行政院國家科學委員會專題研究計畫　成果報告

牙髓斷髓藥物作用於牙髓細胞之反應研究

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中、英文摘要及關鍵詞(keywords)。

中文摘要

臨床上有不同之材料應用於牙髓斷髓術之治療。本研究之目的乃比較不同之配方下，這些材料對於 L929 細胞株之毒性反應。 研究材料乃採用下面幾組配方：1. Zinc oxide powder, eugenol 和 formocresol (FC). 2. Zinc oxide powder, eugenol 和 glutaraldehyde(Glu). 3. Zinc oxide powder, eugenol 和 ferric sulfate (FeS). 4. Calcium hydroxide, distilled water 和 formocresol. 5. Calcium hydroxide ($Ca(OH)_2$), distilled water 和 glutaraldehyde. 6. Calcium hydroxide, distilled water 和 ferric sulfate.。所有材料調製完成後浸泡於細胞培養液中，並將之置備濃度為 10, 20, 40 和 80 μl/ml 之溶液，細胞粒線體呈色反應(MTT assay)用於偵測材料對於L929細胞株之存活率。結果以one way ANOVA 統計分析比較其存活率，事後分析以 Student-Newman-Keul's test 比較。結果顯示六種配方之存活率因所配置之濃度高低有不同之大小，濃度高時細胞之存貨率下降($p<0.05$)。較高之細胞存活率出現於第 3,5,和 6 組。結語：臨床上使用此材料十可以考慮使用下面之組合 Zinc oxide powder, eugenol 和 ferric sulfate; Calcium hydroxide, distilled water 和 glutaraldehyde; 或 Calcium hydroxide, distilled water 和 ferric sulfate。近一步研究則可以於日後進行臨床之研究比較。

英文摘要

Abstract

A number of pulpotomy dressing materials have been applied clinically with various rates of success. The purpose of the present study was to evaluate the toxicity of different medicaments on treated L929 cells. The puplotomy preparations were grouped as follows: 1. Zinc oxide powder, eugenol and formocresol (FC). 2. Zinc oxide powder, eugenol and glutaraldehyde(Glu). 3. Zinc oxide powder, eugenol and ferric sulfate (FeS). 4. Calcium hydroxide, distilled water and formocresol. 5. Calcium hydroxide ($Ca(OH)_2$), distilled water and glutaraldehyde. 6. Calcium hydroxide, distilled water and ferric sulfate. All mixed materials were dissolved in medium and diluted to 10, 20, 40 and 80 μl/ml concentrations. A cell colorimetric assay (MTT) was used to detect the viability of L929 cells. Results were compared using one way analysis of variance (ANOVA). Differences in treatment means were analyzed using Student-Newman-Keul's test and were considered significant at $p < 0.05$. The survival rate of treated L929 cell showed statistical differences as the concentrations of the pulpotomy materials increased ($p<0.05$). The highest survival rates were found in groups 3, 5 and 6. Conclusions: It is recommended that low toxicity formulas such as Zinc oxide powder, eugenol and ferric sulfate; Calcium hydroxide, distilled water and glutaraldehyde; or Calcium hydroxide, distilled water and ferric sulfate be used clinically as pulpotomy dressing materials. Further research with randomized clinical trials is needed to verify these clinical success rates.

Key words: pulpotomy, formocresol, glutaraldehyde, ferric sulphate

Introduction

In extensive dental caries management, a tooth is sometimes treated with a pulpotomy. The goals of pulpotomy intervention can be classified as devitalization [formocresol (FC), glutaraldehyde (Glu), electrocoagulation], preservation [ferric sulphate (FeS), calcium hydroxide ($Ca(OH)_2$), mineral trioxide aggregate (MTA), lasers)] and remineralization (indirect pulp therapy, bone morphogenic proteins, collagen) of the dental pulp in a primary molar with extensive caries (1). Many pulpotomy materials have been clinically applied. There is little data on their toxicity.

Buckley's formocresol was first introduced as a pulp medicament in 1904 (2), and since 1930 (3), it has been the treatment of choice for primary molar vital pulpotomies. A one-fifth dilution of formocresol was as effective as a full strength solution in terms of its initial cytotoxicity on fibroblasts (4,5). Formaldehyde is an ingredient in Buckley's formocresol solution. In June 2004, the International Agency for Research Cancer (IARC) classified formaldehyde as carcinogenic to humans and the dental profession then needed to look for viable alternatives to formocresol (6). Glutaraldehyde was proposed as a new pulp tissue fixative by -Gravenmade in 1975 (7) and has been reported to be a better tissue fixative than formocresol (8). But its systemic distribution from pulpotomy sites, cytotoxicity (9) and mutagenicity (10) have been reported to be similar to formocresol. It has been used clinically as a replacement for formocresol.

Ferric sulphate (15·5%) has been investigated widely and has been used in animal and human studies as a haemostatic agent in pulpotomy procedures. On

contact with blood, a ferric ion protein complex is formed, and the membrane of this complex seals the cut vessels mechanically, producing haemostasis. The agglutinated protein complex forms plugs which occlude the capillary orifices, preventing blood clot formation (11).

Calcium hydroxide has been proposed as an alternative to formocresol for pulpotomies in primary teeth (12). Because fibrous layer and vital pulp tissue are found beyond the calcific bridge (13), calcium hydroxide can be used for either preservation and/or intervention. Thus calcium hydroxide is used in pulpotomies as a base material mixed with formocresol, glutaraldehyde or ferric sulphate.

Because of IARC concerns about formaldehyde carcinogenicity, different medicaments have been selected and applied in pulpotomies. However, cell biocompatibility reports on these materials are lacking, and clinicians are still confused on the best choice of pulpotomy medicaments. Published results on pulpotomy medicaments, included basic and clinical studies, are unclear in material selection. The purpose of the present study was to evaluate cell toxicity from different pulpotomy materials. It is hoped that this study can provide the clinician with further selection criteria for pulpotomy procedures.

MATERIAL AND METHODS

Material and sample preparation

Six different puplotomy materials were prepared and grouped as follows: 1. Zinc oxide powder 6 g : Eugenol 1 ml: Formocresol 1ml. 2. Zinc oxide powder 6 g : Eugenol 1 ml: Glutaldehyde 1 ml. 3. Zinc oxide powder 6 g: Eugenol 1 ml: Ferric sulfate 1 ml 4. Calcium hydroxide 6 g : distilled water 1 ml: Formocresol 1 ml. 5.

Calcium hydroxide 6 g : distilled water 1ml : Glutaldehyde 1 ml. 6. Calcium hydroxide 6 g: distilled water 1 ml: Ferric sulfate 1 ml.

Samples were prepared as follows: freshly mixed materials were placed in glass rings (2 mm in height, 6 mm in diameter) and allowed to set for 24 h at 37 ℃ in a humidified chamber. In the experimental group, five samples of each pulpotomy material were then eluted in 10 ml of cell culture medium at 37 ℃, in air and 5% CO_2 for 24 hours. After that, the materials were centrifuged at 10000rpm for 10 minutes. The supernatant were used to prepare different concentrations of the test materials. The concentrations of the test materials were diluted by adding culture medium to final concentrations of 10, 20, 40 and 80 μl/ml. The pure culture medium without any experimental material served as the control group.

Cell viability test by MTT((3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide colorimetric) assay

The assay procedure was the same as described in our previous study (14). The procedure was as follows: a mouse cell fibroblast cell line (L929) was routinely cultivated in DMEM medium (Sigma Chemical, St. Louis, Mo, USA) supplemented with 5% fetal bovine serum (Sigma Chemical, St. Louis, Mo, USA) at 37 °C in air and 5% CO_2. Single-cell suspensions of L929 cells were obtained from monolayer cell cultures close to confluency after trypsinization. Cell numbers were determined by hemocytometric counting, and 10^4 cells/well were seeded into 96-well plates. Cells were then incubated for 24 h in a humidified atmosphere of air and 5% CO_2 at 37 °C. Cell cultures were exposed to 10, 20, 40 and 80μl/m concentrations of the experimental materials. Exposure of cell cultures was stopped by discarding the exposure medium after 24 h. Viable cells in both treated and untreated cell cultures

were stained with formazan dye MTT (1 mg/ml) (Sigma Chemical, St. Louis, Mo, USA) dissolved in a 200 μl culture medium. After 3 h at 37 °C, the MTT solution was discarded and formazan crystals were solubilized with 200 μl of DMSO. All experiments were performed in triplicate. Optical densities were measured at 570 nm in a multi-well spectrophotometer (Hitachi, Tokyo, Japan). The survival rate was calculated as survival % = absorbance of the treated sample / absorbance of the medium x 100%. Results were compared using one way analysis of variance (ANOVA). Differences in treatment means were analyzed using Student-Newman-Keul's test and were considered significant at $p < 0.05$.

Results

The morphology of L929 cells treated with different materials was observed under a microscope at a magnification of 100X (Figure 1). The control group showed normal L929 growth under routine culture (Fig. 1a). The experimental groups which contained formacresol, such as groups 1 and 4, had decreased L929 cell numbers (Fig. 1 b and 1c). Group 2 also had reduced L929 cell numbers (Fig. 1d). The cell growth in groups 3, 5 and 6 was as good as that in the control group (Fig. 1e, 1f and 1g).

The L929 survival rates after treatment with various pulpotomy materials are shown in the table and in Figures 2-7. The survival rates of all test groups showed statistically significant differences as concentrations changed ($p<0.05$). In group 1, at concentrations above 10μl/ml, the L929 survival rates were below 20% (Fig. 2). In group 2, the L929 survival rates showed dose dependent decreases ($p<0.05$) (Fig.3). In group 4, the L929 cell survival rates were all below 20% at concentrations above 10μl/ml (Fig. 4). In groups 5 and 6, the L929 cell survival rates decreased as the

concentrations increased ($p<0.05$) (Fig. 5,6). In group 3, the L929 cell survival rate was severely decreased at a concentration of 80μl/ml (Fig.7).

Discussion

The present results showed low L929 cell survival rates for materials mixed with formocresol (Fig. 2 and 4). This demonstrates that formocresol is toxic to L929 cells. Previous studies showed different results in experiments with formocresol or formaldehyde (15-18). Small amounts of labeled formaldehyde were detected in the liver, kidney, lung and skeletal muscle of dogs after pulpotomy (15). However, another assessment of the systemic distribution and toxicity of formaldehyde following pulpotomy claimed no toxic effects on the liver and kidneys.(16) Similarily, opposite results were found in two studies of allergic effects (17,18). In a study of embryotoxic and teratogenic effects on chick embryos, formocresol showed mutagnic and carcinogenic effects (19). The results of in vitro and animal studies (in vivo) sometimes do not agree. It is proposed that in vitro tests involve direct contact with the cell, but in animal study chemicals are probably diluted by tissue fluid. In addition, some body organs can detoxify these chemicals, thereby reducing damage. Thus the results of cell culture study and animal study can not be compared. Our in vitro study demonstrated that small amounts of formocresol can cause cell death. Clinicians need to be aware of this when they choose materials for a pulpotomy.

Many base materials have been applied in pulpotomy studies, including calcium hydroxide and zinc oxide power. One clinical report on calcium hydroxide dressings and zinc oxide eugenol dressings with glutaraldehyde showed a 73.6% success rate after 12 months follow-up (20). However, in the present study, the two groups with glutaraldehyde showed different survival results. In Figure 3, the survival rate

decreased to 50% for the 10μl/ml concentrations in zinc oxide eugenol mixed with glutaraldehyde. But in Figure 5 showing calcium hydroxide mixed with glutaraldehyde, the L929 cell survival rate were shown high survival rate except in the high concentrations 80μl/ml which showed low survival rate. The present in vitro survival result was different from the above mentioned clinical success rate.

It is reported that glutaraldehyde is distributed systemically from the pulpotomy site, and its cytotoxicity and mutagenicity have been reported to be similar to formocresol (21-23). Calcium hydroxide has favourable antibacterial effects, is easily resorbed and causes no foreign body reaction. Zinc oxide eugenol paste dressing has been the material of choice for pulpotomies recently, but concerns have been expressed regarding its rate of resorption. Therefore, calcium hydroxide with glutaraldehyde would be better than zinc oxide eugenol with gluaraldehyde. However one clinical assay showed a high success rate (92.9%) was zinc oxide eugenol mixed with glutaraldehyde (20). Therefore, zinc oxide eugenol mixed with glutaraldehyde can be a good pulpotomy material.

Ferric sulphate (15.5%) has been investigated widely and has been used as a haemostatic agent in pulpotomy procedures in human and animal studies. It is used to improve the efficacy of calcium hydroxide. Failure of calcium hydroxide in one study was attributed to persistent extrapulpal blood clots (24). Ferric sulphate and formocresol have produced equivalent successful clinical and radiographic outcomes (25). Thus, in the present study we selected ferric sulphate mixed with calcium hydroxide and zinc oxide eugenol as test materials. The present study showed high or equivalent survival rates for ferric sulphate mixed with either calcium hydroxide or zinc oxide eugenol for all concentrations (Fig 6 and 7, Table 1). The only

exception was for the 80μl/m concentration of zinc oxide eugenol mixed ferric sulphate, which showed a severely decreased survival rate (10.68%) (Fig 7).

It is reported that ferric sulphate appears to be as effective in vital pulpotomies as formocresol, and there is no evidence to date to suggest any adverse effects of this medicament (25). From the present study, the authors propose using ferric sulphate mixed with either calcium hydroxide or zinc oxide eugenol as the treatment of choice for vital pulpotomties.

Conclusions

The results showed high survival rates for a combination of calcium hydroxide or zinc oxide eugenol mixed with ferric sulphate and for calcium hydroxide mixed with glutaraldehyde. These are suggested to be the best choices in pulpotomy medicament. Further research with long term randomized clinical trials is required to evaluate the success rate.

Acknowledge:

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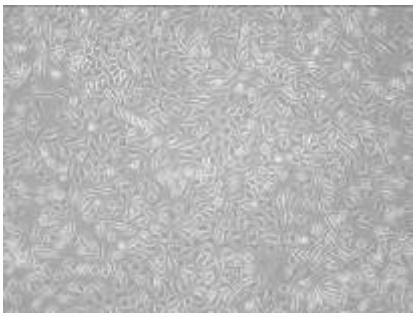
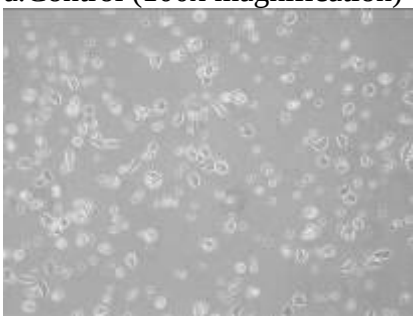
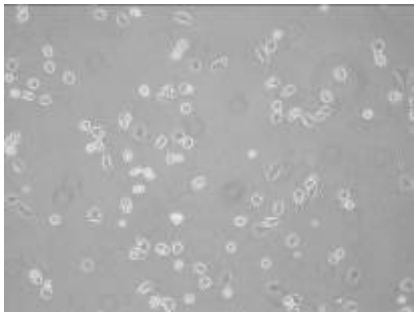


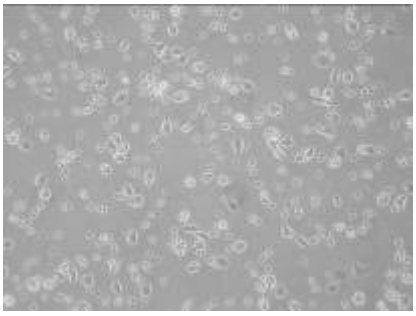
a.Control (100x magnification)



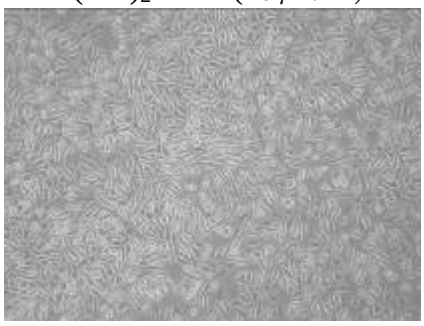
b. ZOE + FC (20 μ l/ml)



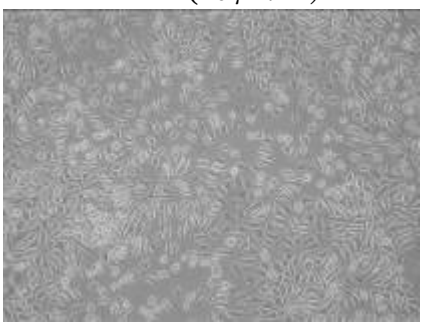
c.$Ca(OH)_2$ + FC (20 μ l/ml)



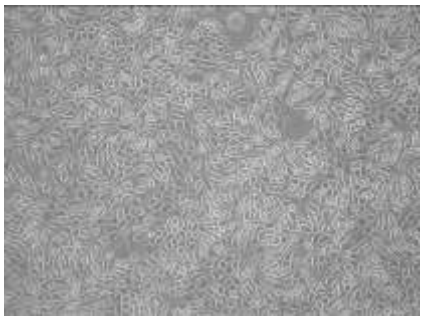
d. ZOE + Glu (20 μ l/ml)



e. $Ca(OH)_2$ + Glu (20 μ l/ml)



f. ZOE + FeS (20 μ l/ml)



g. $Ca(OH)_2$ + FeS (20 μ l/ml)

Figure 1. The morphology of L929 cell treated with different formula pulpotomy materials. The magnificationwas 100x under microscope observation. a. control group. b. ZOE + FC (20 μ l/ml). c.$Ca(OH)_2$ + FC (20 μ l/ml). d. ZOE + Glu (20 μ l/ml).e. $Ca(OH)_2$ + Glu (20 μ l/ml. f. ZOE + FeS (20 μ l/ml). g. $Ca(OH)_2$ + FeS (20 μ l/ml).

Figure 2. L929 cell survival rate for zinc oxide eugenol mixed with formocresol.

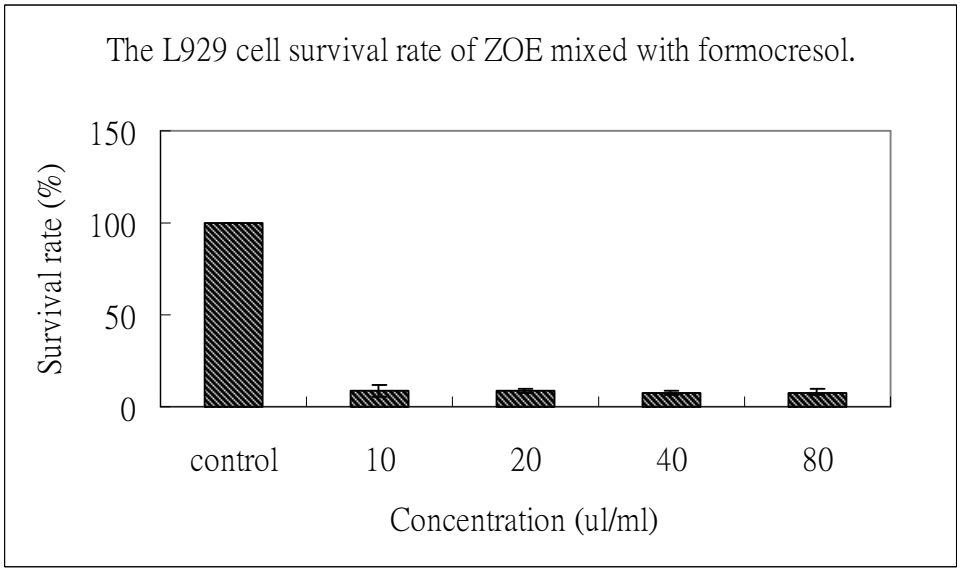


Figure 3. L929 cell survival rate for zinc oxide eugenol mixed with glutaraldehyde.

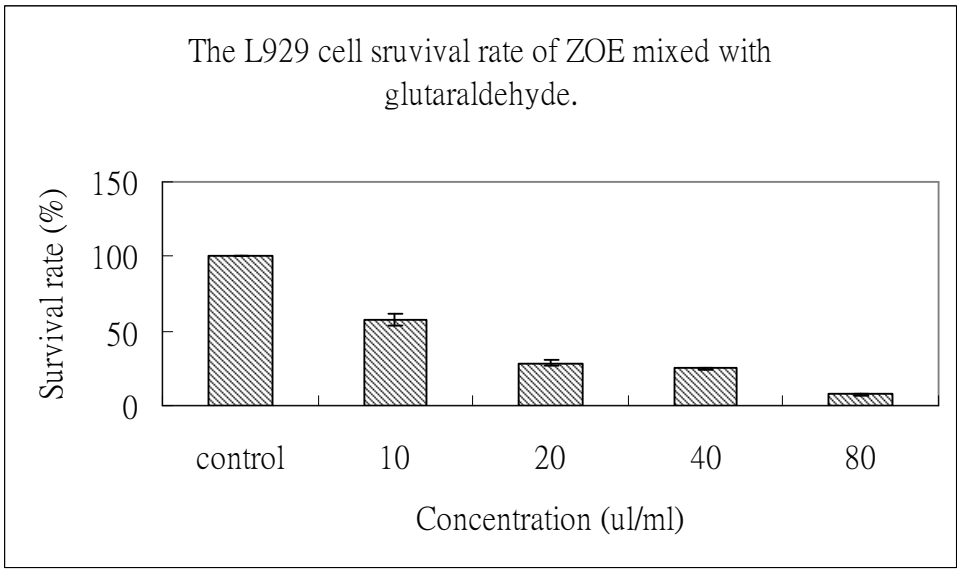


Figure 4. L929 cell survival rate for calcium hydroxide mixed with formocresol

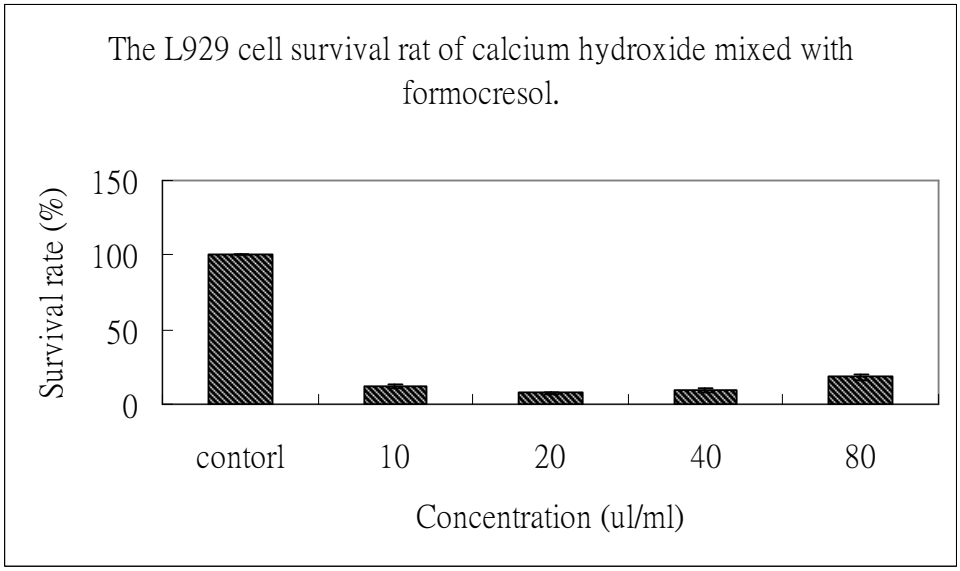


Figure 5. L929 cell survival rate for calcium hydroxide mixed with glutaraldehyde.

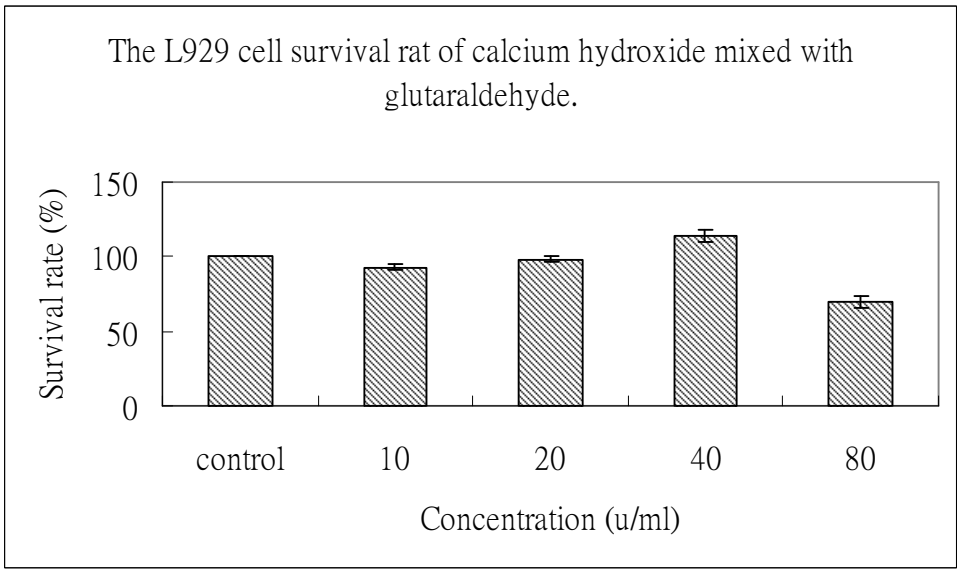


Figure 6. L929 cell survival rate for calcium hydroxide mixed with ferric sulphate.

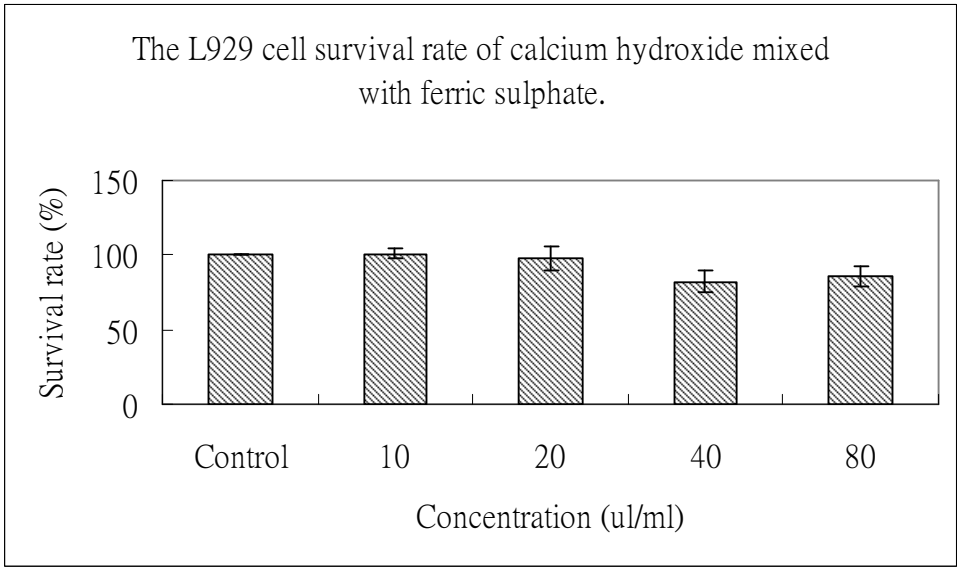


Figure 7. L929 cell survival rate for zinc oxide eugenol mixed with ferric sulphate.

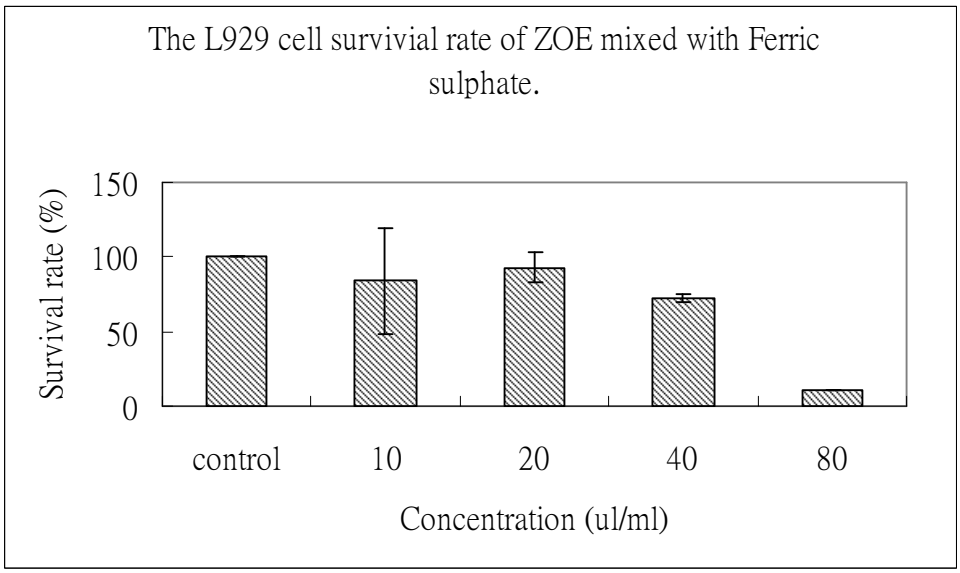


Table I. L929 cell survival rates after treatment with different pulpotomy materials. Aberration: ZOE: zinc oxide eugenol, FC: formocresol, Glu: glutaraldehyde, FeS: ferric sulfate.

		control	10	20	40	80	P<0.05
ZOE+FC	Mean	100	8.918[a]	8.623[a]	7.63[b]	7.997[b]	Yes
	SD	0	3.294	1.222	1.348	1.298	
ZOE+Glu	Mean	100	57.38	28.52	25.15	7.65	Yes
	SD	0	4.192	1.721	0.7876	0.3397	
ZOE+FeS	Mean	100[a]	84.04[b]	92.68[a]	72.5[b]	10.68	Yes
	SD	0	35.81	10.27	2.381	0.6246	
$Ca(OH)_2$+FC	Mean	100	12.54	7.778[a]	9.082[a]	18.35	Yes
	SD	0	1.339	0.8984	0.9753	1.78	
$Ca(OH)_2$+Glu	Mean	100[a]	92.46	98.29[a]	113.8	69.38	Yes
	SD	0	1.989	2.369	4.465	4.189	
$Ca(OH)_2$+FeS	Mean	100[a]	101.1[a]	97.42[a]	82.24[b]	85.41[b]	Yes
	SD	0	3.819	8.226	7.541	6.762	

One-way ANOVA was used to test for significant differences among different concentrations. The Student-Newman-Keul (SNK) multiple comparison of means procedure at $P < 0.05$ was used to show differences. SNK ranking with the same letters do not significantly differ at $P = 0.05$.

(六)計畫成果自評部份，

請就研究內容與原計畫相符程度：內容與計劃大致上相符合

達成預期目標情況：有達到第一年計畫要求

研究成果之學術或應用價值：對不同配方之材料，提供選擇性參考，

已著手投稿期刊

綜合評估:

本研究針對不同配藥方式進行細胞學之初步研究，了解不同配方之細胞毒性，已有初步之結果，日後希望能進行動物試驗，了解於活體內之反應，之後再進行臨床試驗，以提供治療之參考。