# The effect of bead diameter on the accuracy of two current techniques used to quantify bone ingrowth in porous-coated implants

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The effect of the bead diameter on the accuracy of two techniques used in bone ingrowth quantification, microradiography and backscattered electron imaging–scanning electron microscopy (BEI–SEM), was assessed using porous-coated implants. Two groups of seven titanium porous implants (group A: bead size 250–350 μm and group B: 500–700 μm) were implanted for 12 weeks in a canine model. After euthanasia, the same histological slides were prepared for microradiography and BEI–SEM. The percentage of bone, bone ingrowth, bone ongrowth, porosity and bone index were determined by a point counting method using images from both techniques. ANOVA and Tukey's test were used to compare the results from the different bead sizes and techniques. The results showed significant higher bone ingrowth in microradiography groups, and significant lower porosity in only the fine-bead microradiography group (group A size). Microradiography also obtained significantly higher bone ongrowth, but only for the coarse bead size group (group B). From these results it was concluded that microradiography decreases the porosity of the porous coating compared with BEI–SEM. This effect seems to be dependent on the bead diameter. The smaller the diameter, the greater the effect. Furthermore, microradiography increases bone ingrowth which seems to be affected independently of the bead diameter, becoming the most sensitive parameter to increase.

## 1. Introduction
Porous coating of titanium prostheses has been introduced to improve the bone–implant interface strength by increasing the bony reaction around and inside the surface of the implant [1, 2]. Consequently, interest has developed in the methods used to determine bone ingrowth in metallic implants. Two main methods are used to quantify bone ingrowth: microradiography, and backscattered electron imaging–scanning electron imcroscopy (BEI–SEM) because of their ability to detect mature bone.

Previous works have demonstrated the accuracy of BEI–SEM in the histomophometric studies of hydroxylapatite pellets, transcortical beaded porous [3] and fibre mesh titanium implants [4]. The results showed that light microscopy techniques overestimate bone ingrowth and underestimate porosity. The reason for those results seems to be related to the fact that BEI–SEM studies only 1 to 5 μm of the implant surface [5], while the light microscopy techniques require between 35 and 100 μm specimen thickness, which results in projection errors.

However, no information is available on the effect of different bead diameter porous coatings when using the above-mentioned methods of quantification. The purpose of this study is to evaluate the effect of bead diameter on the quantification of bone ingrowth in titanium implants with two different bead sizes using both microradiography and BEI–SEM.

## 2. Material and methods
Fourteen titanium porous coated samples supplied by DePuy USA (Warsaw, IN 45680, USA) were utilized. The samples had a cylindrical shape, 22 mm long and $5 \pm 0.3$ mm in diameter. Two groups of seven implants were used. Bead diameter was 250–350 μm in group A and 500–700 μm in group B. Surface porosity was $47.3 \pm 5.6\%$ in group A, and $39.5 \pm 6.4\%$ in group B.

Seven adult mixed breed dogs of similar size and weight were operated on. General anesthesia was induced and maintained with intravenous pentobarbital sodium (55–65 mg $kg^{-1}$). Prophylactic antibiotics (Cefazolin 20 mg $kg^{-1}$) were given, starting the day before surgery, for three days. One hind limb was selected by randomization in each dog and group A samples were implanted in the proximal femoral cancellous bone using a 3.5 mm drill to a depth of 22 mm. The hole was reamed to a diameter 0.2 mm

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smaller than the sample to be implanted. Samples were press fitted into these holes to lie in the cancellous bone of the medullary canal on the longitudinal axis of the femur. Group B samples were implanted transversely in the distal femoral cancellous bone of the same hind limb using the same technique. Postoperative analgesia was given with Butorphanol (0.2 to 0.4 $mg\,kg^{-1}$ IM).

Twelve weeks after surgery, dogs were euthanized and two implant-bone blocks were obtained from each operated femur. Each block was cut perpendicularly to the longitudinal axis of the implants in order to obtain the samples and discard the ends. Undecalcified cross-sections from samples were cut to 200 μm in thickness with a diamond saw (Leco Vari-Cut VC50, USA; No. 11-4245 diamond wafering blade) and ground to 100 μm. Microradiographies of each section were performed in a habitual technique [6] and images from the same cross-sections were created using a JSM-6400 (JEOL Ltd, Tokyo, Japan) backscattered electron microscope at 39 mm focal distance. Photographs were taken at X30 magnification.

Histomorphometric quantification was performed using a Merz grid [7]. The bone ingrowth area was defined as the section area containing the porous coating, extending 500 μm from the surface of the substrate and including 584 points, and the bone ongrowth area as the section area in the cancellous bone between 500 μm and 720 μm from the surface of the substrate and including 236 points (Fig. 1). The following apparent technique-dependent measurements were calculated to assess the quantity and extent of the real bone ingrowth [8, 9]: the *percentage of bone* in the ingrowth area was defined as the amount of bone in the bone ingrowth area referred to the whole ingrowth area surface, *bone ingrowth* as the percentage of available space for bone formation in the ingrowth area occupied by bone, surface *porosity* of the implant as the percentage of the ingrowth area occupied either by bone or background, *bone ongrowth* as the bone percentage in the ongrowth area, and *bone index* [10] or the ratio between bone ingrowth and bone ongrowth expressed as a percentage (Table I).

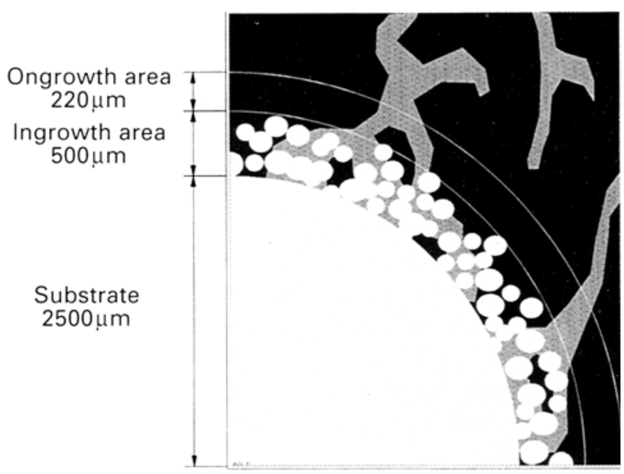


*Figure 1* Schematic representation of the substrate, bone ingrowth including the porous coating and bone ongrowth areas defined for the histomorphometric study. Units are expressed in micrometres.

TABLE I Histomorphometric parameters used in the present study in ingrowth and ongrowth areas defined as in Fig. 1

| Parameter | Description |
|---|---|
| % Bone | $\frac{\text{Ingrowth area bone points}}{\text{Ingrowth area points}} \times 100$ |
| Bone ingrowth | $\frac{\text{Ingrowth area bone points}}{\text{Ingrowth area bone \& background points}} \times 100$ |
| Porosity | $\frac{\text{Ingrowth area bone \& background points}}{\text{Ingrowth area points}} \times 100$ |
| Bone ongrowth | $\frac{\text{Ongrowth area bone points}}{\text{Ongrowth area points}} \times 100$ |
| Bone index | $\frac{\text{Bone ingrowth}}{\text{Bone ongrowth}} \times 100$ |

ANOVA was used to compare the results of both bead size and technique (SAS Software, SAS Institute, Inc., Cary, NC, USA). If a significant difference was observed, Tukey's studentized range test at a confidence level of 95% was used to determine the significant subgroups.

## 3. Results

All animals recovered well after surgery and no significant complication developed. The implants were well tolerated and no lucencies were apparent on radiographic evaluations performed every two weeks throughout the study. Radiographically, all implants appeared to be incorporated at the end of the study.

Both techniques, microradiography and BEI–SEM, were found useful for the study of bone formation achieved in titanium porous implants. In both bead size groups, samples were surrounded by bone in close contact with the implant surface, fitting the definition criteria of osseointegration [11]. New trabecular bone formation, which was differentiated from old trabecular bone on the basis of its density, osteonal arrangement, location and lacunae shape and orientation inside the trabeculae, was more easily observed using the BEI–SEM technique than with microradiography (Figs 2 and 3).

Qualitatively, microradiography was useful for observing fine details on trabeculae shape and distribution, providing quasi three-dimensional insight information on their spacial disposition (Fig. 3). However, BEI–SEM produced sharper, more precise images and provided, at the same time, more information on the internal composition of trabecular bone because of its high resolution power. Fine details on lacunae shape and distribution and bone density were provided only by BEI–SEM (Fig. 2).

Analysis of variance did not show any statistical differences among groups for apparent bone percentage and bone index (Table II). In the remaining three parameters (bone ingrowth, porosity and bone ongrowth) Tukey's test showed significantly different subgroups. Apparent bone ingrowth was significantly higher in both microradiography groups compared with their respective BEI–SEM groups, while there were no differences inside both microradiography and BEI–SEM groups. Porosity was apparently higher in

TABLE II Microradiography and BEI–SEM histomorphometric measurements (Mean ± SD: $n = 7$)

| Measurements | Technique | | | |
|---|---|---|---|---|
| | Microradiography | | BEI–SEM | |
| Group | A | B | A | B |
| Bead diameter (μm) | 250–350 | 500–700 | 250–350 | 500–700 |
| % Bone | $14.7 \pm 5.3$ | $11.0 \pm 4.3$ | $17.8 \pm 5.5$ | $12.6 \pm 3.2$ |
| Bone ingrowth | $54.2 \pm 6.0^{a}$ | $52.5 \pm 12.6^{a}$ | $24.6 \pm 9.5^{b}$ | $32.8 \pm 6.0^{b}$ |
| Porosity | $27.3 \pm 7.2^{a}$ | $33.7 \pm 5.8^{a,c}$ | $45.1 \pm 5.0^{b}$ | $38.6 \pm 6.2^{b,c}$ |
| Bone ongrowth | $37.1 \pm 9.8^{a,b}$ | $44.3 \pm 13.9^{a}$ | $25.3 \pm 7.9^{b}$ | $27.5 \pm 5.1^{b}$ |
| Bone Index | $157.4 \pm 65.3$ | $127.6 \pm 51.0$ | $97.5 \pm 20.2$ | $121.7 \pm 28.3$ |

A = fine beads
B = coarse beads
Superscript characters indicate differences at $p < 0.05$ level (Tukey's test)

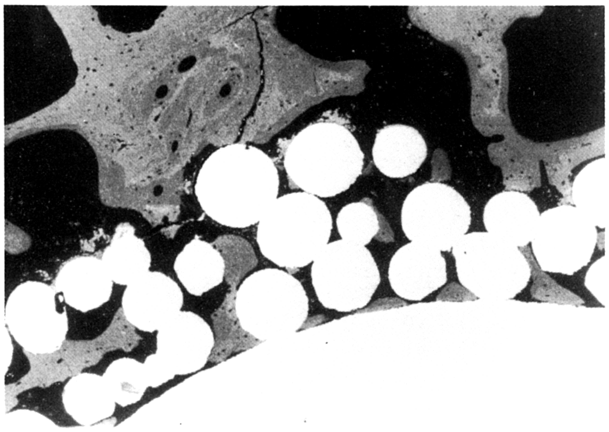

*Figure 2* BEI–SEM from a 250–350 μm bead diameter group A specimen (X30). Fine details on the bone density, osteonal arrangement and lacunae disposition are shown (same specimen and area as in Fig. 3).

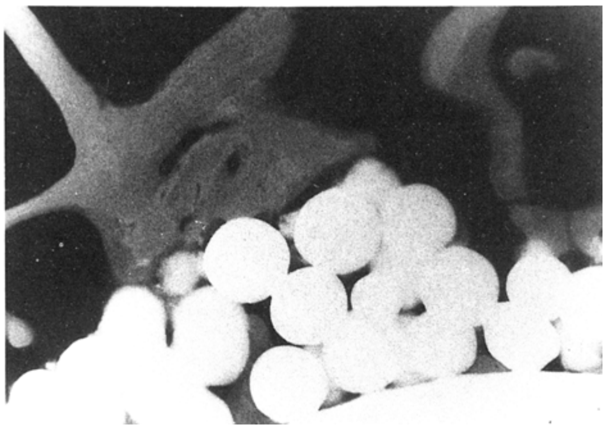

*Figure 3* Microradiography from a group A specimen bead diameter 250–350 μm (X30). Underestimation of porosity (spaces without metal) and overestimation of the area covered by bone are apparent when compared with Fig. 2.

both BEI–SEM groups, but differences were significant only for the fine-bead group (group A) in reference to both microradiography groups. BEI–SEM group B porosity (coarse group) was not different from its homonymous microradiography group. Porosity was not different inside each technique group. Bone ongrowth showed a similar pattern of significance as in bone ingrowth (significant apparent higher results for microradiography) only for coarse bead groups.

## 4. Discussion

Quantified microradiography was introduced by Jowsey *et al.* [6] for the study of turnover of bone, formation and resorption. This technique has been proved useful if some considerations are taken into account. As described by these authors, the section thickness is the most important technical detail affecting fine images. The thicker the section, the denser the specimen will appear to be, so the authors recommended a final section thickness about 100 μm for conventional microradiographies.

This relationship between section thickness and density of the image was recognized in light microscopy many years ago. It was defined as the Holmes effect [12] and many authors tried to calculate it [12–16]. Proposed calculation methods are based on the isotropy or anisotropy of the material object of the study [12]. If isotropic behaviour of bone is assumed, and a mean trabecular width of 150 μm for the thicknesses examined in this study, an error of 42% is predicted for microradiography and 2.1% for BEI–SEM. Isotropic error calculation seems to be an easy and useful estimation of the accuracy of both methods. The role of the microradiography is however, still important in other situations. As Moroni *et al.* [17] have demonstrated, microradiography is useful to determine fine details of trabeculae shape and trabecular fracture patterns after push-out mechanical tests in porous coated implants. In our opinion, this technique is still useful and should not be discarded as a histomorphological laboratory technique.

The use of BEI–SEM for the quantification of bone–implant histomorphometric studies has been widely accepted. BEI–SEM offers higher resolution, easy specimen preparation, and the images it provides are independent of specimen thickness. Therefore, BEI–SEM only differentiates between bone and non-bone (including osteoid) phases, so tissues other than bone are represented as backgroud [18].

As is well known, microradiography increases the apparent bone ingrowth and bone ongrowth, and decreases the apparent implant porosity [3, 4]. Apparent bone ingrowth is affected twice by this effect because microradiography increases the observed bone and reduces the available space for bone ingrowth, as a result of the reduction of the porous coating porosity. However, there is no information available on how different bead diameter porous coatings affect these measurements. The present study indicates that their effect depends on the bead diameter and consequently on the bead number. Thus, the decrease in apparent porosity seems to be more important in the smaller diameter bead size (because of the increase in the number of beads in the porous coating) than could be expected from the diameter reduction. Bone index showed no significant differences between both techniques. The authors believe that this finding results from the bone index defintion itself, where the increase of bone ingrowth and bone ongrowth tend to neutralize each other. Thus, bone index seems to be independent of the method used to quantify the samples.

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