Bull Group Int Rech Sci Stomatol et Odontol - Vol 39 n° 1-2, 1996

Study of behaviour and endurance of Bioapatite® implanted in the periodontium of the dog (closed model)

J.J. BONFIL*, P. TAVITIAN*, G. BERTRAND**, J.L. MOUCHEBŒUF**, M. REMUSAT***, H. TADRIST****

* *Unité de Recherche Evaluative en Thérapeutique et Santé Bucco-Dentaire (Marseille).*
** *Department of Periodontics: Faculty of Dental Surgery of Bordeaux.*
*** *«Interface Matrice Extracellulaire et Bio-Matériaux» Laboratory (Marseille).*
**** *«Système Energétique et Traitements Thermiques» Laboratory (Marseille).*

SUMMARY

The principle purpose of this study was to quantify endurance of the biomaterial Bioapatite® implanted in periodontal structures of the dog (closed model), such quantification being established by means of indices (Endurance Index and Transformed Endurance Index) obtained as a result of data-processed analysis of histologic images. The investigation further aimed at studying the development of new cementum and the reconstruction of an attachment system. The study was conducted on eight dogs and 222 sections. New cementogenesis and the reconstruction of an attachment system are observed both in the test sites and the reference sites. Endurance of the biomaterial is statistically linked with time: the most substantial decrease in the endurance is observed between two and six months. Traces of the material subsist at month 9. The structure of material masses always remains lacunal on a microscopic scale (highest average Endurance Index observed during the investigation: 30.35%). An osteoid deposit can be continually detected as of the second month around crystalline deposits. Furhter, this type of deposit was noted on the periphery of the deposits implanted in ectopic position in the supracrestal connective tissue.

KEY WORDS:

Biomaterial; periodontium; filling material.

RÉSUMÉ

Le but principal de cette étude était de quantifier la rémanence d'un biomatériau, la Bioapatite® implantée dans le parodonte du chien (modèle clos); cette étude quantitative étant basée sur des indices (indice de rémanence et indice de rémanence transformé) issus d'une analyse d'image informatisée des coupes histologiques. De plus ce travail se propose d'étudier la néo-cementogénèse et la reconstruction du système

Animal experiments were performed in accordance with institutional committee (Université Aix Marseille II) and laws applied in France.

d'attache. L'étude a été effectuée sur 8 chiens ayant fourni 222 sections. La néo-cementogénèse et la reconstruction d'un système d'attache sont observées sur les «Sites tests» implantés ainsi que sur les «Sites témoins» (non implantés). La rémanence du matériau est statistiquement liée au temps d'implantation: la décroissance la plus forte de l'indice de rémanence étant observée entre le deuxième et le sixième mois. Des fragments de biomatériau subsistent au neuvième mois. Les amas de biomatériau forment une structure lacunaire à l'échelle microscopique; valeurs maximales de l'indice de rémanence observées durant toute cette expérimentation: 30,35%. Un dépôt ostéoïde peut-être constamment détecté autour des amas cristallins au deuxième mois. Bien plus, ce dépôt était aussi observé à neuf mois à la périphérie des amas cristallins implantés en position ectopique et supracrestale, au sein du tissu conjonctif.

MOTS CLÉS:

Parodonte; biomatériau; matériau de comblement.

INTRODUCTION

Numerous studies (Frank *et al.*, 1987; Ogilvie *et al.*, 1987) have reported the biocompatibility and osteoinductive potential of Bioapatite®. However, no quantifying study has been conducted regarding the disappearance, or even the *in vivo* transformation of this material after being implanted in periodontal structures. Consequently, a method was used which would make it possible to quantify the disappearance of this material following implantation, by evaluating the post-operative endurance of the material in the area treated. Furthermore, data available on the development of a new cementum and the reconstruction of an attachment system when this material has been used are extremely rare. This justifies further investigations.

MATERIALS AND METHODS

General design

Specimens were taken on eight adult «Ariège» dogs, of the same age, each weighing about twenty kilograms. One dog was sacrificed at 1 month post-surgically; 3 at 2 months, 2 at 6 months, 2 at 9 months.

Surgical procedure

Anaesthesia was performed using Nesdonal with ethrane gas maintenance and perfusion with Ringer's lactate. The animals were intubated and connected to an artificial respiration apparatus. A full thickness flap was reflected on one hemi-maxilla. On the buccal side, the flap exposes approximately Icm of buccal alveolar bone. A notch was made perpendicularly to the alveolar bone 4 mm from the collar of the right maxillary cuspid using a 3 mm-diameter trephine manipulated with sterile saline irrigation. The notch being made through the alveolar bone to the dentine consequently involves: alveolar bone, periodontal ligament, cementum and dentine. The notch was filled with Bioapatite® and a contro-lateral notch was performed. The flap was replaced with interrupted 3.0 catgut sutures. After sacrificing the animals at the interval stated above, samples were taken using the block-section technique (Fig. 1).

Histology

The blocks are then processed by routine histologic method: fixed in a 10% formalin solution, decalcified for two months in sodium formate and finally embedded in paraffin. Vertical mesial-distal sections parallel to the long axis of the root are prepared at 5μ (D. profile knife) and serially mounted. Sections are stained with Masson's trichrome.

Histomorphometric techniques

Study of endurance of biomaterial by imaging. (Fig. 2). From each serially mounted specimen (one block-section), sections at 80μm were selected. The selection of 27 to 30 sections was extended from the central of the trephination outward to the peripheral area. The sections selected were photographed in order to obtain black and white prints on 18×24cm paper. The boundary of the trephination was traced on each print, with the baseline and initial borders of the trephination clearly visible at the level of the dentine penetrated by the trephine. A perpendicular line to an A-B segment was traced from each dentinal border of the notch, A and B being defined as the

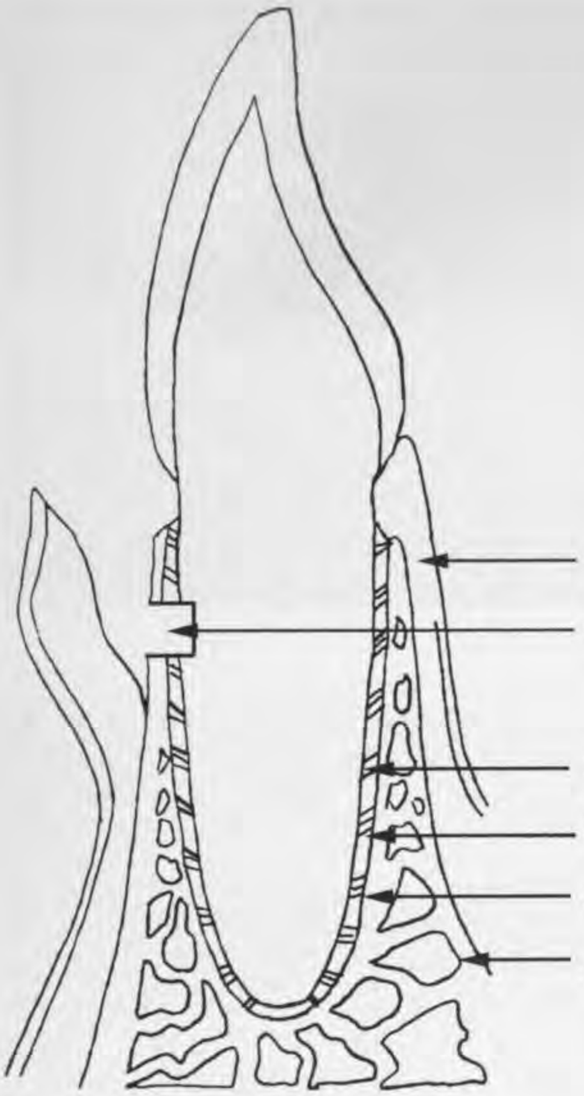


Fig. 1: Section through trephination site: note location of the notch. Trephination has been performed after reflecting a full thickness-flap.

Fig. 1: Coupe de la zone de trépanation: noter la situation de la zone d'encoche. La trépanation a été effectuée après avoir recliné un lambeau de pleine épaisseur.

most buccal points of the residual cortical bone (fig. 2A). Once the trephine surface has been defined on the paper prints, it is examined using an image analysis station (Fig. 2B): reprography mounting bench with high resolution black and white video camera (Sony ×27) connected to a Macintosh II FX computer equiped with a scanning board, hard-disk memory, MCB-34 frame-grabbing software and Optilab image analysis software. The software applications individualised the trephination surface on the one hand, and the crystalline deposits on the other (Fig. 2D). Afterwards, the E.I. (Endurance Index) was measured, for any given image or section, in such a way as to display the ratio of the crystalline deposit surface to the trephination surface.

$$\left(\text{I.E.} = \frac{\text{residual material}}{\text{trephination surface}}\right)$$

Statistical methods

In studying the endurance, the statistical unit is the section selected to which correspond two random variables: Endurance Index (E.I.) and Transformed Endurance Index (T.E.I.). As the Endurance Index is identified by a rate, we deemed it more advisable to use the T.E.I., which is equal to 2 ArchSin $\sqrt{\text{E.I.}}$ There is a greater probability for such a variable to be distributed normally, and the use of parametric tests with this variable are considered by some investigators to be more valid. Statistical analyses were mainly based upon correlation test (Pearson and Spearman), ANOVA and student «t» test. For all these tests, the significance level was set at $\alpha = 0.05$. PCSM software (Deltasoft, 1987) was used.

RESULTS

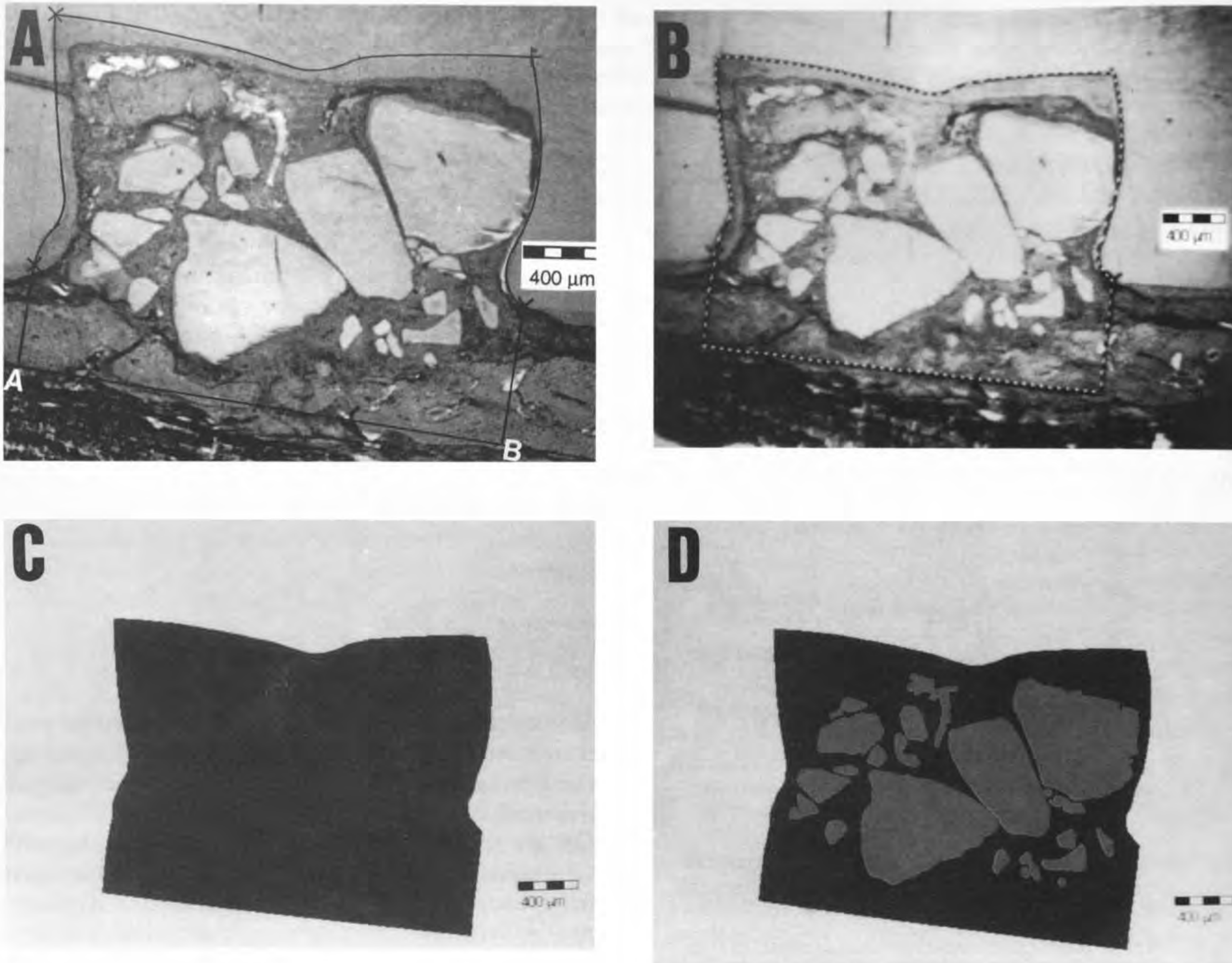
Morphological Data

Month 1 (Fig. 3).

On the control sites, connective fibres, often perpendicular to the major axis of the tooth, reconstitute the periodontal space. In parts, a new bone occupies the trephination surface in the majority of sections. On the test site, the decalcified crystalline deposits are surrounded with non-inflammatory fibrous connective tissue. At some distance from the deposits, newly formed bony trabeculae are observed surrounded with osteoblasts. At this stage there exists neither cementogenesis nor formation of a new attachment system.

Month 2 (Fig. 4).

Substantial regeneration is observed on the control sites. Connective fibres oriented along the major axis of the tooth form a new periodontal space; in some areas secondary repair cementum can be perceived. On the test sites, numerous particle masses are scattered throughout the fibrous connective tissue and, at a distance, newly-formed trabeculae are visible. However, the Bioapatite® is nearly always surrounded with a thin deposit of «bone-like» substance with «cell inclusions», forming an interface between the connective tissue and the particles. The deposit appears more substantial when the particles are small. The Bioapatite® may gather at the surface of the tooth through an attachment system whose constituents are: the above-mentioned peripheral «bone-like» substance, the connective tissue fibres and



▲

Fig. 2: In A, Black and white photo of a selected section. Identification of the trephination surface (T.S.) using method described in text. A black line defines the outline of the T.S. Data processing is used to analyse the negative; in B, the «video» image can be seen with the outline of the surface to be determined; in C, the software application displayed the surface in colour and measured it; in D, the software application measured the surface of the biomaterial masses and displayed it in a different colour.

The ratio $\frac{\text{biomaterial masses}}{\text{trephination surface}}$ was evaluated by the software and yielded the value of the endurance index.

Fig. 2: En A, photographie noir et blanc d'une coupe choisie. Identification de la surface de trépanation, selon la méthode décrite dans le texte. Un traitement informatique analyse le négatif; en B, l'image «vidéo» avec la délimitation de la surface à traiter; en C, le logiciel produit la dite surface en couleur et la mesure; en D, le logiciel mesure la surface des agrégats de biomatériau et la représente dans une autre couleur.

Le rapport $\frac{\text{agrégats de biomatériaux}}{\text{surface de trépanation}}$ *est évalué et fournit l'indice de rémanence.*

▶

Fig. 3: In A, site of Bioapatite® masses at 1 month; in (1), biomaterial masses; in (2), connective healing tissue; in (3), newly formed bone trabeculae; in (4), dentine. In B, control site; in (2), connective healing tissue; in (3) newly formed bone trabeculae surrounded with osteoblasts; in (4), dentine. Further note the presence between connective tissue and dentine of tear artefact.

Fig. 3: En A, site avec agrégats de Bioapatite® à 1 mois; en (1) le biomatériau; en (2), le tissu conjonctif cicatriciel; en (3), les travées d'os néoformé; en (4), la dentine. En B, site «témoin»; en (2), le tissu conjonctif cicatriciel; en (3), travées d'os néoformé bordées d'ostéoblastes; en (4), la dentine. Noter, par ailleurs, entre la dentine et le tissu conjonctif la présence d'un artéfact de déchirure.

cementum. The ligament fibres maintain a non-functional orientation and cementogenesis is variable and often nil.

Month 6 (Fig. 5).

On the control sites, periodontal components have been restored but often presenting little cementum. On the test sites, particles of Bioapatite® subsist. The vestibular osseous wall shows reconstruction in progress. At the heart of the surgical wound, the particles are coated with a material comparable to that observed at two months but thicker. The periodontal ligament may take on a functional appearance. There is a repair cementum.

Month 9 (Fig. 6).

On the control sites, the defects have completely closed and the various periodontal tissues are identifiable. On the test sites, repair is highly advanced. In places, a considerable layer of cellular cementum associated with a thick periodontal ligament is noted. Bioapatite® masses are surrounded by a notable deposit of «bone-like» substance. Occasionally, particles could likewise be observed implanted in ectopic position within the supracrestal connective tissue beneath the epithelial zone. These particles are surrounded by a deposit of bone like substance (Fig. 6C)

Evolution of endurance:

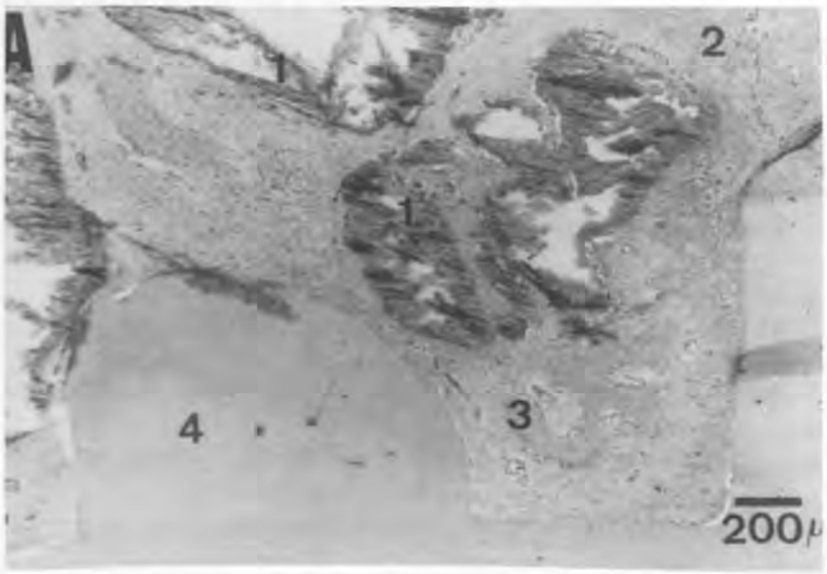
The data relating to the Endurance Index and the Transformed Endurance Index are outlined in Table I and Fig. 7. In the first and second months, Endurance Indices can be observed near 30%, with a drop in the indices in the sixth and ninth months.

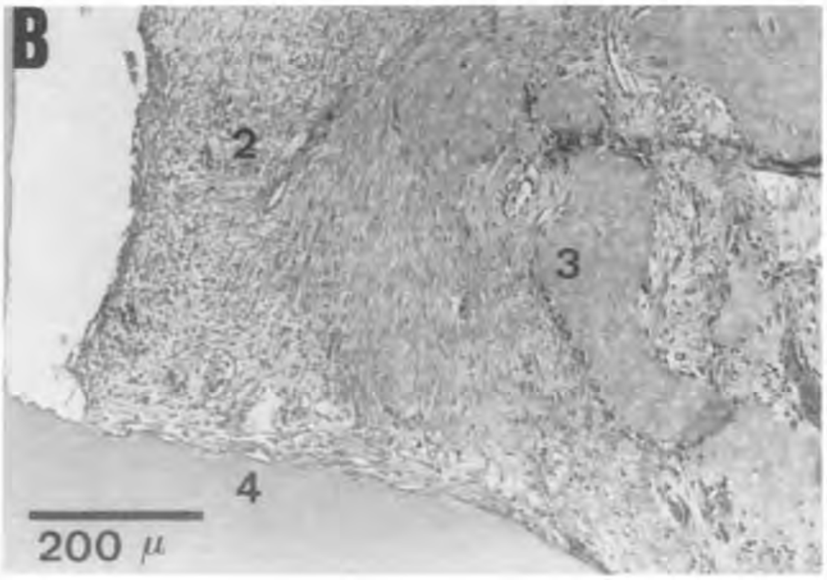
Spearman rank correlation test (between Index and time) is statistically significant (Rho = -0.347, $p < 0.00001$). The pearson correlation coefficient r (between Index and time) is equal to -0.48 with a 95% confidence interval (-0.57; -0.37); it is highly significant ($p < 0.00001$). The determination coefficient r^2 is equal to 23.50%, *i.e. 23.5% of the variance of all the index values can be explained by the time factor.* The Pearson coefficient, between transformed Index and time, is equal to -0.508 with a 95% confidence interval (-0.59; -0.40); it is highly significant ($p = 8.8 \times 10^{-16}$). The determination coefficient r^2

TABLE I: Overall results.
TABLEAU I: Résultats globaux.

	1 month	2 months	6 months	9 months
Number of sections (N) and number of dogs (D)	N = 26 D = 1	N = 84 D = 3	N = 55 D = 2	N = 57 D = 2
Mean endurance index ± SD	0.2608 ± 0.0686	0.2976 ± 0.1196	0.1563 ± 0.1137	0.1496 ± 0.1205
Mean transformed endurance index ± SD	1.0646 ± 0.1597	1.1379 ± 0.2728	0.7583 ± 0.3315	0.7091 ± 0.3799

It may be observed that each dog provides nearly 30 measurements (by image analysis, one measurement corresponds to one section).
On peut observer que chaque chien fournit environ 30 mesures (dans l'analyse d'image à une mesure correspond une coupe).





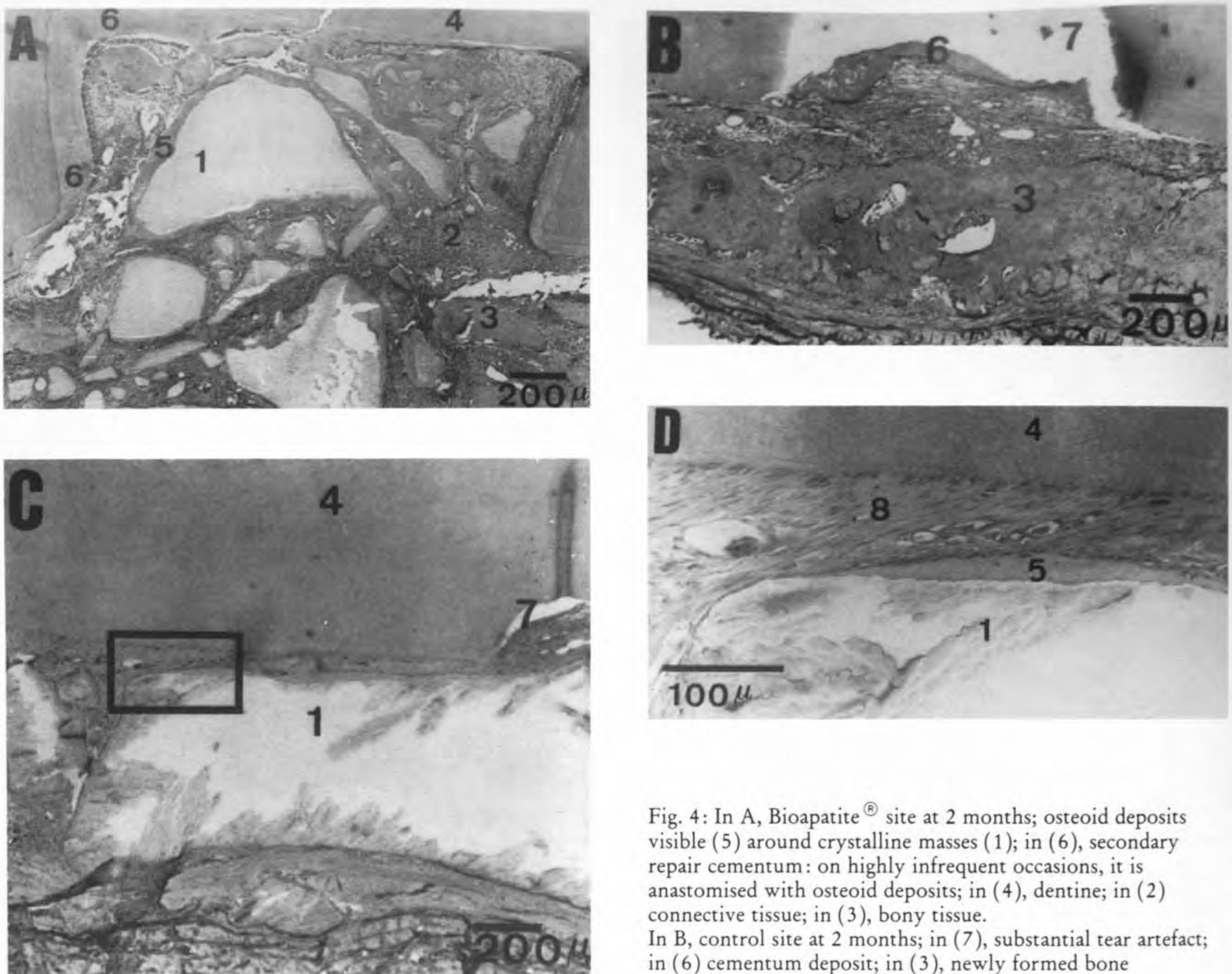


Fig. 4: In A, Bioapatite® site at 2 months; osteoid deposits visible (5) around crystalline masses (1); in (6), secondary repair cementum: on highly infrequent occasions, it is anastomised with osteoid deposits; in (4), dentine; in (2) connective tissue; in (3), bony tissue.
In B, control site at 2 months; in (7), substantial tear artefact; in (6) cementum deposit; in (3), newly formed bone trabeculae.
In C, Bioapatite® site at 2 months: appearance of large Bioapatite® masses extending over the dentinal trephination area; in (1) Bioapatite® masses at the periphery of trephination zone, in (4), dentine; in (7), trephination zone with tear artefact.
In D, higher magnification of zone outlined in C; appearance in (1) of crystalline masses; in (5), osteoid deposit; in (8), fibrous connective bundles fastened to crystalline mass; and in (4), dentine.

Fig. 4: En A, site implanté de Bioapatite® à 2 mois; dépôts ostéoïdes visibles (5) autour des agrégats cristallins (1); en (6), cément secondaire cicatriciel: en de rares cas il peut s'unir avec le dépôt ostéoïde autour des agrégats; en (4), dentine; en (2) tissu conjonctif; en (3), tissu osseux.
En B, site témoin à 2 mois; en (7), artéfact de déchirure; en (6) dépôt cémentaire; en (3), travées d'os néoformé.
En C, site implanté de Bioapatite® à 2 mois, s'étendant au delà des bords de la zone de trépanation; en (1), agrégats de Bioapatite® à la périphérie de la zone de trépanation; en (4), dentine; en (7), zone de trépanation et artéfact de déchirure.
En D, agrandissement de la zone encadrée en C, on voit en (1) des amas de biomatériau; en (5), dépôt de type ostéoïde; en (8), faisceaux de tissu conjonctif s'amarrant aux masses de biomatériau; en (4), dentine.

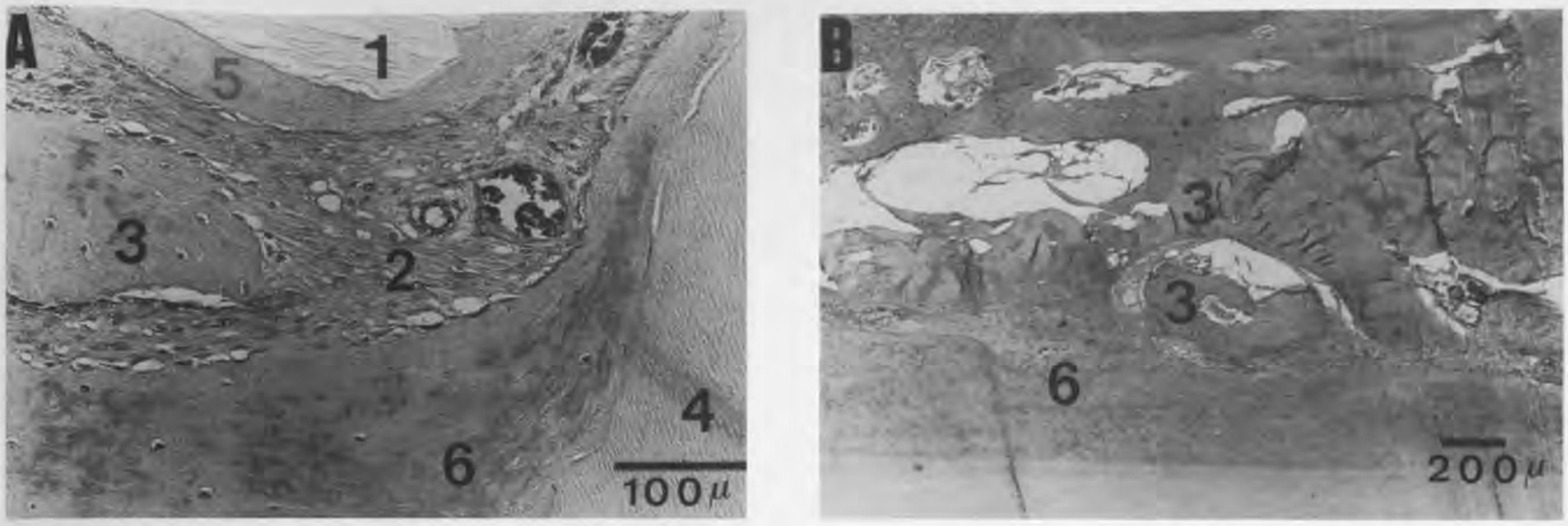


Fig. 5: In A, Bioapatite® site at 6 months; in (1), crystalline mass; in (5), osteoid deposit; in (3), newly formed bone trabeculae; in (2), periodontal ligament-like connective tissue and bonds with secondary cementum (6); in (4), dentine.
In B, control site at 6 months; shallow notch made in primary cementum; in (6) trace deposits of secondary cementum; and in (3), bony-like trabeculae in the process of repair.

Fig. 5: En A, site implanté de Bioapatite® à 6 mois; en (1) les agrégats de matériau cristallin, en (5), le dépôt ostéoïde; en (3), les travées osseuses néoformées; en (2) tissu conjonctif de type desmodontal uni au cément secondaire; en (4), la dentine.
En B, site «témoin» à 6 mois; une mince encoche est visible; en (6), fin dépôt de cément secondaire et en (3), travées osseuses participant aux processus de réparation.

TABLE II: One way A.N.O.V.A. study according to time factor as a qualitative modality (1, 2, 6, 9 months).
TABLEAU II: Analyse de variance, le facteur de temps étant considéré comme une modalité qualitative (1, 2, 6, 9 mois).

Source of variant	Sum of squares	d.f.	Mean square	F.
Factor A (time)	1.0774	3	0.3591	27.776 S*
Rest	2.8186	218	0.0129	
	3.8959	221		

S* = Significant; criterion: endurance index; mean scores for each time differ overall. (*cf.* Fig. 7 and Table II-B).
S = significatif; critère = indice de rémanence; les scores moyens diffèrent dans leur ensemble.*

TABLE II-B: Steps of the protected multiple «t»-test.
TABLEAU II-B: Etapes du test de Student protégé.

Type of comparison	Level of significance
Difference 1 month - 2 months	N.S.
Difference 2 months - 6 months	S.
Difference 6 months - 9 months	N.S.
Difference 1 month - 9 months	S.

N.S. = Non significant; S = Significant; the rest of ANOVA (Table II) and the adequate degree of freedom were used for this test procedure. This table may be consulted in conjunction with Fig. 7.
NS = Non significatif; S = significatif; la valeur résiduelle de l'analyse de variance (Tableau II) et le nombre de d.d.l. correspondant ont été utilisés. Ce tableau doit être consulté au regard de la Figure 7.

is 25.81%, i.e. 25.81% of the variance of all the transformed index values can be explained by time. A one way, A.N.O.V.A. (Table II), while considering the time factor as a quantitative modality (month 1, 2, 6, 9), corroborated that the Index score at various periods significantly differ ($p < 0.0000...$). Analyses based on a protected «t» test, subsequent to one way A.N.O.V.A., produces the result summariced in Table IIB. The same analysis applied to the Transformed Index display similar results.

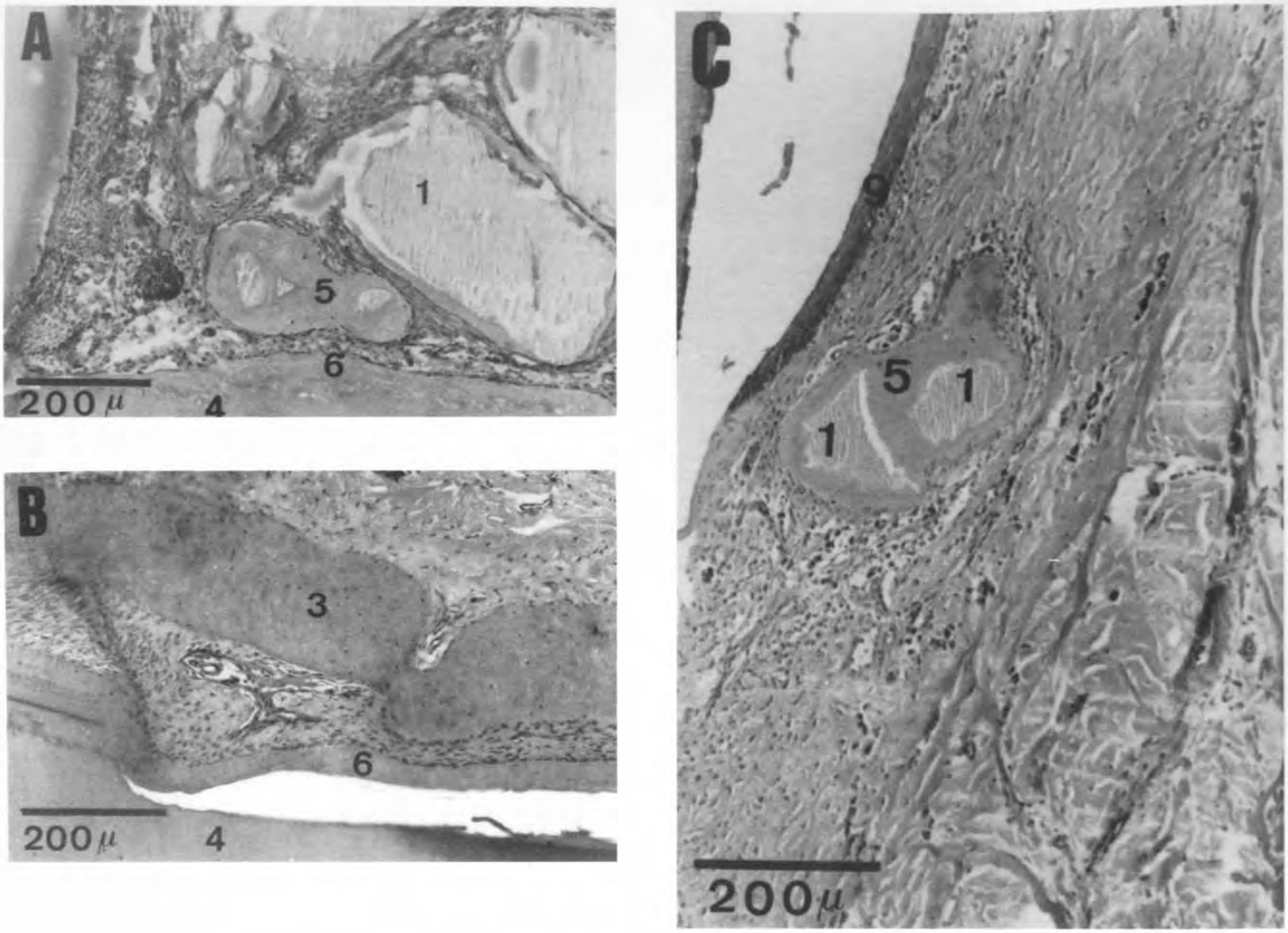


Fig. 6: In A, Bioapatite® site at 9 months; in (1), crystalline masses; in (5), osteoid deposit which may be substantial at the stage; in (6), secondary repair cementum; in (4), dentine.
In B, control site at 9 months; shallow notch; layer of secondary cementum (6) and repaired bone trabeculae (3); in (4) dentine.
In C, crystalline masses in ectopic position (enclosed in flap during suturing). Presence of substantial osteoid deposit (5) around crystalline masses (1); cervicular epithelium in (9); zone situated slighty below the enamel-cementum junction.

Fig. 6: En A, site implanté de Bioapatite® à 9 mois; en (1) les agrégats cristallins, en (5), le dépôt ostéoïde autour du biomatériau qui est important à ce stade; en (6), cément secondaire de réparation; en (4), la dentine.
En B, site « témoin » à 9 mois; une mince encoche; dépot de cément secondaire (6), travées d'os cicatriciel (3); en (4), la dentine.
En C, agrégats de biomatériau implantés en position ectopique (pris dans le lambeau lors de la suture). Présence d'un dépôt ostéoïde (5) autour des agrégats (1); epithélium sulculaire en (9); zone située légèrement en dessous de la jonction émail-cément.

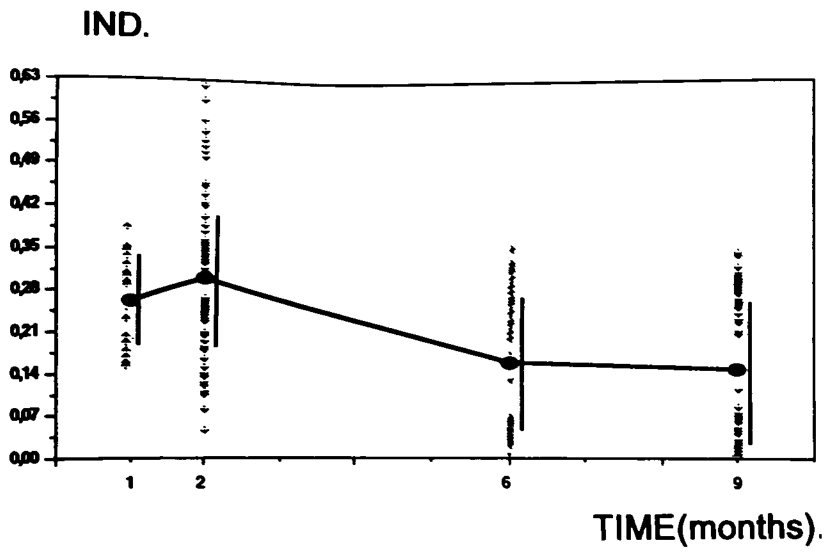


Fig. 7: Bioapatite® from 1 to 9 months, all series pooled. Evolution of endurance index (IND) over time. For each 1 to 9-month period, a «cloud of dots» can be observed, and mean with standard deviation (slighty shifted from cloud of dots) in parallel position. Note overall decrease in index values with implant time. Points represent average values.

Fig. 7: Bioapatite® du mois 1 à 9 toutes séries confondues. Evolution de l'Index de rémanence (IND) en fonction du temps. Pour chaque période entre 1 et 9 mois un nuage de points peut être observé; l'écart type et la moyenne sont figurés, légèrement déportés du nuage de point. Noter la décroissance des valeurs en fonction du temps. Les points représentent la moyenne.

DISCUSSION

Statistical methods

The statistical unit selected for the study of endurance was the section. It would have been possible to select the dog as the statistical unit, however the power of the study would have been lessened in so doing. Situations with «inconstant number of repetitions» were encountered in A.N.O.V.A. analysis. The PCSM software application which was used offers various test options including «unequal numbers of repetitions» or «unequal but nearly-equal numbers of repetitions». The latter option was the one most often validated. An analysis model is found in the litterature concerning two-way analysis of variance with «unequal number of repetitions» (Cetema, 1986), which is an accurate calculating method. This mode was also often applied. Furthermore, the number of sections supplied for each respective dog varies only slightly, and accordingly the weight of each dog in the statistical analysis is not disproportionate.

Surgical methods selected

The closed model with dentinal notches was chosen. Such an experimental model has been previously documented, in particular the study of Bye *et al.* (1987), can be cited; the studies of Frank *et al.* (1987), and Ogilvie *et al.* (1987) approach this model. Image analysis would prove impracticable without such notching. Connective-dentine contact could cause ankylosis and dentine resorption, according to Karring *et al.* (1984). In actual fact, such occurrences of ankylosis were rare: periodontal ligament cells being situated on the perimeter of the surgical wound, it would rapidly colonise the site.

Cavity filling phenomenon

At a very early stage (1 month, 2 months), trephinations were only partially filled with the biomaterial. At this stage, there is still a large connective zone. In this regard, the studies of Frank *et al.* (1987) demonstrate that the Bioapatite® masses at 6 months are largely separated from one another by fibrous connective tissue. Generally on a microscopic scale it would appear highly difficult for a cavity to be completely filled with biomaterial even if it seems to be so to the naked eye. As it has a certain plasticity, the material may be compacted, however there are still substantial gaps in the cavity. This is mentioned to stress the point that the concept by which packing and compacting the material should be avoided in order to leave available space for healing tissue is completely erroneous.

Endurance Indices: effect of «time» factor.

From month 1 to month 9, statistical analysis demonstrates that the Endurance Indices are linked to «time». It should further be pointed out that the indices remain rather stable for months 1 and 2 and that the drop in the indices appears between months 2 and 6. We consider this medium-term resorption, clearly proven in this study, to be a highly interesting property from a surgical point of view. Finally, while endurance indices decrease over time, there is an increase in the thickness of the osteoid deposit around the crystalline masses, as demonstrated by Mouchebœuf (1990) in a preliminary work by our laboratory. The present work, as the above, mentioned preliminary data, underlines the precocity (two months) of the appearance of osteoid deposit. The «time» factor is therefore of the utmost importance as to the endurance of implants.

CONCLUSION

Bioapatite® is a resorbable material. Its endurance is highly linked with time. The most important drop in endurance can be observed between *months 2 and 6*. While the level of filling in the cavities remains stable until the second month, a highly visible gap appearance subsists in all events, and whatever the technique applied. In short, the stability of the material in the early implant stages, its compacting potential combined with lacuna-type filling, favour the osteoinductive function of the material. It is very interesting to note that this function becomes apparent with the development of an osteoid deposit around the crystalline deposits as of the second month. Osteoid deposit can also be observed with implants in ectopic position. Finally, the material does not appear to interfere with cementogenesis and the reconstruction of an attachment system.

REFERENCES

Bye, F.L., Krause, M.E. Regezzi, J.A. and Caffesse, R.G. — Histologic evaluation of periodontal implants in biologically «Closed model». *J. Periodontol., 58:* 110-114, 1987.

Cetema: Statistique Appliquée à l'Exploitation des Mesures. Commission d'Etablissement d'Analyse des Mesures. Commissariat à l'Energie Atomique. Masson, Paris, 1986.

Frank, R.H., Gineste, M., Benque, E.-P., Hemmerle, J., Duffort, J.-F. and Heugebaert, M. — Etude ultrastructurale de l'induction osseuse après implantation d'Hydroxapatite chez l'homme, *J. Biol. Buc., 15:* 125-134, 1987.

Karring, T., Nymam, S. Lindhe, J. and Sirirad, M. — Potentials for root resorption during periodontal wound healing. *J. Clin. Periodontol., 11:* 41-52, 1984.

Mouchebœuf, J.-L. — Etude comparative de divers biomatériaux de comblement utilisés en clinique osseuse parodontale. Expérimentation animale. Thèse d'Université, 1990. Université de Bordeaux.

Ogilvie, A., Frank, R., Benque, E.-P., Gineste, M., Heugebaert, M. and Hemmerle, J.— Biocompatibility of hydroxapatite implanted in human periodontium. *J. Periodontol. Res., 22:* 270-283, 1987.

PCSM: Programme Conversationnel de Statistique pour les Sciences et le Marketing. Deltasoft, Paris, 1987.

Address of correspondence:
Prof. J.J. Bonfil,
Service Odontologie et Unité de Recherche Evaluative en Thérapeutique et Santé Bucco-Dentaire, Hôpital Nord, Chemin de Bourrely, 13015 Marseille, France.