Researchers at the Georgia Institute of Technology, GA, USA, have developed a technique for the noninvasive imaging of cartilage.

Innovative cartilage imaging technique developed to aid osteoarthritis research

A new technique, known as Equilibrium Partitioning of an Ionic Contrasting agent microcomputed tomography (EPIC-microCT), has been developed by researchers at the Georgia Institute of Technology and is reported in the *Proceedings of the National Academy of Science of the United States of America*. It is hoped that this technique will aid research into osteoarthritis.

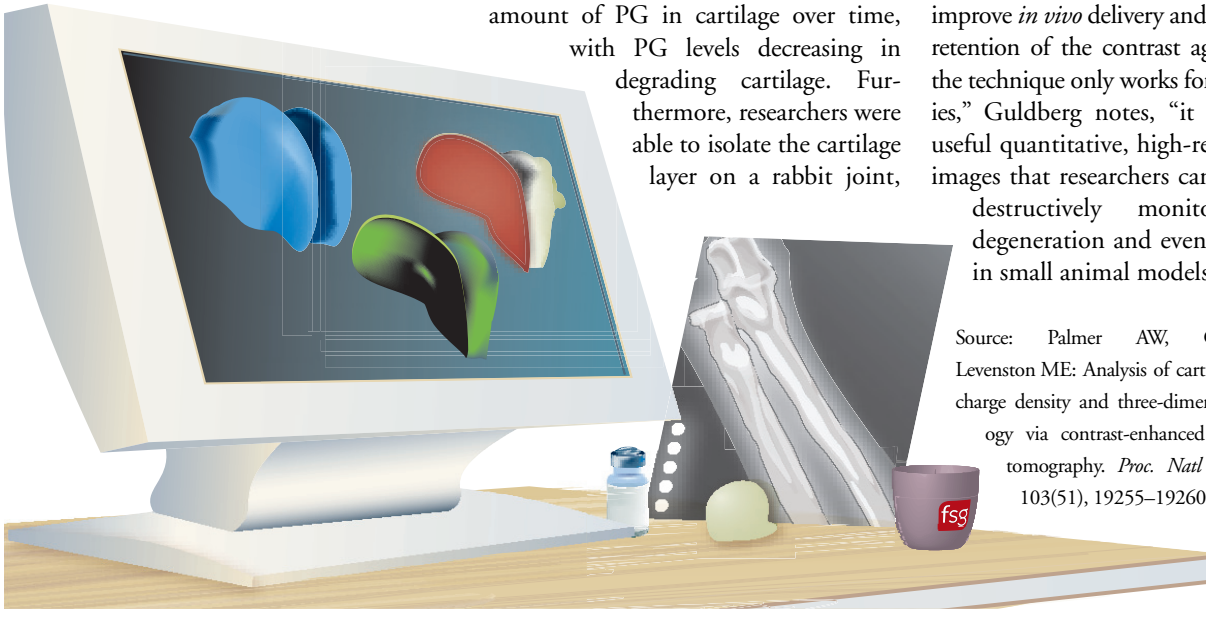
Osteoarthritis is a degenerative, inflammatory disease caused by the wearing away of cartilage in the joints. As the cartilage thins, the surface of the bone is less protected and the patient experiences pain. Although microCT is regularly used in osteoporosis research, it has not previously been used for osteoarthritis imaging since the microCT x-rays are not absorbed by soft tissues, such as cartilage, and thus cartilage does not show up on the scans.

EPIC-microCT involves a combination of existing technologies; microCT is combined with Hexabrix® (Mallinckrodt, MO, USA), an x-ray-absorbing contrasting agent. By using Hexabrix, which is negatively charged, Marc Levenston and colleagues were able to determine the distribution of proteoglycans (PGs), which are also negatively charged and a major component of the cartilage matrix. "By detecting PG content and distribution, the technique reveals information about both the thickness and composition of the cartilage – important factors for monitoring the progression and treatment of osteoarthritis," Levenston explains.

Ashley Palmer determined the principles and the protocol behind this new technique. Samples of cartilage were immersed in the contrast agent, which subsequently diffused into the tissue. Tissues with reduced PG content absorbed more contrast agent compared with cartilage with a high PG content, owing to repulsion between the negative charges on PG and Hexabrix. EPIC-microCT was then utilized to detect the concentration of Hexabrix, thus allowing calculation of the amount of PG present. This technique allows researchers to monitor the changes in the amount of PG in cartilage over time, with PG levels decreasing in degrading cartilage. Furthermore, researchers were able to isolate the cartilage layer on a rabbit joint, owing to differences in the x-ray signals emitted by bone and cartilage, and determine its thickness. This suggests that EPIC-microCT also has the potential to monitor cartilage thinning as osteoarthritis progresses.

Levenston and colleagues are now conducting follow-on research with the aim of studying osteoarthritis progression and monitoring cartilage changes *in vivo*. This study, funded by a 2-year grant from the National Institutes of Health, is important because, "…ultimately, if we can monitor cartilage changes with good resolution, and do it with little or no invasion of the tissue in live animals, then we can track osteoarthritis progression and the effects of drug therapy or other treatments over time," says Robert Guldberg.

Significantly, Hexabrix has already been approved for use in humans by the US FDA, thereby eliminating a major hurdle to the use of this technique. However, researchers still need to determine the optimum concentration for analyzing both cartilage thickness and composition, improve *in vivo* delivery and maximize the retention of the contrast agent. "Even if the technique only works for *in vitro* studies," Guldberg notes, "it still provides useful quantitative, high-resolution, 3D images that researchers can use to non-destructively monitor cartilage degeneration and even regeneration in small animal models".



Source: Palmer AW, Guldberg RE, Levenston ME: Analysis of cartilage matrix fixed charge density and three-dimensional morphology via contrast-enhanced microcomputed tomography. *Proc. Natl Acad. Sci. USA* 103(51), 19255–19260 (2006).

Priority Paper Alerts

Continuous passive motion applied to whole joints stimulates chondrocyte biosynthesis of PRG4.

Nugent-Derfus GE, Takara T, O'Neill JK *et al.*: *Osteoarthritis Cartilage* DOI: 10.1016/j.joca.2006.10.015 (2007) (Epub ahead of print).

The mechanism by which continuous passive motion (CPM) enhances joint rehabilitation is unknown, although it is suggested that it regulates proteoglycan (PRG)-4 metabolism. In this study CPM was applied to bovine stifle joints *in vitro* for 24 h. Variations in PRG4 secretion over the joint surface were observed and regulation of PRG4 biosynthesis by CPM was found to be dependent upon the cycle of cartilage sliding against opposing tissues, such as the meniscus. The study concludes that PRG4 metabolism is stimulated by CPM.

The comparative effectiveness of anti-TNF therapy and methotrexate in patients with psoriatic arthritis: 6-month results from a longitudinal study.

Heidberg MS, Kaufmann C, Rodevand E *et al.*: *Ann. Rheum. Dis.* DOI: 10.1136/ard.2006.064808 (2007) (Epub ahead of print).

Describes the analysis of data from a Norwegian, multicenter study comparing the treatment of psoriatic arthritis (PsA) with tumor necrosis factor (TNF) inhibitors and methotrexate (MTX) monotherapy. 146 patients received anti-TNF therapy and 380 patients received MTX. Disease activity and health-related quality of life measures were compared with baseline at 3- and 6-months post treatment. The study observed greater clinical improvement in PsA patients treated with TNF blockers compared with patients treated with MTX.

Comparison of serum IL-1β, sIL-2R, IL-6, and TNF-α levels with disease activity parameters in ankylosing spondylitis.

Bal A, Unlu E, Bahar G, Aydog E, Eksioglu E, Yorgancioglu R: *Clin. Rheumatol.* 26(2), 211–215 (2007).

This study aimed to determine the relationship between the level of cytokines in serum and disease activity of patients with ankylosing spondylitis (AS). Increased serum levels of interleukin (IL)-6, soluble (s)IL-2 receptor and tumor necrosis factor (TNF)-α were observed in AS patients compared with healthy control subjects. C-reactive protein levels were related to Il-6 serum levels, while sIL-2 receptor, TNF-α and IL-6 correlated with erythrocyte sedimentation rate. Therefore, TNF-α, IL-6 and sIL-2 receptor may be important in AS pathogenesis and the serum levels of these cytokines may act as biomarkers for the diagnosis of AS.

Leukemia drug may provide hope for systemic sclerosis sufferers

Research published in *Arthritis and Rheumatism* suggests that imatinib mesylate, a compound commonly used to treat leukemia, may prove to be effective in treating systemic sclerosis (SSc).

In the USA, approximately 300,000 people suffer from SSc, also known as scleroderma, with approximately 75% of these being women. SSc, an autoimmune disorder, is characterized by excessive collagen deposits and tissue fibrosis, resulting in the thickening and hardening of connective tissue. The fibrosis associated with SSc can lead to death, but as yet an effective antifibrotic therapy has not been developed. This may be a consequence of the lack of knowledge of an underlying cause.

'...as yet an effective antifibrotic therapy has not been developed.'

It has been suggested that transforming growth factor (TGF)-β and platelet derived growth factor (PDGF) stimulate extracellular matrix (ECM) protein synthesis, resulting in fibrosis. Such studies formed the basis of new research aiming to determine the therapeutic potential of inhibiting both these cytokines. Imatinib mesylate, which is a dual inhibitor of the TGF-β and PDGF pathways, has already been demonstrated to have therapeutic efficacy against leukemia and to have a very low-incidence of adverse effects. Therefore, it is an ideal candidate for treating SSc.

Researchers obtained fibroblast cultures from five SSc patients and six healthy age- and sex-matched controls. Real-time PCR was utilized to analyze the expression of ECM proteins following stimulation of the fibroblast cultures with PDGF and TGF-β, and incubation with imatinib mesylate. In addition, proliferation capacity was determined using the MTT assay. The antifibrotic effect of imatinib mesylate was confirmed in mice models of dermal fibrosis.

'...imatinib mesylate appears to be an effective antifibrotic agent at biologically relevant doses.'

Incubation with imatinib mesylate resulted in dose-dependent inhibition of *COL1A1*, *COL1A2* and fibronectin-1 gene expression in SSc and control fibroblasts. In addition, imatinib mesylate suppressed the development of fibrosis in infected mice, using a dose of 50–150 mg/kg. Therefore, imatinib mesylate appears to be an effective antifibrotic agent at biologically relevant doses. Although further research is required to determine the potential of imatinib mesylate for the treatment of SSc, this study provides a starting point for such trials and indicates that this compound may be a promising candidate.

Source: Distler JH, Jungel A, Huber LC *et al.*: Imatinib mesylate reduces production of extracellular matrix and prevents development of experimental dermal fibrosis. *Arthritis Rheum.* 56(1), 311–322 (2006).

New humanized mouse model mimics the rheumatoid arthritis sex bias

Rheumatoid arthritis (RA) is an autoimmune disease, associated with both environmental and genetic factors and with a clear sex bias; women are three-times more likely to be affected by RA compared with men. Despite the known sex bias, until recently, investigation has been limited owing to the lack of a rodent model that mimics this characteristic of human RA.

'...women are three-times more likely to be affected by RA compared with men.'

Research from the Mayo Clinic, MN, USA, published in the January issue of *Arthritis and Rheumatism,* reports the generation of a novel transgenic mouse that displays autoimmune responses and a sex bias similar to those observed in human RA.

The HLA-DRB1*0401 allele has long been associated with RA susceptibility; the gene variant is linked to anticyclic citrullinated peptide (anti-CCP) autoantibodies. Transgenic mice lacking all endogenous mouse class II genes and expressing HLA-DRB1*0401 were created and arthritis was induced.

Arthritis was initiated by injection of Type II collagen. The researchers observed production of rheumatoid factors and anti-CCP autoantibodies similar to those in humans in all transgenic mice that developed arthritis. For example, autoantibodies to Type II collagen were produced, class II molecule expression on T cells and antigen-presenting cells was increased and proinflammatory cytokine production was upregulated. These responses were higher in female mice following challenge with Type II collagen compared with male mice. Furthermore, female mice exhibited more than a threefold greater susceptibility to the development of RA.

'The researchers observed production of rheumatoid factors and anti-CCP autoantibodies...in all transgenic mice that developed arthritis.'

The study concludes that, "…this model may be valuable for studying sex differences observed in humans and for understanding why autoimmunity is increased in women. These mice may also be useful for developing future therapeutic strategies".

Source: Taneja V, Behrrens M, Mangalam A, Griffiths MM, Luthra HS, David CS: New humanized HLA-DR4-transgenic mice that mimic the sex bias of rheumatoid arthritis. *Arthritis Rheum.* 56(1), 69–78 (2006).

Citrullinated fibrinogen antibodies may be involved in the pathogenesis of rheumatoid arthritis

Citrullinated fibrinogen (CitFib) is the major citrullinated protein found in the inflamed synovium of rheumatoid arthritis (RA) patients. Despite the knowledge that cyclic citrullinated peptide (CCP) autoantibodies are sensitive and specific biomarkers for RA, very little research has been carried out to investigate antibody reactivity to CitFib. To this end, researchers at the University of Western Ontario, Ontario, Canada, have conducted a study in 65 RA patients and 63 patients with other rheumatic diseases, with the aim of determining the preponderance of anti-CitFib antibodies in rheumatic diseases.

"...autoimmunity to CitFib is common in patients with RA and may play a role in disease pathogenesis."

David A Bell and colleagues tested both cohorts of patients for the presence of serum immunoglobulin (Ig)M rheumatoid factor (RF), IgG anti-CCP and IgG anti-CitFib antibodies. The sensitivity to the CCP assay was 82% and specificity was 96%. Sensitivity and specificity to the CitFib assay was 75 and 98%, respectively, and the sensitivity and specificity for the RF assay was 80 and 64%, respectively. Antibodies to both CCP and CitFib were present in the sera of the majority of RA patients, with all but one RA, CitFib-positive patient also testing positive for CCP. In addition, just under half of the RF-negative RA patients were positive for CCP and CitFib.

In their report, published in *The Journal of Rheumatology*, Bell and colleagues state that "…these results suggest that autoimmunity to CitFib is common in patients with RA and may play a role in disease pathogenesis". However, in an accompanying editorial in the same issue, Eugen Feist and Gerd Bermester warn that these assays need to be standardized and further tests in larger cohorts of patients are required.

Source: Hill JA, Al-Bishri J, Gladmann DD, CairnsE, Bell DA: Serum autoantibodies that bind citrullinated fibrinogen are frequently found in patients with rheumatoid arthritis. *J. Rheumatol.* 33(11), 2115–2119 (2006).

B-cell depletion therapy may provide hope for systemic lupus erythematosus sufferers

Systemic lupus erythematosus (SLE) is an increasingly common autoimmune disease that predominantly affects the heart, joints, skin, lungs and blood vessels, although any part of the body can be affected. SLE is an example of an autoimmune disease exhibiting a sex bias, with non-Caucasian women being particularly susceptible. Currently there is no cure; however, symptoms are treated with corticosteroids and immunosuppressants.

Researchers from University College London, UK, studied the effects of B-cell depletion therapy (BCDT) based on the monoclonal antibody rituximab. This treatment has yielded promising results in patients with autoimmune diseases, although the mechanism has yet to be determined. Geraldine Cambridge and colleagues administered two doses of rituximab, with or without intravenous cyclophosphamide, 2 weeks apart to 16 SLE patients. Antinucleosome and anti-double-stranded (ds)DNA antibody levels were measured for at least a year following BCDT.

The researchers observed clinical improvement in all patients for at least 3 months following BCDT. At 3 months, the mean values of antinucleosome and anti-dsDNA antibodies had decreased to 71 and 53% of baseline values, respectively. At 6 to 8 months the mean values of antinucleosome and anti-dsDNA antibodies were 64 and 38% of baseline values, respectively. Cambridge and colleagues also assayed for additional autoantibody and antimicrobial antibodies, such as antitetanus toxoid and anti-extractable nuclear antigen; however, BDCT had no effect on the levels of these antibodies. The researchers observed a greater decrease in anti-dsDNA antibodies in patients with a longer clinical response.

Cambridge and colleagues suggest that, "…the efficacy of BCDT in patients with SLE may rely on qualitative features, such as the specificity of autoantibodies, as well as on the quantity of B cells removed".

Source: Cambridge G, Leonardo MJ, Teodorescu M *et al.*: B-cell depletion therapy in systemic lupus erythematosus: effect on autoantibody and antimicrobial antibody profiles. *Arthritis Rheum.* 54(11), 3612–3622 (2006).

Dentists to diagnose osteoporosis?

Hugh Devlin and colleagues (University of Manchester, UK) have developed a unique way of detecting osteoporosis, which they hope "…might even encourage older women to visit the dentist more regularly"!

Osteoporosis predominantly affects women, with the WHO estimating that in the Western world approximately 15, 22 and 38.5% of women aged in their 50s, 60s and 70s, respectively, are affected by osteoporosis. At the age of 80 years or older, up to 70% of women are at risk from this disease. However, the high cost and lack of specialist equipment and staff are currently precluding the implementation of widescale screening for osteoporosis.

Devlin and colleagues have developed a software-based approach to detecting osteoporosis during routine dental x-rays. X-rays are increasingly being used during general dental check ups, with data from 2005 indicating that the number taken has increased by 181% since 1981. These high usage rates, with the aid of 'active shape modeling' technology that detects jaw cortex widths of less than 3 mm automatically, can be utilized to detect osteoporosis. A jaw cortex width of less than 3 mm is a key indicator of osteoporosis.

In the study, 652 women were tested for osteoporosis using the dual energy x-ray absorptiometry (DXA) test – the current test for osteoporosis – and the automated x-ray test. The DXA test identified 140 sufferers and the automated x-ray test detected over half of these. Importantly "the patients concerned may not otherwise have been tested for osteoporosis, and in a real-life situation would immediately be referred for conclusive DXA testing," explains Keith Horner. Other advantages of the test developed by the research team in Manchester include its low cost, simplicity and the fact that it is automated.

With regards to the future prospects of such an approach, Devlin says "…we're extremely encouraged by our findings, and keen to see the approach adopted within the NHS. The next stage will be for an x-ray equipment company to integrate the software with its products, and once it's available to dentists we'd hope that entire primary care trusts might opt in".

Source: Devlin H, Allen PD, Graham J *et al.*: Automated osteoporosis risk assessment by dentists: a new pathway to diagnosis. *Bone* DOI: 10.1016/j.bone.2006.10.024 (2006) (Epub ahead of print).

About the Bulletin Board

The Bulletin Board highlights some of the most important events and launches in rheumatology. If you have newsworthy information, please contact:

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