行政院國家科學委員會專題研究計畫　成果報告

利用磁振造影評估及追蹤以膠原蛋白模板種植後之膝關節半月板軟骨再生過程:動物實驗

計畫類別： 個別型計畫
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執行期間： 93 年 08 月 01 日至 94 年 07 月 31 日
執行單位： 臺北醫學大學醫學系

計畫主持人： 陳榮邦
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計畫參與人員： 林明芳

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中文摘要

目的：一種膠原蛋白模板做為膝關節的半月板代替物已研發成功，人體實驗前必須經由動物實驗以時序觀察半月板的再生過程，利用對軟組織具高解析度之磁振造影(MRI)技術係目前常用的非侵入性觀察方式。**方法：**本實驗包含 20 隻紐西蘭公白兔，分 4 組：(A)半月板切除術合併膠原蛋白模板種植—4 隻兔左膝外側半月板(B) 半月板切除術合併膠原蛋白模板種植—4 隻兔左膝內側半月板(C)半月板切除術 4 隻兔(D)Sham 手術—4 隻兔(E) 不做任何手術—4 隻兔。膠原蛋白模板由本院實驗室製作，術後第 3 至 8 週為 MR 觀察期，最後一次 MRI 實驗後做犧牲性實驗，MR 影像與組織切片以〝一對一〞方式做比較分析。**結果：**B 組有一隻兔子死亡。A 與 B 組 6 隻兔 MRI 影像顯示，半月板前角呈現均勻低訊號，表示組織再生良好，第 7 隻兔出現高訊號，表示再生失敗，與組織學所見吻合。C 組並未出現再生情形。**結論：**我們認為新研發的膠原蛋白模板做為膝關節軟骨的代替物，並以 MRI 觀察及追蹤組織之再生過程，對日後之膝關節軟骨之修復手術具革新性之影響與貢獻。

關鍵詞：膝關節、磁振造影、半月板、膠原蛋白模板、組織再生

Abstract

Purpose. To evaluate regeneration of injured menisci of the knee following reconstituted collagen template implantation in rabbits using magnetic resonance (MR) imaging and correlate these findings with histology. ***Materials and methods***. Twenty adult rabbits were divided into four groups: (**A**) Partial menisectomy with collagen template implantation of *lateral* meniscus of left knee (n = 4 rabbits); (**B**) Partial menisectomy with collagen template implantation of *medial* meniscus of left knee (n = 4 rabbits); (**C**) Partial menisectomy only (n = 4 rabbits); (**D**) Sham operation (n = 4); (**E**) No operation (n = 4). All rabbits received follow-up MR imaging studies with interval of 3 to 8 weeks after surgery. All rabbits were sacrificed after the last follow-up MR imaging for histologic analysis. ***Results.*** One rabbit in group B die. In Group A & B, follow-up MR imaging showed a homogenous low-signal-intensity in 6 of 7 menisci, suggestive of regeneration. The seventh rabbit showed that one meniscus exhibited higher in signal intensity at its anterior horn than that of the posterior horn meniscus, indicating failure of regeneration. The meniscus in group C animal showed no evidence of regeneration. All MR imaging findings were in agreement with histologic findings. ***Conclusion.*** Injured menisci of the knee can regenerate following implantation of a reconstituted collagen template in rabbits. MR imaging can be used to monitor and determine the degree of meniscal regeneration.

Key words: knee, magnetic resonance imaging, meniscus, reconstituted collagen template, regeneration

Introduction

Internal derangement is the most common disorders of the knee in human. Patients who have meniscal tears with persistent symptoms are commonly treated by menisectomy. Recent

advances of implementation of a synthetic meniscal implant or placement of autogenous tissue has been reported. Although autogenous tissue is biocompatible, the resorption of grafts can be unpredictable. The grafts can be difficult to shape and may cause functional and anatomical deformities. For these reasons, a variety of synthetic materials, such as Proplast-Teflon laminate, silicone, Silastic, have been applied. However, the implant material could be unable to resist the wear, shear, and tensile forces; or fibrosis growth around the implant materials that decreases the functional movement of joints; or host-foreign body reaction.

In our laboratory, a new disc regeneration implant material [1], consisting of reconstituted collagen template, has been successfully prepared and implanted in rabbit temporomandibular joint and the temporal healing process of discectomied temporomandibular joint has been reported [2]. Consequently, this study focused on the potential value of MR imaging to assess healing process of the discectomied knee after collagen template implantation in a rabbit model and correlation of MR findings with histology.

Materials and methods

Rabbits: A total of 20 adult New Zealand male rabbits were recruited. There were 3 months old and 2 to 3kg in weight. All rabbits were housed in well-ventilated cages and feed with a regular diet.

All 20 rabbits were divided into four groups as follows: (**A**) Partial menisectomy with collagen template implantation: resection of anterior 1/3 of *lateral* meniscus of left knee (n = 4 rabbits); (**B**) Partial menisectomy with collagen template implantation: resection of anterior 1/3 of *medial* meniscus of left knee (n = 4 rabbits); (**C**) Partial menisectomy only: resection of anterior 1/3 of *medial* meniscus of left knee (n = 4 rabbits); (**D**) Sham operation (n = 4); (**E**) No operation (n = 4). In group A & B, interval to obtain MR imaging were at week 3 and 8 in 4 rabbits, at week 5 and 8 in 2 rabbits, and at day 3 and week 8 in 2 rabbits. In group C, MR imaging was performed in week 3, 8 and 11. Each rabbit was sacrificed after the last follow-up MR imaging.

Surgery: The meniscus of left knee of each rabbit in experimental groups were shaved and prepared with povidone-iodine solution. We used a general anesthesia by using intramuscular injection of chanazine-ketamine (2:1) solution (1c.c./kg/hr) [1-3]. The medial joint line of the knee was incised. The overlying tissue was flapped inferiorly and the meniscus was exposed.

A resection of anterior horn (anterior 1/3 of the meniscus) with partial menisectomy were performed for group A, B, and C. The collagen templates (group A & B) were fixed to the rest of the meniscus using a 4.0 non-resorbable proline suture. The articular capsule was closed with a 4.0 non-resorbable suture. Finally, the skin incision was closed with stainless steel suture clips.

After surgery, the body weight of each rabbit was measured weekly to determine and monitor the health condition. After the last MR imaging, all rabbits were scarified by chanazine and ketamine anaesthesia. All specimens of the knee with or without implants were excised and processed for histologic evaluation.

Templates: The collagen templates were freshly prepared and obtained from pepsinized bovine type I collagen, which were reduced by beta-mecraptoethanol and reconstituted by glutaradehyde. The templates were composed of

collagen-glycosaminoglycan complex that were yield cross-linkage and possess maximum biological activity (Fig. 1). The collagen-based templates were shaped as a "disc" with 5-mm diameter and 1.5-mm thick. The collagen templates were made at Research Center for Biomedical Materials, Taipei Medical University, Taipei.

MR Imaging: MR imaging was performed with use of a commercial surface coil. Imaging parameters included: localizer T1-weighted images (TR=300msec, TE=20 msec); sagittal T1-weighted images (TR=100–320msec, TE=20msec) of left / right knee, 8-cm field-of-view (FOV), 1.0–1.5-mm slice thickness, 192-224 × 224–cm matrix, 3-4 excitations; sagittal T2*-weighted images (TR=450msec, TE=30msec, Flip angle=20^0), 2-cm FOV, 1.0–1.5-mm slice thickness, 224 × 224–cm matrix, 3 excitations.

Two experienced radiologists read all MR images of each rabbit. Parameters for interpretation included MR imaging signal changes on T1- and T2*-weighted images as score 0 = low signal intensity similar to native meniscus; score 2 = signal changes (low signal intensity on T1-weighted images and high signal intensity on T2*-weighted images) suggestive fibrosis or granulation; and score 1 denotes indeterminate. Morphologic changes were defined as follows: tissue regeneration patterns similar to native meniscus in shape and margin (score 0), formless / structureless (score 2), and score 1 denotes indeterminate.

Histology: Each specimen was fixed in formalin, embedded in paraffin and serially sectioned at 5–10microns. Specimens were stained with hematoxylin/eosin and Masson trichrome. One experienced observer interpreted the histology for the regeneration of the meniscus and / or fibroblasts infiltration into implant materials.

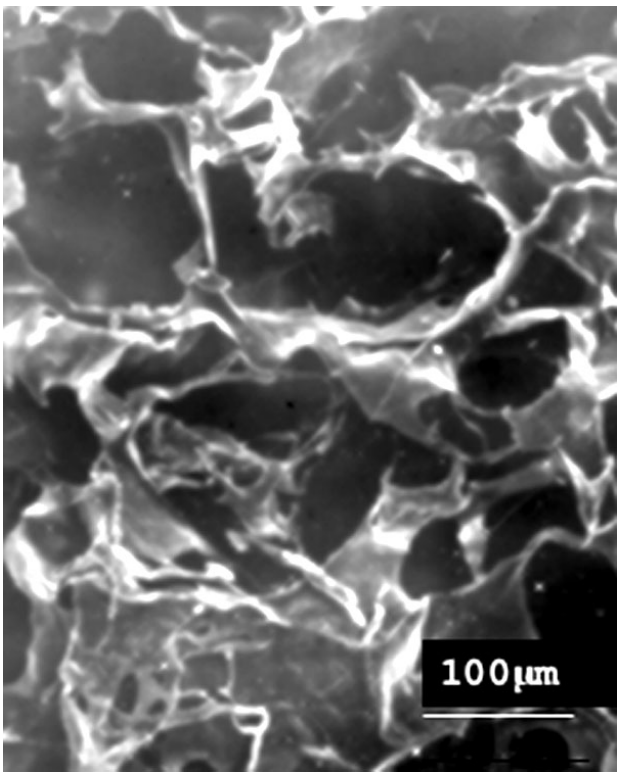

Figure 1. Reconstituted collagen template. The reconstituted collagen forms a nest-like scaffold template after lyophilization, resembling a rounded and randomly arranged network of tissue (scale as indicated).

Results

One rabbit in group B die during the experimental period. In group A & B, the weight of 6 rabbits gained from zero to 53%, one rabbit weight loss 17%. Group C rabbits loss 7.5% weight. In group D & E, MR images showed well-delineated opposite triangular morphology of low-signal-intensity meniscus in sagittal plane. Histology revealed that the meniscus composed of dense connective tissue. There was no difference in group A & B. Their follow-up MR images showed a homogenous low-signal-intensity in 6 of 7 menisci (Fig. 2), suggestive of regeneration. Of these 6 menisci, one meniscus depicted irregular margin at week 8 follow up,

one showed bigger size of the anterior horn with regeneration at week 8 after operation (Fig. 3). The seventh rabbit showed that one meniscus exhibited higher in signal intensity at its anterior horn than that of the control meniscus, indicating failure of regeneration, compatible with its weight loss during the experimental period. Histology revealed that the regenerative menisci had new collagen bundles (Fig. 4). Primitive mesenchymal cells scattered in the new matured collagen bundles. The failure regenerative meniscus showed proliferation of chondroblasts and neovascularization of the meniscus. In group C, MR images showed no detectable anterior horn of the menisci. All MR imaging findings were in agreement with the histologic findings.

Discussion

MR imaging is gaining recognition as an imaging modality of choice for internal derangement of the knee. In human, the MR signal characteristics of menisci of the knee are those of fibrocartilage with very low signal intensity on T1- and T2-weighted images. We have preformed MR imaging of temporomandibular joint discs in rabbits with the use of commercial surface coil. The MR resolution has been promising for both normal discs and regeneration of the discs.

The collagen template material was relatively inert, easy to shape and to adapt to the structure required, and to be sterilized. Results of the present study indicated that the collagen template matrix could guide knee meniscal regeneration after partial menisectomy. The fibrocartilage-like tissue grew into the knee meniscus in the implant groups, whereas fibrosis and no growth of menisci were noted in the menisectomized group. No immunoresponse in the implant groups was observed.

Alteration of the distribution of proteo-

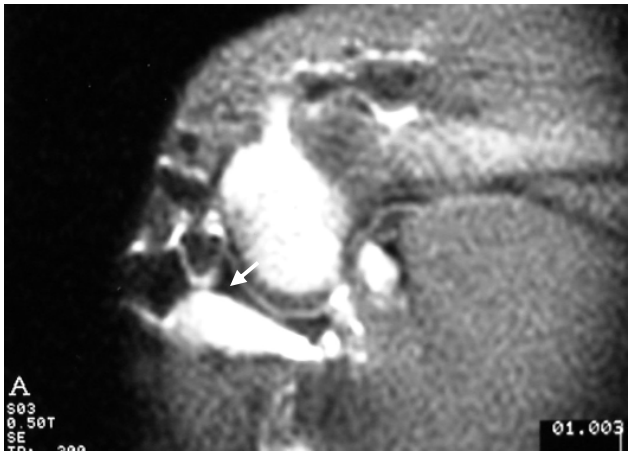

Figure 2. Partial discectomy with collagen template implantation: complete regeneration. Follow-up T1-weighted MR image (TR=300 ms, TE=20 ms) of left knee 8 weeks after partial menisectomy (anterior horn) shows a homogeneous low signal intensity of the regenerative anterior horn of the meniscus (arrow). Note that no signal intensity difference of the regenerative meniscus as compared to the normal posterior horn of the meniscus.

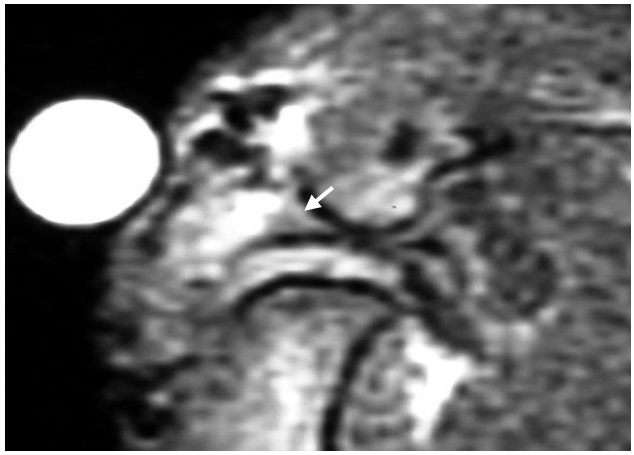

Figure 3. Partial discectomy with collagen template implantation: no regeneration. Follow-up T1-weighted MR image (TR=300 ms, TE=20 ms) of left knee 8 weeks after partial menisectomy (anterior horn) shows a heterogenous high signal intensity of the anterior horn of the meniscus (arrow). Note that signal intensity difference of the failure regeneration of the anterior meniscus as compared to the normal posterior horn of the meniscus.

Figure 4. New collagen bundles exhibit moderate proliferation of chondroblasts and neovascularization in the regenerative meniscus. (H-E stain, magnification ×60)

glycans and water content can contribute to the MR imaging signal changes of the menisci of the knee. Our findings may support, in part, the observation by previous authors [4] that the MR imaging appearance of temporomandibular discs and template correlated well with the increase or decrease of glycosaminoglycans. In our study, all normal menisci depicted low signal intensity indicating predominant proteoglycans. A normal disc is composed of connective tissue with synovial fibroblasts and chondrocytes/ chondroblasts.

In our study, the partially regenerative discs appeared higher signal intensity than the original meniscus, and chondroblast proliferation was also observed. In contrast, the number of new collagen bundles increased and the signal intensity of the regenerative disc decreased. The inflammatory tissue had high signal intensity on T2-weighted images. Further study with dynamic contrast-enhanced MR imaging are needed for differentiation of fibrosis and regeneration.

In summary, menisectomized knee can regenerate following implantation of a reconstituted collagen template in rabbits. MR imaging can be used to monitor and determine the degree of meniscal regeneration.

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研究自評

原申請計畫為 2 年期，因核准一年期因而調整減少兔子數目，但 MRI 追蹤次數仍維持 3 次至 8 週才撲殺。本研究完成一種膠原蛋白模板的製作，並成功植入兔子膝關節半月板切除的空隙，實驗組顯示半月板組織再生與 MRI 影像相符，並已初步達到 100 %預期目標，因此即將投稿 SCI 期刊。同時亦訓練出一名兼具生物與 MRI 技術專業的研究人員，目前已投入本校生物技術實驗室繼續進修中。