科技部補助專題研究計畫成果報告
期末報告

光動力療法提升牙髓幹細胞成骨及脂肪分化潛能之分子機轉探討及應用

計 畫 類 別 ：個別型計畫
計 畫 編 號 ：NSC 102-2314-B-040-012-
執 行 期 間 ：102年08月01日至103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：余權航
共 同 主 持 人：余承佳

處 理 方 式 ：
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中 華 民 國 103年10月29日

中 文 摘 要 ： 近年許多研究陸續證實牙齒所發現的各種幹細胞均極具潛力。牙髓幹細胞生長快速且透過適當引導會表現成骨、脂肪等分化能力，因此引起許多研究學者的興趣。而光動力療法主要是由兩種無毒性的要素所構成，其一是特殊波長的光，另一種則是光感物質。5-氨基酮戊酸（5-aminolevulinic acid, ALA）為新研發的第二代光感藥物（photosensitizer）。本研究計畫發現 5-氨基酮戊酸光動力療法可提昇初代培養牙髓細胞增生力、細胞群落能力、Stro-1 、CD146 表現。在骨分化誘導下，光動力療法也可促使牙髓幹細胞骨分化能力增強及骨分化標記(ALP、DSPP、OCN、BSP)表現。本研究強調基礎研究與臨床應用接軌的重要性，期待能證實光動力治療法有潛力提升牙髓幹細胞分化潛能， 提供未來口腔再生醫學之應用。

中文關鍵詞： 光動力療法； 牙髓幹細胞； 再生醫學

英 文 摘 要 ：

英文關鍵詞：

科技部補助專題研究計畫成果報告

（□期中進度報告/■期末報告）

光動力療法提升牙髓幹細胞成骨及脂肪分化潛能之分子機轉探討及應用

計畫類別：■個別型計畫　□整合型計畫
計畫編號：MOST 102-2314-B-040-012
執行期間：　102 年 8 月 1　日至 103　年 7　月 31　日

執行機構及系所：中山醫學大學口腔醫學院牙醫系(所)

計畫主持人：余權航
共同主持人：周明勇、余承佳
計畫參與人員：周筱鈞、陳心茹

本計畫除繳交成果報告外，另含下列出國報告，共 ___ 份：
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□出席國際學術會議心得報告

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中　華　民　國　103　年　10　月　29　日

一、中文摘要

近年許多研究陸續證實牙齒所發現的各種幹細胞均極具潛力。牙髓幹細胞生長快速且透過適當引導會表現成骨、脂肪等分化能力，因此引起許多研究學者的興趣。而光動力療法主要是由兩種無毒性的要素所構成，其一是特殊波長的光，另一種則是光感物質。5-氨基酮戊酸（5-aminolevulinic acid, ALA）為新研發的第二代光感藥物（photosensitizer）。本研究計畫發現5-氨基酮戊酸光動力療法可提昇初代培養牙髓細胞增生力、細胞群落能力、Stro-1 、CD146表現。在骨分化誘導下，光動力療法也可促使牙髓幹細胞骨分化能力增強及骨分化標記(ALP、DSPP、OCN、BSP)表現。本研究強調基礎研究與臨床應用接軌的重要性，期待能證實光動力治療法有潛力提升牙髓幹細胞分化潛能， 提供未來口腔再生醫學之應用。

二、Abstract

Stem cells populations in teeth have the ability of self-renewal and multipotent differentiation. Dental pulp stem cells (DPSCs) are characterized by its ability of self-renewal, as in colony forming capacity or its high proliferation rate, and multipotent differentiation. Photodynamic therapy (PDT) involves two individually non-toxic components, light and photosensitizer. In the study, we first showed PDT enhanced Stro-1 and CD146 expression in dental pulp cells (DPCs). PDT treatment also promoted osteogenic induction differentiation capability, and expression of osteogenic induction differentiation markers (ALP、DSPP、OCN、BSP) in DPCs. Taken together, through the proposed studies, the PDT in enhancing self-renwal and differentiation in DPCs will be thoroughly studied and the results will certainly shed new lon regenerative medicine.

三、 前言

Dental pulp stem cells (DPSCs) are a recently developed technology that holds promise for stem cell biology and regenerative medicine. DPSCs are well known to be multi-potent cells capable of differentiation along multiple lineages (1). DPSCs have the remarkable potential for multi-lineage differentiation capacity including osteoblast (2), cartilage (3), adipocyte (4), muscle (5), hepatocyte (6), and neurons (7). Therefore, an improved comprehension of the cellular and molecular mechanisms which modulate self-renewal and differentiation properties of DPSCs could be pursued to bring forth future progress in regenerative medicine.

Photodynamic therapy (PDT) involves two individually non-toxic components, light and photosensitizer (8). PDT is a new treatment and holds considerable promise for many diseases (9). Nevertheless, PDT mediated phenotypes and molecular mechanisms in regulating DPSCs are still unclear.

四、 研究方法

Cultivation of primary cells from dental pulps tissues

Dental pulp tissues were freshly derived from a caries-free intact premolar that was extracted for orthodontic treatment purpose. All of the clinical samples in this study were collected after obtaining written informed consent and this study was reviewed and approved by the Institutional Review Board in Chung Shan Medical University Hospital (CSMUH No: CSI13108). Dental pulp tissue was immersed in phosphate buffered saline (Caisson, North Logan, UT) solution and enzymatically dissociated into single cells. These cells were grown in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin-G and 100 μg/ml streptomycin. (Grand Island, NY).

Identification of cell phenotypic markers by FACS

Dental pulp cells were used for phenotypic marker identification by flow cytometry. 10^5 cells were resuspended in 100 μl PBS and incubated with primary STRO-1 and CD146 antibodies at 4^0C for 1 h with 1: 100 dilutions. The labeled cells were suspended in 100 μl PBS with 1μl goat anti-mouse IgG conjugated with FITC (Chemicon, AP124F) at 4^0C for 1 h, then examined with a FACSCalibur apparatus (Becton Dickinson).

Quantitative real-time reverse-transcriptase (RT)-PCR

Briefly, total RNA (1 μg) of each sample was reverse-transcribed in a 20-μL reaction using 0.5 μg oligo(dT) and 200 U Superscript II RT (Invitrogen, Carlsbad, CA). The amplification was carried out in a total volume of 20 μL containing 0.5 μM of each primer, 4 mM MgCl2, 2 Ml LightCyclerTM–FastStart DNA Master SYBR green I (Roche Molecular Systems, 15 Alameda, CA) and 2 μL of 1:10 diluted cDNA. PCR reactions were prepared in duplicate and heated to 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 10 seconds, annealing at 55°C for 5 seconds, and extension at 72°C for 20 seconds. Standard curves (cycle threshold values versus template concentration) were prepared for each target gene and for the endogenous

reference (GAPDH) in each sample. Quantification of unknown samples was performed using LightCycler Relative Quantification Software version 3.3 (Roche Molecular Systems, Alameda, CA).

Assays for cell proliferation

An MTT assay kit (Sigma-Aldrich) was used to analyze the cell proliferation. Specifically, 1 x 10^3 cells were seeded in each well of a 24-well plate, and then 10 μL of MTT solution was added to the cells which were then incubated at 37°C for 3 hours. The supernatant was removed, and 200 μL of DMSO were added directly to the cells. The MTT color reaction was analyzed using a microplate reader set at A560 nm.

In vitro osteogenic differentiation

Cells were cultured in DMEM-LG (Invitrogen) supplemented with 15% FBS, 50 μg/mL ascorbate-2-phosphate, 10 nmol/L dexamethasone, and 10 mmol/L β-glycerophosphate (Sigma, St. Louis, MO) for 2 weeks. At the end of osteogenic induction, cells were washed twice with PBS, fixed for 10 min at room temperature with 3.7% paraformaldehyde, and stained with Alizarin red to assess osteogenic differentiation.

Statistical analysis

The statistical package of Social Sciences software (version 13.0) (SPSS, Inc., Chicago, IL) was used for statistical analysis. Student's t test was used to determine the statistical significance of differences between experimental groups; p values less than 0.05 were considered statistically significant.

五、 研究目的

The overall study will illustrate the molecular mechanisms of PDT in maintaining self-renewal and promoting differentiation properties and evaluate the possibility of the PDT for a potential regenerative medicine.

六、研究成果

Fig.1. PDT enhanced proliferation rate in and colony formation ability in DPC cells.

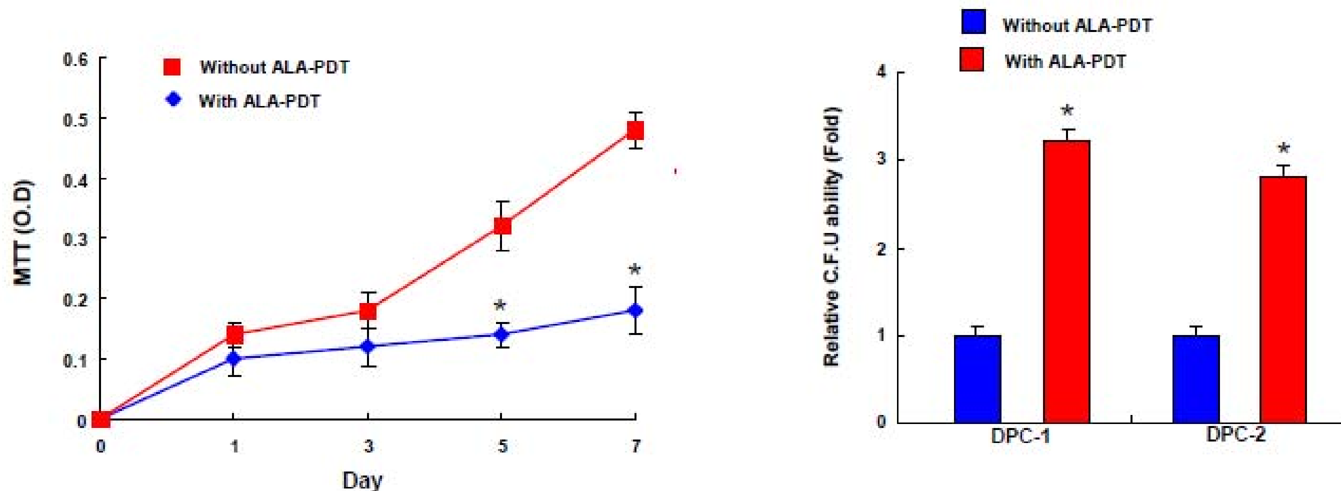


Fig.2. PDT enhanced STRO-1 and CD146 expression in DPC cells.

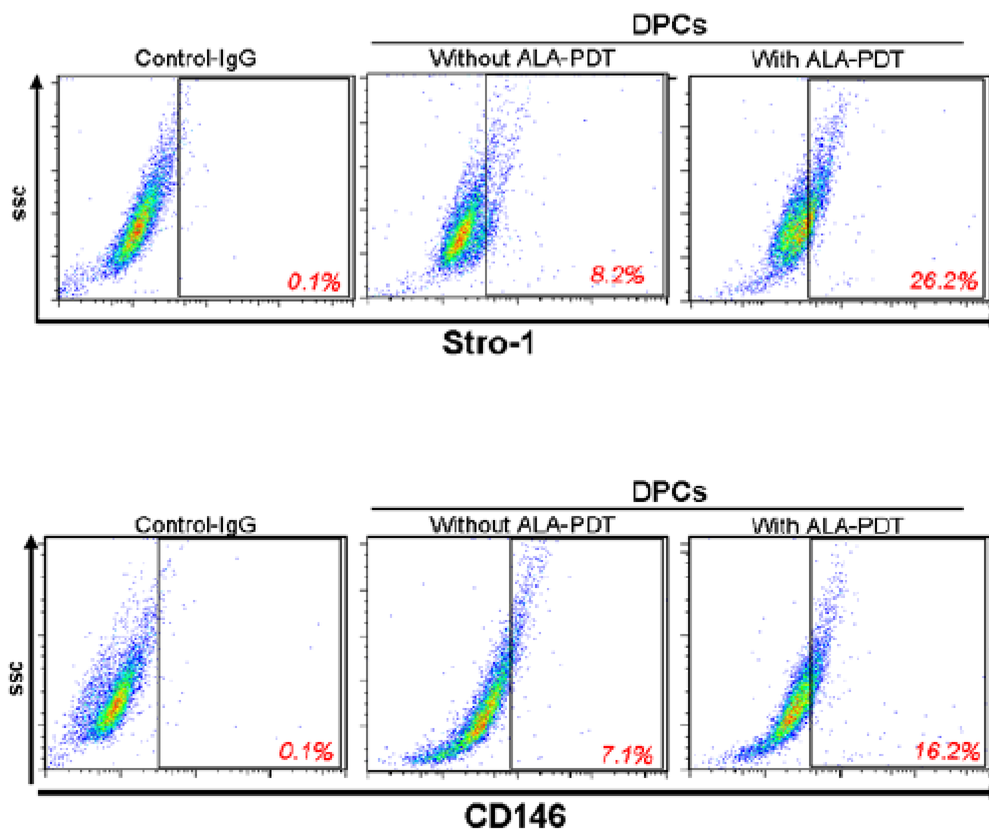


Fig.3. PDT enhanced osteogenic properties in DPC cells

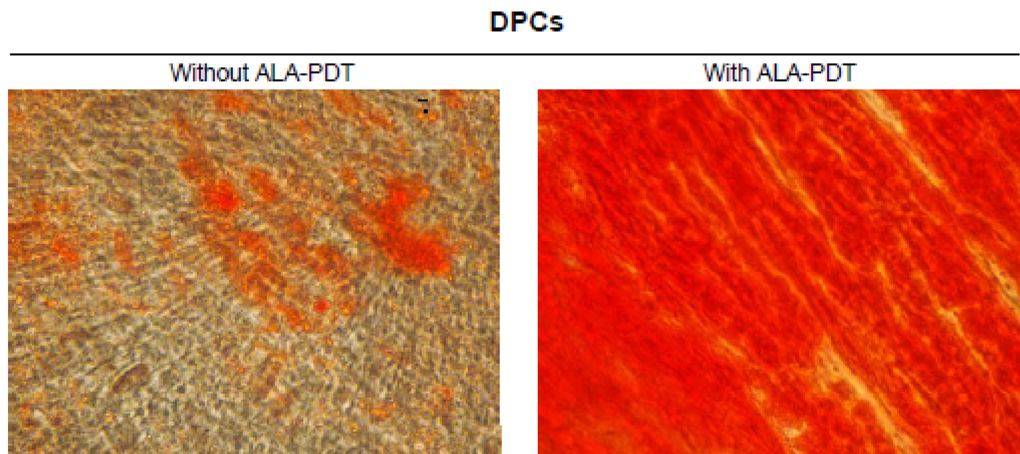
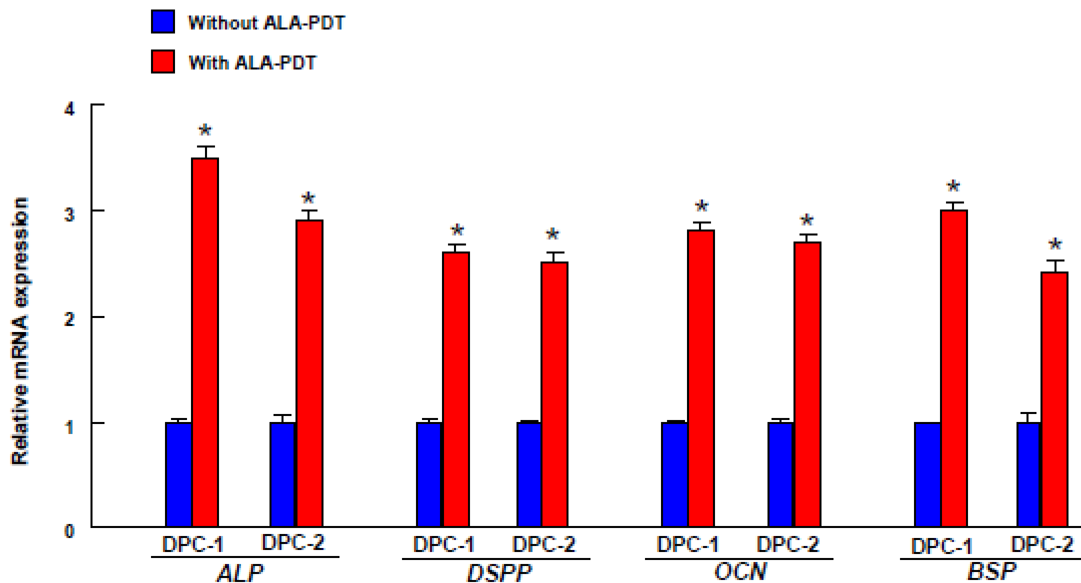


Fig.4. PDT enhanced osteogenic markers (ALP, DSPP, BSP, and OCN) in DPCs



七、參考文獻

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cells changes during cell passaging. BMC cell biology. 2010;11:32.

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科技部補助計畫衍生研發成果推廣資料表

日期:2014/10/28

<table>
<tr><td rowspan="3">科技部補助計畫</td><td>計畫名稱: 光動力療法提升牙髓幹細胞成骨及脂肪分化潛能之分子機轉探討及應用</td></tr>
<tr><td>計畫主持人: 余權航</td></tr>
<tr><td>計畫編號: 102-2314-B-040-012- 學門領域: 牙醫學</td></tr>
<tr><td colspan="2">無研發成果推廣資料</td></tr>
</table>

102年度專題研究計畫研究成果彙整表

計畫主持人：余權航			計畫編號：102-2314-B-040-012-			
計畫名稱：光動力療法提升牙髓幹細胞成骨及脂肪分化潛能之分子機轉探討及應用						
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	1	2	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%	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。)	部分相關研究已發表於 Int J Mol Sci. 2014 Oct 15；15(10):18623-39.部分資料持續整理後 將投稿於國際期刊

	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	

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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） 證實光動力治療法有潛力提升牙髓細胞分化潛能， 提供未來口腔再生醫學之應用。