divided into two types with respect to their biochemical characteristics and the nature of the process of their formation. The nanotubes of type I are shorter, more dynamic and contain actin filaments. They are formed when cells explore their surroundings in order to make contact with another cell. The nanotubes of type II are longer, more stable and have cytokeratin filaments. They are formed when two already connected cells start to move apart. On the nanotubes of both types small vesicles were found as an integral part of the nanotubes (i.e. dilatations of the nanotubes). The dilatations of type II nanotubes do not move along the nanotubes, while the nanotubes of type I have frequently dilatations (gondolas) that move along the nanotubes in both directions and are formed in different ways. We suggested theoretical models that may explain how these nanotubes are created and stabilised.

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1445-Pos Board B289

Simulation Of Particle Diffusion Across Gap Junction Channels Based On Their Pore Geometry Explains Unidirectional Fluxes

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Connexin proteins form gap junction channels that allow intercellular communication with distinct perm-selectivity properties. Mono-heteromeric and heterotypic combinations of cardiac connexin43 (Cx43) and Cx45 induce a preferential flux based on molecular size. For Lucifer Yellow or Rhodamine123, preferential flux was 3x larger from homomeric to heteromeric connexons. For heterotypic combinations, fluxes from homomeric Cx45 to homomeric Cx43 connexons were 4x larger. This favored direction was not expected. **Our objective was to use computational simulation of particle diffusion across gap junction channel pores to find if geometrical parameters can explain our in vitro permeability data.**

HeLa cells were stained with red or green dyes to differentiate expression and co-cultured on glass cover-slips for 8 hours. A Nikon epifluorescent Eclipse7000 microscope helped quantifying diffusion. Fluorescent dyes were iontophoretially injected into a single red cell surrounded by green cells. Coupling coefficient (cells touching the injected cell/touching cells with dye) was determined 3 min after.

3D geometric model of the pore was mathematically modeled combining cylinders, cones and an ellipsoid. Particle position, velocity, acceleration and force vectors were calculated after every time step (10^{-14} s), considering wall-particle and particle-particle elastic interactions, inter-particle electrostatic, Brownian and other forces. Particles' paths were recorded and those crossing the pore were counted. Without electric field, number of particles crossing increased linearly as mouth radius increased. With an electric field, number of particles crossing varied non-linearly with a maximum when radius was around 21.4 Å. **Our computer simulations predict that changes in phosphorylation, voltage or connexin recombination yield to changes in pore structure which in turn affects large molecules' permeability.**

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Diffusion-Sensing versus Quorum Sensing in a Model Biofilm

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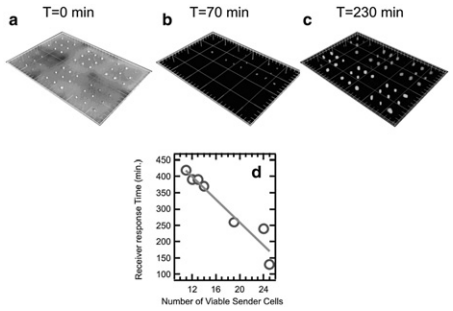
Cell interactions through soluble signaling molecules control differentiation, immune response and other physiologically vital processes in everything from tissue to biofilms. We tested a model of a bacterial biofilm, which uses an autoinducer (AI), N-acyl-L-homoserine lactone (AHL) for signaling, to discriminate quorum-sensing (QS) and diffusion-sensing (DS). The AI induces the transcription of a set of genes that includes the gene-encoding the AI-producing enzyme, promoting a positive feed-back. We creating a synthetic biofilm using a microfluidic network, to convey cells to an assembly area where multiple, time-shared optical tweezers are used to array them. The cells are encapsulated in a 30μm×30μm×45μm volume of hydrogel mimicking an extra-cellular matrix. To extend the size, shape and constituency of the array, we then step to an adjacent location while maintaining registration with the reference array, and repeat the process as illustrated in Figure 1(a). Using this step-and-repeat method, we formed arrays of *E. coli* engineered to produce AHL, which is functionally linked to a fluorescence reporter. As shown in Fig. 1(d), the threshold to induce AI production and fluorescence depends on the number of cells and the mass-transfer, indicating that QS is a side effect of DS.



1447-Pos Board B291

Identification of Transmembrane Helix 2 (M2) as the Main Pore-Lining Helix of Connexin 43 Gap-Junctional Hemichannels (GJH)

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GJH are connexin hexamers that dock head-to-head to form gap-junctional channels. Current GJH models are based on low-resolution structural data showing 4 transmembrane α helices per connexin monomer, with insufficient detail for helix assignment; helices have been named A, B, C and D, with B and C as pore-lining. Here, we used luminescence resonance energy transfer to calculate distances between same-position residues in diametrically-opposed monomers of purified GJH formed by functional single-cysteine Cx43 mutants. Mutants were labeled with one donor (chelated Tb^{3+})-labeled and one or more fluorescent acceptor-labeled monomers, and distances between donor-acceptor probes were calculated from sensitized acceptor emission lifetimes. The distances allowed for the assignment of M2 (~45 Å) and M3 (~41 Å) as pore-lining, and M1 (~57 Å) and M4 (~60 Å) as peripheral helices. On the extracellular side (narrower side of the pore), the distances were ~23 and ~40 Å, for M2 and M3 residues, respectively. The shorter M2 extracellular-side distance is evidence of tilting and suggests that M2 is helix C, the primary pore-lining helix. Single-Cys mutants of M1, M2 and M4 (8 per helix) were labeled with BADAN, a probe that displays emission maxima at longer wavelengths in hydrophilic environments. BADAN emission peaked at $\geq$ 480 nm in five of the M2 residues studied, none of the M1 and only one M4 residue position (probably exposed to the cytoplasm). From these and previous results, we built a new GJH model with the following helix assignment: M1 = A, M2 = C, M3 = B and M4 = D. This work was supported in part by NIH grants DC007150, GM068586 and GM79629, American Heart Association, Texas Affiliate grant 0755002Y, and Texas Advanced Research Program grant 010674-0046-2007.

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Bridging Cadherin-mediated Cell Adhesion to Multicellular Pattern Formation by Multiscale Modeling and Simulation

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The difficulty to study the cadherin-mediated cell adhesion lies in the complexity from cadherin binding specificity to multi-cellular pattern formation. Cadherins are the main adhesion molecules on the cell membrane to hold cells together. They play important roles in many biological processes, such as cell sorting during embryonic development, acting as tumor-suppressors and also mediating cell signaling. Cadherin binding specificities have been shown to be primarily homophilic, but can be more promiscuous. On the other hand, cell-cell adhesion specificity is not simply correlated with molecular-binding specificity within the cadherin family. It has been suggested that cellular binding specificity also arises from differences in overall cadherin cell surface concentration. In order to understand how these different factors lead to various multi-cellular pattern formation results, we present a computational strategy to model the relation between cadherin binding and cell adhesion in multiscale. Three levels of simulation schemes in different resolutions are constructed to model the multiple cellular system, cell membrane interface and cadherin molecular binding, respectively. Results generated from higher level of simulation are used as input parameters for the simulation in lower level, which combine different modeling into a comprehensive and hierarchic computational strategy. Results from different experimental methods can further be

used to guide or parameterize our modeling, and also can be used to compare with our simulation results. Current simulation results show that different adhesion energetic parameters can lead to different multi-cellular patterns, consistent with the experimental data. Future improvements and potential applications are also discussed.

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Relating Electrical Conductance, Connexin 43 Immunostaining, and Cell Shape in Micropatterned Cardiac Cell Pairs

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Gap junctions are responsible for cell-cell electrical coupling and maintaining normal cardiac conduction patterns. Connexin 43 (Cx43) is the dominant gap junction protein in ventricular myocytes. Although the relationship between Cx43, conduction, and tissue structure have been extensively studied in engineered monolayers of cardiac tissue, there have been few studies comparing conductance, Cx43, and cell shape on the cell-cell level. We have used micropatterning to control the dimensions of myocyte pairs and study electrophysiological properties at very high resolution. We hypothesized that conductance and Cx43 immunostaining would be directly correlated. We also sought to relate our electrophysiological measurements to cell shape. Using a dual voltage clamp system, we measured the conductance of micropatterned ventricular myocyte pairs, and subsequently fixed and immunostained the same cells for Cx43. Thus, we compared conductance and Cx43 immunostaining serially in the same cell pair. The volume of Cx43 immunostaining was determined using confocal microscopy and quantitative software programs. Using brightfield images, we measured cell dimensions and 2-dimensional cell-cell contact. We studied three types of rectangular cell pairs with varying length to width aspect ratios (3.33:1, 5:1, 6.67:1). The average length of the cell-cell junction ($R2=0.99$, $n=23$), average conductance ($R2=0.92$, $n=22$), and average Cx43 immunostaining ($R2=0.85$, $n=22$) increased linearly relative to the aspect ratio. We found a linear relationship between Cx43 immunostaining and conductance ($R2=0.70$, $n=22$). A weaker linear relationship was found between conductance and 2-dimensional length of the cell-cell junction ($R2=0.51$, $n=23$). Our results suggest that cell pairs nearly maximize their contact area, which contributes to increases in both Cx43 and conductance. Cell pairs with higher length-width aspect ratios have more cell-cell contact and therefore higher Cx43 density and conductance. However, Cx43 density is the most important determinant of conductance.

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Role of connexin 32 hemichannels in ATP release from Schwann cells

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The X-linked Charcot Marie Tooth (CMTX) is an inherited disease due to mutations in connexin 32 (Cx32) gene expressed in Schwann cells (SC) of peripheral nerves. In SC, Cx32 localizes in the paranodes, in the Schmidt Lanterman incisures and in the cell surface. Cx32 can form "reflexive" gap junction channels as well as functional hemichannels open upon membrane depolarization. We have explored the permeability of Cx32 hemichannels to ATP, in SC and in a heterologous expression system. Murine sciatic nerve trunks release ATP under electrical or mechanical stimulation, as determined by the luciferase reaction. Luminescence imaging revealed that ATP release is especially intense at the SC paranodes, which contain the highest immunofluorescent label for Cx32. Cultured adult SC have a high expression of Cx32 and under mechanical stimulus release ATP being insensitive to exocytosis blockers like brefeldin A. In *Xenopus* oocytes expressing human Cx32, we measured simultaneously the hemichannel currents and the release of ATP elicited by a square depolarizing pulse up to +100 mV. Depolarizing pulses induced characteristic slowly activating outward currents and when the membrane potential returned to the holding voltage tail currents coinciding with the peak of ATP release. The deconvolution of the light signal revealed that the time courses of the tail current and the ATP release were coincident. We established a direct relationship between the amount of ATP released and the amplitude of tail current. Applying positive voltages closer to the ATP reversal potential during the tail current reduced the amount of ATP released. Five different single amino acid mutants of Cx32, described in CMTX, affecting intracellular, extracellular or transmembrane domains, were tested. Those mutations deeply inhibited or abolished the hemichannel currents and the ATP release.

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Regulation Of Neuronal Connexin-36 Channels by pH

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Neurotransmission through electrical synapses plays an important role in the spike synchrony among neurons and oscillation of neuronal networks. Indeed, electrical transmission has been implicated in the hypersynchronous electrical activity of epilepsy. We have investigated the influence of intracellular pH (pHi) on the strength of electrical coupling mediated by connexin36 (Cx36), the principal gap junction protein in the electrical synapses of vertebrates. In striking contrast to other connexin isoforms, the activity of Cx36 channels decreases following alkalosis rather than acidosis when it is expressed in Xenopus oocytes and N2A cells. This uncoupling of Cx36 channels upon alkalinization occurred in the vertebrate orthologues analyzed (human, mouse, chicken, perch and skate). While intracellular acidification caused a mild or moderate increase in the junctional conductance of virtually all these channels, the coupling of the skate Cx35 channel was partially blocked by acidosis. The mutational analysis suggests that the Cx36 channels may contain two gating mechanisms operating with opposing sensitivity to pH. One gate, the dominant mechanism, closes for alkalosis and it probably involves an interaction between the C- and N-terminal domains, while a secondary acid sensing gate only causes minor, albeit saturating, changes in coupling following acidosis and alkalosis. Thus, we conclude that neuronal Cx36 channels undergo unique regulation by pHi since their activity is inhibited by alkalosis rather than acidosis. These data provide a novel basis to define the relevance and consequences of the pH-dependent modulation of Cx36 synapses under physiological and pathological conditions.

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Propagation of Fast and Slow Intercellular Calcium Waves in Primary Cultured Smooth Muscle Cells

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Tissue blood flow is controlled by the changes in the diameter of the arteries and arterioles due to the coordinated contraction and relaxation of smooth muscle cells (SMCs) within the vascular wall. The contractile state of SMCs is regulated primarily by the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). The increase in $[Ca^{2+}]_i$ in response to hormonal stimuli propagates from cell to cell along the vessel wall as a wave, and activates the process of contraction. The mechanism underlying this phenomenon, however, is not yet fully revealed.

In this work, we study the onset and propagation of intercellular calcium waves through gap junctions in primary cultured vascular SMCs. For imaging intercellular Ca^{2+} waves, SMCs seeded along a collagen line and loaded with the fluorescent Ca^{2+} indicator Fluo-4 were locally stimulated mechanically or chemically. The stimulation evoked two distinct calcium waves: 1) a fast Ca^{2+} wave (several mm/s), and 2) a much slower Ca^{2+} wave (few tens of μm/s); both waves propagated to neighboring cells. The fast Ca^{2+} wave was caused by the propagation of membrane depolarization and subsequent Ca^{2+} influx through voltage operated channels. This fast wave facilitated the onset and propagation of a slow, but higher amplitude Ca^{2+} wave that started from the stimulated cell and propagated to neighboring cells. Our results suggest a possible mechanism for intercellular Ca^{2+} wave propagation through gap junction channels in SMCs.

1453-Pos Board B297

Connexin Pore Block By ABG-Sugars

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Pore blockers are valuable for structure-function study of membrane channels. Prior work shows novel anthranilamide moieties (ABG) derivatized to maltosaccharides of different lengths (Gn: n-glucose) are size-indexed pore blockers of connexin channels: block occurs with size-match with a segment of the pore lumen, not if the lumen or blocker is too narrow or wide. Permeation studies using the same maltosaccharides derivatized with an uncharged fluorescent group (PA-sugars) show the narrowest part of the pore (size-selective filter) decreases $Cx32>Cx26\approx Cx26/Cx32$, the last being heteromeric. Efficacy studies of ABG-sugar block from each side of the pore reveal new information about