

## CELL TO CELL COMMUNICATION:

# Implications for Oral Health, Pathogenesis, and Drug Therapies

BY JANYCE HAMILTON

Humans talk so much that strategic interruption is a required part of conversation. Animals vocalize or thump out their own language. Now it turns out that molecularly, communication between cells is not so different. As Alice in Wonderland said on her strangely plausible trip through a rabbit hole, it just gets “curiouser and curiouser.”

## AUTHOR

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**S**ure cells respire, have a nucleus, and make waste as if little critters. But in recent years, a surprisingly personified trait has been cocking the heads of research scientists — it has been shown that a cell can *talk* to another cell. Better yet, the other cell hears and responds. Among the known cell types that communicate throughout their communities are bacterial, animal, and human.

This report talks about how scientists continue to understand what was once so mysterious: cell-cell communication. Also explained is exactly how cells communicate and how questions are raised about whether or not cells have a sentient or “knowing” quality. Examples of how these molecular communication processes are being studied by university and government oral craniofacial researchers are provided. Novel pharmacotherapeutic approaches to one day molecularly dissuade colonization of biofilms and periodontal pathogens are not far-fetched. Strange stuff is going on, too, including fooling cells into thinking nothing is wrong, while the clinically dead are revived, and trying to prevent “back talk” so communities of cells accept a newly implanted cell that will contain the first artificially created chromosome.

## How it Was Discovered That Cells Talk

Bonnie Bassler, PhD, is an investigator with the Howard Hughes Medical Institute, Chevy Chase, Md., and professor of molecular biology, Princeton University, Princeton, N.J. She has been dubbed “The Bacteria Whisperer” for her entertaining and affectionate lectures about how bacterial cells talk.<sup>1</sup> She credits the Hawaiian bobtail squid as “ground zero” in which molecular detail of cell-cell communication was first comprehended.<sup>2</sup>

Explained Bassler, the 2-inch long squid lives buried in the sandy floor of the coastal Pacific shallow salt water by day and emerges at night. It’s different than most sea creatures in that it has a glowing light organ filled with luminescent bacteria known as *Vibrio fischeri*. In the 1960s and ‘70s microbiologists K. Nealson and J.W. Hastings of Harvard University studied the properties of *V. fischeri* bacte-

ria. That team's first clue to bacterial cells communicating among each other was that if the bacteria were outside of the organ, they "knew it" and didn't glow; there had to be a dense number of them together first. The team theorized that a chemical molecule, which they dubbed an "autoinducer" (AI), was released so the cells got the message that they had amassed in sufficient numbers and so it was time to flip the gene switch on to light up.

Nealson and Hastings published a paper in 1973 that they had found the autoinduction phenomenon and, furthermore, that they isolated, characterized, and identified the signal molecule (autoinducer).

Another piece of the mystery came in during the 1980s when Dr. Mike Silverman and grad student Joanne Engebrecht, from the Agouron Institute, La Jolla, Calif., chopped up the glowing chromosome — the structural genetic enzymes for luminescence and the density sensing mechanism — from the *V. fischeri* bacterial cell, injected it into *Escherichia coli* to see if it also glowed on a piece of DNA. It did and that part was the AI, the density-sensing mechanism. Yet, more proof there was communicating going on was that when the squid was propelling itself around at night to hunt for food, it used biochemical sensors on its back to detect how much moon and starlight were hitting it. To counter-illuminate the light shining upon it through the water's surface, it would open its skin flap covering the light organ, and the *V. fischeri* cells needed to project the precise same amount of light from its bottom side, would communicate "the light has changed," and begin to amass. Now the amount of glow emitted from the bottom side matched that hitting the topside so that the squid didn't cast a shadow on the sandy seafloor. The Hawaiian bobtail squid propelled

about, covertly evading predator detection while sneaking up on shrimp.

Knowing all of this, Bassler commenced upon new research and nailed things down. In another glowing sea creature, *Vibrio harveyi*, she identified not one but two signal circuitry systems and published the definitive proof of bacterial cell-cell talk and quorum sensing.

**Quorum sensing.** The "butterfly effect" goes like this: Will the flap of a butterfly's

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wings affect a tornado in Texas? Likewise, is there such an effect with cells? If one cell's receptors receive a signal at the cell surface, they dive through the cytoplasm and to the brain and genes, which translate and activate a response (even if it's a nonresponse). One little cell kicking out an inflammatory agent may not be perceived as arthritic knee pain, but cells make sure they "call all the butterflies in Texas" for the biggest effect on the tornado. Turns out that a bacterial cell knows, said Bassler, that one cell dribbling out toxins isn't going to amount to much kick, so the bacteria "wait" and count heads. They sense when they are alone versus together via "quorum sensing" AI molecules, and when they know there are enough of them together, then they all secrete their toxins at the same time (**FIGURE 1**).

Said Bassler, "Bacteria are using a rich

chemical lexicon to coordinate population-wide behaviors and carry out tasks in groups that they could never manage if they simply acted as individuals."

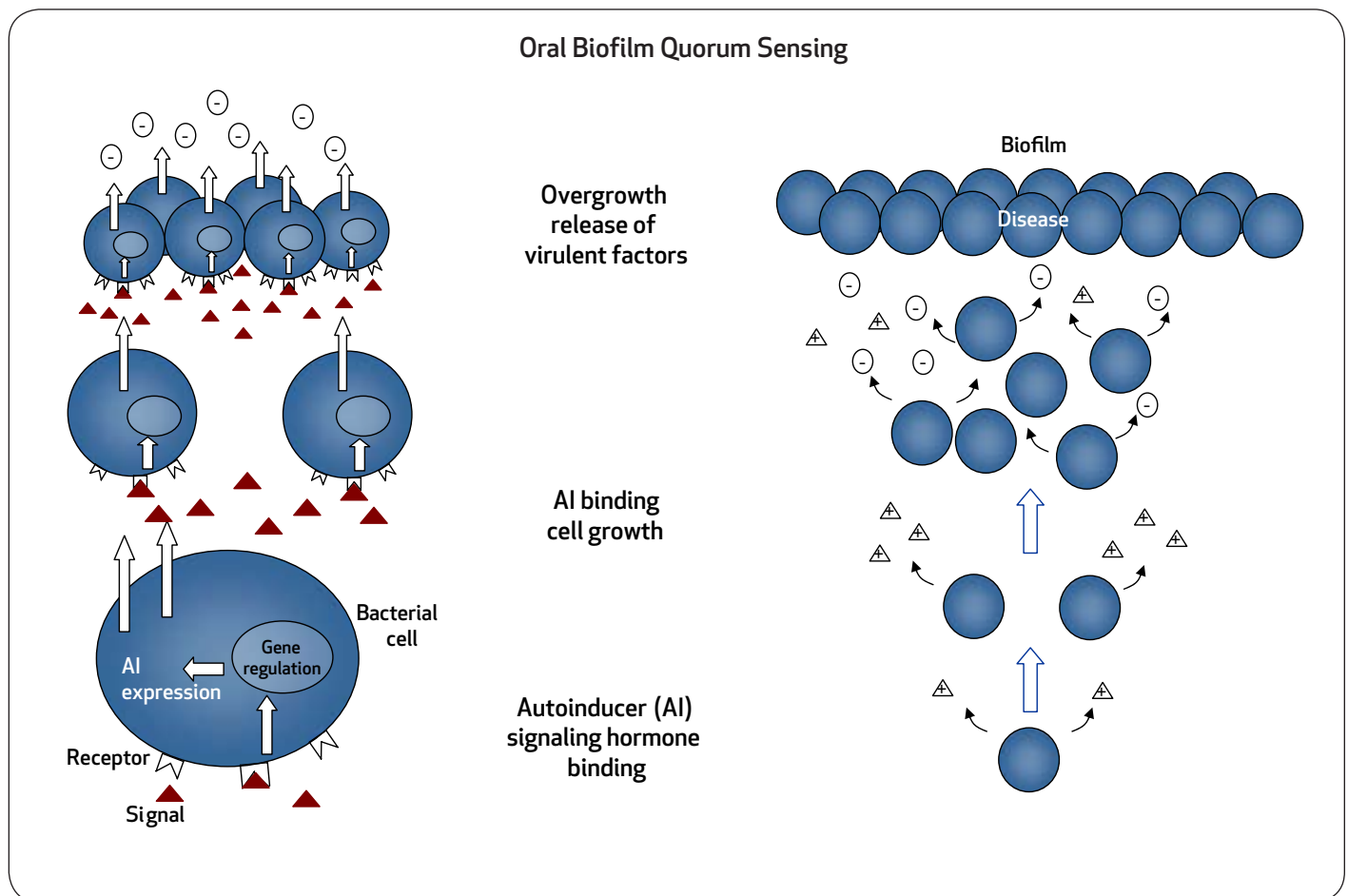
### How One Cell Talks to Another Cell

Bassler said cells are not socially reclusive loners, but have a language for their own species, and are in effect multilingual for communicating between species. They aren't psychic and they don't have "eyes," so within the dark confines of intercellular broth, they talk to tell each other when they are there and who is there. "They coordinate gene expression, which ultimately coordinates behavior so they can all act as one, as if multicellular organisms," she said.

Many questions remain that science still needs to work out. For example, how is information preserved, where is it stored in the cell circuitry? Physicists work on this too. Also, how do bacterial cells decode mixes of signals and know to tweak the functions of multiple genes given various messages simultaneously? What can impede, stop, or introduce noise-to-signal flow out of and in between cells — can the signals be eaten, tricked, eavesdropped, and tattle-tailed upon?

Chemical signaling used to be thought a trait of only higher organisms, but it's not. With the talking talents of multicellular organisms, signaling makes cells function much like the functions of a human body, where our brains signal and chemically coordinate the whole population so things get done that couldn't get done alone, such as metabolism.

Therapy to treat oral pathogens one day might involve taking bacterial mutants that are of the same species found in a biofilm but have enhanced virulence and that can't "talk" or "hear." If they are injected into the oral cavity (or through a dentifrice or rinse) and dominate the nor-



**FIGURE 1.** Oral biofilm quorum sensing. Quorum sensing — releasing and receiving chemical signaling molecules called autoinducers between bacterial cells — allows the cells to coordinate information to control gene expression as population density of cells increases or decreases. While no one has found or demonstrated the bacterial cell quorum-sensing system in oral biofilms yet; it is, however, suspected partly because monospecies, which do not alone form biofilm (in vitro) do so in vivo where there are multiple species. Since the precise signaling molecule has not been identified in oral biofilm production, it has been theorized that they are likely types of autoinducers “talking” and “hearing” each others’ messages to coordinate population-wide gene expression. At low concentrations, bacterial cell growth remains low, as are the numbers of autoinducer molecules released. Growth rates of all climb steadily. When the cell density results in a threshold of sufficient autoinducer numbers (“+”) released, the cells sense this and the behavior/functions of clusters of cells begin acting in sync, indicating gene expression is being coordinated. This may alter the release of autoinducers and begin the release of other factors (host-damaging virulence factors) (“-”). As the cell population reaches a critical threshold, a biofilm forms which can result in disease. (Illustration courtesy of Robert Dorsam and Janyce Hamilton.)

mal bacterial population — voila! Nothing gets done as planned as mutants rule — the good kind, the therapeutic “Franken cells” our molecular researchers created.

### Is Cell-Cell Communication Chemical Mimicry of Talking and Listening, or in Fact Proof of ‘Consciousness’?

In Dr. Seuss’ book, *Horton Hears a Who!*, an elephant hears a speck of dust trying to get his attention. The tiny speck of dust is a miniature planet, upon which is a town called “Whoville” with microscopic residents called “Whos.”

Horton the elephant befriends them, and repeats “a person’s a person, no matter how small” throughout the book. Well, all this “talking” going on between cells makes them sound personified as if sentient, “knowing” little Whos. Certainly, these terms explain a theory of “behavior” or action of a cell so that we can understand processes more easily. But is this just analogy in using these descriptors, or is there evidence that cells have “thoughts,” or an intelligence that drives them to fulfill their functions and change course when conditions warrant?

Neurologists have long explained the coordinated interactions among nerve cells in directing muscle cell contraction (and other activities) as akin to little electrochemical “children” switching games on the playground. This theory is that a group of cells — operating as if pixels, switches, and or transistors — together make up the whole of conscious thought. But just because schools of fish zigzag in unison isn’t to say each minnow doesn’t have its own thought on the matter. Likewise, molecularly, some cognitive neurophysicist researchers are

questioning if each cell has “thought” too — that is, could each cell be recognizing and thereby decoding visuals?

*Neural cell-cell cognition example.* Neurons are the “building blocks of cognition” according to Itzhak Fried, a physicist and professor of neurosurgery and psychiatry and biobehavioral sciences at the University of California, Los Angeles, Medical Center. He also is director of the epilepsy surgery program and co-directs the seizure disorder center there as well as heads the cognitive neurophysiology laboratory and does studies through the Brain Research Institute. For a 2007 study published in prestigious *Journal of Cognitive Neuroscience*, Fried was part of a team of researchers reporting on their nine patients having brain depth electrodes inserted in order to evaluate their seizures for subsequent surgery.<sup>3</sup> While subjected to electrodes in their brains anyway, the team took advantage of the rare invasive opportunity to gauge single neural cell response (measured using local field potentials [LFP] while being exposed to images of objects, landmarks, animals or individuals — some chosen per patient preference). The reaction of each nerve cell monitored was recorded. For this study, as well as one a few years earlier by Fried and colleagues, it was found that a lone neural cell in effect “lit up” both when the subject looked at an image he or she recognized, and when he or she closed their eyes and recalled the image from memory. A single neuron’s activity spikes selective to each category of visual stimuli and it is invariant to “different views of the same person.”<sup>4</sup> The findings of these studies are among the small body of evidence that, at minimum, visually perceived image and imagining an image have the same shared neural circuits.

If preliminary investigations that



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have quantified amplitude and spiking measured in LFP continue to replicate findings and increase in number, it will be reckoning day. The neuroscientists pooh-poohing the concept of a single neural cell recognizing, say, the dog of its human host? Well, let’s just say that those neuroscientists should keep working out their alternative explanations.

*Pain and malignancy cell-cell talk example.* How signals are used by a cell to respond to real pain versus phantom pain stimuli is interesting. Also, how is cell-cell communication relevant to cancer cells, tumor formations, and decisions to metastasize? How do cells know where to form secondary site tumors? Why are there clinical patterns that are reproducible — for example, certain cancers of the breast always spread to specific bones?

All good questions that researchers pick away at continuously.

Brian L. Schmidt, DDS, MD, PhD, oncology fellowship director, Department of Oral and Maxillofacial Surgery, University of California, San Francisco, and a federally funded oral cancer researcher,

said cell communication and signaling is not an area in *oral cancer* research that has been studied as extensively as it has been in other more common cancers such as breast, prostate, and lung cancer. “Gene swapping, molecular vaccines, and cell communications are not something reported on widely in the dental journals because oral researchers have only begun looking at these questions during the last few years,” he said.

Oral mucosal cells use an intricate form of communication to produce an intact epithelial barrier in the mouth. As the carcinogenesis process is initiated, cells change their cell-to-cell signaling and receptor communication. Once this change occurs, he said, the cancerous mucosal cells no longer cooperate to maintain a mucosal barrier.

“Soon an ulcer appears and the patient is in the dental office complaining of pain. The cancerous mucosal cells also change their communication with the deeper connective tissue cells. Like a moving front of migrating geese, the cancerous cells descend into the connective tissue,” Schmidt explained, adding that once the epithelial mucosal cells invade beyond the basement membrane into connective tissue, the diagnosis of squamous cell carcinoma is made. Next, the carcinoma cells “talk” about how they can survive free of the main tumor. The cancerous cells identify the lymphatic system and metastasize to the neck.

Schmidt said not all oral cancers metastasize but there is currently no way of determining which ones will. His NCI-supported study is focused on identifying molecular markers in oral SCC that can be used to predict metastasis.

“Looking at quality of life studies, the head and neck cancer patient’s pain is the highest across all cancers,” Schmidt explained, because oral cancers produce

proteins that sensitize nerve fibers, lowering the pain threshold and leading to debilitating pain just to talk, eat, or drink. Schmidt's creation of the UCSF Oral Cancer Pain Questionnaire may be used in his NIDCR-supported study looking for ways to test experimental analgesics to directly target oral cancer pain.<sup>5,6</sup> Little doubt that he'll be reading the literature on studies of head, neck, and oral cancer pain signaling with interest as this is an emerging frontier of experimental analgesics.

### Cell-Cell Communication in Healthy Versus Diseased States

The existing molecular study model in science had been "reductionism" — looking at one cell, and isolating and identifying smaller and smaller aspects of its independent traits. In recent years, however, that model has shifted to "holism" — seeing how one cell intermingles with others in health and pathological processes. These ideas are new to biology and medicine, so for dentistry it is not surprising there are few dental journal articles and books yet on cell-cell communication (confirmed by library staff at the American Dental Association in Chicago for this report). Instead, academic and government medicodental researchers, often with PhD and MD degrees, are thus far publishing their work first in medical and science journals. The pioneer author of overall cell behavior in the dental discipline is the former NIDCR director, and current University of Southern California School of Dentistry Dean Harold C. Slavkin, DDS (see sidebar).

"Cell talk (with scientists and clinicians listening) is a very intriguing topic in molecular biology as well as many human diseases and disorders. It is the cornerstone of modern dentistry and medicine and the cutting edge for

pharmaceutical advances," he said.

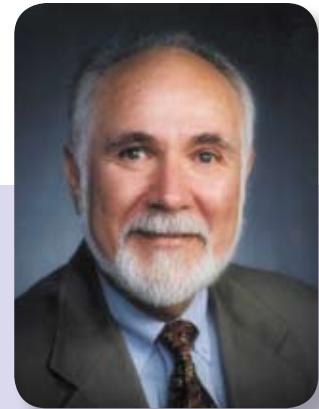
Even Slavkin is excited to know what he's suspected of several oral bacteria all along is now being proven — they communicate. Examples of bacteria using the universal lexicon of AI signaling are *Helicobacter pylori*, *Porphyromonas gingivalis*, and *Streptococcus mutans*.

(On this note, Bassler commented that AI "communication helps oral bacteria adhere to surfaces and take up iron ... [and] is involved in the formation of mixed species biofilms. Bacteria without the ability to 'talk' with the (universal language) can form mono-species just fine, but they cannot form the mixed species biofilms that are present on teeth").

But Slavkin says cell-cell communication is not just about the formation of biofilm, this signaling directs the virulence levels of oral pathogens, the oral mucosal immune response, the inflammatory response of the periodontal tissues, and subsequent tissue destruction.

Interspecies bacterial cell talk and interaction is just now coming into its own with relatively new publications and databases, such as the *Journal of Molecular Signaling*, *Signal Transduction Knowledge Environment Database of Cell Signaling*, and the *Journal of Cell to Cell Communication and Signaling*, struggling less and less for manuscripts, contributors, and reviewers. In fact, an entire Fall 2007 issue of the mainstream magazine, *Science*, was devoted to cell signaling.<sup>7</sup>

"This is a new field, and not many people are working on it," admitted Wenyuan Shi, PhD, from his desk at the Section of Oral Biology, UCLA School of Dentistry, who is working on it. Shi is a coauthor with K.H. Kuramitsu; X. He; R. Lux; and M.H. Anderson from UCLA; State University of New York, Buffalo; and a biotech company called C3 Jian, of the article "Interspecies Inter-



### Dental Pioneer's History of Cell-Cell Signal Research

"In the late 1960s, I was captivated by the problems related to cell-cell communication — How do cells recognize self and nonself, how do they 'choose' to assemble or not assemble into tissues/organs, and how do they sustain specific phenotypes? I chose the developing mammalian tooth organ as my model. I cultured rat teeth in artificial environments, such as the chick chorioallantoic membrane. I dissected these teeth, dissociated into cell isolates, and performed studies to define what, when, and how these cells reorganize to form teeth. Some 30 scientific papers and perhaps 15 years later, we learned that the signals for communication were often ions or small polypeptides termed 'growth factors' and the reception or receptors were specific to bind the growth factor ligand. We later learned that once the signal was bound to a specific receptor the process invoked an intracellular mechanism termed 'signal transduction' by which signals were propagated to specific regions of the genome and acted to regulate gene expressions including positive and negative controls. For me it began with my paper in *Nature* in 1967."

— HAROLD C. SLAVKIN, DDS

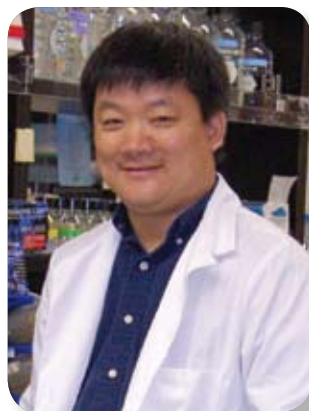
actions within the Oral Community” in the December 2007 issue of *Microbiology and Molecular Biology Reviews*. The article is an important publication by privately and NIH grant-funded researchers from dental schools collaborating with those from multidisciplinary departments of microbiology, molecular genetics, and immunology. Such “multidoctoral” collaboration is breaking down barriers between professions while unearthing fresh ground to understand how communities of bacterial cells talk and otherwise interact.

“Bacterial cells are not a bunch of things just sticking together. If you read the literature in detail, their ‘brains’ are truly functional. They actually are talking with extensive signal transduction,” Shi said, adding “I think of them as a different species of animals but instead of on earth, in their own ecological micro-community.”

The basis for ecology on earth involves extensive interactions between different organisms. The similar rules apply to different microorganisms in the oral cavity, Shi explained.

Same species cells speak one language, while other species of cell types speak another, he said. “So some cells might speak English, and some French, but there is also a universal language so different species can cross talk — something like hand signals,” Shi explained of autoinducers. Interspecies conversing would be necessary for a complex oral biofilm of some 700 species to form. There’s no denying that gunky plaque contains a high degree of organization. Emitted are very specific directions regarding who goes where so each cell “sits” akin to one another in a mosaic pattern, building into a grand scheme that either magically or with maestro-like intention confers adherence or growth advantage — you be the judge.

Whatever it is termed and whomever/



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whatever is at the helm, the behavior of the same cell differs in liquid suspension in vitro versus on a solid surface in vivo because, conceivably, they “sense” and communicate amongst themselves these distinctions regarding their surroundings. The positioning as biofilms aggregate is ordered not randomly distributed. Cells in effect know their group’s size, they know who — cell-wise — is there around them and what they are all doing, and that is the only way it makes any sense that their behavior is purposeful. To create oral biofilm, they “select their neighbors” as if school students lining up by height in rows for a class photo. This shuffling and choosing of location can and does respond to change if, say, a mouthrinse disrupts the colony and repairing/rebuilding processes must commence all over again, or the football player doesn’t brush his teeth all weekend so nutrients and metabolism are on overdrive. The community adapts and balances in the oral ecosystem by cell-cell communication and then action. Extraordinary.

### Proteomic and Other Pharmacotherapies Targeting Oral Pathogens That Involve Cell-Cell Communication

It is no surprise that Shi and his colleagues are viewing cell community-wide communication and behavioral interaction as the next frontier for pharmaceutical advances.

The old model is to use antibiotics to kill off all bacteria, which often creates conditions for the return of pathogenic bacteria with virulence. Meanwhile, scientists are beginning to wonder if ecology-based therapy could be applied to control oral microbial infections. A quorum sensing regulatory molecule known as autoinducer-2 (A-2) is needed for some interspecies bacterial cells to be able to form oral biofilms. A cell type that can inactivate A-2 in oral biofilm hasn’t been identified. A gene known as luxS, if taken away in *S. mutans*, abolished its ability to produce a mutacin (a virulence factor), even at high cell density. There has been signaling observed between two dental plaque species in vitro, but the identity of the precise signaling molecule is not determined.

Work at the Los Alamos National Laboratory with its oral pathogens sequence database is interjecting that an “inducible repressor may be used as a suppressor” of a mutacin gene. Could *S. mutans* tinkering impact the quorum sensing system and bacteriocin production? Maybe Shi and colleagues will find out. Their published review looks at several areas that could have potential or are in development to be products, like mouthwash with gene modified bacteria, for dentistry:

■ *Selectively inhibiting adherence of pathogenic bacterial cells in oral colonies by producing antagonist bacteria that generate “bacteriocins” also called “mutacins,” to inhibit the growth of certain other bacteria.* Examples are *S. mutans* to inhibit *S. sanguis*, and *S. salivarius* to

inhibit biofilm formation by *S. pyogenes*;

- *Passive or active immunization with antibodies in a vaccine that may penetrate the matrix of oral biofilm to interfere with the normal properties of a functional pathogenic bacterial cell;*

- *Bacterial cell replacement therapy — rinsing with mouthwash containing bacteria to overtake or knock down virulence capabilities of existing bacterial populations.* It is now likely that genes are being naturally horizontally transferred between bacterial cells in dental plaque. Although not proven yet, this is highly plausible because *S. mutans* strains have diversely different genes, thus have likely swapped DNA from other *S. mutans* strains or maybe even other species to get what they need. Researchers can either inject more of a bacteria that has genes either naturally or therapeutically altered, which, as a source, will dilute the population from fully functioning pathologically. For example, a noncariogenic *S. mutans* strain (with bacteriocin against other more harmful *S. mutans* strains) could be introduced to grow abundantly and largely render the latter pathologically insignificant in number or function. In theory, this just might reduce colonization of the harmful *S. mutans* to protect enamel. This showed promise in animal studies and awaits approval for human studies.

- *Probiotic approaches.* Instead of killing problem bacteria, try aiding the helpful bacteria in the microbial balance. Bacterial supplementation is an example; in human diet probiotics, this includes eating yogurt to improve bacterial flora population for improved gut function.

- *Interference (for example, messing with metabolization or transporting) with signaling mechanisms of one cell that would normally be received by another to tell it to grow or to stay sensitive to a harmful agents.* For example, while it is

not yet proven in vivo for oral biofilm cells, in vitro it seems that *S. gordonii* can inactivate the competence sensing peptide of *S. mutans* to antagonize its quorum-sensing-dependent abilities, leaving them more vulnerable and sensitive to antimicrobials agents like histatins.

■ **Targeted antimicrobial therapy via a novel specific targeted antimicrobial peptide (stamp) technology.** This is something Shi's lab team is working on. This is to be the "smart bomb" of oral bacteriology therapy. He described it as instead of applying a broad-spectrum herbicide to the lawn that kills the dandelion and grass, applying a STAMP-type agent would kill the dandelion, spare the grass, and also encourage the grass to flourish and fill in the empty space. Said Shi, "We are working with companies to put our product on the market in a few years."

DNA separation techniques using PCR can look at a bacterial cell population profile in dental plaque. Genomic pharmacotherapeutic researchers can excise a band of DNA, clone it, and sequence it to determine which bacterial species it is. T-RFLP is helpful too, and goes well beyond the existing culturing and 16S reran analyses methods, for assessing the cell identities in communities of cells too, as dentistry remains at the biofilm composition discovery phase. This is why looking at cell-cell communication is a lofty high specificity pursuit and not yet priority — we don't even know all the cell types that are present in healthy versus disease oral aggregated bacterial communities and tissues yet.

But we're getting there.

### First New Artificial Life Form on Earth Nearly Complete

A synthetic chromosome based on a DNA bacterium *Mycoplasma genitalium*, which reportedly has one-fifth of



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J. SILVIO GUTKIND, PHD

its genetic makeup removed and synthetically replaced — dubbed *Mycoplasma laboratorium* by the Nobel Laureate Hamilton Smith's team at the J. Craig Venter Institute, Rockville, Md., is slated to be finished during 2008, according to an institute representative contacted in late 2007. How will its signal be sensed? Will its receptors hear normally and respond appropriately? Will it replicate its synthesized DNA into new cells?

The fake chromosome is said to be "watermarked with inks" for easy microscopic identification. Once it is implanted into a live bacterial cell, things get interesting. It may or may not take control of the cell and in effect become a new life form. It may or may not normally metabolize and replicate itself, but the team hopes it will. These scientists already completed a genome transplant of one type of bacterium into the cell of another, an engineered change over from one to another cell species.

The new life form will depend for its ability to replicate itself and me-

tabolize on the molecular machinery of the cell into which it has been injected, and in that sense it will not be a wholly synthetic life form. However, its DNA will be artificial, and it is said that the DNA that controls the cell is credited with being the building block of life.

The spontaneous issues that could erupt during molecular cell-cell communication and signaling to be encountered when it is inserted into a bacterial cell (knowing there will be community-wide sensing of the newly engineered *M. laboratorium*, whether or not it will be distinctively differentiated by the nonsynthetic cells in the Petri dish) will be better than watching the Superbowl. Like the old days of testing a farm animal organ transplant, gene geeks are gambling on whether the natural bacteria will be fooled or not into being controlled by artificial DNA bacteria. If they are, the team has plans to engineer, over time, simple artificial-cellular hybrid life and organisms.

And so it goes.

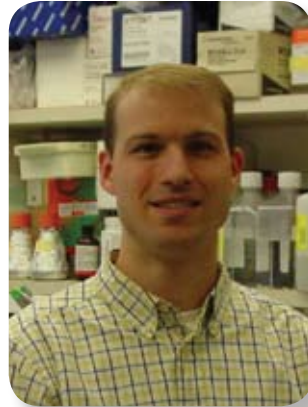
**Oral cancer cell-cell communication example.** While Schmidt of UCSF toils away at finding molecular markers for cancer metastasis and studies cancer pain, the cell-cell communications between a healthy and a cancer cell are being scrutinized by others. In few other diseases are there so many action verbs that connote a brain at work, a malignant cell *manipulates* the signaling network, *attracts* chemicals, *instructs* cells, and *alerts* immune response to drop their weapons. Even the phrase "crosstalk" is used in highly technical papers when it comes to cell-cell signaling in cancer.

Scientists at the Oral and Pharyngeal Cancer Branch of the National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Md., are no exception. A 2007 review, "G-Protein-

Coupled Receptors and Cancer,” by Robert Dorsam, PhD, and J. Silvio Gutkind, PhD, of the NIDCR, is being widely disseminated as part of the body of knowledge that is used to piece together the mysteries of cell signaling and circuitry, including whether interfering with these signals may help prevent and treat cancer.<sup>8</sup>

What the coauthors of the paper learned is that G-protein-coupled receptors, GPCRs, make up the bulk of cell surface molecules whose role is signal transmission (send and receive messages). Recently, Dorsam and Gutkind highlighted that malignant cells, which take command of their environment to promote cancer cell proliferation and migration, can “hijack” the functions of GPCRs, thereby disrupting normal inter- and intracellular communications. The havoc wreaked by the malignant hijacker starts with its chemical invisibility, it “flies under the radar,” remaining undetected by the immune system. The malignant invader simulates GPCR function to increase the supply of blood, oxygen, and nutrients, too. It then sends trick signals to naïve surrounding tissue telling it to loosen up their tight structure and make the blood vessels permeable so the malignant cells can stream inside and proliferate. Since the malignant cell has now taken over the virtual “air traffic control system” of multiple cells’ GPCRs, it sends signals and even a second round of messengers acting on GPCRs in distant organs telling them to make a safe haven for migrating invading cancer cells that would be arriving soon on a lymphatic current.

Like the wartime strategy of jamming the signal of the enemy’s radio-broadcasted propaganda, Gutkind and Dorsam are part of a task force exploring the effects of running interference on GPCRs and their downstream targets. Changes in the interactions and cancer



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progression would be informative.

The team also reported on the findings of researchers detailing how chronic inflammation of tissues can increase metastasis likelihood by releasing chemicals to stimulate the conditioning of “pre-metastatic niches” in lymph nodes and secondary organs favorable for tumor colonization.<sup>9,10</sup> While those studies reported on colon and lung inflammation-cancer research, no one knows yet whether or not chronically inflamed sites of gingival, periodontal and mucosal epithelial tissues are rife with similar interactions.

Commented Gutkind, “We are now learning why cancer cells grow unrestrained and subvert surrounding healthy cells to satisfy their increasing need for nutrients and oxygen. These processes involve a constant communication among cells, and deciphering the molecular complexity of the underlying mechanisms provides golden opportunities for the early diagnosis and new preventative strategies and treatment options for human malignancies.” Dorsam discussed that, “Oral health care clinicians

should be aware of the very complex network of processes underneath a seemingly innocuous oral lesion, that there is something beneath the surface. Now because of increasing knowledge about communication within and among cells, we’ll be able to treat malignant processes in a new way.”

Both molecular biologists agreed that dental school pharmacology classes could benefit from more fully addressing cell communication. Dental students are not taught what happens and what is involved in cell-cell signaling, just why disease happens and what medicine is needed. But dentists need new drug therapies, and now those drugs are in the alpha phase of development. Thus, in the next five to 10 years, it is going to become important for the dental students to be taught the basic molecular principles behind oral health and disease so they know how the new drugs coming their way work.

For now, among the emerging uses of GPCR-targeting agents, drug delivery using radio-labeled and binding peptides are candidates for targeting malignant cells that overexpress GPCRs. The result could be that cancer treatments would have fewer side effects. With GPCR communicating such a core part of aberrant signaling and metastatic commanding, drug developers will be eyeing these cell-surface molecules as lead candidates for getting the job done.

### Temporarily Tricking Cells, Systems and Organs in the Dead to Stall for Emergency Resuscitation

Since it has been shown that a cell has receptors to “hear” and signals to “send out” messages, then another question becomes in what ways can researchers try to fool cellular surveillance mechanisms to not switch on a gene function. For example, investigators are pursuing repressing the cell

apoptosis process — programmed initiation of cell death — for “an hour or more” so surgeons can repair heart and lungs of the newly dead. This stunningly strange medical research (near) breakthrough is in the works and involves distracting normal cell reactions to delay or circumvent functions like apoptosis. In the United States, one such effort comes from the director of the recently established Center for Resuscitation Science at the University of Pennsylvania in Philadelphia.

In a 2007 report, Lance Becker, MD, an emergency medicine physician researcher at U Penn, explained that contrary to the belief that four to five minutes after a “fatal” heart attack where the failed pump means noncirculating blood flow causes the brain to “begin dying,” turns out it’s just in shut-down mode to conserve oxygen that cells are also still alive.<sup>11</sup> They looked at heart cells under a microscope in their “very fresh cadaver” and found, although starving for oxygen since they were cut off from a circulating blood supply, they were alive. Only hours later did they die. But you cannot resuscitate a person who is clinically dead for an hour from a heart attack, Becker’s team learned, because when the blood supply resumes, the cells die from the suddenly resumed oxygen supply. It’s too much of a shock.

In fact, emergency room protocol of administering an oxygen mask to heart attack sufferers, administering epinephrine and shocking the heart muscle to rush in the flow of blood to restore oxygen supply may be a backward approach, in a sobering theorem put forth from the Center. The oxygen flush may be misinterpreted as a tsunami of a terrible problem such as a cancer attack, and a cell is programmed to kill itself rather than compromise to this sudden change of status.

A four-site study published in 2006 showed using a heart-lung bypass machine (to keep the brain fed blood mechanically circulated and the heart in suspended animation until its restarting was worked out) had a 70 percent higher survival rate than traditional methods of resuscitation.<sup>11</sup>

Becker’s center will be supporting the investigation and information sharing of a host of novel principles for newly “dead”:

### WHAT IF THE chemical alphabet of signals is in effect, decoded? Will we one day have transcripts of dialogues between cells?

- slowing down metabolism through methods such as cooling blood with ice and salt or other hypothermia-induction strategies;
- continuing trials of circulation with heart-lung bypass to keep brain oxygen levels going;
- molecular approaches to blocking chemical communications of genetically programmed switch-on of mitochondria that initiate apoptosis;
- acting as an information hub in publishing and soliciting/reviewing papers and study proposals for additional approaches in alternative resuscitation ideas; and
- training investigators who are willing to take risks, and accepting such patients willing to submit to such unorthodox end-of-life treatments to help advance science, and maybe even increase their odds of not making it over to the other side.

Knowing that all types of cells communicate with each other, and that investigators are intent on not only understanding how they do this, but knowing that they can control it regarding apoptosis, will no doubt bring therapeutics to a level of biomolecular tricksterism and passive means to faux-signal the body’s trillions of cells into a nitrous-oxide like stupor: “You are getting sleepy, all is well, relaaaaxxxx ...” as your body sits dead on a gurney, blood corpuscles kept viable by flowing via a machine-pumped circuit like some lazy river, while perhaps a cloned human heart muscle is retrieved, jump-started, and surgically sewn into your arteries by the fastest resuscitation cardio ER team in the history of medicine.

Science moves fast, and there are no flies on Dr. Lance Becker.

### Conclusion

The general concepts of cell signaling and receptoring — cell to cell communicating — are the same in all cell types. But, signaling pathways between different cell species, whether inside the common housefly or the stench of “morning breath” tongue, are distinctively different. Unlocking one door, leads to 10 more. There’s no stopping now, so while interference with cell signaling will be the first round of therapeutics, what if the chemical alphabet of signals is in effect, decoded? Will we one day have transcripts of dialogues between cells? Then can we script artificially encoded signals that we can broadcast to “turn on” and to command gene functions? Imagine if, tailored to the patient’s disease progression, molecular surgeons will one day use a wireless mouse chairside to click away at instructing the DNA function to switch on/off and to horizontally share chromosomes for customized therapy?

No one thought cloning would happen in our lifetime either. ■■■■

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