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Investigation of deer antler as a potential bone regenerating biomaterial

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Bone regeneration using biomaterials has become a widely used technique for surgical restoration of bone defects. No biomaterial in use displays ideal characteristics and efficacy for bone formation. In search for new biomaterials, interest was focused on the remarkable regeneration properties of deer antler. The study compares the structural units identified in deer antler tissue, a tissue with unique capacity of self-regeneration, with clinically used Gen-Os biomaterial in order to evaluate the potential use of the deer antler as a bone regeneration biomaterial. These results can be the premises of a new development in the field of grafting materials, opening new perspectives towards future possible combinations of this material with other biomaterials used as bone substitutes.

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1. Introduction

One of the most stringent problems in human medicine is the challenge of bone reconstruction. Situations of bone loss are frequently encountered for various reasons related to trauma, bone diseases and infections, bone tumors, congenital anomalies, as well as age-related degeneration and atrophy.

Bone regeneration is promoted by mechanisms of osteoconduction (bone growth from existing bone by stimulation of osteoblasts to form new bone), osteoinduction (stimulation of mesenchymal cells to differentiate into osteoblasts) and osteogenesis, i.e. bone formation in sites where bone did not exist previously.

The search for a higher quality biomaterial is currently investigating new potential alternatives to the products on the market, as no biomaterial in use displays ideal characteristics and efficacy for bone formation.

The study focused on the remarkable properties of deer antler. The pattern of deer antler growth has initiated a multitude of scientific debates and research over its unique natural model of rapid and complete bone regeneration [1]. Research has been focused on various parameters triggering and guiding antler growth in order to understand it and subsequently influence the adult human healing pattern, which consists of cicatrisation (healing), rather than regeneration and (re-)growth [2].

The purpose of the investigation was to examine the ultrastructure of the deer antler versus clinically validated biomaterial Gen-Os, a collagenized porcine bone heterograft, in order to determine whether there are structural correlations between these biological materials of animal origin, with the intention that after gamma sterilization deer antler bone could be further evaluated in order to assess its structural similarity and differences from other conventional bone regeneration biomaterials.

2. Experimental

Antler tissue from male red deer (Cervus elaphus carpaticus) was prepared in powder samples. Gen-Os® (Tecnoss, Torino, Italy) samples of clinical use is sterilized with gamma rays and for this reason the investigated deer antler bones were also gamma irradiated (for 33 hours, average absorption dosis 32,7 ± 1,9 kGy). The irradiation effect was studied by electron spin resonance (EPR). EPR spectra were recorded at room temperature in X band, with 4 G modulation amplitude, on an ADANI spectrometer.

Scanning electron microscopy and energy dispersive X-ray spectroscopy (SEM/EDX) were used to explore the morphology and to determine the elemental composition of the investigated samples (LEO 1530 microscope).

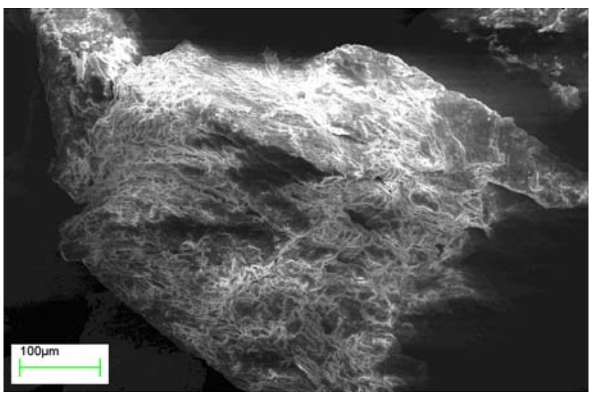
Structural units and functional groups entering the samples were investigated by attenuated total reflection Fourier transform infrared (ATR- FTIR) and by Raman spectroscopy. ATR- FTIR spectra were recorded with a resolution of 2 cm^{-1} on a Bruker spectrophotometer at room temperature. Raman spectra of bone powders were recorded on a BrukerRaman spectrometer FRA 106/9 using 1064 nm excitation laser source.

3. Results and discussion

Modern surgical bone restoration relies on three basic techniques: autologous transplants, guided bone regeneration techniques using biomaterials and guided bone distraction (callus elongation) [3]. There is a large requirement of developing methods to increase the self-healing capacity of the human bone [4].

For this reason, experimental works have been conducted on the use of biomaterials as scaffolds for living cells promoting bone regeneration [5-7].

Research attempted to understand the properties of the deer antler regeneration, the only mammalian tissue capable to fully regenerate annually at a tremendous growth rate. Regeneration of the antler is a stem-cells based process, almost a recapitulation of the initial antler formation [8]. Antler growth is regulated by various growth factors, enzymes, hormones [2], by regulated mechanisms of cell growth and cell death and by changes in the hormonal balance.



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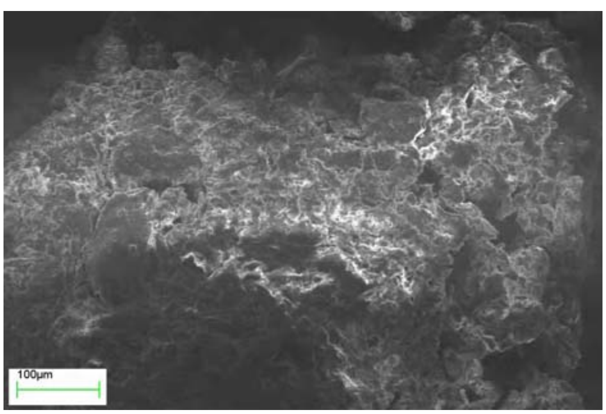

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Fig. 1. SEM picture of deer antler (a) and pork (b) bones.

The preliminary findings in our research group represent strong indications that with adequate preparation, the antler tissue can be subjected to further experimental testing of its bone-inducing capacity in animals [9, 10]. The acquired information can enrich medical knowledge in two directions: *i)* to transpose the growth pattern to increase the capacity for bone self-regeneration and *ii)* to establish the role of deer antler tissue as a bone-growth inducing biomaterial.

The microstructure of the deer antler bone is characterized by larger pores than the pork bone (Fig.1) and seems more fibrous.

The porous structure is a remnant demonstrating the presence of spaces filled with richly vascularised bone marrow in the living animal. This in turn reveals its high turn-over and regeneration capacity.

High porosity is generally accepted to be influencing faster resorption in biomaterials transplanted in vital bone for defect reconstruction.

It is also a favorable parameter of a biomaterial for promoting osteoconduction in living bone.

The pore size is used to assess the capacity of bone regeneration biomaterials to be used as scaffolds for multiplication of living cells. A large pore size allows for a good cell attachment, as well as spread and proliferation of the osteoblastic-like cells [11].

The high porosity of the deer antler could also stimulate cell colonization and multiplication.

The ratio between the number of calcium and phosphorus atoms in hydroxyapatite, the mineral phase of the bone is 1.67. The EDX data for Ca/P ratio is 1.47 for deer antler bone and 1.93 for pork bone, evidencing the higher phosphorus content in the first case.

The influence of the phosphorus content on the crystallization and bioactivity of biomaterials has been demonstrated [11]. It has been shown that phosphorus induces crystallization of calcium phosphate phases, the stabilization of the wollastonite phase at high temperature and produces a heterogeneous distribution of defects in the pieces. The biomaterials containing high levels of phosphorus show the fastest formation rate of an apatite layer in a simulated body-like environment.

Neither deer antler nor pork bones evidence any EPR signal before gamma irradiation. The EPR spectra recorded from Gen-Os and irradiated deer antler bone are shown in Fig. 2. Gamma irradiation induces paramagnetic defects. The two spectra are very similar and consist of a doublet with $g_{\parallel} = 2.03$ and $g_{\perp} = 2.04$ and the hyperfine constant $A \approx 5$ G. The doublet could be attributed to an electron trapped at a nonbridging oxygen vacancy [12]. The doublet structure is the result of the electron interaction with the nearest neighbour ^{31}P nucleus (100 % natural abundance, $I = 1/2$). The defect electron state is assumed to be the nonbonding phosphorous *sp* hybrid orbital. On the basis of this assumption the hyperfine isotropic interaction is attributed to the neighbourhood of the phosphorous ion and the oxygens to which it is bonded.

The calculated value for such doublets was found [13] to range from 2.005 to 2.040, which is in good agreement with the obtained values from the EPR spectra of the investigated bone samples.

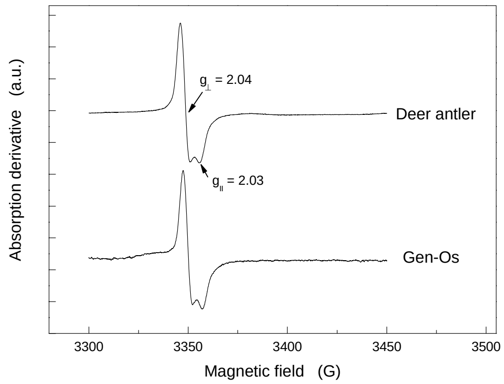

Fig. 2. EPR spectra of Gen-Os and gamma irradiated deer antler bone.

Infrared and Raman spectroscopy evidence features both for organic and inorganic components of bone samples. Some of these features are better evidenced in IR spectra, some of them in the Raman ones. The absorption bands occurring in IR spectra are shown in Fig 3. The intense bands around 3420 cm^{-1} are due to the water content of bone samples. The IR bands centered at 1646, 1535 and 1239 cm^{-1} are assigned to the amide groups [14].

The antisymmetric ν_3(P-O) stretching mode leads to the absorption peak at 1020 cm^{-1} and a shoulder around 1100 cm^{-1} and a second one around 960 cm^{-1}. These vibrations inform on PO_4 structural units of the inorganic bone phase, i.e. hydroxyapatite phase [15]. The amides I (around 1600 cm^{-1}) and II (around 1535 cm^{-1}) bands as well as the CH_2 and CH_3 peaks (1410 and 1440 cm^{-1}) occur in different ratios in the investigated samples.

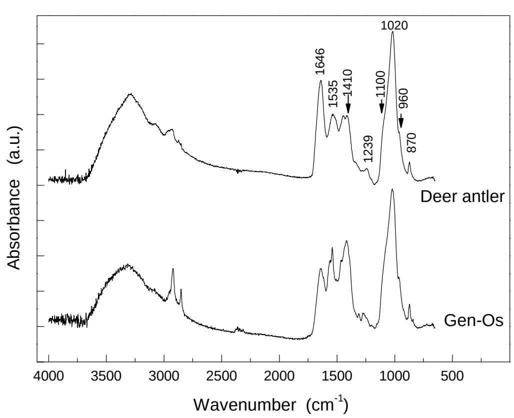

Fig. 3. ATR-FTIR spectra of deer antler bone and Gen-Os.

At the same time, this change is also observed in the Raman spectra (Fig. 4). On the other hand, the ν(CH) bands, characteristic to vibrations of methyl and methylene groups, in the 2800-3000 cm^{-1} domain, are better evidenced in the Raman spectra. The ratio between the area of these bands and that of $\nu_1[PO_4]^{3-}$ band centered at 960 cm^{-1} is lower in Gen-Os than in deer antler bone, that denotes a higher organic phase content in deer antler bone.

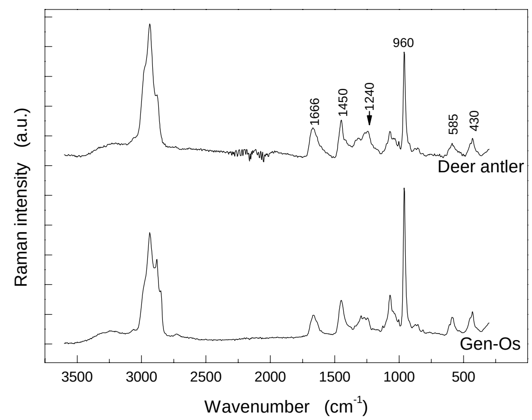

Fig. 4. Raman spectra of deer antler bone and Gen-Os.

The influence of heat treatment on biomaterials [16] is used to enhance their cristallinity and biologic behavior (bone regeneration). Previous studies show that deer antler maintains a cristallinity value similar to the one of human bone after heat treatment [9].

4. Conclusions

The gamma irradiation imposed to potential biomaterials induces in deer antler bone the same type of stable defects evidenced in homologated clinically used bone regenerating biomaterials.

The high porosity of the deer antler could also stimulate cell colonization and multiplication. Similar structural units of inorganic phase are evidenced in the comparatively studied samples. According to Raman signatures the deer antler bone contains a higher content of organic phase than Gen-Os biomaterial. A larger pore size is evidenced for deer antler bone that enhances the cell attachment, as well as spread and proliferation of the osteoblastic-like cells. The structural similarity of deer antler bone with bone regeneration biomaterials, as well as its favorable behavior under gamma sterilization circumstances suggest that deer antler is worth being analyzed in the attempt to understand the mechanisms of bone growth stimulation for its use as a biomaterial.

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