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Morphometric computerized analysis on the dentinal tubules and the collagen fibers in the dentine of human permanent teeth

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SUMMARY

A morphometric analysis has been performed on important components of human dentine using an image computerized analyzer. The dentinal tubule diameter and their area percentage were calculated. Moreover the area percentage of the collagen fibers in the dentinal matrix was measured. These parameters have been evaluated in different areas of the coronal and the radicular dentine in permanent teeth.

Measurements have been performed on undecalcified and decalcified teeth and on teeth treated with enzymatic digestion to remove the organic non collagen matrix and to evidentiate the collagen fiber network.

The values obtained in different areas of the tooth and in samples sumitted to different treatments were evaluated by statistical analysis.

Dentinal tubule diameter and area percentage significatively decrease from the inner to the peripheral dentine both in the undecalcified teeth as in the decalcified ones and in the samples undergone to enzymatic digestion. The collagen fiber percentage in the organic matrix is significatively lower in the mantle dentine.

KEY WORDS:

Computerized morphometric analysis, dentinal tubules, collagen fibers, diameter, area percentage.

RÉSUMÉ

Il a été procédé à une recherche morphométrique relative à des composants importants de la dentine à l'aide d'un analyseur d'images computérisé. Le diamètre des tubules dentinaires et le pourcentage de la surface qu'ils occupent dans la dentine ont été mesurés. En outre, le pourcentage de la surface occupée par les fibres collagènes dans la matrice dentinale a été également mesuré. Ces valeurs ont été relevées dans des surfaces différentes de dentine, dans la racine et dans la couronne de dents permanentes. Les mesures ont été effectuées sur des dents décalcifiées ainsi que sur des dents traitées par digestion enzymatique afin d'enlever, après décalcification, la composante organique non collagène et séparer la trame des fibres collagènes.

Les valeurs obtenues dans les différentes surfaces de la dent et dans des dents soumises à divers traitements ont été étudiées et confrontées au moyen d'analyse statistique.

Le diamètre des tubules et leur pourcentage de surface diminuent significativement de la dentine la plus interne à celle périphérique, aussi bien dans les dents non décalcifiées que dans les dents décalcifiées et dans celles qui sont soumises à digestion enzymatique. Le pourcentage de la surface des fibres collagènes dans la matrice organique est significativement inférieure dans la dentine périphérique.

MOTS CLÉS:
Analyse d'images computérisés, tubules dentinaires, fibres collagènes, diamètre, aire percentage.

INTRODUCTION

Computerized morphometric analysis is an investigating method that can give some advantage in the quantitative evaluation of biological structures. In fact the sample examination is carried out by means of an image analyzer that allows more correct and accurate measurements than those obtained with other current methods.

As far as dentine is concerned literature gives different and sometimes conflicting data on size and area percentage of dentinal tubules (Ketterl, 1961; Fromme and Riedel, 1970; Tronstad, 1973; Garberoglio and Branstrom, 1976; Wittaker and Kneale, 1975; Franck and Nalbandian, 1989, Sogaard-Pedersen et al., 1990).

No quantitative data exist on the collagen fibers that represent a fundamental component of dentine. Collagen fibers can be isolated from the other components of this tissue through demineralization and some treatments removing the organic matrix (Sogaard-Pedersen, 1990; Marchetti et al., 1992). In samples undergone to this method we have previously observed a variation in the collagen fiber density in different areas of the tooth (Marchetti et al., 1992).

In the present investigation we have carried out quantitative evaluation of collagen fiber density and of dentinal tubule diameter and area percentage using a computerized morphometric analyzer.

MATERIAL AND METHODS

18 permanent teeth, premolar and molar, from adult subjects, have been employed. Six teeth were decalcified in formic acid and sodium formate; six teeth were decalcified and treated for isolating the collagen fiber network of dentine by using H_2O_2, trypsin and EDTA to remove the non collagen matrix (Marchetti et al., 1992). Samples submitted to these treatments were routinely processed for transmission electron microscopy (TEM). Six teeth, untreated with decalcifying agents, were assigned to scanning electron microscopy (SEM).

Dentinal tubule diameter and area percentage were measured on all the teeth. Area percentage of collagen fibers in the organic matrix has been calculated on the teeth decalcified and treated with enzymatic digestion.

Measures have been performed in different areas of crown and root of the teeth in order to evaluate possible variations in the parameter values. These areas were selected in the circumpulpal, inner (2 mm from the pulp) and peripheral dentine.

Quantitative evaluation of area percentage of dentinal tubules and measurements of their diameter in demineralized teeth and in teeth demineralized and submitted to enzymatic digestion of non-collagen organic matrix have been performed on light microscopic sections. The computer camera was directly connected to the microscope.

The same parameters on dentinal tubules were evaluated on undecalcified teeth and measures were performed on SEM micrographs (1000 ×).

Morphometric analysis on the area percentage of collagen fibers has been performed on TEM micrographs (5000 ×).

Measures on TEM and SEM micrographs have been performed by submitting the same micrographs to the computer camera with a further magnification of the pictures.

45 measures have been made on each selected area of crown and root dentine.

Morphometric analysis was made on the images of the specimens, displayed on a TV color monitor and elaborated with an image analyzer program. The following steps were carried out on processing all the preparations: (1) image input and storage in computer memory; (2) calibration of the measuring

system; (3) normalization of the image grey levels in a range of 255 levels: black(0)-white(255); segmentation of the image grey levels by a dynamic discrimination in order to obtain binary images suitable for measurements; selection and measurement of parameters; storage of measured data.

Basic statistic and analysis of variance were performed on data obtained for each parameter in the selected areas.

Values derived from measurements in the three different areas of crown and root dentine of teeth treated with the same procedure were compared.

Finally a comparison among the values obtained in the same dentine area in teeth undergone to the different treatments was made.

RESULTS

The different samples employed for measurements are represented in Figs. 1, 2, 3. The mean values of measures and related standard deviations are referred in Tables 1, 2, 3.

Dentinal tubule diameter and area percentage decrease from circumpulpal to peripheral dentine. No significative differences have been observed between the values obtained for these two parameters in corresponding areas of crown and root; therefore they have been grouped together. On the contrary the area percentage of collagen fibers shows different values in corresponding areas of crown and root; these values have been evaluated separately.

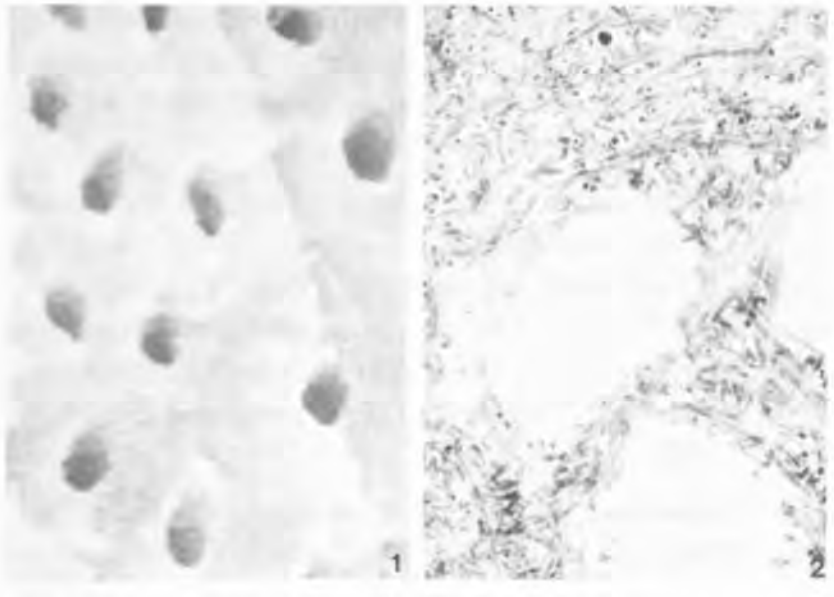

Fig. 1: Dentinal tubules at SEM. Undecalcified tooth
⊢⊣ 3 μm
Fig. 1: Tubules dentinaires au M.E.B. Dent non décalcifiée.

Fig. 2: Collagen fibers at TEM. Tooth decalcified and treated with enzymatic digestion
⊢⊣ 1 μm
Fig. 2: Fibres collagènes au M.E.T. Dent décalcifiée et soumise à digestion enzymatique.

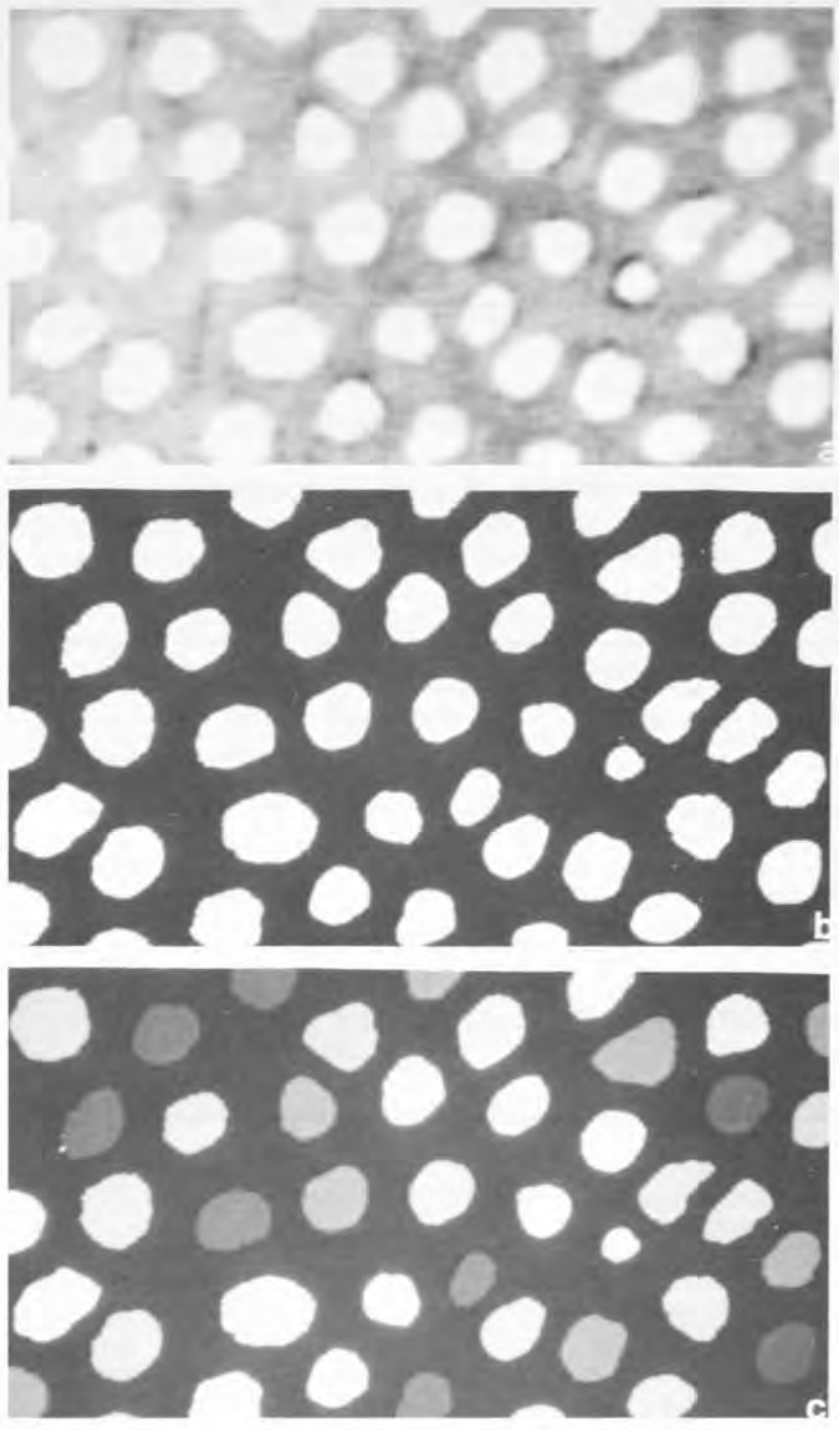

Fig. 3: Some steps of the image elaboration program by means of a computerized image analyzer.
a) Normalized image of dentinal tubules at light microscopy from a decalcified tooth;
b) binary image obtained by a dynamic discrimination of the grey levels;
c) image were the structures to be measured have been identified.
⊢⊣ 3 μm
Fig. 3: Quelques phases du programme d'élaboration des images au moyen d'un analyseur d'images computérisé.
a) Image normalisée de tubules dentinaires examinés au microscope photonique dans une dent décalcifiée.
b) Image binaire obtenue grâce à une sélection convenable des niveaux de gris.
c) Image où les structures à mesurer (tubules dentinaires) ont été identifiées.

TABLE 1

Mean diameter (μm) ($\pm$ SD) of dentinal tubules			
	Areas of dentine		
	circumpulpal	interior	peripheral
undecalcified teeth	2.37 $\pm$ 0.28 *	1.30 $\pm$ 0.12 *	0.63 $\pm$ 0.09 *°
decalcified teeth	2.63 $\pm$ 0.35 **	1.62 $\pm$ 0.18 **	1.24 $\pm$ 0.11 **°
teeth decalcified and undergone to enzymatic digestion	2.54 $\pm$ 0.31	1.57 $\pm$ 0.16	1.31 $\pm$ 0.12 °

* $P<0.01$
** $P<0.05$
° $P<0.01$

TABLE 2

Area percentage ($\pm$ SD) of dentinal tubules			
	Areas of dentine		
	circumpulpal	interior	peripheral
undecalcified teeth	25 $\pm$ 3.82 *	16 $\pm$ 2.09 *	3 $\pm$ 0.63 *
decalcified teeth	27 $\pm$ 4.91 **	17 $\pm$ 2.12 **	6 $\pm$ 0.83 **
teeth decalcified and undergone to enzymatic digestion	28 $\pm$ 5.42 ***	19 $\pm$ 2.41 ***	5 $\pm$ 0.71 ***

* $P<0.01$
** $P<0.05$
*** $P<0.05$

TABLE 3

Area percentage ($\pm$ SD) of collagen fibers in the dentinal matrix in teeth decalcified and undergone to enzymatic digestion			
	Areas of dentine		
	circumpulpal	interior	peripheral
crown	77.1 $\pm$ 9.9	71.3 $\pm$ 6.8	61 $\pm$ 4.6
root	77.3 $\pm$ 10.9	74.2 $\pm$ 8.2	71.2 $\pm$ 6.6

* $P<0.05$.

Statistical analysis on values obtained in the specific different areas of dentine shows a significative difference in tubule diameter if we compare the peripheral dentine values to the interior and circumpulpal dentine ones, both in undecalcified ($p<0.01$) and in decalcified teeth ($p<0.05$).

Significative differences are also present in the area percentage of the tubules among the peripheral dentine and the other dentinal areas in undecalcified ($p<0.01$), in decalcified ($p<0.05$), in decalcified and treated teeth ($p<0.05$).

The comparison of tubule data obtained in the same areas of dentines treated in different ways shows a significative difference in tubule diameter of the peripheral tissue by comparing undecalcified teeth and the sample undergone to the other two methods ($p<0.01$).

The area percentage of collagen fibers in dentinal matrix decreases from circumpulpal to peripheral dentine. A significative difference exists among the values of peripheral (mantle) dentine and the values of the other two areas in the crown.

DISCUSSION

Literature data on diameter and area percentage of dentinal tubules are various and sometimes conflictual. This has generally been ascribed to the different characteristics of the samples: undecalcified and decalcified teeth. We think that some discrepancies could also be indebted to the different measuring systems that the Authors applied. Therefore comparisons and evaluations among the different results are sometimes problematic.

Our results differ from those obtained by some Authors in decalcified (Ketterl, 1961) and in undecalcified teeth (Tronstad, 1973; Wittaker and Kneale, 1979) but they are in agreement with data presented by others (Garberoglio and Branstrom, 1976; Frank and Nalbandian, 1989; Sogaard-Pedersen et al., 1990).

The comparison of our data on tubule diameter in decalcified and in undecalcified teeth confirm that demineralizing process causes the loss of peritubular dentine, greatly mineralized and the increase of tubule diameter. Moreover the comparison of values on tubule diameter obtained in the same areas in teeth submitted to different treatments shows that peritubular dentine, that gets last with decalcification, is thicker in the peripheral than in the circumpulpal dentine.

The values obtained in decalcified teeth and in teeth treated with enzymatic digestion are not significativelly different. This datum confirms that treatment to remove organic non-collagen matrix from dentine does not modify the collagen component organization and the tubule structure. The measurements carried out on teeth undergone to this enzymatic digestion show that collagen fibers are less dense in the peripheral dentine than in the other areas of tooth. This characteristic is significatively evident in mantle dentine nevertheless here the collagen fibers have a larger diameter (Marchetti et al., 1992). It seems interesting to note that collagen fibers decrease in area percentage while their diameter increases from circumpulpal to peripheral dentine. These morphological differences in the collagen component could probably influence the functional characteristics of dentine.

Our study confirms that automatic computerized morphometric analysis is an efficacious and correct method in investigating on biological structures. Particularly it could be assumed as particularly useful for objectifying further morphometric investigations on dental tissues.

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