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(54) Title: INDUCED REMINERALISATION OF HUMAN TOOTH ENAMEL

(54) Bezeichnung: INDUIZIERTE REMINERALISATION VON HUMANEM ZAHNSCHMELZ

(57) Abstract: The invention relates to the induced remineralisation of human tooth enamel and in particular the build up of apatite on tooth material.

(57) Zusammenfassung: Die vorliegende Anmeldung betrifft die induzierte Remineralisation von humanem Zahnschmelz und insbesondere den Aufbau von Apatit auf Zahnmateriale.

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Zur Erklärung der Zweibuchstaben-Codes und der anderen Abkürzungen wird auf die Erklärungen ("Guidance Notes on Codes and Abbreviations") am Anfang jeder regulären Ausgabe der PCT-Gazette verwiesen.

Induced remineralization of human tooth enamel

Description

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The present application relates to the induced remineralization of human tooth enamel and in particular the building up of apatite on tooth material.

10

Teeth are composite materials formed from apatite and proteins. They are very hard biomaterials based on calcium and phosphate. The tooth enamel, the outer layer of the crown of the tooth, is the hardest part of the tooth and contains no living cells. Tooth enamel consists of inorganic crystals, which have typical highly oriented arrangements. Tooth enamel is a tissue which, as soon as it is formed, remains unchanged almost for life, since the cells which are involved in the construction of the teeth die as soon as tooth formation is complete. Finished tooth enamel consists of approximately 95% by weight of apatite, approximately 3% by weight of proteins and lipids and approximately 2% by weight of water.

15

In order to avoid or to repair damage to teeth, in particular by caries, it has been attempted for a long time to employ remineralizing systems. It was initially attempted here to improve the condition of the teeth by 20 application of calcium phosphate compounds. Such single-component systems in which it is attempted to apply tooth material which has already been previously prepared, for example apatite, hydroxyapatite or other calcium phosphate compounds, to the teeth are 25 described, *inter alia*, in EP 0 666 730 B1 or WO 01/95863. The problem of such systems is that treating tooth material with calcium phosphate compounds does not lead to growth of apatite which is

structurally similar to the tooth material, but rather to a mere addition of apatite crystals to the tooth material, the apatite crystals having a morphology which is completely different from the tooth material.

5 No strengthening of the tooth enamel or permanent filling of lesions is thus brought about, since the apatite crystals added do not have sufficient similarity and adhesion to the natural tooth material.

10 It was furthermore attempted with two-component systems to obtain a remineralization of teeth, the systems customarily comprising a calcium phase and a phosphate phase. Two-component systems are described, for example, in WO 98/10736 and DE 33 03 937 A1. It is
15 disadvantageous with the procedures described there that the method described in WO 98/10736 combines calcium and phosphate solutions before application such that a metastable solution is formed from which apatite should crystallize out on the tooth. The method does
20 not allow localized treatment on the tooth, since the reagent is employed as a mouthwash or gel which is massaged in using a toothbrush. Furthermore, the composite nature of the native enamel is not taken into account, since no organic component is contained in the
25 system. The formation of tooth enamel-like crystallites is accordingly improbable. DE 33 03 937 describes a process in which calcium and phosphate ions are applied separately to the tooth in succession by immersing this in a cap which contains the corresponding ions in a
30 gelatin matrix. With a recommended time of action of only two minutes, it is not to be expected that really larger amounts of apatite can be formed on the tooth surface. It is not confirmed using image material that the newly formed apatite layer has enamel-like
35 structures.

In further studies (S. Busch et al., Eur. J. Inorg. Chem. (1999), 1643-1653; S. Busch et al., Chem. Mater.

13 (2001), 3260-3271; S. Busch, Zahnärztliche Mitteilungen [Dental Communications] 91, No. 10 (2001), 34-38; R. Kniep et al., Angew. Chem. 108, No. 22 (1996), 2787-2791), the biomimetic morphogenesis of fluoroapatite gelatin 5 composites was investigated. Biomimetic growth and self-organization of fluoroapatite aggregates by means of diffusion in denatured collagen matrices were observed here. The fundamental principles of fluoroapatite formation in gelatin gels was investigated here by means of 10 double diffusion experiments of calcium and phosphate solutions in a U-tube. These studies describe the formation of fluoroapatite beads within the gel used.

Advantageously, one or more embodiments of the present invention may provide a process with which defects on tooth 15 material can be repaired by remineralization. One or more embodiments of the invention may serve to cover tooth material with a protective apatite layer.

Thus in a first aspect, the present invention provides a process for the growth of apatite on tooth material, 20 comprising the steps:

- (i) applying to a tooth a first gel comprising gelatin and phosphate ions,
- (ii) applying a second gel which is free of phosphate ions, calcium ions and fluoride ions, the first 25 layer of gel being covered with this second gel, and
- (iii) applying a medium containing calcium ions.

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The invention further provides a kit when used in accordance with the process of the invention, comprising:

- 5 (a) a first gel which comprises gelatin and phosphate ions,
- (b) a second gel which is free of phosphate ions, calcium ions and fluoride ions as well as
- (c) a medium containing calcium ions.

10 The invention relates in particular to the use

- (i) of a first gel which comprises gelatin and phosphate ions,
- (ii) of a second gel which is free of phosphate ions, the first layer of gel being covered with this

15 second gel and

(iii) of a medium containing calcium ions,
for the production of an agent or of a kit for the
growth of apatite on tooth material.

5 According to the invention, it is surprisingly possible
to achieve a real growth of tooth enamel-like material.
A significant advantage consists in the fact that a
high order of small apatite needles is obtained, which
structurally have a great similarity to native tooth
10 enamel. With appropriate substrate orientation,
virtually no difference can be detected between apatite
which has grown and original tooth material.

Further advantages of the invention are that a real
15 growth of the fluoroapatite crystallites on the tooth
substrate can be assumed, as the SEM images show. The
Vickers hardness of this novel layer corresponds to
that of natural enamel. Carrying out the individual
20 steps is so simple that the remineralization of tooth
enamel can in principle be carried out by the patient
himself. The gel can be applied locally to the damaged
sites and solidifies there. Since the warmed gel cools
very rapidly, waiting times between the individual
steps are barely necessary.

25 Since the softening temperature of the gel is somewhat
above normal body temperature (38 to 42°C), melting of
the gel during the period of action is prevented.
Uncontrolled mineralization can thus be avoided.

30 By addition of fluoride ions to the phosphate-
containing gel, the resistance of the layer to acids
can be increased.

35 According to the invention, it is possible by means of
induced remineralization to regenerate tooth enamel
defects. By the use of a two-layer gel which is solid
at body temperature and can be applied locally to the

affected site on the tooth, and by the use of a mouth rinsing as a medium containing calcium ions, mineralization conditions are created which bring about the formation of a tooth enamel-like substance which grows
5 directly on the tooth. In the case of the previously published double-diffusion method, it was shown only that fluoroapatite which results by countercurrent diffusion of calcium and phosphate ions in a gelatin gel forms spherical aggregates whose organic proportion
10 by weight corresponds to that of mature, human tooth enamel. The double-diffusion method, however, has neither opened up a possibility of making possible the remineralization of tooth enamel in man nor implied this possibility in any manner. The experimental setup
15 employed in the double-diffusion method brings about the formation of small beads and does not allow the growth of uniform layers of apatite material on a substrate. This is only possible by means of the procedure according to the invention.

20 The invention can be applied, in particular, in man. It is possible here, for example, to cure relatively small carious defects by induced remineralization or to cover sensitive sites on the tooth with a protective apatite
25 layer. The procedure for treatment here is preferably as follows: The carious site is initially painted with a thin layer of the phosphate-containing gel warmed to approximately 50°C or this is applied with a suitable syringe, which can be warmed. The gel solidifies
30 immediately on the surface of the tooth and is covered with the protective gel according to the same method. 1 to 3 times a day, an approximately 10-minute mouth rinsing is then carried out with the calcium solution. Between the rinsings, the tooth is covered with a
35 suitable cap, which can be made of plastic or metal, such that the patient is not incapacitated and the remineralization can take place undisturbed. If many teeth are affected, the whole row of teeth can also be

protected with a rail, such as is employed, for example, against odontoprisis. Every two days, the gel is changed, and at this opportunity the affected tooth is cleaned and disinfected.

5

According to the invention, initially a first gel is applied to the tooth material. This gel contains gelatin and phosphate ions and optionally further constituents. The content of gelatin in the first gel 10 is preferably from at least 15% by weight, more preferably from 25% by weight, up to 40% by weight, more preferably up to 30% by weight. The gelatin, in particular, has a function in the development of the morphology of the apatite formed. It was surprisingly 15 discovered that when using gelatin, an apatite material is deposited on the surface of the tooth material which has a great similarity with native tooth enamel. When using other organic matrices, however, other morphologies of the apatite crystallizes were 20 observed, such that a building up of apatite on the surface of the tooth material, as desired according to the invention, does not occur.

Gelatine is a polypeptide which can be obtained, in 25 particular, by hydrolysis of the collagen contained in the skin and bone of animals. Gelatine customarily has a molecular weight of 15,000 to over 250,000 g/mol and can be obtained from collagen under acidic or alkaline conditions. According to the invention, the following 30 gelatins are preferably employed: Acid-hydrolyzed gelatin types (type A), e.g. prepared from pigskin or calfskin having a high Bloom value, e.g. 250 to 350 Bloom (the Bloom value is understood as meaning a parameter which characterizes the gel solidity, in 35 general it is true that the higher the Bloom value, the higher the proportion of long-chain molecules in the gelatin and the higher the gel solidity).

Beside gelatin, which is contained for the formation of the desired morphology of the apatite and the building up on the surface of the tooth material, the first gel furthermore comprises phosphate ions. These phosphate ions represent a basic constituent of the apatite built up from calcium phosphate. The concentration of the phosphate ions in the first gel is preferably at least 0.01 mol/l, more preferably at least 0.05 mol/l, and up to 0.5 mol/l, more preferably up to 0.2 mol/l, and in particular 0.08 mol/l.

The first gel preferably has a softening temperature which lies above normal body temperature such that the gel is solid at body temperature. The softening temperature of the first gel preferably lies in the range from 38 to 45°C, more preferably from 38 to 42°C. The first gel is preferably applied in warmed form, for example warmed to 45 to 55°C. After applying, the gel cools and becomes solid.

According to the invention, a second gel, a so-called protective gel, is applied in a further step. The first gel layer, in particular, is covered with this second gel. The protective gel, which functions as a gel covering layer, surprisingly causes the mineralization, that is the formation of apatite, to take place mainly or exclusively on the surface of the tooth and not on the gel-liquid boundary layer. By means of the two-layer gel construction which is achieved in the process according to the invention, surprisingly a building up or growth of apatite on the tooth material and not a crystallization or formation of apatite spheres within the gel occurs, as is described in the prior art. Only by means of the two-layer construction is a practicable and technically useful remineralization of the teeth thus possible.

The pH and the gel concentrations of the second gel

typically correspond to those which are indicated herein for the first gel. The second gel also preferably has a softening temperature of 38 to 45°C, in particular of 38 to 42°C and is preferably applied 5 warmed to 45 to 55°C.

In a third step, a medium containing calcium ions is finally applied. The medium containing calcium ions provides the basic structural material further needed 10 for the formation of apatite, namely calcium ions. These calcium ions diffuse through the protective gel and the first gel layer as far as the surface of the tooth material and are deposited there as apatite. The concentration of the calcium ions in the medium 15 containing calcium ions is preferably at least 0.01 mol/l, more preferably at least 0.05 mol/l, and up to 0.5 mol/l, more preferably up to 0.2 mol/l, and in particular 0.13 mol/l.

20 It was discovered that according to the invention surprisingly a uniform layer of apatite crystallites which are parallel or radial can be formed. Furthermore, this layer exhibits no peripheral gap or only a submicrometer-large peripheral gap to the native 25 tooth material. The direction of growth of the apatite crystallites takes place perpendicular to the substrate, independently of the orientation of the enamel prisms, such that on suitable orientation of the enamel prisms the longitudinal orientation of the 30 artificially grown crystals proceeds substantially identically to the crystals in the prisms. The order of magnitude of fusion crystals and grown fluoroapatite is identical. Within the layers, a tight and uniform packing can be observed. Furthermore, the apatite layer 35 applied has a Vickers hardness which corresponds to that of the native tooth enamel. The apatite layers applied according to the invention in particular have a Vickers hardness in the range from 250 to 400 HV.

According to the invention, it is possible to apply apatite layers in any desired thickness, since the layer thickness achieved is dependent on the frequency 5 of the change of gel. Per change of gel, up to now layer thicknesses of up to 1 μm can be achieved.

In a preferred embodiment, a gelatin-glycerol gel is employed as the first gel. The weight ratio of gelatin 10 to glycerol is preferably 1:5 to 5:1 here, in particular 1:2 to 2:1. Glycerol has the effect that the softening point of the gel is raised above the normal human body temperature. The gel solidity achieved is necessary in order to obtain the two-layer system 15 during the mineralization such that a specific, controlled crystal deposition is made possible. In a liquid gel, a spontaneous precipitation of finely crystalline material would occur, which does not grow on the tooth.

20 The first gel preferably furthermore contains fluoride ions. The fluoride can be added, for example, as sodium fluoride or ammonium fluoride. In this embodiment, fluorine-rich apatite or fluoroapatite can be grown on 25 the surface of the tooth material. Fluoroapatite is, in particular, more acid-resistant than the carbonate-containing hydroxyapatite of the natural tooth enamel, the morphology of the layers of fluoroapatite forming nevertheless having a great similarity to native tooth 30 enamel.

The growth rate of the apatite or fluoroapatite is determined, inter alia, by the pH of the first gel. Preferably, the first gel has a pH of 2.0 to 6.0, in 35 particular of 4.0 to 6.0, more preferably of 5.0 to 5.5.

A significant feature of the present invention is the

use of a protective gel as the second gel. The first gel layer containing phosphate ions is covered with this protective gel. By use of this protective gel layer, surprisingly the apatite formation takes place 5 exclusively on the surface of the tooth material and a spontaneous crystallization of apatite crystallites or composite aggregates does not occur, such as is observed in the case of the procedures known in the prior art. In contrast to the investigations with 10 double-diffusion chambers, a coating of tooth material surfaces can thus be obtained in a controlled manner. The second gel preferably contains no materials which are to be incorporated into the apatite, and is thus, in particular, free of phosphate ions, calcium ions 15 and/or fluoride ions. Gelatine can likewise be employed for the formation of the second gel, a gelatin-glycerol gel being preferred. The second gel used can, however, also be another gel, e.g. selected from polysaccharides, for example agarose or carrageen, and also 20 carboxymethylcellulose.

According to the invention, the tooth material coated with a first gel and protective gel is finally treated with a medium containing calcium. The medium containing 25 calcium employed can be, for example, a solution containing calcium ions and/or a gel containing calcium ions. The medium containing calcium ions is preferably prepared here using a water-soluble salt containing calcium ions, for example from CaCl_2 .

30 The medium containing calcium ions preferably has a pH of 6 to 8.

According to the invention, the two constituents of 35 apatite, namely phosphate ions and calcium ions, are in each case separately supplied as an individual component, the calcium phosphate formation then only taking place on the tooth material surface.

In order to prevent a local overacidification on the mineralization front by means of the proton release during the apatite formation, the phosphate gel is 5 preferably treated with a buffer system, preferably an acetic acid buffer or α,α,α -tris(hydroxymethyl)methyl-amine buffer.

Before the treatment with the first gel, the tooth 10 material can be pretreated, in particular defatted, etched or/and rinsed. For example, for better efficacy the tooth surface can initially be defatted with ethanol and etched with phosphoric acid and subsequently rinsed with deionized water.

15 The process according to the invention is suitable, in particular, for the treatment of human teeth or tooth enamel. Carious defects can be treated here by remineralization or else the tooth material can also be 20 covered prophylactically with a protective apatite or fluoroapatite layer. The apatite layers are formed both on tooth enamel and on dentine as a substrate.

The invention furthermore relates to a composition 25 and/or a kit which, in particular, is suitable for the use described above and comprises
a) a first gel which comprises gelatin and phosphate ions,
b) a second gel which is free of phosphate ions, and
30 c) a medium containing calcium ions.

The preferred embodiments of the first and second gel and of the medium containing calcium ions are as described above here.

35 The invention is further illustrated by the attached figures and the following examples.

Figures 1 to 6 show SEM-micrographs of apatite grown on tooth material.

Examples

5

Example 1

Step 1. Preparation of the tooth material

A human tooth (arbitrary) was separated from its root
10 and the crown was sawn into disks approximately 0.5 mm wide, namely such that the longitudinal axis of the enamel prisms was orientated obliquely to the direction of the cut. The disks were immersed for 30 s in a 30% strength phosphoric acid solution, washed with
15 deionized water and dried.

Step 2. Preparation of the gel

A homogeneous gel whose pH was 5.0 was prepared at 80°C with stirring from 8.56 g of gelatin, 8.24 g of 85%
20 glycerol solution, 7.26 g of H₂O, 1.8 ml of 2N NaOH, 2.7 ml of 2N HAc, 13.8 mg of NaF and 236 mg of Na₂HPO₄. A further gel was prepared from 8.56 g of gelatin, 8.24 g of 85% glycerol solution and 11.76 g of H₂O. A 0.133 molar calcium solution was prepared from CaCl₂ salt.

25

Step 3. Induced mineralization on the tooth surface

The surface of the tooth disks was painted with approximately 0.5 ml of the phosphate-containing gel.
30 After its solidification, covering was carried out with approximately 0.5 ml of the additive-free gel. The tooth disk was inserted into a plastic tube closed on one side and stored at 37°C in a calcium solution. The gel and the solution were renewed every 7 days, altogether 16 x. For the appraisal of the layer grown,
35 a fracture of the sample perpendicular to the cut surface was carried out in order to be able to measure the layer thickness. As the SEM-micrograph in figure 1 confirms, a uniform layer of elongated crystallites

having a layer thickness of 7.2 μm is formed. This corresponds to a growth rate of approximately 450 nm/week.

Example 2

5 The procedure is carried out analogously to example 1, but the direction of the cut of the tooth disks was largely perpendicular to the longitudinal axis of the enamel prisms. Moreover, the sample was stored at 36°C and exposed to the calcium solution for only 60 min
10 daily. The gel was replaced every 2 days. The sample was investigated after 10 replacement cycles. The SEM-micrograph (figure 2) shows a uniform layer of crystals arranged in parallel, whose orientation and order of magnitude corresponds to that of the native enamel. A
15 submicrometer-large peripheral gap can be detected. The layer thickness is 2.7 μm . This corresponds to a growth rate of 135 nm/day.

Example 3

20 The tooth preparation was carried out analogously to example 1, but the direction of the cut of the disk was largely parallel to the longitudinal axis of the enamel prisms. The sample was stored at 37°C and the replacement cycles correspond to example 2, but the gel
25 was renewed 36 x altogether. The SEM-micrograph (3a) allows a uniform, 15 μm thick layer to be detected. Ray-like intergrown structures result here, which guarantee a good sealing of the native tooth surface. The peripheral gap to the tooth is minimal; very small,
30 morphological peculiarities of the native surface are represented by the growing material (analogously to an impression). The size and morphology of enamel crystals and grown fluoroapatite are identical (figure 3b). The growth rate is approximately 210 nm/day.

35

Example 4

The procedure corresponds to example 2, but 755.2 mg were employed for the phosphate gel and the gel was

replaced only 5 x. Analogously to example 2, the SEM-micrograph (figure 4a) shows the corresponding orientation of enamel crystallites and grown fluoroapatite. The crystallites do not yet appear, 5 however, to be completely matured. The layer thickness is 5 μm ; this corresponds to a growth rate of 500 nm/day.

Example 5

10 The procedure corresponds to example 4a, but the direction of the cut was carried out parallel to the longitudinal axis of the enamel prisms and the sample was investigated only after 10 gel exchange cycles. At 9 μm , the layer is almost twice as thick as that from 15 example 4 (cf. Figure 5), which points to the linearity of the growth rate. It appears more mature than the layer in example 4. The lacking peripheral gap between native and grown material is clear. Figure 4b shows the top view of this layer, which is morphologically 20 largely representative of all layers.

Example 6

In order to adapt the synthesis conditions even more to the real problem in caries-related hard tooth tissue 25 destruction, a cavity having the dimensions (H x W x L) 3 x 3 x 2 mm was bored into the chewing surface of a premolar. The further treatment corresponds to that of example 3. The calcium solution, however, contained 30 0.233 mol/l. The tooth sample was then fractured for the investigation such that the outline of the cavity can be detected. Figures 6a + b show clearly that both the cavity base and the side walls are covered.

All synthesis methods mentioned lead to firmly adhering 35 fluoroapatite layers, which on the basis of the crystal morphology and arrangement have great similarity to native tooth enamel. The orientation of the growing crystallites basically runs perpendicularly to the

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surface of the substrate. By means of suitably frequent repetition of the replacement cycles, in principle arbitrarily thick layers can be produced.

5

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of 10 a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference in this specification to any prior 15 publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general 20 knowledge in the field of endeavour to which this specification relates.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A process for the growth of apatite on tooth material, comprising the steps

5 (i) applying to a tooth a first gel comprising gelatin and phosphate ions,
(ii) applying a second gel which is free of phosphate ions, calcium ions and fluoride ions, the first layer of gel being covered with this second gel,
10 and
(iii) applying a medium containing calcium ions.

2. The process of claim 1, wherein the first gel employed is a gelatin-glycerol gel.

15 3. The process of any of the preceding claims, wherein the first gel furthermore contains fluoride ions.

4. The process of any of the preceding claims,
20 wherein the first gel has a pH of 2.0 to 6.0.

5. The process of any of the preceding claims, wherein the second gel is selected from gelatin-glycerol gels, polysaccharide gels or carboxymethyl-cellulose gels.

25 6. The process according to any of the preceding claims, wherein the medium containing calcium ions employed is a solution containing calcium ions or a gel containing calcium ions.

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7. The process according to any of the preceding claims, wherein the medium containing calcium ions has a pH of 6 to 8.

5 8. The process according to any of the preceding claims, wherein the tooth material is defatted or/and rinsed before the application of the first gel.

10 9. The process according to any of the preceding claims, wherein the tooth material is human teeth or/and tooth enamel.

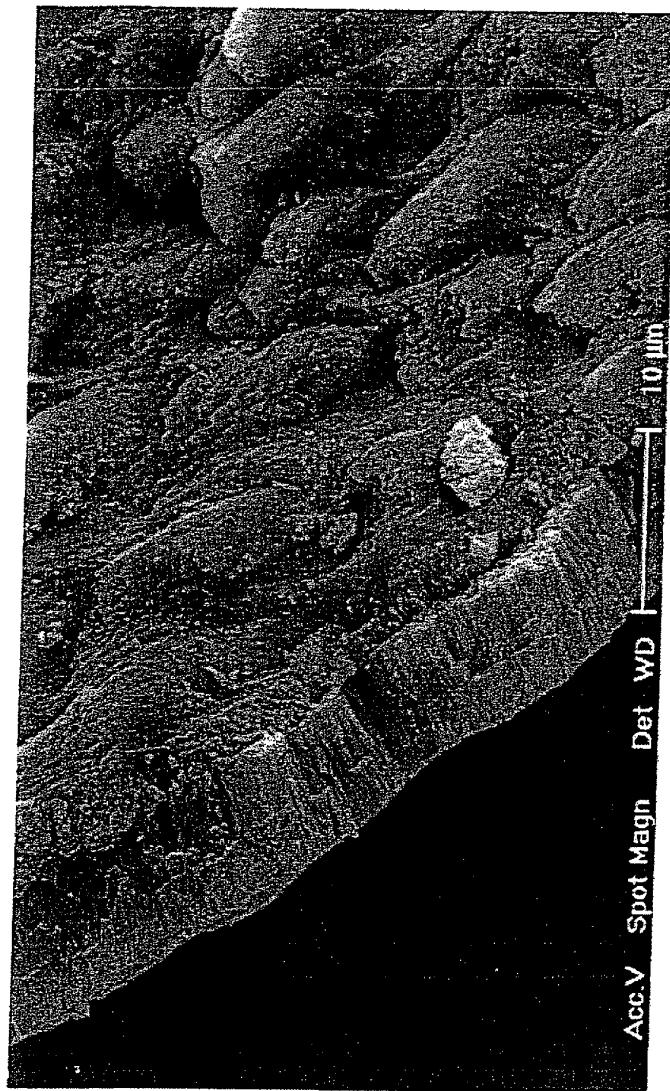
15 10. The process according to any of the preceding claims, wherein components (i), (ii) and (iii) are intended for the treatment of carious defects by remineralization.

11. A kit, when used according to any of claims 1 to 10, comprising:

20 (a) a first gel which comprises gelatin and phosphate ions,
(b) a second gel which is free of phosphate ions, calcium ions and fluoride ions as well as
(c) a medium containing calcium ions.

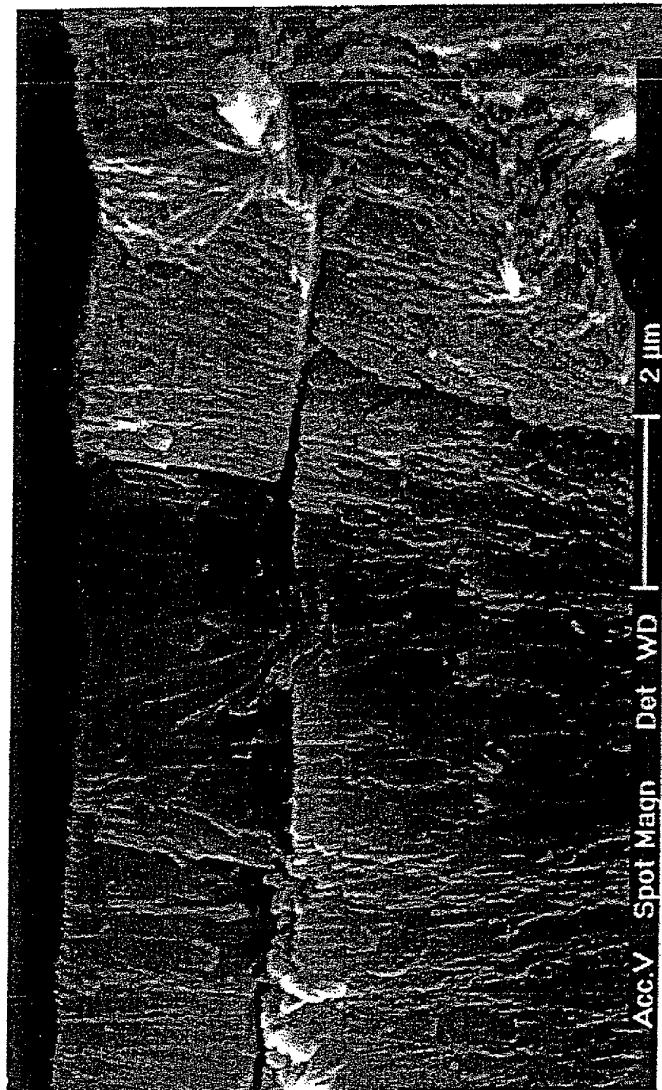
25 12. The kit according to claim 11, wherein components (a), (b) or/and (c) are furthermore defined as in any of claims 2 to 7.

13. A process according to claim 1, or a kit according 30 to claim 11 substantially as hereinbefore described and/or exemplified and/or illustrated in the Figures.



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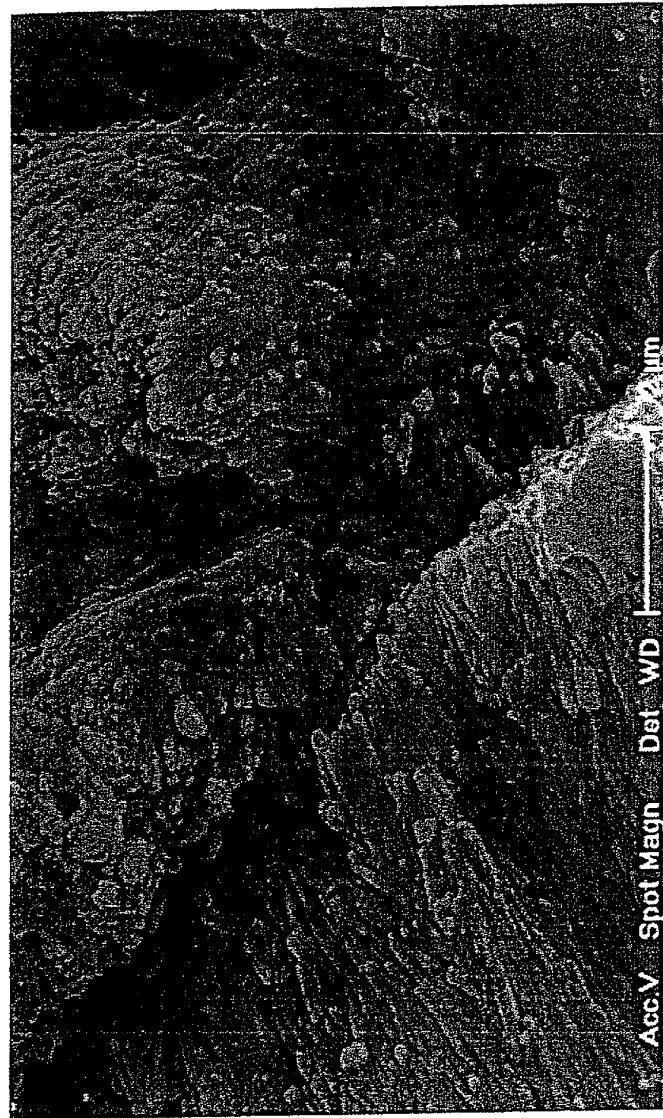


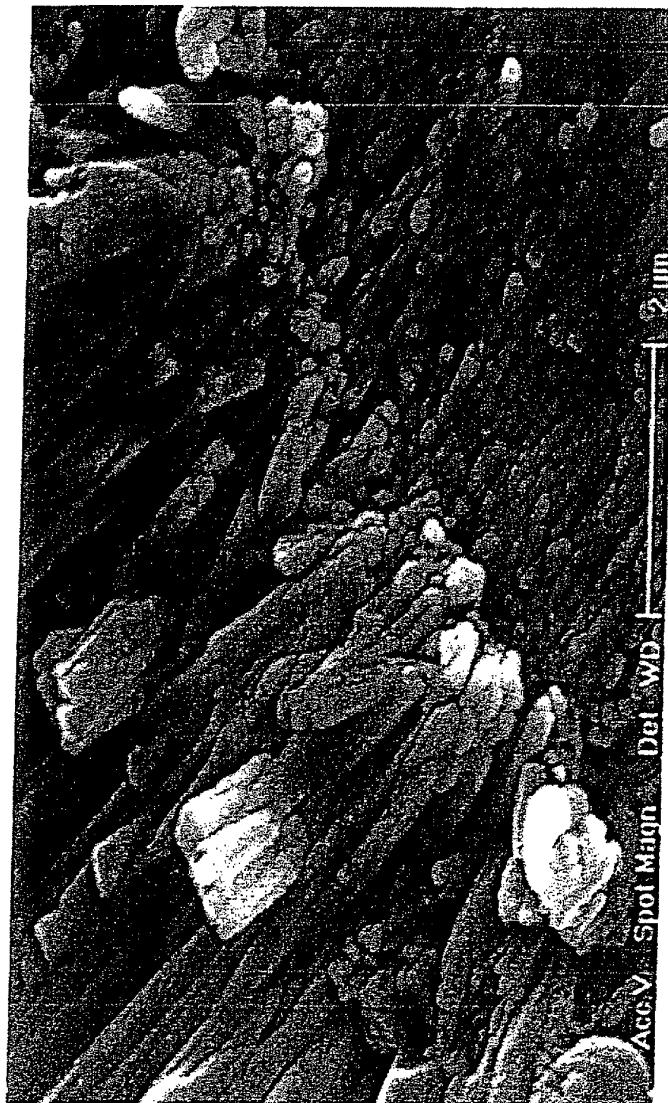
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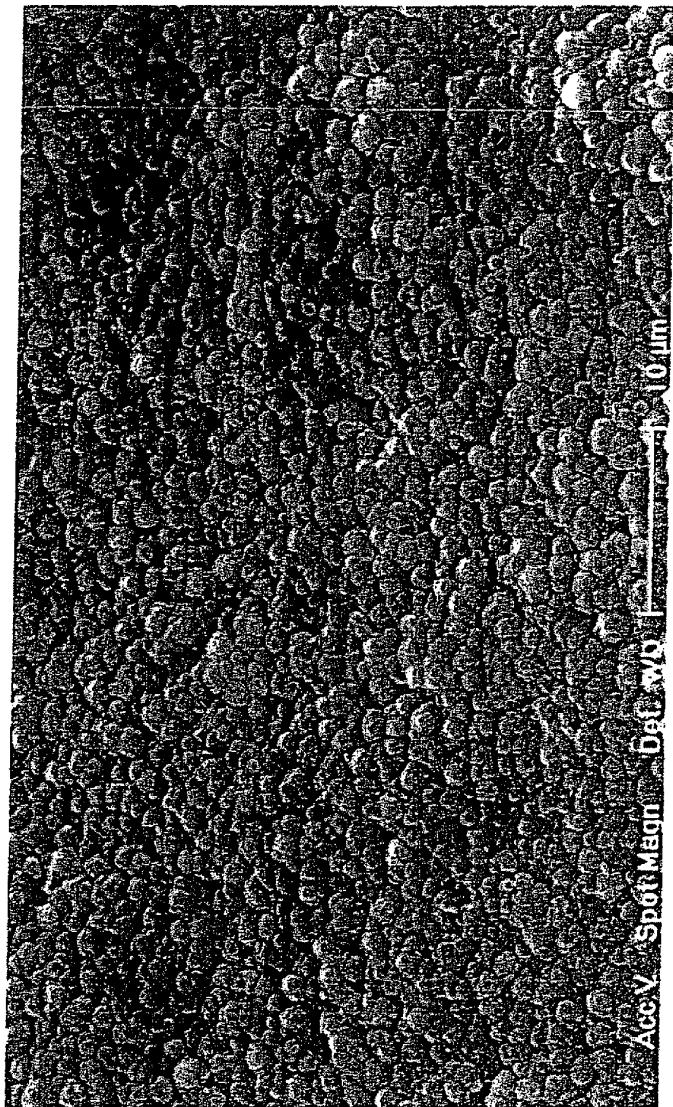
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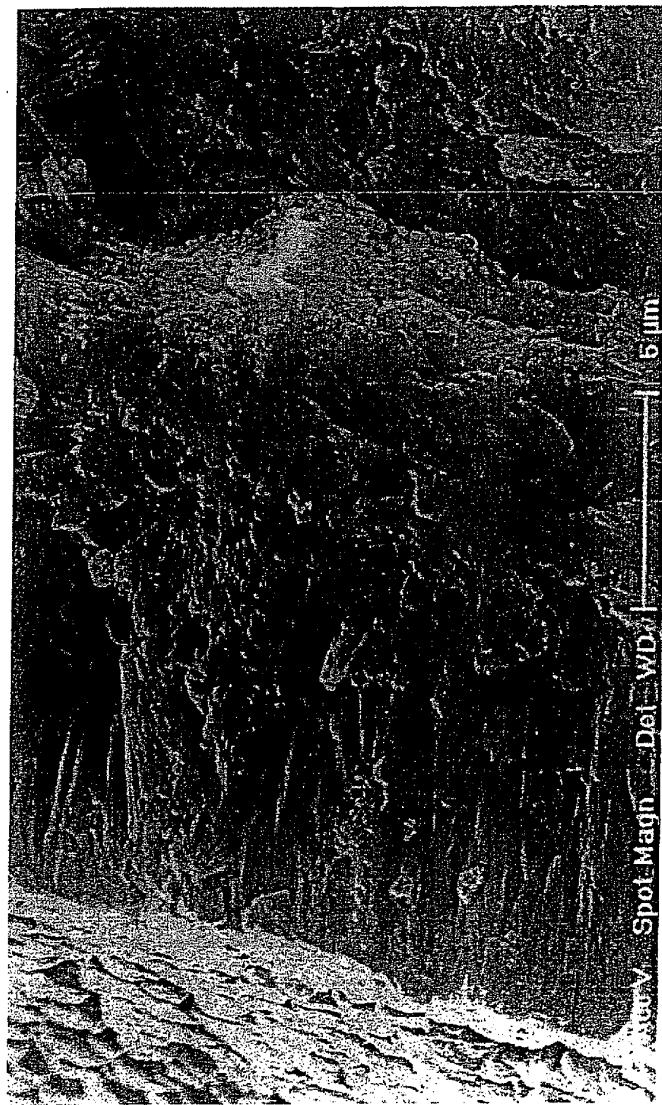
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