Laporan Akhir Projek Penyelidikan Jangka Pendek

Tajuk Projek:

An *In vitro* Study on the Sealing Ability of Nano Hydryapatite and Standard Hydroxyapatite on Dentinal Tubules

Penyelidik:

Dr. Sam'an Malik Masudi
Prof. Dr. Ab. Rani Samsudin
Dr. Karima Akool Al Salihi
Nor Shamsuria Omar, BSc.

PUSAT PENGAJIAN SAINS PERGIGIAN
UNIVERSITI SAINS MALAYSIA

BAHAGIAN PENYELIDIKAN & PEMBANGUNAN
CANSELORI
UNIVERSITI SAINS MALAYSIA

Laporan Akhir Projek Penyelidikan Jangka Pendek

1) Nama Penyelidik: Dr. Sam'an Malik Masudi

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Nama Penyelidik-Penyelidik
Lain (Jika berkaitan) : 1. Prof. Dr. Ab. Rani Samsudin

2. Dr. Karima Akool Al Salihi

3. Nor Shamsuria Omar

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2) Pusat Pengajian/Pusat/Unit : School of Dental Sciences USM

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3) Tajuk Projek: An *In vitro* Study on the Sealing Ability of Nano Hydryapatite and Standard Hydroxyapatite on Dentinal Tubules

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4) (a) Penemuan Projek/Abstrak

(Perlu disediakan makluman di antara 100 – 200 perkataan di dalam Bahasa Malaysia dan Bahasa Inggeris. Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Pembangunan sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti).

Abstract

Dentin hypersensitivity is a common clinical problem; with incidence of 15% to 30% of the population at the age between 20 and 40 years. Previous study revealed that dentin hypersensitivity is closely accompanied with the open dentinal tubules structures. The presence of open dentinal tubules is the main condition seen in the sensitive area of the exposed dentine and is in agreement with the hydrodynamic theory proposed by Brännstrom. In fact, new material technology allowed chemist to synthesize nano structured Hydroxyapatite (nano HA). Nano HA-based materials showed high degree of surface activity, reactive and have capability in reducing mechanically the functional diameter of the tubules in order to minimize the dentinal permeability and therefore sensitivity as well. The aim of this study is to evaluate the closure of dentinal tubules after the aplication of nanostructured and standard HA. Sixteen extracted human maxillary permanent premolars are used in this study. The teeth are divided into 2 groups randomly. Eight dentin discs with 3 mm thickness were prepared and cut under the DEJ of the occlusal area (four discs) and cervical area (four discs). Each group has eight dentin discs. All the dentine samples were treated with an EDTA solution (pH 7.4) for 1 minute to remove the all smear layer. Half discs of Group I were treated for 5 minutes with carboxymethylcellulose (CMC) gel containing nano structured HA and Group II with standard HA. The other half discs were remaining untreated as a control. All specimens then washed in running water for 5 minutes. All specimens were examined under SEM at magnifications of x1500 to x2000. The percentage of dentinal closure of each specimen will be calculated from the Photomicrograph of representative using an image analyzer. The study showed that the percentage of dentinal closure treated with nano structured HA was higher than standard HA. As a conclusion of this study, nano structured HA were superior compared to standard HA in the closure of dentinal tubules.

Abstrak

Hipersensitif dentin adalah permasalahan utama yang ditemui di klinik pergigian dengan insidensi 15% sampai 30% dari pada populasi antara umur 20 dan 40 tahun. Hipersensitif dikeranakan oleh adanya dentin yang terbuka serta berhubung dengan kaedah hidrodinamik dari Brannstrom. Teknnik dalam bidang bahan memungkinkan ahli kimia untuk mensintesa Hidroksi apatit dalam bentuk Nano (Nano HA) yang mempunyai aktifitas permukaan tinggi, lebih reaktif dan dapat masuki serta menutup ataupun mengurangkan diameter dari tubuli dentin. Penyelidikan ini bertujuan untuk melihat penutupan dari tubuli dentin setelah aplikasi Nano HA dan Standar HA. Enam belas gigi yang telah dicabut dibahagi kepada dua kumpulan secara random. Selanjutnya dibuat spesimen dentin dengan memotong bahagian oklusal gigi dibawah DEJ dan membentuk disk dentin setebal 3 mm, sehingga secara keseluruhan terdapat 20 disk. Semua spesimen dentin dibasuh dengan larutan EDTA (pH 7.4) selama 1 minit untuk membuang 'smear layer'. Setengah bahagian dari disk pada kumpulan pertama diaplikasi paste yang mengandungi Nano HA selama lima minit, sedangkan setengah bahagian dari disk kelompok kedua dengan standar HAP. Sedangkan setengah bahagian lagi dari kedua kelompok disk tidak diberi apa-apa aplikasi sebagai kontrol. Specimen selanjutnya dibasuh dengan aliran air selama 5 minit. Selanjutnya kedua kumpulan diperiksa dibawah SEM dengan pembesaran 1500 sampai 2000 kali dan Fotomikrograf dibuat pada daerah representatif. Peratusan penutupan tubuli dentin kemudian dikira pada mikrograf dan di analisis. Terlihat peratusan penutupan tubuli dentin yang lebih luas pada dentin yang di aplikasikan Nano HAP. Berdasarkan hasil penyelidikan ini terlihat bahwa Nano HA dapat menutup tubuli dentin dengan lebih baik, sehingga dapat digunakan untuk rawatan gigi dengan dentin hipersensitif.

(b) Senaraikan Kata Kunci yang digunakan di dalam abstrak:

Bahasa Malaysia	Bahasa Inggeris
- hidroksiapatit berstruktur nano	- nanostructured hydroxyapatite
- tubules dentin	- dentinal tubules
- dentin hipersensitif	- hypersensitive dentin
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....	....
....	....
....	....
....	....

5) Output Dan Faedah Projek

(a) Penerbitan (termasuk laporan/kertas seminar)
(Sila nyatakan jenis, tajuk, pengarang, tahun terbitan dan di mana telah diterbit/dibentangkan).

1. Paper Presentation: Study on dentinal closure of nano hydryapatite and microcrystalline hydroxyapatite.
   Author: SAM'AN MALIK MASUDI*, AB. RANI SAMSUDIN*, ROHANA bt. ADNAN**, ROZITA bt A. RAMLI**.
   School of Dental Sciences USM (*) and School of Chemical Sciences (**), USM
   Presented at: Forum Ilmiah (FORIL) VIII, Faculty of Dentistry, Univ. Trisakti, Jakarta. 7-9 Juli 2005.

2. Published Article: Perbandingan Efek Hidroksiapatit Berukuran Nano dan Hidroksiapatit Standar Terhadap Penutupan Tubuli Dentin Secara *In vitro*.
   Author: SAM'AN MALIK MASUDI*, AB. RANI SAMSUDIN*, ROHANA bt. ADNAN**.
   School of Dental Sciences USM (*) and School of Chemical Sciences (**), USM
   Published in Majalah Ilmiah Kedokteran Gigi / Indonesian Scientific Journal in Dentistry. Tahun 20, No. 61, Juli 2005.

3. Paper Presentation: Preparation and Characterization of Hydroxyapatite-Chitosan Nano Composite Blending Process.

   Author: R.A. RAMLI*, R. ADNAN*, S. MASUDI**, M. ABU BAKAR*
   School of Chemical Sciences (*), USM and School of Dental Sciences USM (**).

   Presented at: The 1st USM-Penang International Postgraduate Convention and 1st Penang International Conference for Young Chemist: Blazing a New Frontier in Chemical Sciences. Universiti Sains Malaysia Main Campus, Penang- Malaysia. 24-27 May 2006.

4. Publishing Article (in process) : Preparation and Characterization of Hydroxyapatite-Chitosan Nano Composite Blending Process.

   Author: R.A. RAMLI*, R. ADNAN*, S. MASUDI**, M. ABU BAKAR*
   School of Chemical Sciences (*), USM and School of Dental Sciences USM (**).

   Will be published in Microscopy and Analysis, Willey Journal.

5. Publishing Article (in process) : An *In vitro* Study on the Sealing Ability of Nano Hydryapatite and Standard Hydroxy-apatite on Dentinal Tubules

   Author: MASUDI, S.M.*, SAMSUDIN, A.R.*, ADNAN, R.**, OMAR, N.S.
   School of Dental Sciences USM (*) and School of Chemical Sciences (**), USM

   Will be published in Dental Materials, Elsevier Journal.

6. Paper Presentation: An *In vitro* Study on the Sealing Ability of Nano Hydryapatite and Standard Hydroxyapatite on Dentinal Tubules.

   Author: MASUDI, S.M. School of Dental Sciences USM

   Presented at: Weekly CPC Seminar at School of Dental Sciences USM. 30 May 2006.

(b) Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten.
(Jika ada dan jika perlu, sila guna kertas berasingan)

i) Further Study: - Cytotoxicity study on nano hydroxyapatite
- Genotoxicity study on nano hydroxyapatite
- Animal study of nano hydroxyapatite

ii) Product development:

- Nano hydroxyapatite based desensitizing agent for dentin hypersensitive treatment.
- Nano hydroxyapatite based root canal sealant for RCT.
- Nano hydroxyapatite dental implant coating material.

iii) Comercialization Prospect: -

iv) Patent: -

(c) Latihan Gunatenaga Manusia

i) Pelajar Siswazah:

1. Rohana bt. Adnan (PPSKimia-USM)
MSc. Research Topic: Preparation and Characterization of Hydroxyapatite-Chitosan Nano Composite Blending Process.

2. Dr. Jalal Ja'far Alshakhshir (PPSG-USM)
MSc. Research Topic: An evaluation of sealing ability of a new nano-hydroxyapatite endodontic sealant: *in vitro* study

3. Dr. Shadi Mohammad Ali Al-Omari (PPSG-USM)
MSc. Research Topic: The coronal sealing ability of a new Hydroxyapatite based sealer after post space preparation.

ii) Pelajar Prasiswazah: ....

iii) Lain-Lain : Pegawai Sains di Craniofacial Laboratory

....

....

6. Peralatan Yang Telah Dibeli:

- Tiada -

UNTUK KEGUNAAN JAWATANKUASA PENYELIDIKAN UNIVERSITI

Memenuhi syarat-syarat geran USM.
Dimajukan kepada RCMO melalui J/K Pelantar Penyelidikan Klinikal

13/02/07.

T/TANGAN PENGERUSI
J/K PENYELIDIKAN
PUSAT PENGAJIAN

An *In vitro* Study on the Sealing Ability of Nano Hydryapatite and Standard Hydroxyapatite on Dentinal Tubules

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Abstract

Dentin hypersensitivity is a common clinical problem; with incidence of 15% to 30% of the population at the age between 20 and 40 years. Previous study revealed that dentin hypersensitivity is closely accompanied with the open dentinal tubules structures. The presence of open dentinal tubules is the main condition seen in the sensitive area of the exposed dentine and is in agreement with the hydrodynamic theory proposed by Brännstrom. In fact, new material technology allowed chemist to synthesize nano structured Hydroxyapatite (nano HA). Nano HA-based materials showed high degree of surface activity, reactive and have capability in reducing mechanically the functional diameter of the tubules in order to minimize the dentinal permeability and therefore sensitivity as well. The aim of this study is to evaluate the closure of dentinal tubules after the aplication of nanostructured and standard HA. Sixteen extracted human maxillary permanent premolars are used in this study. The teeth are divided into 2 groups randomly. Eight dentin discs with 3 mm thickness were prepared and cut under the DEJ of the occlusal area (four discs) and cervical area (four discs). Each group has eight dentin discs. All the dentine samples were treated with an EDTA solution (pH 7.4) for 1 minute to remove the all smear layer. Half discs of Group I were treated for 5 minutes with carboxymethylcellulose (CMC) gel containing nano structured HA and Group II with standard HA. The other half discs were remaining untreated as a control. All specimens then washed in running water for 5 minutes. All specimens were examined under SEM at magnifications of x1500 to x2000. The percentage of dentinal closure of each specimen will be calculated from the Photomicrograph of representative using an image analyzer. The study showed that the percentage of dentinal closure treated with nano structured HA was higher than standard HA. As a conclusion of this study, nano structured HA were superior compared to standard HA in the closure of dentinal tubules.

Keywords: nanostructured hydroxyapatite, dentinal tubules, hypersensitive dentin

Introduction

Dentin hypersensitivity is a common clinical problem, and when dentin is exposed by the abrasion of enamel or the gingival retraction with periodontitis, sudden severe pain is elicited by external stimuli such as an air blast, cold water, or scratch.

Although dentin hypersensitivity is commonly encountered in dental clinics, the details of the mechanisms are still poorly understood, and no consistent treatment has been established.. Dentin hypersensitivity is characterized by short, sharp pain arising from exposed dentin in response to stimuli typically thermal, evaporative, tactile, osmotic or chemical and which cannot be ascribed to any other form of dental defect or pathology. Surveys, which examined for dentin hypersensitivity showed 15% to 30% of the study population are affected and the age range for dentin hypersensitivity is broad, spanning early teenage to more than 70 years (Fischer *et al.*, 1992). However, peak incidence is between 20 and 40 years (Flynn *et al.*, 1985). The wear of enamel and retraction of gums frequently cause the exposure of dentine and the appearance of dentin hypersensitivity (Chabansky *et al.*, 1997; Absi *et al* 1987; Johnson and Brannstrom 1974; Masson *et al.*, 1991 and Pashley 1990).

Previous study revealed that dentin hypersensitivity is closely accompanied with the open dentinal tubules and the tube-like (process-like) structures The presence of open dentinal tubules is the main condition seen in the sensitive area of the exposed dentine (Yoshiyama *et al.*, 1989, 1990) and is in agreement with the hydrodynamic theory proposed by Brannstrom (1966); Johnson and Brannstrom (1974) and by Pashley (1990).

In fact, new material technology allowed chemist to synthesize nano structured Hydroxyapatite (nano HA) with a high degree of surface activity and with average crystal diameter in a nano size (Dolci *et al.*, 2001; Bonetti *et* al., 1993 and Valdre *et al..*, 1999).

Hydroxyapatite (HA) with the chemical formula $Ca(PO4)_6(OH)_2$ is the main component of the bone and teeth. HA is known to be biocompatible, bioactive, osteoconductive, non inflammatory. Recently, synthetic HA prepared at nano level (1-100 nm), to mimic the mineral component and the microstructure of natural bone.

Synthetic HA prepared plays a significant role in various biomedical applications. Its unique functional properties such as height surface are and ultra fine structure of the synthetic HA is similar to synthetic apatite. Therefore, synthetic HA has been widely applied in a variety of biomedical applications such as, bone substitutes materials, constituent implants, and dental material.

These nano structured HA-based materials are therefore a promising material that may have future and considerable clinical dental applications. The materials are biocompatible, reactive and have capability in reducing mechanically the functional diameter of the tubules in order to minimize the dentinal permeability and therefore sensitivity as well. As shown in Fig. 1, the smaller scaled of material, would decrease a gravity cohesion, but increased van-der Walls adhesion charging hydrogen bridges that leads to the higher surface activity. This phenomenon will explain that the nano structured HA are more reactive and adhesive compared to micro structured (standard) HA. Nano structured HA, which is completely similar to that of dentine and/or enamel, can significantly reduce dentinal sensitivity, protecting the dentin from acid attack by creating an acid-resistant layer inside and outside the dentinal tubules.

New structure processing of HA for the treatment of dentin hypersensitivity can be mixed in gel form to be used by dental professional in the clinic as well as mixed in the toothpaste for patient home treatment. The nanometer-sized grains also have been found to increase osteoblast adhesion, proliferation and mineralization. Study conducted by Dolci *et al.*, (2000) has shown that Nano HA materials that are totally biocompatible due to their chemical and physical nature.

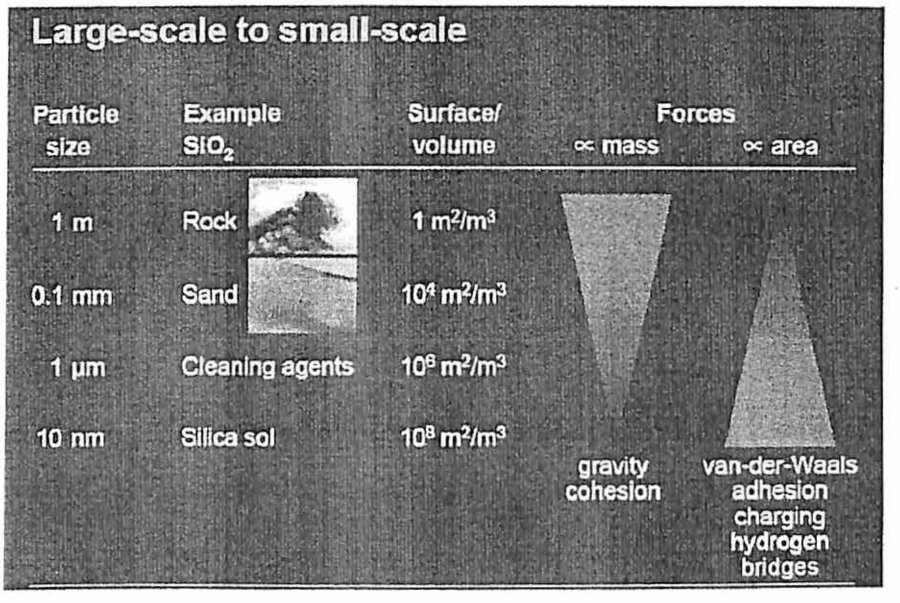

Fig. 1. The small-scale of material shows lower gravity cohesion and higher van-der-Walls adhesion charging hydrogen bridge.

School of Chemical Sciences Universiti Sains Malaysia (USM) have developed nano structured HA for biomedical application. The HA nanocrystal were synthesized by wet chemical method using $Ca(OH)_2$ and H_3PO_4 as Ca and P precursors, respectively. TEM microgram shows the size of nano HA crystal were between 30 to 80 nm as shown in Fig. 2. The HA nanocrystals were found to be rod-like and a little agglomerate. X-ray diffraction analysis of nano HA confirmed that this material was the typical characteristic of the carbonate type-B hydroxyapatite as shown in Fig. 3.

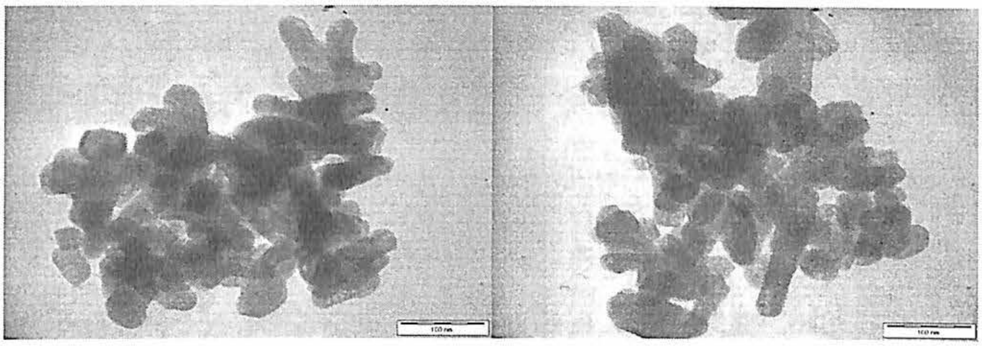

Fig. 2. TEM micrograph of nano structured HA. The HA nanocrystals were found to be rod-like with the size of 30 to 80 nm.

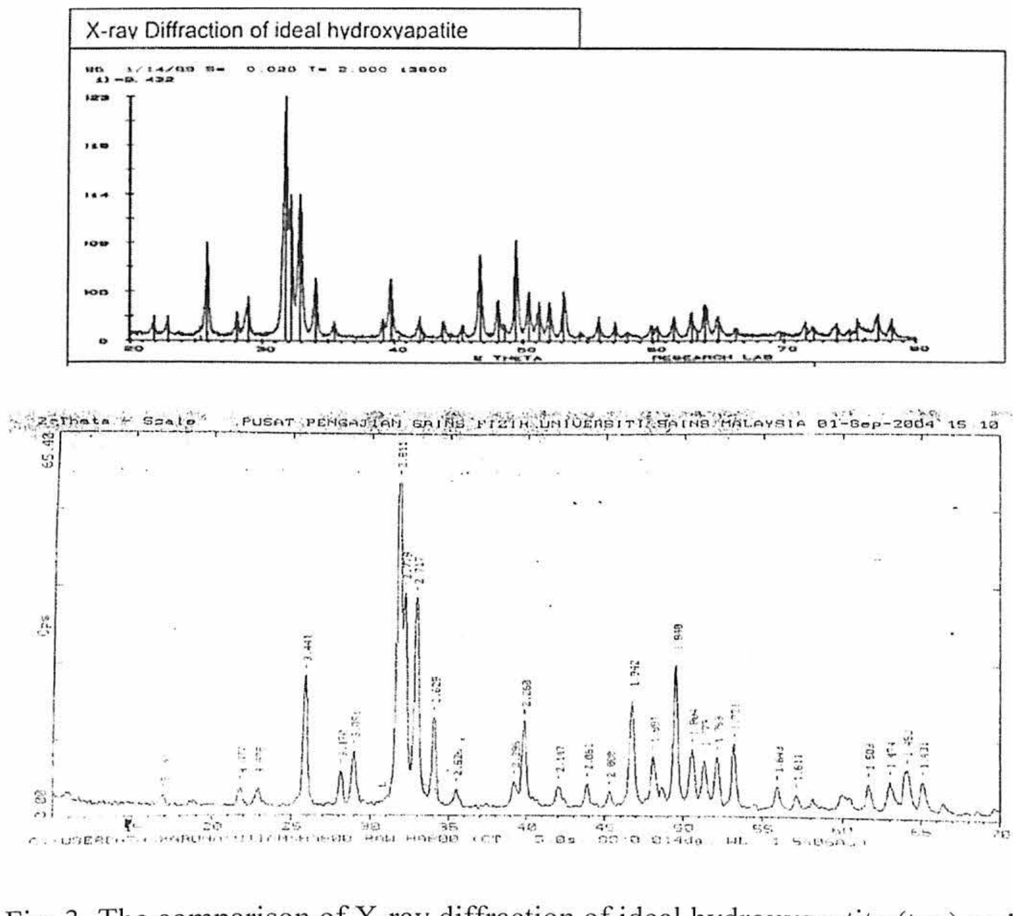

Fig. 3. The comparison of X-ray diffraction of ideal hydroxyapatite (top) and nano structured HA (bottom).

The objective of this study was compare quantitatively the closure of dentinal tubuli after application of nano HA and standard HA materials, by using Scanning electron microscope (SEM).

Materials and Methods

Sixteen erupted human maxillary permanent premolars were use in this study. The teeth are to be collected from the dental clinic in School of Dental Sciences, Universiti Sains Malaysia (USM). The teeth were carefully clean and then examined at low magnification. No surface showing a development fault or crack or white-spot lesion is used for the experiment. The teeth were preserved in normal saline solution at 37^0 C not more than one month. The effect of the HA gel were study on sound occlusal dentin and cervical dentin.

One dentin disc per tooth (subtotal eight discs) with 3 mm thickness were prepared and cut under the dentino-enamel-juntion of occlusal area of the tooth for occlusal specimens. The other eight dentin discs also with 3 mm thickness were cut under dentino-enamel-junction of cervical area of the tooth for cervical specimens as illustrated in figure 4. Total samples of sixteen dentin discs were divided into 2 (two) groups randomly. Each group was eight dentin discs, which contain four discs of occlusal specimens and four discs of cervical specimens.

All the dentine samples were treated with an EDTA solution (pH 7.4) for 1 minute to remove the all smear layer and then cut into 2 halves discs. The first half discs were treated for 5 minutes with gel containing nano HA and standard HA and examined under SEM. The other half discs were examined under SEM (before nano and standard HA treated) as a control.

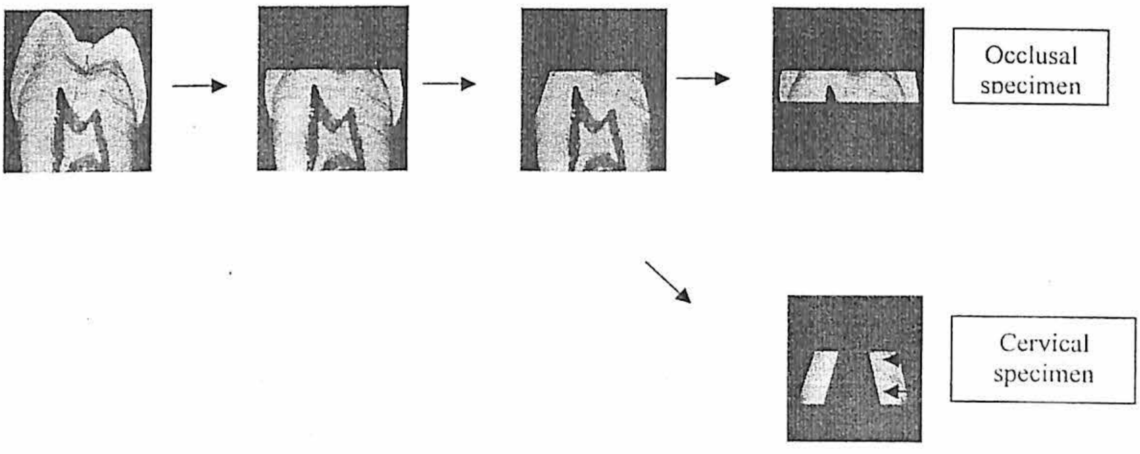

Fig. 4. Diagram of dentin for occlusal and cervical specimens

Carboxymethylcellulose (CMC) gel was prepared for use as vehicle of nano structured HA and standard HA as a desensitizing agent. The formulation of Carboxymethylcellulose (CMC) gel based on the method described by Dolci *et al.* (2001). CMC in aqueous solution containing parabens to 0.2% (0.02 g of methylparahydroxybenzoate and 0.02 g of propylparahydroxybenzoate were used in 20 ml solution) to better conserve the gel. The parabens were made soluble in aqueous solution at temperature of 70° C and the CMC was then added once a temperature of 50° C has been reached, then it was stirred until the right consistency and transparency was obtained.

The mixture of nano structured HA and standard HA were prepared with CMC gel. The amount of 0.6 g of nano structured HA were incoporated in a quatity of 3 ml of CMC gel, resulting in a concentration of 20% by weight to form nano HA gel. The same amount of standard HA were incoporated in a quatity of 3 ml of CMC gel also to form standard HA gel.

The first half discs specimens (eight discs which contain four discs of occlusal specimens and four discs of cervical specimens), were divided randomly into two groups. In group one, dentin discs were treated with gel containing nano structured HA for 5 minutes and then washed in running water for 5 minutes. The same procedures were repeated for group two with standard HA gel.

Scanning Electron Microscope (SEM) Observation

Before observation under SEM, all specimens were left to stand for 12 hours in glutaraldehyde at 2 % buffered with sodium cacodylate 0.1 M (pH = 7.3) in a refrigerator at a temperature of 4° C. The dentin discs specimens are then left to dry for an hour on blotting paper (controlled dehydration phase) then the samples were fixed to SEM metal stub using double-sided adhesive tape and were placed in an evaporator where they were coated with a 200 Å layer of gold and observed under the Scanning Electron Microscope (SEM). Photomicrographs are taken of representative areas at magnifications of x1500 to x2000. The percentage of dentinal closure of each sample were calculated from the Photomigrograph and image analyzer.

Results

Scanning electron microscope images reported in figures 5 to 7 were the observation of sound occlusal and cervical dentin before gel treatment and also specific observation after the application of nano HA and standard HA gels.

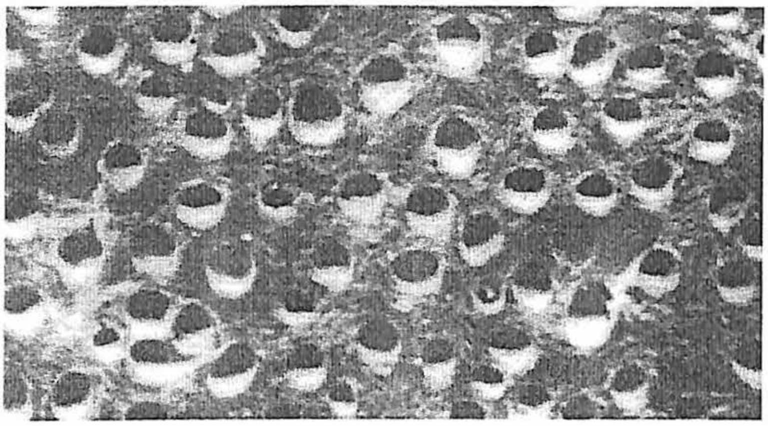

Fig 5. SEM micrograph of the dentin surface after treated with EDTA solution to remove smear layer. In this case dentinal tubuli resulted completely open.

Figure 5 shows SEM image of the occlusal dentin surface after treated with EDTA solution to remove the smear layer. In this case, dentinal tubuli resulted completely open.

The SEM morphologies of the dentin surface after application of CMC gel containg standard HA (microcristalline) for 5 minutes and then washed in running water for 5 minutes. The SEM micrograph exhibited the dentinal tubuli resulted partially closed in the percentage of 48% (Fig. 6).

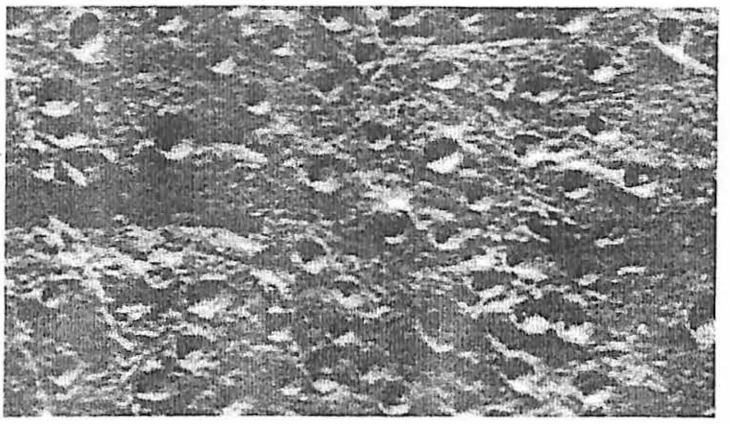

Fig. 6. SEM micrograph of the occlusal dentin surface after application of standard HA gel (microcristalline) for 5 minutes and then washed in running water for 5 minutes. The SEM micrograph exhibited the dentinal tubuli resulted partially closed in the percentage of 48%

Figure 7a reports the occlusal dentin surface treated with CMC gel containing nano structured HA for 5 minutes and then washed in running water for 5 minutes. To be noted in the SEM micrograph that the majority of the dentinal tubuli are highly closed in the persentage of 89%. Figure 7b on right side exhibits in higher magnification and better detail, the morphology of dentin surface treated with CMC gel containing nano structured HA for 5 minutes. SEM micrograph in figure 7a shows the border between a treated dentin zone with a CMC gel containing nano structured HA (on the right) and an untreated dentin surface zone on the left side. In figure 4b, SEM micrograph shows higher magnification of SEM image also in the border between a treated dentin zone with a CMC gel containing nano structured HA (on the bottom) and an untreated dentin surface zone on the top side.

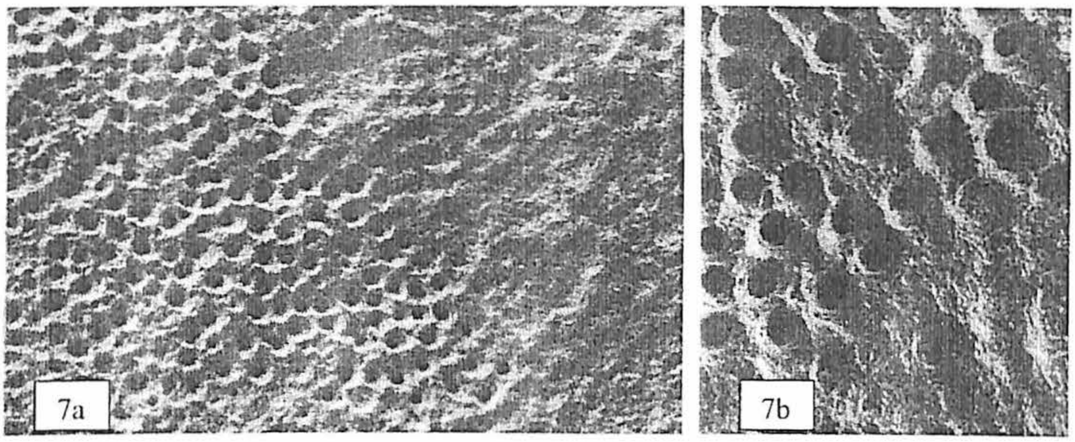

Fig. 7. SEM micrograph of : 7a The dentin surface treated with CMC gel containing nano structured HA for 5 minutes and then washed in running water for 5 minutes. To be noted in the SEM micrograph that the majority of the dentinal tubuli are highly closed in the persentage of 89%. Fig. 7b on right side exhibits in higher magnification and better detail of dentin surface treated with CMC gel containing nano HA.

Discussion

From the data presented in this *in vitro* study suggested that the nano HA synthesized by wet chemical method using $Ca(OH)_2$ and H_3PO_4 as Ca and P precursors, may be an effective and active material for reducing dentinal hypersensitivity. In the fact the application of CMC gels containing nano structured HA are able to reduce dentinal sensitivity with the higher closure percentage (89%) of the dentinal tubuli. On the contrary, it has been shown in this in vitro test that the percentage of dentinal closure in CMC gel containing standard micro crystalline HA, that was only 48%.

Our study is in agreement with those found by Dolci *et al.*, (2001) that used nano HAP with different processing method. They used defective nano HA produced by lattice destabilization method and they found from application of nano HA with gel, aqueous suspensions and mixtures of tooth paste, were able to reduce the dentinal permeability by

50 to 55% of the maximum dentin permeability value and showed high closure of dentinal tubuli. Another similar work by Braunbarth *et al.* (2002) found that application of nanoscaled calcium phosphate protein composite (Nanit®active) on dentin leads to the formation of an apatite layer closely bonded to the natural dentin and occluded of dentinal tubuli. A process of occlusion of dentinal tubuli was labeled as 'neomineralisation'.

In addition, from SEM study on nano HA particle size suggested that with the high penetration and reaction efficiency of nano HAP gel to dentin surface leading to the reducing of agglomerate mean diameter of nano structured HA. SEM observations of dentin surfaces have shown the precence of a thin layer of nano crystals or agglomerate after the applications of CMC gel containing nano HA, also after washing in water. Nano HA as shown in Fig. 2, have the tendency to agglomerate as a result of high degree of nano HA crystals affinity. The application of nano HA gel should be treated with high penetration to the dentinal surface and reaction efficiency for the occlusion of dentinal tubuli in sensitive exposed dentin.

Under clinical conditions the exposure of the dentin to food and/or acid drinks and saliva is in the fact influencing dentin sensitivity. Through clinical study, the effect of nano HA application for the solution of dentin hypersensitive problem is now being extensively studied.

These nano structured HA are biocompatible because their chemical and physical nature that is completely similar to dentin or enamel and bone. Further study should be done to ensure biocompatibility of this material.

Nano HA can be used in toothpaste, gel and root canal sealer formulations for professional use in Dentistry. This material can be considered as a promising material for the future of the in office (gel form) and home (toothpaste) application for dentin hypersensitive treatment.

Conclusion

In conclusion, nano structured HA were superior compared to standard HA in the closure of dentinal tubules based on *in vitro* study..

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THE 8th SCIENTIFIC FORUM

FACULTY OF DENTISTRY - TRISAKTI UNIVERSITY

FORIL

Certificate Of Attendance

Presented to

Dr. Sam'an Malik

As

Short Lecturer

IN RECOGNITION OF PARTICIPATION

IN THE 8th SCIENTIFIC FORUM

FACULTY OF DENTISTRY - TRISAKTI UNIVERSITY

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PERBANDINGAN EFEK HIDROKSIAPATIT BERUKURAN NANO DAN HIDROKSIAPATIT STANDAR TERHADAP PENUTUPAN TUBULI DENTIN SECARA *IN VITRO*

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Dentin hypersensitivity is a common clinical problem; with incidence of 15% to 30% of the population at the age between 20 and 40 years. Previous study revealed that dentin hypersensitivity is closely accompanied with the open dentinal tubules structures. The presence of open dentinal tubules is the main condition seen in the sensitive area of the exposed dentine and is in agreement with the hydrodynamic theory proposed by Brannstrom. In fact, new material technology allowed chemist to synthesize Nano Hydroxyapatite (Nano HA). Nano HA-based materials showed high degree of surface activity, reactive and have capability in reducing mechanically the functional diameter of the tubules in order to minimize the dentinal permeability and therefore sensitivity as well. The aim of this study is to evaluate the closure of dentinal tubules after the aplication of Nano and Standard HA. Twenty extracted human maxillary permanent premolars are used in this study. The teeth are divided into 2 groups randomly. One dentin disc per tooth (Total 20 discs) with 3 mm thickness were prepared and cut under the DEJ of the occlusal area. Each group has 10 dentin discs. All the dentine samples were treated with an EDTA solution (pH 7.4) for 1 minute to remove the all smear layer. Half discs of Group I were treated for 5 minutes with paste containing Nano HA and Group II with Standard HA. The other half discs were remaining untreated as a control. All specimens then washed in running water for 5 minutes. All specimens were examined under SEM at magnifications of x1500 to x2000. The percentage of dentinal closure of each specimen will be calculated from the Photomigrograph of representative areas and further quantitative analysis will be done using an image analyzer. The study showed that the percentage of dentinal closure treated with Nano HA was higher than Standard HA. As a conclusion of this study, Nano HA were superior compared to standard HA in the closure of dentinal tubules.

PENDAHULUAN

Hipersensitif dentin adalah permasalahan yang umum ditemui di klinik dokter gigi. Pasien biasanya mengalami sakit yang mendadak akibat adanya rangsangan dari luar seperti udara, air dingin atau mekanik. Walaupun sering ditemukan, mekanisme secara terperinci dari hipersensitif dentin belum sepenuhnya diketahui dan belum terdapat perawatan yang konsisten terhadapnya.

Hipersensitif dentin memiliki karakteristik dengan adanya sakit yang tajam berasal dari dentin yang terbuka sebagai respons dari rangsangan panas, taktil, evaporatif, osmotik ataupun patologis yang berbeda dengan kerusakan ataupun patologi gigi lainnya. Survei menunjukkan bahwa 15% sampai 30 % dari populasi studi mengalami hispersentif dentin, dengan kisaran umur yang luas, dari umur belasan tahun sampai 70 tahun (Fischer dkk., 1992). Pada penelitian lain oleh Flynn dkk. (1985) didapati bahwa usia yang paling banyak menderita hipersensitif dentin adalah antara 20 dan 40 tahun. Keausan enamel dan retraksi gusi merupakan penyebab yang paling sering terjadinya ekposur dentin dan hipersensitif dentin (Johnson & Brannstrom, 1974; Absi dkk., 1987; Pashley, 1990; Masson dkk., 1991 dan Chabansky dkk., 1997). Studi yang dilakukan oleh Yoshiyama dkk., 1989 and 1990) menunjukkan bahwa hipersensitif dentin berhubungan erat dengan terbukanya tubuli dentin dan struktur dentin yang berbentuk pipa (*tube-like* or *process-like*). Terdapat banyaknya tubuli dentin yang terbuka terlihat pada daerah sensitif dari dentin dan hal ini sesuai dengan teori hidrodinamik yang dinyatakan oleh Brannstrom pada tahun 1966 (Johnson & Brannstrom, 1974; Pashley, 1990).

Berbagai cara pengobatan telah di perkenalkan untuk menutup tubuli dentin secara mekanis, namun banyak dari metoda ini tidak memberikan hasil yang memuaskan ataupun hanya efektif untuk sebagian kasus. Perawatan dengan fluoride pada pasta gigi ataupun gel akan memberikan hasil pada penggunaan dalam jangka waktu yang lama. Aplikasi potasium oksalat ternyata memberikan efek toksik. Penggunaan pasta gigi dengan hidroksi apatit standar (*microcrystalline HA*) juga pernah di test secara *in vitro*, namun memberikan hasil yang kurang efektif. (Prati dkk., 1994).

Perkembangan teknologi bahan memungkinkan ahli kimia untuk mensintesa Hidroksi apatit berukuran nanometer (Nano HA). Bahan Nano HA mempunyai aktifitas permukaan yang tinggi dengan diameter kristal berukuran nano atau 10^{-9}m. (Bonetti dkk., 1993; Dolci dkk., 1999 and Valdre dkk., 1999). Bahan yang mengandungi Nano HA merupakan bahan yang mempunyai prospek dimasa yang akan datang termasuk penggunaan dibidang kedokteran gigi. Bahan ini biokompatibel, reaktif dan mempunyai kemampuan untuk mengurangi secara mekanik, diameter fungsional dari tubuli dentin yang pada gilirannya akan meminimalkan permeabilitas dentin serta efek sensitif. Nano HA yang merupakan bahan dengan struktur yang sama dengan dentin atau enamel dapat mengurangi sensitivitas dentin serta melindungi dentin dari asam dengan pembentukan lapisan tahan asam di bagian dalam maupun luar dari tubuli dentin. Hidroksiapatit berukuran nanometer terbukti dapat meningkatkan adhesi, proliferasi dan mineralisasi dari odontoblast (Dolci dkk., 2001).

Bahan Nano HA untuk perawatan hipersensitif dentin dapat dibuat dalam bentuk gel untuk digunakan dokter gigi di klinik ataupun dalam bentuk pasta gigi untuk pemakaian pasien di rumah.

BAHAN DAN CARA

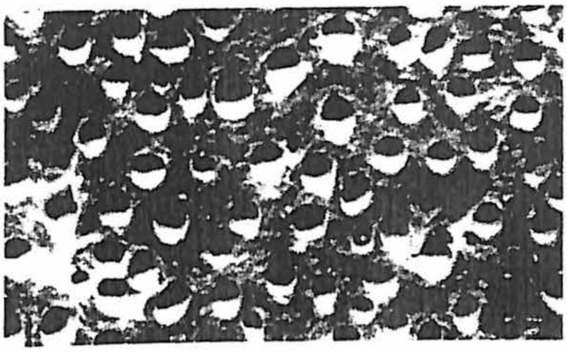
Penelitian ini adalah penelitian eksperimental secara *in vitro*. Tujuan penelitian adalah untuk melihat penutupan dari tubuli dentin setelah aplikasi Nano HA dan Standar HA dengan menggunakan Scanning Electron Microscope (SEM).

Bahan Standar Hidroksiapatit yang digunakan dalam penelitian ini adalah diproduksi dan didapatkan dari *School of Material Engineering*, Universiti Sains Malaysia (USM). Sedangkan bahan Nano Hidroksiapatit diproduksi dan didapatkan dari *School of Chemical Sciences*, USM. Bahan Hidrosiapatit ukuran standar dan standar terdapat dalam bentuk bubuk dan pada penelitian ini dibuat dalam bentuk gel dengan menambahkan *Carboxymethylcellulose Gel (CMC gel)*.

Dua puluh gigi premolar atas yang telah ekstraksi untuk keperluan perawatan ortodontik digunakan dalam penelitian ini. Gigi tersebut dibersihkan dan diperiksa dibawah mikroskop pada pembesaran rendah untuk melihat adanya lesi *white-spot, crack* atau cacat pertumbuhan pada gigi-gigi tersebut. Hanya gigi yang *sound* dan bebas dari lesi *white-spot, crack* atau cacat pertumbuhan yang digunakan dalam penelitian. Gigi-gigi tersebut disimpan dalam larutan normal salin pada suhu 37° C tidak lebih dari 1 bulan. Samel gigi dibagi dua kelompok secara random. Selanjutnya dibuat spesimen dentin dengan memotong bagian oklusal gigi tepat dibawah DEJ sehingga berbentuk disk dentin setebal 3 mm. Semua spesimen dentin di cuci dengan larutan EDTA (pH 7.4) selama 1 menit untuk menghilangkan *smear layer*. Setengah bagian dari disk pada kelompok pertama diaplikasikan gel CMC yang mengandungi Nano HAP selama lima menit, serta setengah bagian dari disk kelompok kedua dengan gel CMC yang mengandungi standar HAP. Sedangkan setengah bagian lagi dari kedua kelompok disk tidak diberi perlakuan sebagai kontrol. Spesimen selanjutnya dicuci dengan air mengalir selama 5 menit. Sebelum diperiksa dengan SEM, semua spesimen selanjutnya disimpan pada larutan glutaraldehyde 2% beserta buffer sodium cacodylate 0.1 M (pH=7.3) selama 12 jam di dalam lemari es dengan suhu 4° C. Semua spesimen dikeringkan selama 1 jam pada kertas *blotting* untuk mengontrol fasa dehidrasi. Selanjutnya specimen dilekatkan pada stub logam dari SEM dengan pita adhesif dua-sisi, untuk seterusnya ditempatkan pada evaporator untuk pelapisan emas setebal 200 Å. Semua spesimen dari kedua kelompok diperiksa dibawah SEM dengan pembesaran 1500 sampai 2000 kali dan Fotomikrograf dibuat pada daerah representatif. Persentase penutupan tubuli dentin kemudian dihitung pada mikrograf dan di analisis.

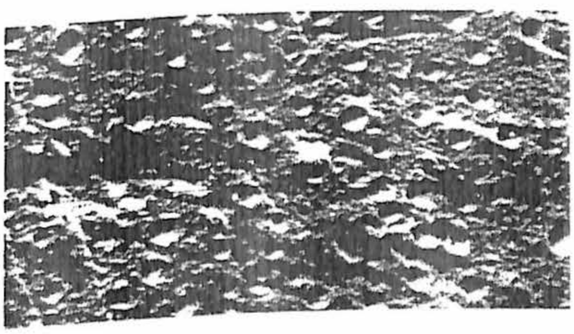
HASIL

Gambar 1 menunjukkan gambaran SEM dari permukaan dentin setelah di cuci dengan larutan EDTA untuk menghilangkan *smear layer*. Tampak seluruh permukaan tubuli dentin terlihat terbuka.



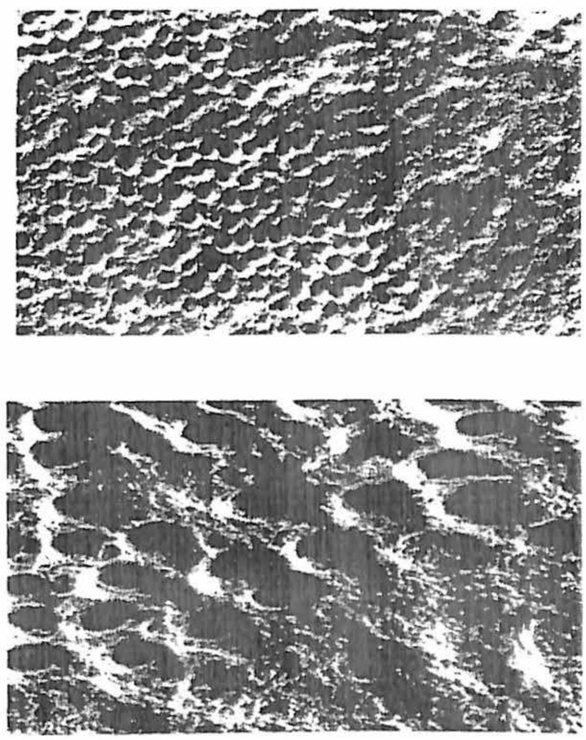
Gambar 1. Gambaran SEM dari permukaan dentin setelah di cuci dengan larutan EDTA untuk menghilangkan *smear layer*. Tampak seluruh permukaan tubuli dentin terlihat terbuka.

Pada hasil test dengan Standard Hidroksiapatit terlihat gambaran SEM dari permukaan dentin setelah aplikasi Standar Hidroksiapatit (*microcristalline*) yang telah dicuci dengan air mengalir selama 5 menit. Dari hasil analisa fotomikrograf tampak permukaan tubuli dentin tertutup sekitar 60 persen, seperti pada gambar 2.



Gambar 2. Gambaran SEM dari permukaan dentin setelah aplikasi Standar Hidroksiapatit (*microcristalline*) dan telah dicuci dengan air mengalir selama 5 menit. Tampak permukaan tubuli dentin tertutup sekitar 60 persen.

Pada gambar 3 terlihat gambaran SEM dari permukaan dentin pada zona yang berbatasan setelah aplikasi NanoHidroksiapatit dan telah dicuci dengan air mengalir selama 5 menit. Tampak permukaan tubuli dentin tertutup sekitar 90 persen pada dentin yang di aplikasikan Nano Hidroksiapatit dan tubuli dentin yang seluruhnya terbuka pada zona kontrol. Gambar sebelah kanan menunjukkan pembesaran yang lebih tinggi pada zona yang sama.



Gambar 3. Gambaran SEM dari permukaan dentin pada zona yang berbatasan setelah aplikasi Nano Hidroksiapatit dan telah dicuci dengan air mengalir selama 5 menit. Tampak permukaan tubuli dentin tertutup sekitar 90 persen pada dentin yang di aplikasikan Nano Hidroksiapatit, dan tubuli dentin yang seluruhnya terbuka pada zona kontrol. Gambar sebelah kanan menunjukkan pembesaran yang lebih tinggi.

PEMBAHASAN

Dari data yang terlihat dalam hasil penelitian antara Standar Hidroksiapatit dan Nano Hidroksiapatit terlihat bahwa tubuli dentin yang mendapatkan aplikasi Nano Hidroksiapatit menunjukkan persentase penutupan yang lebih tinggi (90 persen) dibandingkan dengan Standar Hidroksiapatit dengan persentase penutupan sebesar 60 persen. Nano hidroksi apatit kemungkinan dapat digunakan untuk mengurangi pengaruh sensitif dentin pada gigi dengan dentin yang terbuka (*exposed dentin*).

Sebagai tambahan, dari gambaran SEM dapat terlihat adanya pengurangan ukuran penggumpalan partikel Nano Hidroksiapatit setelah di aplikasikan pada dentin, menunjukkan adanya penetrasi yang tinggi serta efisiensi dari partikel Nano hidroksiapatite ke dalam tubuli dentin. Perlu diketahui, Nano Hidroksiapatite ini mempunyai karakteristik untuk membentuk *agglomerate* (mengumpul), yang disebabkan oleh afinitas yang tinggi.

Nano Hidroksiapatit dapat digunakan dalam bentuk gel atau pasta sesuai dengan keperluan dokter gigi ataupun pasien dalam menanggulangi hipersensitif dentin. Dolci dkk. (2001) menyatakan bahwa Nano Hidroksiapatit adalah biokompatibel secara keseluruhan, dikarenakan memiliki sifat kimia dan fisik yang sama secara natural dengan dentin dan enamel, namun perlu dibuktikan apakah bahan tersebut terbukti biokompatibel dengan penelitian lanjutan, mengingat suatu bahan akan mengalami perubahan sifat fisik, kimia maupun toksisitas apabila dibuat dalam ukuran nanometer.

KESIMPULAN

Terlihat tingkat persentase penutupan tubuli dentin yang lebih tinggi pada dentin yang di aplikasikan Nano Hidroksiapatit dibandingkan dengan persentase penutupan dentin yang diaplikasikan Standar Hidroksiapatit. Berdasarkan hasil penelitian ini terlihat bahwa Nano Hidroksiapatit dapat menutup tubuli dentin dengan lebih baik, sehingga dapat digunakan untuk perawatan gigi dengan dentin hipersensitif.

SARAN

Penelitian lebih lanjut dari penggunaan Nano Hidroksiapatit sebagai menutup tubuli dentin dengan penelitian *in vitro* dalam hal biokompatibilitas terhadap sel serta jaringan dalam rongga mulut, dilanjutkan dengan penelitian pada binatang *(animal study)* dan pada giliran selanjutnya apabila terbukti aman, dapat dilanjutkan dengan penelitian klinis pada pasien untuk alternatif pengobatan hipersensitif dentin.

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PREPARATION AND CHARACTERIZATION OF HYDROXYAPATITE-CHITOSAN NANOCOMPOSITES BY BLENDING PROCESS

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1.0 INTRODUCTION

Hydroxyaptite, HA with the chemical formula $Ca(PO_4)_6(OH)_2$ is the main component of bone and teeth. HA is known to be biocompatible, bioactive, osteoconductive, non-toxic and non-inflammatory [1]. Recently, synthetic HA prepared at nano level (1-100 nm) plays a significant role in various biomedical applications. Its unique functional properties such as high surface area and ultra fine structure of the synthetic HA is similar to biological apatite. Therefore, synthetic HA has been widely applied in a variety of biomedical applications such as dental materials [2], bone substitutes materials [3] and constituent implants [4]. However, the application of pure HA is limited and cannot be used as load bearing implants materials due its brittleness and poor mechanical properties. Therefore, many efforts have been made to modify HA by polymers such as PHB [5], collagens [6], PMMA [7], polyethylene [8] and chitosan [9]. Among these polymers, the biopolymer chitosan (CS) has gained greater attention due to their excellent biocompatibility and biodegradability [10]. Therefore, the combination of HA and CS is expected to show an increase the osteoconductivity and biodegradation together with improved mechanical properties for biomedical applications. In this article, we report a very simple technique to synthesize HA-CS nanocomposite by using inexpensive precursors at low temperature through blending process. The interaction between hydroxyapatite and chitosan were investigated.

2.0 METHOD

The HA nanocrystals were prepared by wet chemical method using $Ca(OH)_2$ and H_3PO_4 as Ca and P precursors, respectively. Meanwhile, the HA-CS composites were synthesized by blending method. The ratio compositions of HA-CS composites were shown in Table 1. The phase crystallinity and composition of the HA nanocrytals and HA-CS nanocomposites were analysed by powder X-ray diffraction (XRD) technique. Meanwhile, the FT-infrared spectra (KBr disc) were used to identify the functional group. The particle size and morphology the of synthesized HA and HA-CS composite were observed using transmission electron microscope (TEM) and field emission scanning electron microscope (FESEM).

Table 1: Composition of HA-CS nanocomposite

HA (g)	CS(g)	Composition of HA-CS weight ratio (wt%)
0.500	0	100/0
0.425	0.075	85/15
0.300	0.150	70/30
0.250	0.250	50/50
0.100	0.400	20/80

2.0 RESULT AND DISCUSTION

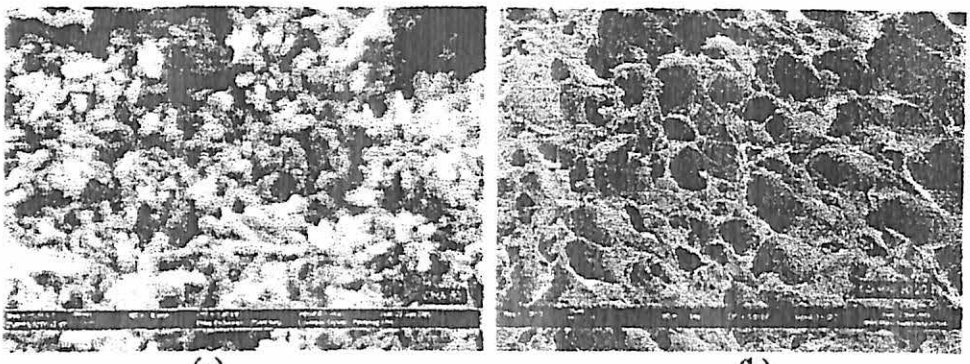
IR analysis of HA and HA-CS nanocomposites confirm the typical characteristics peaks of the carbonate type-B hydroxyapatite. Meanwhile, the peaks in the region 2929-2879 cm^{-1} represent of the aliphatic C-H stretching band of the Chitosan molecule. Another major peaks at 1655, 1599 and 1263 cm^{-1} represented the amino group (NH_2) which is the major functional group of Chitosan.

All the composites exhibit a typical apatite crystal structure according to the standard reference JCPDS 9-432 [11]. The broad peaks found at the region near to 32° (2θ) indicated that HA prepared was poor crystallinity due to the low temperature preparation. Peaks detected around 26° being 002 diffraction and the region near to 32° overlapping diffraction of (300), (211), and (112) were attributed to HA crystal structure. Meanwhile, the peak found at around 20° was assigned to CS molecules. Consistently, the CS peak increased with decreasing HA content. The fraction of crystalline phase (X_c) of the samples can be determined as: $X_c = 1 - (V_{112/300} / I_{300})$ where $V_{112/300}$ is intensity of hollow between (112) and (300) reflections and I_{300} is the intensity of (300) reflection [12]. The crystallinity values of the composites were given in Table 2. The obtained results suggest that the higher the amount of CS in the composite the lower the crystallinity. This phenomenon is expected to the incresed interaction between CS molecules with decreasing HA content. Sample with low crystallinity are much preferred for the bone regeneration due to their resorbability properties [13].

Table 2: The crystallinity value of HA and HA-CS nanocomposites

Samples	Crystallinity, X_c (%)
HA	0.302
HA/CS 85/15	0.296
HA/CS 70/30	0.256
HA/CS 50/50	0.199
HA/CS 20/80	0.180

The surface morphologies of HA and HA-CS nanocomposites are shown in Fig. 1. The SEM micrograph of the HA crystals (Fig.1a) exhibited nano sized crystals with almost uniform particles size. The HA particles with regular shape still present in the composite with low CS (HA-CS 85/15) content. However, porous structure was found for the composite HA-CS 20/80. One of the possible reason may be due to the CS molecule providing the scaffold for the HA crystals to grow. According to the literature, the composite containing porous structure will be more beneficial for bone regeneration.



(a) (b)

Fig.1. SEM micrograph of: (a) HA and (b) HA-CS 20/80.

Fig. 2 shows the TEM micrograms of HA and HA-CS nanocomposites. The HA nanocrystals (Fig.2a) were found to be rod-like and well disperse. Similar to the SEM result above, the agglomeration of the HA-CS nanocomposites increased with the increasing CS content.

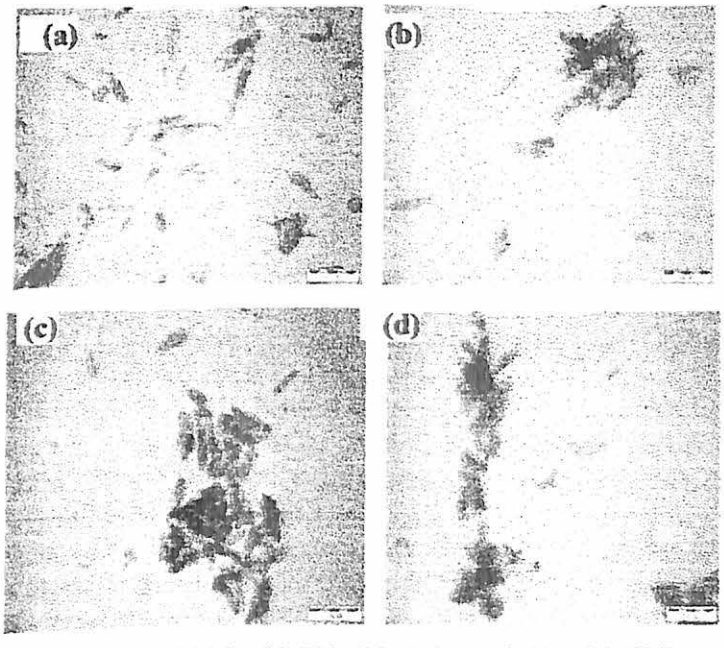


Fig.2, TEM micrograph of: (a) HA; (b) HA-CS 85/15; (c) HA-CS 70/30; and (d) HA-CS 50/50.

In conclusion, HA-CS nanocomposites with various weight ratios were successfully prepared using blending method. Both SEM and TEM observation indicate that the aggregation and agglomeration of HA crystals in the composite increased with increasing CS content. The porous structure obtained for the composite with high CS ratio.

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PENYATA PERBELANJAAN SEHINGGA 31 DISEMBER 2006

Jumlah Geran:	RM	Tiada rekod	Ketua Projek:	DR. SAM'AN MALIK MASUDI
Peruntukan 2004 (Tahun 1)	RM	10,000.00	Tajuk Projek:	An In vitro Study on the Sealing Ability at Nano Hydroxyapatile and Standard Hydroxyapatile on Dentinal Tubules
Peruntukan 2005 (Tahun 2)	RM	9,530.00		
Peruntukan 2006 (Tahun 3)	RM	0.00	Tempoh:	01 April 04- 30 Sept 06
			No.Akaun:	304/PPSG/6131301

Kwg	Akaun	PTJ	Projek	Donor	Peruntukan Projek	Perbelanjaan T'kumpul Hingga Tahun Lalu	Peruntukan Semasa	Tanggungan Semasa	Bayaran Tahun Semasa	Belanja Tahun Semasa	Baki Projek
304	11000	PPSG	6131301		-	-	-	-	-	-	-
304	14000	PPSG	6131301		-	-	-	-	-	-	-
304	15000	PPSG	6131301		-	-	-	-	-	-	-
304	21000	PPSG	6131301		5,500.00	3,174.05	2,325.95	-	713.00	2,305.05	1,612.95
304	22000	PPSG	6131301		-	-	-	-	-	-	-
304	23000	PPSG	6131301		400.00	-	400.00	-	-	-	400.00
304	24000	PPSG	6131301		-	-	-	-	-	-	-
304	25000	PPSG	6131301		-	-	-	-	-	-	-
304	26000	PPSG	6131301		-	-	-	-	-	-	-
304	27000	PPSG	6131301		8,460.00	2,454.80	6,005.20	-	9,376.00	1,195.80	(3,370.80)
304	28000	PPSG	6131301		-	-	-	-	-	-	-
304	29000	PPSG	6131301		4,470.00	1,325.00	3,145.00	-	50.00	930.00	3,095.00
304	32000	PPSG	6131301		-	-	-	-	-	-	-
304	35000	PPSG	6131301		700.00	-	700.00	-	-	-	700.00
					19,530.00	6,953.85	12,576.15	-	10,139.00	4,430.85	2,437.15