that evolved from the medial anterior boundary to the central region. The scoring method showed excellent discrimination between disease stages: the pattern-based score was significantly different between all knee groups and the correlation across groups indicated a strong monotonic score increase with worsening of disease severity (Fig.2). In contrast, medial compartment thickness did not show significant differences until an advanced disease state.

Conclusions: This study identified a characteristic progression in thickness map with increasing medial knee OA and the scoring method allowed for excellent differentiation between disease severities. The fact that thickness patterns can be statistically associated with increased OA severity is not trivial as thickness is known to vary among asymptomatic individuals and response to OA is partially subject-specific. In fact the novel method introduced in this study might constitute a shift in the analysis of cartilage thickness. While a discrete reference (KLG) was used to assess the pattern-based score, the method provides continuous scoring and thus has the potential to detect more subtle differences. Clearly the method will need to be tested with larger cohorts and other OA conditions. However, the robust statistical findings here are very promising given the limited size of the training datasets used for this study.

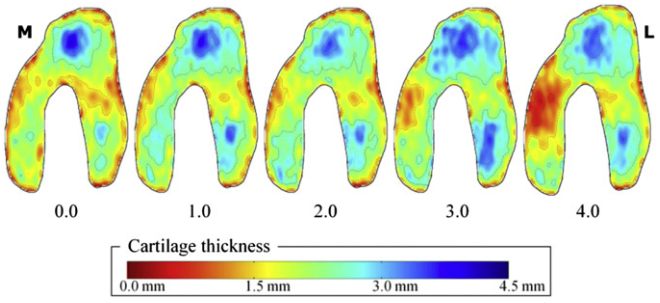

Figure 1. Characteristic progression in cartilage thickness with increasing medial knee OA (left to right) identified using the method introduced in this study.

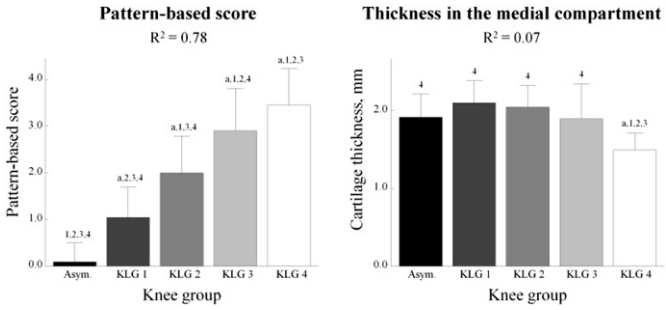

Figure 2. Pattern-based score was significantly different between all knee groups (left plot), whereas mean compartment thickness was less sensitive to increases in OA severity (right plot). Data are presented as mean ± SD, Superscripts indicate significant differences ([a]significantly different from asymptomatic; [1]significantly different from KLG 1; [2]significantly different from KLG 2; [3]significantly different from KLG 3; [4]significantly different from KLG 4).

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BONE MECHANOTRANSDUCTION VIA T-TYPE VOLTAGE SENSITIVE CALCIUM CHANNEL PLAYS A KEY ROLE IN INDUCTION OF THE OSTEOARTHRITIC PHENOTYPE

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Purpose: Mechanotransduction in bone is partially mediated through activation of voltage sensitive calcium channels (VSCC). Mice deficient in $Ca_v3.2$ (alpha1H), the pore forming subunit of T type-VSCC (T-VSCC), exhibit reduced new bone formation response to mechanical loading. It is believed that soluble factors released by mechanically stressed subchondral osteoblasts can activate catabolic pathways in adjacent cartilage tissue. The objective of this study is to understand the role of T-VSCC in mediating the mechanotransduction signals in osteoblasts and test whether the selective inhibition of T-VSCC in mechanically stimulated osteoblasts prevents the development of an osteoarthritic phenotype in chondrocytes.

Methods: The response of murine pre-osteoblastic cells (MC3T3) subjected to either static or fluid shear stress (FSS) (~3.5 dynes/cm^2 for 2 hours) conditions was compared using real-time PCR and Western blot analyses. T-VSCCs were inhibited by NNC 55-0396 in cells treated under the same conditions. Phenotypic changes of primary mouse chondrocytes grown in 3D micromass cultures and treated for one week with conditioned media (CM) collected from MC3T3 (sheared or static) were assessed by real-time PCR, immunolabeling, alcian blue and alizarin red staining.

Results: While cyclooxygenase2 (COX2) and osteopontin are significantly increased following FSS in MC3T3 cells, responses to FSS are reduced by half in the presence of NNC 55-0396. Additionally, treatment of primary chondrocytes with CM from sheared MC3T3 cells induced expression of hypertrophy markers (Collagen X and Alkaline Phosphatase). This catabolic effect was nearly abolished when primary chondrocytes were treated with CM obtained from MC3T3 sheared in the presence of NNC 55-0396.

Conclusions: Inhibition of T-VSCC's reduces the response of osteoblasts to FSS. Sheared osteoblasts secrete factors capable of inducing hypertrophy and potentially pro-inflammatory pathways in chondrocytes. The observed inhibitory effect of NNC 55-0396 indicates that T-VSCC is upstream to the release of soluble catabolic factors from osteoblasts and therefore can be a potential target for blocking subchondral bone response to abnormal mechanical loading and subsequent cartilage degeneration in OA. To test this hypothesis, we are currently comparing OA progression and subchondral bone response between $Ca_v3.2$ knockout and wild type mice using a noninvasive in vivo knee loading model.

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AUTOMATIC CARTILAGE SEGMENTATION AND MEASUREMENT FOR DIAGNOSIS OF OA USING 3D BOX AND GAUSSIAN FILTERS

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Purpose: This paper presents an automatic cartilage segmentation using 3D Box filter and multi-level Gaussian filters for diagnosis of Osteoarthritis (OA) with gradient multi-echo Magnetic Resonance Images (MRI). The 3D Box filter is applied for automatically detecting the Volume of Interest (VOI) of bone, and the multi-level Gaussian filters are applied for accurately computing the 3D normal vectors of cartilage. Then, the measurement of cartilage thickness and volume is made along the direction of the normal vectors in the VOI. Experimental results demonstrate the robustness and efficiency of the proposed approach to 3D cartilage segmentation in the gradient multi-echo MRI.

Methods: In the gradient multi-echo MR images, separating cartilage and muscle based on intensity is a difficult job because they are very similar in intensity. This paper firstly introduces a 3D box filter for rough selection of the bone VOI where cartilage is attached. In order to speed up the processing time, a 3D integral image is computed. Secondly, a 3D hybrid level set segmentation algorithm is applied to the detected bone VOI for fine segmentation of bone from other tissues. Traditional hybrid level set algorithms need to manually set an initial seed volume. However, this paper proposes an automatic initial position estimation method by computing the minimum energy in the 3D space, which corresponds to the center position of the bone. Then, the 3D multi-level Gaussian filters are developed for computing the Bone Cartilage Interface (BCI) by finding 3D normal vectors from the 3D bone surface. Finally, the border between cartilage and other tissues such as muscle or fat is determined based on local and global histogram information of intensity from the bone up to 5mm region along the normal vectors (The maximum cartilage thickness is assumed 5mm). The thickness and volume of local cartilage in the 3D VOI are measured along the 3D normal vectors.

Results: In this research, six gradient multi-echo MRI's from three different patients are used in order to demonstrate the performance of the proposed method. It is able to segment the cartilage and measure the thickness efficiently for all the test data. Fig. 1 and Fig.2 show the segmentation and measurement results with gray and color types. The

"Result window" shows the cartilage information with gray (or color) intensity over the 3D bone shape. The developed program can display and save color DICOM MRI as shown in Fig. 2, and provide the statistical information (Mean, Min, Max of thickness and Volume) of the local VOI of "Lateral Condyle" and "Medial Condyle" as shown in the figures.

Conclusions: This paper proposes fully automatic cartilage segmentation and measurement method without any training process. The proposed method can visualize the whole cartilage distribution with the 3D bone shape. The experimental results demonstrate that it can be used for inspecting cartilage damage or loss directly. The developed software is therefore applicable in clinical knee OA diagnosis systems. Future studies include more tests with new MRI data for confirming the accuracy of the measurements.(Acknowledgment: This study was supported by a grant of the Korean Health Technology R&D project, Ministry for Health, Welfare & Family Affairs, and Republic of Korea. (A091120))

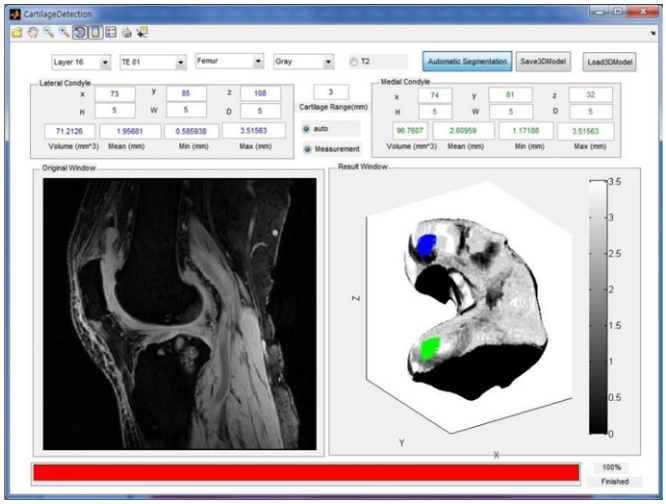

Fig. 1. Cartilage segmentation and measuring software for diagnosis of knee OA (gray).

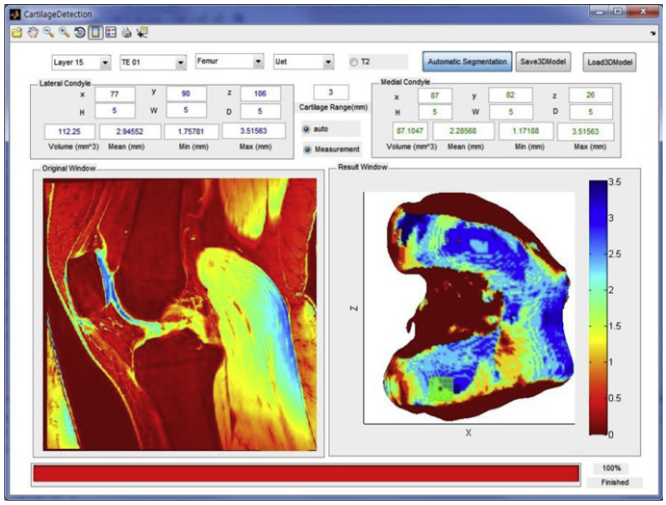

Fig. 2. Cartilage segmentation and measuring software for diagnosis of knee OA (color).

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LITHIUM CHLORIDE - A NOVEL TREATMENT FOR OSTEOARTHRITIS?

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Purpose: Lithium chloride (LiCl) is currently being used for the treatment of bipolar disorder because of its ability to interact with various receptors and affect major signaling pathways. In addition, it has been suggested that LiCl may inhibit matrix metalloprotease (MMP) expression in interleukin (IL)-1-treated articular chondrocytes. Based on its ability to modulate signaling pathways, we hypothesized that LiCl inhibits cartilage degradation during osteoarthritis (OA). Therefore, the purpose of this study was to determine the effects and mechanism of action of LiCl on cartilage degradation during OA.

Methods: The expression of catabolic genes and the activation of NF-κB in mouse and human articular chondrocytes cultured in the absence or presence of 10ng / ml IL-1, 50ng / ml IL-6, 10mM LiCl, and 10μM glycogen synthase kinase 3 (GSK-3) inhibitor SB-216763 (SB) were determined by real time PCR and luciferase reporter assays. Cell lysates from chondrocytes treated with IL-1, IL-1 / LiCl, or IL-1 / SB were analyzed for mitogen activated protein kinase (MAPK) and signal transducer and activator of transcription 3 (STAT3) activities by immunoblotting. Cartilage degradation in mouse femoral head explants cultured in the absence or presence of IL-1, LiCl and SB was determined by safranin O staining, and measuring the release of glycosaminoglycan (GAG) and IL-6 into the culture media by ELISA. Cartilage degradation in a post-traumatic OA mouse model using the transection of the medial collateral ligament and partial medial meniscetomy (PMX) after treatment with vehicle or LiCl (weekly intraarticular injections for 8 weeks) was analyzed histologically by toluidine blue staining.

Results: Cartilage degradation was markedly reduced in LiCl-treated mice 8 weeks after PMX surgery compared with vehicle-treated mice despite the fact that Li+ as a potent inhibitor of GSK-3 stimulates Wnt/β-catenin signaling, a well-known signaling pathway that stimulates cartilage degradation in surgical-induced mouse OA models. To determine the mechanisms of how LiCl protects articular cartilage against degradation, we determined the effects of LiCl and the GSK-3 inhibitor (SB) on catabolic events in IL-1-treated mouse articular chondrocytes. Treatment with LiCl, but not SB, resulted in decreased mRNA levels of catabolic markers, including ADAMTS-5, COX-2, IL-6, iNOS, and MMP-13, in articular chondrocytes treated with IL-1. LiCl markedly reduced the proteoglycan loss and the release of IL-6 in IL-1-treated mouse articular chondrocytes or femoral head explants, whereas SB further increased proteoglycan loss and IL-6 release in IL-1-treated chondrocytes or explants. LiCl treatment markedly reduced NF-κB and p38 MAPK signaling activities in IL-1-treated chondrocytes. As a consequence of reduced IL-6 expression in LiCl and IL-1-treated chondrocytes, LiCl markedly reduced Jak/STAT3 signaling activity in IL-1-treated chondrocytes. LiCl directly reduced Jak/STAT3 signaling activity in IL-6-treated articular chondrocytes.

Conclusion: LiCl protects articular cartilage against degradation during OA via the inhibition of major signaling pathways involved in OA pathology, including NF-κB, p38 MAPK and STAT3 signaling. Our findings suggest that LiCl as a modulator of the activities of major signaling pathways involved in OA pathology may act as novel compound for the treatment of OA. The advantage of LiCl as a potential novel compound for the treatment of OA is that LiCl is already approved for the treatment of patients with bipolar disorders.

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UTILIZATION OF HIGH THROUGHPUT MECHANICAL SCREENING FOR THE EVALUATION OF MECHANICAL PROPERTIES AND COMPRESSIVE INJURY MODELS

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Purpose: Restoration of cartilage after damage remains a perplexing clinical issue. High throughput (HT) assays enable rapid screening of chemical libraries for the discovery of novel compounds relevant to tissue development and healing. For musculoskeletal tissue engineering applications, HT technologies should also enable measurement of construct mechanical properties, as the success of these repair therapies will likely depend on their mechanical functionality. Similarly, given the mechanical origin of certain diseases, such as post-traumatic osteoarthritis (PTOA), HT methods for mechanical perturbation of numerous constructs might prove beneficial for the identifying novel factors that modulate disease progression. Here we demonstrate that a novel HT mechanical screening (HTMS) device can enable measurement of compressive mechanical properties of biomaterials and induce reproducible injury in engineered cartilage in a HT manner.

Methods: HTMS Testing of Hydrogels: 15% polyacrylamide (PA) and 4% agarose (Ag) gels (Ø:4mm,H:2.25mm; n=24/material) were tested using a step-compression protocol in the 48 well device. Gels were compressed to 10% strain over 200s and held for 1000s per step, for