CLINICAL EVALUATION OF PREFORMED BONE SERIES BIOPLANT – BIOPLANT ELASTA – OSTEOGEN

INTRODUCTION

The correct clinical evaluation of a medical surgical presents considerable difficulty of standardizing the method of exposure, due to considerable heterogeneity of products that make up this category and also for the substantial differences in the method of preparation of products of similar categories.

I personally think that in this Annex should also be included in Annex X (risk analysis according to ISO 14971:2012) which it contains many useful elements to provide a complete clinical evaluation such as residual risk the possible contraindications.

Given what is described in the introduction I believe that the elements to be considered for this type of product are as follows:

1. Morphological analysis structural highlighting the mechanical characteristics as a function of the biological environment of grafting.
2. For bone tissues of animal origin applicable to humans, the level of deantigenizzazione standardized reached in preparation.
3. The interaction of the product with the biological system reference: Angiogenesis - Osteoblasts - Osteoclasts.
4. The type of rehash (physiological / non-physiological)
5. The timing of the biological response
6. Clinical cases confirming the steps 1 to 5.

For the evaluation of the points listed above will make use of publications and laboratory analysis with appropriate explanatory comments. Being very often these works extended and include most purposes, the most relevant parts are highlighted.

1 Morphological analysis highlighting the structural mechanical properties as a function of the biological graft.

Istituto Superiore di Sanità
Scanning electron microscope and 3D microtomography observations of equine bone.
Rossella Bedini, Pietro Ioppolo, Raffaella Pecci, Perla Filippini, Salvatore Caiazza, Alessandra Bianco, Gioele Columbro
2005, 38 p. Rapporti ISTISAN 05/37 (in Italian)
The aim of this work was to analyze some grafts for bone reconstructions from equine bone tissue, industrially producted, by means of 3D micro-tomography and Scanning Electron Microscope (SEM) images. These samples have been treated by the manufacturer in a suitable way: they have been deantigenized and some of them have been demineralized. In order to evaluate the quality of the deantigenization and demineralization system the analysis was made, to measure the porosity of the samples and to estimate the new micro-tomographyc method. The 3D microtomography technique with Skyscan instrumentation allows to perform structural observations without any particular treatment, alteration or damaging of the sample. Moreover it is possible to perform an acquisition with a 3D reconstruction of the sample, both before and after a test or an implant, for example, into an animal, because this technique does not alter the tested object in any way. Results show that the 3D micro-tomography technique, with Skyscan 1072 instrumentation, could be considered as a valid and effective alternative to the SEM observation.
Key words: Equine bone, Bone reconstructions, Micro-tomography, SEM

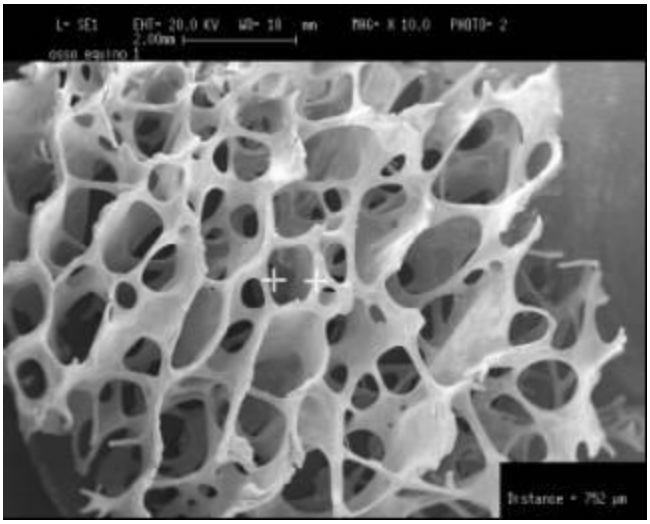


Figure 1. SAMPLE 1: SEM micrograph of bone equine Deantigenizzato at a magnification of 10X

Figure 1 shows the bone equine deantigenizzato at low magnification (10X). Already at this magnification can highlight precisely the trabecular bone. In this case was chosen a section of bone with a rather high porosity in order to observe the internal structure

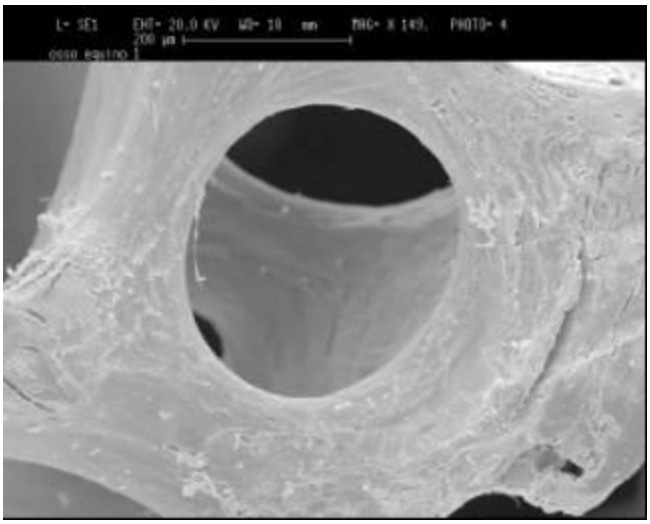


Figure 2. SAMPLE 1: SEM micrograph of bone equine deantigenizzato at a magnification of 149x

Figure 2 shows a detail of the bone section previously chosen. Magnification 150 X allows us to highlight the single bone porosity and any changes in its structure and also a possible presence of residues.

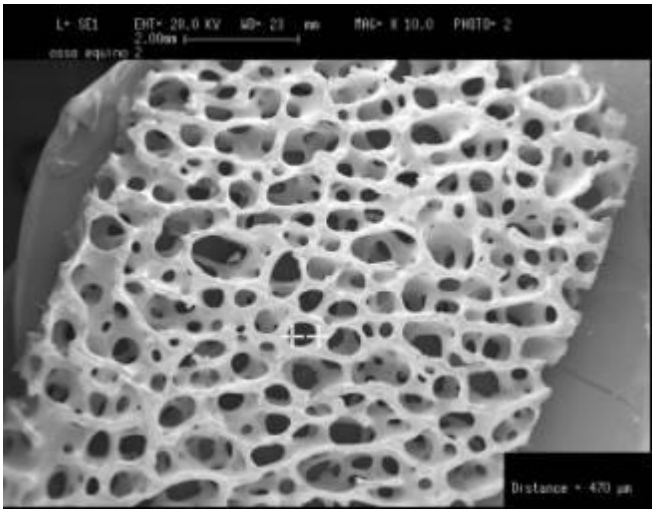


Figure 3. SAMPLE 2: SEM micrograph of bone equine Deantigenizzato at a magnification of 10X

The photomicrograph of Figure 3, at low magnification (10 X), there is a section of bone with porosities well below that shown in Figures 1 and 2. Even in this case is It has been possible to make a measurement of the average size of the porosity that results to beof 435 + 58 microns.

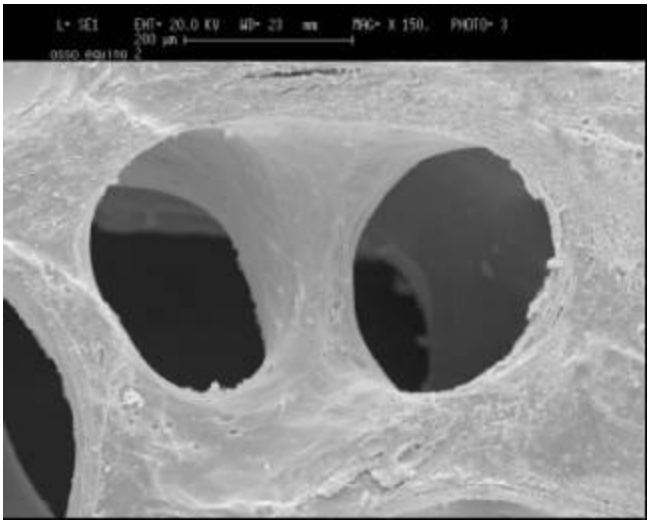
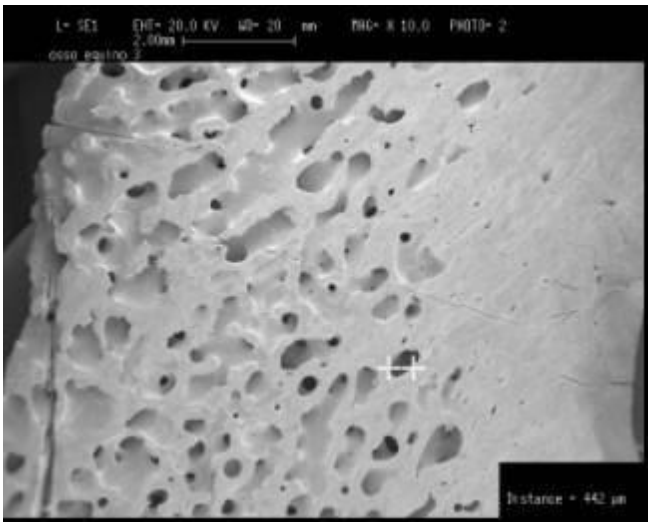


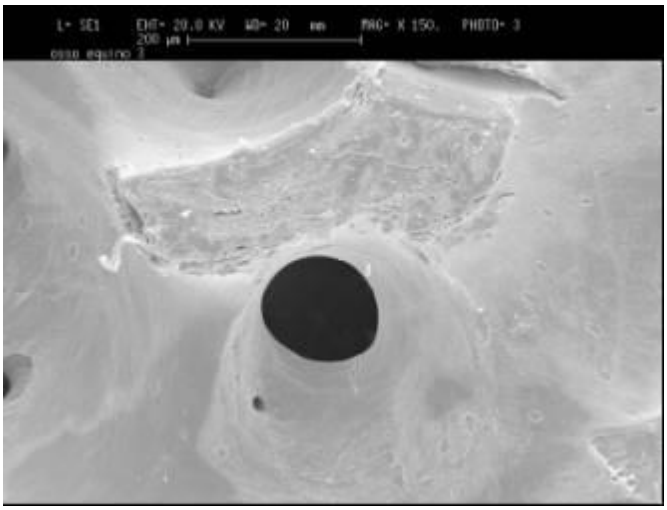
Figure 4. SAMPLE 2: SEM micrograph of bone equine deantigenizzatoat a magnification of 150

n Figure 4, at a magnification comparable with the detail shown in Figure 2 (150X), due to the greater compactness of the bone of this sample, it was possible evaluate a greater number of pores simultaneously.



In this image of Figure 5 it was observed, at 10 X magnification, a section of bone particularly compact, in which it was possible to highlight the gradual transition between bone Compact and spongy bone. To the measurements, the size of porosity maintain however, an average diameter very similar to that of the sample analyzed previously, resulting in this case of 439 + 32 microns.

Figure 5. SAMPLE 3: SEM micrograph of bone equine deantigenizzatoat a magnification of 10X



The detail that can be observed in Figure 6, at high magnification (150 X) puts in evidence, despite the size of the porosity still high, the extreme compactness bone. It is a lower presence of holes, and then the distances between the porosity are more than the structures of the two samples previously photographed and analyzed.

Figure 6. SAMPLE 3: SEM micrograph of bone equine Deantigenizzato at a magnification of 150X

Discussion :

The technology in the design of prosthetic aids and medical devices has reached today a high technical level can restore the loco-motor functions of patients in time fast and with very satisfactory results.
The adoption of these systems often, however, is hampered by unfavorable conditions of the tissues that must accommodate them.
For these reasons, a careful analysis of the device must be done before implantation in the body the aim of our experimental analysis is to verify the status of mineralization, to measure the porosity and to assess the quality of deantigenizzazione sample.
These three assessments are all three fundamental for the success of the system; in fact, the state of mineralization of the sample affects its mechanical strength, the deantigenizzazione is important because a three-dimensional structure free from residues organic enables quick and effective formation of new vessels (angiogenesis) carriers apparatus cell act to bone remodeling, an accurate estimate of the size of the pores it is useful because the pores are too small (diameter less than 100 microns) prevent the regrowth of the fabric.
For these analyzes were produced of SEM images and images micro-CT using adequate instruments.
Were analyzed by SEM three different samples (Figures 1, 3 and 5). It is noted that each has a porosity different from the other, and in particular is very visible that the third sample of tiny pores.
In Figures 2, 4 and 6 have been reported of the details of the samples at a magnification appropriate to analyze the three-dimensional structure (enlargements are 150 X and, of numerous facts, it shows only one sample for illustrative purposes).
In all three samples it is possible to appreciate a three-dimensional structure devoid of residues organic (on trabecular bone does not appear any trace of contaminant).
Furthermore, always from the previous picture, it is possible, in the first analysis, say that the pores have a diameter much greater than 100 microns.
For a complete analysis of its internal structure, it is necessary to change instrumentation and analyze the sample using X-ray microtomography.
Looking at the images similar to those taken by SEM shown in Figure 1-6, but produced by microtomography for comparison (Figure 7-12), you can draw the same conclusions listed first. However, for the Figures 1-6 we had to cover the samples with a layer of conductive material, making them unavailable for medical and surgical applications for which they were designed, while Figures 7-12 they have been obtained without any change inso the test samples. Furthermore, from a comparison between Figures 1-6 and 7-12 it is possible appreciate the nature of 3D micro-CT images.
But what we propose to do with this further analysis is, as mentioned, an examination of the internal structure of samples without damaging the same.
For each slide you can make some further enlargements without loss of quality picture and from these it is possible to appreciate an internal structure free of organic residues and with a pore size that meets the conditions of implantation; Furthermore, it is seen that the porosity in the three samples is uniform and that the third is visibly less porous.
From previous images, also, we note that the trabecular bone appear less dark in the first and second sample compared to the third. From here it is possible to conclude that the first two they were demineralized, while the third is still mineralized.

Furthermore, the uniformity of the color of the trabeculae is seen that in the first and second sample there are no accumulations of residues apatites.
From these images it can be concluded that all the samples meet the criteria of the project and

implantability conditions:

- **the first two samples were deantigenizzati successfully, They have a high porosity and are demineralized; for these characteristics can be used for reconstructions skeletal not subject to functional load and for applications in which it is requiredadaptability of the structure of the support;**
- **the third sample was deantigenizzato successfully, has a low porosity and is mineralized; for these characteristics can be used for reconstructions skeletal subject to functional load.**

These features comply with those listed in the technical product and therefore they can be used for the purposes for which they were designed.

2. Level deantigenizzazione standardized reached in preparation.

LABORATORIO CHIMICO
CAMERA COMMERCIO TORINO
Azienda Speciale della Camera di commercio di Torino

Rapporto di prova **2013/6734**
Torino, lì 10/01/2014
Data di arrivo 11/12/2013
Pagina 1/1

Spett.le
MAGGI srl
Via Tetti Castagno n° 5/A
ANDEZENO
10020 TO

CAMPIONE: **TESSUTO OSSEO BOVINO OSTEOGEN**
tipologia: granulato spngioso 0,5/1 mm.
lotto: 13001
CAMP.TORE: Committente

Parametri determinati	Valore rilevato	Unità di misura	Valore limite	Metodo di prova	Inizio - Fine Analisi
* SOSTANZA GRASSA	N.R.< 0,50	g/100 g		UNI ISO 1443:1991	08/01/2014 10/01/2014

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LAB N° 0064

3. The interaction of the product with the biological system reference: Angiogenesis -Osteoblasts - Olsteoclasts.

Osteoplant acts on stem cells derived from peripheral blood.

Sollazzo V[1], Palmieri A, Girardi A, Zollino I, Brunelli G, Spinelli G, Carinci F.
Author information

Abstract

OBJECTIVES:

The osteoplant is an equine, flexible, heterologous, deantigenic, cortical, and spongy bone tissue, totally reabsorbable, used for implantation of bone tissue, to restore skeletal, even weight-bearing structures. However, how the osteoplant alters osteoblast activity to promote bone formation is poorly understood.

MATERIALS AND METHODS:

To study how the osteoplant induces osteoblast differentiation in mesenchymal stem cells, the expression levels of bone-related genes, and mesenchymal stem cell markers are analyzed, using real time Reverse Transcription-Polymerase Chain Reaction (RT-PCR).

RESULTS:

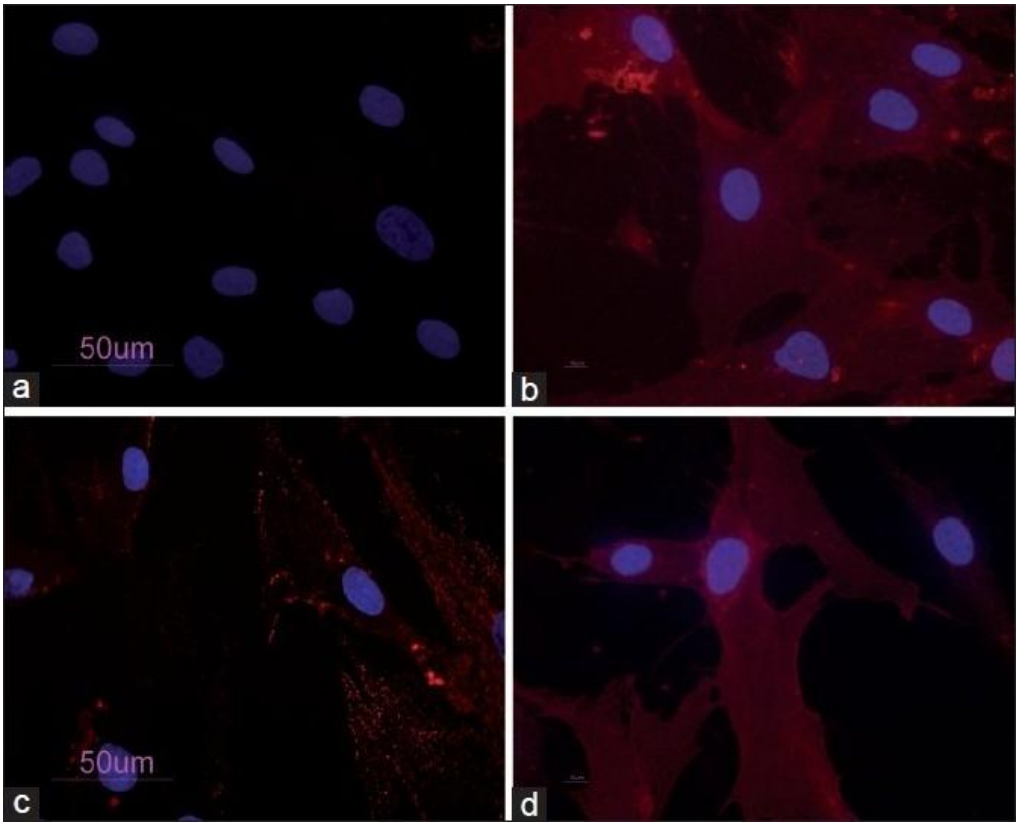
The osteoplant causes induction of osteoblast transcriptional factors such as osterix (RUNX2), and of bone-related genes such as osteopontin (SPP1) and osteocalcin (BGLAP). In contrast the expression of ENG (CD105) is significantly decreased in stem cells treated with osteoplant, with respect to untreated cells, indicating the differentiation effect of this biomaterial on stem cells.

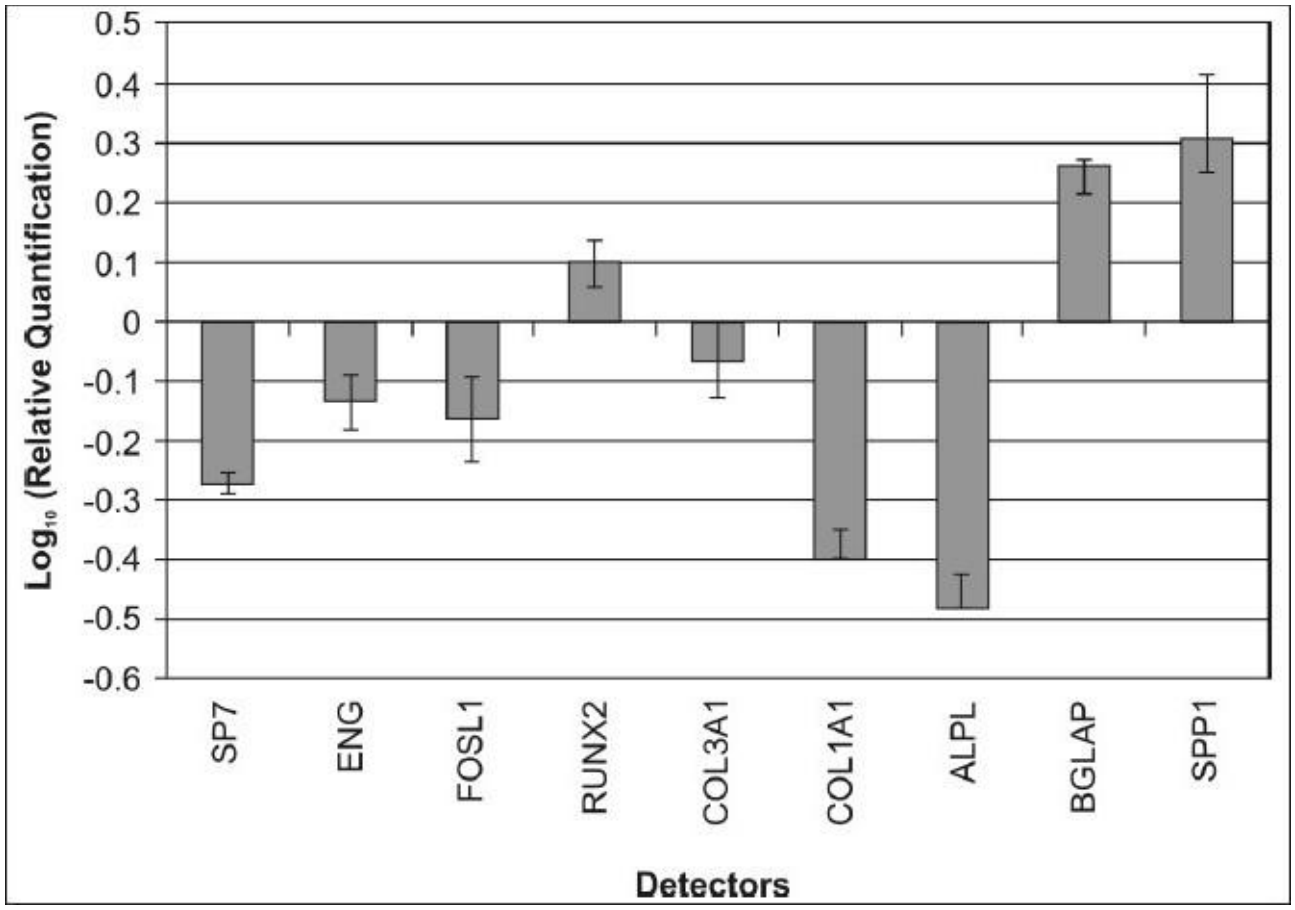
CONCLUSION:

The obtained results can be relevant to better understand the molecular mechanism of bone regeneration and as a model for comparing other materials with similar clinical effects.

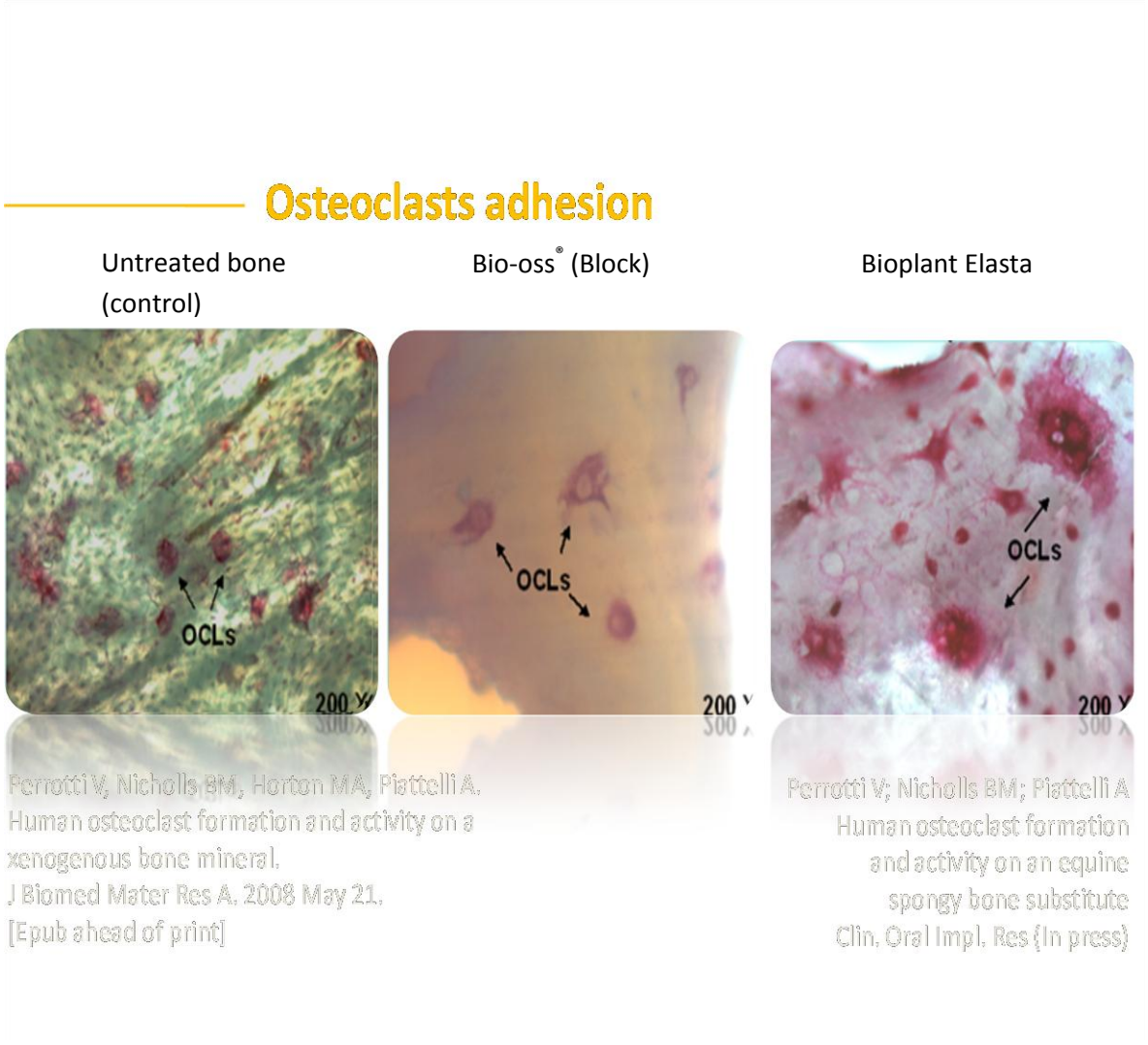
KEYWORDS:

Gene expression; bone; osteoplant; stem cell




0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
Log10 (Relative Quantification)
SP7
ENG
FOSL1
RUNX2
COL3A1
COL1A1
ALPL
BGLAP
SPP1
Detectors

4. La type of rehash (physiological / non-physiological)



Perrotti V, Nicholls BM, Horton MA, Piattelli A.
Human osteoclast formation and activity on a xenogenous bone mineral.

J Biomed Mater Res A. 2008 May 21.
[Epub ahead of print]

5. Biological response times

MOMENTI E ASPETTI DEL RIMANEGGIAMENTO OSSEO

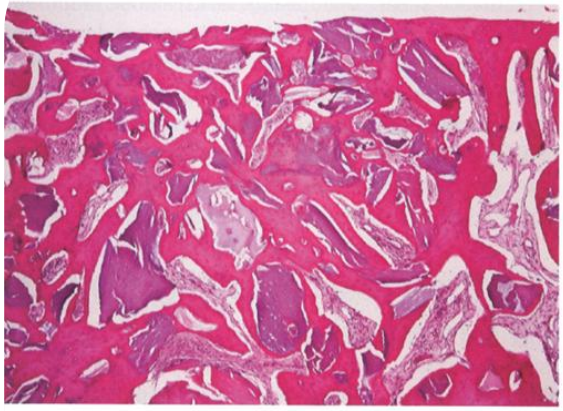


Fig. 7.20 Tessuto osteoide con materiale eterologo collagenato di origine equina.

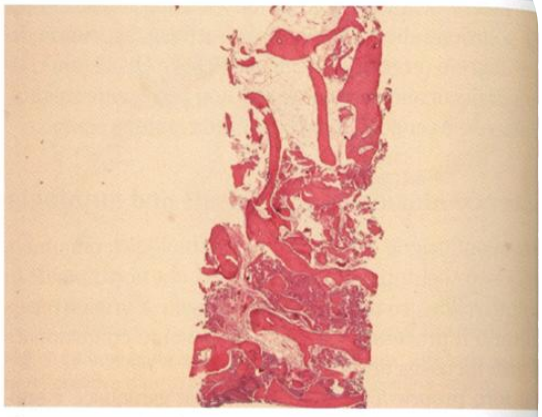
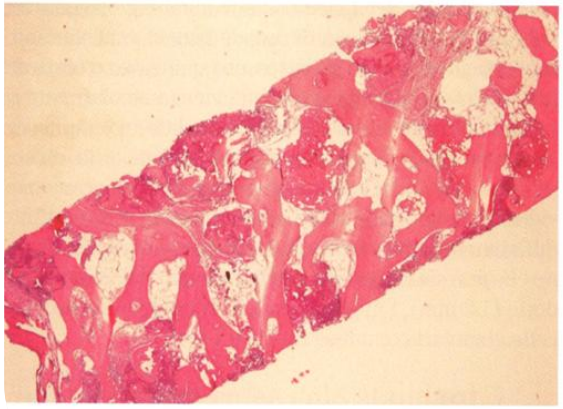


Fig. 7.21 Esame istologico a 3 mesi: granuli di osso eterologo pr senti in grande quantità.



ig. 7.22 Esame istologico a 6 mesi: presenza di osso neoformato inore quantità di osso eterologo.

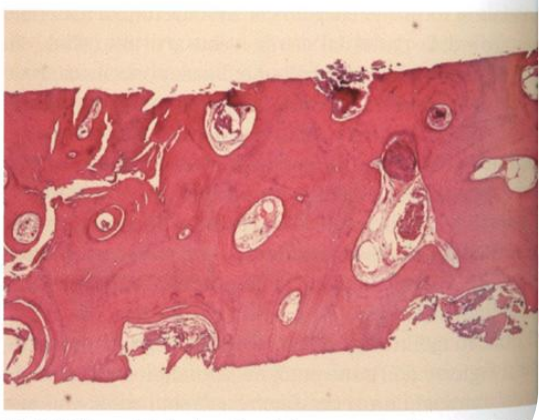


Fig. 7.23 Esame istologico a 9 mesi: fase avanzata di maturazione presenza di osso neoformato e scarsa quantità di osso eterolo