Etiology and Management of Osteoarthritis

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Osteoarthritis (OA) represents failure of the diarthrodial (synovial) joint. Although the pathologic hallmark of OA is the progressive lost of articular cartilage over the habitually loaded areas of the joint surface, OA is not a disease of only tissue cartilage, it affects all of the tissues of the joint, including the synovium, subchondral bone, capsule, ligaments, periarticular muscles and the sensory nerves whose termini lie within these tissues. Furthermore, OA may be result from abnormalities in any of the above tissues.

OA is nearly ubiquitous – most people over the age of 65 show significant deterioration of at least several joints. In a sense the real problem is not OA, but *painful* OA; even though only 25-30% of those who exhibit pathologic changes of OA are symptomatic, because of the aging of our population and the fact that age is the most powerful risk factor for OA, the burden of OA as a public health problem will increase steadily over the coming year. In addition to age, a number of other risk factors for have been identified, including major joint trauma, repetitive stress, overload of the joint, obesity, female gender, genetic factors, congenital/developmental defects, quadriceps weakness (knee OA), inflammatory joint disease and several metabolic/endocrine disorders. Notably, some of these risk factors are preventable.

Overwhelmingly, the complaint that leads the person with OA to seek medical attention is joint pain. A variety of pharmacologic interventions are used to treat the symptoms of OA (e.g., simple analgesics, nonsteroidal anti-inflammatory drugs (NSAIDs), including selective cyclooxygenase-2 inhibitors (coxibs); opioids and intraarticular (IA) injection of glucocorticoids and hyaluronan (HA). It has become clear, however, that nonpharmacologic measures e.g., education of the patient in principles of joint protection; weight loss (if the patient is obese); thermal modalities; strengthening of periarticular muscles and orthotics are the *keystone* of OA therapy. Pharmacologic agents should be employed as adjuncts to such nonpharmacologic measures.

Significant expansion of the overall NSAID market for OA has occurred recently, driven chiefly by the availability of coxibs, which the manufacturers promote heavily to physicians and consumers. Although these agents were touted as "super-aspirins," they are no more effective for OA pain than acetaminophen; on average, nonselective NSAIDs and coxibs decrease OA pain by only 20-25%. The level of satisfaction with NSAID therapy in OA is not great; < 20% of patients with hip or knee OA in whom NSAID treatment was initiated remained on the same NSAID 12 months later, due largely to lack of efficacy or nonspecific gastrointestinal (GI) adverse effects. The GI tolerability of coxibs appears to be slightly, but not strikingly greater than that of nonselective NSAIDs. The chief advantage of coxibs, relative to nonselective NSAIDs, resides not in their efficacy but in their safety, relative to serious GI adverse events, such as ulcers and ulcer

complications (hemorrhage, bleeding, obstruction, perforation and death). The elderly exhibit an increased risk for these. Both celecoxib and rofecoxib appear to be associated with a decreased risk of serious GI adverse events. However, the decreased incidence of ulcer complications seen after 6 mos. of treatment with celecoxib was not apparent in subjects treated for 12 months and concomitant use of low-dose aspirin appeared to negate the gastroprotective benefits of celecoxib. The rofecoxib GI safety study raised a concern about the possibility that coxibs increase the risk of thrombosis, although the sample size was inadequate to address this question and no information was provided about comparability of the treatment groups with respect to risk factors for cardiovascular disease. Opioid therapy for OA remains controversial; concerns about tolerance and addiction in the elderly OA patient may have been exaggerated, but real problems exist with regard to opioid side effects in the elderly. Although HA therapy enjoys some popularity, claims that it provides "viscosupplementation" are not substantiated by the data and it is questionable whether the response to HA is superior to that seen after IA injection of saline. The greatest chance of producing benefit in OA lies with total joint arthroplasty (for knee OA and hip OA). However, uniform criteria for performance of these procedures do not exist, they are often performed only after years of ineffective medical therapy, and their cost has a major impact on the national health care budget. Clearly, more effective, safer and less expensive treatments for OA are needed.

NO in Experimental Joint Inflammation: Benefit or Detriment?

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The host response to infection or injury initiates a cascade of events involving recruitment of leukocytes and the release of multiple inflammatory mediators. One of these mediators, nitric oxide (NO), not only represents an important microbicidal agent in host defense, but also functions as a biological signaling and effector molecule in inflammation and immunity. However, overproduction of NO can be autotoxic and contribute to tissue damage and has been implicated in pathogenesis of tumors, and infectious, autoimmune and chronic degenerative diseases. NO is generated via constitutive and inducible nitric oxide synthases (NOS) which catalyze the oxidation of a guanidino nitrogen associated with L-arginine. Whereas endothelial NOS (eNOS) and neuronal NOS (nNOS) are constitutively expressed, iNOS is transcriptionally induced by bacterial constituents and inflammatory mediators, including TNF and IL-1. In an experimental model of bacterial component-induced joint inflammation and tissue degradation, functionally distinct roles of the constitutive and inducible NOS were demonstrated. Following systemic delivery of an arthritogenic dose of streptococcal cell walls (SCW), these bacterial peptidoglycan-polysaccharide complexes disseminate and target the peripheral joints, liver and spleen of the treated animals. Following deposition of the SCW in the peripheral joints, an initial innate inflammatory response to the bacterial components progresses into an adaptive immune response with the recruitment and activation of mononuclear phagocytes and T lymphocytes. With the release of cytokines and inflammatory mediators, there is an upregulation of gene expression for iNOS, but not the constitutive nNOS or eNOS. Nonetheless, the constitutive NOS isoforms, regulated by calcium fluxes and interaction with calmodulin, may also enhance NO production. Increased release of NO was detected not only in the synovium, but also in the circulation, and plasma levels of nitrate plus nitrite, the stable products of NO reactions, correlated with disease progression. Following inhibition of NO production with nonspecific NOS inhibitors, such as L-NMMA, which target all three isoforms, there is a striking therapeutic benefit with reduced signs and symptoms of erosive arthritis. In contrast, selective targeting of iNOS with L-NIL resulted in exacerbation of the synovial inflammation and degradation of joint structures. Based on these data, it appears that the constitutive isoforms of NOS contribute to the pathophysiology of the arthropathy, and that induced NOS and NO may function, in part, in a protective pathway. Moreover, the suppression of NO following treatment with TNF antagonists results in reduced inflammation and the associated synovial pathology. Collectively, these data implicate discrete roles for the NOS isoforms in the emergence of local tissue pathology and underscore the need to define the specific pathways that are being targeted for interventional strategies.

Genetics of Bone Development and Predisposition to Osteoarthritis

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The increased incidence of osteoarthritis (OA) with age, the existence of genetic risk factors in the form of population polymorphisms, the association with OA of variations in genes encoding structural cartilage matrix proteins, and the identification of mutations in several such structural genes in families with early-onset hereditary OA, are strong indications that there is a large genetic component to OA. The disorder therefore, like cancer, belongs in the family of multifactorial genetic diseases. Consequently, studies of hereditary OA may provide insights into pathogenetic mechanisms that are highly relevant to many forms of OA.

Hereditary OA can be subdivided into (a) early-onset OA associated with an underlying chondrodysplasia, (b) conditions associated with metabolic joint diseases including crystal-associated arthropathies, and (c) primary generalized OA with mild dysplasia. Among the osteochondrodysplasias are diseases caused by mutations in genes encoding collagen II, collagen IX, collagen XI, cartilage oligomeric matrix protein (COMP), matrilin-3, and lubricin (proteoglycan 4). Degenerative joint disease is a consequence of all these mutations and good mouse models exist for many.

Although most attention has been focused on degenerative changes in large joints such as knees and hips in cases of hereditary OA, age-dependent changes in the temporo-mandibular joint (TMJ) are likely to occur as well. For example, in mice *(cho*/+) that are heterozygous for a loss of function mutation in *Col11a1*, the gene encoding one of the three polypeptide subunits of collagen XI, degenerative joint disease following the same pattern of evolution is clearly seen both in the knee joints and the TMJ. The sequence of pathological changes are also those seen at different stages of OA in humans: An increased accumulation of cartilage proteoglycans (at 3 months in the TMJ of the mutant mice) and increased cellular proliferation generating characteristic clusters of chondrocytes, followed by loss of proteoglycans and loss of cartilage matrix and cells (at 6 months in the mutant mice). The *cho* mice represent a good model for Stickler-like syndromes in humans and it is therefore quite possible that TMJ involvement is part of these syndromes, but has been overlooked or ignored.

The existence of several common environmental and physiological risk factors for OA (occupation, trauma, aging, obesity, and gender) raises the question of whether OA caused by structural gene mutations is the result of alterations in cartilage that are fundamentally different from those that occur in more common forms of the disease. We suggest that this is not the case, and believe that structural changes caused by specific gene mutations simply lower the threshold at which normal biomechanical stresses on a joint results in the cascade of cellular and molecular events that define the OA process. Thus, whether OA in a joint, including the TMJ, is associated with an inherited gene

mutation, a somatic mutation affecting cellular function in or around a joint, a developmental anomaly associated with polymorphisms in several interacting genes, or trauma to the joint, the underlying cause consists of multiple contributory components. This opens the possibility that OA can be prevented or effectively treated through strategies that minimize the effects of some of these contributory components without necessarily “curing” an inherited mutation or somatic gene defect.

The Role of Friction and Adhesive Forces in TMJ Dysfunction

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The paper presents a model for the pathogenesis of TMJ internal derangement focusing on the process of lubrication impairment and its possible involvement in TMJ dysfunction. The model was constructed using accumulated clinical data as well as results obtained from laboratory investigations regarding TMJ lubrication, overloading, friction, adhesive forces and their relation to TMJ disc displacement with and without reduction, disc anchorage and osteoarthritis.

Free sliding of the disc tightened to the condyle in the TMJ is enabled due to the presence of phospholipids and hyaluronic acid which constitute an efficient lubrication system. Collapse of the lubrication system in the presence of uncontrolled oxidative stress produces friction between the smooth, high-energy denuded elastic sliding disc and the fossa. On such occasions, on opening of the mouth the condyle is pulled forward away from the lagging disc and the condyle-disc connection slackens gradually. The loosened disc becomes the initial stage in the process of disc displacement. Correspondingly, in the presence of thin fluid film (sub-boundary lubrication) adhesive forces may be generated between the mating surfaces totally preventing the disc from sliding, resulting in 'anchored disc phenomenon' associated with severe limitation of mouth opening. This phenomenon explains the unforeseen instantaneous release of the disc and rehabilitation of maximal mouth opening following arthrocentesis. This phenomenon is distinct from the commonly held cause for closed lock – where the condyle sliding is restricted by the non–reducible disc.

Understanding joint function and the pathogenesis of its dysfunction enables the development of more conservative treatment modalities with minimal complications. Our results challenge the overuse of surgical intervention, and in turn provide information necessary for improving the construction of the TMJ artificial joint.

References

1. Dan P, Nitzan DW, Dagan A, Yadgar S: H2O2 degrades cell surface proteoglycans and exposes the cell to lysis by phospholipas A2. FFBS letters 383:75-78,1997
2. Nitzan DW, Marmary Y: The " anchored disc phenomenon": a proposed ethiology for sudden onset severe and persisent closed lock of the TMJ. J Oral Maxillofac Surg 55 (8):797-802, 1997
3. Nitzan DW: The process of lubrication impaiment and its involvement in temporomandibular joint disc displacement: A theoretical concept. J Oral & Maxillofac Surg. 59(1):36-45, 2001
4. Rachamim E. Better H, Dagan A and Nitzan D: Electron microscopic demonstration and Biochemical Extraction of phospholipids from TMJ synovial fluid and articular surfaces. J Oral & Maxillofac Surg 59:1326-1332, 2001

5. Nitzan DW, Nitzan U, Dan P and Yadgar S: Hyaluronic acid protects surface-active phospholipids from lysis by exogenous Phospholipase A2. Rheumatol 40 (3) 336-340, 2001
6. Nitzan DW, Gati I and Kohen R: Changes in the Reducing Power of the synovial fluid from Temporomandibular Joint presenting 'Anchored Disc Phenomenon'. J Oral Maxillofac Surg 2002
7. Nitzan DW: Temporomandibular Joint "Open –lock" Vs Condylar Dislocation: Diagnosis, treatment and pathogenesis. J Oral Maxillofac Surg 2002
8. Nitzan DW, Etsion Y: Adhesive force – the underlying cause of the "anchored disc phenomenon". J Oral & Maxillofac Surg 2002

Glycosaminoglycan Profiling by FACE-
Monitoring Joint Tissue Degeneration and Repair

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Regeneration of a functional extracellular matrix (ECM) following joint tissue injury due to mechanical or metabolic/inflammatory insult, is an essential process to ensure long-term functionality of the tissue. However, degenerative diseases, including knee and hip OA and TMJ disorders, are initiated and overlaid on endogenous homeostatic repair responses; thus articular cartilages, ligaments, menisci and synovial membranes continuously modulate anabolic and catabolic pathways to protect the major tissue components such as the collagens and proteoglycans (PG). On the other hand, tissue repair responses can often lead to chronic stimulation of the cellular processes that underlie severe and irreversible tissue destruction. Therefore, collagen and PG metabolism have become important pharmacological targets for modifying progression of degenerative joint diseases, with the hope that such treatments protect the collagenous network and regenerate the tissue proteoglycan contents and preserve the mechanical 'strength' of the affected tissue.

Joint tissues contain chondroitin/dermatan sulfate (CS/DS), keratan sulfate (KS) PGs and the glycosaminoglycan (GAG), hyaluronan (HA). These provide tissue hydration for fluid flow and molecular transport, charge interactions for intermolecular spacing and polyanionic domains for cell-matrix interactions during adhesion, migration and proliferation. In contrast to the relatively inert collagenous network, the PG composition of a tissue is rapidly adaptable throughout life, and results from changes in core protein synthesis and degradation as well as altered activation of glycosyl- and sulfotransferases that regulate type, molecular size and oligosaccharide-repeat structure of the GAGS. As a result, monitoring changes in GAG content and composition of both joint tissues and synovial fluids has been a commonly used indicator of tissue remodeling in degenerative joint diseases. However, the commonly used GAG assay procedures, for example immunoassays with MABs to sulfated sequences on the polymer or HPLC quantitation of di- and oligosaccharide products generated by depolymerization with GAG-specific lyases/hydrolase, generally lack specificity and sensitivity. Therefore, a Fluorophore based carbohydrate electrophoresis procedure (FACE) for GAG quantitation and fine structure analyses was developed and shown to overcome many of the above-mentioned methodological limitations. As an example we report the application of this procedure for detecting ACL transection-induced remodeling of CS/DS PGs and HA in menisci and synovial membranes biopsied from rabbit knee joints. Such data clearly showed that injury resulted in a marked increase (~2 fold) in the CS/DS contents (per mg dry weight), of both medial and lateral menisci and the synovial membrane, and the latter also had a markedly (~ 1.5 fold) increased HA content. Importantly, we were able to detect changes

in the anabolic response when tissues were harvested from animals that had been given dietary glucosamine supplementation for 12 weeks post-surgery. Firstly, for medial menisci, CS/DS contents was ~ 50 % greater than in placebo-fed animals, secondly, for lateral menisci it was the same or somewhat lower and thirdly, post-surgical increases in CS/DS and HA in synovial membranes were abrogated by dietary glucosamine. Together these quantitative tissue compositional data provide a basis for examining the cell-biological mechanisms underlying the much-reported in vivo effectiveness of oral glcNH2 on pain relief in human degenerative joint diseases.

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References

1. Luyten FP. A scientific basis for the biologic regeneration of synovial joints. ***Oral Surg Oral Med Oral Pathol Oral Radiol Endod (1997) 83:167-9.***
2. Tanaka A, Kawashiri S, Takatsuka S, Narinobou M, Nakagawa K, Tanaka S. Expression of matrix metalloproteinase-2 in osteoarthritic fibrocartilage from human mandibular condyle. ***J Oral Pathol Med (2000) 29:314-20.***
3. Axelsson S, Holmlund, A, Hjerpe, A Glycosaminoglycans in normal and osteoarthrotic human temporomandibular joint disks. ***Acta Odontol Scand (1992) 50:113-9***.
4. Fujita S,Iizuka, T, Iizuka T, Dauber, W Localization of keratan sulphate and chondroitin-6-sulphate on the anteriorly displaced human temporomandibular joint disc--histological and immunohistochemical analysis. ***J. Oral Rehabil (2001) 28:962-70.***
5. Ratcliffe A, Israel, H A, Saed-Nejad F, Diamond B J) Proteoglycans in the synovial fluid of the temporomandibular joint as an indicator of changes in cartilage metabolism during primary and secondary Osteoarthritis. ***Oral Maxillofac Surg (1998) 56:204-8.***
6. Shibata T, Murakami KI, Kubota E, Maeda H J. Glycosaminoglycan components in temporomandibular joint synovial fluid as markers of joint pathology. ***Oral Maxillofac Surg (1998) 56:209-13***
7. Plaas AH, West L, Midura RJ, Hascall, VC. Disaccharide Composition of HA and CS/DS. Analyses by FACE ***Methods in Molecular Biology (2001) 17: 117-28***
8. Plaas AH, West L, Midura RJ. Keratan Sulfate Disaccharide Composition Determined by FACE Analyses of Keratanase II and Endo-b-galactosidase Digestion Products. ***Glycobiology (2001) 11:779-90***
9. Calabro A, Midura RJ, Wang A, West L, Plaas AH, Hascall VC. FACE Analyses of Glycosaminoglycans. **Osteoarthritis and Cartilage (2001) 9: S16-22**
10. Nguyen P, Mohamed, S E, Gardiner D, Salinas T. A randomized double-blind clinical trial of the effect of chondroitin sulfate and glucosamine hydrochloride on temporomandibular joint disorders: a pilot study. ***Cranio (2001) 19:130-9***

11. Harper, R P, Kerins, C A, McIntosh JE, Spears R. Modulation of the inflammatory response in the rat TMJ with increasing doses of complete Freund's adjuvant ***Osteoarthritis Cartilage (2001) 9:619-24***.

Molecular Mechanism of the Induction of Metalloproteinases 1 and 3 in Human Fibroblasts by Calcium Phosphate Crystals* Role Of Calcium-Dependent Protein Kinase C- .

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Crystalline calcium pyrophosphate dihydrate (CPPD) and basic calcium phosphate (BCP) are the two commonest forms of pathologic articular mineral. Each occurs frequently in osteoarthritic joints including TMJ joints, and each may be phlogistic, causing acute attacks of pseudogout in the case of CPPD crystals and acute calcific periarthritis in the case of BCP crystals. There is compelling in vitro evidence that these crystals engender multiple biological effects that would promote joint degeneration, and clinical observations support a relationship of both crystal types to osteoarthritis.

In vitro biological effects of these crystals include induction of mitogenesis, oncogene expression and matrix metalloproteinase (MMP) synthesis and secretion in human fibroblasts. To date, crystal-elicited signal transduction pathways have not been completely studied. Since protein kinase C (PKC) is known to play an important role in signal transduction, we investigated the participation of this pathway in the BCP crystal induction of MMP-1 and MMP-3 mRNA and protein expressions in human fibroblasts. Using reverse transcription/polymerase chain reaction (RT/PCR), northern and western blotting techniques, we show here that BCP crystal stimulation of MMP-1 and MMP-3 mRNA and protein expressions in human fibroblasts is dependent upon the calcium-dependent PKC signal transduction pathway and that the PKC- isozyme is specifically involved in the pathway. We have previously shown that BCP crystal induction of MMP-1 and MMP-3 is also dependent on the P44/42 mitogen activated protein kinase (P44/42 MAPK) signal transduction pathway. We now show that these two pathways operate independently and seem to complement each other. This leads to our hypothesis that the two pathways initially function independently, ultimately leading to an increase in mitogenesis and MMP synthesis, and may converge downstream of PKC and P44/42 MAPK to mediate BCP crystal induced cellular responses.

References

1. Ryan LM, **Cheung HS**. The role of crystals in osteoarthritis. In: Osteoarthritis Rheumatic Disease of America, Volume 25, (K.D. Brandt, Ed.) W.B. Saunders Co., Philadelphia, 1999, pp. 257-267.
2. Brogley MA, Cruz M, **Cheung HS**: Basic calcium phosphate crystal induction of collagenase-1 and stromelysin expression is dependent on a p42/44 mitogen-activated protein kinase signal transduction pathway. J. Cell. Physiol. 180:215-224, 1999.
3. Cheung HS: The role of crystals in articular tissue degeneration. Current Rheumatology Reports 1:128-131, 1999.

4. **Cheung HS**: Calcium crystal effects on the cells of the joint: Implications for pathogenesis of the disease. Current Opinion in Rheumatology, 12(3): 223-227, 2000.
5. Wierzbicki A, **Cheung HS**: Molecular modeling of inhibition of hydroxyapatite crystal by phosphocitrate. J Molecular Structure (Theochem) 529:73-82, 2000.
6. Bai G, Howell DS, Roos BA, Howard GA, **Cheung HS**. BCP Crystals Upregulate Metalloproteinases and downregulate Tissue Inhibitor of metalloproteinase in human fibroblasts. Osteoarthritis & Cart. 9: 416-422, 2001.
7. **Cheung HS**. Phosphocitrate as a potential therapeutic strategy for crystal deposition disease. Current Rheumatology Reports 3:24-28, 2001.
8. Reuben PM, Wenger L, Cruz M, **Cheung HS**. Induction of metalloproteinase-8 in human fibroblasts by basic calcium phosphate crystals: Effect of phosphocitrate. Conn. Tissue Res. 42:1-12, 2001.
9. Sun Y, Wenger L, Brinckerhoff CE, Misra R. **Cheung HS**. BCP crystals induced matrix metalloproteinase-1 through RAS/MEK1/SRF/c-fos/AP-1/MMP-1. Pathway: Involvement of transcription factor binding sites: PEA-3 and AP-1. J. Biol. Chem, 277:1544-1552, 2002.
10. Reuben PM, Brogley MA, Sun Y, **Cheung HS**. Molecular Mechanism of the Induction of Metalloproteinases 1 and 3 in Human Fibroblasts by Basic Calcium Phosphate Crystals. Role of calcium-dependent protein kinase C-. Accepted for publication by JBC.

Sub-Chondral Bone and Bone Resorption in the TMJ: Is There a Role for Anti-Resorbing Agents such as 17-ß-Estradiol?

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Several tissues are involved in TMJ (temporomandibular joint) health, including synovial fluid, the TMJ disc, articular cartilage, and subchondral bone. The focus of this presentation is to review evidence which points to loss of subchondral bone in TMD (temporomandibular joint disorders) and to discuss whether there is a role for anti-resorbing agents (such as 17-ß-estradiol) in potential TMD therapy. Bone is constantly undergoing turnover, even in an adult, so that bone formation and bone resorption are going on simultaneously. With aging, however, the balance between formation and resorption changes, and there is a net bone loss and resulting osteopenia. Bony changes occur in both the condyle and temporal components of the TMJ with aging. It appears, however, that there has been no comprehensive analysis of aging changes which occur in the temporal and condylar sites, and idiopathic condylar resorption is a poorly understood progressive disease that affects the TMJ. Osseous degenerative changes in TMD have previously been evaluated in patients with chronic connective tissue diseases (such as ankylosing spondylitis and rheumatoid arthritis) and in arthritis. In previous studies, radiographic examinations revealed that 33-85% of patients showed signs of bony degeneration, including cortical erosion, condylar flattening and joint space alterations. Bone destruction can be severe in the condyle and temporal components. Studies have reported radiographic erosions in up to 51% of TMD patients. There is evidence that subchondral bone changes may precede cartilage changes in arthritis and in TMD. The pathophysiology of TMD may be further complicated by concomitant osteoporosis, and by our lack of knowledge concerning the relationship between bony degenerative changes and symptomatology. Osteoporosis is an important and statistically significant risk factor for the long-term success of surgical procedures commonly employed in TMD. In a previous study which assessed several risk factors, osteoporosis proved to carry the highest risk of potential failure; a 50% failure rate for surgical TMD procedures was seen in patients with osteoporosis confirmed by lumbar QCT measurement. In this presentation, clinical examples of the spectrum of resorption patterns in TMJ-TMD transpharyngeal and panoramic tomography x-rays of condyles will be presented from studies of patients of various ages. Basic science studies which used anti-resorptive agents, including estrogen, to alter mandibular bone turnover, and reports on the effects of these agents on cartilage, will also be reviewed. Since bone resorption is important in TMD and since osteoporosis poses a threat for approximately 55% of the US population aged 50 and older, a discussion of the potential role of anti-resorbing agents in TMJ bone health is both timely and important.

Selected References

1. Flygare L, Hosoki H, Petersson A., Rohlin M, Akerman S. Bone volume in human temporomandibular autopsy joints with and without erosive changes. Acta Odontol. Scand. 1997;55:167-172.
2. Hunziker J, Wronski TJ, Miller SC. Mandibular bone formation rates in aged ovariectomized rats treated with anti-resorptive agents alone and in combination with intermittent parathyroid hormone. J. Dental Res. 2000;79:1431-1438.
3. Kirk WS Jr. Risk factors and initial surgical failures of TMJ arthrotomy and arthroplasty: A four to nine year evaluation of 303 surgical procedures. J Craniomandibular Practice 2002;16:154-161.
4. Malinin T, Ouellette EA. Articular cartilage nutrition is mediated by subchondral bone: a long-term autograft study in baboons. Osteoarthritis Cartilage 2000;8:483-491.
5. Marcus R, Wong M, Heath H, III, Stock JL. Antiresorptive treatment of postmenopausal osteoporosis: comparison of study designs and outcomes in large clinical trials with fracture as an endpoint. Endocr. Rev. 2002;23:16-37.
6. Ng MC, Harper RP, Lee CT, Wong BS. Effects of estrogen on the condylar cartilage of the rat mandible in organ culture. J. Oral Maxillofac. Surg. 2002;57:818-823.
7. Nordahl S, Alstergren P, Appelgran A, Appelgren B, Eliasson S, Kopp S. Pain, tenderness, mandibular mobility, and anterior open bite in relation to radiographic erosions in temporomandibular joint disease. Acta Odontol. Scand. 1997;55:18-22.
8. Ong TK, Franklin CD. A clinical and histopathological study of osteoarthrosis of the temporomandibular joint. Br. J. Oral Maxillofac. Surg. 1996;34:186-192.
9. Pedersen TK, Jensen JJ, Melsen B, Herlin T. Resorption of the temporomandibular condylar bone according to subtypes of juvenile chronic arthritis. J. Rheumatology 2001;28:2109-2115.
10. Riggs BL. The mechanisms of estrogen regulation of bone resorption. J. Clin. Invest. 2000;106:1203-1204.
11. Rodan GA, Martin TJ. Therapeutic approaches to bone disease. Science 2000;289:1508-1514.
12. Smith JA, Sandler NA, Ozaki WH, Braun TW. Subjective and objective assessment of the temporalis myofascial flap in previously operated temporomandibular joints. J. Oral Maxillofac. Surg. 1999;57:1058-1065.
13. Teitelbaum SL. Bone resorption by osteoclasts. Science 2000;289:1504-1508.
14. Yasuoka T, Nakashima M, Okuda T, Tatematsu N. Effect of estrogen replacement on temporomandibular joint remodeling in ovariectomized rats. J. Oral Maxillofac. Surg. 2000;58:189-196.

Subchondral Bone, Microdamage And Trabecular Microfracture

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Most studies show an association between increased bone density and degenerative joint degeneration (DJD), but the role of subchondral density and stiffness is not clear [1-3]. Some have proposed that initiation of joint degeneration is caused by variations in stiffness in subchondral bone, perhaps caused by accelerated remodeling or focal variations in subchondral thickness, and that progression is promoted by the increased stiffness itself [4]. Some studies, however, show decreased subchondral density associated with joint degeneration [5-7]. The reasons for these differences between studies is twofold: (1) bone's apparent density (bone mass/total tissue volume) increases with DJD, whereas bone's material properties (bone mass/bone volume) may decrease due to accelerated subchondral remodeling. The apparent density is a structural property that increases in response to either an increase in mineralization of the tissue or an increase in bone volume. The material density can decrease with increased bone volume if the mineralization of a unit of tissue has decreased, for example in response to increased turnover. (2) Studies typically do not discriminate between the cortical bone of the subchondral plate, and subchondral trabecular bone, yet these are different architecturally, spatially, mechanically and physiologically [8,9].

There may also be changes to the material properties of the calcified cartilage that contribute to the subchondral sclerosis characteristic of DJD. Conventional wisdom holds that calcified cartilage provides a layer of intermediate stiffness between the relatively compliant articular cartilage and the much stiffer subchondral bone [10]. However, a combination of tidemark advancement and the potential for calcified cartilage to become more heavily mineralized than bone may both detract from the longer-term health of the joint.

The accelerated remodeling which is now documented to accompany DJD may in part be stimulated by the accumulation of microdamage that requires repair [11,12]. This occurs in both subchondral bone and in calcified cartilage, and provides evidence that microdamage, at least in calcified cartilage, may play a role in the pathogenesis of DJD, although cause and effect has never been demonstrated.

Trabecular microfractures are distinct from microcracks in subchondral bone and calcified cartilage [13,14]. Healing trabecular microfractures were implicated early as increasing bone stiffness and contributing to degeneration of the overlying cartilage. However, although the incidence of trabecular microfractures increases coincidentally with the age-related increase in prevalence of DJD, it is now clear that they are not involved in the pathogenesis or progression of disease.

Each of these observations has implications for understanding appropriate treatments to prevent or delay DJD.

References

1. Hannan, M.T., Anderson, J.J., Zhang, Y., Levy, D., and Felson, D.T. (1993). Bone mineral density and knee osteoarthritis in elderly men and women. The Framingham Study. *Arthritis and Rheumatism*, **36**, 1671–80.
2. Arden, N.K., Griffiths, G.O., Hart, D.J., Doyle D.V., and Spector, T.D. (1996) The association between osteoarthritis and osteoporotic fracture: The Chingford study. *British Journal of Rheumatology* **35**, 1299-1304.
3. Fazzalari, N., and Parkinson, I.H. (1997) Fractal properties of subchondral cancellous bone in severe osteoarthritis of the hip. *Journal of Bone and Mineral Research*, **12**, 632-640.
4. Radin, E.L. and Rose, R.M. (1986). Role of subchondral bone in the initiation and progression of cartilage damage. *Clinical Orthopaedics and Related Research*, **213**, 34–40.
5. Li, B. and Aspden R.M. (1997) Mechanical and material properties of the subchondral bone plate from the femoral head of patients with osteoarthritis or osteoporosis. *Annals of the Rheumatic Diseases*, **56**, 247-254.
6. Li, B. and Aspden, R.M. (1997) Composition and mechanical properties of cancellous bone from the femoral head of patients with osteoporosis or osteoarthritis. *Journal of Bone and Mineral Research*, **12**, 641-651.
7. Grynpas, M.D., Alpert, B., Katz, I., Lieberman, I., and Pritzker, K.P.H. (1991). Subchondral bone in osteoarthritis. *Calcified Tissue International*, **49**, 20–6.
8. Burr, D.B. and Schaffler, M.B. (1997) The invovlement of subchondral mineralized tissues in Osteoarthrosis: Quantitative microscopic evidence. *Microscopy Research and Technique,* **37**, 343-357.
9. Burr, D.B. (1998) Subchondral bone. In: Osteoarthritis (K.D. Brandt, M. Doherty and L.S. Lohmander, eds). Oxford: Oxford University Press. Chapter 7.3, pp. 144-156.
10. Mente, P. and Lewis, J.L. (1994). The elastic modulus of calcified cartilage is an order of magnitude less than that of subchondral bone. *Journal of Orthopaedic Research* **12**, 637-647.
11. Mori, S., Harruff, R., and Burr, D.B. (1993). Microcracks in articular calcified cartilage of human femoral heads. *Archives of Pathology and Laboratory Medicine*, **117**, 196–8.
12. Sokoloff, L. (1993). Microcracks in the calcified layer of articular cartilage. *Archives of Pathology and Laboratory Medicine*, **117**, 191–5.
13. Fazzalari, N., Vernon-Roberts, B., and Carracott, J. (1987) Osteoarthritis of the hip: Possible protective and causative roles of trabecualr microfractures in the head of the femur. *Clinical Orthopaedics and Related Research,* **215**, 224-233.
14. Radin, E.L., Parker, H.G., Pugh, J.W., Steinberg, R.S., Paul, I.L. land Rose, R.M. (1973) Response of joints to impact loading. III. Relationship between trabecular microfractures and cartilage degeneration. *Journal of Biomechanics*, **6**, 51-57.

Angiogenesis in Arthritis

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Angiogenesis is important in a number of rheumatic diseases such as rheumatoid arthrititis (RA). Cytokines and growth factors such as vascular endothelial cell growth factor (VEGF) may play a role in angiogenesis. In arthritis models, angiogenesis inhibitors have resulted in decreased severity of arthritis and serum VEGF production. In another model, the soluble VEGF receptor flt-1 resulted in reduction of arthritis. These results indicate that modulation of angiogenesis may modulate arthritis, at least in animal models. In human RA, several groups have found VEGF in the joints. Recent interest has focused on VEGF production by RA fibroblasts. In RA patients treated with anti-TNF-alpha, vascular deactivation occurs so that serum levels of VEGF fall along with clinical improvement. Hence, VEGF is likely an important mediator of angiogenesis in the RA joint.

What is the mechanism of action of angiogenic mediators like VEGF and basic fibroblast growth factor (bFGF)? Particularly important in mediating angiogenesis are the alpha v beta 3 and alpha v beta 5 integrins. It appears that cytokines like TNF-alpha or bFGF act via alpha v beta 3 integrin while VEGF acts via alpha v beta 5 integrin. In an animal model, inhibition of alpha v beta 3 integrin resulted in amelioration of a model of lapine arthritis.

Other important angiogenic mediators in arthritis include soluble endothelial adhesion molecules, such as E-selectin. We have shown that soluble E-selectin is a potent angiogenic mediator and that it acts on endothelial cells via the src and PI3 kinase pathways. Moreover inhibitors of these pathways block soluble E-selectin mediated angiogenesis, pointing to novel ways to inhibit angiogenesis in arthritis.

Opposing the action of angiogenesis inducers, are angiogenesis inhibitors. We have shown that another cytokine, IL-4, is a potent anti-angiogenic factor. Moreover, when an adenoviral vector bearing the IL-4 gene was given to rats prior to development of adjuvant-induced arthritis, these animals showed less severe arthritis and their joints exhibited decreased inflammatory infiltrate and decreased angiogenesis compared to controls.

Another emerging paradigm is that angiogenesis inhibitors often reside within other proteins. The idea that endothelium is quiescent for long periods of time and yet can be induced to sprout new capillaries in a matter of hours in response to an angiogenic stimulus, suggested that angiogenesis regulators might be stored for expedient use. Examples of this include angiostatin and endostatin. It is likely that inhibitors of angiogenesis are present in the RA synovium, but are outweighed in effect by angiogenesis inducers.

Can angiogenesis regulation help us in the therapy of patients with RA? There are a number of endogenous and exogenous inhibitors of angiogenesis which have been identified to date. These include a cartilage-derived factor, troponin, angiostatic corticosteroids, minocycline, fumigillin, choloroquine, sulfapyridine, methotrexate, penicillamine and thiol containing compounds such as gold compounds.

Can we use angiogenic markers to help guide our therapy in diseases like RA? Some preliminary data from several centers indicate that VEGF and bFGF are increased in RA vs. normal serum, especially in early erosive RA and in patients who are anti-Sa and anti-native collagen positive. Serum VEGF is increased in early RA. Early RA synovial fluid VEGF and MMP-9 correlate with each other and with arthroscopic synovitis and vascularity scores. Evidence is mounting that markers of angiogenesis may help us in early RA.

Can we use angiogenic markers to predict RA disease outcome? One study presented in abstract form indicated that serum vascular markers predicted disability and radiological changes in RA. Serum VEGF was associated with greater disease activity and soluble selectins were associated with increased disability. Hence, it is tempting to speculate that angiogenic markers may help guide us in therapy of rheumatic diseases such as RA in the future.

References

1. **Koch AE**, Polverini PJ, Kunkel SL, Harlow LA, DiPietro LA, Elner VM, Elner SG, Strieter RM: Interleukin-8 as a macrophage-derived mediator of angiogenesis. *Science* 258: 1798-1801, 1992.

2. **Koch AE**, Halloran MM, Haskell CJ, Shah M, Polverini PJ: Soluble forms of E-selectin and vascular cell adhesion molecule-1 are angiogenic mediators. *Nature* 376:517-519, 1995.

3. **Koch AE**: Angiogenesis: implications for rheumatoid arthritis *Arthritis Rheum*.41: 951-962, 1998.

4. Szekanecz Z, Szegedi G, **Koch AE**: Angiogenesis in rheumatoid arthritis: pathogenic and clinical significance. *J. Invest. Med.* 46:27-41, 1998.

5. **Koch AE**: The role of angiogenesis in rheumatoid arthritis: recent developments. *Ann. Rheum. Dis.* 59 (Suppl.1): 165-171, 2000.

Vascular Endothelial Growth Factor (VEGF) Stimulates Bone Repair by Promoting Angiogenesis and Bone Turnover

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Several growth factors are expressed in distinct temporal and spatial patterns during fracture repair. Of these, vascular endothelial growth factor, VEGF, is of particular interest because of its ability to induce vascularity. To determine whether VEGF is required for bone repair, we inhibited endogenous VEGF activity during bone healing. Treatment of mice with a soluble, neutralizing VEGF receptor decreased angiogenesis, bone formation, and callus mineralization in femoral fractures. Inhibition of VEGF also dramatically inhibited healing of a tibial cortical bone defect, consistent with our discovery of a direct autocrine role for VEGF in osteoblast differentiation. In separate experiments, slow release of exogenous VEGF enhanced blood vessel formation, ossification, and callus maturation in mouse femur fractures, and promoted bony bridging of a rabbit radius segmental gap defect. Our results indicate that a slow-release formulation of VEGF, applied locally at the site of bone damage, may prove to be an effective therapeutic to promote bone repair.

The Role of Genetic Factors in Angiogenesis

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Alterations in the number of microvessels in tissue are an essential physiological adaptation. Growth of blood vessels is an important part of the response to exercise, wound repair, inflammation, adaptation to hypoxia and pregnancy. Reductions in microvessel density (rarefaction) have been observed in hypertension, diabetes, high sodium intake and numerous other physiological and pathological conditions. In many situations, both rarefaction and angiogenesis coexist in the same vascular bed resulting in a dramatic remodeling of the microcirculation. One such case occurs in response to elevations in sodium intake in which suppression of the renin-angiotensin system leads to rapid reductions in vessel density followed by a rebound angiogenesis and a final chronic state of reduced vessel density. Many factors influence the ability of tissues to undergo the process of angiogenesis. Among these factors, genetic background may play a crucial role in how an individual responds to angiogenic stimuli.

In a series of studies using rat models we have used techniques of genomic manipulation to clarify several important angiogenic pathways. Several existing strains of rats were assayed to determine their ability to undergo angiogenesis in response to a chronic electrical stimulation paradigm and the strains were ordered based on the magnitude of their responses. The Brown Norway rat and the Dahl Salt Sensitive rat, two inbred strains at the extremes of the distribution for this response, were selected for further study. Analysis of the angiogenic response in consomic animals of the SS background revealed that introgression of the BN chromosome 13 restored an angiogneic response to the SS rat. Further studies in which only a small portion of chromosome 13 was substituted revealed that a region containing the renin gene was responsible for this restoration. The role of the renin-angiotensin system in this restored response was confirmed by the use of pharmacological inhibitors of this pathway in the congenic and consomic animals. Finally, analysis of the angiogenic pathway further revealed an unexpected association between activation of the renin angiotensin system and VEGF expression. This series of studies demonstrates the power of such techniques for illumination of both known pathways and unknown relationships and provides a starting point for the use of physiological genomics techniques in the study of angiogensis in muscle as well as in other tissues.

Angiogenesis: Necessary and Sufficient for Inflammatory Arthritis?

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Angiogenesis, the formation of new blood vessels from the pre-existing vasculature, is a hallmark and one of the earliest pathological findings of the inflamed joint. Angiogenesis directly contributes to arthritic pathology, since the addition of angiogenic cytokines such as bFGF augment arthritis severity. Importantly, inhibition of angiogenesis thru blockade of integrin $\alpha v\beta 3$, a selective marker and crucial regulator of the angiogenic process, ameliorates arthritic disease in both early and late stage arthritis in a rabbit model of inflammatory arthritis. Notably, angiogenesis inhibition was superior to cyclosporine, a T-cell suppressant, in reducing arthritis severity, although it is clear that combined therapies may offer a superior approach. Angiogenesis may be sufficient for the generation of arthritides, since gene delivery of the angiogenic cytokine VEGF produces an inflammatory synovitis in the absence of T-cells in the athymic mouse. Thus, strategies which target angiogenesis offer a promising new approach for the treatment of inflammatory arthritis.

Mediator Mechanisms Behind Symptoms and Signs of Temporomandibular Disorders in Chronic Arthritis

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Knowledge of the biological mechanisms is of outmost importance for the understanding of acute and chronic pain in the temporomandibular joint (TMJ). The arthritic conditions which develop in this joint, and any other joint, may be based on local or systemic inflammatory processes and may cause tissue destruction as well as pain. One important aspect in the diagnosis of disorders of the TMJ is to determine whether there is an active inflammatory process and whether this process has a destructive nature. Another aspect is to find treatments that specifically block mediators of pain and inflammation peripherally or centrally.

Peripheral terminals of primary afferent nociceptors not only respond to noxious stimuli by pain mediation, but also release inflammatory mediators (neuroendocrine peptides) causing neurogenic inflammation. The sympathetic nerves of the peripheral nervous system contribute to joint inflammation by release of mediators from the postganglionic sympathetic nerve fibres.

Another mediator of nociceptive pain is serotonin (5-HT), which has long been known to be an important endogenous mediator of inflammation in peripheral tissues and to sensitize or excite peripheral sensory nerve endings including those of the TMJ.

Prostaglandin E_2 (PGE_2) acts as a potent proinflammatory molecule, which stimulates bone resorption and promotes sensitization of peripheral nociceptors of the TMJ.

Cytokines, among them tumor necrosis factor alpha (TNF) and interleukin 1 (IL-1) participate in the development of pain and tissue destruction in the TMJ. Blocking of the production of TNF has been introduced as a new therapeutic approach.

References

1. Buck SH, Walsh JH, Yamamura HT: Neuropeptides in sensory neurons. Life Sci 1982;30:1857-1866.
2. Alstergren P, Appelgren A, Appelgren B, Kopp S, Lundeberg T, Theodorsson E: Co-variation of neuropeptide Y, calcitonin gene-related peptide, substance P and neurokinin A in joint fluid from patients with temporomandibular joint arthritis. Arch Oral Biol 1995;40:127-135.
3. Birrell G J, McQueen DS, Iggo A, Grubb BD. The effects of 5-HT on articular sensory receptors in normal and arthritic rats. Br J Pharmacol 1990;101:715-721.

4. Alstergren P, Kopp S. Pain and synovial fluid concentration of serotonin in arthritic temporomandibular joints. Pain 1997;72:137-143.
5. Murakami KI, Shibata T, Kubota E, Maeda H. Intra-articular levels of prostaglandin E_2, hyaluronic acid, and chondroitin-4 and -6 sulfates in the temporomandibular joint synovial fluid of patients with internal derangement. J Oral Maxillofac Surg 1998;56:199-203.
6. Kubota E, Imamura H, Kubota T, Shibata T, Murakami K. Interleukin 1 beta and stromelysin (MMP3) activity of synovial fluid as possible markers of osteoarthritis in the temporomandibular joint. J Oral Maxillofac Surg 1997;55:20-27.
7. Nordahl S, Alstergren P, Kopp S. Tumor necrosis factor in synovial fluid and plasma from patients with chronic connective tissue disease and its relation to temporomandibular joint pain. J Oral Maxillofac Surg 2000;58:525-530.
8. Nordahl S, Alstergren P, Eliasson S and Kopp S. Radiographic signs of bone destruction in the temporomandibular joint with special reference to markers of disease activity - A longitudinal study. Rheumatology 2001;40:691-694.
9. Maini RN, Brennan FM, Williams R, Chu CQ, Cope AP, Gibbons D, Elliott M, Feldman M. TNF- in rheumatoid arthritis and prospects of anti-TNF therapy. Clin Exp Rheum 1993;11:173-175.

Pathophysiological Mechanisms in Osteoarthritis Lead to Novel Therapeutic Strategies

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Osteoarthritis (OA) is a debilitating, progressive disease of diarthrodial joints that is associated with aging (for review, see ref.1). The aging process in synovial joints and OA pathology overlap particularly with respect to synovial joint remodeling. At the molecular level, OA can be characterized as an imbalance between anabolic (i.e. extracellular matrix [ECM] biosynthesis) and catabolic pathways (ECM degradation) in which articular cartilage is the principal site of tissue injury responses. The contribution of synovium to the OA process must also be considered. The pathophysiology of OA synovium may involve elements of a so-called "non-classical" inflammatory response of the synovial joint (2). The role of chondrocytes is critical in the OA process and progression of OA can be judged by the vitality of OA chondrocytes and their capacity to resist apoptosis (programmed cell death). The pathways resulting in ECM biosynthesis are important in replenishing cartilage ECM and include synthesis and integration of proteoglycans, various collagen isoforms and accessory proteins into the ECM. These events may be regulated by growth factors, such as insulin-like growth factor-I (IGF-I), its binding proteins (IGFBPs) and transforming growth factor-β (TGF-β) among others. Catabolic events are regulated by cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α). These cytokines regulate both the synthesis of ECM proteins and the transcription of metalloproteinases (MMPs) which are activated in the OA process. Therapeutic strategies which focus on modulating the activity of these cytokines (which have proven to be clinically efficacious in rheumatoid arthritis) may ultimately be employed in OA. These strategies may involve neutralizing the activity and synthesis of Il-1 and TNF-α or their receptors on chondrocytes and synoviocytes. In addition, signaling pathways involving mitogen-activated protein kinases (MAPKs) whose activity appears critical for MMP expression in fibroblast-like synoviocytes and synovium (3) and inflammation in general (4) also appears germane to the OA process.

References

1. Malemud CJ: Front Biosci 4:d659-d661, 1999
2. Attur MG *et al*: Osteoarthritis Cartilage 10: 1-4, 2002
3. Han Z *et al*: J Clin Invest 108: 73-81, 2001
4. Singh R *et al*: submitted, 2002

Osteoarthritis of the Temporomandibular Joint: Bringing Research Findings to Clinical Practice

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This presentation will focus on the transfer of information from research into the clinical practice of managing patients with temporomandibular disorders. In the 1990's studies involving synovial fluid analysis helped clinicans shift emphasis away from restoring gross anatomic relationships, such as disc displacement. Research findings indicated that the temporomandibular joint, as a synovial joint, is susceptible to cartilage degradation and synovial inflammation when loads exceed the adaptive capacity of the tissues. The response of joint tissues to external loads lead to biochemical changes in synovial fluid which precede gross anatomic abnormalities. Transfer of this information led to significant changes in clinical practice with renewed emphasis on reduction of excessive joint loading, maintenance of joint mobility and reduction of inflammation and pain.

Examples of how information transfer from research on ostearthritis of the temporomandibular joint directly led to changes in clinical practice will be provided. Furthermore, examples of how information transfer based on inadequate research or commercial enterprises led to flawed treatment principles will also be demonstrated. How current and future research initiatives in areas such as tissue engineering may influence clinical practice in the future will be discussed.

Mesenchymal Stem Cells and Their Use For Regenerating TMJ Tissues

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Mesenchymal Stem Cells (MSCs) are found in a variety of locations in the body and, in adults, function to provide replacement cells for those that expire in a number of skeletal tissues. MSCs can be isolated from bone marrow; culture expanded and delivered back into the body in suitable delivery vehicles to regenerate bone, cartilage, muscle, marrow stroma, tendon and fat. The principles of Tissue Engineering needed to regenerate these tissues are now becoming known. It is this approach that holds the promise to repair or regenerate skeletal tissues especially those associated with the TMJ.

Analgesic Effect of Elastoviscous Hyaluronan Solutions and the Treatment of Arthritic Pain

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Elastoviscous hyaluronan solutions have an analgesic effect when injected intra-articularly in animal and human joints. This was first discovered using animal behavioral models and later confirmed in neurophysiological studies in cat and rat joints. These studies on both normal and experimentally produced arthritis in joints confirmed that only elastoviscous solutions of hyaluronan or certain of its derivatives (hylans) have a desensitizing effect on nociceptive sensory receptors. Recently, this desensitizing effect of elastoviscous hyaluronan solutions was also demonstrated on intact or on isolated patches of oöcyte cell membranes.

Viscosupplementation, the exchange of pathological synovial fluid in arthritic joints with pure elastoviscous solutions of hyaluronan or hylans is a widely accepted therapeutic modality used to provide long lasting analgesia in human knee joints. Viscosupplementation for the treatment of temporomandibular joint pain has only been used experimentally and only few studies have been published. The rationale for using viscosupplementation in this joint will be discussed.

References

1. Balazs EA, Denlinger JL (1984) The synovial cell. In *Modern Aging Research 4, Comparative Pathobiology of Major Age-Related Diseases. Current Status and Research Frontiers* (Eds. Scarpelli, D.G. and Migaki, G.), Alan R. Liss, Inc., New York, 129-143.
2. Kopp S, Wenneberg B, Haraldson T, Carlsson GE (1985) The short-term effect of intra-articular injections of sodium hyaluronate and corticosteroid on temporomandibular joint pain and dysfunction *J Oral Maxillofac Surg* 43:429-435.
3. Kopp S, Carlsson GE, Haraldson T, Wenneberg B (1987) Long-term effect of intra-articular injections of sodium hyaluronate and corticosteroid on temporomandibular joint arthritis *J Oral Maxillofac Surg* 45:929-935.
4. McCain, JP, Balazs EA, De la Rua, H (1989) Preliminary studies on the use of a viscoelastic solution in arthroscopic surgery of the temporomandibular joint *J Oral Maxillofac Surg* 47:1161-1168.
5. Bertolami CN, Gay T, Clark GT, Rendell J, Shetty V, Liu C, Swann DA (1993) Use of sodium hyaluronate in treating temporomandibular joint disorders: a randomized, double-blind, placebo-controlled clinical trial *J Oral Maxillofac Surg* 51:232-242.

6. Pozo MA, Balazs EA, Belmonte C (1997) Reduction of sensory responses to passive movements of inflamed knee joints by hylan, a hyaluronan derivative. *Exp Brain Res* 116:3-9.

7. Belmonte C, Pozo MA, Balazs EA (1998) Modulation by hyaluronan and its derivatives (hylans) of sensory nerve activity signaling articular pain. In: T. Laurent (ed) *The Chemistry, Biology and Medical Applications of Hyaluronan and its Derivatives (Proceedings of the Wenner-Gren Foundation International Symposium held in honor of Endre A. Balazs, September 18-21, 1996, Stockholm, Sweden)*. Portland Press, London, 205-217.

8. Alpaslan C, Bilgihan A, Alpaslan GH, Güner B, Yis Ö, Erbaş D (2000) Effect of arthrocentesis and sodium hyaluronate injection on nitrate,, nitrate, and thiobarbituric acid-reactive substance levels in the synovial fluid *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 89:686-690.

9. Sato S, Oguri S, Yamaguchi K, Kawamura H, Motegi K (2001) Pumping injection of sodium hyaluronate for patients with non-reducing disc displacement of the temporomandibular joint: two year follow-up *J Cranio-Maxillofac Surg* 29:89-93.

Gene Therapeutic Targets in the Treatment of TMJ Arthritis

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Identification of a small animal model that undergoes pathological temporomandibular joint (TMJ) degeneration would represent a significant research tool. To date however, only one transgenic animal has been described that may have features characteristic of TMJ arthritis. It is a transgenic mouse that over expresses the human form of TNFα and undergo changes that resemble the arthridites of temporomandibular dysfunction. In this study we show that the disc, articular and synovial cells in this animal cause destruction of fibrous tissue, cartilage and bone and that the cells in the disc and articular cartilage express MMP9, IL-6 and IL-1. Furthermore, our recent cell findings indicate that the TMJ disc cells, themselves, produce matrix metalloproteinases that are regulated by cytokines and prostaglandins.

In an attempt to devise a therapeutic regimen to halt the progression of arthritis in the TMJ we have developed an adeno-associated viral vector delivery system that can transfect cells in a joint space. Injection of the virus into the knee joint of the TNFα transgenic mouse will allow for transformation of synovial lining cells, articular chondrocytes and meniscal cells. Transfection of cells occurs 5-6 cell layers deep and remains active for 3-4 weeks. Introduction of specific therapeutic gene targets into these cells may be one way to arrest degeneration of key joint structures.

Sex Differences in Masseter Muscle Function

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The masseter muscle of several mammalian species is sexually dimorphic (1, 2, 4-7). In the rabbit, adult females contain nearly equal proportions of fibers of slow or fast phenotypes, but under the influence of testosterone, in adult males, nearly 80% of masseter fibers express fast-related proteins (4). In males, masseter fibers of the fast, IIA phenotype are 65% larger than fibers of the same phenotype in females. In females, fibers containing slow myosin heavy chain isoforms are nearly one-third larger than fibers of the same phenotype in males. To assess whether these anatomical differences underlie functional sex differences, we measured the torques produced about the ipsilateral temporomandibular joint in response to electrical activation of different masseter regions in adult males and females (3). Masseter compartments in males produced torques more rapidly and of greater overall magnitude than the same compartments of females. The same compartments in males and females produced torques whose directions were similar. Similar measurements were made of the torques produced by stimulation of single motor units. Even though very few motor units were encountered in either sex that contracted as slowly as motor units in hindlimb muscles (>40 msec rise time), masseter muscles of both male and female rabbits contain small, relatively slowly contracting (20-40 msec rise time) motor units. Muscles of females contain fewer very fast contracting (< 20 msec rise time) motor units than muscles of male rabbits, and only males were found to contain motor units producing large (>30 mN) forces. In contrast to predictions from anatomical observations, slower contracting motor units in females do not produce larger forces than the same units in males. During cortically-evoked rhythmic activation of the masticatory muscles, similar activation patterns were found in both males and females, but the forces produced were of larger magnitude in males. These results are interpreted as evidence that the teeth, jaws, and temporomandibular joint are loaded more intensely by the masticatory muscles in males than in females.

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References

1. d'Albis, A., Couteaux, R., Janmot, C., and Mira, J. C. 1993. Opposite regulations by androgenic and thyroid hormones of V1 myosin expression in the two types of rabbit striated muscle: skeletal and cardiac. *FEBS Letters* **318:** 53-6.
2. Eason, J., Schwartz, G., Pavlath, G., and English, A. 2000. Sexually dimorphic expression of myosin heavy chain isoforms in adult mouse masseter muscles. *J. Appl. Physiol* **89:** 251-258.
3. English, A., Carrasco, D., and Widmer, C. 1999. Torques Produced by Different Compartments of the Rabbit Masseter Muscle. *J Appl. Biomech.* **15:** 347-359.

4. English, A., Eason, J., Schwartz, G., and Shirley, A. 1999. Sexual dimorphism in the rabbit masseter muscle: myosin heavy chain composition of neuromuscular compartments. *Cells Tissues Organs* **164:** 179-191.
5. Gutmann, E., Hanzlikova, V., and Lojda, Z. 1970. Effect of androgens on histochemical fibre type. Differentiation in the temporal muscle of the guinea pig. *Histochemie* **24:** 287-91.
6. Lyons, G. E., Kelly, A. M., and Rubinstein, N. A. 1986. Testosterone-induced changes in contractile protein isoforms in the sexually dimorphic temporalis muscle of the guinea pig. *J Biol Chem* **261:** 13278-84.
7. Maxwell, L. C., Carlson, D. C., McNamara, J. A., and Faulkner, J. A. 1979. Histochemical characteristics of the masseter and temporalis muscles of the rhesus monkey (*Macaca mulatta*). *Anat. Rec.* **193:** 389-402.

How Do Hind Limb Skeletal Muscles Adapt to Chronic Increases in Mechanical Stress?

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In order to determine the temporal patterns in which limb skeletal muscle adapts to chronically imposed mechanical stress, we used a unilateral functional overload model in which the relatively small slow-twitch soleus and fast-twitch plantaris muscles were overloaded to increase their weight bearing activity by the surgical removal of the large medial/lateral gastrocnemius muscle. Control and overloaded muscles were obtained after 1,2,4,8,16, 24, and 35 days of overload and examined for a number of markers of protein translational capacity, as well as growth factor expression. While there was evidence that the total protein content (mg/muscle) of the muscle was increased early on and throughout the various time points in response to the overload, the concentration (mg/g) of the myofibril fraction was significantly reduced during the early stages of overload suggesting that the contraction apparatus likely had a reduced functional capacity. This deficit was corrected after 24 days in the case of the soleus muscle and by 8 days in the plantaris muscle. These deficits in myofibril concentration were mimicked to a large extent by reductions in the total myosin heavy chain (MHC) and actin mRNA concentration relative to total RNA. These changes at the myofibril level occurred in spite of the fact that total RNA content increased throughout the overload period, which is indicative that there was sufficient translational machinery in the muscle. Expression of muscle-specific growth factors (IGF-1 and mechano-growth factor) and IGF-1 binding protein 4 were increased relative to control muscles suggesting that an anabolic stimulus had occurred. Further, there were increases in the phosphorylation state of P70 S6 Kinase and of 4E binding protein, which are markers of protein initiation activity. Thus, while there was ample evidence that the muscle demonstrated activity of being in a true anabolic state throughout the overload stimulus, it is surprising that the expression of the myofibril fraction clearly lagged behind the growth the muscles as a whole during the first week of overload stress. One possible explanation for this deficit in the fractional growth of the myofibril fraction is that the muscle experiences a level of trauma/inflammation at the onset of the overload stimulus, which specifically impairs the growth of the myofibril fraction during the initial stages. Once this initial trauma is overcome, the muscle then is capable of resuming its normal growth potential.

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IGF-I Restores Satellite Cell Proliferative Potential in Immobilized Old Skeletal Muscle with Aging

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One of the key factors responsible for the age-associated reduction in muscle mass may be that satellite cell proliferation potential (number of doublings contained within each cell) could become rate limiting to old muscle regrowth. No studies have tested whether repeated cycles of atrophy-regrowth in limb muscles of aged animals deplete the remaining capacity of satellite cells to replicate or what measures can be taken to prevent this from happening. We hypothesized that there would be a pronounced loss of satellite cell proliferative potential in gastrocnemius muscles of aged rats (25- to 30-mo-old FBN rats) subjected to three cycles of atrophy by hind limb immobilization (plaster casts) with intervening recovery periods. Our results indicated that there was a significant loss in gastrocnemius muscle mass and in the proliferative potential of the resident satellite cells after just one bout of immobilization. Neither the muscle mass nor the satellite cell proliferation potential recovered from their atrophied values after either the first 3-wk or later 9-wk recovery period. Remarkably, application of insulin-like growth factor I onto the atrophied gastrocnemius muscle for an additional 2-wk after this 9-wk recovery period rescued approximately 46% of the lost muscle mass and dramatically increased proliferation potential of the satellite cells from this muscle.

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References

1. Chakravarthy, M.V., B.S. Davis, and F.W. Booth. IGF-I restores inactivity-depressed satellite cell proliferation in old skeletal muscle. J. Appl. Physiol. 89:1365-1379, 2000.

2. Chakravarty, M.V., T.W. Abraha, R.J. Schwartz, M.L. Fiorotto, and F.W. Booth. IGF-I extends in vitro replicative life-span of skeletal muscle satellite cells by enhancing G1/S cell cycle progression via the activation of PI3'-kinase/Akt signaling pathway. J. Biol. Chem. 275:35942-35952, 2000.

3. Chakravarthy, M.V., M.L. Fiorotto, R.J. Schwartz,, and F.W. Booth. Long-term insulin-like growth factor-I overexpression in skeletal muscles depletes satellite cell replicative reserves and does not protect against age-associated muscle atrophy. Mech Ageing Dev 122:1303-20, 2001.

4. Chakravarthy, M.V., E.E. Spangenburg, and F.W. Booth. Culture in low
levels of oxygen enhances in vitro proliferation potential of satellite cells from old skeletal muscles. Cell. Molec. Life Sci. 58:1150-1158, 2001.

Human Extraocular, Jaw-closing and Laryngeal Muscles: How Different are they from their Counterparts in Limb and Abdominal Muscle?

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Mammalian skeletal muscle fibers can be classified by the heavy chain (MyHC) and light chain (MyLC) isoforms of myosin (the primary motor protein) that they contain. Some highly-specialized muscle fibers in human extraocular and jaw-closing muscles express either novel myosins or unusual combinations of isoforms of unknown functional significance. Extrinsic laryngeal muscles may express the extraocular MyHC isoform for rapid contraction and a tonic MyHC isoform for slow tonic contractions. In jaw-closing muscles fiber phenotypes and myosin expression have been characterized as highly unusual. Human skeletal muscle contains fiber types and myosin isoforms I, IIA and IIX. The jaw-closing muscles of most carnivores and primates have tissue-specific expression of the type IIM or "type II-masticatory" MyHC. Human jaw-closing muscles, however, do not contain IIM myosin. Rather they express type I, IIA and IIX (like other muscles), and myosins typically expressed in developing or cardiac muscle. Fiber morphology is also unusual in that the type II fibers are of smaller diameter than type I.

Given the profound variability in contractile protein expression in the highly-specialized cranial muscles we have begun to look at the relationship of gene message to isoform content. Monoclonal antibodies have not yet detected IIB MyHC protein in large mammals including man. Given that human jaw-closing muscles express a diversity of MyHC isoforms, masseter muscle may be a likely candidate for the presence of IIB. Using the in situ hybridization technique with MyHC – specific mRNA riboprobes we can now demonstrate abundant expression of IIB MyHC mRNA, but no IIB MyHC protein in a subset of masseter fiber types. Further demonstrating highly unusual gene regulation in this muscle.

By combining physiologic and biochemical techniques it is possible to determine the maximum velocity of unloaded shortening (V_o) of an individual skeletal muscle fiber and subsequently determine the type and amount of myosin isoform content. When analyzed, some laryngeal fibers shorten at much faster rates than type II fibers from limb and abdominal muscle. Yet some type I fibers in masseter show an opposite trend towards speeds ten fold slower than type I fibers of limb muscle. These unusual shortening velocities are regulated by MyHC isoforms in laryngeal fibers and by MyLC isoforms in masseter. For the jaw-closing muscles, this finding represents the first case in human muscle of physiologic regulation of kinetics by light chains.

For investigating how muscle tissue composition may vary among human subjects and serve as clinical markers for craniofacial growth, some investigators have begun to determine fiber type characteristics of human masticatory muscles. Daniels et al. (2001) have determined the fiber type distribution in masseter muscle biopsies take from 11 patients with an orthodontic diagnosis of "severe skeletal Class II" (small mandible

correctable only by surgical repositioning) and 16 patients with "severe skeletal Class III" (large mandible correctable only by surgical repositioning). Morphology was further characterized for vertical dimension of the face as possessing either a normal, open or deep bite. Fibers were classified as belonging to one of four possible fiber type groups; type I, type transitional, type II or type neonatal/atrial. Significant differences were found for fiber type distribution and mean area of fiber types, but variability between individuals was the most common characteristic. Similar work is necessary to determine how muscle cell function contributes to temporomandibular disorders.

References

1. Darab DR, Minkoff R, Sciote JJ, Sulik KK. Midfacial clefts in mice treated with methotrexate. Teratology. 1988; 36:77-86.
2. Sciote JJ. Fibre type distribution in the muscle spindles of cat jaw-elevator muscles. Arch Oral Biol. 1993;38:685-688.
3. Sciote JJ. Fiber types in cat jaw-closing muscles. Thesis. 1992;Univ of Michigan Press.
4. DelGaudio J, Sciote JJ, Carroll WR, Esclamado RM. Rat laryngeal muscle contains an atypical myosin heavy chain. Ann Otol Rhinol Laryngol. 1995;104:237-245.
5. Sciote JJ, Rowlerson AM, Hopper C, Hunt NP. A fiber type classification scheme for the human masseter muscle. J Neurol Sci. 1994;126:15-24.
6. Sciote JJ, Rowlerson AM, Carlson DS. Myosin expression in the jaw closing muscles of the domestic cat and american opossum. Arch Oral Biol. 1995;40:405-413.
7. Sciote JJ, Rowlerson A, Hopper C, Hunt NP, Fiber type classification of human masseter muscle fibres. In, Brain and Oral Functions. Oral Motor Function and Dysfunction, (Eds;Morimoto T, Matsuya T, Takada K), Elsevier Science, Amsterdam, 1995;439-449.
8. Bosley M, Burhanudin R, Rowlerson A, Sciote JJ. Muscle spindles in the masticatory muscles of the cat and opossum. In, Alpha and Gamma Motor Systems (Eds;Taylor A, Gladden M, Durbaba R). Plenem Press, New York, 1995;240-253.
9. Sciote JJ, Kentish J. Unloaded shortening velocities of rabbit masseter muscle fibres expressing skeletal or -cardiac myosin heavy chains. J Physiol. 1996;492:659-667.
10. DelGaudio J, Sciote JJ. Changes in myosin expression in denervated laryngeal muscle. Ann Otol Rhinol Laryngol. 1997;106:153-159.
11. Al-Syabi H, Studen-Pavlovich D, Morris T, Sciote J. Educational opportunities for non U.S. citizens in American dental schools. Beruit Dent News 1998;5(3):11-15.
12. Sciote JJ, Rowlerson A. Skeletal fiber types and spindle distribution in limb and jaw muscles of the adult and neonatal opossum, Monodelphis domestica. Anat Rec. 1998;251:548-562.
13. Sciote JJ, Morris T. Skeletal muscle function and fiber types: The relationship between occlusal function and the phenotype of jaw-closing muscles in humans. J Orthod. 2000;27:15-30.

14. Shashi V, Berry MN, Shoaf S, Sciote J, Goldstein D, Hart T. Unique form of mental retardation with a distinctive phenotype maps to Xq26-q27. Am J Hum Gen. 2000;66:469-479.
15. Sherwood TF, Mooney MP, Sciote JJ, Smith MP, Cooper GM, Siegel MI. Cranial base growth and morphology in second trimester normal and cleft lip human fetuses. Cleft Palate Craniofac J. 2001;38:587-596.
16. Sciote JJ, Horton MJ, Zyman Y, Pascoe G. Differential effects of diminished oestrogen and androgen levels on development of skeletal muscle fibers in hypogonadal mice. Acta Physiol Scand. 2001;172:179-187.
17. Horton MJ, Brandon CA, Morris TJ, Yaw KM, Sciote JJ. Abundant expression of from myosin heavy-chain IIB RNAs in a subset of human masseter fibres. Arch Oral Biol. 2001;46:1039-1050.
18. Morris TJ, Brandon CA, Horton MJ, Sciote JJ. Maximum shortening velocity and myosin heavy-chain isoform expression in human masseter fibers. J Dent Res 2001;80:1845-1848.
19. Sciote J, Morris T, Brandon C, Horton M, C. Rosen C. Unloaded shortening velocity and myosin heavy chain variations in human laryngeal fibers. Ann Otol Rhinol Laryngol. 2002;(in press).
20. Tavajohi H, Kapur R, Sciote JJ. Hypodontia and craniofacial morphology in an orthodontic population. Am J Orthod Dentofacial Orthop. 2002;(in press).
21. Kapur R, Sciote J. Variability in third order control. Angle Orthod. 2001; (first revision).
22. Lamparski D, Sciote JJ, Rinchuse DJ, Close JC. Comparison of skeletal and dental changes between two-point and four-point rapid palatal expanders. Am J Orthod Dentofacial Orthop. 2001;(first revision).
23. Poisson E, Mooney MP, Sciote JJ, Koepsel R, Cooper GM. TGF isoform expression in the perisutural tissues of craniosynostotic rabbits. J Bone Miner Res. 2001 November;(submitted).
24. Brandon CA, Rosen C, Georgelis G, Horton MJ, Sciote JJ. Muscle fiber type composition and effects of vocal fold paralysis on the two compartments of the human posterior cricoarytenoid. Ann Otol Rhinol Laryngol. 2001 November;(submitted)
25. Alsyabi H, Sciote J, Braun T, Mooney M, Mao J, Oberoi, S. Mechanical stresses in craniofacial bones in response to surgical fixation and orthopedic loading. J Orthod. 2001 November;(submitted).
26. Kwon H, Kapur R, Sciote J. Frictional resistance in esthetic brackets with different slot material design. Am J Orthod Dentofacial Orthop. 2001 Nov; (submitted).
27. Kwon H, Kapur R, Sciote, J. Frictional resistance in esthetic and metal brackets. Am J Orthod Dentofacial Orthop. 2001 November;(submitted).
28. Friel H, Kapur R, Sciote J. Analysis of human dental eruption in Hypophosphastasia. Eur J Orthod. 2001 December;(submitted).
29. Friel H, Kapur R, Sciote J. Timing of dental eruption in hypophosphatasia: case reports. Am J Orthod Dentofacial Orthop. 2001 December; (submitted).
30. Brandon CA, Rosen C, Georgelis G, Horton MJ, Sciote JJ. Muscle fiber type composition in human thyroarytenoid muscle. Ann Otol Rhinol Laryngol 2001 December; (submitted).

Neuromuscular Strategies for Control of Tongue Movement

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Fundamental to understanding motor systems physiology is identification of the principles by which motor units (MU, a motoneuron and the muscle fiber it innervates) are selected into activity. Typically, movement is thought to be achieved through the selection and differential activation of individual muscles. It is apparent, however, that in many motor systems and in many movements, the muscle is not the unit to which neuronal integration is addressed. This is most obvious in cases in which some MUs of a muscle are active in one behavior, whereas other MUs of the same muscle are active in another. The bases for this selective activation of MUs are not known. Recognition of within-muscle diversity of fiber type composition and neuromuscular compartment organization has led to the suggestion of type-based or compartment-based strategies of MU selection.

Observations of independent movements of anterior and posterior regions of the tongue body during oromotor behaviors suggest an additional possibility: in some muscle systems, strategies of MU selection may be based on MU location. To determine whether location may be a basis for MU selection, we have characterized motor endplate distribution, muscle fiber morphology and MU organization in the intrinsic longitudinal muscles of the rat tongue. In both superior and inferior intrinsic longitudinal muscles multiple motor endplate bands are present between the posterior tongue border and the tongue tip. By analogy to other mammalian muscles, these findings suggest that intrinsic longitudinal muscles of the rat tongue are composed of short muscle fibers that originate and insert within a localized region of the tongue body and thus overlap in-series to span the posterior-to-anterior tongue length. Dissection of individual muscle fibers directly confirms an in-series organization of intrinsic longitudinal muscles. Muscle fibers isolated *in situ* taper at both ends and are < 6 mm long, i.e., occupy < 20% of muscle length. Glycogen depletion study allows the direct reconstruction of muscle fiber organization with respect to individual MUs and is necessary to determine whether MUs themselves are organized in-series. Following stimulation of isolated intrinsic longitudinal muscle motor axons, territories of individual MUs are localized to < 25% of antero-posterior tongue body length.

We conclude that intrinsic longitudinal muscles of the rat tongue are of in-series design and that intrinsic longitudinal MUs are localized antero-posteriorly within the tongue body. Thus differential activation of intrinsic longitudinal MUs according to location could contribute to the independent movement of anterior and posterior tongue regions observed in oromotor behaviors. Because MUs are spatially localized in many muscles, strategies of MU selection may be based on MU location in other muscle systems.

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