Gap Junctions in Human Synovial Cells and Tissue

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Introduction

We previously reported the existence of gap junctions between cultured rabbit synovial fibroblasts (HIG-82, ATCC) (O.V. Kolomytkin, A.A. Marino, K.K. Sadasivan, R.E. Wolf and J.A. Albright. Interleukin-1ß switches electrophysiological states of synovial fibroblasts. *Am. J. Physiol.* 273 (Regulatory Integrative Comp. Physiol. 42):R1822–R1828, 1997). This study was undertaken to establish the presence of functional gap junctions in human synovial cells and synovial tissue.

Theoretical Considerations

Measurement of Gap Junctions Based on Analysis of Transient Current

Assume that the measured cell in an aggregate is surrounded by *n* identical adjacent cells and that gap junctions are present between adjacent cells (Figure A). If a voltage step V is applied to the electrode, the resulting current, I, satisfies

$$RC\,\ddot{I} + \{[n + 1 + (r/R_c)](R/r) + 1\}\dot{I} + [1/(rC)][(r/R_c) + 1]I = [1/(rC)][n + 1 + (r/R_c)](V/R_c)$$

where *C* is the capacitance of the membrane of each cell, *R* is the resistance of the perforated patch, *r* is the resistance of the gap junctions between two adjacent cells in the aggregate, and R_c is the resistance of the cell membrane (Figures B and C).

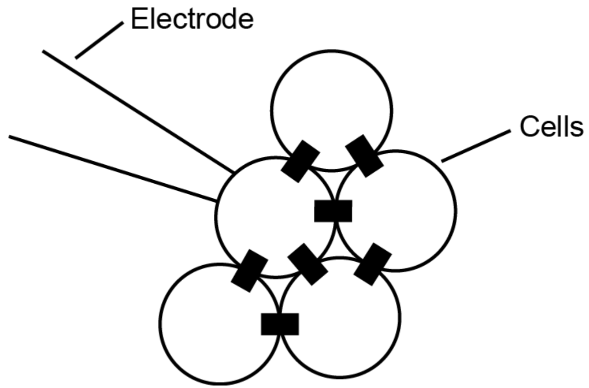
The solution is $I(t) = C_1 exp[k_1 t] + C_2 exp[k_2 t] + I_0$, where

$k_{1,2} =$
$\left\{-[n + 1 + (r/R_c)](R/r) - 1 \pm \sqrt{\{[n + 1 + (r/R_c)](R/r) + 1\}^2 - 4(R/r)[1 + (r/R_c)]}\right\}/2RC$, C_1 and C_2 are constants, and t is time.

$I_0 = V\,[n + 1 + (r/R_c)]/R_c[1 + r/R_c]$, where I_0 is the current for $t \to \infty$.

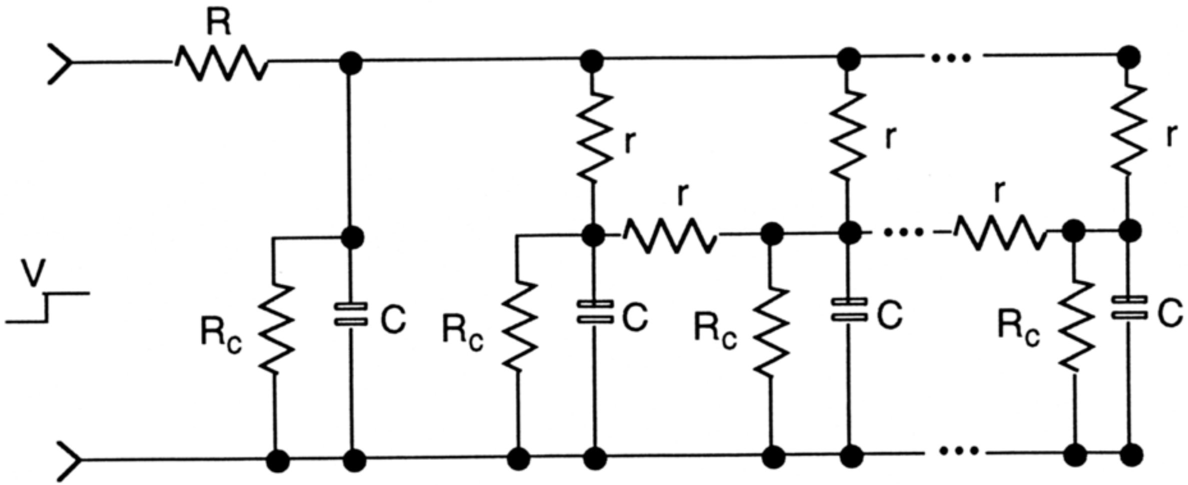
For the case of a single cell ($n = 0$, $t \to \infty$), $k = 1/RC$. R was found from the initial condition, $R = V/I(0)$, with V = 2 mV. C was obtained by fitting the solution $I(t) = I(0)\, exp\,[-t/RC]$ to the experimental curves.

Figure A



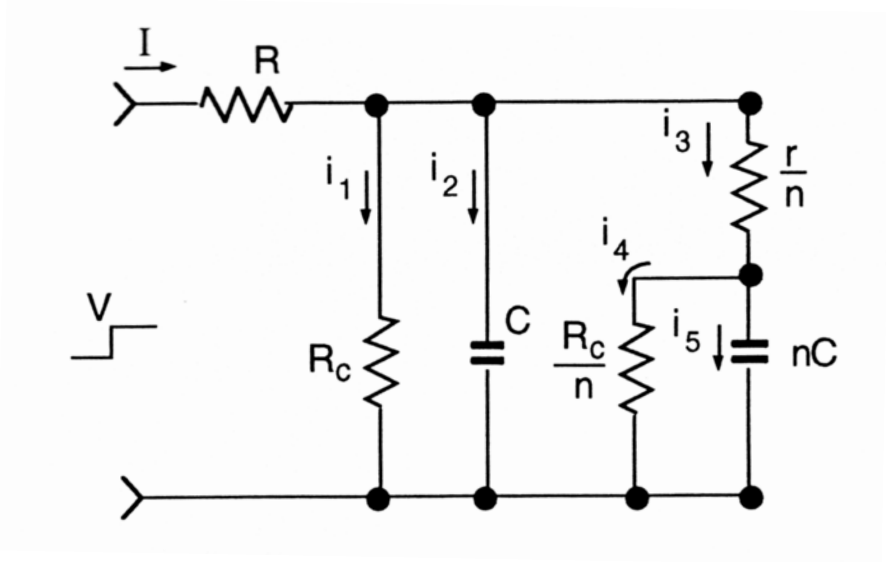
The patched cell is assumed to form gap junctions with adjacent cells.

Figure B



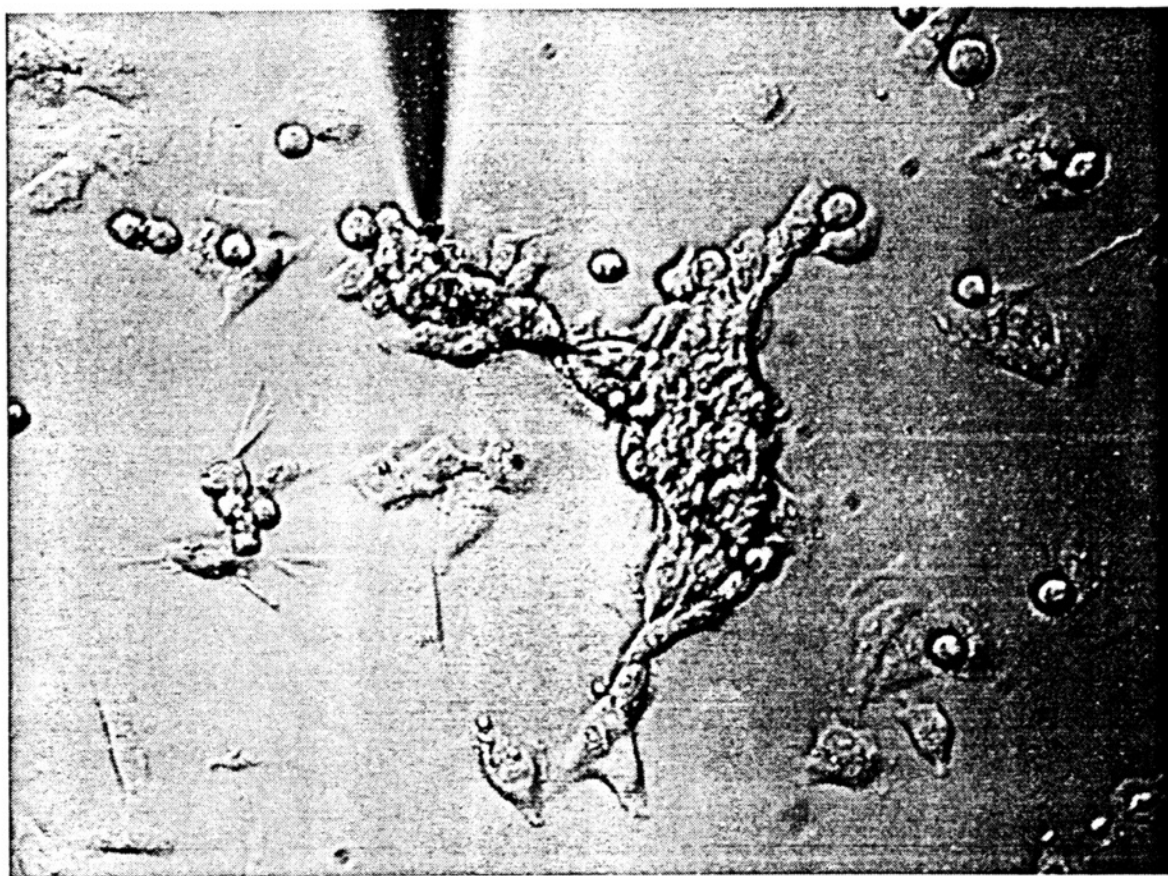
Equivalent circuit for a cell aggregate of arbitrary size.

Figure C



Simplified equivalent circuit for use in calculation.

10 μm



20 µm

Methods

Cells and Tissue

Synovial tissue was obtained from 19 patients who underwent total joint reconstruction or other surgical procedures. The tissue was transported from the operating room to the laboratory in phosphate buffer solution within 10-60 minutes after surgery. Gap junction determinations were made directly on the tissue, on primary cell cultures obtained from the tissue, and on cultured cells obtained from primary cultures.

For measurements on synovial tissue *in situ*, a small piece of the synovium was fixed to the bottom of a petri dish using a fiberglass net glued to a stainless-steel ring. The weight of the ring held the tissue on the bottom of the dish, and the gap in the net (about 3 mm) was sufficient to permit visualization of the tissue and placement of the micropipette. The measurements were made in bath solution.

For measurements in primary cell cultures, the synovial tissue was washed with bath solution and treated with 1 mg/ml collagenase and 1 mg/ml hyaluronidase. After 15 minutes' treatment, cells became detached from the synovial surface and began aggregating on the bottom of the petri dish. Fifteen minutes later (30 minutes after addition of the enzymes) the synovial tissue was removed, the dish with the cells was washed with bath solution, and the cells were incubated in medium for 40 minutes at 37° to allow recovery from the enzyme treatment. The medium was then replaced with bath solution, and all gap junction measurements were made in bath solution, employing the cell aggregates that remained adherent to the bottom of the petri dish.

Synovial cell cultures were established from cells obtained by enzymatic digestion of minced synovial tissue. Following enzymatic dissociation for 30-60 minutes at 37°C, using 3 mg/ml collagenase in F12 medium, the resulting cells were centrifuged, washed twice in PBS, resuspended in F12 medium with 10% heat-inactivated FCS containing penicillin (100 units/ml) and streptomycin (100 µg/ml). Adherent cells were grown to confluence and passaged with 0.25% trypsin. The procedure yielded a morphologically homogeneous population after the second passage.

Electrical Measurements

The nystatin perforated-patch method was used to measure the gap junctions at voltage clamp. **This method was used because it permits use of the whole-cell configuration for measuring electrical properties of the cell while preventing diffusion of small signaling molecules from the cell into the electrode. The nystatin method therefore preserves intracellular regulation.**

Glass capillaries 1.0 mm in diameter were pulled in two steps (PB-7, Narishige) and fire-polished in a microforge (MF-9, Narishige). The resistance of the electrodes was 7-9 MΩ in bath solution. The pipette salt solution was (in mM): K-aspartate (monopotassium salt), 125; KCl, 30; NaCl, 4; HEPES-KOH, 10; pH, 7.2, 318 milliosmoles/liter. The composition of the bath solution was (in mM): NaCl, 145; KCl, 5.4; $CaCl_2$, 1.5; $MgCl_2$, 1.0; HEPES-NaOH, 5.0; glucose, 5.0; pH, 7.3, 328 milliosmoles/liter. Because nystatin interfered with giga-seal formation, the tip of the pipette was filled with a nystatin-free solution prior to the addition of pipette solution containing 0.3 µg/ml of nystatin. The giga-seal was formed during the time needed for the nystatin to diffuse to the tip of the micropipette.

Giga-seals (≈10 GΩ) were formed under negative pressure (5-10 cm, H_2O), typically within 0.5-5 minutes; the success rate was greater than 50%. After giga-seal formation the negative pressure was removed and the nystatin channels formed within 5-15 minutes; the resistance of the perorated-patch membrane was 40±20 MΩ. Giga-seals and nystatin pores usually remained stable for hours.

Transient currents were recorded using a patch-clamp amplifier (Axopatch 200B, Axon Instruments). The amplifier was connected to a computer (TL-1 DMA Interface, Axon Instruments), and commercial software (pCLAMP 6, Axon Instruments) was used to control the amplifier, and to collect and analyze the experimental data.

Gap Junction Resistance

After formation of a perforated patch, a 2-mV step was applied and the transient current passing through the electrode was measured in aggregates of different sizes. Theoretical analysis showed that the time constant of the transient current decay depended strongly on the resistance between adjacent cells in an aggregate. If cells in an aggregate were electrically separated from each other, the resistance would be high and the time constant would be low regardless of the number of cells in the aggregate, because the voltage step would charge the membrane capacitance of only one cell. However, if the cells were connected to each other by gap junctions, the resistance would be relatively low and the time constant of the current decay would increase with increasing number of cells in the aggregate, because the voltage step would charge the membrane capacitance of all cells in the aggregate.

Results

Typical results for the transient current response from aggregates of cultured synovial cells are shown in the Figure. Curve 1 shows the transient current decay for a single human synovial cell in culture. After applying the 2-mV step, the transient current charged the capacitance of the cell membrane (about 1 μF/cm^2), resulting in the rapid current decay shown (time constant (t) ≈1 msec).

Curve 2 shows the transient current for a human synovial cell aggregated with a small number of other cells (≈3). The transient current decay was slower (t ≈ 4 msec) in comparison with that of a single cell, indicating that the capacitance of the cells was in parallel and therefore additive. This could occur only if the cells connected to each other by gap junctions.

Curve 3 shows the transient current for a human synovial cell in a large cell aggregate (more than 20 cells) in culture. The transient current decay was slower (t ≈ 47 msec) than the cell in the small aggregate, as would be expected if the cells in the large aggregate also formed gap junctions.

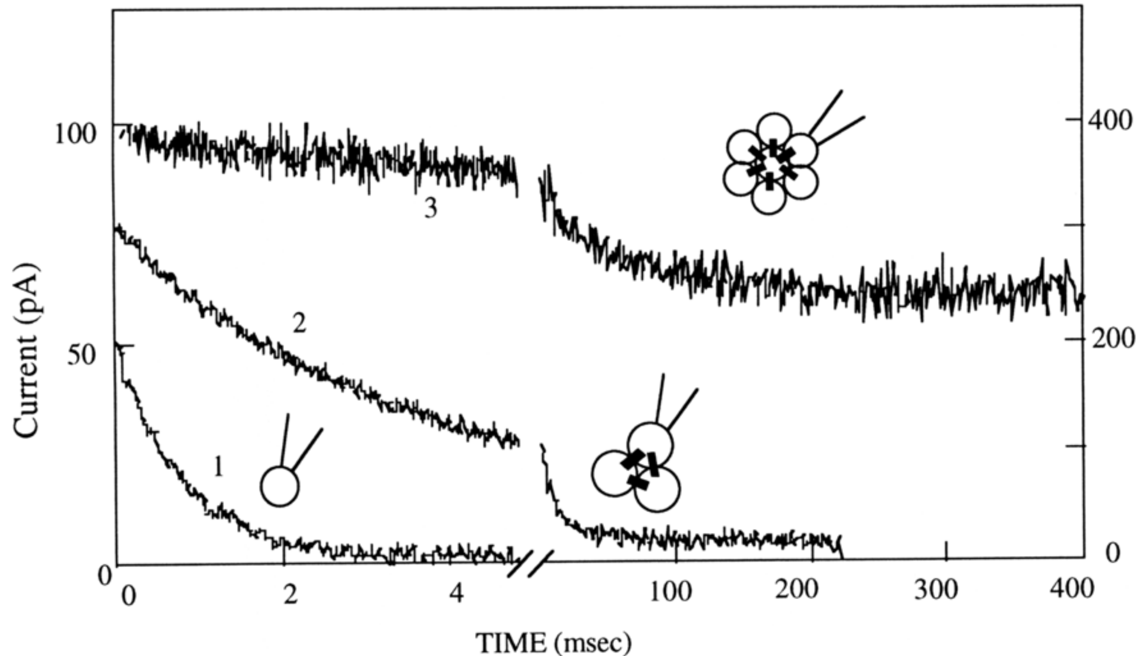
The results shown in the Figure were observed in the cultured cells of all 19 patients studied (passages 2-10). In each instance, the time constant of the current decay lengthened as the number of cells in the aggregate was increased.

To provide evidence that the connexins forming the gap junctions were present in the tissue, and were not solely a result of culturing, measurements were made on cells released from synovial tissue by enzyme treatment. Measurements on aggregates of various sizes made within 90 minutes of the time of removal of the tissue from the patient were identical to the results shown in the Figure (N=3 patients).

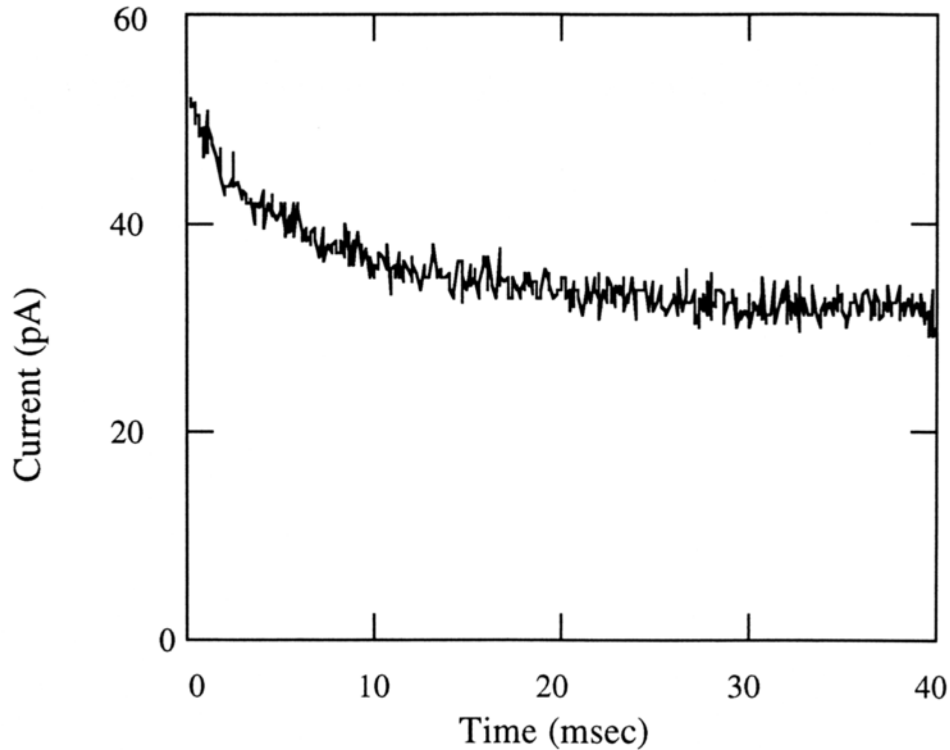
The gap junction resistance between adjacent cells was found from measurement of transient current by the method described in Theoretical Considerations to be 300±100 MΩ in measurements for 7 different aggregates of 2-3 cells.

Further evidence that functional gap junctions existed in synovial tissue was sought by attempting to patch synovial cells in tissue explants. In most instances it was not possible to make a nystatin patch to the synovium because of the unavoidable presence of cellular debris and intercellular substances. in 5 instances, however, involving cells from 3 patients, patches were formed, and it was therefore possible to employ the electrical technique to ascertain whether the patched cell formed gap junctions. In each instances, long time constants for decay of the current were observed, suggesting that the patched cell formed gap junctions (Figure).

Transient Current Responses for Human Synovial Cells in Culture to a Voltage Step

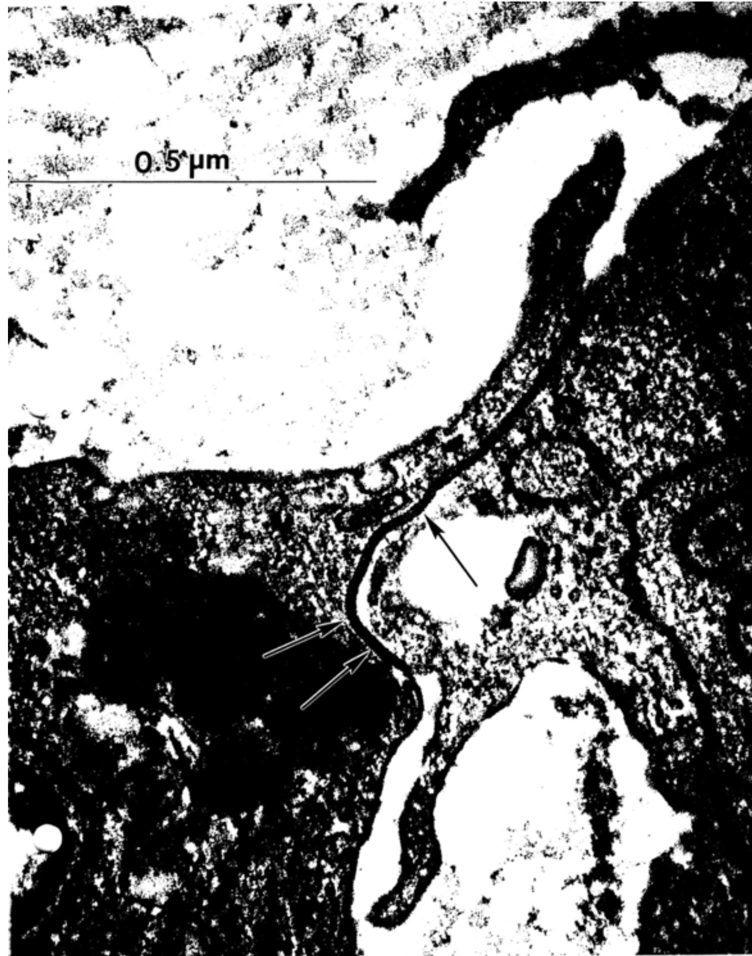


After perforated patch formation, a 2-mV step was applied to the electrode at zero time. Curve 1, patch formed on an isolated cell; curve 2, patch formed in a cell that was aggregated with 3 other cells; curve 3, aggregate of more than 10 cells. Individual records were filtered with a 10-kHz low-pass 4-pole Bessel filter; each curve was produced by averaging 10 voltage steps. Scale for curves 1, 2 is shown on the left side, and for curve 3 on the right side.



After perforated patch formation, a 2-mV step was applied to the electrode. The curve was produced by averaging 10 voltage steps. Individual records were filtered with a 10-kHz low-pass 4-pole Bessel filter. The long current decay proved the existence of gap junctions between cells in the tissues.

Synovial Cells From an Osteoarthritis Patient



Two synovial cell processes are united by a lengthy gap junction (arrows). (Courtesy of Dr. William Meek, Oklahoma State University.)

Discussion

We previously found that gap junctions between aggregated synovial cells were necessary to permit the cells to respond to IL-1ß (2). Because the synovial cells were connected by gap junctions, it is possible that the consequences of a ligand-receptor interaction in one or a few cells in an aggregate could spread either actively or passively through the aggregate, effectively propagating the ability of cells to transition from the high to the low V_m state. If such a mechanism operated *in vivo*, it would permit a response by large regions of the synovium (in either normal or pathological cases) following ligand-receptor interactions in localized regions.

Our observation that the synovial fibroblasts formed gap junctions raises the possibility that electrical coupling between the synoviocytes might be an important aspect of synovial physiology. For example, based on morphological evidence, it appears that the number of synovial gap junctions is altered in arthritic patients (3). Thus it is possible that changes in gap junctions might play a causative role in development of arthritis.

Advantages of the Electrical Method

1. Permits determination of gap junctions without disturbing intracellular signaling. (Determining gap junctions with fluorescent dyes requires use of impaling electrode.)

2. Permits quantitative measurement of resistance of gap junctions between two cells in an aggregate of cells.

3. Measurements of gap junction resistance are rapid (0.2-2.0 sec).

4. The method is applicable to small cells (~10 μm).

5. Only one measuring electrode is needed. (Previous electrophysiological methods required two measuring electrodes.)

References

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2. Kolomytkin, O.V., Marino, A.A., Sadasivan, K.K., Wolf, R.E. and Albright, J.A.: Interleukin-1ß switches electrophysiological states of synovial fibroblasts, Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42):R1822-R1828, 1997.

3. Meek, W.D., Raber, B.T., McClain, O.M., McCosh, J.K. and Baker, B.B.: Fine structure of the human synovial lining cell in osteoarthritis: Its prominent cytoskeleton, Anat. Rec. 231:145-155, 1991.

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