Methods:  These findings suggest that Sox9 may be an ideal candidate to differentiate into chondrocytes and express chondrocyte marker genes. Human articular chondrocytes with adenoviral vectors expressing Sox9 were efficiently infected with the adenoviral vectors expressing Sox9 and GFP. Type II collagen production was measured using RT-PCR and immunohistochemical analyses. We hypothesize that an adenoviral vector expressing Sox9 will efficiently infect articular chondrocytes in vitro, leading to increased type II collagen production. To test this hypothesis, we infected cultured human articular chondrocytes with adenoviral vectors expressing Sox9 and GFP. Type II collagen production was measured using RT-PCR and immunohistochemical analyses.

Discussion: The data reported in this study provide important insights into the use of Sox9 to augment the healing of articular cartilage injuries. Earlier investigations have generated a large body of evidence that attributes to the role that Sox9 plays as a "master regulator" of chondrogenesis, and specifically, as a stimulator of type II collagen synthesis. The observation that Sox9 expression is increased in the early stages of articular cartilage injury, and appears to be expressed only in those chondrocytes undergoing proliferation, is further evidence that Sox9 is a critical mediator of the healing response mounted by articular chondrocytes in response to injury.

Previous attempts to augment articular cartilage healing have focused on the use of bone morphogenetic proteins (BMPs), particularly BMP2. While BMP2 has potent chondrogenic properties, it is also known to induce bone formation. Furthermore, BMPs are secreted proteins that generate their effect by binding to cell-surface receptors. Thus, if first be secreted into the extra-cellular environment, raising concerns that they could extravasate into the joint space, affecting other local tissues such as the synovium. Although mineralization has not been identified in animal studies using BMPs for articular lesions, ossification within the articular cartilage has occurred after cases of ACI procedures, where periosteal patches are used to seal the defect after implantation. This occurs even when BMPs are not used, suggesting that the addition of osteogenic agents may lead to unacceptable rates of ossification within the cartilage that could compromise patient outcomes.

The use of Sox9 may help to allay these concerns. As a member of the Sry-type HMG-box gene family, Sox9 is a transcription factor that acts within the nucleus to promote the expression of genes critical to chondrogenic differentiation and therefore, is not secreted. Among its target genes are type II collagen and aggrecan, both of which are key components of normal hyaline cartilage. It has not been shown to promote osteogenesis, and actually appears to be down-regulated in osteoblasts. Interestingly, although Sox9 is a probable downstream target of many BMPs, it appears to orchestrate BMP-mediated chondrogenesis only, rendering its effect more targeted than that of the BMPs.

The natural history of articular cartilage injuries has been studied in detail, and one of the prominent features of many healed lesions is the preponderance of fibrocartilaginous repair tissue. The cells which populate this fibrous repair tissue are primarily spindle-shaped, and synthesize type I collagen. The resulting tissue is, therefore, distinct from surrounding normal hyaline cartilage both with respect to its viscoelastic properties and its behavior under compression. In a long term follow-up of 101 patients after ACI procedures, Peterson et al. found that most graft failures of the medial femoral condyle occurred in lesions that were fibrous in appearance. He performed immunohistochemical staining for type II collagen in both fibrous and hyaline-like healed lesions, and found that significant type II collagen could be detected only in lesions that healed with relatively normal-appearing hyaline cartilage. None of the fibrous lesions demonstrated type II collagen on immunohistochemical staining. Of greater importance, the presence of type II collagen in healed lesions was associated with more favorable functional scores on long term follow-up, leading Peterson et al. to suggest that type II collagen was highly correlated with good to excellent long-term outcomes.

To the extent that Sox9 is able to induce type II collagen synthesis and apparently preserve the chondrocytic appearance of cells in vitro, gene therapy utilizing this chondrogenic factor may hold great promise in the treatment of articular cartilage injuries, and warrants further study. While other factors have been successfully delivered to articular chondrocytes and studied in vitro, this is the first study to our knowledge that has demonstrated an increase in type II collagen in response to gene therapy in articular chondrocytes. In spite of this, it is important to note that Sox9 alone may not be as effective as a combination of factors, including local PTHrP, IHH and/or selected BMPs, in the treatment of articular cartilage defects. In general, though, gene therapy approaches that incorporate Sox9 may help serve as important adjuncts to current surgical treatments, helping to assure better, more reliable, long-term outcomes in patients with articular cartilage injuries.