Distinct Gene Expression Patterns in Surface, Middle, and Deep Zones of Bovine Articular Cartilage
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Introduction
The regeneration of articular cartilage remains a challenge and is an
unmet clinical need in orthopaedic surgery. Since hyaline articular
cartilage will not heal spontaneously, these lesions eventually lead to
degenerative joint disease. Cartilage abrasion, subchondral drilling, and
microfracture techniques stimulate articular cartilage healing by
introducing endogenous “stem cells” from the bone marrow to the
damaged area; however, they result in fibrocartilaginous healing and do
not offer durable surgical repair.(1-3) The osteoarticular transfer system
(OATS) harvest articular cartilage plugs with intact subchondral bone
from a donor site and provide partial hyaline articular cartilage coverage
to an area of damaged articular cartilage.(4-6) However, this technique
has a significant challenges in terms of donor site morbidity, especially
with large defects, and yields only fibrocartilaginous healing.
Autologous chondrocyte implantation (ACI) expands harvested
chondrocytes in vitro and reimplants them into an area of damaged
articular cartilage under a periosteal flap.(7) Recently, allograft juvenile articular cartilage (deNovo® NT, Zimmer, Inc., Warsaw,
Indiana) has been utilized to avoid the technical requirements as well as
donor site morbidity of ACI and OATS. However, this technology results
in poor integration with recipient cartilage especially in the
superficial zone.(8, 9) A complete understanding of the responsive cells, inductive signals, and extracellular scaffolding required for the
recaptulation of embryonic development and morphogenesis (i.e.,
regeneration) remain elusive.(10-12) Our hypothesis is that distinct sets of
gene expression defines the different zones of the articular cartilage.
We have investigated gene expression patterns in the superficial, middle,
and deep zones by microarray analysis of bovine articular cartilage in an attempt to better understand the gene expression patterns and
determine potential signaling pathways for optimal articular cartilage
regeneration and homeostasis.

Materials and Methods
The superficial zone of bovine stifle joint femoral condyle articular
cartilage (~100 μm) was harvested using a dermatome. An
osteochondral plug was removed using a coring reamer and middle and
depth zone articular cartilage (1.25 mm) were removed from each plug
using a custom jig. (13-14) Total mRNA was extracted from 25 million
chondrocytes, and after cRNA probe labeling, Affymetrix GeneChip®
Bovine Genome Array Analysis was performed. Data analysis was
performed using the dChip MFC Application v1.0.0.1. We considered
genes in one cartilage zone as elevated if their expression was greater
than 2-fold higher than the comparison genes in another cartilage zone.
In addition, the elevation was required to be statistically significant (p <
0.05) and non-random via ANOVA analysis (p < 0.01) in both samples
of the same zone. The result of the dChip comparison between the gene
expression of the superficial and middle zone articular cartilage was
input into the Ingenuity Pathway Analysis Software v8.5 to evaluate the
changes in canonical signaling pathways as well as any cellular
processes and networks involved. Fisher’s exact test (p < 0.05) was used
to determine statistical significance.

Results
Affymetrix microarray analysis of superficial and middle zone articular
cartilage reveal 52 differentially expressed genes greater than 10-fold
and 114 differentially expressed genes greater than 5-fold. However,
there were no genes identified with a greater than 5-fold change in
expression when comparing middle and deep zone articular cartilage.

Extracellular Matrix - There were large changes in genes responsible for
the composition of the cartilaginous extracellular matrix. MMP13, MMP9, and MMP2 expression were increased 72-, 12.4-, and 3.5-fold
in middle zone articular cartilage, respectively. Laminin-β1, and Collagen X α1 expression were increased in the superficial layer by
approximately 3-fold. However, there were numerous collagen genes
that displayed increased expression in the middle zone as well as
Aggrecan and Matrilin 1.

Signaling - There are changes in the cell-matrix and cell-cell signaling
between each articular cartilage layer. There is a modest 2.8-fold
increase in the expression of integrin-β5 and -α2 in the superficial zone
articular chondrocytes. Alternatively, there is a modest 4.2-fold increase
in the expression of plakoglobin in the middle zone articular
chondrocytes. Correspondingly, there were changes in the cytokines
as well. Two tubulin genes were increased in the superficial layer while
three actin genes and glial fibrillary acidic protein (GFAP) were
increased in the middle layer.

Morphogens - Changes in the gene expression of the TGF-β superfamily
pathway play a critical role in cartilage differentiation. The expression of
two bone morphogenetic proteins (BMPs), BMP4 and BMP5, were
reciprocal between the superficial and middle zones of articular
cartilage. The expression of inhibin, beta a (INHBA), a member of the
TGF-β superfamily and traditional inhibitor of FSH, as well as
TGF-β3 were elevated 17.1- and 2.2-fold, respectively, in the superficial
zone of articular cartilage. Changes in the gene expression of the Wnt
pathway play a critical role in cartilage differentiation. The expression
of known GSK3β inhibitory DKK1 was increased in superficial zone
articular chondrocytes by 2.4-fold. The middle zone articular
chondrocytes had an increased expression of several Wnt pathway
inhibitors, including, FRZB, FZD1, and FZD9. Interestingly, we found
not changes in the gene expression of genes involved in the hedgehog
(Hh) pathway.

Pathway Analysis - There were statistically significant changes in 9
canonical pathways: valine, leucine, and isoleucine degradation (12 of
111 genes; p < 0.004); atherosclerosis signaling (12 of 112 genes; p <
0.010); coagulation system (7 of 37 genes; p < 0.011); LPS/IL-1
mediated inhibition of RXR function (19 for 215 genes; p < 0.023);
ATM signaling (8 of 53 genes; p < 0.029); VDR/RXR activation (10 of
80 genes; p < 0.033); sulfur metabolism (3 of 61 genes, p < 0.037);
glutathione metabolism (8 of 98 genes, p < 0.040); aryl hydrocarbon
receptor signaling (15 of 154 genes; p < 0.046).

Discussion
The changes in gene expression between the superficial, middle, and
depth zones of articular cartilage reveal several key findings that will be
critical to articular cartilage tissue engineering and regeneration.(15-16)
However, comparing the middle and deep zones of articular cartilage
only 7 genes were changed out of 23,000 genes on the Affymetrix
bovine genome array and no gene was changed more than 5
fold suggesting these two zones appear more similar than anticipated.
In contrast, when comparing the middle and superficial zones of articular
cartilage 1,471 genes were changed suggesting that these zones are
remarkably distinct. Our results characterize the superficial and middle
zone of articular chondrocyte as highly specialized cell types and
suggest that these cell types are distinct and that the specific analysis
of these highly specialized cells must be required before routine cartilage
regeneration becomes a reality. In conclusion, the regeneration of
articular cartilage must be based on ensuring the gene expression in
superficial and middle zones of articular cartilage.

References