行政院國家科學委員會專題研究計畫 成果報告

研究根管充填劑對造牙骨質細胞的影響
研究成果報告(精簡版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 100-2314-B-040-006-
執 行 期 間 ：100 年 08 月 01 日至 101 年 07 月 31 日
執 行 單 位 ：中山醫學大學牙醫學系（所）

計 畫 主 持 人：黃富美
共 同 主 持 人：張育超
計畫參與人員：此計畫無其他參與人員

報 告 附 件 ：出席國際會議研究心得報告及發表論文

公 開 資 訊 ：本計畫涉及專利或其他智慧財產權，2 年後可公開查詢

中 華 民 國 101 年 11 月 16 日

中 文 摘 要 ： 根管治療成功與否，是建立在是否能夠完全清除發炎齒髓組織和建立無菌的根管， 以及根管完全密閉充填，且經常為確保根管及側根管完全密閉充填，將根管充填劑擠壓至根管尖外或由側根管擠壓至根管外。雖然過一段時間會慢慢被組織所吸收，但與根尖組織仍有長時間直接接觸，根尖組織包括骨細胞與造牙骨質細胞(cementoblast)，研究根管充填劑對人類骨髓幹細胞、人類骨細胞(U2OS) 與造牙骨質細胞(murine cell line OCCM.30) 的體外毒性實驗。

中文關鍵詞： 根管充填劑, 造牙骨質細胞, 人類骨髓幹細胞

英 文 摘 要 ： The success of the root canal treatment, is built on the ability to completely clear the inflammation of the tooth pulp and establish aseptic root canal, as well as restorations of root canal completely airtight, and often to ensure that the root canal and lateral root canal filling completely airtight, root canal filling agent extrusion to root canal apex, or extrusion to lateral root canal. Although over time will slowly be absorbed by tissues, but still with apex tissues have long time in direct contact with the apex tissues including bone cells and makes cementoblast.

We want to study cytotoxicity of endodontic sealers on human bone marrow stem cells, human osteoblasts U2OS cells and cementoblast (murine cell line OCCM.30).

英文關鍵詞： endodontic sealers, cementoblast human bone marrow stem cells

行政院國家科學委員會補助專題研究計畫 □期中進度報告 ■期末報告

（計畫名稱）

研究根管充填劑對造牙骨質細胞的影響

計畫類別：■個別型計畫　□整合型計畫
計畫編號：NSC　100-2314-B-040-006-
執行期間：　100 年 8 月 1 日至 101 年 7 月 31 日

執行機構及系所：中山醫學大學牙醫學系（所）

計畫主持人：黃富美
共同主持人：張育超
計畫參與人員：

本計畫除繳交成果報告外，另須繳交以下出國報告：
□赴國外移地研究心得報告
□赴大陸地區移地研究心得報告
■出席國際學術會議心得報告及發表之論文
□國際合作研究計畫國外研究報告

處理方式：除列管計畫及下列情形者外，得立即公開查詢
□涉及專利或其他智慧財產權，□一年■二年後可公開查詢

Introduction

Teeth with severe pulpal or periapical inflammation can be successfully treated with the established techniques of cleaning and shaping the root canals, followed by obturation of the root canal system. A large variety of root canal sealers have been advocated for use in conjunction with solid or semi-solid filling materials. Currently, root canal sealers are available based on various formulas such as expoxy resin, calcium hydroxide and zinc oxide-eugenol.

Ideally, root canal sealers should be biocompatible and have satisfactory physico-chemical properties. They should also be well tolerated by the periradicular tissues. Indeed, since these materials will be in direct contact with periapical tissues for prolonged periods of time, their biocompatibility is of primary importance. A biocompatible sealer should neither prevent nor hinder tissue repair but should aid or stimulate the reorganization of injured structures.

One method of testing the biological compatibility of root canal sealers is to use an *in vitro* model to determine the cellular response. This has the advantage that many factors and variables can be controlled and the cytotoxicity can be determined with reliability and reproducibility.

Osteoblasts are considered the cells primarily concerned with providing physical barriers and structural components in the periapical tissues. To date, there has been very little data on the cytotoxicity of various types of root canal sealers in different culture systems. The purposes of this investigation were to study the cytotoxic effects of elutes of different types of root canal sealers on Immortalized cementoblasts (OCCM) human bone marrow stem cells and human osteoblastic cell line U2OS cells.

Materials and Methods

Sealers

Three type root canal sealers were evaluated: MM-sealer epoxy resin-base root canal sealer, Tubliseal (Kerr, Romulus, MI, USA), and Canals (Showa, Tokyo, Japan) zinc oxide eugenol based sealer, Apexit Plus calcium hydroxide based sealer. The component of each root canal sealer was shown in table 1.

Table 1. Composition of the tested root canal sealers as given by the manufacturers

Material	Compositions	Source
Tubliseal	40% Zinc oxide, 2.75% barium sulfate, 25% Oleo resins, 7.5% thymol iodide, 22.75% eugenol, 2% modifiers	Kerr, Romulus, MI, USA
Canals	Powder: Zinc oxide, barium sulfate, bismuth bibcarbonate, rosin. Liquid: olive oil, eugenol.	Showa, Tokyo, Japan

Apexit Plus

Base：Calcium hydroxide / Calcium oxide 36.9,Hydrated collophonium 54.0,Fillers and other auxiliary materials (highly dispersed silicon dioxide, phosphoric acid alkyl ester) 9.1

Catalyst：Disalicylate 47.6, Bismuth hydroxide / Bismuth carbonate 36.4, Fillers and other auxiliary materials (highly dispersed silicon dioxide, phosphoric acid alkyl ester)16.0

MM-sealer epoxy resin-base root canal sealer

- Base：epoxy oligomer resin29%, ethylene glycol salicylate18%,calcium phosphate17%, bismuth subcarbonate26%, zirconium oxide10%
- Catalyst ： polyaminobenzoate31% 、 triethanolamine5%, calcium phosphate29%, bismuth subcarbonate21%, zirconium oxide10%, calcium oxide4%

Sample fabrication

The cements were mixed according to the manufacturers' instructions. Triplicate sample disks of the root canal sealers were fabricated in sterile cylindrical glass molds 10 mm in height and 3 mm in

diameter. Excess flash was removed with a sterile scalpel. The specimens were placed in polyethylene vials directly after mixing.

Elute preparation

The fresh samples were those tested immediately after disk transfer. Each specimen was placed in 10 mL of fresh culture medium and transferred into fresh media after 48 hr. All samples were extracted four times consecutively in culture medium. After each elution period, the extracts were removed, and the vials were filled again with fresh medium. Cytotoxicity was determined after 24 h incubation of the cells and elutes. Cells without addition of elutes acted as untreated control.

Cell cultures

Human bone marrow stem cells (human mesenchymal stem cell-bone marrow, catalog number: 7500) were obtained from ScienCell Research Laboratories. Cell were maintained in mesenchymal stem cell medium (MSCM, cat. No. 7501, ScienCell Research Laboratories). U2OS cells (American Tissue Type Collection HTB 96) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY) supplemented with 10 % fetal calf serum (FCS) (Sigma Chemical Co., St. Louis, MO), 100 μg/ml of streptomycin, 100 mg/ml of penicillin at 37℃ in humidified incubator under ambient pressure air atmosphere containing 5 % CO_2. Confluent cells were detached with 0.25 % trypsin and 0.05 % EDTA for 5 min, and aliquots of separated cells were subcultured. The cells were subcultured at 1:4 splits every 3rd day.

Cytotoxicity assay

Cytotoxicity was evaluated by the almar blue dye assay. Almar blue is an oxidation-reduction indictor for eukaryotic cells. It is a recently developed extension of the cytotoxicity assay base on the

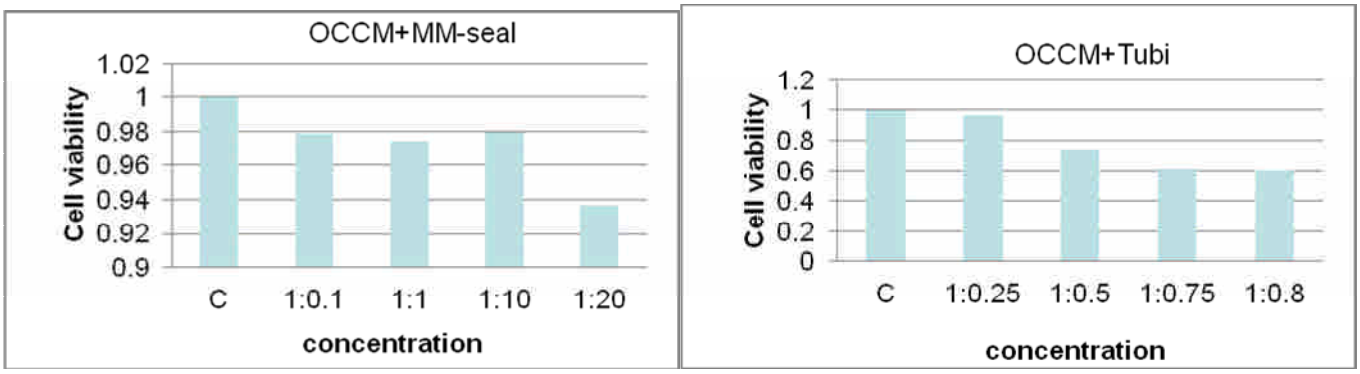
reduction of tetrazolium salts by the mitochondrial cytochromoxidase system. Briefly, The culture cell line seeded 5×10^4 cells per well into 24-well culture plates and incubated to attach for 24 h. The culture medium was replaced with fresh Dulbecco's modified Eagle medium and various concentrations of halothane. After trypsining, alamar blue dye was added to a 250 µl alquot of cell suspensions for 2 h at 37 °C. The colorimetric determination was done at 570 nm and 600 nm on a plate reader (CytoFluor™ 4500, Millipore, Bedford, USA). The percent inhibition of mitochondria activity in response to a test agent as compared to untreated cells was calculated by the formula:

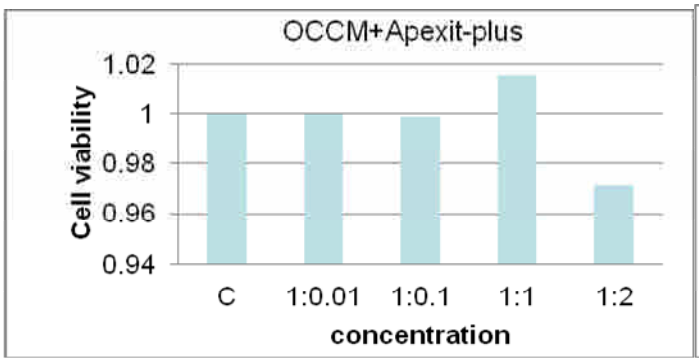
$$\frac{100 - (OD_{570} - OD_{600}) \text{ of test agent dilution}}{(OD_{570} - OD_{600}) \text{ of untreated control}} \times 100$$

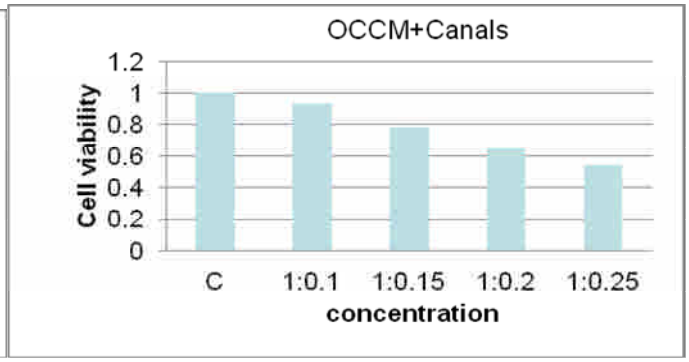
Statistical analysis

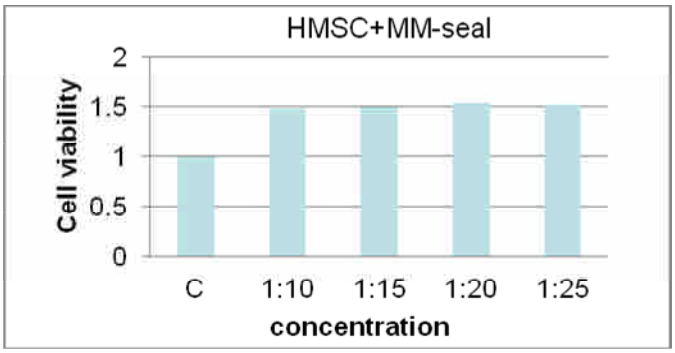
Five replicates of each concentration were performed in each test. All assays were repeated 3 times to ensure reproducibility. Statistical analysis was conducted by one-way analysis of variance. Tests of differences of the treatments were analyzed by Duncan's test and a value of $P<0.05$ was considered statistically significant.

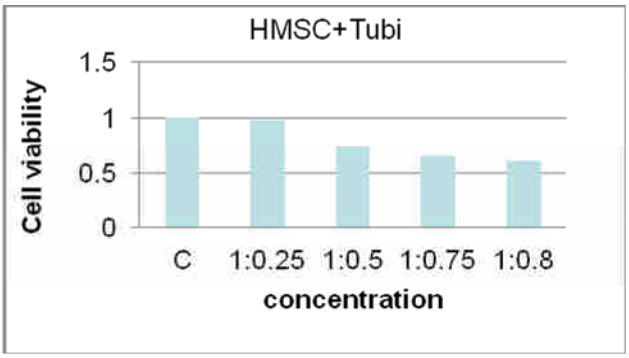
Results

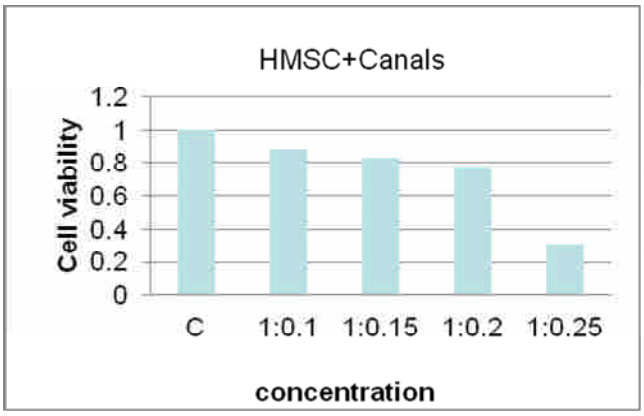


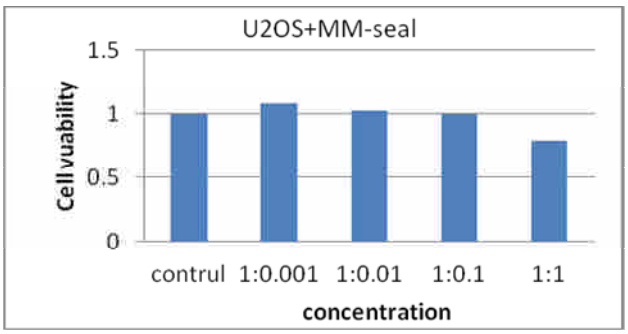

OCCM+Apexit-plus
Cell viability
1.02
1
0.98
0.96
0.94
C
1:0.01
1:0.1
1:1
1:2
concentration

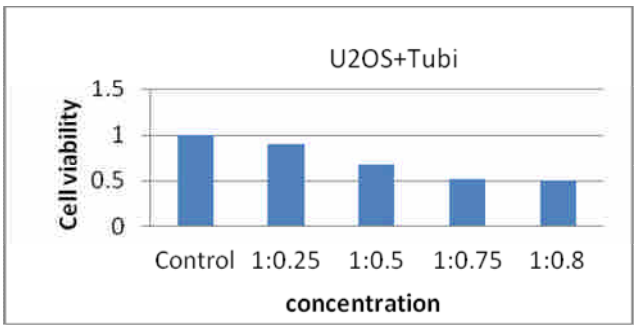

OCCM+Canals
Cell viability
1.2
1
0.8
0.6
0.4
0.2
0
C
1:0.1
1:0.15
1:0.2
1:0.25
concentration

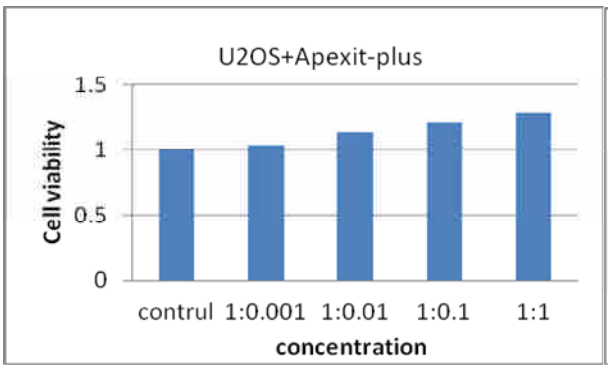

HMSC+MM-seal
Cell viability
2
1.5
1
0.5
0
C
1:10
1:15
1:20
1:25
concentration

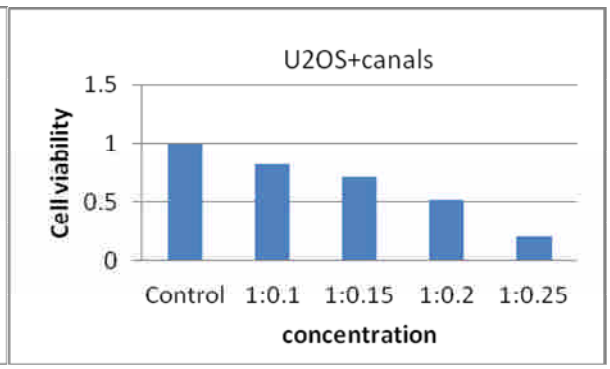

HMSC+Tubi
Cell viability
1.5
1
0.5
0
C
1:0.25
1:0.5
1:0.75
1:0.8
concentration

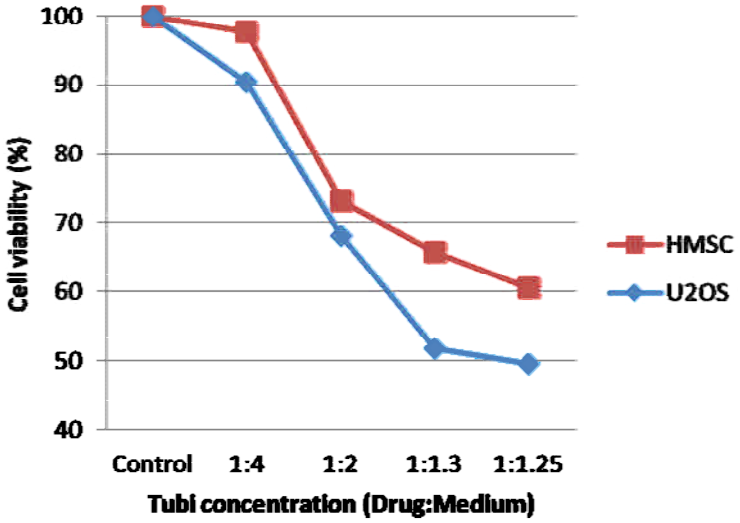

HMSC+Canals
Cell viability
1.2
1
0.8
0.6
0.4
0.2
0
C
1:0.1
1:0.15
1:0.2
1:0.25
concentration

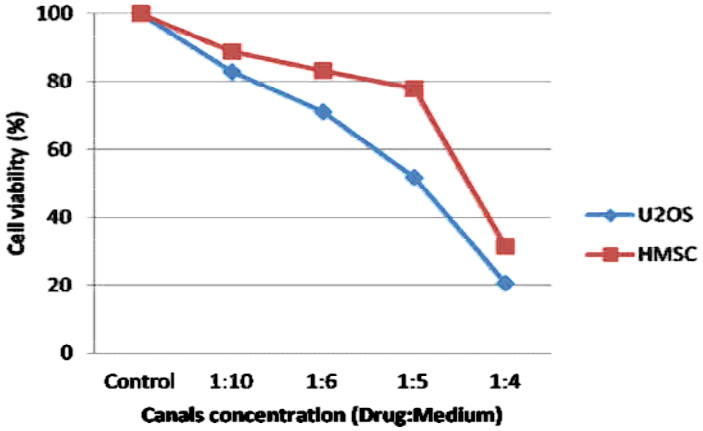

U2OS+MM-seal
Cell vuability
1.5
1
0.5
0
contrul
1:0.001
1:0.01
1:0.1
1:1
concentration


U2OS+Tubi
Cell viability
1.5
1
0.5
0
Control
1:0.25
1:0.5
1:0.75
1:0.8
concentration


U2OS+Apexit-plus
Cell viability
1.5
1
0.5
0
contrul
1:0.001
1:0.01
1:0.1
1:1
concentration


U2OS+canals
Cell viability
1.5
1
0.5
0
Control
1:0.1
1:0.15
1:0.2
1:0.25
concentration





Conclusions

This *in vitro* study examined the cytotoxic effects of resin based, zinc oxide eugenol based, and calcium hydroxide based root canal sealers on human bone marrow stem cells and Immortalized cementoblasts (OCCM) results confirm that components that leach out of root canal sealers after setting for extended periods, caused moderate or severe toxic reactions to cultured cells.

行政院國家科學委員會補助國內專家學者出席國際學術會議報告

101 年 10 月 8 日

報告人姓名	黃富美	服務機構 及職稱	中山醫學大學 教授
時間 會議 地點	2012/06/20~2012/06/23 巴西（Brazil ）伊瓜蘇瀑布 (Iguacu Falls)	本會核定 補助文號	NSC-100-2314-B-040-006
會議 名稱	（中文）第 90 屆國際牙醫研究學會年會 （英文）90th General Session & Exhibition of the international Association for Dental Research		
發表 論文 題目	（中文）根管充填劑對人類骨髓幹細胞的體外毒性實驗 （英文）Cytotoxicity of endodontic sealers on human bone marrow stem cells		

報告內容應包括下列各項：

一、　參加會議經過

今年的國際牙醫研究學會年會在於巴西（Brazil ）伊瓜蘇瀑布 (Iguacu Falls)舉行，因位於南美洲，沒有直飛的班機，因此路途遙遠轉機過程較為辛苦，因油價飆漲機票超貴，因此臺灣參加人數較少，大多數是南美洲國家論文發表。同時又遇上二十一國經濟會議，因此在里約旅館安排較為困難，巴西治安較差，但遇上經濟會議，觀光景點都有加強警備，但晚上逛街時也目睹有人被搶。

國際牙醫研究學會年其論文發表形式分為 Hand-on Workshop 、Oral presentation、Poster presentation 三種。筆者今年以 Poster presentation 方式發表。

報告的論文題目為：根管充填劑對人類骨髓幹細胞的體外毒性實驗。

摘要內容： Objectives: Root canal sealers are frequently placed in direct contact with living tissues. Therefore, root canal sealers should have good biocompatibility. The purpose of this study was to determine the cytotoxicity of resin based sealer Tubli-Seal (Kerr, Romulus, MI, USA), zinc oxide eugenol based sealer Canals (Showa, Tokyo, Japan), and calcium hydroxide based sealer Apexit Plus (Ivoclar Vivadent, Liechtenstein) on human bone marrow stem cells.

Methods: Freshly mixed materials were filled in glass rings (4 mm height and 10 mm in diameter) and eluted in 10 ml of culture medium for 1 day. Subsequently, various dilutions (final dilution: 1/2, 1/4, and 1/8) of these extraction media were prepared for this study. Cytotoxicity was judged using alamar blue dye reduction assay on human bone marrow stem cells. Statistical analysis was conducted by one-way analysis of variance. Tests of differences of the treatments were analyzed by Duncan's test and a value of $P<0.05$ was considered statistically significant.

Results: The results showed that elutes from root canal sealers were cytotoxic to human bone marrow stem cells in a dose-dependent manner ($p<0.05$). The rank orders with respect to cytotoxicity were found to be as follows: Canals > Tubli-Seal > Apexit Plus.

Conclusions: Taken together, root canal sealers demonstrated cytotoxic effects to human bone marrow stem cells. The sensitivity of toxicity depended on the materials tested.

國際牙醫研究學會年會是當今牙醫界最大且地位最高的學術會議，目前共分為 Behavioral Sciences & Health Services Research、Cariology Research、Craniofacial Biology、Dental Materials、Diagnostic Systems、Dental Anesthesiology Research、Education Research、Geriatric Oral Research、Implantology Research、Microbiology/Immunology、Mineralized Tissue、Neuroscience、Nutrition Research、Oral Health Research、Oral & Maxillofacial Surgery、Oral Medicine & Pathology、Periodontal Research、Pharmacology/Therapeutics/Toxicology、Prosthodontics Research、Pulp Biology、Salivary Research 等 21 個組別。其官方出版的期刊 Journal of Dental Research 是牙科 impact factor 最高的期刊。

二、　與會心得

本屆年會因為在南美洲巴西（Brazil ）伊瓜蘇瀑布舉行，雖然臺灣參加人數較少，大多數是南美洲國家論文發表，但南美洲人熱情，與當地學者閒聊不論在研究或南美洲風俗人情上收穫良多，吸取了許多寶貴的經驗及目前研究的新方向，對於往後的研究裨益良多，同時也對南美洲國家 風土人情、生活習俗與地理景觀有更為深入了解；再此亦非常感激國科會予以經費補助參與此次國際牙醫研究學會年會。

ACCEPTED

Friday, March 23, 2012

Fu-Mei Huang
Chung Shan Medical University
Taichung, Taiwan

Abstract ID#: 161364

Abstract Title: Cytotoxicity of endodontic sealers on human bone marrow stem cells

Dear Fu-MEi Huang,

It is a pleasure to inform you that your abstract has been ACCEPTED as a POSTER PRESENTATION at the IADR General Session (June 20-23, 2012). The meeting will take place in Igua 蕞 Falls, Brazil.

Please note that some students/co-workers have provided an alternate e-mail address for notification, so if this letter is addressed to a colleague, please forward it to his/her attention. E-mail notifications are sent only to the address provided for the presenter when the abstract was submitted; it is the presenter responsibility to notify co-authors.

PRESENTATION INFORMATION

Presentation Date: Thursday, June 21, 2012 Session Title: Biocompatibility and Biologic Effects

Session Time: 9:00 AM - 10:15 AM

Location: Convention Center, Poster Hall

Poster Viewing Time: 9 a.m. ?5 p.m.

Poster Set-up Time: 8:30 a.m. ?9 a.m.

Poster Tear-down Time: 5 p.m. ?5:15 pm

POSTER SIZE

Dimensions of the poster board are 1m x 1.98m (3.28 ft x 6.5 ft). The board will be used VERTICALLY. These are the maximum dimensions to follow when creating your poster but you may make your poster smaller. You are only required to be at your poster board during the session time listed above and not the entire poster viewing time. For further information please go directly to the meeting home page on the IADR website at www.iadr.org/iags.

Thank you for submitting your paper. We look forward to your presentation at the meeting and note that we have scheduled a full conference so we hope you stay for the duration of the meeting. If you have any questions, please send a message to meetings@iadr.org. Every attempt will be made to respond as soon as possible.

Sincerely,

Mary MacDougall, PhD
IADR Annual Session Committee Chair

IADR GENERAL SESSION
IGUAÇU FALLS, BRAZIL • JUNE 20-23, 2012

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Cytotoxicity of endodontic sealers on human bone marrow stem cells

FM Huang*, and YC Chang (School of Dentistry, Chung Shan Medical University, Taichung, Taiwan)

Introduction

Root canal sealers are frequently placed in direct contact with living tissues. Therefore, root canal sealers should have good biocompatibility. The purpose of this study was to determine the cytotoxicity of zinc oxide eugenol based Tubliseal (Kerr, Romulus, MI, USA), and Canals (Showa, Tokyo, Japan) on human bone marrow stem cells.

Table I. Composition of test materials according to manufacturers' descriptions

Material	Compositions	Source
Tubliseal	40% Zinc oxide, 2.75% barium sulfate, 25% Oleo resins, 7.5% thymol iodide, 22.75% eugenol, 2% modifiers	Kerr, Romulus, MI, USA
Canals	Powder: Zinc oxide, barium sulfate, bismuth bibcarbonate, rosin. Liquid: olive oil, eugenol.	Showa, Tokyo, Japan

Methods

Freshly mixed sealer were filled in glass rings (4 mm height and 10 mm in diameter) and eluted in 10 ml of culture medium for 1 day. Subsequently, various dilutions of these extraction media were prepared for this study.

Cell cultures

Human bone marrow stem cells (human mesenchymal stem cell-bone marrow, catalog number: 7500)

U2OS cells (American Tissue Type Collection HTB 96)

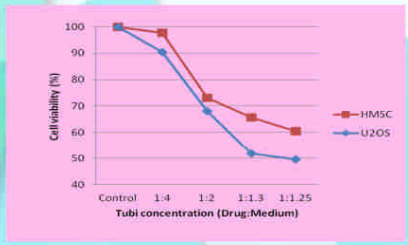
Cytotoxicity assay--- almar blue dye assay

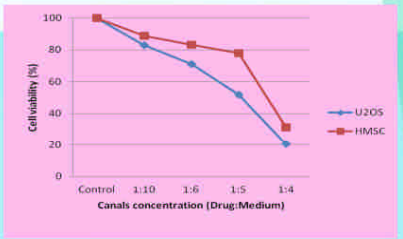
The colorimetric determination was done at 570 nm and 600 nm on a plate reader (CytoFluor™ 4500, Millipore, Bedford, USA). The percent inhibition of mitochondria activity in response to a test sealer as compared to untreated cells was calculated by the formula:

$$\frac{100 - (OD_{570} - OD_{600}) \text{ of test sealer dilution}}{(OD_{570} - OD_{600}) \text{ of untreated control}} \times 100$$

Results

The results showed that elutes from root canal sealers were cytotoxic to human bone marrow stem cells and U2OS cells in a dose-dependent manner ($p<0.05$). The rank orders with respect to cytotoxicity were found to be as follows: Canals > Tubli-Seal.





Conclusions

This *in vitro* study two kinds zinc oxide eugenol based root canal sealers on human bone marrow stem cells and U2OS cells, caused moderate or severe toxic reactions.

國科會補助計畫衍生研發成果推廣資料表

日期:2012/11/14

國科會補助計畫	計畫名稱: 研究根管充填劑對造牙骨質細胞的影響
	計畫主持人: 黃富美
	計畫編號: 100-2314-B-040-006- 學門領域: 牙醫學

無研發成果推廣資料

100 年度專題研究計畫研究成果彙整表

計畫主持人：黃富美	計畫編號：100-2314-B-040-006-
計畫名稱：研究根管充填劑對造牙骨質細胞的影響	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	1	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	無

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估 ■達成目標 □未達成目標（請說明，以 100 字為限） □實驗失敗 □因故實驗中斷 □其他原因 說明：
2. 研究成果在學術期刊發表或申請專利等情形： 論文：□已發表 □未發表之文稿 ■撰寫中 □無 專利：□已獲得 □申請中 ■無 技轉：□已技轉 □洽談中 ■無 其他：(以 100 字為限)
3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)