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Biotechnology & Bioengineering II

3354-Pos Board B459

Recombinant MG53 Binds Lipid Signals on Damaged Cell Membranes to Increase Membrane Repair Capacity

Noah Weisleder, Norio Takizawa, PeiHui Lin, Tao Tan, Pinjung Chen, Rosalie Yan, Xiaoli Zhao, Moonsun Hwang, Hiroshi Takeshima, Jianjie Ma.

Plasma membrane repair is a highly conserved mechanism that appears in most eukaryotic cells. While a simple lipid bilayer will reseal through thermodynamic principles, the presence of a cytoskeleton results in the native plasma membrane being held under tension, thus some disruptions the plasma membrane cannot spontaneously reseal. Previous studies established that plasma membrane repair is an active process involving translocation of intracellular vesicles to the injury site and fusion of these vesicles to form a repair ''patch''. An emerging concept in cell biology establishes this intrinsic membrane repair/regeneration process is a fundamental aspect of normal physiology and that disruption of this mechanism underlies the progression of many human diseases, including cardiovascular disease, neurodegeneration, ischemic injury and muscular dystrophy. We recently discovered that mitsugumin 53 (MG53/TRIM72) is an essential component of the cell membrane repair. In this study we tested the translational value of targeting MG53 function in tissue repair and regenerative medicine using in vitro cell based assays and animal models. While native MG53 protein is restricted to striated muscle, beneficial effects against cellular injuries are present in non-muscle cells with overexpression of MG53. In addition to the intracellular action of MG53, acute injury of the cell membrane leads to exposure of lipid signals that can be detected by MG53, allowing recombinant MG53 to repair membrane damage when provided in the extracellular space. Human MG53 protein retains dose-dependent protection against membrane disruption in both muscle and non-muscle cells. Intravenous delivery of MG53 protein is safe in rodent models, with suitable pharmacokinetic properties that are highly effective in restoring or preventing localized damage to muscle tissues. Our data indicate the MG53 protein is an attractive biological reagent for restoration of membrane repair defects in human diseases.

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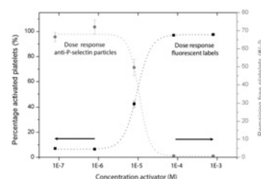
Measurement of Platelet Activation with Anti-P-Selectin Coated Magnetic Microparticles

Loes van Zijp, Arthur M. de Jong, Nona Jongmans, Thijs C. van Holten, Mark Roest, Menno W.J. Prins.

Under normal conditions, platelets have very little interaction with each other or with other cells. However platelets become activated for instance, when they are exposed to collagen by vascular damage. Upon activation, several molecules get exposed on the cell membrane to support adhesion, spreading and aggregation of the platelets onto the damaged vessel. Platelet activation is involved in cardiovascular diseases. Traditionally, platelet activation is quantified with fluorescent markers in combination with flow cytometry. However flow cytometry requires complex and expensive equipment.

We are studying novel technologies for platelet activation biosensors that are inexpensive and require minimal sample preparation. We present a proof of principle measurement of platelet activation with magnetic particles. Particles coated with anti-P-selectin were used to remove activated platelets from samples stimulated with different concentrations of activation agonists. We analyzed the platelet activation level by measuring the remaining unbound cells in the solution. We compared our new approach to traditional flow cytometry and found good agreement (figure).



Our cell removal assay has the potential to be used in integrated cell biosensors which don't rely on fluorescent labeling and detection of cells.

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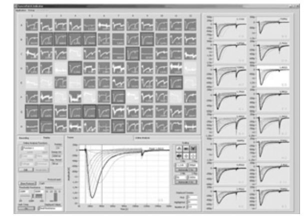
Highly Parallel Planar Patch Clamp for Ion Channel Screening

Andrea Brüggemann, Claudia Haarmann, Cecilia Farre, Juergen Steindl, Christian Patzig, Timo Stengel, Christian Grad, Johannes Stiehler, Michael George, Niels Fertig.

To meet the ever increasing demand for higher throughput in ion channel screening and safety testing, new platforms are being introduced allowing for yet more throughput whilst retaining data quality. A new platform recording from 96 cells at a time with giga-seals, with continuous recording during compound application and addition of multiple compounds to each of the 96 cells being recorded will be described. This new platform vastly increases throughput, while reducing the cost per data point. Borosilicate glass substrates containing microstructured apertures are used for parallel patch clamp experiments. In the figure below, a screenshot of an experiment on 96 cells in a parallel is displayed. Shown are raw current responses of $Ca_V3.2$ channels expressed in HEK293 cells to a current voltage relationship step protocol.



Recordings from various ligand- and voltage-gated ion channels on this platform will be shown.

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Piezoelectric Planar Patch Clamp System for Mechanically Actuating Ion Channels

Eric Stava, Minrui Yu, Hyuncheol Shin, Jonathan Rodriguez, Robert H. Blick.

Piezoelectric substrates enable the generation of mechanical forces with Angstrom resolution via an applied voltage. When using such a substrate for planar patch clamp experiments, these deformations will induce tension in the lipid membrane. This will, in turn, affect the conductive properties of any mechanosensitive ion channels in the membrane, as well as mechanically perturb any other proteins embedded in the cell membrane. We show that an applied electric field induces structural changes in our piezoelectric quartz substrates and, thus, to the micron-sized pores in the quartz. By affecting the electric field across the quartz substrate, we can precisely control the deformation of the pore. We also find that this field does not have an effect on the electric field across the pore. Therefore, it will not directly affect the electrical properties of the ion channels under test. Further, we have incorporated ion channels into this system, and demonstrate that the applied electric field alters the conductive properties of the ion channels. We, thus, show a device capable of actuating ion channels under a range of complex, finely-tunable mechanical stimuli.

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Multisuction Electrode Arrays to Investigate Multi-Sensory Integration in Neural Tissue

John M. Nagarah, Pieter Laurens Baljon, Daniel A. Wagenaar.

We are studying the neuronal basis of multisensory integration in the medicinal leech, a model animal with a well-characterized and relatively simple nervous system. We aim to discover how the leech's nervous system combines biologically relevant mechanical and visual sensory cues in order to come to a coherent decision about subsequent motion. One goal is to characterize the neural computation that occurs in the leech ganglion, where processing for most described leech behaviors occurs, while visually and mechanically stimulating the leech preparation. Specifically, we will interrogate all the neurons in the ventral side of the ganglion simultaneously at the single cell level by combining multielectrode array (MEA) recordings with voltage sensitive dye (VSD) imaging. The excellent spatial resolution of the VSDs combined with the temporal resolution of MEAs will provide a high level of detail of this computation in the ganglion. We enhanced the MEA design by fabricating optically transparent multi-suction electrode arrays (MSEAs). Each electrode in an array of 60 is constructed around a microfabricated suction pore. Directed suction holds neurons closer to the electrodes and immobilizes tissue without physical distortion, resulting in more stable recordings. The MSEA fabrication protocol builds upon a previously described batch-fabrication technique to make planar patch-clamp electrodes; thus, an entire array of an arbitrary number of pores and devices can be created simultaneously. We are exploiting this by making devices for our collaborators in parallel with our MSEA devices in order to study network behavior in mouse hippocampus slices, and ion channels in giant unilamellar vesicles (GUVs) and mammalian cells.

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Dual Micropores in Glass Substrates for Ion Channel and Gap Junction Recording

Brandon Bruhn, Stefan Schuhladen, Ran An, Michael Mayer.

Despite the commercial success of automated high-throughput patch clamp technologies, instruments on the market typically cannot match the quality of single-channel recordings obtained by traditional patch clamp experiments with glass micropipettes. In automated approaches that use suction to position a cell to a micropore, the inner surface of the pore can be contaminated with proteins in solution prior to seal formation. Traditional patch clamping avoids this problem by applying a positive pressure to the micropipette prior to contacting the cell membrane, which enables significantly higher electrical seal resistances. This research aims to develop a universal platform for high-quality transport assays on glass chips that circumvents the problem of surface contamination and takes advantage of the high seal resistances and low capacitive