行政院國家科學委員會專題研究計畫 期末報告

低能量雷射應用於牙周細胞之生物訊息反應與對實驗動物之牙齒矯正移動速率研究(第 3 年)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 99-2314-B-040-014-MY3
執 行 期 間 ：101 年 08 月 01 日至 102 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：高嘉澤
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計畫參與人員：碩士班研究生-兼任助理人員：許瑛祺
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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 102 年 10 月 15 日

中 文 摘 要 ： 矯正治療除了維持口腔健康外，咬合功能與穩定性為矯正醫師所期望的，如何迅速完成牙齒移動更是醫師與患者期盼的。過去許多研究也提出用藥物或是化學物質或是機械力等各種方式，嘗試找出最佳之工具或模式。低能量雷射為近年被提出之方式之一，本研究計畫以三年時間，第一年研究細胞之基本反應，細胞生物學上訊息之表現。第二年研究依第一年研究得到之雷射劑量參數照射後，比較牙周韌帶細胞株與骨細胞株之骨訊息因子，結果顯示 5 , 10 J 之雷射處理其細胞生長效果有明顯表現。因此第三年之研究目的則以前二年之參數值作用於兔子動物，觀察牙齒移動之速率與骨頭 remodeling 之變化。研究方法：以紐西蘭兔子於麻醉下，將彈簧裝置裝置餘下顎門牙區，以 25g 力量大小作用於牙齒，經過 3, 6 週，當中以 5 J 低能量雷射每週照射一次，期間分別紀錄牙齒之變化量，並於兔子犧牲後取牙齒周圍組織，以切片分析細胞之變化。結果顯示：牙齒之移動速度並未有明顯之改變，切片顯示 Collagen I 表現最顯著出現於移齒經矯正力作用後，以雷射處理過之組別上；VonKossa stain 顯示一樣之反應。結語：低能量雷射對於矯正下之牙齒移動可以有幫助牙齒之周圍組織生長與骨頭 remodeling。

中文關鍵詞： 低能量雷射、組織切片、動物試驗、骨生成

英 文 摘 要 ： The purpose of the orthodontic is function, health, esthetic and stability. How to achieve the faster orthodontic therapy time is the expectation of most orthodontists. Many researches and efforts are trying to reach the reducing treatment time. Such as using the chemical, medicine, mechanical method etc were reported. The low level dose laser (LLDL) is mentioned recently in orthodontic therapy. This is a three project, the first and second years project were done, and have a good basic cellular data and laser reference value. The third year project is to apply these reference data on animal study, to evaluate the LLDL effects on orthodontic tooth and investigate the rate and histology of underlying bone changes. The closed coil spring with 25 g force were applied on lower anterior teeth of rabbit. The LLDL diode laser with 5J and 10 J were treated on the underlying alveolar one a week and last for three and six weeks. The tooth movement distance and rate were evaluated. After scarified rabbits, the lower

jaw were sectioned and performed the histology analysis. The results showed the group with LLDL treated tooth, its moving distance did not show statistical difference. But the histology of slide with the collagen I stain and VonKossa stain showed the LLDL group has prominent ostoid and collagen I appearance. Conclusion: the LLDL treated on animal orthodontic tooth can enhance the bone proliferation and remodeling.

英文關鍵詞： low level laser, animal study, biopsy, remodeling

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

低能量雷射應用於牙周細胞之生物訊息反應與對實驗動物之牙齒矯正移動速率研究

The study of low energy laser on periodontal cells signal expression in vitro and evaluate low energy laser effects on orthodontic tooth movement rate in vivo

計畫類別：√個別型計畫　□整合型計畫

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計畫主持人：高嘉澤

共同主持人：黃翠賢

計畫參與人員：謝婉玉、謝明佑、許瑛祺

本計畫除繳交成果報告外，另含下列出國報告，共 _1__ 份：

□移地研究心得報告

√出席國際學術會議心得報告

□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

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中　華　民　國103　年 10　月 01　日

中、英文摘要及關鍵詞

中文摘要

關鍵詞:　低能量雷射、組織切片、動物試驗、骨生成

矯正治療除了維持口腔健康外，咬合功能與穩定性為矯正醫師所期望的，如何迅速完成牙齒移動更是醫師與患者期盼的。過去許多研究也提出用藥物或是化學物質或是機械力等各種方式，嘗試找出最佳之工具或模式。低能量雷射為近年被提出之方式之一，本研究計畫以三年時間，第一年研究細胞之基本反應，細胞生物學上訊息之表現。第二年研究依第一年研究得到之雷射劑量參數照射後，比較牙周韌帶細胞株與骨細胞株之骨訊息因子，結果顯示5 , 10 J之雷射處理其細胞生長效果有明顯表現。因此第三年之研究目的則以前二年之參數值作用於兔子動物，觀察牙齒移動之速率與骨頭remodeling 之變化。研究方法：以紐西蘭兔子於麻醉下，將彈簧裝置裝置餘下顎門牙區，以25g力量大小作用於牙齒，經過3, 6 週，當中以5 J低能量雷射每週照射一次，期間分別紀錄牙齒之變化量，並於兔子犧牲後取牙齒周圍組織，以切片分析細胞之變化。結果顯示：牙齒之移動速度並未有明顯之改變，切片顯示Collagen I表現最顯著出現於移齒經矯正力作用後，以雷射處理過之組別上；VonKossa stain顯示一樣之反應。結語：低能量雷射對於矯正下之牙齒移動可以有幫助牙齒之周圍組織生長與骨頭remodeling。

Abstract

Key words: low level laser, animal study, biopsy, remodeling

The purpose of the orthodontic is function, health, esthetic and stability. How to achieve the faster orthodontic therapy time is the expectation of most orthodontists. Many researches and efforts are trying to reach the reducing treatment time. Such as using the chemical, medicine, mechanical method etc were reported. The low level dose laser (LLDL) is mentioned recently in orthodontic therapy. This is a three project, the first and second years project were done, and have a good basic cellular data and laser reference value. The third year project is to apply these reference data on animal study, to evaluate the LLDL effects on orthodontic tooth and investigate the rate and histology of underlying bone changes. The closed coil spring with 25 g force were applied on lower anterior teeth of rabbit. The LLDL diode laser with 5J and 10 J were treated on the underlying alveolar one a week and last for three and six weeks. The tooth movement distance and rate were evaluated. After scarified rabbits, the lower jaw were sectioned and performed the histology analysis. The results showed the group with LLDL treated tooth, its moving distance did not show statistical difference. But the histology of slide with the collagen I stain and VonKossa stain showed the LLDL group has prominent ostoid and collagen I appearance. Conclusion: the LLDL treated on animal orthodontic tooth can enhance the bone proliferation and remodeling.

報告內容：

壹、前言

矯正治療技術與觀念之發展非常迅速，治療上除要求矯正裝置之品質精良外，更要求患者治療之時間縮短，因此為加速矯正治療牙齒移動速度，治療上有使用藥物注射[1]、電刺激[2]或是超音波之應用[3]等方法加速骨頭之變化。然而這些方法應用上有其缺點如藥物之處理為侵入性，電刺激會造成患者不適等。過去矯正應用雷射之範圍為減低牙齒移動之疼痛或做局部牙齦之切除與漂白為主。Cruz et al.於2004年提出低能量二極體雷射處理的矯正患者，其牙齒之移動速率較未經低階雷射處理者移動速率快。[4]但Cruz et al.於研究中所用之測試樣本少只有十一位矯正患者，且其結果發現並無解釋低能量二極體雷射處理造成牙齒移動速率較快速結果之機轉。

本研究室曾設計一模式探討對低能量雷射作用於發炎之牙齦纖維細胞，比較細胞發炎之癒合狀況。研究結果發現，二極體雷射以5 J/cm^2 以及10 J/cm^{2}射劑量刺激下會促進 L929細胞株的增生並且促進其貼附能力。RT-PCR 的結果發現二極體雷射以5 J/cm^2 以及10 J/cm^{2}照射劑量刺激已經發炎的細胞,會降低兩個發炎指標TNF-α以及IL-1的基因表現。Western blotting 結果也顯示 iNOS 蛋白表現在雷射照射後表現量下降，另一方面在相同的劑量照射後，也發現磷酸化ERK蛋白有增加的趨勢。基於此，回顧牙齒移動時，壓力側骨會有細胞發炎反應而張力側則出現細胞生長，此引發本研究計畫之動機，即由低能量二極體雷射處理後之牙周韌帶細胞或骨細胞其細胞生物學上究竟發生了何種變化；如果應用於動物試驗是否可以更清楚的比較出低能量二極體雷射處理後之牙齒移動速度差異。

二、生物刺激(biostimulatory effects)

生物刺激近年來應用較多的概念，有許多生物刺激之模式如：雷射(laser)生物刺激、磁式(magnetic) 生物刺激或autologous生物刺激等。生物刺激之效應包括促進傷口癒合[27,28]、纖維細胞增生[29]、膠元蛋白生成[30]、神經修復等功能[31]。本計劃預計以雷射作為刺激源。

雷射英文名是 Laser，即 Light Amplification by the Stimulated Emission of Radiation 的縮寫。雷射的發

展有很長的歷史，它的原理早在 1917 年已被著名的物理學家愛因斯坦發現，但要直到 1958 年激光才被首次成功製造。

雷射基本躍遷機制為一種受激輻射(Stimulate Emission)，即光子射入物質誘發電子從高能階躍遷到低能階，並釋放光子。

雷射介質可以分為三類:固體、液體以及氣體。不同的介質所發出的雷射光波長也不同。有以下三大特性 ：

1. 雷射是單色的，在整個產生的機制中，只會產生一種波長的光。這與普通的光不同，例如陽光和燈光都是由多種波長的光合成的，接近白光。
2. 雷射是相干的，所有光子都有相同的相，相同的偏振，它們疊加起來便產生很大的強度。而在日常生活中所見的光，它們的相和偏振是隨機的，相對於激光，這些光就弱得多了。
3. 雷射的光束很狹窄，並且十分集中，所以有很強的威力。相反，燈光分散向各個方向轉播，所以強度很低。

按波長分類，可分為紫外線雷射、可見光雷射及紅外線雷射等三種。牙科雷射主要介於500-10000奈米之間，以可見光與紅外線雷射居多。目前牙科雷射種類以活性介質不同來命名計有：CO2雷射、Nd-YAG釹雅克雷射雷射、Argon雷射、二極體雷射Diode Laser(GaAL As)、鉺雅克雷射(Erbium Yttrium-Aluminum-Garnet Laser)Ho-YAG雷射、鉺鉻YSGG雷射Er, Cr:YAGG和Er:YAG雷射。

以下就本研究欲採用之二極體雷射作一介紹：

半導體雷射是所有雷射機型當中體積最小的，它的優點是重量輕、耐用、消耗低而且壽命長。一般二極體雷射的壽命是105小時，是He-Ne雷射壽命的十倍。以壽命而，二極體雷射比較佔有優勢,比較適合長時間的操作。如果依照波長及操作功率來細分的話，二極體雷射可以再細分成數類:

(一)、390nm-550nm: 此波段的二極體雷射其發光波長分別為390-440nm與520nm左右,最大的應用是在超高密度的儲存系統例如HD-DVD光碟機或高解析度的印表機等。

（二)、635-670nm:5mW以下低功率的二極體雷射主要用於雷射指示器、條碼閱讀機、以及唯讀型光資訊存取系統，例如DVD-ROM，而操作功率100mW以上的二極體雷射則用於雷射印表機、固態雷射激勵源以及醫學上。

(三).750-950nm： 5mW以下的780nm二極體雷射是最早被商業上大量生產的二極體雷射，已經被廣泛用於CD-ROM、CD-game等商品。10mW-1W中級操作功率方面，30mW的780nm的二極體雷射可用於讀寫型存取系統,例如CD-R。500mW-1W的808nm二極體雷射常做為Nd:YAG雷射的激勵源,用於舞台秀。1W以上的高功率二極體雷射大多用於醫學上的治療、數位印刷。

三、低功率雷射臨床應用

低功率雷射（或稱之低能量雷射）為最常使用於牙科之雷射之一，其波長介於500 和 1100 nm，操作區產生約1–4 J/cm2熱量，輸出功率為10 and 90 mW之間。牙周軟組織手術例如牙齦切除術、繫帶切斷術、黏膜表皮病灶移除、黏膜牙齦手術以及藥物引發牙齦腫大的切除，皆可採用雷射[5-8]，可用的雷射包括二氧化碳雷射、釹雅克雷射、二極體雷射及鉺鉻YSGG雷射。

其優點如下

一、雷射切割後的癒合比用電刃來得快。二、可快速止血，且手術區域乾燥， 視野清楚。 三、可能降低因血液傳染的機會。 四、CO2 雷射可能藉由汽化微生物而消毒手術區, 這可歸因於它對潮濕組織的親和性。對於擔憂有菌血症發生可能的病患,雷射的使用可降低冒險性。五、因為對血管、淋巴管和神經纖維的封閉, 雷射可將術後腫脹及疼痛降到最低。六、雷射可加以調整對組織做切割、汽化、或擬結。

四、雷射對於生物體之反應

矯正治療時之牙齒，一方為壓力側即產生骨吸收現象，為一發炎破壞之現象，另一側為張力側，即骨頭生成，牙齒之反應須等骨頭生理反應吸收與沉積(remoldeling)後牙齒才可以移動。一般在組織中之傷口癒合反應通常包括四個時期，即Hemostasis stage, Inflammation stage，Proliferation stage和Remodeling stage。低功率之雷射會影響細胞之代謝過程，增進組織再生之功能，抗發炎之反應以及止痛。[5-8] 由於雷射強調對組織具有促進傷口癒合之功能，然而至目前研究結果，仍有分歧，因為不同之暴露時間，與功率對於纖維細胞可產生促進或是抑制性的變化。[8]

Loevschall & Arenholt-Bindslev 1994, 以low-power irradiation 處理結締組織細胞，結果發現可促進DNA合成。[9] Balboni et al. 1986 則提出會使細胞之Collagen增加；[10] Abergel et al. 1987, Skinner et al. 1996，則發現會使Procollagen 分泌增加，增加細胞之增值率(proliferation rate)；[11] Bednarska et al. 1998, Webb et al. 1998 則認為以雷射處理後會增加細胞之移動能力(cell migration)。[12] Neiburger 1995 提出雷射作用後可增加傷口癒合(Wound healing)；[13] Shimizu et al. 1995, Sakurai et al. 2000 [14] 研究更指出經雷射處理後，會使組織之發炎過程(inflammatory process)效應減小；[15] 另外Whitters et al. 1995 [16]，Renton-Harper & Midda 1992 [17]，對於牙髓之止痛或敏感性牙齒之改善也都於臨床上有報告出現。Kawasaki et al. 於老鼠之上顎牙齒作一牽引動作，並以低能量雷射照射牙齒觀察其牙齒移動速率，發現經照射後牙齒移動加速並可以使骨頭發生remodeling 現象。[18]

五、細胞組織學上變化

以二極體雷射 (Ga-Al-As)刺激人類牙髓細胞，觀察細胞之反應發現，MAPK/ERK蛋白有增加之現象，代表細胞有增生分化之效應。[19] 以低劑量雷射處理大鼠顱骨細胞(Calvarial cell)，結果發現藉由刺激Insulin like growth factor I 之出現可促進骨之生長。[20] 纖維細胞對不同波長之雷射效應也產生不同之結果，860nm 可刺激細胞增生，[21] 812nm可增加細胞之DNA合成，[22] 660nm可調控纖維母細胞之生長因子。[23] 632.8nm波長下更可刺激肌肉纖維母細胞之生長，[24] 並刺激分泌IL-1和IL-8，[25] 並增加細

胞之移動性(motility rate)。[26] 大鼠的內皮細胞和纖維母細胞都對低密度雷射照射的促進細胞分裂訊息有反應。不過，並不是每一種波長的光都有同樣的效果，而且近紅外光甚至可能會有抑制的效果。這種波長對於細胞增生的影響，以及內皮細胞和纖維母細胞的不同反應，或許可以解釋之前文獻當中，對於雷射刺激生物體內及生物體外細胞增生，所得到的結果各有不同。

由上可知，不論於臨床上之效果或是於實驗室做出的結論，大多數文獻均報導雷射對於組織生物體反應是有助益的，顯示低能雷射可提供臨床醫師對於組織的產生正向的功用，但是雷射的波長，使用的劑量，何謂適當的狀況下操作，促進癒合之生理機轉(physiological mechanism)，到目前為止仍無法有明確定論。

六、牙齒在矯正移動時之骨頭變化

依牙齒之矯正移動原理，當牙齒位於骨頭中，牙齒受力傾斜，於骨頭上，牙齒壓迫骨頭之一方稱之為壓力側，反稱為張力側。壓力側之骨頭會有破骨細胞出現，造成骨頭之吸收，張力側則有成骨細胞出現，形成新的骨頭，此稱之為機械性骨生理反應。

如前所述，目前之矯正治療，不論是醫師或是患者都希望療程能迅速完成，且過程中不會產生疼痛。因此治療上有使用特殊矯正托架，藥物注射、電刺激或是超音波刺激之應用等方法加速骨頭之變化。然而這些方法應用上有其缺點如藥物之處理為侵入性，且容易造成全身性反應，和電刺激會造成患者不適、害怕等問題。甚至也有利用作齒周皮質骨鑽洞術(Cortication technique, 即Accelerated osteogenic orthodontic觀念)，[24,25] 利用牙周翻瓣手術處理骨頭後，再拉牙齒，以加速牙齒之移動。然總觀這些方法都有其缺點，患者接受度不強。而 Cruz et al.於2004年提出低能量二極體雷射處理的矯正患者，發現其牙齒之移動速率較未經雷射處理者之移動速率快。[4] 過去研究有關於骨細胞受到張力或壓力之研究顯示，當使用彎曲壓力裝置（Flexercell) 來製造週期性張力並將之作用於培養的鼠顱頂細胞，結果顯示在0.04% 張力下會促進細胞增生，PGE2 生成增加，但ALP 活性與膠原合成則下降。[26,27] 而當以1000

μ strain 之週期性張力作用於人的成骨細胞則會造成細胞增生、alkaline phosphatase (ALP)及carboxyl-terminal collagen type I (ColI)分泌增加，但osteocalcin (OC)則減少其釋放。[28,29] 由於成骨母細胞與破骨骨細胞均會分泌MMPs 。當進行骨吸收時，成骨母細胞所分泌的MMPs ，先分解骨表面的未礦化骨頭，以方便破骨骨細胞與下層的鈣化基質產生鍵結，進而進行骨吸收，破骨骨細胞進行骨吸收時，包括去礦化及分解有機基質，而分解基質主要藉助二種酵素，cystein proteinase (CPs)和MMPs，此時細胞分泌的結締組織生長因子mRNA會增加。[30]

研究目的

本研究計畫為設計三年之研究，

第一年研究目的，以低能量雷射於不同條件下，比較不同劑量雷射處理時間下，探討對於牙周韌帶細胞株與骨細胞株之細胞生物學上訊息之表現，作為研究加速牙齒矯正移動之基礎。

第二年研究目的，於體外模擬活體矯正之狀況，將牙周韌帶細胞株與骨細胞株細胞分別培養在具壓力的環境下，依第一年研究得到之雷射劑量參數照射後，比較牙周韌帶細胞株與骨細胞株之骨訊息因子變化。

第三年研究目的，以動物試驗，模擬牙齒之矯正模式下，比較有、無低能量雷射照射後，被矯正牙齒之移動速度變化與動物之骨細胞反應。

貳、研究方法與材料

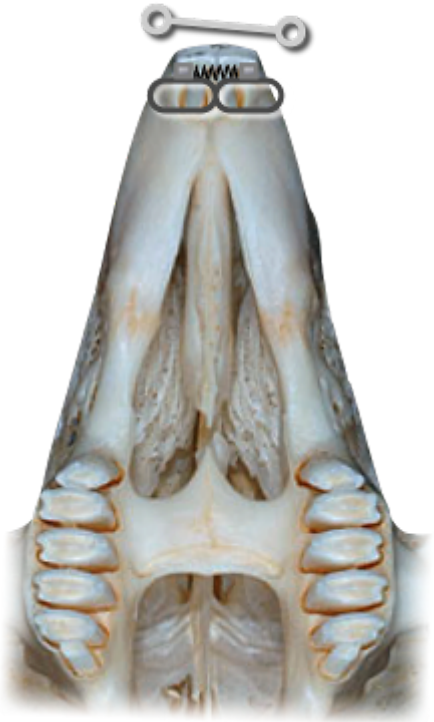
以動物試驗，模擬牙齒之矯正模式下，比較有、無低能量雷射照射後，被矯正牙齒之移動速度變化與動物之骨細胞反應。

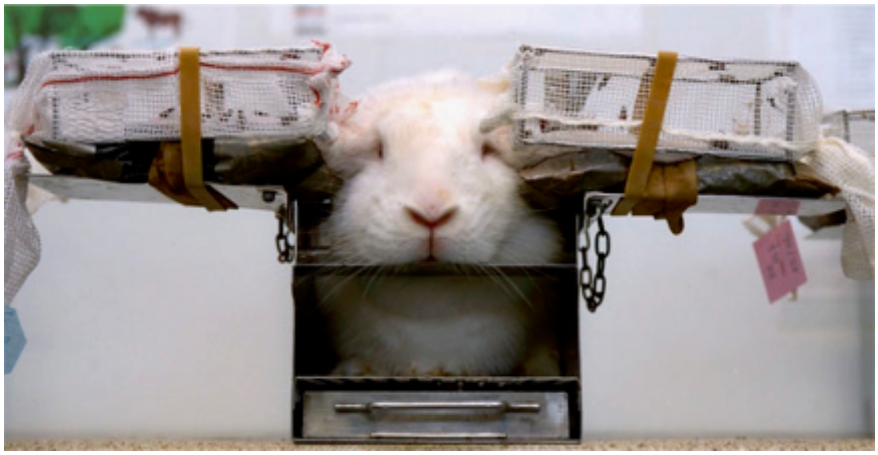
一、動物飼養與準備、 矯正裝置設計與安置

紐西蘭兔最大的特點是早期生長發育快，2月齡体重達2.0kg左右。成年母兔体重4.5~5.4kg，公兔

4.1~5.4kg。母兔繁殖力强，最佳配种年龄5~6月龄，年產5窩以上，每窩產7~8隻。紐西蘭兔適應性和抗病性强，性情溫順，易于管理 。

九十六隻七週大的雄性紐西蘭兔， 購自國家動物飼養中心，取得本校動物中心動物試驗許可，飼養於本校之動物中心。動物之麻醉以Ketamine (90 mg/kg body weight)之合適劑量注射後，進行實驗之操作。參考Kobayashi et al.之設計[35]，如下面圖以兔子為試驗對象，於口內裝上彈簧矯正裝置，施力後，同隻動物一側為實驗組，分別接受低能量雷射照射，另一側為對照組，無接受雷射照射。





二、 低能量雷射之處理與牙齒間空間變化紀錄

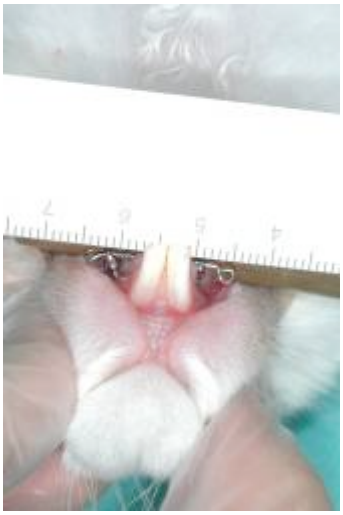
雄性紐西蘭兔口中之雷射照射位置為大白齒與門齒之近心與遠心端，劑量採用前二年之參數，用5 J之低能量二極體雷射照射， 收樣之試驗期間設定為 3, 6 weeks（每次約十隻）。牙齒移動之觀測，於大白齒與門牙同一水平處，作一凹痕，分別3, 6 weeks 作二齒之間牙齒空間之記錄。

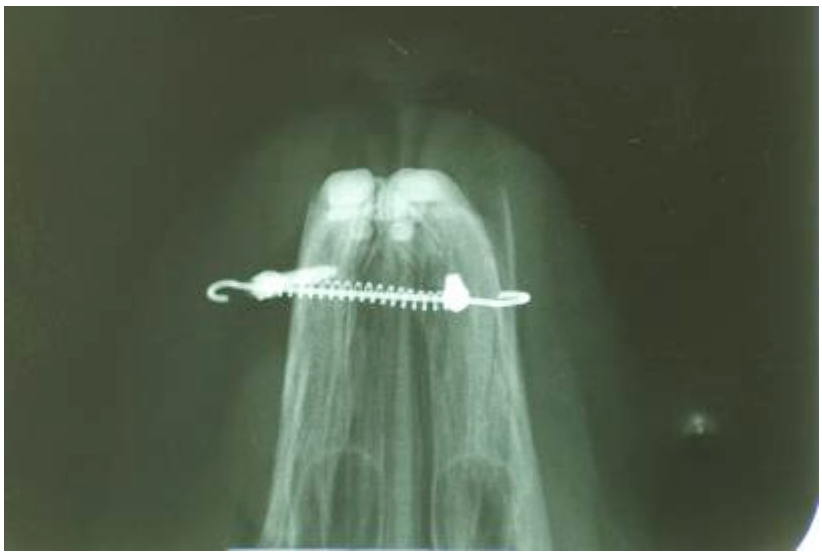
三、動物犧牲、 組織切片觀察

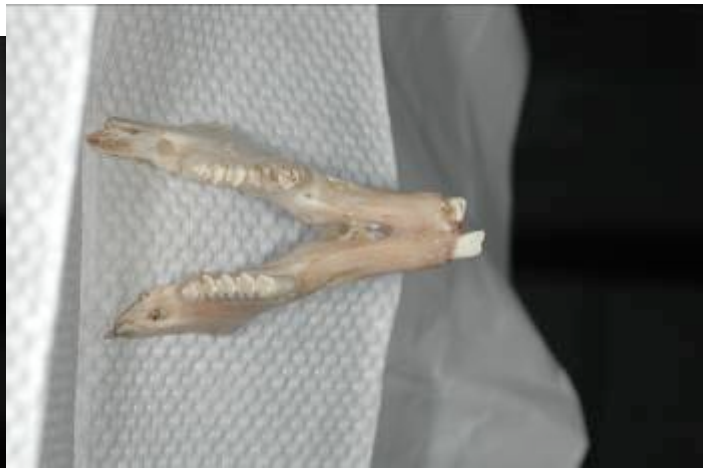
動物將以二氧化碳吸附法作犧牲動作。犧牲後，以高速鑽石鋸片，切割選取大白齒與門齒區周圍之骨

頭和牙根，大小約4mm3的體積。將取下之組織以液態氮冷凍，存於-70 °C 。

以含4% paraformaldehyde之Phosphate buffered saline溶液固定組織；於-4 °C 下，以10% ethylenediaminetetraacetic acid之diethylpyrocarbonate , phosphate buffer saline 溶液脫鈣十天。之後將組織包埋於paraffin wax，作約4um大小之切片，再將切片作Hematolylin and esoin (HE stain) , Vonkossa stain, Toluidin stain Trichrome stain and Colalgen stain染色觀察取下之牙齒與骨頭組織變化。[36]







四、資料統計

所有結果均記錄下(mean +/- SD)，以 SPSS 10.0 (SPSSFW, SPSS, Chicago, IL, USA) 軟體，ANOVA做統計分析。 而實驗組與控制組數據之間的差距再以非配對 t 試驗 (Unpaired Student's t-test) 分析，結果以 $p<0.05$ 代表具有統計學上的顯著性差。

參、結果

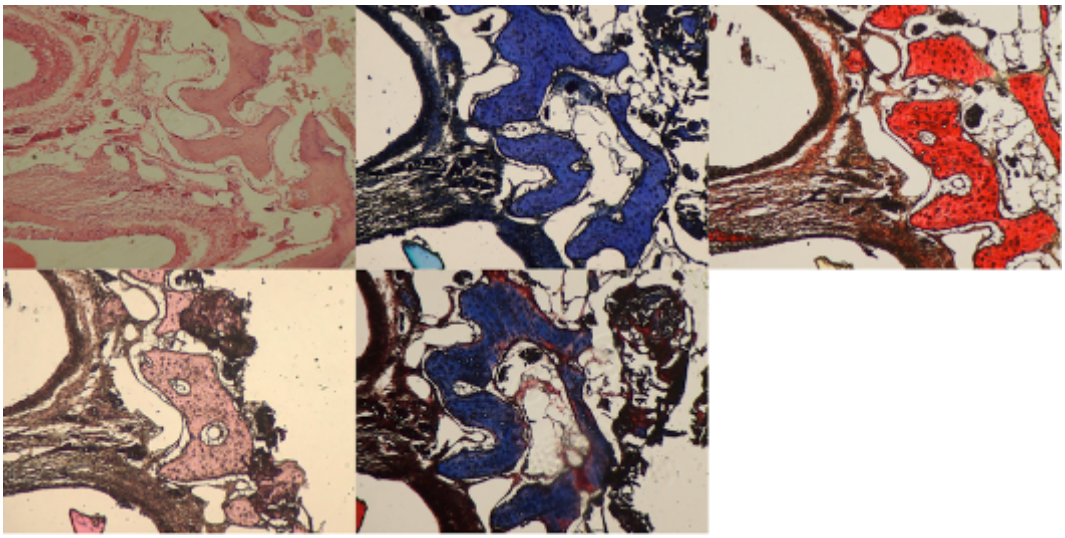
一、牙齒間距離經矯正力作用後之變化如下。

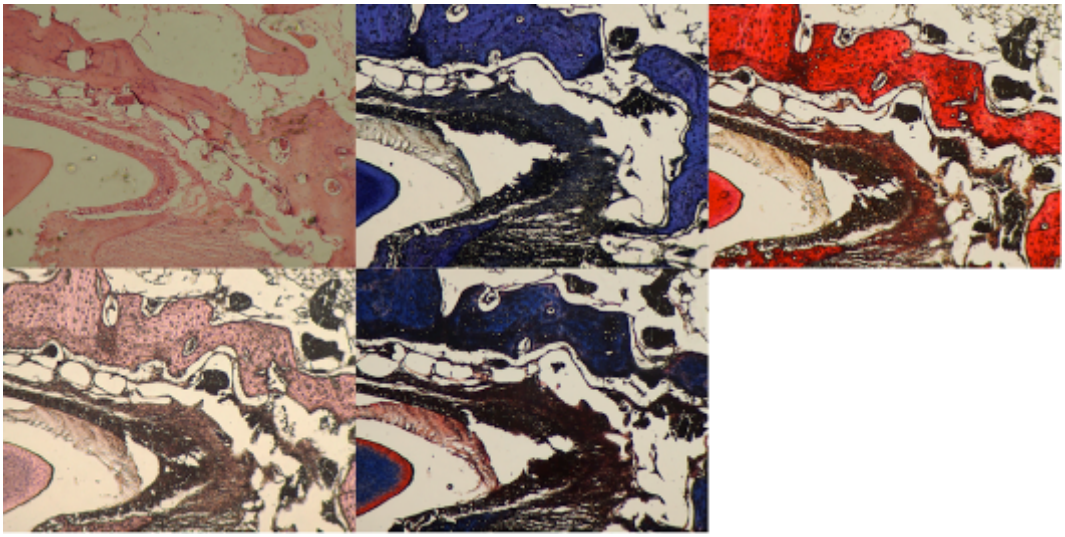
表 一、

	3w Laser	3w Non-L	6w Laser	6w Non-L
Diastema 1	1.96	1.12	2.73	1.98
2	2.33	0.93	1.89	2.43
3	2.75	1.32	1.70	2.10
4	2.04	1.16	2.02	3.41
5	1.76	1.86	2.07	1.27
average	2.17 +/- 0.23	1.28+/-0.41	2.08+/-0.26	2.24+/-0.31
Incisor 1	2.68	2.43	2.93	2.07
2	2.74	1.05	2.26	2.79
3	3.07	1.78	2.79	3.68
4	2.00	1.18	2.65	3.16
5	2.42	1.88	2.66	2.21
average	2.58+/-0.33	1.66+/-0.25	2.66+/-0.31	2.78+/-0.26
Molar 1	1.60	1.74	1.45	1.69
2	0.89	1.70	2.07	1.53
3	1.80	1.68	1.58	1.75
4	1.40	1.02	1.19	3.89
5	1.22	1.88	2.95	1.44
average	1.38+/-0.41	1.60+/-0.39	1.85+/-0.24	2.06+/-0.25

二、組織切片觀察

A、控制組之組織，未有施力或雷射處理 （圖 一）

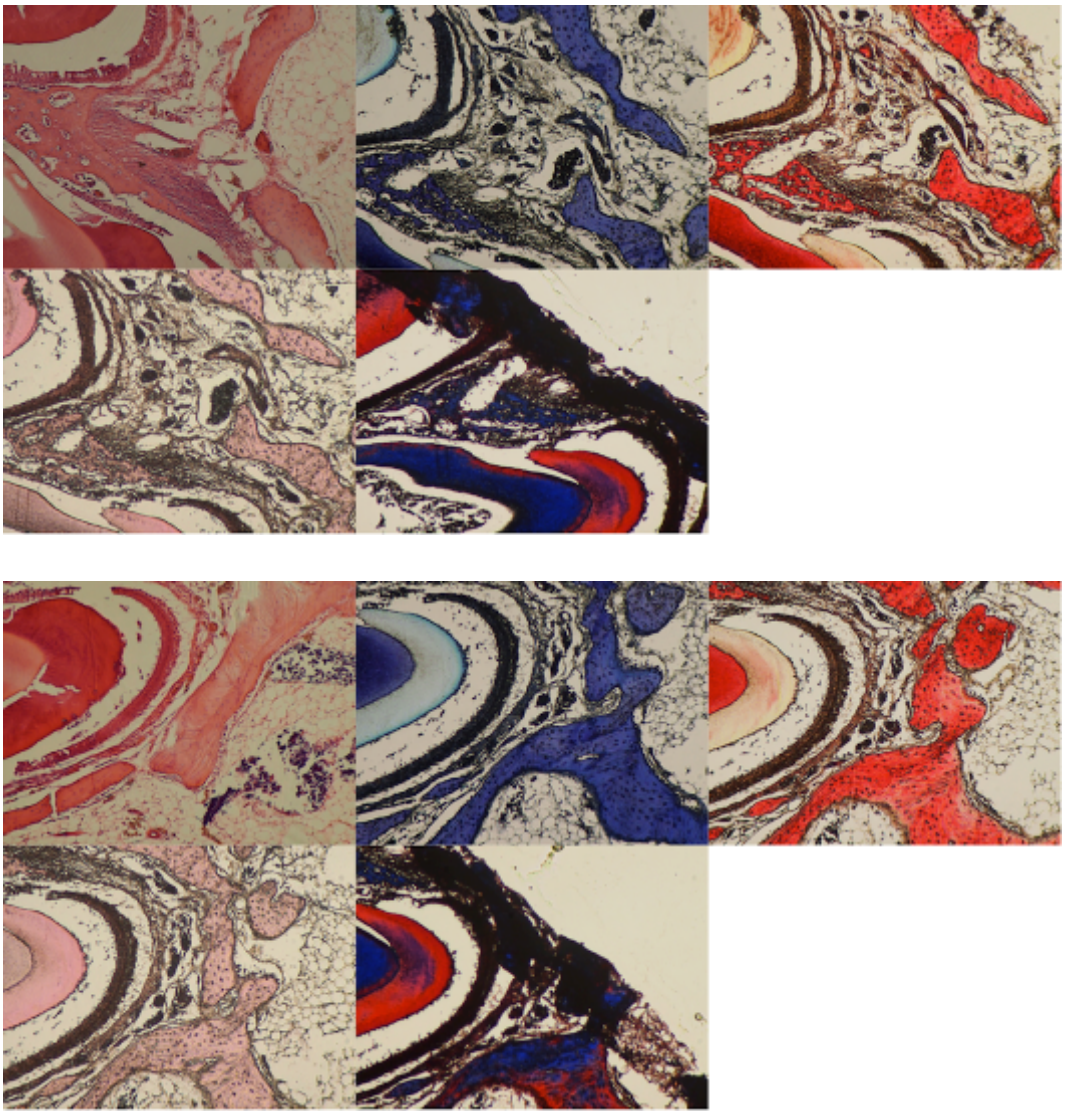




H&E stain	Toludine stain 黑bone 藍 cytoplasma, nuclei. acidic carbohydrates are metachromatic (pink to purple)	Collagen stain
Von Kossa stain 咖啡 骨頭 粉紅 cytoplasma	Trichrome stain 藍 clooagen 紅 soft tissue 黑radiopaque 無機質	3 week, no ortho, no laser

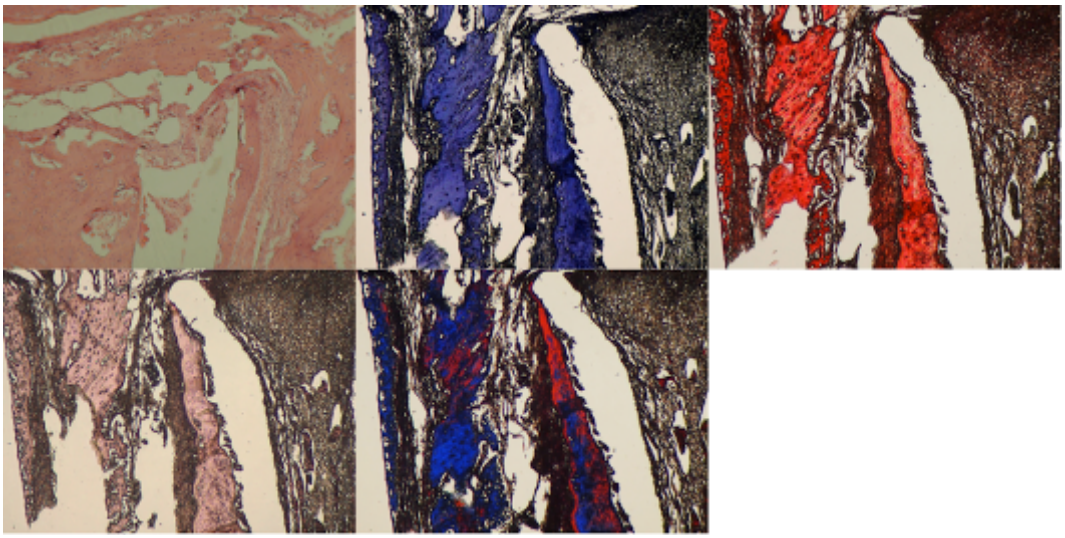
3

B、控制組之組織，未有施力但有雷射處理 （圖 二）



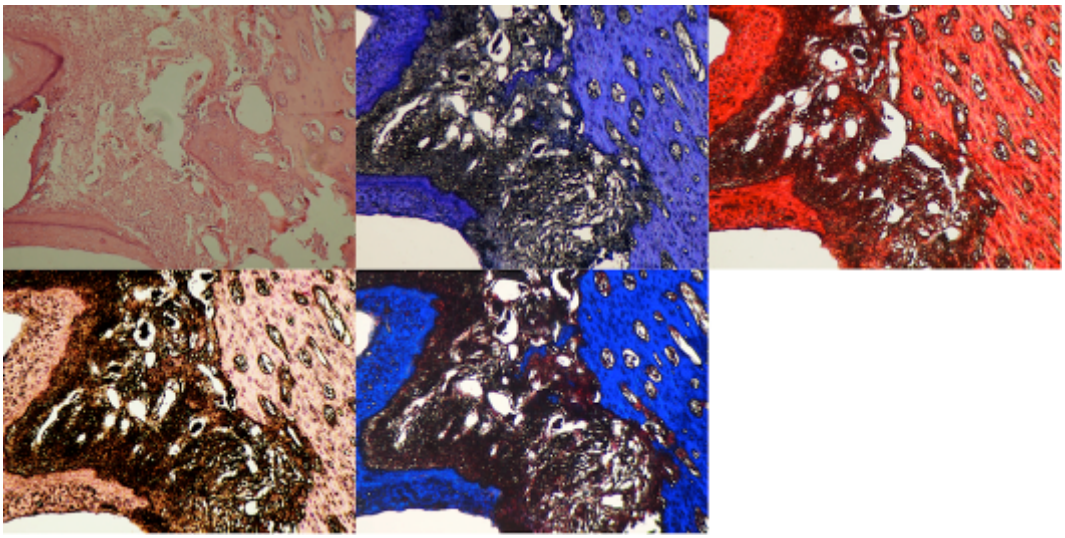
H&E stain	Toludine stain	Collagen stain
Von Kossa stain	Trichrome stain	3 week, no ortho, laser

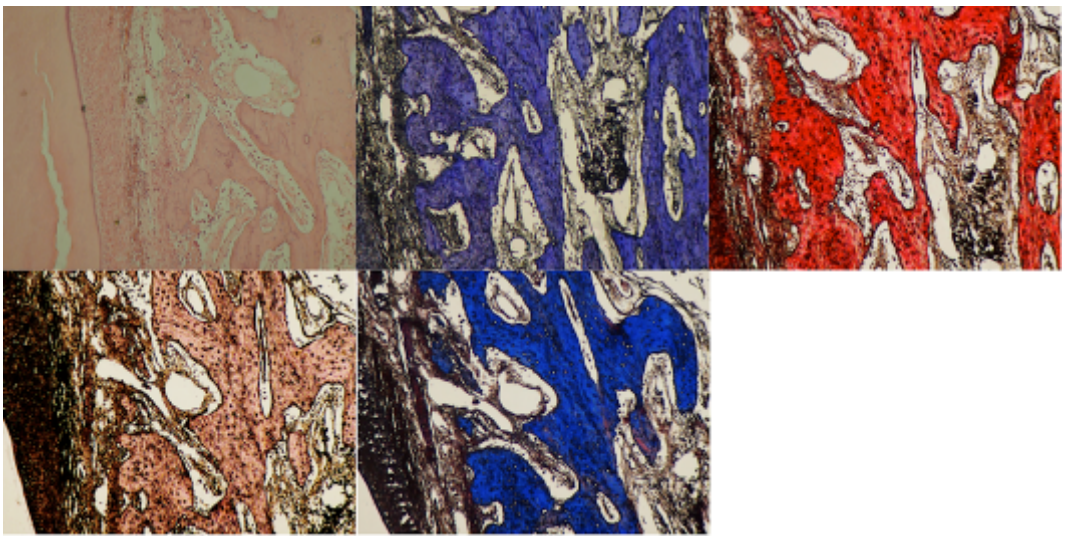
C、 控制組之組織，有施力但未有雷射處理（圖 三）



H&E stain	Toludine stain	Collagen stain
Von Kossa stain	Trichrome stain	3 week, ortho, no laser

D、 控制組之組織，有施力並雷射處理（圖 四）

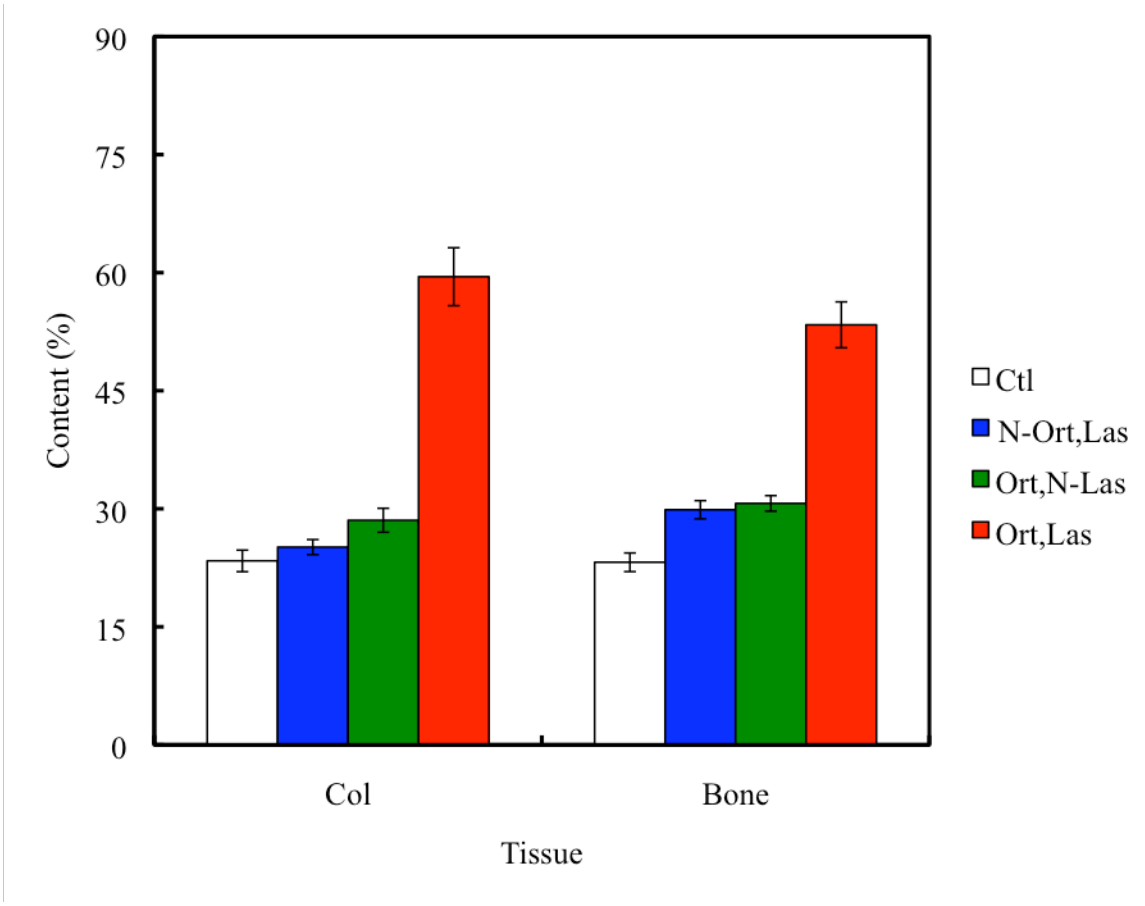




H&E stain	Toludine stain	Collagen stain
Von Kossa stain	Trichrome stain	3 week, ortho, laser

經過電腦量化以後，結果如下：（**圖　五**）

施力下之組織經過雷射照射後，其組織切片中collagen stain和VonKossa stain表現量較控制組高。



肆、討論

本計劃之動物試驗結果顯示經雷射處理之組別，在矯正施力後，其骨組織之分化生成效果較未施力與未有雷射處理組別表現高。過去前面二年之研究顯示，低能量雷射5J, 10 J對於細胞階層之反應呈現，細胞會有增生，與骨分化現象出現。證據顯示5J或10 J略具差異性，為本年度之計畫究以5J作為參數，所呈現結果觀察到collagen I 與 osteoid stain結果均較控制組好，似乎表示有bone remodeling increase 現象，此結果與2012年Altan等發表結果相仿，他們以20g 力量作用於 albino Wistar rats，使用820nm Ga-AL-As二極體雷射，100mW強度照射，實驗經過八天後犧牲動物觀察切片之細胞變化，結語認為驚雷射處理後之骨細胞有remodeling效果，並且識經過osteoblast differentiation達成，但是作者也認為應該

有更加之參數值可作為日後繼續研究之方向。(37)

本研究計畫原本計畫以大鼠作為試驗對象，但是經過初步試驗，發現所養之大鼠死亡率太高，而且不易將矯正裝置安置，最後更改對象以兔子作為目標，但由本研究結果發現，雖與Altan之時驗對象不一樣，但是結果相似，即雷射有助於骨細胞骨化反應，但是否對於牙齒之移動可以增加速度或是量。在本研究中尚未發現，原因為所測得的牙齒距離變化並不顯著，雖然本研究觀察達48 天，但未有差異性出現。

2000 年 Kawasaki等(38)同樣以LLLT處理老鼠後觀察牙齒之移動研究顯示，LLLT處理後牙齒移動速率有增加，免疫化學染色切片顯示，LLLT處理之張力側細胞有增生與分化現象，即骨細胞有remodeling 現象出現。 Bone remodeling結果與目前研究LLLT作用於兔子之切片之collagen I 增加結果相似。Kawasaki 研究終始用之雷射劑量顯示9分鐘之累積照射，共達54.0J能量，平均一分鐘也是5 J之能量。由於照射模式不同，所得結果也可能出現差異，本研究乃每週照射一次，共計3-6 次之照射，累計能量約為15-30J。差異為本研究之牙齒移動量似乎與正常組相較之下，無太明顯差別。

再者，矯正施力之差異上，本研究使用約25g力量，作為門牙間之空隙移動，而Kawasaki研究牙齒移動之起始力量約10g，是否不同動物之牙齒移動量會因力量大小有差異，日後仍可以繼續研究。

另外，於研究顯示，作者利用 Ga-Al-As diode LLL 處理老鼠，劑量約為4.98J/cm2，七天之後研究發現fibronection 和collagen type I 有明顯增加，作者認為LLLT可以讓移動牙齒時，促進結締組織之快速轉換而達到牙齒快速移動。(39) 由圖五結果可以發現，本研究之collagen I 表現量也明顯大於控制組之表現。

結論與建議

本計劃經三年之研究發現，二極體低能量雷射對於細胞階層之研究可以發現有很有意義的正向促進功能，即可以刺激細胞之增殖生長，加速分化現象，對於動物之牙齒周圍細胞反應也呈現正面之效果，

對於牙齒支頤動是否可以加速，本計劃結果則未有發現，唯對於最佳之LLLT參數值，則無法於現況下確認。因此，如果有由研發端一開始設計雷射，在接續所有生物醫學上之測試，相互modify，應可以找到最佳之參數值，因此，日後有機會將與工學院人才一起合作。

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附件二

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）

Tsui Hsien Huang, Yu Chuan Lu, **Chia Tze Kao.** Low-level diode laser therapy reduces lipopolysaccharide (LPS)-induced bone cell inflammation. Lasers in Medical Science: Volume 27, Issue 3 (2012), Page 621-627(SCI 2.337)

Tsui-Hsien, Huang., Shiau-Lee Liew, Chih-Lin, Chen, Ming-You Shie, **Chia-Tze Kao**. The Low Level Laser Effects on Simulated Orthodontic Tension Side Periodontal Ligament Cells. Photomedicine Laser Surgery. 2013 Feb;31(2):72-7.

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）

本計劃探討以低能量雷射加速牙齒移動之研究結果顯示於細胞研究上有加速生長效果，於分子階層上，結果也相互呼應細胞之反應結果，但是於動物試驗上，所用之參數值卻未見到預期之結果，其原因可能是因所用之參數值不是用於動物或是所選動物個體上差異造成。收集以發表文獻，似乎均顯示低能量雷射加速牙齒移動有其效果，我們將繼續再進行，期望能找到最合適之參數，將結果轉譯為臨床尚有用之技術或是發展為專用之工具。

國科會補助專題研究計畫出席國際學術會議心得報告

日期102_年03_月30_日

計畫編號	NSC 99-2314-B-040-014-MY3		
計畫名稱	低能量雷射應用於牙周細胞之生物訊息反應與對實驗動物之牙齒矯正移動速率研究		
出國人員姓名	高嘉澤	服務機構及職稱	中山醫學大學 牙醫系教授
會議時間	102年　03月20日 至 102年03月　23日	會議地點	美國西雅圖
會議名稱	(中文)2013年國際牙醫學會學術年度大會 (英文)2013, International Association of Dental Research Annual meeting		
發表題目	(中文)二極體雷射對張力下牙周韌帶細胞之影響 (英文)Laser Effects on Simulated Orthodontic Tension Side Periodontal Ligament Cell		

一、參加會議經過

本次會議於三月十九日搭飛機，經過十二小時飛行抵達西雅圖，也是晚間時刻，過去聽聞西雅圖入境是最嚴格的，真的是不虛傳，不知何原因，領行李後居然同班機所有人於出關處站等一個小時，此也不知是嚴格或是效率差。隔天一早（三月二十日），前往conventional center報到，由於事先已經由網路作業，因此憑單據條碼，迅速取得報到資料。當天有bone biology與material science演講，因此前往聆聽。三月二十一日會場上人數較第一天多，本次會議因建築物結構關係，分有幾個樓層舉辦不同之會場，有些會場空間小，但較熱門之場地則有安排大型場地，台灣有台大醫師受邀keynote演講，值得喝彩。晚上受邀參加中國夜，與其它同好見面。三月二十二日當天一早前往會場將海報佈置好，併參觀會場之exhibition，似乎越來越多之廠商已不參加，取代的是不同之大學介紹該校之入學招生與研究方向。下午開始海報之展示，本人海報益友多位參與者前來交流討論。三月二十三日，因有同校老師之報告，因此也前往會場給予支持鼓勵，這天人數顯然變少。當天也選擇性聽取一些有關臨床試驗之演講，並完成了這次IADR會議的參與。

二、與會心得

由於氣候不同，西雅圖此時仍非常寒冷，溫度約30F-35F，會場內顯得溫暖許多。台灣參加知人數，據大家彙整約有30-40人參與，反觀大陸則許多重點大學紛紛有時人以上團對參與，並有許多研究發表，值得台灣學術

界注意。大陸也於此舉辦中國夜，邀請國際上之重量級人物與會，來彰顯實力，這也是我們更要關心。不過重量級演講，台灣也有代表，且或好評，這也值得我們稍寬心些。

三、發表論文全文或摘要

Abstract

Objectives: The purpose of this study was to analyze proliferation, inflammation and osteogenic effects on periodontal ligament (PDL) cells after low level laser therapy (LLLT) under simulated orthodontic tension conditions. **Background data:** Low level lasers affect fibroblast proliferation and collagen synthesis and reduce inflammation. Few studies have focused on the LLLT changes in the PDL due to moving teeth. **Materials and Methods:** A human periodontal ligament (PDL) cell line was cultured in a -100 kPa tension incubator. The PDL cells were treated with a 670 nm low level diode laser, output power of 500 mW (continuous wave modus) for 2.5 or 5 seconds, spot area 0.25 cm^2, corresponding to 1.25 J and 2.5 J at an energy density of 5 J/cm^2 or 10 J/cm^2 respectively**.** PDL cell viability was assayed by detecting the ability of the cells to cleave tetrazolium salt to formazan dye. Inflammation and osteogenic markers were analyzed by western blot analysis. **Results:** PDL cell viablity increased in the experimental group, based on the ability of the cells to cleave tetrazolium salt at day 7 ($p<0.05$). The experimental group showed no difference in PDL cellular morphology compared with the control group. The inflammation markers iNOS, COX-2 and IL-1 showed stronger expression in $5J/cm^2$ and 10 J/cm^2 therapy at day 1 and day 5, but decreased in expression at day 7. The osteogenic marker osteocalcin (OC) expression level was significantly higher at day 7 ($p<0.05$) than in the control cells. **Conclusion:** The LLLT significantly increased PDL cell proliferation, decreased PDL cell inflammation and increased PDL OC activity under the tension conditions used in this study.

Introduction

Orthodontic tooth movement is a process that combines both pathologic and physiologic responses to externally applied forces. Recently, how to enhance the rate of tooth movement without causing harmful effects to periodontal tissue has become an issue of significant interest to patients as well as orthodontists. Efficient tooth movement may be accomplished by mechanical, biomechanical or bio-stimulatory methods. The mechanic methods are focused on the bracket structure design, such as self-ligating brackets or materials involving metal or ceramic. The biomechanical methods involve medicine such as prostaglandin E2, a parathyroid hormone.[1,2] Biostimulation involves the modification of the environment to stimulate existing bacteria capable of bioremediation. With the introduction of therapeutic lasers, biostimulation also refers to the application of photon energy to injured tissue, in order to achieve a stimulatory and regenerative effect at the molecular level. The one of bio-stimulatory method is performed using sub-500 mW ranges of low level laser therapies (LLLTs). [3]

Previous studies have shown that low level lasers affect fibroblast proliferation and collagen synthesis and reduce inflammation.[4-6] LLLT irradiation facilitates the reorganization of the connective tissues during tooth movement in rats. [7] In orthodontics, LLLTs induce an enhancement of rapid palatal expansion and rapid tooth movement.[8,9]

The purpose of the present study is to analyze the proliferation, inflammation and osteogenic effects in PDL cells after LLLT in simulated orthodontic tension conditions.

Materials and Methods

Periodontal ligament cell line culture

A PDL was obtained from the Bioresource Collection and Research Center of Taiwan (ATCC 33277; DSM 20709). PDL cells were cultivated in two different media. Medium A (MA) is composed of DMEM medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 1 mM HEPES. Medium B (MB), differentiation medium, is composed of DMEM medium supplemented with 10 μl/μL 10^{-5} M dexamethasone, 0.05 g/μL *L*-Ascorbic acid and 2.16 g/μL glycerol 2-phosphate disodium salt hydrate. All incubations were at 37 °C, 95% humidity and 5% CO_2.

Tension incubator and diode laser on PDL cells

The PDL cells were seeded at a density of $1x10^4$ cells/well in 96-well plates. After 24 hours of cultivation, the media were changed either MA or MB. The culture plates were transferred to a tension incubator (TI, Model. 3618P, Lab-Line Instrument - Thermolyne Co. IL., USA) for one day under the following conditions: -100 kPa (1 Pa= 1/100,000 kg/cm^2, equal to a negative force of 101 g/mm^2), 37 ^{0}C and 5% CO_2.

The PDL cells were irradiated with a 670 nm diode laser (Ga-Al-As, Arts-Laser Model 970, Arts International Biotechnology, Inc., Germany) using directly mounted fiber optics at an output power of 500 mW (continuous wave modus) for 2.5 or 5 seconds, spot area 0.25 cm^2, corresponding to 1.25 J and 2.5 J at an energy density of 5 J/cm2 or 10 J/cm2 respectively [5]. In the experimental groups, the laser beam was adjusted to exactly cover the bottom of one culture well. After laser treatment, the plates continued incubating in the TI for one, five or seven days. The experiment was performed in triplicate. The extracts of mineral trioxide aggregate (MTA), which have osteoinduction ability, were used as a positive control group.[10] After treatment, cells were analyzed for their proliferation and expression of cellular proteins.

PDL cell viability assay

PDL cell viability was determined using the mitochondria tetrazolium bromide (MTT) colorimetric assay. Briefly, the control and experimental PDL cells were harvested after one, five or seven days in the TI. Then, 10 µl MTT (5 mg/ml in phosphate-buffered saline [PBS]) was added to each well, and the plate was incubated at 37 °C for 4 h to produce formazan. The medium was then removed from the culture wells, and the cells were gently rinsed three times with PBS. Following this step, a 100:1 dilution of 0.04 N HCl (stop solution) was added to each well of the tissue-culture plate to halt further reduction of MTT. This stop solution was removed, the cells were rinsed three times with PBS, and the formazan in the cells was extracted with isopropanol. The formazan extracts were transferred, and their absorbance was measured at a wavelength of 570-600 nm in a spectrophotometer (U 2000 type, Hitachi Co., Tokyo, Japan). The results were compared using analysis of variance (ANOVA). Differences in treatment means were analyzed using the Student-Newman-Keuls test and were considered to be significant at $P<0.05$.

Cellular protein expression analyzed by western blot

According to our previously described western blot analysis method,[11] the harvested cells were

lysed in 50 ml of lysis buffer (1% Triton X-100, 0.5% NP40, 10 mM EGTA, 0.2 mM Na_3VO_4, 0.2 mM NaF, and 0.2 mM PMSF). Cell lysates were cleared at 15,000 rpm for 15 min at 4 ^{0}C. Twenty-five micrograms of protein from each sample was boiled for 5 min after adding 1 µl SDS gel-loading buffer (125 mM Tris, pH 6.8, 5% glycerol, 28 mM SDS, 1% beta-mercaptoethanol, and 0.006% brompophenol blue).

Proteins were separated by 12.5% SDS-PAGE and transferred onto polyvinylidend difluoride membranes. The membranes were blocked for 1 h at room temperature in 3% BSA, 5% nonfat dried milk, 10 mM Tris, pH 7.5, 100 mM NaCl, and 0.1% Tween 20. To detect the proliferation, inflammation and osteoblastic effects of the experimental PDL, the appropriate antibodies [iNOS, IL-1, COX-2, MMP-3 alkaline phosphatase (ALP), osteocalcin (OC)] were used to immunoblot the membrane. After four washes, the membrane was overlaid with a secondary antibody for 1 h, then washed with TBS-T buffer for 20 min. The resultant films were scanned and quantified using a densitometer and the SCION image program.

Results

PDL cell viability (Fig. 1) and morphology (Fig. 2)

The LLLT-treated PDLs cultured in MA or MB and incubated under -100kPa demonstrated similar trends in behavior. PDL cells under -100kPa and LLLT showed a significantly lower viability rate than the control group at day 1 ($p<0.05$). PDL cell viability in MA under -100kPa incubation was lower than in the control group at day 1 and day 5 ($p<0.05$). However, in MB, the PDL cell viability was lower at day 1 only. There was no difference in PDL cell viability between the 5 J/cm^2 and 10 J/cm^2 laser in the MA group under -100 kPa incubation ($p>0.05$). PDL cell viability in the MB group at 10 J under -100 kPa incubation showed higher viability than the 5 J/cm^2 condition at day five and day seven ($p<0.05$) (Fig.1).

The PDL cell morphologies under the different treatment condition are shown in figure 2. Normal PDL cell morphology takes on a spindle shape, and this morphology was observed with no changes in all conditions tested.

Inflammation expression

Inflammation markers in PDL cells cultured in MA and MB showed similar patterns of expression (Fig. 3). iNOS, COX-2 and IL-1 expression appeared highest at day 1 and decreased in expression by day 7 ($p<0.05$). MMP-3 expression increased from day 1 to day 7 ($p<0.05$).

(Figure 3b, c). The PDL cells cultured under -100 kPa incubation with 10 J/cm^2 showed the highest MMP-3 expression of all the treatment conditions ($p<0.05$).

Osteogenic reaction

Osteogenic markers in PDL cells cultured in MA and MB showed similar patterns of expression (Fig. 4). In the western blot analysis, the bands corresponding to ALP and OC in the MB group were stronger than those in the MA group. Expression of the bone formation marker ALP was highest in PDL cells cultured in MB at day 5 ($p<0.05$). The OC expressed in PDL cells cultured in MB was higher in the 10 J/cm^2 treatment group than in the 5 J/cm^2 group at day 7 ($p<0.05$). The PDL cells cultured in MB had higher ALP and OC expression than PDL cells cultured in MA ($p<0.05$) (Fig. 4).

Discussion

The orthodontists are interesting in alveolar bone change under orthodontic force. To fasten the tooth movement at orthodontic pressure side and increase the orthodontic tooth stability are still in working. Until recently, the mechanism for osteogenesis at tension sites in tooth movement was not well understood, but reasonable inferences could be made from various mechanotransduction models.[12] Periodontal ligament cells respond to force by increasing cell viability and apoptosis. The relative extent to which these two competing processes occur controls the proportion of the various cell populations in the periodontal ligament and reflects the specific biomechanics of osteogenesis. [13]

The present in vitro study aimed to simulate the tension site of tooth movement and apply LLLT to PDL cells to evaluate whether this procedure could promote osteogenesis. The results of this study reveal that LLLT caused PDL cell viability to decrease at day 1 and day 5 but increase at day 7 in both experimental groups (grown in either MA or MB) incubated under -100 kPa. After LLLT was applied, the PDL cells cultured in a tension incubator showed higher viability at both 5 J/cm^2 and 10 J/cm^2 than the control group at day 7 (Fig. 1). The inflammation markers iNOS, COX-2, MMP-3 and IL-1 showed higher expression in PDL cells cultured in the -100kPa incubator than in control cells (Fig. 3). There was a statistically significant increase in the expression of these markers at day 1 and day 5 but expression decreased at day 7. Similar studies have shown that inflammatory responses to tension might be strain-dependent because low magnitude tensile strains are anti-inflammatory and induce magnitude-dependent anabolic signals in osteoblast-like periodontal ligament cells, culminating in the regulation of inflammatory gene

transcription. [14]

PDL cell inflammatory markers decreased after LLLT at day 1 and 5 in MA or MB groups (Fig. 3 b, c). This finding is similar to another study, which compared the TNF-α and IL-1 expression upon LLLT-induced inflammation in rats.[15] The results showed that LLLT has anti-inflammatory activity. [15] Similarly, LLLT reduced inflammation in our present study, which indicates that LLLT can be beneficial to overcome cellular inflammation. After cellular inflammation subsided, initiation of the osteogenic effect could be observed. It is reasonable that the tension side of the alveolar bone has osteogenic capabilities.

The application of MTA in the present study was to provide osteoinduction material. [10] Both the MTA-positive control group and the experimental laser treatment group under -100 kPa tension induced similar cellular reaction patterns. Morphological evidence of PDL cell disruption at tension sites in tooth movement has also been described after only 5 minutes of tension loading, further suggesting the involvement of an inflammatory mechanism. [16,17] In the present study, the lack of any PDL cell morphology changes may be due to the 5 min delay between loading and imaging (Fig. 2).

In vivo, normal cells are in differentiation. To add differentiation medium in the test group was to promote that the cell was under differentiating. It provided more information on experimental conditions that were similar with in vivo condition. ALP expression in the MA group showed no significant difference in growth at any time interval of culture (Fig. 4 b). LLLT did not increase ALP expression in PDL cells grown in MA. In contrast, ALP expression in PDL cells was higher in the MB group at day 5 but showed no difference at days 1 and 7. Differentiation medium (MB), which has been optimized to promote preferential differentiation, seemed to enhance the ALP expression in the present study.

The OC expression in PDL cells was higher, compared to control cells, in the MA group treated with LLLT 10 J/cm^2 at day 7, while the PDL cells in the MB group treated with LLLT had higher, compared to control cells, OC expression at day 5 and 7 (Fig. 4b). OC is known to express late in the process of bone formation. In the present study, the presence or absence of differentiation medium did not affect OC expression. These results indicate that the LLLT-applied tension in PDL cells can promote osteogenesis.

The clinical relevance of present study may be more important with regard to the stability of post-orthodontic tooth movement. It is because bone formation can be quickly initiated at the tension site of orthodontic tooth movement, thus increasing post-orthodontic stability. The animal model to set up and test with present experimental conditions will be the future study.

Conclusion

The LLLT significantly increases cellular viability, decreases cellular inflammatory markers expression and increases OC activity in PDL cells in present study. Although not yet proven by our studies, LLLT may promote the tooth movement rate under clinical conditions.

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四、建議

一、 由於經濟油價問題，機票顯得非常貴，年輕人參與意願降低，政府單位可以多增取預算，鼓勵補助國內更多人員參加此類研究型會議。

二、 補助國內大型學術團體舉辦晚會，提升國家知名度。

五、攜回資料名稱及內容

由於大會非常重視環保，本次帶回資料只有IADR program book 與含摘要之USB。

六、其他

無。

國科會補助計畫衍生研發成果推廣資料表

日期:2013/09/19

國科會補助計畫	計畫名稱: 低能量雷射應用於牙周細胞之生物訊息反應與對實驗動物之牙齒矯正移動速率研究
	計畫主持人: 高嘉澤
	計畫編號: 99-2314-B-040-014-MY3　　學門領域: 牙醫學

無研發成果推廣資料

99 年度專題研究計畫研究成果彙整表

計畫主持人：高嘉澤	計畫編號：99-2314-B-040-014-MY3
計畫名稱：低能量雷射應用於牙周細胞之生物訊息反應與對實驗動物之牙齒矯正移動速率研究	

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	2	0	100%	人次	
		博士生	1	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	2	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%	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）

本計劃探討以低能量雷射加速牙齒移動之研究結果顯示於細胞研究上有加速生長效果，於分子階層上，結果也相互呼應細胞之反應結果，但是於動物試驗上，所用之參數值卻未見到預期之結果，其原因可能是因所用之參數值不是用於動物或是所選動物個體上差異造成。收集以發表文獻，似乎均顯示低能量雷射加速牙齒移動有其效果，我們將繼續再進行，期望能找到最合適之參數，將結果轉譯為臨床尚有用之技術或是發展為專用之工具。