn's Multiple Comparisons Test* ($p < 0.05$). All measurements were expressed as average \pm standard deviation.

Results

Immunohistochemical data

MVD

A high MVD value was measured in all groups where autologous bone was grafted. The MVD measurement was performed in the areas surrounding the new bone tissue formation.



Immunohistochemical sections for MVD evaluation.
Autologous bone (a) autologous bone + equine bone (b).

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

VEGF

VEGF is expressed in all groups examined with varying intensity. The evaluation was carried out at the endothelial cells lining the vessels.

	HIGH (Average ± SD)	LOW (Average ± 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

NOS3

NOS3 is expressed in all groups examined with different intensity. The evaluation was carried out at the endothelial cells lining the vessels.

	HIGH (Average ± SD)	LOW (Average ± 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

NOS1

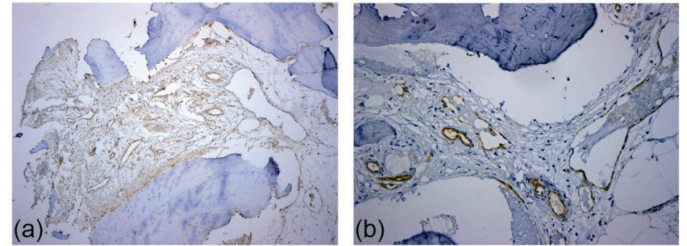
NOS1 is expressed in all examined groups. The evaluation was performed on endothelial cells lining the vessels. There are no significant differences ($p > 0.05$) in NOS1 expression in the groups considered.

Discussion and conclusions

From an immunohistochemical point of view, using a combination of autologous and equine bone or autologous bone only produces comparable results. This evidence, jointly with the observation that equine grafts are biocompatible and their implant is attended by progressive angiogenesis, indicates that equine bone may provide a valid alternative to autologous bone in sinus lift.

Autologous vs Autologous + Equine ns $p > 0.05$
 Autologous vs Control*** $p < 0.001$
 Autologous + Equine vs Control*** $p < 0.001$

* significant ns non significant



Strep ABC VEGF 10x: (a) VEGF Control group (b) VEGF Autologous bone.

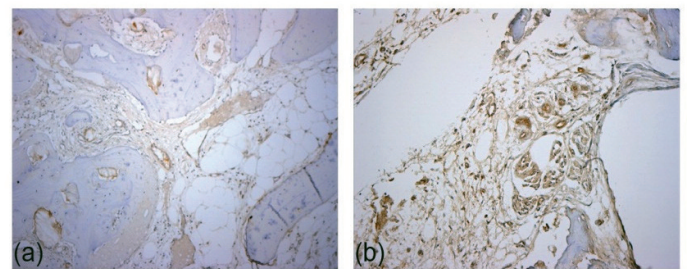
HIGH INTENSITY:

Autologous vs Autologous + Equine ns $p > 0.05$;
 Autologous vs Control* $p < 0.05$;
 Autol. + Equine vs Control ns $p > 0.05$

LOW INTENSITY:

Autologous vs Autologous + Equine ns $p > 0.05$;
 Autologous vs Control* $p < 0.05$;
 Autologous + Equine vs Control ns $p > 0.05$

* significant ns non significant



Strep ABC NOS3 10x: (a) NOS3 Control group (b) NOS3 Autologous bone.

HIGH INTENSITY:

Autologous vs Autologous + Equine ns $p > 0.05$;
 Autologous vs Control* $p < 0.05$;
 Autologous + Equine vs Control ns $p > 0.05$

LOW INTENSITY:

Autologous vs Autologous + Equine ns $p > 0.05$;
 Autologous vs Control * $p < 0.05$;
 Autologous + Equine vs. Control ns $p > 0.05$

* significant ns non significant