Introduction: The healing potential of articular cartilage in response to injury is poor (1). Because of the lack of blood vessels and perichondrium. Unlike articular cartilage, costal cartilage is covered with a vascularized perichondrium. Perichondrium is well known to have a chondrogenic potential (2). In skeletal development, undifferentiated mesenchymal cells derived from the perichondrium condense, proliferate and differentiate into cartilage. Therefore, costal cartilage is thought to have a better healing potential than articular cartilage. However, little is known about the biological responses of costal cartilage to mechanical injury. Several studies have investigated cartilage matrix damage and chondrocyte death in response to mechanical injury (3)(4). CD44 is a cell-surface receptor for hyaluronic acid that plays a crucial role in cell migration (5). Whether costal cartilage can recombine at the site of a dissection injury is unknown. The aim of the present study was to investigate the healing process in costal cartilage subjected to mechanical injury in mice.

Methods: ICR mice aged 5 weeks were used. Under the anesthesia, the left tenth costal cartilage was sharply dissected using microscissors. At 1 day, 1 week, 2 weeks, 3 weeks, and 12 weeks after injury, the mice were sacrificed, and the cartilage from ribs 9 to 11 were fixed with 4% paraformaldehyde overnight. After demineralization with EDTA for 3-6 days, paraffin sections were prepared by standard histological procedures. The samples were coronary sectioned at thickness of 4µm. The sections were stained with safranin O fast green hematoxylin. Type II collagen mRNA expression was examined for in situ hybridization. Cell proliferation was detected using the BrdU monoclonal antibody. TUNEL method was performed using an Apop Tag Kit. Immunohistochemical staining for CD44 was performed using an ABC Kit.

Results: One day after the injury, a gap was detected at the injury site. BrdU-positive cells and CD44 were detected in perichondrium and surrounding tissues. TUNEL-positive cell were detected at the ends of the cartilage fragments.

One week after the injury, granulation tissue containing numerous cells was observed between the dissected cartilage fragments (Fig 1A). BrdU-positive cells were detected in the perichondrium and the granulation tissue (Fig 1B). TUNEL-positive cells and CD44 were also detected in the granulation tissue.

Two weeks after the injury, repair tissue was seen at the site of the injury, and both ends of the dissected cartilage fragments had combined. The tissue was stained with safranin O and was covered with a thick membrane-like perichondrium (Fig 2A). Type II collagen mRNA was strongly expressed in the cells of the safranin O-positive repair tissue (Fig 2B). BrdU-positive cells were detected in the perichondrium (Fig 2C). TUNEL-positive cells were detected in the newly formed cartilaginous tissue (Fig 2D).

At three and twelve weeks after the injury, the amount of repair tissue had decreased; the cartilage ends had not recombined, but formed a pseudo-articulation with a cavity (Fig 3A). A few TUNEL-positive cells were detected at the ends of the cartilage fragments. CD44 was detected in the cells on the cavity surface (Fig 3B).

Discussion: Two weeks after the induction of costal cartilage injuries in mice, the dissected cartilage fragments had combined with newly formed safranin O-positive tissue. Type II collagen mRNA was strongly expressed in the cells of the newly formed tissue. These results indicate that costal cartilage is capable of healing through the formation of new cartilaginous tissue.

The perichondrium was thickened, and several BrdU-positive cells were visible from one day to two weeks after the injury. These results suggest that the perichondrium may have an important role in cartilage formation after costal cartilage injury.

Despite the early biological response, the newly formed cartilaginous tissue was not sufficient to enable the cartilage fragments to recombine by 3 to 12 weeks after the injury. CD44 was detected locally on the surface of the cavity between the cartilage fragments. CD44 expression can be induced by mechanical stress. The ends of the fragments may have been subjected to abnormal movement, resulting in the formation of a pseudoarticulation. These results indicate that the newly formed cartilaginous tissue was insufficient to support the recombination of the dissected ends.

TUNEL-positive cells were detected in chondrocytes in the newly formed cartilaginous tissue from 2 to 3 weeks after injury. These results indicate that the number of chondrocytes in the newly formed tissue can be decreased by apoptosis, preventing the cartilage injury from healing and leading to pseudo-articulation.