

Gap Junctions in Osteoarthritis

David D. Waddell¹, Andrew A. Marino², Oleg V. Kolomytkin², William D. Meek³, Robert E. Wolf², Kalia K. Sadasivan², and James A. Albright².

¹Orthopaedic Research Specialists, Shreveport, LA, USA.

²LSU Health Sciences Center, Shreveport, LA, USA.

³University of Oklahoma, Tulsa, OK, USA.

Presented at American Academy of Orthopaedic Surgeons 2002 Annual Meeting; Dallas, Texas, February 2002.

Abstract

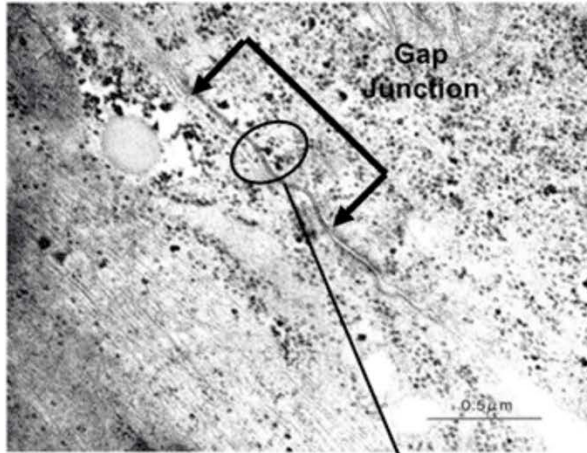
Synovial cells are electrically and metabolically coupled through gap junctions (GJs) composed of connexin43 protein, but their specific role in synovial tissue is unknown. We hypothesized that synovial GJs were correlated with the presence of joint disease. Tissue biopsies were obtained from the fat pad of 11 patients with grade-four osteoarthritis (OA), and from 10 non-arthritic patients, and analyzed by SDS-PAGE for connexin43 using Western blots and by electron microscopy (EM).

Connexin43 was 50% greater in the OA patients (relative intensities of Western blots, 12.4 ± 2.0 compared with 8.2 ± 0.08 , $P < 0.05$). In the EM study, regular 2-nm intermembrane gap separations characteristic of GJs were found in all tissues. The structures were about 1 μm in length, and usually occurred in cell processes. GJ hemichannels could frequently be resolved on the basis of periodic density changes. A minimum of 500 cells from each patient were examined, and the number of GJs in the OA patients was more than 4 times that of the controls (4.41 ± 2.21 compared with 1.00 ± 0.71 GJ/100 cells, $P < 0.05$). Considering that the arthritic synovial layer was generally thicker than normal, it can be concluded that the absolute number of gap junctions in the arthritic knees was far greater than normal. Whether GJs are a cause or a consequence of the disease remains to be determined. In either case, however, it is reasonable to suppose that GJs could serve as therapeutic targets to interrupt disease progression.

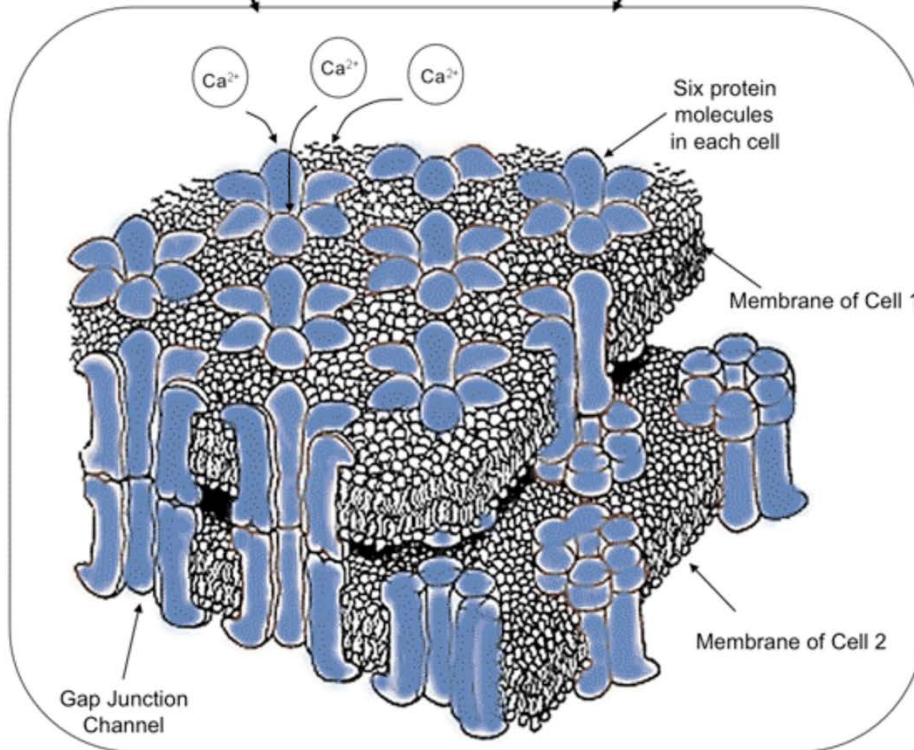
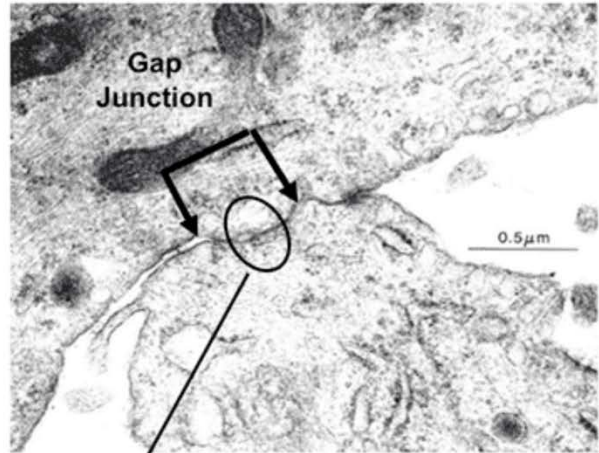
In summary, gap junctions were more prevalent in patients with osteoarthritis, suggesting that gap junctions might be effective therapeutic targets.

We Discovered that Anatomical Structures Known as Gap Junctions Form between Synovial Cells in Culture and in Tissue

Gap junction between two cultured HIG-82 rabbit synovial cells.

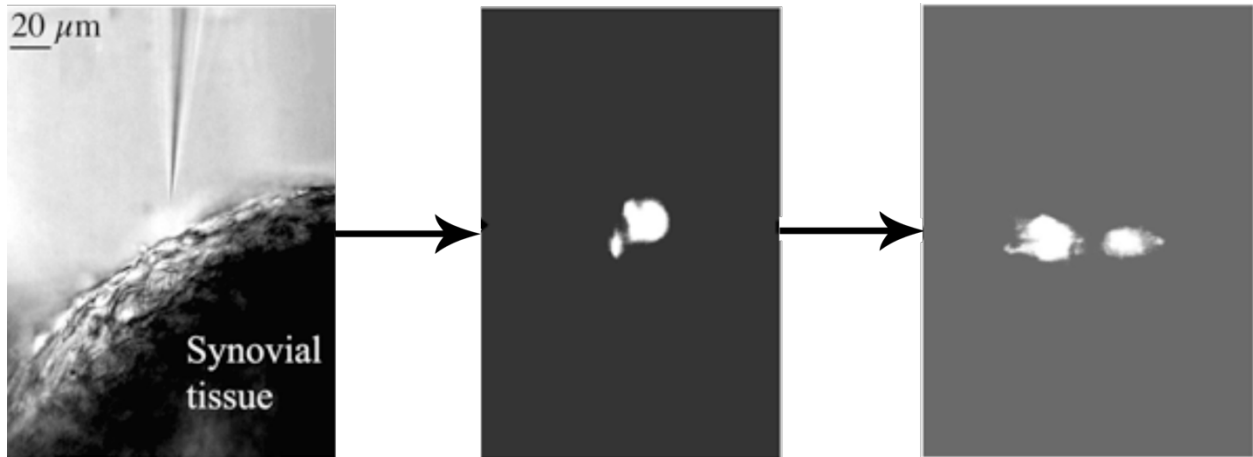


Gap junction between two synovial lining cells from the human knee joint.



Gap junctions are composed of aligned assemblies of protein pores that permit the exchange of ions and small molecules between the cytoplasm of adjacent cells.

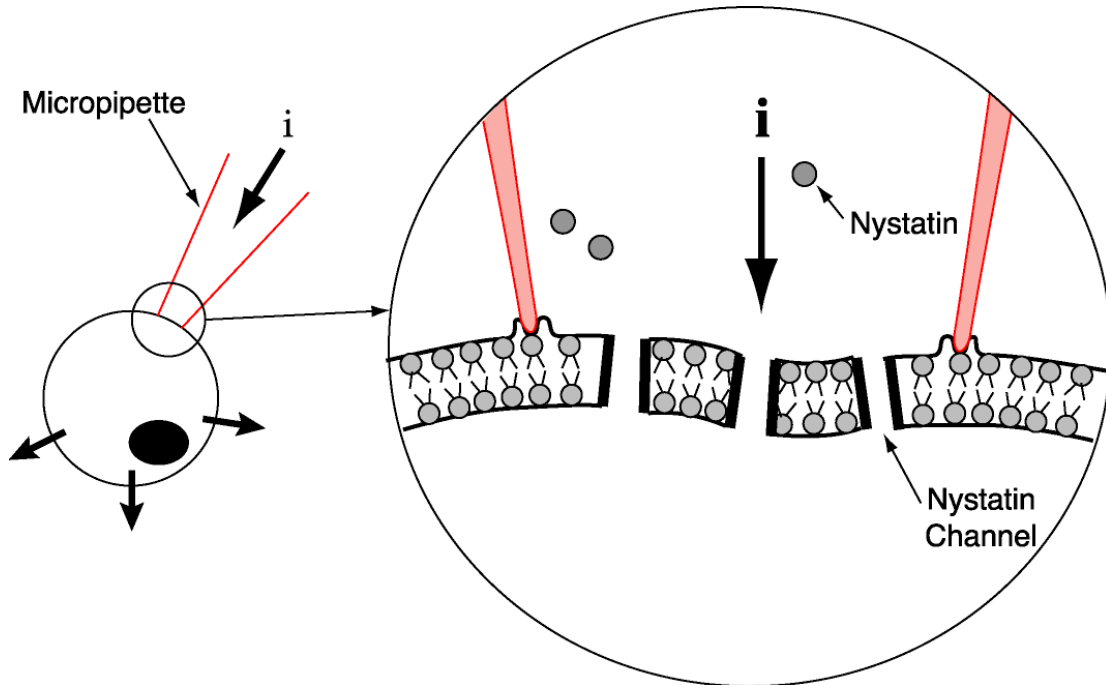
Use of Dye Transfer to Verify Functionality of Gap Junctions



Gap-junction channels are regulated (like membrane ion channels). This experiment demonstrates that adjacent synovial lining cells are functionally connected. It shows that a fluorescent dye injected into one cell can appear in an adjacent cell, which could happen only if the interiors of the two cells were in communication with one another

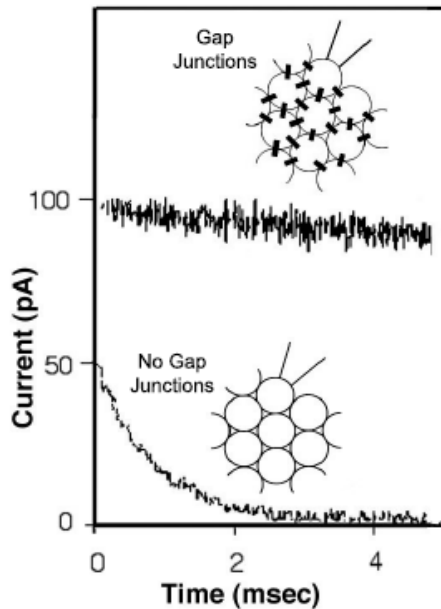
The Patch-Clamp Method: An Improved Technique for Demonstrating Gap Junctions in Synovial Cells

Nystatin Patch-Clamp



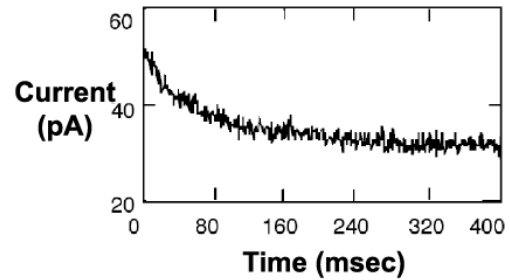
When a glass micropipette is placed against the plasma membrane of a cell, it is possible to measure the electrical properties of the cell. If a voltage is applied to the cell through the micropipette, the resulting flow of current (i) can be measured. The antibiotic nystatin is placed in the micropipette, from where it diffuses into the cell membrane to produce channels which permit the current to flow into the cell.

Electrical Behavior of Gap Junctions



When a patched cell is connected to adjacent cells by gap junctions (shown as black rectangles), then the current that flows through the micropipette when a voltage is applied to the cell has a long time constant (takes a long time to decay to 0). However, when adjacent cells are not connected by gap junctions, the current decays to 0 quickly (bottom curve).

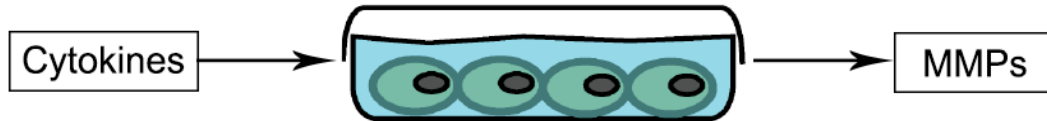
Gap Junctions in Synovial Tissue



The electrical response of synovial lining cells took a long time to decay toward 0. This result unequivocally indicated that the SLCs were connected by gap junctions. From an analysis of the details of the curve it was possible to show that at least 50 cells were connected together by gap junctions.

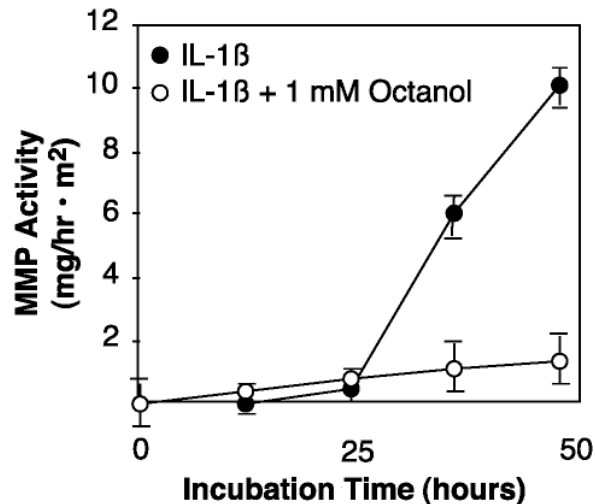
Possible Role of Intercellular Communication Through Gap Junctions in Progression of Osteoarthritis

In Vitro Model for Regulation of Synthesis and Secretion by Synovial Cells



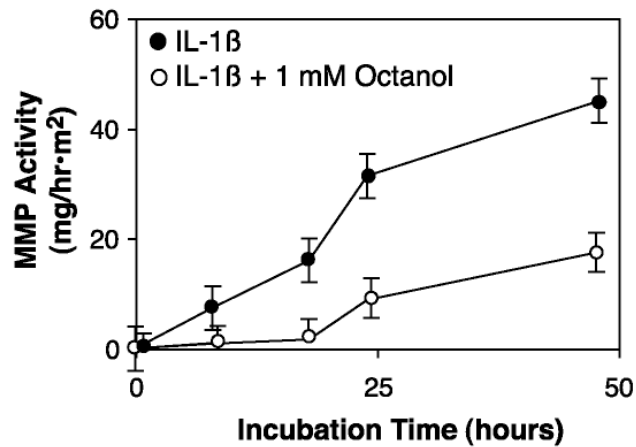
IL-1 β and TNF α can stimulate synovial cells in culture to produce matrix metalloproteinases (MMPs) and other substances. Overexpression of MMPs is believed to be an important process in the development of osteoarthritis. The model system can be used to study the mechanisms involved in stimulated secretion of MMPs.

Effect of Gap-Junction Inhibitor on MMP Production by HIG-82 Synovial Cells Stimulated by IL-1 β



We developed an assay to measure the amount of MMP activity present in the supernatant of synovial cells exposed to IL-1 β . As expected, the MMP activity increased following exposure to the cytokine. When the gap-junction inhibitor octanol was added to the cell medium, the IL-1 β -induced increase in MMP activity was blocked, indicating that gap junctions were critical to the ability of IL-1 β to trigger MMP production. Similar results were found with other gap-junction inhibitors. The results showed that gap junctions were critical to the ability of IL-1 β to trigger the release of MMPs.

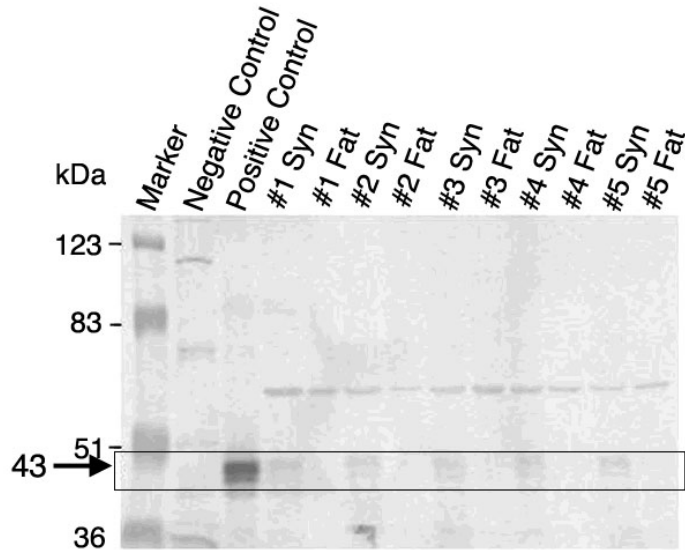
Effect of Gap-Junction Inhibitors on MMP Production by Human Synovial Tissue Stimulated by IL-1 β



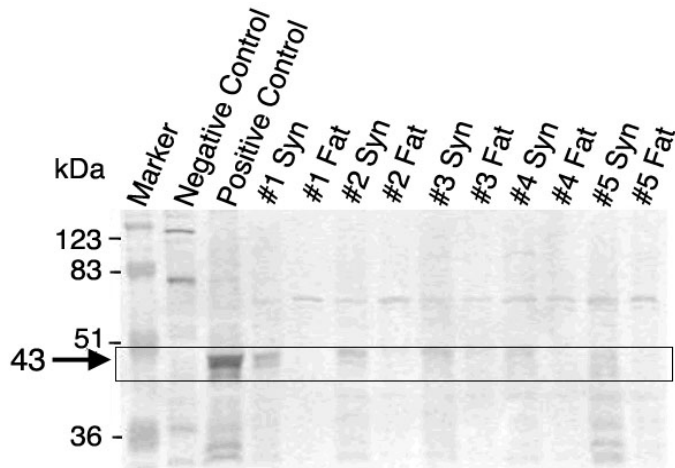
Synovial tissue was obtained from four patients who had OA Grade IV. When the tissue was exposed to IL-1 β in culture, the synovial lining cells were stimulated to produce MMP activity. Again, the process could be blocked using a gap-junction inhibitor, thereby showing the importance of gap junctions in mediating the response. Similar results were obtained with other gap-junction inhibitors.

Evidence that Gap Junctions Are Related to Osteoarthritis

Normal Synovium

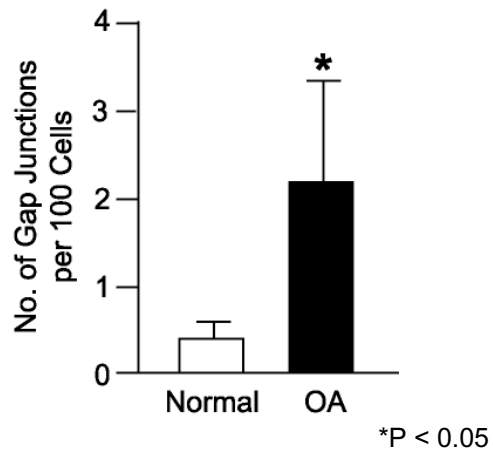


Osteoarthritic Synovium



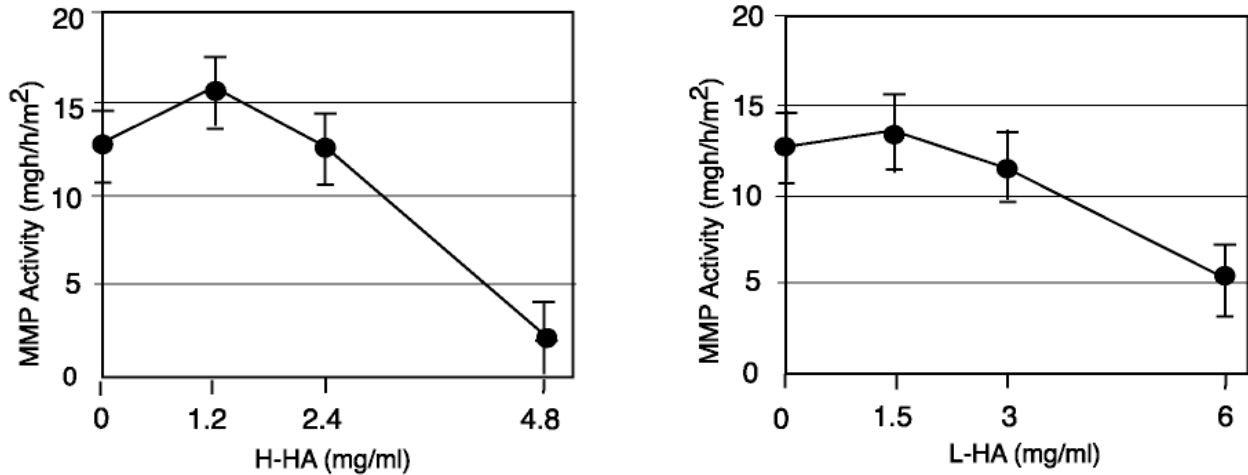
A connexin43 protein was found in the synovial biopsies from each of 5 patients who had OA Grade IV. Analysis of the densitometric traces of the normal and OA blots confirmed that there was statistically significantly more connexin protein in the synovial lining cells from the OA patients. Histological examination of all synovial biopsies revealed that the number of synovial cells did not differ between normal and OA (1–3 cell thick layer). Together, the results suggest that there is an increased amount of connexin43 protein **per cell** in OA, compared with normal.

Electron Microscopy



Synovial biopsies from 6 patients with OA and 5 patients who had no OA were analyzed using electron microscopy to ascertain whether gap junction structures occurred more frequently in OA. A minimum of 500 cells were examined in each patient. The results demonstrated unequivocally that, on average, the frequency of gap junctions was significantly greater in OA.

The Effect of Low Molecular Weight Hyaluronan (L-HA) and High Molecular Weight Hyaluronan (H-HA) on IL-1 β -Induced MMP Activity in the Supernatant of Synovial Cells



A confluent cell layer of HIG-82 synovial cells was cultured for 48 hours in serumless medium containing IL-1 β (100 ng/ml) and varying concentrations of either H-HA (Synvisc) or L-HA (Hyalgan). At the lower concentrations, hyaluronan had no effect on the ability of IL-1 β to stimulate the synovial cells to produce MMPs. At the higher concentrations, however, hyaluronan blocked the effect of IL-1 β . The hyaluronans alone (without IL-1 β) had no effect on MMP activity (data not shown for clarity). The results suggest that a possible mechanism to explain the clinical benefit of hyaluronan is that it antagonizes the gap-junction mediated pathway triggered by proinflammatory cytokines.

Summary

Synovial lining cells are connected by gap junctions, thereby allowing the cells to communicate with one another. The extent of the intercommunication is greater in patients with osteoarthritis, as judged by (1) an increased number of gap junctions and (2) an increased amount of gap-junction protein. *In vitro* studies on synovial cells demonstrated that intercommunication between the cells is critical to their ability to secrete proteases in response to stimulation by inflammatory cytokines. Overproduction of proteases is partly responsible for the degenerative changes associated with osteoarthritis. Consequently, our observations suggest that the development of agents that antagonize intercellular communication might be a useful therapeutic approach to the treatment of the disease.