Effect of Gelatin Hydrogel Incorporating Fibroblast Growth Factor 2 on Human Meniscal Cells in an Organ Culture Model

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INTRODUCTION

The meniscus has a low healing potential, and in some cases, primary repair has been unsuccessful. Fibroblast growth factor 2 (FGF 2) is well known to stimulate fibroblast proliferation and enhancement of collagen synthesis. However, tissue regeneration attempts using FGF 2 alone are not always successful, because the half-life of FGF 2 is too short to sustain biological activity. To correct for this problem, we developed a controlled release system using gelatin hydrogel [1]. FGF 2 is immobilized in gelatin hydrogel through a physicochemical interaction with gelatin molecules, and the immobilized FGF 2 is released from the hydrogel as a result of hydrogel degradation. The purpose of this study was to investigate the effects of gelatin hydrogels incorporating FGF 2 on meniscal cells in organ culture.

MATERIALS AND METHODS

This study was performed after institutional review board approval was obtained. 25 total knee arthroplasties were performed in 20 patients for medial compartment osteoarthritis. Mean age of the patients was 75.7 years. The 25 lateral menisci were sampled aseptically. Each meniscus was cut into small pieces, 6 mm in width. 3 consecutive pieces taken out from each meniscus constituted a single set (Fig. 1). The middle piece of each set was assigned to the “non-culture group”, one of the remaining two pieces was assigned to the “FGF(+) culture group”, and the remaining piece was assigned to the “FGF(-) culture group”. There were 93 pieces constituting 31 sets. The 31 FGF(+) pieces and the 31 FGF(-) pieces were treated with meniscal suture and organ culture. The 31 non-culture group pieces were evaluated histologically to assess meniscal degeneration. If a sample had severe degeneration (distinct cleavage, fibrocartilaginous separation), all three specimens in that set were excluded from further evaluation. A 2-9 braided nylon thread was coated with gelatin hydrogel, as previously reported [2]. Meniscal suture was performed on the FGF(+) culture groups. The gelatin hydrogel-coated thread was soaked in 400 μg/mL of either FGF 2 solution (FGF(+) culture group) or physiologic saline (FGF(-) culture group). The meniscal suture was performed according to the inside-out technique. The histological sections are oriented horizontally, parallel to the plane of the thread (Fig. 2). Organ culture initiated immediately after the meniscal suture. Each of the meniscal specimens was placed into a well on a 12-well culture plate and cultured in Ham’s F12 medium containing 10% fetal bovine serum. The samples were cultured for 4, 7, and 14 days. The cell density, the number of PCNA-positive cells and TUNEL positive cells in the FGF(+)/(−) culture groups was measured 1 mm from the peripheral rim of the meniscus, as shown in Fig. 3. In both culture groups, we tested four “contact areas” touching the thread, and one “distant area” distant from the thread. Statistical analyses of the data were carried out using Student’s t-test. Differences of P < 0.05 were considered to be significant.

RESULTS

In histological evaluation of the non-culture group, severe degeneration was observed in 5 pieces. Thus 15 specimens comprising the 5 sets were excluded from further evaluation. Of the remaining 26 sets undergoing further study, 10 sets of FGF(+)/(−) groups had been cultured for 4 days, seven sets for 7 days, and nine sets for 14 days. After culture for 4 days, the cell density of the contact area was significantly higher in the FGF(+) group (107.8±34.8/mm²) than in the FGF(−) group (81.6±51.8/mm²) (Fig. 4, 7). After 7 days, the cell density of the contact area was significantly higher in the FGF(+) group (103.4±40.6/mm²) than in the FGF(−) group (76.9±33.9/mm²). Within the FGF(+) group, the cell density was significantly higher in the contact area than in the distant area (74.6±13.8/mm²). The number of PCNA-positive cells tended to be slightly higher in the FGF(+) group than in the FGF(−) group. After culture for 7 days, the number of PCNA-positive cells in the contact area was significantly higher in the FGF(+) group than in the FGF(−) group (Fig. 5).

The number of TUNEL-positive cells tended to be slightly lower in the FGF(+) group than in the FGF(−) group. After 4, 7 and 14 days, the number of TUNEL-positive cells in the contact area was significantly lower in the FGF(+) group than in the FGF(−) group (Fig 7, 8).

DISCUSSION

Our results suggest that FGF 2 stimulates the proliferation of meniscal cells, thereby increasing their number, and inhibits DNA fragmentation. It is also suggested that FGF 2 incorporated in gelatin hydrogel-coated thread maintains biological activity and has the potential to promote the repair of human meniscus in vitro. Further studies are needed to clarify the optimal method of using growth factors in the treatment of human meniscal repair, although we believed that FGF 2 incorporated gelatin hydrogels is an effective biomaterial for meniscal repair.

REFERENCES