Bioelectricity Buzz

Recent Bioelectricity-Related Articles Selected by Ann M. Rajnicek, Media Editor of *Bioelectricity*.

The *Buzz* for this Special Issue on the *Bioelectricity of the Tumor Microenvironment* includes reviews and primary articles to whet your appetite on topics related to tumor cells (of course!) but also encompasses topics on the nano-scale (ions, small molecules, membranes and nanoparticles), micro-scale (electroactive microorganisms), electrode-tissue interfaces (in spinal cord, brain and tumor microenvironments) and ion transporting tissues (organoids). Enjoy!

Cells versus tissues: The potential for cancer

There is growing interest in controlling tumors by changing the bioelectrical traits of cancer cells. This paper describes a bioelectric model underpinning cancer progression and treatment that reflects the dynamic interaction of the bioelectrical properties of cells and tissues.

> *Joao Carvalho*. A bioelectric model of carcinogenesis, including propagation of cell membrane depolarization and reversal therapies. *Sci Rep*. (2021) 11:13607. https://www.nature.com/articles/s41598-021-92951-0.

The long-standing idea that cancer arises from a succession of genetic mutations, known as the 'somatic mutation theory', fails to explain carcinogenic consequences of exposure to non-genomic toxins. An alternative, the tissue organization field theory (TOFT) considers the origin of cancer as a tissue-scale event, with the bioelectrical state of cells and tissues central to its progression.

Carvalho presents a computational model consistent with TOFT principles, incorporating membrane potential, ionic conduction, cellular gap junctions and cell-cell communication at tissue level. The model builds on one proposed by Cervera et al. in 2020 (doi.org/10.1103/PhysRevE.102.052412), revising it to simplify cell automata and to incorporate the stochastic nature of tissues, whilst permitting consideration in three dimensions, taking tissue domains into account.

The simulations confirm that the cellular neighborhood, not just the state of the individual cell, is key to controlling fate. A polarized tissue with cells in a quiescent state will be stable until perturbed sufficiently by a carcinogenic event that propagates a depolarization wave through the tissue, driving it toward a cancer state. By this model the depolarized state can be reversed, repolarizing the tissues if cell conductivity or ion pump activities change appropriately.

Carvahlo concludes that this observation, which should be framed in conjunction with other (e.g. genetic and biomechanical) considerations, contributes to cancer progression and that it can be targeted pharmacologically (or otherwise) to prevent or treat cancer. Indeed, cancer therapies based on manipulation of the bioelectric state of cells are being developed in several laboratories. This model adds to the resources that may aid identification of new electrical/ion transport-based therapies.

Ironing out cancer: Targeted anti-tumor properties of zero-valent iron nanoparticles.

Moving from ions to iron. Zero-valent iron nanoparticles (ZVIs) were initially developed for magnetic resonance imaging to improve the magnetic properties of iron-based nanoparticles used as contrast agents. Here Wu et al. review ZVI properties and propose mechanisms by which ZVIs can selectively and lethally target cancer cells, perhaps exploiting the acidic extracellular tumor microenvironment to aid efficiency.

Ya-Na Wu, Li-Xing Yang, Pei-Wen Wang, Filip Braet, and Dar-Bin Shieh. From microenvironment remediation to novel anti-cancer strategy: The emergence of zero valent iron nanoparticles. Sci Pharmaceutics. (2022) 14:99. https://doi.org/10.3390/pharmaceutics14010099.

Compared to iron oxide nanoparticles, when inside cells zero-valent iron (ZVI) nanoparticles generate stronger reactive oxygen species by the Fenton reaction, and in cancer cells they alter mitochondrial membrane potential, mitochondrial structure, and energy metabolism. Consequently, they can kill cancer cells selectively, while sparing nearby non-cancer cells. There is *in vitro* and *in vivo* evidence to support this idea (e.g. lung/oral carcinoma, leukemia and breast cancer).

Several mechanisms for inducing anti-tumor activity have been proposed for ZVIs. 1) I*n vivo*, ZVIs within the tumor microenvironment (TME) are thought to modulate tumor-associated macrophages; inhibiting tumor growth and metastasis by polarizing pro-tumoral M2 macrophages to anti-tumoral M1 macrophages. 2) I*n vitro* studies suggest a role for modulation of membrane potential. Although ZVI nanoparticle uptake was the same for control and oral or colorectal cancer cells, only cancer cells showed an irreversible loss of membrane potential in mitochondria. 3) The Fenton reaction is triggered in cancer cells by ZVIs. In the acidic microenvironment of cancer, rapid ionization of iron nanoparticles enables release of ferrous ions, which react further with hydrogen peroxides. The resulting hydroxyl radicals are toxic to cancer cells.

These mechanisms are likely to co-exist. For example, attenuated immune responses are associated with acidic TME, where the ZVI nanoparticles degrade into 'iron ion bombs' that modulate tumor-associated immunity. Since blood vessels in TME are often more permeable, ZVI particles may enter tumor tissues more readily via leaky blood vessels. The ability to target 'iron ion bombs' that activate specifically in the tumor microenvironment (and inside cancer cells) offers an advantage over conventional small molecule compounds (e.g. doxorubicin).

A caveat to ZVI nanoparticle therapies is that some cells demonstrate ZVI-resistance, attenuating ZVI-induced oxidative stress and displaying different genetic and metabolic profiles for mitochondrial function compared to non-tumor cells. However, profiling tumors may allow personalized therapy to optimize ZVI based outcomes.

ZVI nanoparticles offer further possibilities to exploit their magnetic properties combined with therapies such as thermodynamic therapy and computational tomography, meaning tumor localization and treatment efficacy can be monitored.

Molecular multitasking: an organic molecule to sense and modulate electric fields

The ability to both detect and modulate electric fields using a single molecule has far -reaching utility for bioelectricity research. Excitingly, Zhang and colleagues have designed and tested an organic molecule that can perform both functions.

> *Yingmu Zhang, Jinghan He, Patrick J. G. Saris, Hyun Uk Chae, Subrata Das, Rehan Kapadiac, and Andrea M. Armani*. Multifunctional photoresponsive organic molecule for electric field sensing and modulation. *J Mater, Chem*. *(2021). DOI: 10.1039/d1tc05065f*.

Molecules or particles with fluorescence properties are familiar tools for biological research. They provide information about cell structure and function and have emerging roles as indicators in optoelectronic devices to detect specific molecules. Designing a molecule that has dual functions (e.g., sensing and controlling an electric field) presents many challenges. For example, there is a need to isolate different excitation mechanisms to reduce crosstalk between domains within the single molecular entity.

Zhang and co-workers have designed the first dual-purpose molecule that has an electric field sensor module and an electric field modulator module. They call it "NAI–TPE-PyS" because the sensor module is derived from the photo-induced electron transfer dye tetraphenylethylene (TPE) modified with 3-(4-methylpyridin-1-ium-1-yl)propane-1-sulfonate (PyS), and the modulator module is the organic photoconductor naphthalimide (NAI). A long non-interacting alkyl chain separates the modules and further prevention of crosstalk is provided because TPE and NAI have little overlap in their absorption and emission spectra.

The reporter module was modelled and tested *in silico* to optimize molecule design. It was found to have appropriate properties to facilitate voltage-dependence. Importantly, the authors predict that the molecule will retain voltage sensitive luminescence even if its motion is restricted by biological constraints, such as insertion in a cell membrane in an aligned form.

The two modules can be controlled separately. Stimulating with 350 nm light activates the NAI-based module, emitting light at 400 nm wavelength but stimulating with 390 nm light emits light at 585 nm, the emission spectrum for the TPE-based dye without emission from the NAI module. Therefore 390 nm light is used for voltage imaging and 350 nm light is used for voltage modulation. This was demonstrated directly. When photoconductivity of a film of NAI–TPE-PyS deposited onto electrodes was assessed experimentally, current vs voltage measurements with 19 mW of 350 nm illumination revealed a stable, real time, increase in voltage upon illumination, which decreased sharply when the light was switched off.

The elegance and potential utility of this system will be clear to *Bioelectricity* readers. The technology builds on fluorescence microscopy technologies already found in most laboratories. If the molecules can be shown to be safe in biological systems, the mind boggles with possible experiments. I just wish it had a snappier name!

Striking the right (spinal) cord: Improved electrical stimulation parameters in spinal cord injury.

Spinal cord injury with functional deficits is often accompanied by significant physical, psychological, and economic challenges. Unfortunately, few effective treatments are available. Rowald and colleagues report rapid improvement of functional outcomes in three patients as part of a clinical trial using an improved modality for electrical stimulation of the damaged cord.

Andreas Rowald, Salif Komi, Robin Demesmaeker, Edeny Baaklini… + 64 others…, and Grégoire Courtine. Activity-dependent spinal cord neuromodulation rapidly restores trunk and leg motor functions after complete paralysis. *Nature Medicine* 28:260–271 (2022). https://doi.org/10.1038/s41591-021-01663-5.

Injury to the spinal cord can cause devastating loss of sensory and motor function, and the human spinal cord is notoriously poor at spontaneous repair. However, electrical stimulation therapies aiming to improve function of spared neuronal circuitry have been gaining prominence. Epidural electrical stimulation (EES) of the lumbosacral spinal cord, coupled with intense physical training has shown promise, with restoration of supported walking in some individuals. Existing protocols are based on the idea that targeted stimulation of specific dorsal root nerve fibers using defined stimulation patterns can recapitulate the natural spatiotemporal activation patterns used for walking.

The paddle type electrodes used for previous studies were designed for epidural positioning on the dorsal column, which would not target motor functions optimally. Rowald and colleagues reasoned that targeting the dorsal roots involved specifically in leg and trunk movements would be more likely to restore motor control.

The team designed a 16-electrode EES array using a computational model to optimize precise electrode arrangements. The modelling data were coupled with anatomical scans of each participant and intraoperative single EES pulse stimulation and accompanying electromyograms to guide electrode placement for each patient. A wireless biomimetic EES implant pulse generator was configured that could deliver up to 10 stimulation waveforms. The impulse generator was implanted in the abdomen and controlled by a touchscreen interface using custom software that provided real time EES waveforms, kinematics, and muscle recordings.

Optimized EES stimulation protocols for each patient were then delivered with pulse frequencies of 20 Hz for extensor muscles and 100 Hz for flexor muscles, to reflect differences in natural neuron activation frequencies.

Before the EES implant procedure each of the 3 participants had complete sensorimotor paralysis and all were unable to take any steps. Impressively, on the first day all participants could step independently on a treadmill, but with poor gait and extension. By the third day, gait patterns improved, and participants could walk on level ground with a weight supporting aid, as well as cycle on a recumbent bike, swim and control trunk movements.

Importantly, the improvement, with continued training, permitted independence in real world settings, with the ability to fully support standing weight by 5 months. This is crucial to quality of life, permitting one participant to climb stairs and walk over uneven terrain. Previous studies have

demonstrated that electrostimulation for spinal cord injury is generally effective only during episodes of stimulation. Importantly, Rowald and colleagues found that two participants recovered the ability to activate proximal muscles without needing EES. This represents an exciting step forward for spinal cord repair using bioelectricity. I look forward to the next chapter.

Boning up on biomaterials: An electroactive material for bone repair in diabetic conditions.

The report by Wu et al. (2022) elsewhere in this edition of *Buzz* describes modulation of macrophage activity by iron nanoparticles to target cancer. Here, Dai and colleagues drive macrophage function using a ferroelectric composite material in the context of diabetic bone repair.

Xiaohan Dai, Boon Chin Heng, Yunyang Bai, Fuping You, Xiaowen Sun, Yiping Li, Zhangui Tang, Mingming Xu, Xuehui Zhang, Xuliang Deng. Restoration of electrical microenvironment enhances bone regeneration. *Bioactive Materials* 6 (2021) 2029–2038. https://doi.org/10.1016/j.bioactmat.2020.12.020.

Diabetic individuals have increased incidence of bone fracture, delayed fracture resolution, and higher risk of bone graft failure. This is attributable in part to diabetes being a chronic inflammatory condition. Macrophages are key regulators of the immune response which can display a pro-inflammatory M1 phenotype or a pro-healing M2 phenotype. Since bone has inherently electrical properties and electric fields drive tissue (including bone) repair, it follows that electroactive materials may stimulate bone repair. However, how electrically active materials impact diabetic osteogenesis has been underexplored.

Dai and colleagues prepared a ferroelectric $BaTiO_3$/poly (vinylidene fluoridetrifluoroethylene) (BTO/P(VDF-TrFE)) nanocomposite membrane to recapitulate the electrically active microenvironment within bone. Corona poling treatment creates a dipole, polarizing the material, which can be implanted in a bone defect with the negatively charged side facing the defect to create an enclosed electrical microenvironment. Under physiological conditions the material approximated the electrical properties of bone, with a stable piezoelectric coefficient of ~8.19 pC/N.

The ability of the polarized material to influence macrophage function was tested *in vitro* using a human monocytic THP-1 cell line cultured for 2 days under normal glucose (NG; 5.5 mM) or high glucose (HG; 25 mM) conditions (19.5 mM mannitol was used in an osmotic control group). THP-1 cells grown under NG or mannitol control conditions displayed hallmarks of a non-activated M0 state, whereas under HG conditions they resembled the M1 pro-inflammatory phenotype, including elevation of inducible nitric oxide synthase (iNOS) and an array of pro-inflammatory cytokine genes.

When grown in NG medium *in vitro* on the unpolarized, electrically inert membrane (UP-NG) macrophages displayed an M0 phenotype but in HG medium on a polarized, electrically active membrane (P-HG) they had an M1 phenotype. Flow cytometry revealed a predominantly M2 macrophage phenotype in the P-HG group. Collectively, the data suggest that the electrically active membrane attenuated the HG-induced shift to M1 phenotype and promoted the M2 phenotype.

To determine whether altering macrophage phenotype induced bone formation, human bone marrow mesenchymal stem cells (BM-MSCs) were grown for 7 days *in vitro* using macrophage-conditioned medium and osteogenic differentiation markers were quantified. Osteogenic gene and protein expression were downregulated for BM-MSCs in medium from UP-HG conditions, but

conditioned medium collected from macrophages under P-HG conditions induced osteogenic markers in BM-MSCs.

The ability of the material to induce bone formation was tested in a rat model of Type 2 diabetes. Bilateral skull bone defects (5 mm) were made in male adult mice. Polarized, electrically active membrane was implanted over the defect on one side with unpolarized material over the contralateral injury site. Micro CT scans at 4 weeks and 8 weeks post-surgery revealed more bone formation on the polarized, electrically active material side, which was confirmed by histological analysis.

The ability of an electrically active material to stimulate bone growth is a welcome addition to therapies to treat healing disorders in diabetes. What would be valuable in conjunction with this study is evidence for how diabetes impacts the natural electrical properties of bone.

Spelling it out: a brain computer interface for communication by a completely 'locked in' person.

Progressive neurodegeneration and loss of muscle control in conditions such as amyotrophic lateral sclerosis (ALS) may eventually remove all possible modes of communication, leaving the individual in a 'locked in' state, particularly after external ventilation becomes necessary. Chaudhary and colleagues demonstrate for the first time that a person with chronic locked in state can communicate solely using a brain-based communication interface.

Ujwal Chaudhary, Ioannis Vlachos, Jonas B. Zimmermann, Arnau Espinosa, Alessandro Tonin, Andres Jaramillo-Gonzalez, Majid Khalili-Ardali, Helge Topka, Jens Lehmberg, Gerhard M. Friehs, Alain Woodtli, John P. Donoghue, & Niels Birbaumer. Spelling interface using intracortical signals in a completely locked-in patient enabled via auditory neurofeedback training. *Nat Comm*. (2022) 13:1236. https://doi.org/10.1038/s41467-022-28859-8.

This field has attracted some controversy! Although there has been success using cursor control and sentence formation for patients in locked in state (LIS), those modes have not been successful at the level of volitional sentence communication for chronic LIS patients. One idea was that communication was prevented by fronto-temporal brain degeneration in combination with chronic lack of sensory input, motor output and cognitive dysfunction. This study set out to determine whether volitional brain-based communication was retained in LIS individuals.

Two intracortical 64 microelectrode arrays were implanted in the brain precentral gyrus and superior frontal gyrus of a male patient with ALS leading to chronic LIS, who had lost all purposeful eye movement, his last remaining communication mode. On the 86th day after electrode implantation a neurofeedback paradigm was used to provide the patient with an auditory feedback tone relating to his neural activity. He could modulate the neural firing rate (by a mechanism that remains mysterious) to change the pitch of the tone. From day 106 he could select letter groups or individual letters presented by a synthesized voice to free spell by modulating the tone up or down to indicate 'yes' or 'no'. He produced intelligible output on 44 of the 107 days when the speller was used.

Although this study demonstrates proof of concept that a person with chronic LIS can communicate via a brain interface, significant improvements are required. The system is invasive and slow; the

rate of character production in intelligible spelling episodes averaged only 1.08 characters per minute over the study period, and even then, only on some (41%) days. It is also possible that as neurodegeneration progresses further even this mode of communication would prove ineffective. However, this patient was able to communicate with carers, family, and the study team using the device to provide personal messages, statements regarding needs, guidance for his physical care and to suggest improvements to the system. For someone in chronic LIS, their carers and loved ones, this has the potential, with refinement, to offer valued improvement of quality of life.

Extremely small: growth and enrichment of extreme electroactive microorganisms.

In addition to basic bioelectricity research interest, electroactive microbes are gaining attention for their potential utility in microbial fuel cells and other emerging technologies. Most studies have used microbes from non-extreme environments due to their relative ease of culture, but Singh and co-workers offer a way to extend this work, describing detailed methods for growing and characterizing electroactive microorganisms from extreme environments.

Ramandeep Singh, Srishti Chaudhary, Sukrampal Yadav, Sunil A. Patil. Protocol for bioelectrochemical enrichment, cultivation, and characterization of extreme electroactive microorganisms. *STAR Protocols 3,101114*. (2022). https://doi.org/10.1016/j.xpro.2021.101114.

Electromicrobiology is the study of microorganisms that undergo extracellular electron transfer (EET) processes to or from a solid-state electron acceptor or donor. The microbes are categorized based on their EET traits as exoelectrogens (if they transfer electrons to a solid-state electron acceptor) or as electrotrophs (if they oxidize a solid-state electron donor). Singh et al present a detailed protocol for a process to enrich, cultivate and analyse the electrical properties of nitrate-enriching electrotrophs from a lake with extremely high salinity and pH.

The procedure is broken down into six steps: 1) Preparation of media, reactor equipment, and inoculation with microbes (1-2 days). 2) Electrochemical enrichment of target microbes on electrodes and monitoring of bioelectrocatalytic current production (2-8 weeks). 3) Cyclic votammetry to check for presence of redox compounds (4-6 hours). 4) Analysis of redox metabolites and soluble redox mediators. (5-6 hours). 5) Microscopic confirmation of microbial growth at the electrode surface and bulk phase (2-3 days). 6) Identification of the enriched microbial communities (40-60 days).

The ability to culture and characterize electroactive microbes from extreme environments may reveal populations with properties superior to those already targeted for bioelectrochemical reactors. This methodology has the potential to be modified for requirements of other extreme microbes.

Easy to swallow: an *in vitro* model for study of esophageal ion transport mechanisms.

Esophageal inflammation and cancer may arise from altered ion transport across esophageal tissues, but preclinical studies have been hampered by lack of an appropriate *in vitro* model. Korsós and colleagues have developed a three-dimensional mouse organoid model to overcome that barrier and identify ion transport activity in the organoids for the first time.

Marietta Margaréta Korsós, Tamás Bellák, Eszter Becskeházi, Eleonóra Gál, Zoltán Veréb, Peter Hégyi, and Viktória Venglovecz. Mouse organoid culture is a suitable model to study esophageal ion transport mechanisms. *Am J Physiol Cell Physiol* 321: C798–C811 (2021). https://journals.physiology.org/doi/full/10.1152/ajpcell.00295.2021.

Although normal and esophageal adenocarcinoma cell lines can be used in classical Ussing chamber studies for ion transport, technical issues and cell line instability hamper reproducibility. A better solution is an organoid model in 3 dimensions (3D) that more faithfully recapitulates the esophageal tissues using non-cancerous, native cells. Esophageal organoid models exist in which undifferentiated stem cells at the basal layer proliferate and migrate to the lumen, differentiating as they move to replace the suprabasal cells. However, ion transporter activities have not been reported for such organoids.

Korsós and colleagues used two different mouse strains to create 3D organoids. Esophageal tissues were dissociated and plated in a Matrigel matrix. A cell-fill organoid structures 250-300 µm in diameter formed 3 to 4 days after plating that continued to grow, with peak diameter between 7 to 9 days after plating. Organoids were characterised by immunomicroscopic and flow cytometric methods, confirming epithelial stem cell origin by expression of the stem cell marker leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5) and the cytoplasmic keratin (CK) marker CK14, which is expressed in basal epithelial cells.

Further analysis using PCR and immunochemistry demonstrated the presence of the most common ion transporters in esophageal organoids, including the Slc26a6 Cl^-/HCO_3^- anion exchanger (AE), the Na^+/H^+ exchanger (NHE), the $Na^+/HCO3^+$ cotransporter (NBC), the cystic fibrosis transmembrane conductance regulator (CFTR), and anoctamin 1 Cl^- channels. Functionally, although microfluorimetric studies showed low CFTR activity, NHE, AE and NBC activities were relatively high.

The ability to freeze and passage such organoids offers experimental flexibility and consistency. Consequently, this model will find use in pre-clinical studies of the causative roles for ion-transport in esophageal immunoinflammatory conditions and cancer. It could thus aid development of therapies that target ion transport in these conditions.

From both sides now: Bipolar electrodes for sensing and actuating electrical activities in cells.

This paper emphasizes the underappreciated potential of bipolar electrochemistry as a tool for controlling and detecting redox reactions at the nanoscale inside cells.

Andie J. Robinson, Akhil Jain, Ruman Rahman, Sidahmed Abayzeed, Richard J. M. Hague, and Frankie J. Rawson. Impedimetric characterization of bipolar nanoelectrodes with cancer cells. *ACS Omega* (2021) 6, 44, 29495–29505. https://doi.org/10.1021/acsomega.1c03547.

Stimulation and recording electrodes used *in vivo* are usually connected (hardwired) to an external power source. However, an alternative approach uses an external EF and redox reactions in a conductive material to produce an EF wirelessly in the material by bipolar electrochemistry. Robinson and team are working to harness such systems that operate remotely and non-invasively to report electrical input on a nanoscale.

The basic principles of bipolar electrochemistry are intuitive, but often underappreciated. Consider any conductive material (electrode or particle) of any shape (sphere, flat plane) immersed in an electrolyte (cell cytoplasm, saline solution, culture medium, body fluid). When the electrolyte is subjected to an EF from external (feeder) electrodes, redox reactions at the edges of the electroconductive material poles induce an EF (potential difference) within it. The EF induced in the material bestows properties of a bipolar electrode (BPE), with an opposite polarity to the EF within the electrolyte itself. BPE properties arise spontaneously in the material with no direct connection to the external electrodes needed.

The example describes a direct current BPE, but Robinson et al. propose that alternating BPEs offer advantages due to the ability to tune them by altering frequency. The authors further propose that electrochemical impedance spectroscopy could allow assessment of BPE systems on the nanoscale. To demonstrate this, they modelled the behavior of 125 nm gold nanoparticles (AuNPs) bathed in electrolytes (saline and deionized water) positioned between feeder electrodes. Thus, it could be shown theoretically that current could be driven through AuNPs in a high impedance electrolyte, making them act as nano-PBEs.

The ability of AuNPs as PBEs to impact cell function was tested experimentally by incubating malignant brain cancer cells (U251 cell line) with AuNPs BPEs. Excitingly, the particles (500µg/ml) were successfully internalized by the cells. Additionally, the AuNPs were proven to act as PBEs within the cells. Electrochemical impedance spectroscopy measurements identified changes in cells loaded with AuNP BPEs compared to cells alone (in both water and saline) that suggested that current was being driven through them.

Loading cells with nanoparticle BPEs offers many exciting experimental opportunities. For example, controlling cell behaviors by applying current with BPE NPS or obtaining electrical readout from discrete cell populations loaded with them. It is tempting to imagine that multiple populations of BPE NPs could be tuned for specific purposes in cells or tissues controlled remotely and non-invasively.

It was a challenge to pare down the number of featured papers for this issue since so much interesting and important work is being published across a variety of topics. Thank you to everyone who is working so hard to advance this work. I am confident that you will continue to make my task of selecting articles even more difficult for the next issue. I look forward to it.

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