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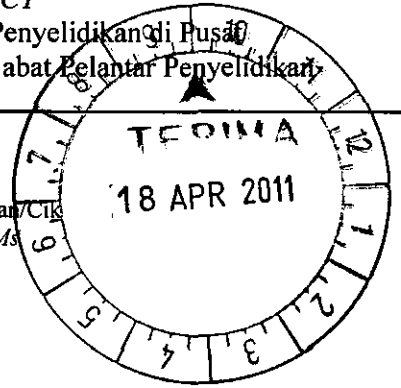
# **AN IN-VITRO STUDY TO COMPARE ANTIMICROBIAL ACTIVITY OF ENDODONTIC SEALERS**

**DR. ZAIHAN ARIFFIN  
PUSAT PENGAJIAN SAINS PERGIGIAN  
(304/PPSG/6139027)**

**LAPORAN AKHIR PROJEK PENYELIDIKAN JANGKA PENDEK**

**FINAL REPORT OF SHORT TERM RESEARCH PROJECT**

Sila kemukakan laporan akhir ini melalui Jawatankuasa Penyelidikan di Pusat Pengajian dan Dekan/Pengarah/Ketua Jabatan kepada Pejabat Pelantar Penyelidikan.



**1. Nama Ketua Penyelidik: Zaihan Ariffin**

*Name of Research Leader*

Profesor Madya/  
*Assoc. Prof.*

Dr./  
*Dr.*

Encik/Puan/Cik/  
*Mr/Mrs/Ms*

**2. Pusat Tanggungjawab (PTJ):**

*School/Department*

Pusat Pengajian Sains Pergigian

**3. Nama Penyelidik Bersama:**

*Name of Co-Researcher*      *Asso... Prof Madya Dr Sama'an Malik Masudi*

**4. Tajuk Projek:**

An in vitro study to compare antimicrobial activity of endodontic sealers

**5. Ringkasan Penilaian/Summary of Assessment:**

	Tidak Mencukupi <i>Inadequate</i>		Boleh Diterima <i>Acceptable</i>	Sangat Baik <i>Very Good</i>	
	1	2		3	4
i) Pencapaian objektif projek: <i>Achievement of project objectives</i>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
ii) Kualiti output: <i>Quality of outputs</i>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
iii) Kualiti impak: <i>Quality of impacts</i>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
iv) Pemindahan teknologi/potensi pengkomersialan: <i>Technology transfer/commercialization potential</i>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
v) Kualiti dan usahasama : <i>Quality and intensity of collaboration</i>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
vi) Penilaian kepentingan secara keseluruhan: <i>Overall assessment of benefits</i>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>

**6. Abstrak Penyelidikan**

(Perlu disediakan di antara 100 - 200 perkataan di dalam Bahasa Malaysia dan juga Bahasa Inggeris. Abstrak ini akan dimuatkan dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & masyarakat luar).

**Abstract of Research**

(An abstract of between 100 and 200 words must be prepared in Bahasa Malaysia and in English).

This abstract will be included in the Annual Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

Sila lihat lampiran 1

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**7. Sila sediakan laporan teknikal lengkap yang menerangkan keseluruhan projek ini.**

[Sila gunakan kertas berasingan]

Applicant are required to prepare a Comprehensive Technical Report explaining the project.

(This report must be appended separately)

Sila lihat lampiran 2

**Senaraikan kata kunci yang mencerminkan penyelidikan anda:**

List the key words that reflects your research:

Bahasa Malaysia

Bahasa Inggeris

Antibakterial \_\_\_\_\_

antibacterial \_\_\_\_\_

Mikro-organisma \_\_\_\_\_

micro-organism \_\_\_\_\_

Endodontik sealant \_\_\_\_\_

endodontic sealer \_\_\_\_\_

**8. Output dan Faedah Projek**

*Output and Benefits of Project*

(a) \* **Penerbitan Jurnal**

*Publication of Journals*

(Sila nyatakan jenis, tajuk, pengarang/editor, tahun terbitan dan di mana telah diterbit/diserahkan)

(State type, title, author/editor, publication year and where it has been published/submitted) -Sila lihat lampiran 3

An agar diffusion study comparing the antimicrobial activity of Nanoseal with some other endodontic sealers

- (b) **Faedah-faedah lain seperti perkembangan produk, pengkomersialan produk/pendaftaran paten atau impak kepada dasar dan masyarakat.**  
*State other benefits such as product development, product commercialisation/patent registration or impact on source and society.*

*Product Nanoseal sedang dalam proses pengkomersialan*

\* Sila berikan salinan/Kindly provide copies

- (c) **Latihan Sumber Manusia**  
*Training in Human Resources*

- i) Pelajar Sarjana: \_\_\_\_\_  
*Graduates Students*  
(Perincikan nama, ijazah dan status)  
(Provide names, degrees and status)

*Dr Ali Burak Kamal AAl- Saraj BDS (Master of Science [Restorative])*

- ii) Lain-lain: 1) Menghadiri : 14<sup>th</sup> National Conference on Medical and Health Sciences 2009 -USM  
*Others*  
2) Menghadiri APDSA – speaker and judges

9. **Peralatan yang Telah Dibeli:**  
*Equipment that has been purchased - lampiran 4*

*Membeli bahan-bahan kajian guna buang*

*Canon laser Printer –*

  
**Tandatangan Penyelidik**  
*Signature of Researcher*

*10/4/11*  
**Tarikh**  
*Date*

**Komen Jawatankuasa Penyelidikan Pusat Pengajian/Pusat**  
*Comments by the Research Committees of Schools/Centres*

The research project has been completed successfully. Outcome of research project include:

1. 1 International publication - Australian Endodontic Journal
2. 1 Oral conference presentation ✓
3. 1 MSc graduate ✓



**TANDATANGAN PENERUSI**  
**JAWATANKUASA PENYELIDIKAN**  
**PUSAT PENGAJIAN/PUSAT**

*Signature of Chairman*

*[Research Committee of School/Centre]*

**PROFESSOR ZULKIFLI AHMAD**

Profesor Perubatan Masyarakat

Timbalan Dekan

(Penyelidikan & Pengajian Siswazah)

Pusat Pengajian Sains Pergigian

USM Kampus Kesihatan

16150 Kubang Kerian, Kelantan

14.4.2011

**Tarikh**  
*Date*

**AN *IN- VITRO* AGAR DIFFUSION STUDY COMPARING THE ANTIMICROBIAL  
ACTIVITY OF NANOSEAL WITH SOME OTHER ENDODONTIC SEALERS**

**ABSTRACT**

Complete elimination of microbes in teeth with an infected dental pulp is one of the main objectives of endodontic treatment. Endodontic sealers should have a wide antimicrobial spectrum and action time because of the polymicrobial characteristics of endodontic infections and to eliminate residual microorganisms unaffected by the effects of chemomechanical preparation and intracanal medication of endodontic treatment.

The objectives of this study were

- 1) To evaluate the antimicrobial activity of NanoSeal <sup>™</sup>, AH26 <sup>™</sup>, Tubli-Seal <sup>™</sup>, Sealapex <sup>™</sup> and Roeko-Seal <sup>®</sup> against *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Streptococcus sobrinus*, and *Escherichia coli* in three incubation periods of 24 hours, 48 hours and 7 days .
- 2) To compare the antimicrobial activity of the five sealers towards each type of microorganism in the three periods.
- 3) To compare the antimicrobial activity between the tested sealers towards each type of microorganism in the three periods.

Agar diffusion test (ADT) was used. In this study 50 Muller- Hinton agar plates were punched with 5 standard wells (4 mm in diameter and 5 mm in depth) on each a plate. Ten replicates were made for each type of microorganism. Two hundred microliters of bacterial suspension was made of appropriate turbidity for each type of microorganism and all plates were streaked with sterile cotton swab. Freshly mixed endodontic sealers were dispensed in

the wells of each plate. The plates were observed after 24 hours, 48 hours and 7 days incubation for each type of microorganism.

Zones of inhibition produced were measured and recorded. Data were analysed by using Kruskal-Wallis test.  $P$  value  $<0.05$  was considered statistically significant of difference. Mann-Whitney test (*Post hoc* test for Kruskal-Wallis test) was used to compare the zones of inhibition between each two sealers towards each type of microorganism.  $P$  value  $< 0.005$ , based on *Bonferroni correction* to avoid type 1 error rate, was considered statistically significant of difference.

Five MH agar plates were prepared and inoculated (one plate for each type of microorganism) to serve as a positive control group and tested for their susceptibility to antibiotics and another five plates were prepared to serve as negative control as the bacteria were incubated and allowed to grow alone without any additives.

All tested materials exhibited inhibition zones towards the tested microorganisms for all the tested periods except for Roeko-Seal® as it showed no inhibition zones. Significant difference was found between the tested sealers ( $P<0.001$ ) towards each microorganism at all times with regard to their antimicrobial activities. There was no significant difference (based on *Bonferroni correction* to avoid type 1 error rate) observed between NanoSeal™ and AH26™ ( $P>0.005$ ). Tubli-Seal™ showed the greatest inhibitory effect towards the tested microorganisms followed by Sealapex™, NanoSeal™  $\approx$  AH26™, and Roeko-Seal®. For *S.mutans* and *S.sobrinus*, NanoSeal™  $\approx$  AH26™ showed greater effect than Sealapex™. With regard to the tested microorganisms, *P.aeruginosa* and *E. faecalis* were the most resistant strains to NanoSeal™ and AH26™ then followed by *E.coli*, *S.mutans*, and

*S.sobrinus*. On the other hand, *E. faecalis* was more resistant to Tubli-Seal™ than the other microorganisms. While for Sealapex™, it showed the greatest inhibitory effect towards *P.aeruginosa* then *E. faecalis*, *E.coli*, *S.mutans*, and *S.sobrinus* respectively.

In conclusion, the antimicrobial inhibitory activity of the new experimental sealer NanoSeal™ was comparable to AH26™ and as good as other sealers, except for Roeko-Seal®. However, Tubli-Seal™ exhibited the greatest inhibitory effect and Roeko-Seal® exhibited no inhibitory effect against all test strains. The order of antimicrobial inhibitory activity of the tested materials could be expressed in the following sequence: Tubli-Seal™ > Sealapex™ > NanoSeal™ ≈ AH26™ > Roeko-Seal® for *E.faecalis*, *P.aeruginosa*, and *E.coli*. On the other hand it was Tubli-Seal™ > NanoSeal™ ≈ AH 26™ > Sealapex™ > Roeko-Seal® for *S.mutans* and *S.sobrinus*.



**KAJIAN IN-VITRO PERBANDINGAN DIFUSI AGAR AKTIVITI  
ANTIMIKROBIAL BAHAN NANOSEAL DENGAN BAHAN SEALANT  
ENDODONTIK YANG LAIN**

**ABSTRAK**

Penghapusan mikrob secara menyeluruh pada pulpa gigi yang dijangkiti adalah menjadi salah satu objektif utama dalam rawatan endodontik. Bahan sealant endodontik seharusnya mempunyai spektrum antimikrob yang luas dan masa tindakan yang lama disebabkan oleh ciri-ciri polimikrobial jangkitan endodontik dan ini juga penting untuk penghapusan sisa-sisa mikroorganisma yang tidak dapat dihapuskan oleh kesan penyediaan kemomekanikal dan penggunaan ubat intrakanal endodontik.

Matlamat kajian:

- 1) Untuk menilai aktiviti bahan antimikrobial bahan sealant endodontik -NanoSeal™ , AH26™ , Tubli-Seal™, Sealapex™ dan Roeko-Seal® keatas *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Streptococcus sobrinus*, dan *Escherichia coli* dalam tiga tempoh inkubasi iaitu 24 jam, 48 jam dan 7 hari.
- 2) Untuk membandingkan aktiviti antimikrobial kelima-lima bahan sealant tersebut terhadap setiap mikroorganisma itu dalam ketiga-tiga tempoh inkubasi.
- 3) Untuk membandingkan aktiviti antimikrobial setiap bahan sealant tersebut dengan mikroorganisma dalam ketiga-tiga tempoh inkubasi.

Bagi kajian *in-vitro* ini, ujian Difusi Agar (ADT) telah digunakan. 50 plet Agar Muller-Hinton (MH) telah ditebuk sebanyak 5 lurahan (berdiameter 4mm dan sedalam 5mm) setiap jenis satu dengan setiap jenis microorganism diberikan 10 ulangan. 200 µl kultur bakteria telah disediakan mengikut kesesuaian setiap jenis mikroorganisma. Kesemua plet kultur

tersebut akan disapu dengan kapas kesat yang steril berserta dengan setiap jenis mikroorganisma yang telah disediakan. Bahan sealant endodontik yang telah disediakan kemudiannya diisi kedalam setiap satu lurahan pada plet tersebut. Plet-plet itu diperhatikan dalam tempoh inkubasi 24 jam, 48 jam dan 7 hari, bagi setiap jenis mikroorganisma.

Zon-zon antimikrobial yang terhasil telah diukur dan direkodkan. Kesemua data dianalisa dengan menggunakan ujian Kruskal-Wallis dan Mann-Whitney. Lima daripada plet MH (satu bagi setiap jenis mikroorganisma) itu telah diinokulasi sebagai sampel kumpulan kawalan positif dan diuji kerecatannya terhadap antibiotik dan lima plet lagi telah disediakan untuk kawalan negatif dan setiap jenis bakteria ini telah diinkubasi dan dibiarkan untuk berpoliferasi tanpa sebarang aditif.

Kesemua bahan yang telah diuji menunjukkan zon-zon antimikrobial aktiviti terhadap kesemua jenis mikroorganisma dalam tempoh masa kajian kecuali Roeko-Seal® yang tidak menunjukkan apa-apa zon aktiviti antimikrobial aktiviti. Terdapat perbezaan yang signifikan ( $P < 0.001$ ) wujud di antara bahan –bahan sealant endodontik yang telah diuji dari segi aktiviti antimikrobial pada mikroorganisma dalam tempoh ujian ini. Namun begitu, tidak ada perbezaan yang signifikan yang diperhatikan antara NanoSeal™ dan AH26™ ( $P > 0.005$ ). Tubli-Seal™ menunjukkan kesan yang paling tinggi terhadap jenis mikroorganisma yang telah diuji diikuti dengan Sealapex™, NanoSeal™ dan AH26™. Tetapi bagi kumpulan *S. mutans* dan *S. Sobrinus*, NanoSeal™  $\approx$  AH26™ menunjukkan kesan yang lebih kuat berbanding Sealapex™. Bagi setiap jenis mikroorganisma yang telah diuji, *P. aeruginosa* dan *E. faecalis* telah menunjukkan kesan penentangan mikroorganisma yang paling kuat pada NanoSeal™ dan AH26™ diikuti pula oleh *E. coli*, *S. mutans* dan *S. sobrinus*. Namun begitu, *E. faecalis* telah menunjukkan kesan lebih tinggi penentangannya terhadap Tubli-Seal™ jika

dibandingkan dengan mikroorganisma yang lain. Bagi Sealapex™ pula, ia menunjukkan kesan rencatan yang paling tinggi terhadap *P.aeruginosa* diikuti *E. faecalis*, *E.coli*, *S.mutans*, dan *S.sobrinus*.

Kesimpulannya, aktiviti antimikrobial bahan sealant eksperimen NanoSeal™ adalah setanding dengan AH26™ dan bahan sealant lain kecuali Roeko-Seal®. Namun begitu, Tubli-Seal™ telah menunjukkan kesan penentangan yang paling kuat. Manakala Roeko-Seal® tidak menunjukan sebarang penentangan kepada semua strain mikroorganisma yang telah diuji. Turutan aktiviti rencatan antimikrobial bagi setiap bahan yang telah diuji adalah seperti turutan berikut: Tubli-Seal™ > Sealapex™ > NanoSeal™ ≈ AH26™ > Roeko-Seal® untuk *E.faecalis*, *P.aeruginosa*, dan *E.coli*, manakala Tubli-Seal™ > NanoSeal™ ≈ AH26™ > Sealapex™ > Roeko-Seal® untuk *S.mutans* and *S.sobrinus*.