

Influence of osteoporosis on osseointegration: an experimental study

**C. Clemente de Arriba, J. Tabuenca, R. Rodríguez-Torres,
R. Fernández-Valencia, J. Carrascosa-Sánchez and L. Gómez-Pellico**

Department of Morphological Sciences and Surgery, School of Medicine, University of Alcalá. Alcalá de Henares, 28871-Madrid, Spain

SUMMARY

The purpose of the present study was to evaluate the quality of osseointegration in osteoporotic animals, receiving implants with hydroxyapatite coating. Wistar rats, aged 20 weeks were used. Two groups, control and experimental, were established. The second group was subjected to bilateral ovariectomy to induce osteoporosis. Both groups received intramedullary implants in one of their femora at the age of 36 weeks, the other femur remaining without implant. 12 weeks later, all the animals were sacrificed. Densitometric, histological and morphometric analyses were performed. All the results confirmed a poorer bone response to the implant in the osteoporotic group. However, the bone mineral density and morphometrical values for the implanted osteoporotic group were higher than those observed for the osteoporotic group without the implant, suggesting that the implant stimulates new bone formation and slows down the process of osteoporosis.

Key Words: Bone – Histomorphometry – Densitometry – Interface – Osteoporosis – Rat

INTRODUCTION

Implantological treatment into bone tissue in any part of the organism faces a common problem: -bone loss as time passes- which leads to implant loss. The causes vary; it may be a

reaction against the foreign body, bone remodelling with a predominance of resorption due to the physical characteristics of the implant, the patient is age or previous host illnesses facilitating a decrease in bone mass.

Currently, hydroxyapatite coatings over metallic biomaterials, mainly titanium and its alloys, are used with a view to accelerating bone formation on the implant surface. Osseointegration is thus made easier and the risk of implant loss becomes minimal. However, for this to be achieved the environment of the host and his/her metabolic situation must be suitable.

Implantological treatment is generally needed in middle or advance age, when osteoporosis frequently appears as a metabolic disease, its frequency being eight times higher in women. 20-30% women over 65 suffer from the disease (Lane, 1994), since bone mass maintenance is related to oestrogen levels, which decrease dramatically after menopause; bone turn-over is modified and resorption becomes predominant over formation. Such important bone changes logically affect the processes of osseointegration. This is why experimental studies must find a model animal, simulating menopause, with a bone loss pattern similar to that observed in humans. Riggs and Melton (1983) considered two types of osteoporosis depending on their menopausal or senile cause.

The purpose of the present study was to assess bone behaviour when a biomaterial coated with hydroxyapatite was used in situations of evident osteoporosis, in an experimental model animal, simulating menopausal osteoporosis.

Correspondence to:

Dra. Celia Clemente de Arriba. Departamento de Ciencias Morfológicas y Cirugía.
Facultad de Medicina, Universidad de Alcalá. 28871 Alcalá de Henares, Madrid, Spain.
Phone: 91-885 48 79; Fax: 91-885 45 93. E-mail: celia.clemente@alcala.es

Submitted: July 16, 2000

Accepted: September 13, 2000

MATERIAL

- 1.1: Implants: Titanium alloy implants (Ti-6Al-4V) 2.5 cm long and 1.7 mm in diameter, with a 100 micron-thick hydroxyapatite coating applied by the plasma-spray technique, and provided by *Industrias Quirúrgicas del Levante, Merck Biomet*, were used.
- 1.2: Animals: Ten Wistar rats, aged 20 weeks, weighing 240-280 g each, and stabled at the circadian rate of light and with access to food and water ad libitum were used. The legal requirements (R.D. 223/1998, B.O.E. of 18th March, and order of 13th October 1989, B.O.E. of 18th October) concerning the protection of experimental animals were respected.
- 1.3: Technical equipment:
 - a) Dual energy X-ray absorptiometry densitometer: NORLAND XR-26 (*Norland Corp Fort Atkinson*).
 - b) Cutting-grinding system, EXAKT (*EXAKT-Apparatebau, Norderstedt, Germany*) for hard tissues.
 - c) Software for image analysis: MIP-4 connected to light microscope.
 - d) Software for statistical analysis: SPSS V 10.0.

METHOD

- 2.1. Animals: The 10 rats were divided in two groups: control and osteoporotic.

Characteristics of the control group: An intramedullary implant was placed in the right femurs of the animals at 36 weeks of age, according to the technique described below; their left femurs remained without implant. Intramedullary placement allows the evaluation of bone response to implant without any added factors, such as load support in the case of transcortical implants.

Characteristics of the osteoporotic group: Bilateral ovariectomy was performed at 20 weeks of age and the animals were implanted at 36 weeks of age following the same procedure as in the control group.

Both groups were sacrificed 12 weeks after surgical treatment.
 - 2.2: Surgical technique: All the rats were anaesthetised with diazepam, atropine and ketamine in addition to local anaesthesia on the surgical wound and antibiotic prophylaxis with 4 mg/kg cefazoline. Five animals were subjected to bilateral ovariectomy following the common veterinary practice used to induce osteoporosis. After 16 weeks both groups, by using a sterile surgical technique, underwent a medial parapatellar arthrotomy; the intercondylar notch of their right femur was pierced and the medullary cavity ground. Later, an implant was introduced along the cavity, filling the whole of the intramedullary space. The left femur remained without implant as a control in both groups.
 - 2.3. Sample collection: The animals were sacrificed with sodium pentobarbital (i.v.) at lethal doses and their femora obtained for histological processing. The material was fixed in 10% formaldehyde buffer at pH=7 to avoid sample decalcification.
 - 2.4. Bone densitometry: Bone mineral density was determined in g/cm². Densitometric assessments were made in two areas:
 - a) BMD assessment of an area of 0.5cm x 0.5cm over the implant. BMD was also assessed at the same level in the contralateral femur (left, without implant).
 - b) BMD was determined in the whole femur, deducting the equivalent to the implant.
- The following abbreviations were used:
- RSFBMD = bone mineral density of right femur (superior part)
- LSFBMD = bone mineral density of left femur (superior part)
- TRFBMD = bone mineral density of total right femur
- TLFBMD = bone mineral density of total left femur.
- Figure 1 shows the densitometric images obtained with the areas corresponding to the BMD of the superior part of either the right or left femora.
- 2.5. Histological preparations: After densitometric evaluations, the femora were subjected to a process of dehydration and embedding in methacrylate, using increasing concentrations according to the technique described by Donath and Breuner (1982), with modifications. The method affords histological sections thin enough to be observed at light microscopy and without it being necessary to decalcify the sample or remove the implant, which facilitates the study of the interface and avoids the risk of mistaken histological interpretation. Four sections 70µ thick were obtained and dyed with the normal substances (Masson trichromic, hematoxylin-eosin, Junqueira picrosirius). Qualitative assessments were made with conven-

tional light microscopy for each section, along with quantitative assessments by means of image analysis.

2.5.1. Qualitative evaluation: 3 levels were considered, as shown on figure 2.

- a) Analysis of tissue response localised around the implant, differentiating the presence of bony or fibrous tissue. This was termed "peri-implantary response".
- b) Analysis of modifications on the whole cortical bone, evaluating remodelling, resorption and bone formation. This was called "cortical response".
- c) Changes in the medullary cavity, with the presence or absence of bone trabeculae filling the space not occupied by the implant. This was called "medullary response".

2.5.2. Quantitative evaluation: This was performed by image analysis in the four preparations from each animal and the mean per animal was taken. Bone areal density (Aa) was evaluated with morphometric analysis. Sections parallel between each other and perpendicular to the long axis of the femur were made (type Cavalieri sections) (Cruz-Orive, 1997) and each preparation was analysed as follows:

- a) Total area assessment (At), including bony tissue, the medullary cavity and bone formation.
- b) Calculation of bone tissue area, i.e., total area less medullary area (Ao).
- c) Bone areal density (Aa), as the Ao/At ratio.

Figure 3 shows the above calculations.

Statistical analysis of results was performed using of the SPSS V 10.0 system with non-parametric tests.

RESULTS

Densitometric results:

Table 1 shows the results obtained for both regions studied, evaluating bone densitometry (g/cm^2) in the upper region of the femur, either for the right femur over the implant (RSFBMD) or the left without implant in the same region (LSFBMD) and both groups of study, control and osteoporotic. The densitometric results relating

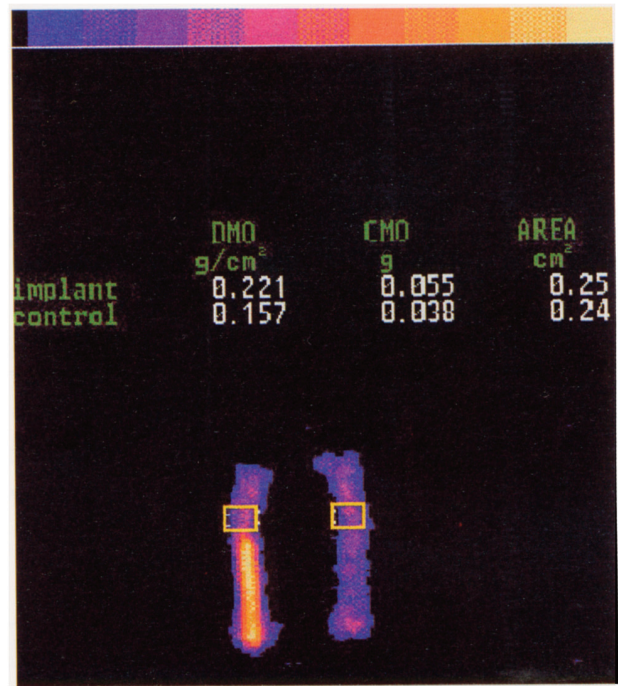


Fig. 1.- Densitometric image of femora with and without implant. The box corresponds to the studied area: over the implant.

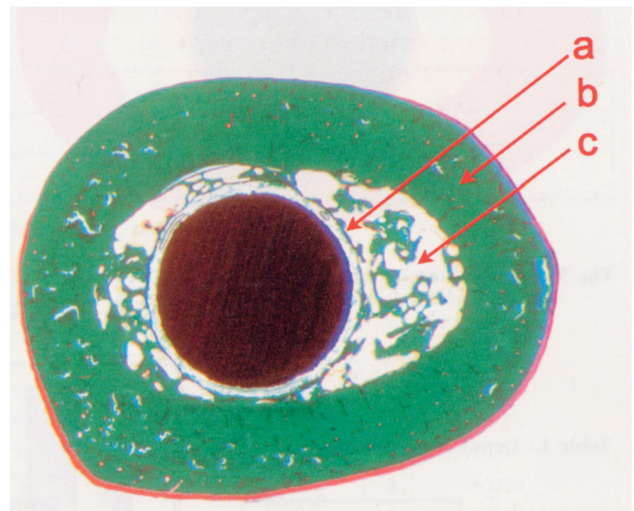


Fig. 2.- Implant into the medullary cavity. Arrows point to the three areas of histological study, Masson, x2.5.
a = peri-implantary response
b = cortical response
c = medullary response

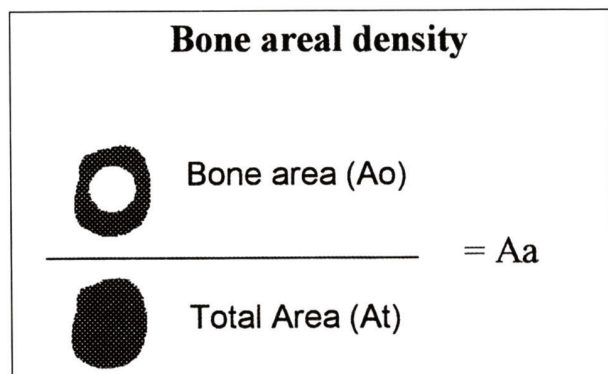


Fig. 3.- Chart representing the morphometric study. Bone areal density; bone area/total area.

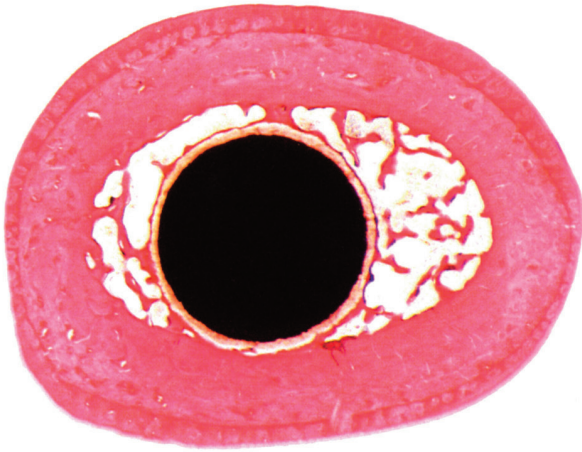


Fig. 4.- Aspect of control group preparation.. Bone trabeculae can be observed inside the medullary cavity; implant with hydroxyapatite coating. Sirius. x2.5.

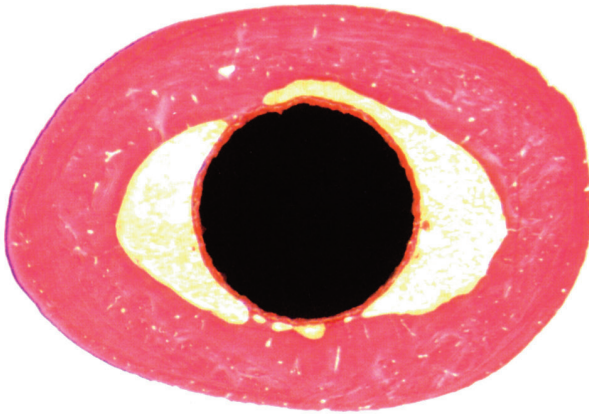


Fig. 5.- Aspect of osteoporotic animal preparation. An absence of trabeculae inside the medullary cavity can be observed. Sirius. x2.5.

to the whole femur, on either the right (TRFBMD) or left sides (TLFBMD), and for both groups, are also shown.

The statistical analysis within the same group confirmed a higher BMD of implanted bones when compared to their contralateral homologues without implants. This occurred in both healthy animals and in those with experimental osteoporosis. However, the difference was only significant for the upper area of the implant, not around it (Table2).

On comparing the control and osteoporotic groups, a higher BMD was found in the control group with implant, although significant values were only obtained for the total region and not for the upper part (Table 3).

On comparing the left sides (without implant) of both groups, there was higher BMD in the control group in both regions studied, although the differences with the osteoporotic group were not significant in any case (Table 4).

Qualitative histological results:

In the control group there was active cortical remodelling, new bone tissue appearing at the expense of cortical periosteum, with trabeculae perpendicular to the native cortical and the aspect of young bone, visible all along the bone outline. Around the metallic implant, the hydroxyapatite coating layer was differentiated and on it, a layer of laminar —type bone tissue, starting mainly from the endosteum closer to the implant (anterior or posterior faces) and tending to surround it, was visualised. In the medullary cavity, the presence of bone trabeculae could be observed (Figure 4).

Table 1.- Densitometry in both groups of study.

| | Region | Control ± (ESM) (g/cm ²) | Osteoporotic ± (ESM) (g/cm ²) |
|-----------------|--------|--------------------------------------|-------------------------------------------|
| BMD upper part | RSFBMD | 0.2244 ± 0.0088 | 0.2048 ± 0.0051 |
| | LSFBMD | 0.1688 ± 0.0066 | 0.184167 ± 0.0042 |
| BMD whole femur | TRFBMD | 0.1851 ± 0.0061 | 0.1674 ± 0.0066 |
| | TLFBMD | 0.17448 ± 0.0068 | 0.1673 ± 0.0036 |

Densitometry in both regions of both groups (for explanation see text).

Graphic of densitometry

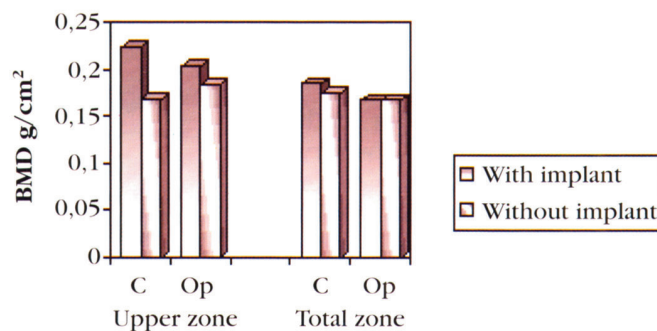


Table 2.-

| | |
|-------------------------------------------|----------------|
| RSFBMD control > LSFBMD control | p = 0,042 |
| RSFBMD osteoporotic > LSFBMD osteoporotic | p = 0,013 |
| TRFBMD control > TLFBMD control | p = 0,225 (ns) |
| TRFBMD osteoporotic > TLFBMD osteoporotic | p = 0,753 (ns) |

Left and right BMD behaviour.

Table 3.-

| | |
|--------------------------------------|---------------|
| RSFBMD control > RSFBMD osteoporotic | P = 0,08 (ns) |
| TRFBMD control > TRFBMD osteoporotic | P = 0,043 |

Behaviour in both implanted groups (control and osteoporotic).

Table 4.-

| | |
|--------------------------------------|----------------|
| LSFBMD control > LSFBMD osteoporotic | P = 0,279 (ns) |
| TLFBMD control > TLFBMD osteoporotic | P = 0,345 (ns) |

Behaviour in both groups without implant (control and osteoporotic).

Table 5.-

| Side | Control ± ESM | Osteoporotic ± ESM |
|-------|---------------|--------------------|
| Right | 0,675 ± 0,014 | 0,638 ± 0,027 |
| Left | 0,612 ± 0,050 | 0,406 ± 0,050 |

Behaviour in both groups without implant (control and osteoporotic).

Graphic of bone areal density

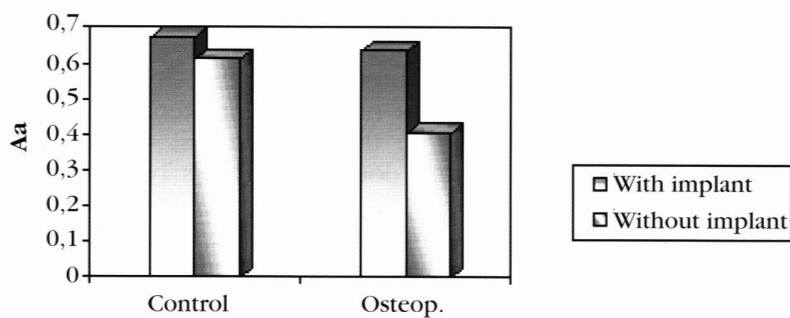


Table 6.-

| | |
|----------------------------------------------------------|----------------|
| Control with implant > Control without implant | P = 0,14 (n.s) |
| Osteoporotic with implant > Osteoporotic without implant | P = 0,0011 sig |

Aa behaviour within the same group.

Table 7.-

| | |
|--------------------------------------------------------|---------------|
| Control with implant > Osteoporotic with implant | P = 0,027 sig |
| Control without implant > Osteoporotic without implant | P = 0,007 sig |

Aa behaviour in femora with and without implant of different groups.

In the osteoporotic group there was also cortical remodelling, but this was less intense; periimplant bone tissue was rare and sometimes appeared inserted with fibrous tissue; there were no bone trabeculae within the medullar cavity (Fig. 5).

Histomorphometric results:

Table 5 shows the bone areal density (Aa) results, providing the quantity of bone tissue in relation to the total. Statistical analysis within a group confirmed the presence of a higher bone areal density (Aa) in the implanted femora as compared to their contralateral homologues without implant, and this occurred in both the osteoporotic and healthy animals. However, the difference was only significant in the osteoporotic group, not in the control one (Table 6).

When the osteoporotic and control groups were compared with each other, a higher Aa was found in the implanted control group, the values being significant. The left sides (without implant) of both groups also proved to have a higher Aa in the control group, with statistically significant differences (Table 7).

DISCUSSION

Our animal experimentation model for the study of osteoporosis was spurred by the studies made by other authors (Hayashi et al., 1989; Kalu, 1991; Patlas et al., 2000). These authors showed that ovariectomy performed at 20 weeks of age resulted in skeletal and metabolic changes, similar to peri- and postmenopausal human osteoporosis. Ovariectomy in very young animals (Kalu, 1991; Patlas et al., 2000) is not useful as an experimental model, due to the longitudinal growth of rats, which logically does not occur in adult women.

In 1983, Rigs and Melton differentiated two types of osteoporosis: I, or postmenopausal, in which the loss of trabecular bone is predominant, and type II, or senile, with loss of both bone types, trabecular and cortical.

Ovariectomy in rats over 20 weeks of age causes (Williams, 1996) changes precisely in trabecular bone, not in the cortical type. For this reason it is not a useful model to study senile osteoporosis, although it is suitable for the study of postmenopausal osteoporosis.

When an implant was placed in the diaphyseal cavity, the presence of trabecular bone in the cavity and around the implant was confirmed in the control groups. This neoformation of trabecular bone was clearly lower in the osteoporotic group with diaphyseal implants. Other authors (Hara et al., 1999) found

loss of trabecular bone at the level of the metaphyses, while the cortical bone remained unchanged in the osteoporotic animals. However, although osteoporotic animals do not respond with the same intensity as healthy ones, they are still capable of a partial response, since there is some bone inside the cavity and around the implant due to the biomaterial used.

Quantification of the results was in agreement with the histological impression in all aspects. Both densitometry, prior to the collection of histological sections, and morphometry, performed upon the sections, revealed a lower response in the osteoporotic group, as expected. The osteoporotic group showed a poorer reaction to the implants; however, their bone mass improved significantly, as judged by both densitometric and morphometric analyses, when compared to their contralateral femurs without implants. Previous studies had confirmed these aspects, since the authors (Okazaki et al., 2000) found BMD to be higher in the implanted osteoporotic animals than in those without implant.

Although it is true that osteoporosis shows some improvement, this is not enough for implant acceptance. As in our study, in a study with SEM other authors (Hayashi et al., 1994) also found less trabecular bone in the metaphyses of ovariectomized animals and a lower quality interface bone-implant.

Hydroxyapatite coating with metallic biomaterials has proved efficient, promoting osseointegration during the first weeks (Hayashi et al., 1994). It also shows greater affinity than other coating biomaterials (Hayashi et al., 1989). In 1994, working with rats Hayashi obtained higher affinity indices for biomaterials coated with hydroxyapatite than for titanium biomaterials without coating. This was so during the first 4 weeks after surgery, after which the indices become equal. Such indices were also higher in osteoporotic animals, in a comparison with biomaterials without coating, and always remained lower than in the control group.

In our study, bone mineral density and bone areal density were always higher in the osteoporotic animals with hydroxyapatite-coated implants. These may promote osseointegration, but the values are always below those of the control group (Soballe, 1993). Other authors (Hara et al., 1999; Okazaki et al., 2000) also found higher BMD and affinity indices in their control groups than in the ovariectomized animals.

In sum, the poorer response to implant of the osteoporotic group can be said to take place at all levels of the femur, since the same trend is present in all the cases, although with densitometry, statistically significant differences are only found in the upper implant region. With

morphometry, the same differences are found, although with different levels of significance.

The evaluation of femora without implant confirms the higher Aa in the control group and points to the same trend, although significance is not reached with densitometry.

As a final conclusion, it is confirmed that titanium biomaterial with hydroxyapatite coating improves bone response in osteoporotic animals, although the observed values remain below those of the control group.

REFERENCES

- CRUZ-ORIVE LM (1997). Stereology of single objects. *J Microscopy*, 186: 93-107.
- DONATH K and BREUNER G (1982). A method for the study of undecalcified bones and teeth with attached soft tissues. *J Oral Pathology*, 11: 318-326.
- HARA T, HAYASHI K, NAKASHIMA Y, KANEMURU T and IWAMOTO YJ (1999). The effect of hydroxyapatite coating on the bonding of bone titanium implants in the femora of ovariectomised rats. *Bone Joint Surg*, 81-B: 705-709.
- HAYASHI K, UENOYAMA K, MASHIMA T and SUGIOKA Y (1994). Remodelling of bone around hydroxyapatite and titanium in experimental osteoporosis. *Biomaterials*, 15: 11-16.
- HAYASHI K, UENOYAMA K, MATSUGUCHI N, NAKAGAWA S and SUGIOKA Y (1989). The affinity of bone to Hydroxyapatite and Alumina in experimentally induced osteoporosis. *J Arthroplasty*, 4: 257-262.
- KALU DN (1991). The ovariectomized rat model of postmenopausal bone loss. *Bone Mineral Research*, 15: 175-192.
- LANE JM (1994). Osteoporosis: cause of geriatric fractures. In: Brighton CT, Friedlaender GE and Lane JM (eds.). *Bone formation and Repair*. American Academy of Orthopaedic Surgeons. North River Road, pp 471-481.
- OKAZAKI A, KOSHINO T, SAITO T and TAKAGI T (2000). Osseous tissue reaction around hydroxyapatite block implanted into proximal metaphysis of tibia of rat with collagen-induced arthritis. *Biomaterials*, 21: 483-487 R.
- PATLAS N, ZADIK Y, YAFFE P, SCHWARTZ Z and ORNOY (2000). Oophorectomy-induced osteopenia in rats in relation to age and time post-oophorectomy. *Cells Tissues Organs*, 166: 267-274.
- RIGGS BL and MELTON LJ (1983). Evidence for two distinct syndromes of involutional osteoporosis. *Am J Med*, 75: 899-901.
- SOBALLE K (1993). Hydroxyapatite ceramic coating for bone implant fixation. Mechanical and histological studies in dogs. *Acta Orthop Scand*, 255 (suppl): 1-58.
- WILLIAMS S, WAKISAKA A, ZENG Q, BARNES J, MARTIN G, WECHTER WJ and LIANG CT (1996). Minocycline prevents the decrease in bone mineral density and trabecular bone in ovariectomized aged rats. *Bone*, 19: 637-644.