Self-assembling β-hairpin peptide hydrogel scaffold for meniscal defect

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INTRODUCTION

Meniscal tear is the most common intra-articular knee injury. As it is poorly vascularized, the inner portion of the meniscus has limited healing capacity[1, 2]. Partial meniscectomy has been a widely accepted therapy for meniscal tear. However, biomechanical changes are inevitable after meniscectomy. Multiple meniscal repair techniques andscaffolds have been developed. Therefore, new approaches are required for meniscal repair and regeneration. Significant progress in tissue engineering has generated useful biomaterials. Self-assembling peptide scaffolds (SAPS) are candidates for meniscus repair and regeneration. Hydrogels are preferred in meniscus tissue engineering studies as they fill in meniscal lesions of various shapes[3].

We had improved SAPS stiffness and developed KI24RGDS (IKIKIKIKIK-RGDS-KIKIKIKIKI), a β-hairpin peptide with the amino acid sequence, RGDS[4,5].

The purpose of this study was to determine whether KI24RGDS stays in a meniscal defect and facilitates meniscal repair and regeneration in a rabbit model.

METHOD

KI24RGDS peptide was synthesized on Alko-PEG resin using a standard manual Fmoc-protocol
with a DMT-MM activation procedure. The crude KI24RGDS peptide was purified and analysed by high-performance liquid chromatography.
To prepare a KI24RGDS hydrogel, pure KI24RGDS peptide was dissolved in MilliQ water, then an equal volume of phosphate-buffered saline was added to adjust the peptide concentration. All rheological studies were performed on a rheometer at 25 °C.

The design of the present study was approved by the Animal Research Committee of Osaka Medical College(No.30122). All medial menisci were photographed before fixation with paraformaldehyde. Each specimen was sliced in the
radial plane and then stained with hematoxylin-eosin (H&E) and safranin O.

RESULTS

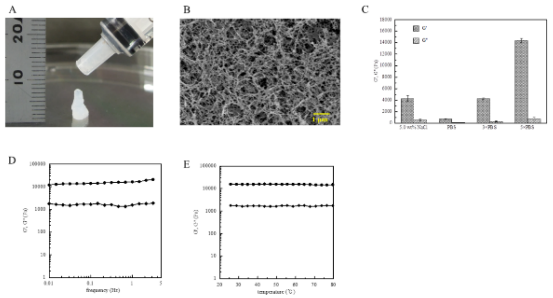

Figure 1 (A) Gross observation of KI24RGDS. It is readily injectable by syringe yet viscous enough to stay at the site of injection.
(B) SEM image of 3% (w/v) KI24RGDS hydrogel. Scale bar = 1 μm.
(C) Mechanical properties of KI24RGDS hydrogel under several solvent conditions.
(D) Relationship between modulus ratio (G'/G") and frequency sweep for 3% (w/v) KI24RGDS hydrogel in 5× PBS (●: G'; ◆: G"). (E) Relationship between modulus ratio (G'/G") and temperature for 3% (w/v) KI24RGDS hydrogel in 5× PBS (●: G'; ◆: G").

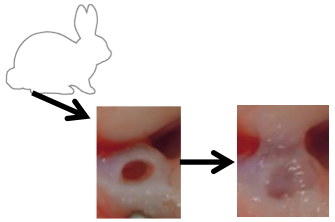

Figure 2 A 2.0 mm-diameter cylindrical defect was created by biopsy punch in the inner 2/3rds of the anterior portion of rabbit medial meniscus (left knee).

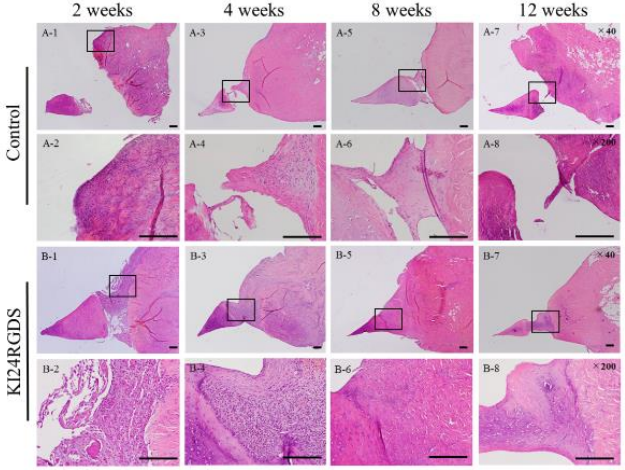

Figure 3 Representative H&E (A, B) at 2 wks, 4 wks, 8 wks, and 12 wks post-surgery. Microphotograph of the entire meniscus at ×40 magnification. Boxed areas are ×200 magnification. Scale bar = 200 μm. In the control group, there was no reparative tissue in the defect at 2 wks post-surgery (A-1, 2). Cells migrated from the peripheral slowly , and regenerated at 4 wks and 8 wks post-surgery (A-3–6). However, the meniscal defect was still only partially filled even at 12 wks after surgery (A-7,8). In the KI24RGDS group, the SAPS remained in the defect and was visible along with reparative tissue at all evaluation time points (B). Cell migration from the peripheral tissue and proliferation were observed at 2 wks post-surgery (B- 1, 2). Reparative tissue gradually increased at 4 wks and 8 wks post-surgery (B-3–6).

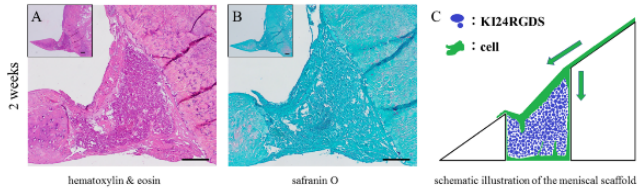

Figure 4 Histology of the KI24RGDS group at 2 wks after implantation (A, B). The cylindrical defect was filled with KI24RGDS, which served as a meniscal scaffold for cell migration. (A) H&E staining, (B) safranin O staining, and (C) schematic diagram of the meniscal scaffold and cell migration from the peripheral tissue. Scale bar = 200 μm.

CONCLUSIONS

This in vivo study demonstrated that KI24RGDS remained in the meniscal lesion and facilitated the repair and regeneration of a rabbit meniscal defect model. KI24RGDS is highly biocompatible and biodegradable, has strong stiffness, and is safe. Thus, it is feasible for clinical use in meniscal repair and regeneration. Future efforts to improve SAPS stiffness of SAPS could broaden the scope of its clinical applications in the treatment of human organ and tissue injury and disease.

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