

行政院國家科學委員會專題研究計畫 成果報告

組織免疫化學染色法決定轉型生長因子、組織麩胺酸轉胺酶、細胞基質與細胞凋亡於鼠犬蛔蟲症肉芽腫肌炎

計畫類別：個別型計畫

計畫編號：NSC91-2314-B-002-221-

執行期間：91年08月01日至92年07月31日

執行單位：國立臺灣大學醫學院寄生蟲學科

計畫主持人：蘇霸霸

計畫參與人員：范家#22531；林永和 姚俊賢

報告類型：精簡報告

處理方式：本計畫可公開查詢

中 華 民 國 93 年 2 月 10 日

行政院國家科學委員會補助專題研究計畫  成果報告  
 期中進度報告

以組織免疫化學染色探討鼠犬蛔蟲症肉芽腫肌炎與轉型生長因子  
 $\beta 1$  型、組織麩胺酸轉胺酶二型、細胞基質及細胞凋亡之相關性

計畫類別： 個別型計畫  整合型計畫

計畫編號：NSC 91-2314-B-002-221

執行期間：91 年 8 月 1 日至 92 年 7 月 31 日

計畫主持人：台大醫學院 寄生蟲學科 蘇 霽 靄 副教授

共同主持人：台北醫學大學寄生蟲學科 范家堃 助理教授

計畫參與人員：台北醫學大學 病理學科 林永和 講師

姚俊賢 先生

成果報告類型(依經費核定清單規定繳交)： 精簡報告  完整報告

本成果報告包括以下應繳交之附件：

- 赴國外出差或研習心得報告一份
- 赴大陸地區出差或研習心得報告一份
- 出席國際學術會議心得報告及發表之論文各一份
- 國際合作研究計畫國外研究報告書一份

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫  
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

執行單位：台大醫學院 寄生蟲學科

中 華 民 國 92 年 10 月 30 日

## 摘 要

本實驗以犬蛔蟲幼蟲侵犯ICR株鼯鼠骨骼肌引致的肉芽腫性肌炎為一研究模式，在感染犬蛔蟲後的第一、三、五、七、十四、二十八、五十六和八十四天犧牲鼯鼠，血清利用柯達乾式生化儀檢測感染犬蛔蟲之ICR株鼯鼠血清中激酸激酶(CK)、乳酸去氫酶(LDH)、丙胺酸轉胺酶(ALT)及天門冬胺酸轉胺酶(AST)以監控肌肉及肝臟損傷的情形，結果顯示其生化數值在感染組之鼯鼠有高於控制組的情形，此可能與犬蛔蟲幼蟲於鼯鼠肝臟及肌肉移行而造成細胞之損傷有關。並以組織免疫化學染色探討骨骼肌中肌肉實質區與疏鬆結締組織之肉芽腫中貝它一型轉型生長因子(TGF- $\beta$ 1)、組織麩胺酸轉胺酶 (TG 2)、細胞基質(extracellular matrices, ECMs)與細胞凋亡(apoptosis)之表現情形；最後利用影像分析軟體(ImagePro, USA)，於400倍視野(HPF)下隨機挑選約15-20個病灶區加以定量分析。結果顯示TGF- $\beta$ 1之總表現量於感染後第二週達最高之表現量，而後隨著感染時間增加而遞減；TG 2之總表現量於第二週達最高，隨後稍降低其表現量，但仍保持一定的表現，而細胞基質中的collagen 2及collagen 4的總表現量，於感染後第四週達到最高，並隨著感染時間增加而遞減；Masson-Trichome stain的結果顯示collagen 1的總表現量於第四週開始出現，並有增加纖維化的趨勢。細胞凋亡之染色TUNEL的結果顯示細胞凋亡主要於第二週及第四週的肉芽腫病灶處，除了發炎細胞，部分肌細胞也有凋亡的現象。此研究顯示了犬蛔蟲所引致的肉芽腫性肌炎，由TGF- $\beta$ 1與TG 2之相互調控，來調節細胞基質、發炎細胞的浸潤及膠原的包圍覆蓋，來增加肉芽腫之限制蟲體及毒殺蟲體的能力，並藉著巨噬細胞來清除肉芽腫中細胞凋亡之嗜伊紅性白血球以達到降低發炎反應之目的。

## 圖解說明

圖一. ICR 株鼯鼠感染犬蛔蟲第一週後疏松結締組織及肌肉實質區中肉芽腫的病理變化和纖維化的情形(A, B, E 及 F, H&E stain; C, D, G 及 H, Masson trichrome stain; A, C, E 及 G scale bar=100  $\mu\text{m}$ ; B, D, F 及 H scale bar=20  $\mu\text{m}$ ) 圖 F 箭號(  $\rightarrow$ )所指為嗜伊紅性白血球。

圖二. ICR 株鼯鼠感染犬蛔蟲第二週後疏松結締組織及肌肉實質區中肉芽腫的病理變化和纖維化的情形(A, B, E 及 F, H&E stain; C, D, G 及 H, Masson trichrome stain; A, C, E 及 G scale bar=100  $\mu\text{m}$ ; B, D, F 及 H scale bar=20  $\mu\text{m}$ ) 圖 B 箭號( $\rightarrow$ )所指為嗜伊紅性白血球。

圖三. ICR 株鼯鼠感染犬蛔蟲第四週後疏松結締組織及肌肉實質區中肉芽腫的病理變化和纖維化的情形(藍綠色的部位代表纖維化)(A, B, E 及 F, H&E stain; C, D, G 及 H, Masson trichrome stain; A, C, E 及 G scale bar=100  $\mu\text{m}$ ; B, D, F 及 H scale bar=20  $\mu\text{m}$ ) 圖 B.箭號( $\rightarrow$ )所指為嗜伊紅性白血球，箭頭( $\blacktriangleright$ )蟲體切面；圖 E.箭號( $\rightarrow$ )為細胞外基質(ECMs)。

圖四. ICR 株鼯鼠感染犬蛔蟲第八週後疏松結締組織及肌肉實質區中肉芽腫的病理變化和纖維化的情形(藍綠色的部位代表纖維化)(A, B, E 及 F, H&E stain; C, D, G 及 H, Masson trichrome stain; A, C, E 及 G scale bar=100  $\mu\text{m}$ ; B, D, F 及 H scale bar=20  $\mu\text{m}$ ) 圖 B.箭頭( $\blacktriangleright$ )蟲體切面；圖 E.箭號( $\rightarrow$ )為細胞外基質(ECMs)；圖 F.箭號( $\rightarrow$ )所指為嗜伊紅性白血球。

圖五. ICR 株鼯鼠感染犬蛔蟲第十二週後疏松結締組織及肌肉實質區中肉芽腫的病理變化和纖維化的情形(藍綠色的部位代表纖維化)(A, B, E 及 F, H&E stain; C, D, G 及 H, Masson trichrome stain; A, C, E 及 G scale bar=100  $\mu\text{m}$ ; B, D, F 及 H scale bar=20  $\mu\text{m}$ ) ; 圖 B. 箭號(  $\rightarrow$ )所指為類上皮細胞。

圖六. 免疫組織化學染色之陽性控制組: A. 皮膚真皮層之 TGF- $\beta$ 1 陽性組, B. 乳癌細胞之 TG 2 陽性控制組, C. 腎臟基底膜之 collagen 4 陽性控制組, D. 軟骨細胞之 collagen 2 陽性控制組。陽性結果呈棕色 (scale bar =20  $\mu\text{m}$ )

圖七. ICR 株鼯鼠感染犬蛔蟲第一週後疏松結締組織(A-D) 及肌肉實質區(E-H)肉芽腫病灶處, TGF- $\beta$ 1(A, E), TG 2(B, F) Collagen 4(C, G) 及 Collagen 2(D, H)之免疫組織化學染色結果; scale bar=20  $\mu\text{m}$ , 棕色表示陽性反應。

圖八. ICR 株鼯鼠感染犬蛔蟲第二週後疏松結締組織(A-D) 及肌肉實質區(E-H)肉芽腫病灶處, TGF- $\beta$ 1(A, E), TG 2(B, F) Collagen 4(C, G) 及 Collagen 2(D, H)之免疫組織化學染色結果; A-D scale bar=50  $\mu\text{m}$ ; E-H scale bar=20  $\mu\text{m}$ , 棕色表示陽性反應。

圖九. ICR 株鼯鼠感染犬蛔蟲第四週後疏松結締組織(A-D) 及肌肉實質區(E-H)肉芽腫病灶處, TGF- $\beta$ 1(A, E), TG 2(B, F) Collagen 4(C, G) 及 Collagen 2(D, H)之免疫組織化學染色結果; A-D scale bar=50  $\mu\text{m}$ ; E-H scale bar=50  $\mu\text{m}$ ), 棕色表示陽性反應。

圖十. ICR 株鼯鼠感染犬蛔蟲第八週後疏松結締組織(A-D) 及肌肉實質區

(E-H)肉芽腫病灶處，TGF- $\beta$ 1(A, E)，TG 2(B, F) Collagen 4(C, G) 及 Collagen 2(D, H)之免疫組織化學染色結果；A, B, E 及 F scale bar=50  $\mu$ m；C, D, G 及 H scale bar=50  $\mu$ m，棕色表示陽性反應。

圖十一. ICR 株鼯鼠感染犬蛔蟲第十二週後疏鬆結締組織(A-D) 及肌肉實質區(E-H)肉芽腫病灶處，TGF- $\beta$ 1(A, E)，TG 2(B, F) Collagen 4(C, G) 及 Collagen 2(D, H)之免疫組織化學染色結果；scale bar=50  $\mu$ m，棕色表示陽性反應。

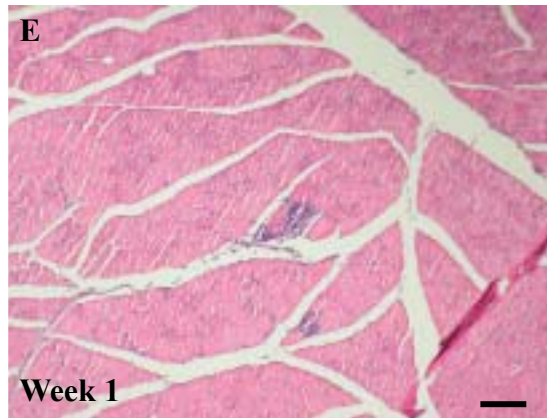
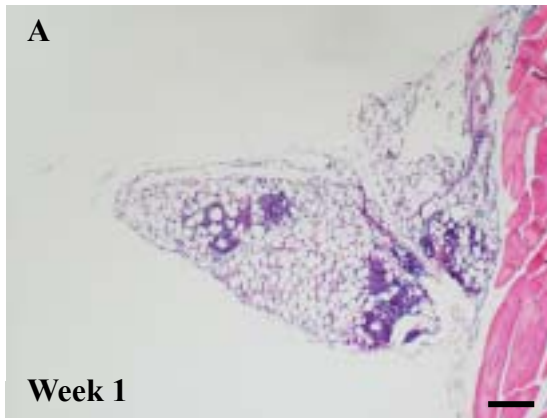
圖十二. ICR 株鼯鼠感染犬蛔蟲，疏鬆結締組織第一週至第十二週肉芽腫 TUNEL stain 結果(A 及 B scale bar=20  $\mu$ m; C-F scale bar=50  $\mu$ m)；F.肌肉實質區感染後第四週 TUNEL stain 結果呈現陰性的結果；G 及 H 肌細胞於感染犬蛔蟲後第二週及第四週可發現細胞凋亡的現象；(G 及 H scale bar=20  $\mu$ m)；箭號(➡)所指為陽性細胞。

圖十三. TUNEL 細胞凋亡染色之控制組: A.以 DNase I 處理之淋巴細胞陽性控制組，箭頭所指為陽性之染色結果，B.為反應中不加 TdT 酵素之淋巴細胞陰性控制組，C.以 DNase I 處理之肌細胞陽性控制組，箭頭所指為陽性之染色結果，D.為反應中不加 TdT 酵素之肌細胞陰性控制組。(Scale bar=20  $\mu$ m)

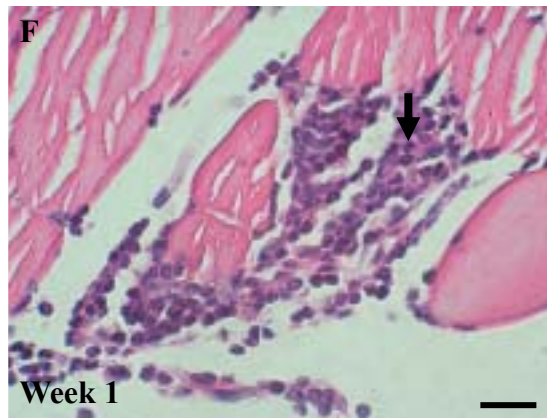
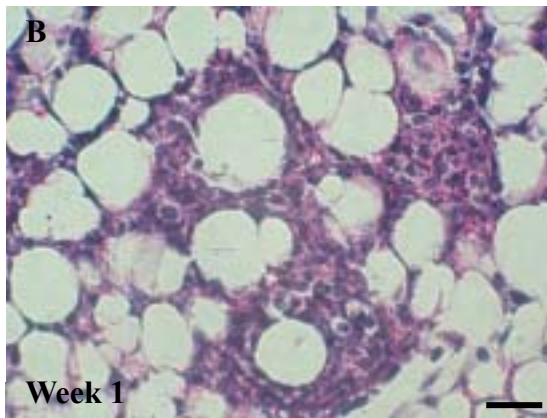
圖一. Loose connective tissue

Muscular area

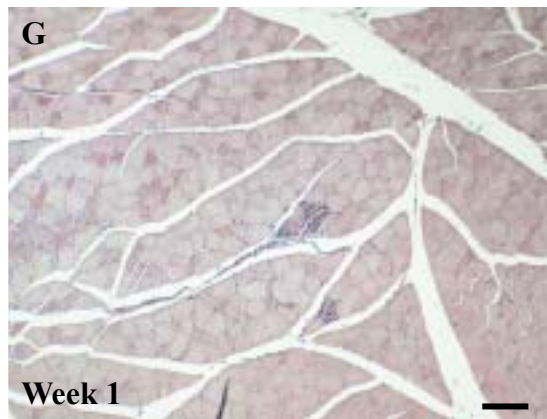
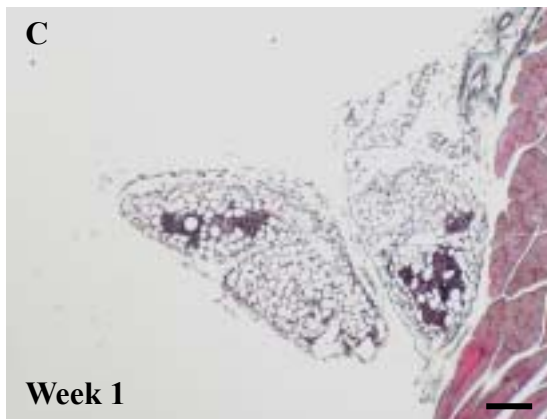
H & E stain



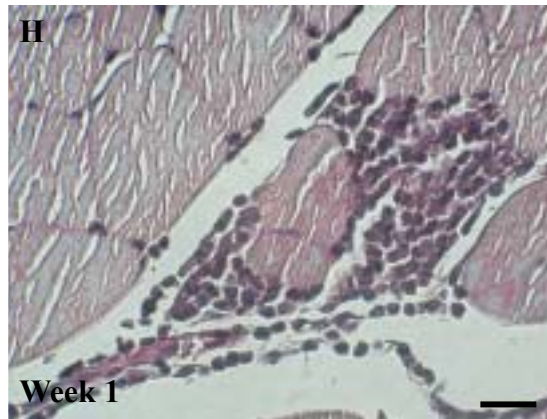
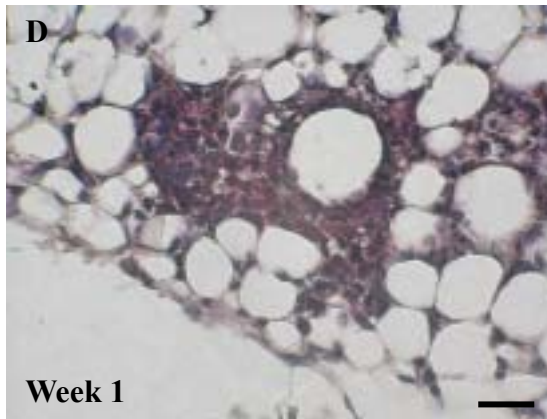
H & E stain



Masson trichrome stain



Masson trichrome stain

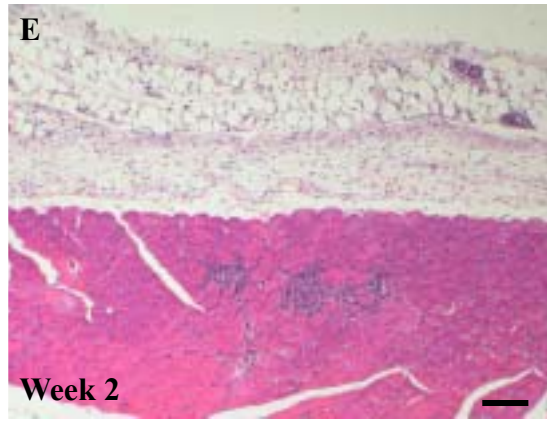
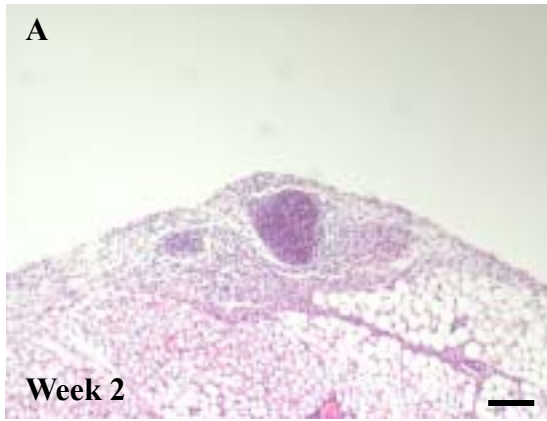


A, E, C and G scale bar = 100  $\mu$  m ; B, F, D and H scale bar = 20  $\mu$  m

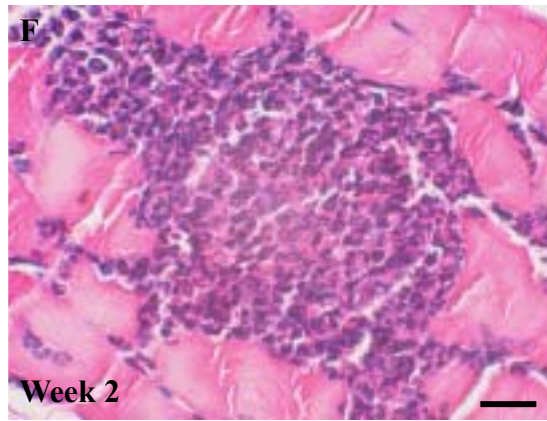
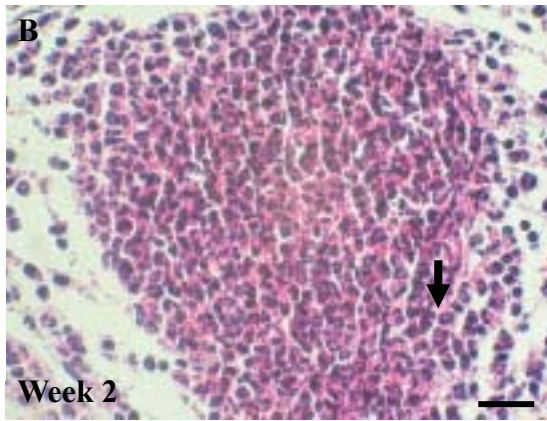
**圖二. Loose connective tissue**

**Muscular area**

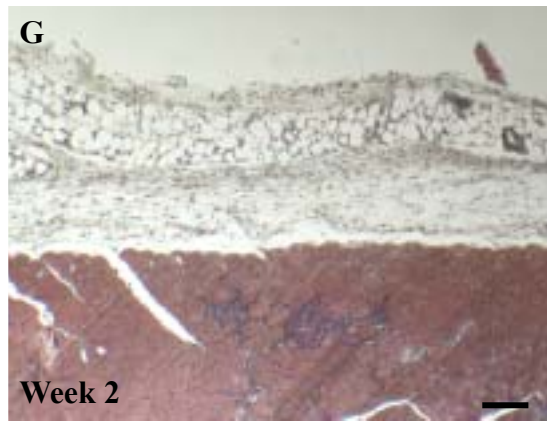
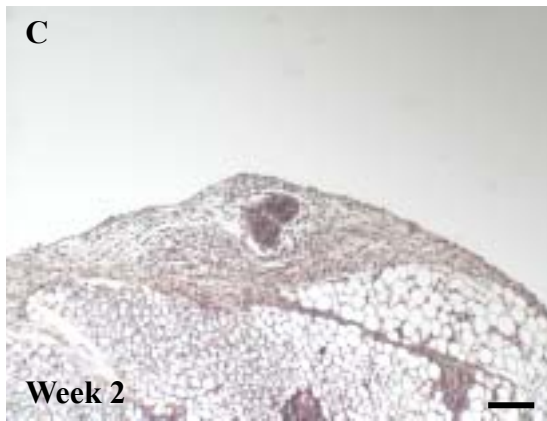
**H & E stain**



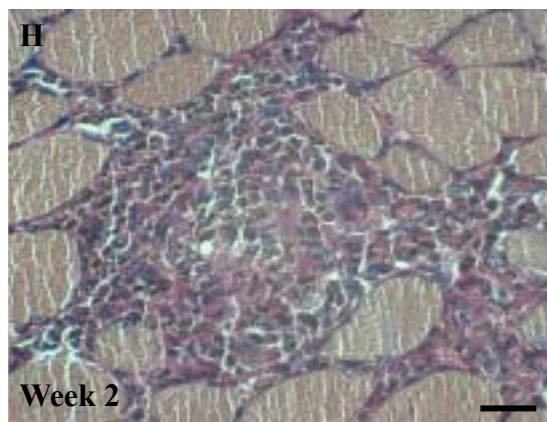
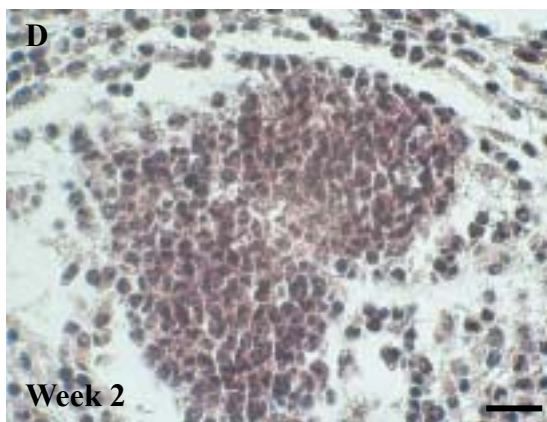
**H & E stain**



**Masson trichrome stain**



**Masson trichrome stain**



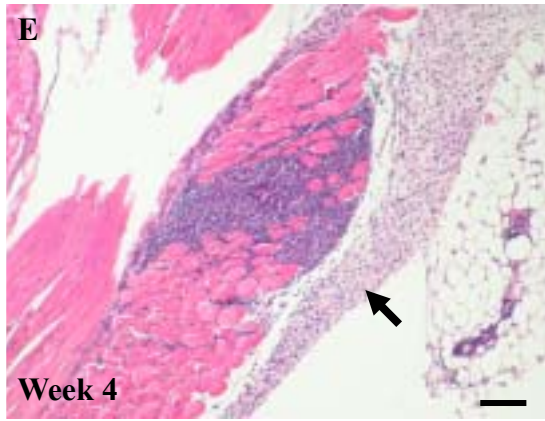
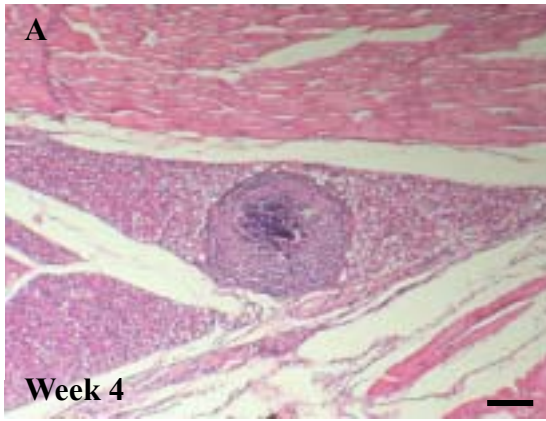
**A, E, C and G scale bar = 100  $\mu$  m ; B, F, D and H scale bar = 20  $\mu$  m**



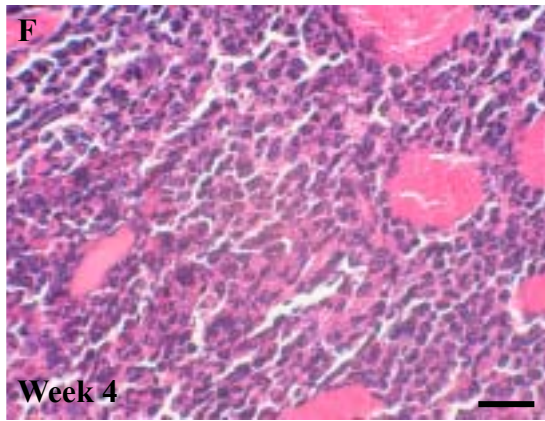
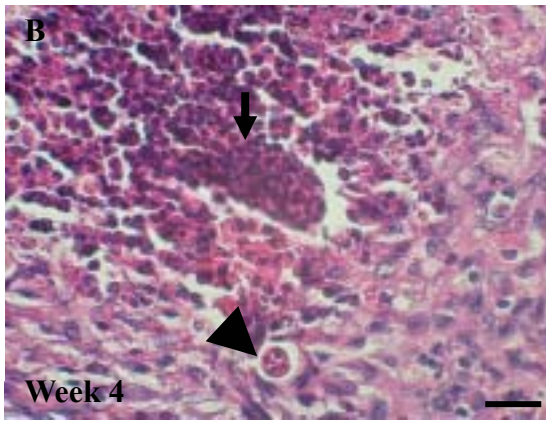
圖三. Loose connective tissue

Muscular area

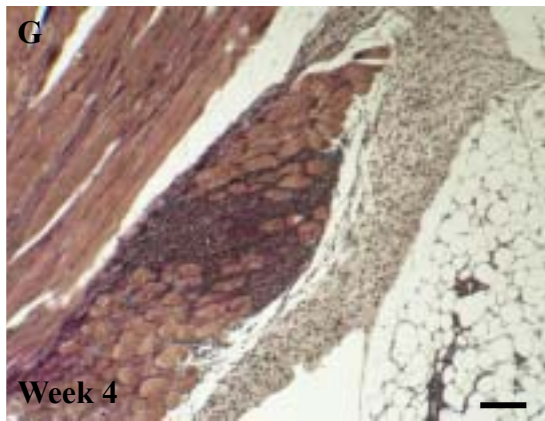
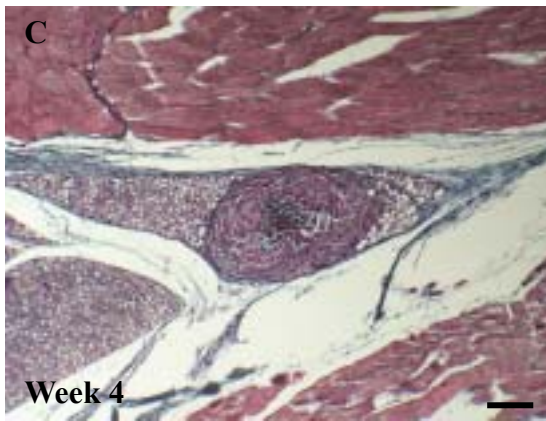
H & E stain



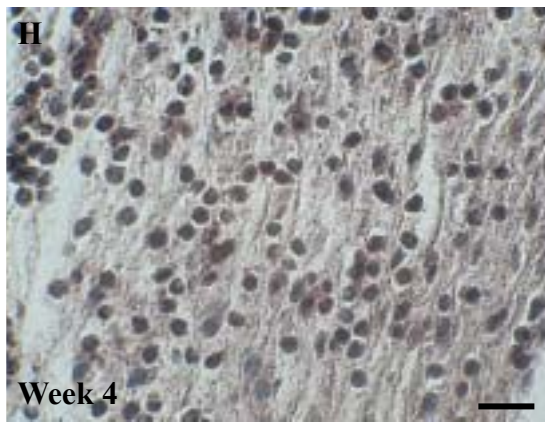
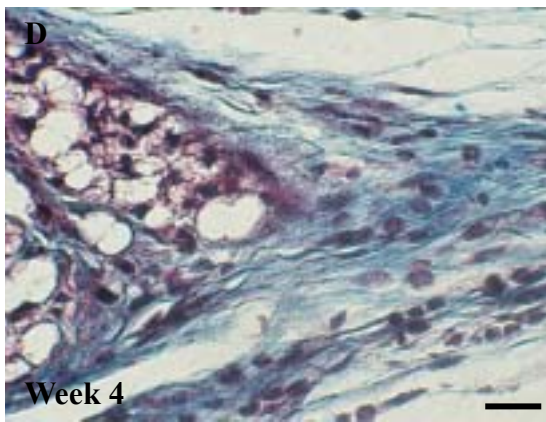
H & E stain



Masson trichrome stain



Masson trichrome stain

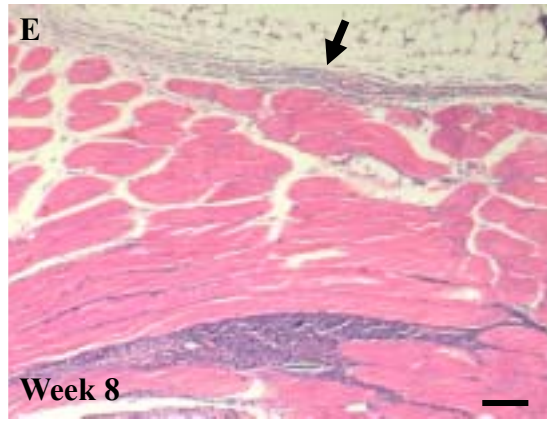
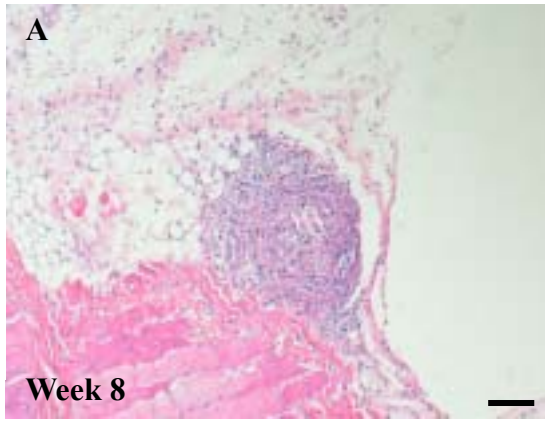


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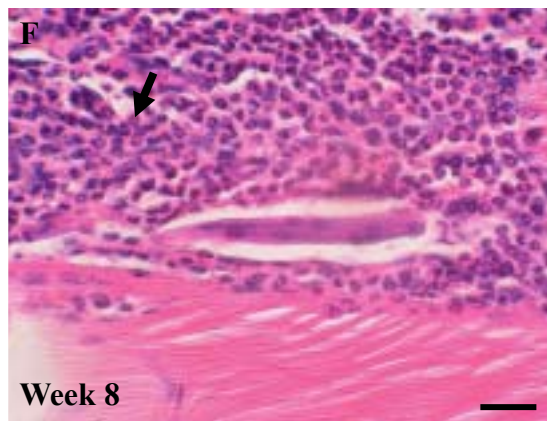
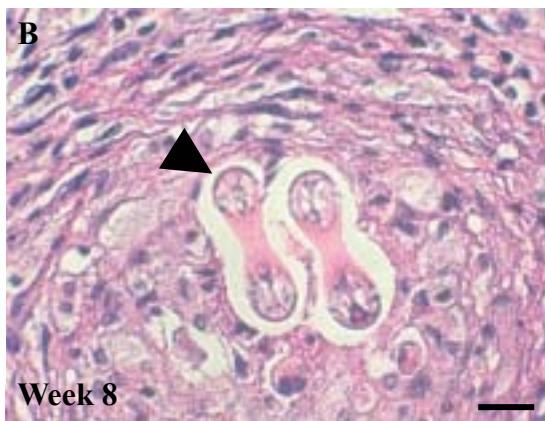
圖四. Loose connective tissue

Muscular area

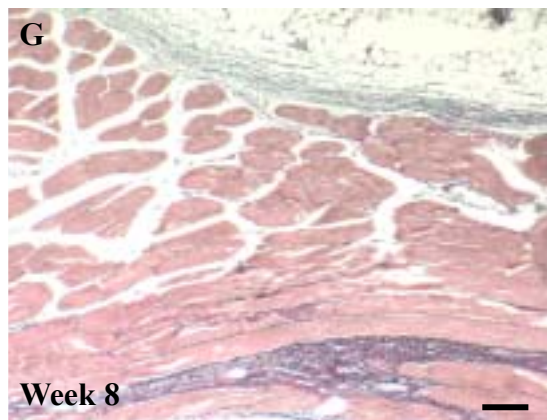
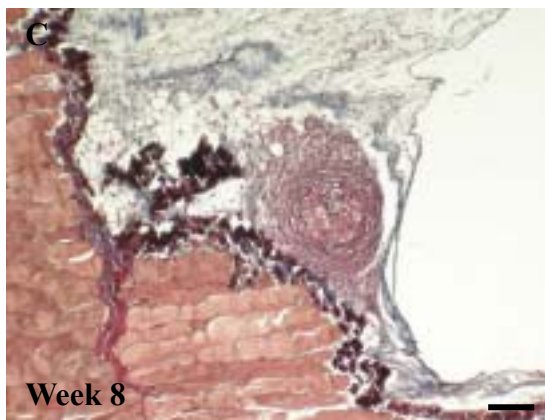
H & E stain



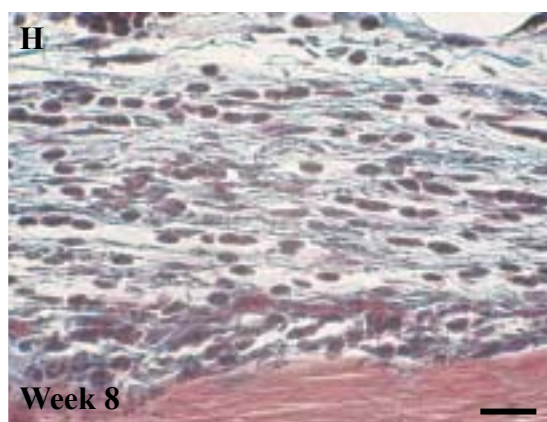
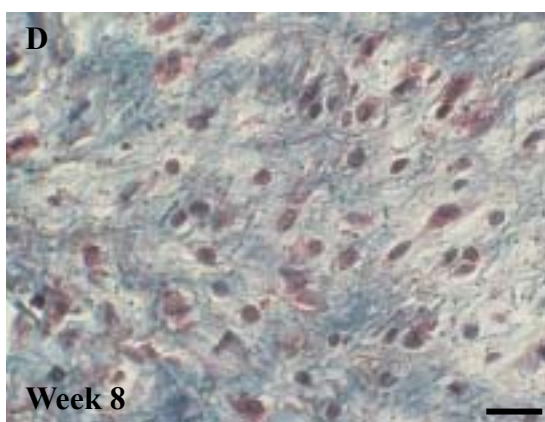
H & E stain



Masson trichrome stain



Masson trichrome stain

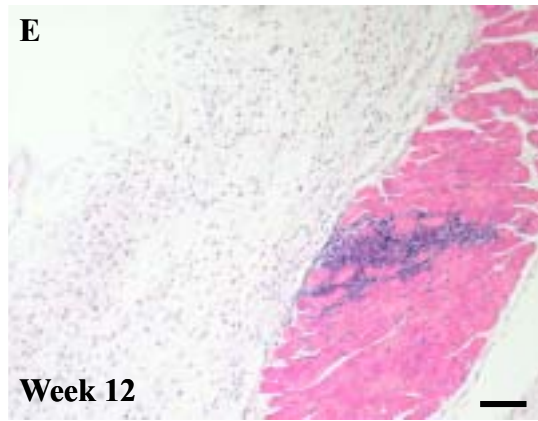
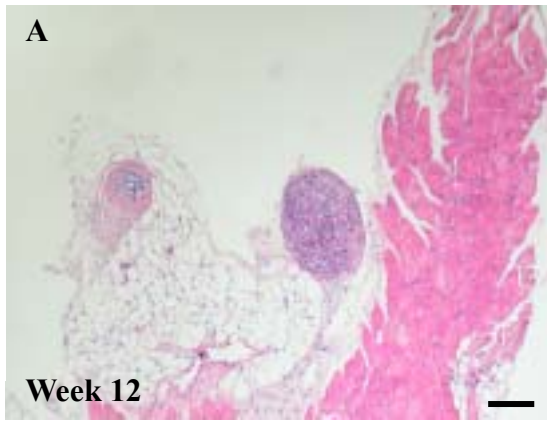


A, E, C and G scale bar = 100  $\mu$  m ; B, F, D and H scale bar = 20  $\mu$  m

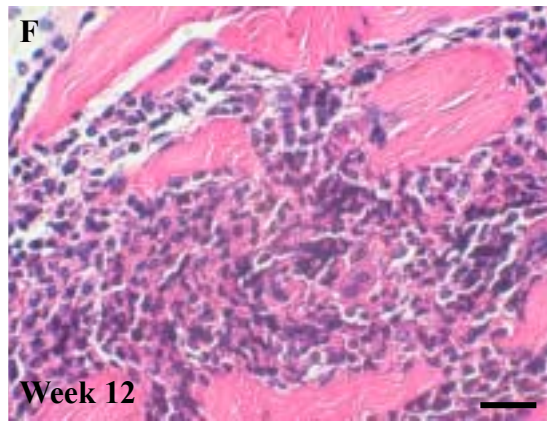
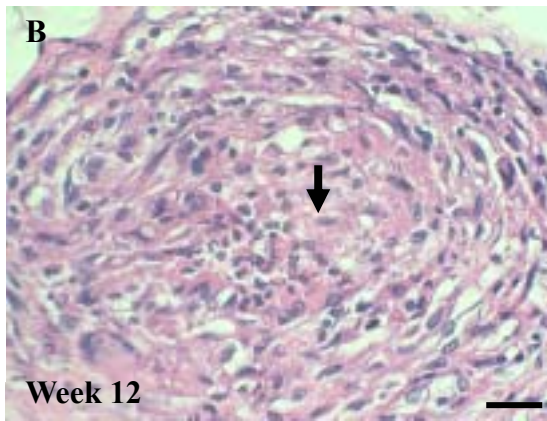
圖五. Loose connective tissue

Muscular area

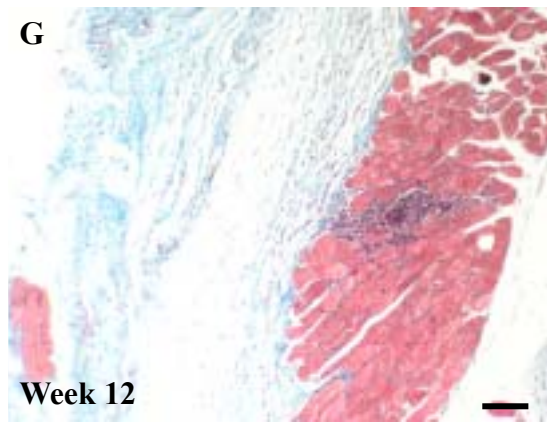
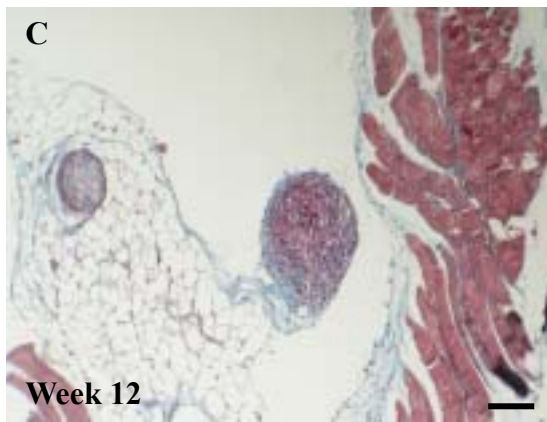
H & E stain



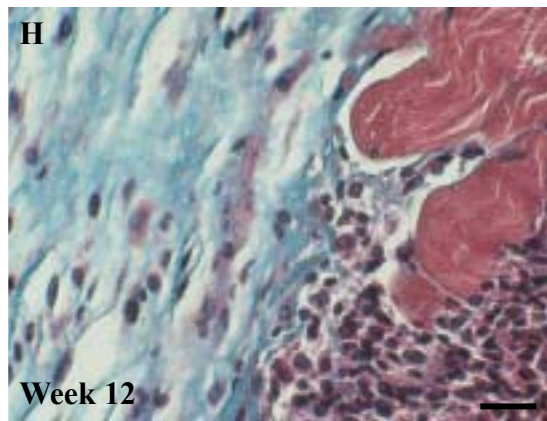
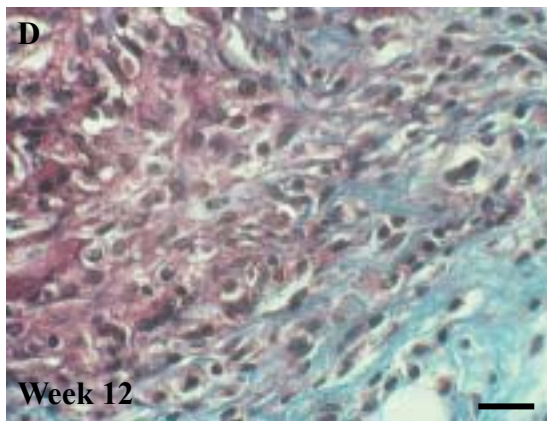
H & E stain



Masson trichrome stain

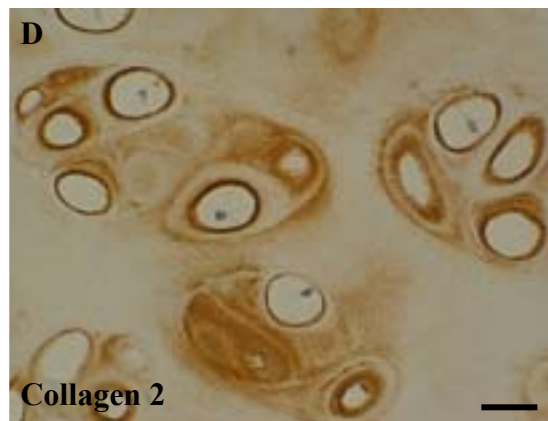
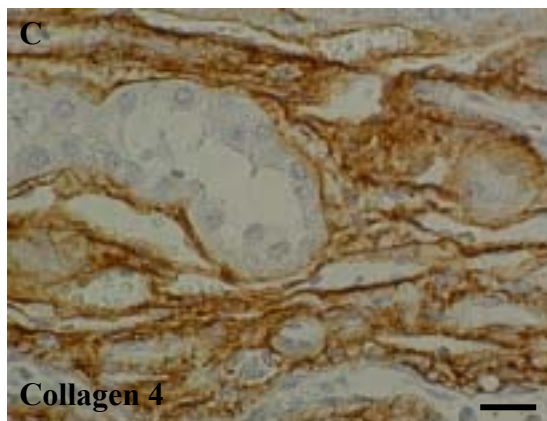
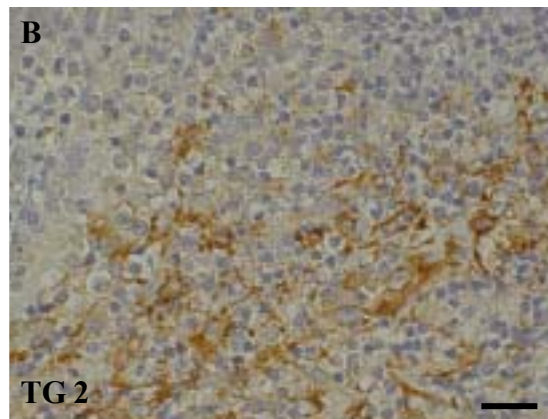
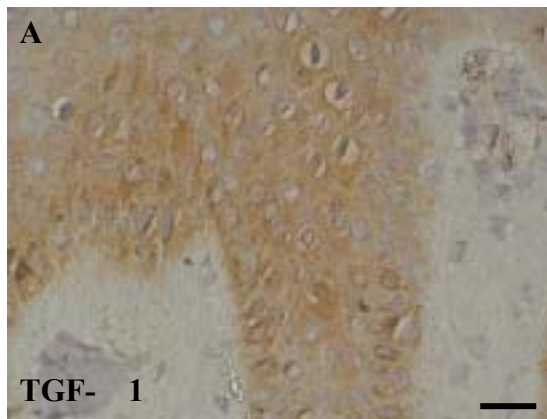


Masson trichrome stain



A, E, C and G scale bar = 100  $\mu$  m ; B, F, D and H scale bar = 20  $\mu$  m

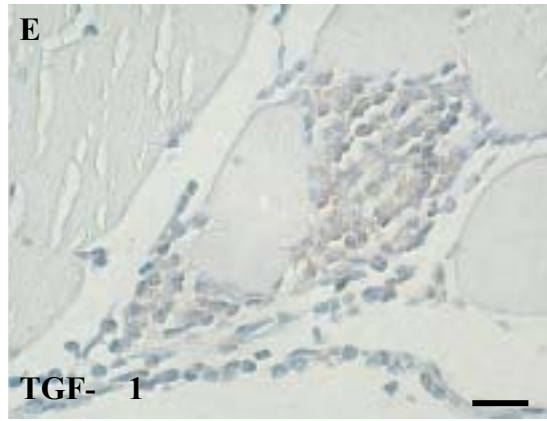
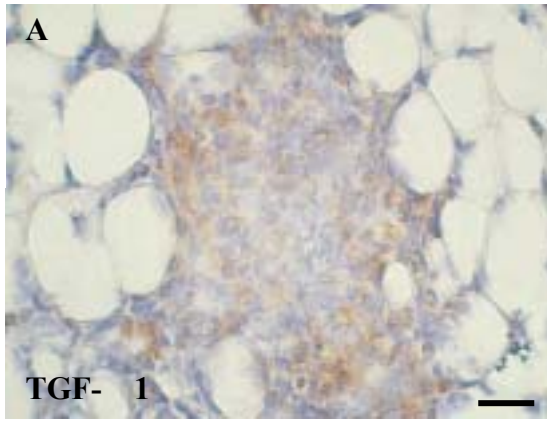
圖六.



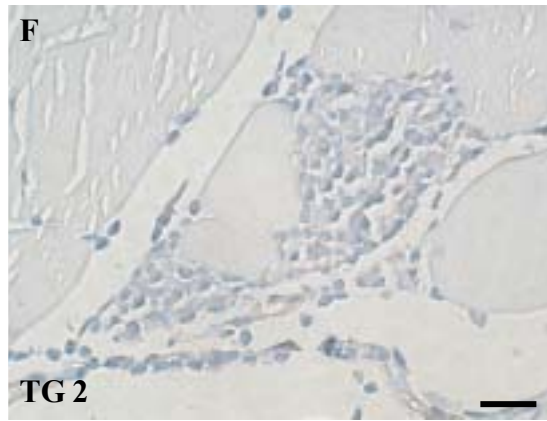
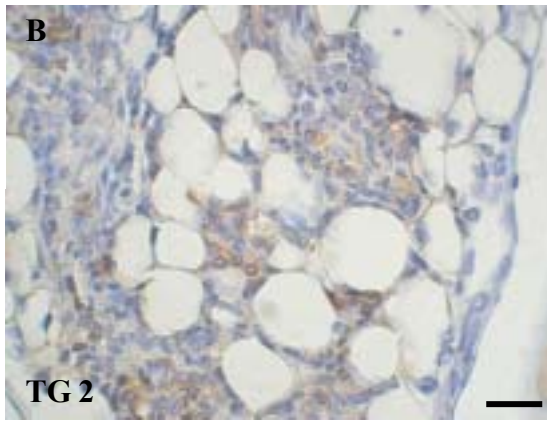
圖七. Loose connective tissue

Muscular area

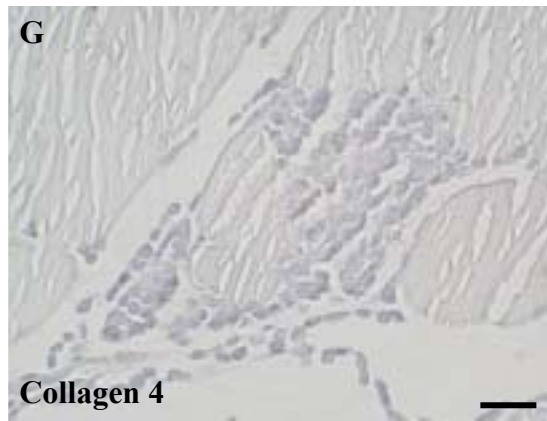
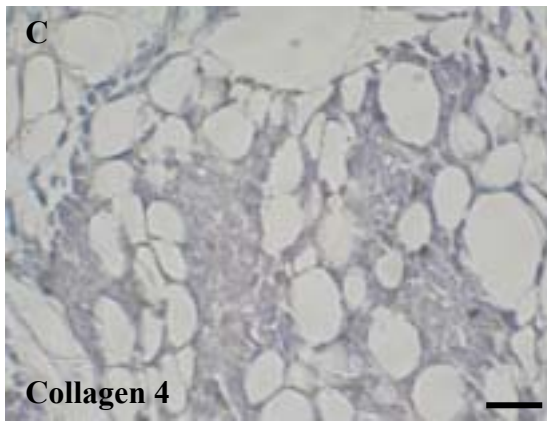
Week 1



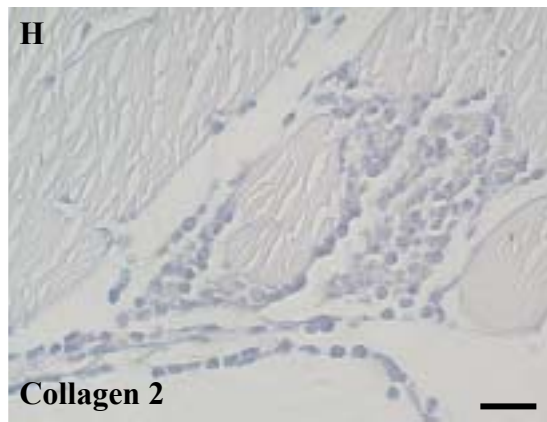
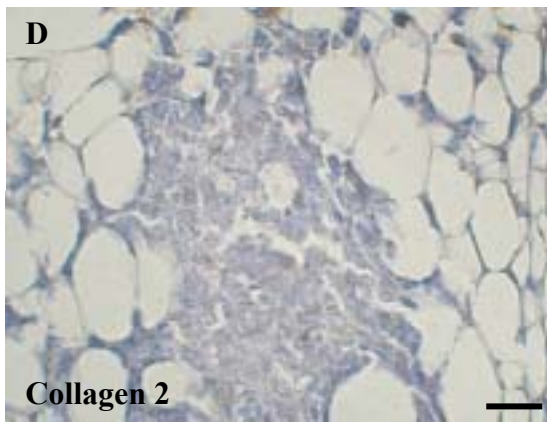
Week 1



Week 1



Week 1

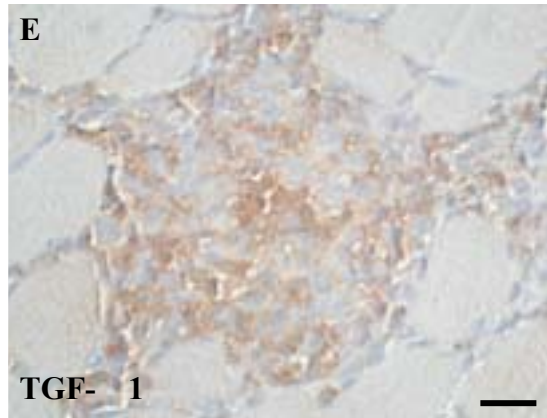
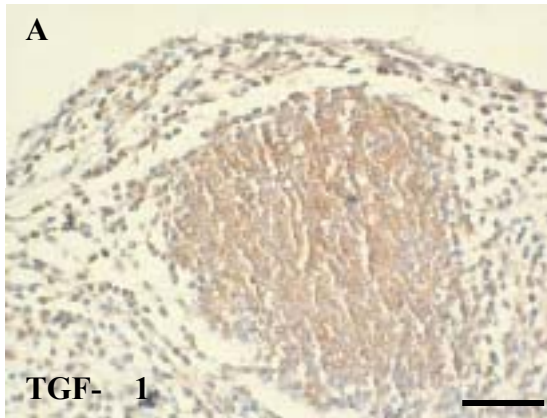


Scale bar= 20  $\mu$ m

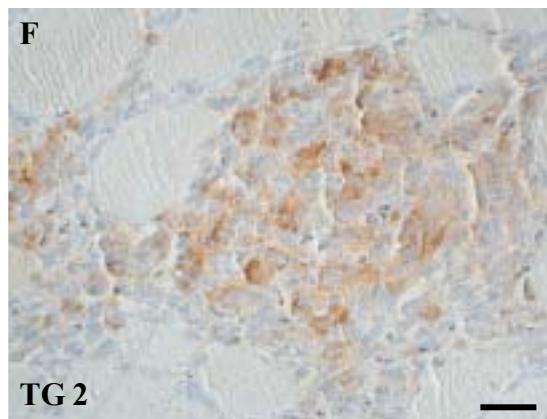
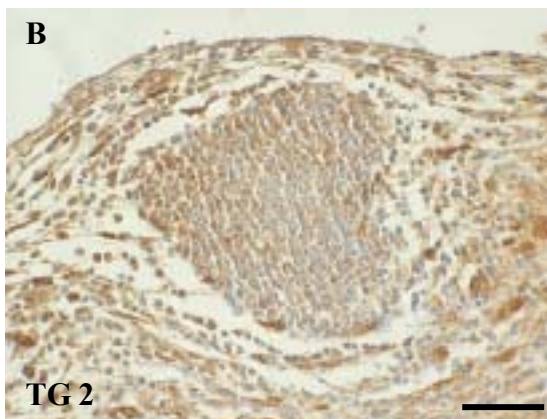
圖八. Loose connective tissue

Muscular area

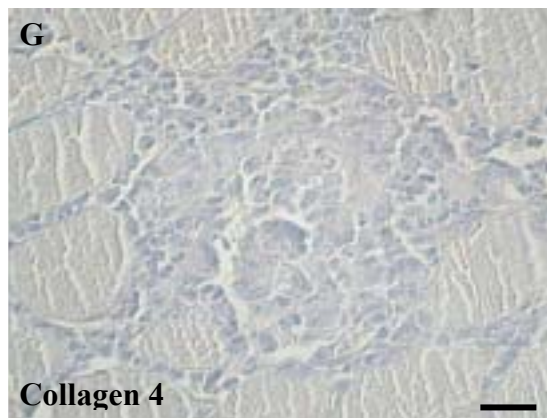
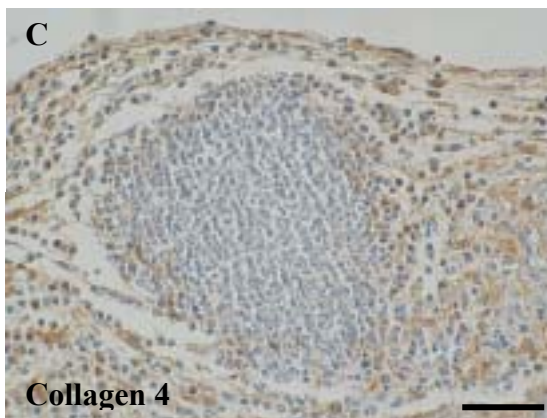
Week 2



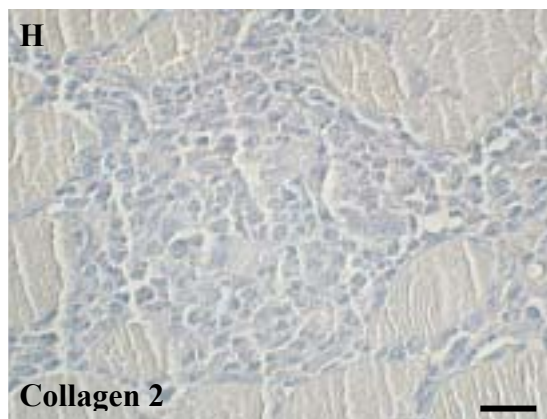
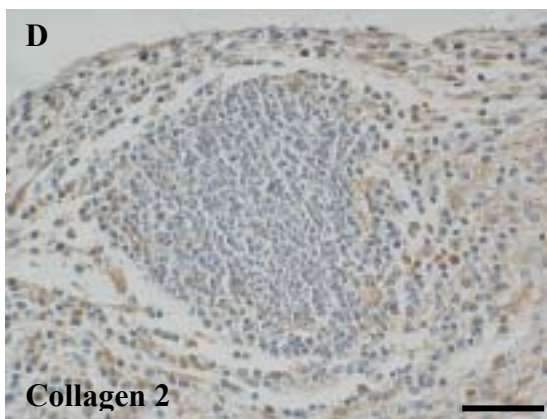
Week 2



Week 2



Week 2

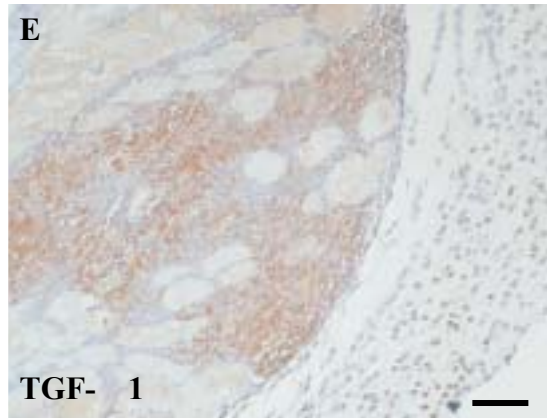
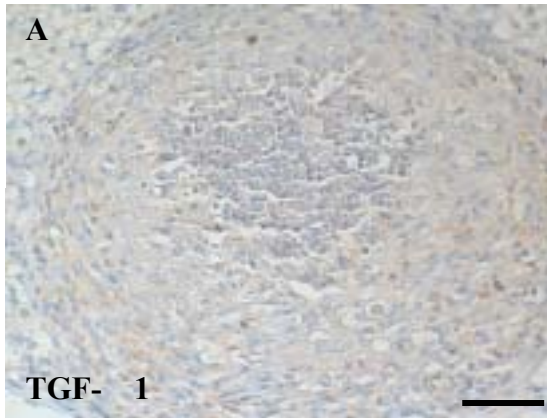


A-D scale bar=50  $\mu$ m; E-H scale bar= 20  $\mu$ m

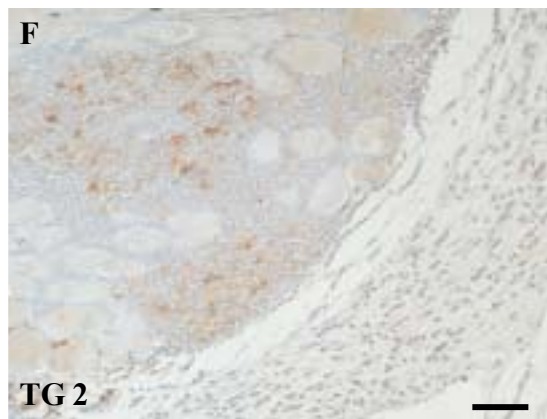
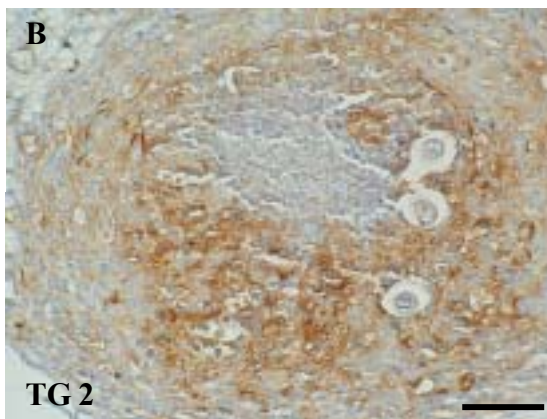
圖九. Loose connective tissue

Muscular area

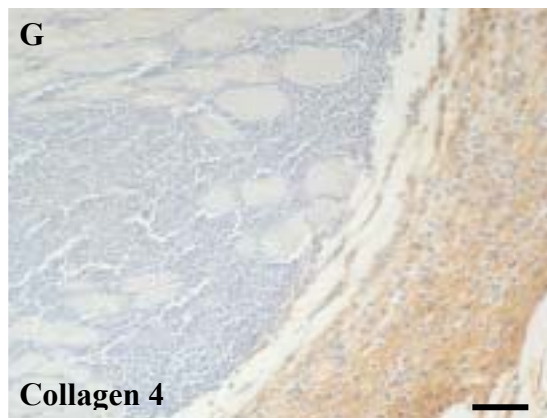
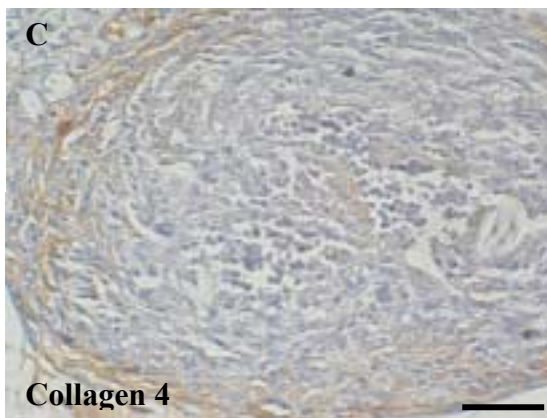
Week 4



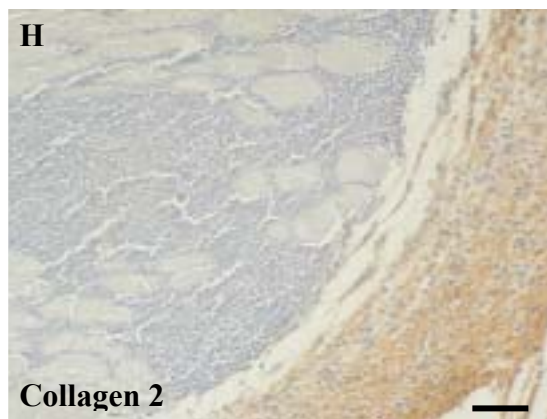
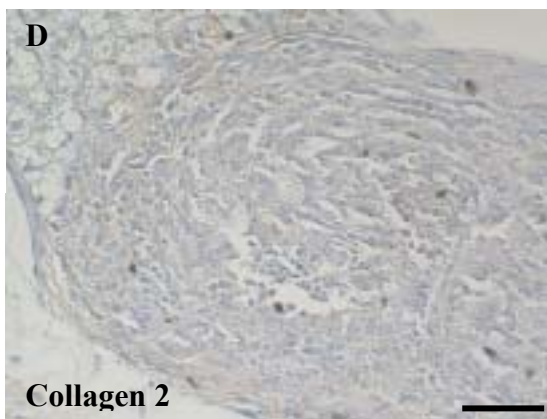
Week 4



Week 4



Week 4

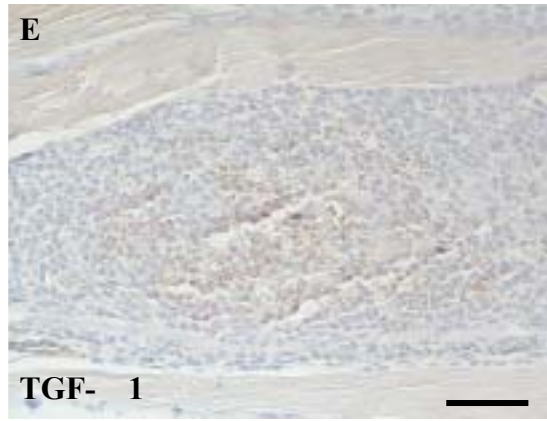
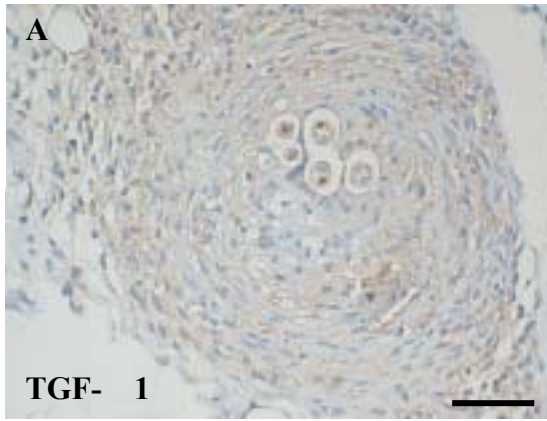


Scale bar=50  $\mu$ m

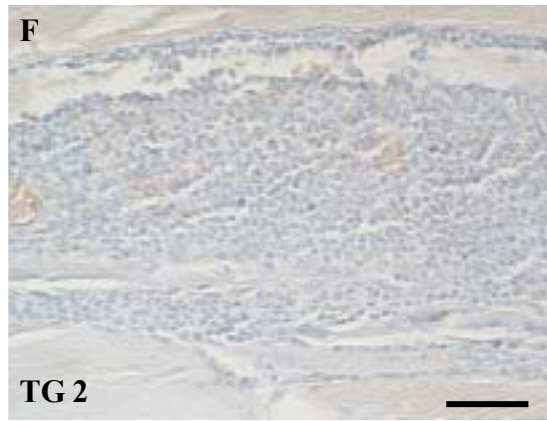
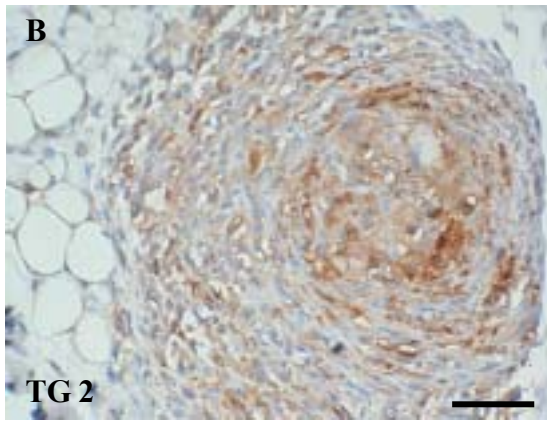
圖十. Loose connective tissue

Muscular area

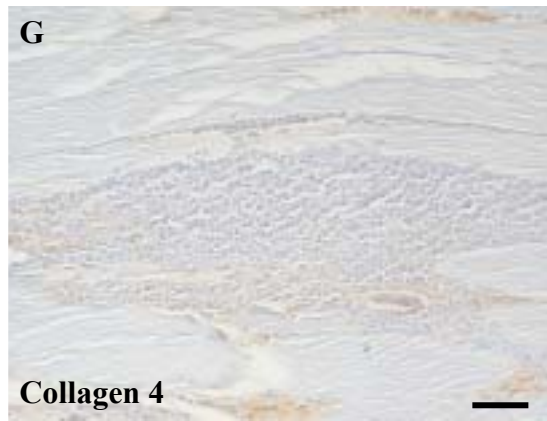
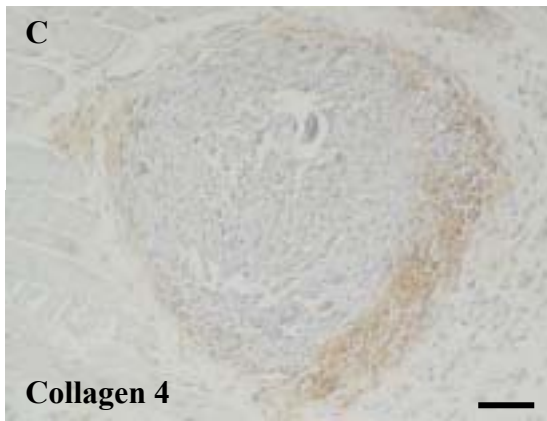
Week 8



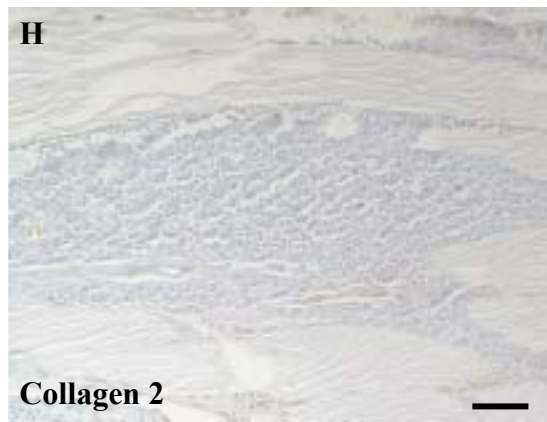
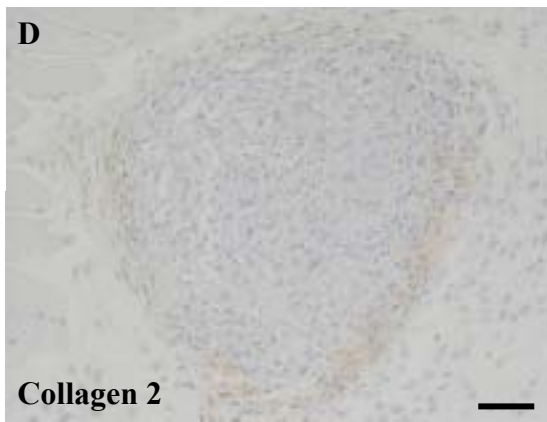
Week 8



Week 8



Week 8



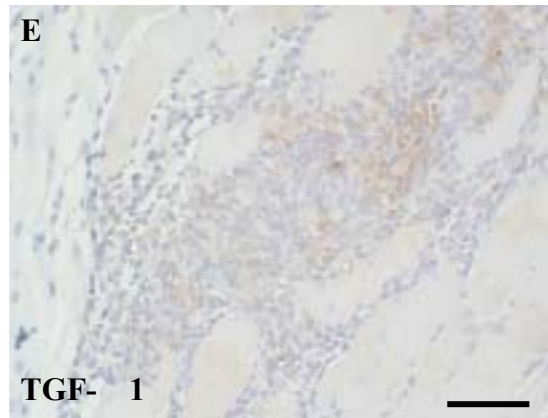
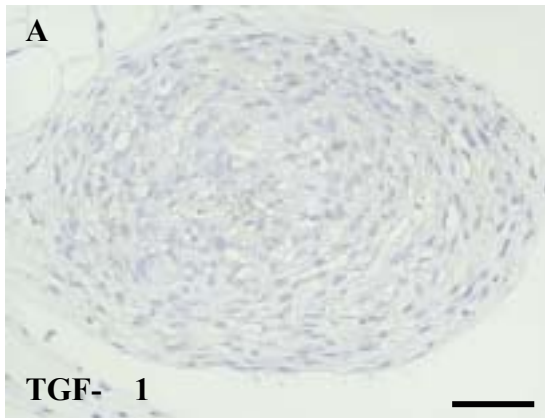
Scale bar=50  $\mu$ m



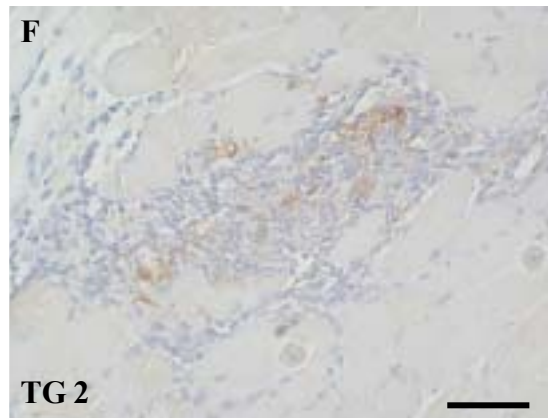
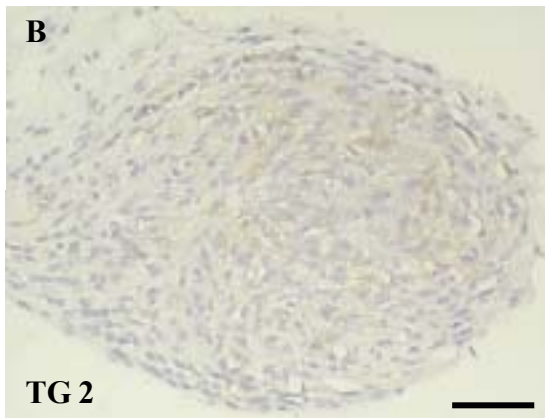
圖十一. Loose connective tissue

Muscular area

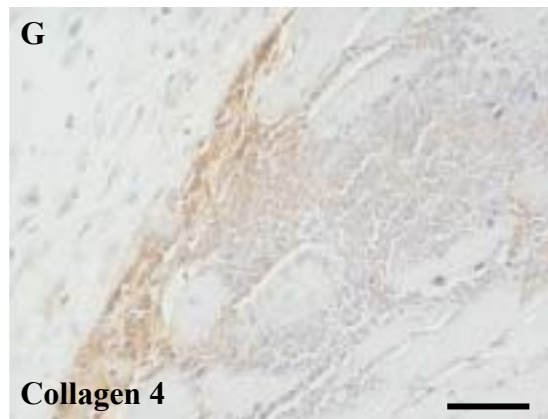
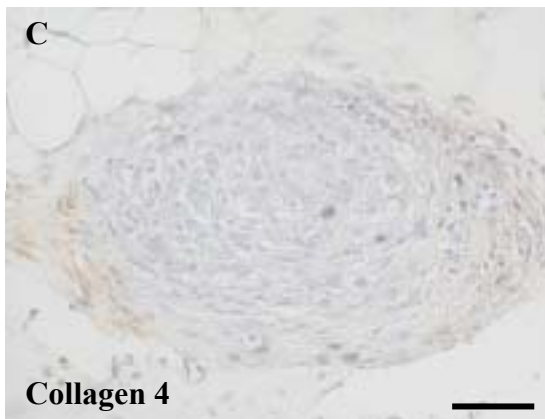
Week 12



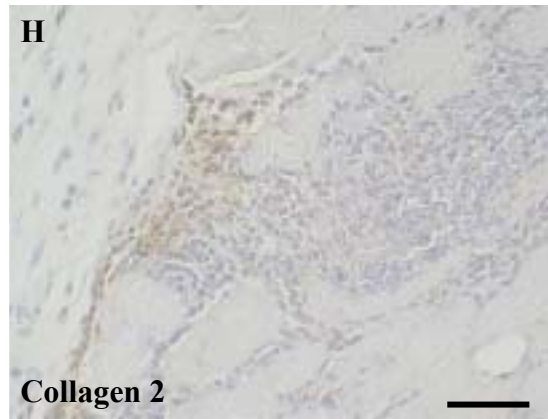
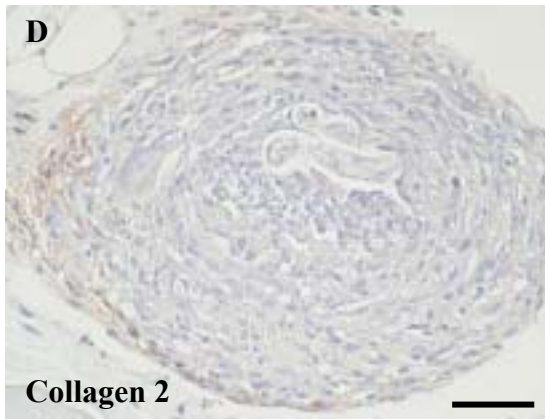
Week 12



Week 12

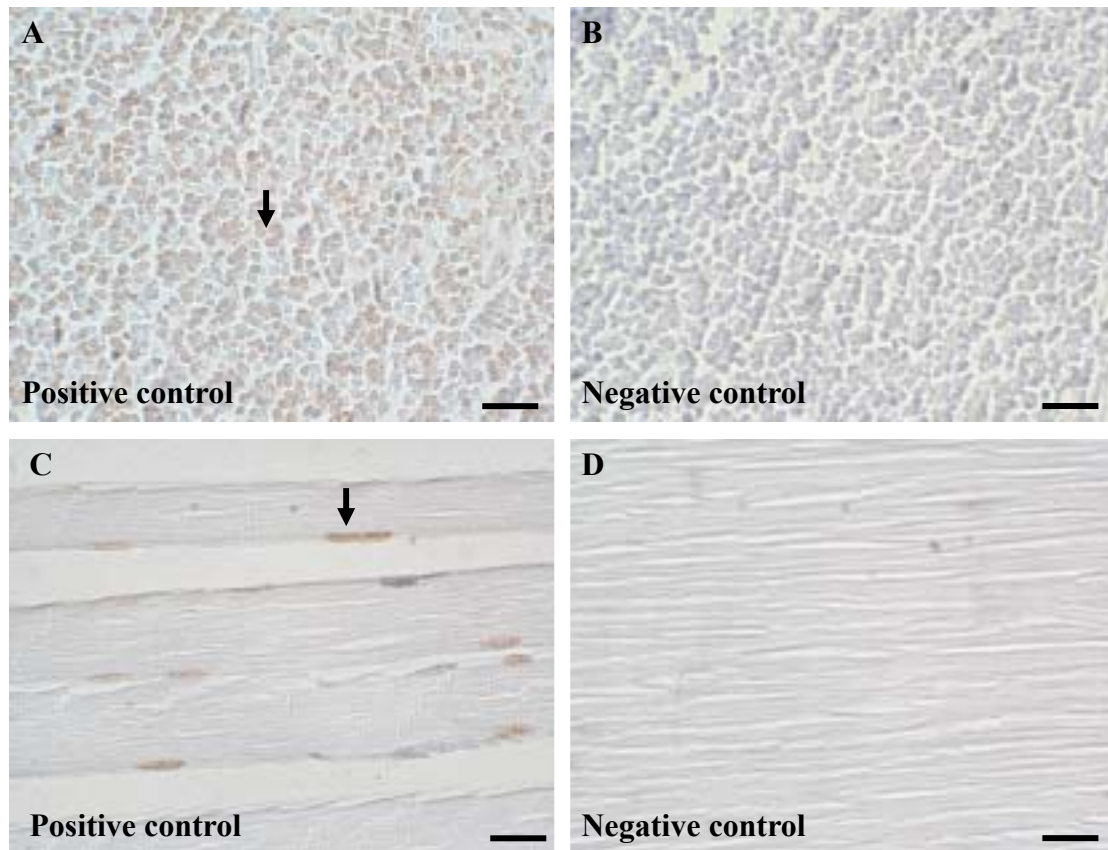


Week 12

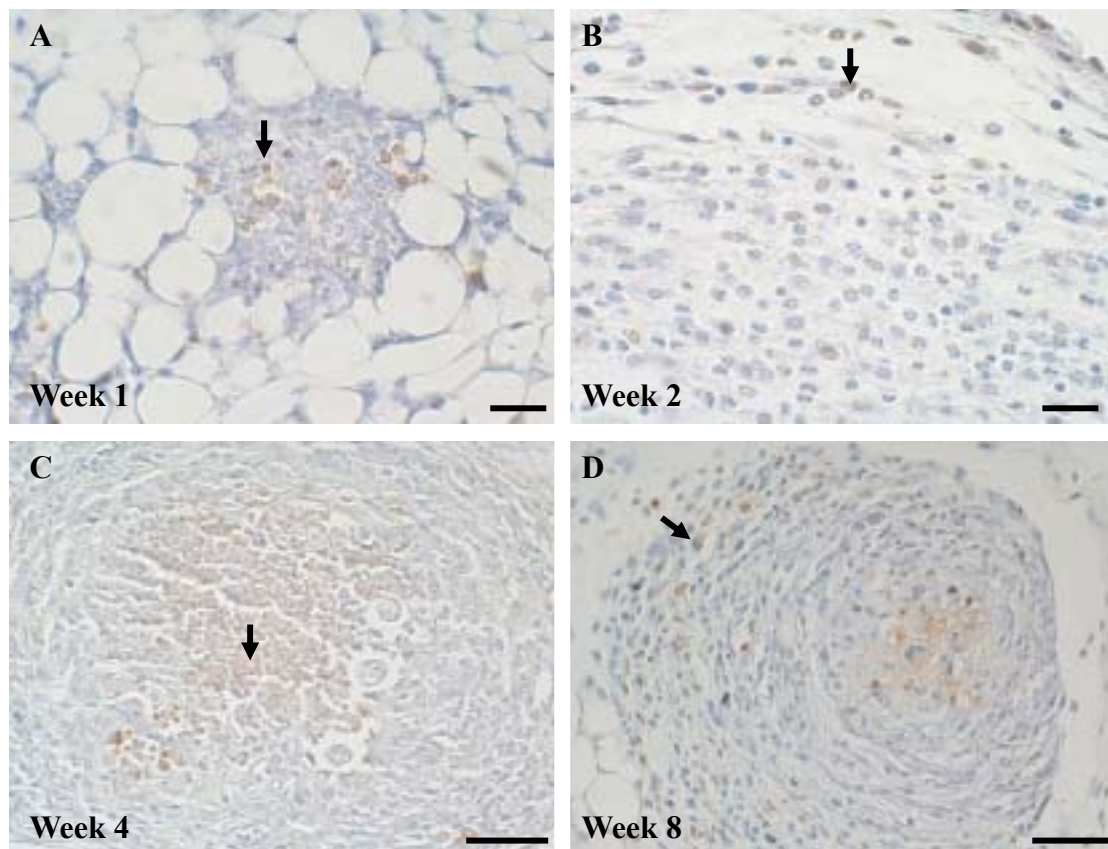


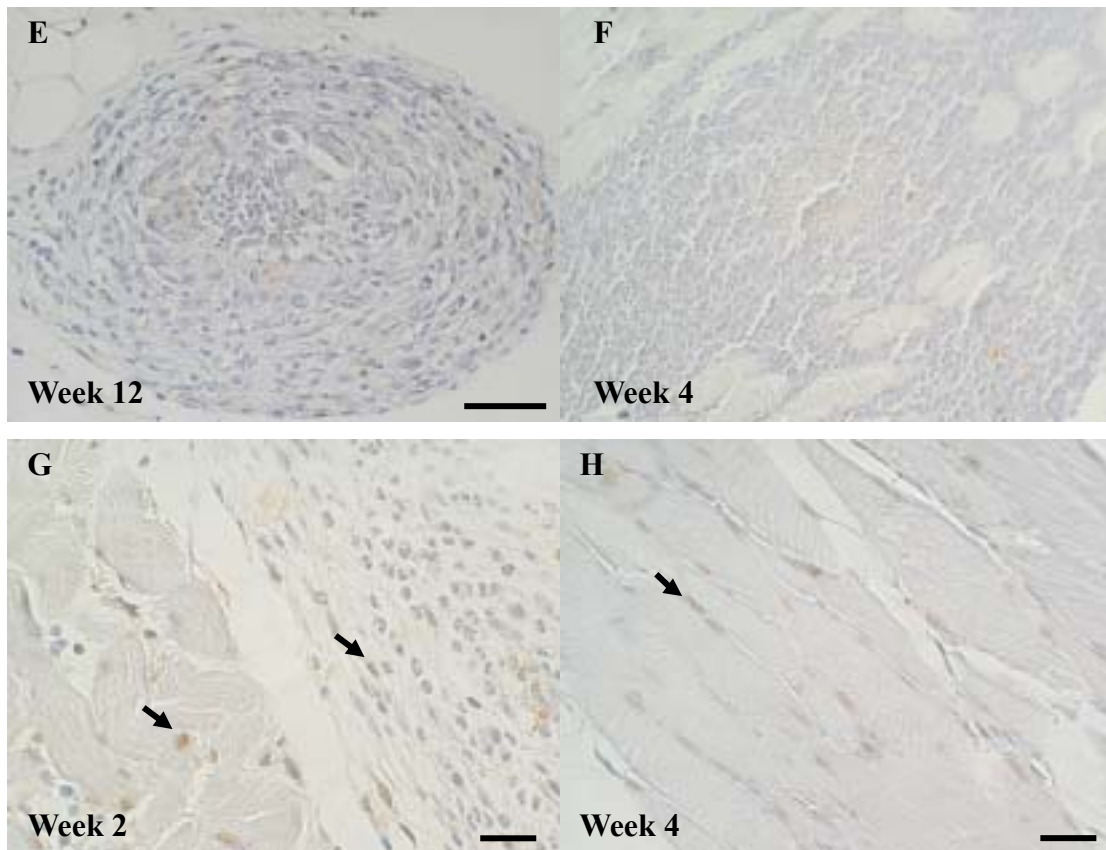
Scale bar=50  $\mu$ m

圖十二.



圖十三.





A, B, G and H scale bar=20  $\mu\text{m}$ ; C, D, E and F scale bar =50  $\mu\text{m}$