

Applications of Biophysics in the Understanding and Treatment of Cellular Diseases

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Abstract:

Biophysics is one of the modern technologies used in various biological applications, as biophysics deals with biological functions that depend on physics parameters such as mechanical force or electricity, and with the interaction of living organisms with these parameters such as ionizing radiation, light or sound, and with the interaction of living organisms and their environment. Topics include organic molecules. This technique also works as a treatment for many cellular diseases, including cancer. The basic techniques used are (X-ray Crystallography, Nuclear Magnetic Resonance Spectroscopy, Fluorescence Microscopy, Atomic Force Microscopy). These techniques have demonstrated important results, as they are considered high-precision techniques that are easy to use in treatment and biological diagnostics. In addition, they can be used in medical applications.

Keywords: *Biophysics, Cellular Diseases, X-ray Crystallography, Nuclear Magnetic Resonance Spectroscopy, Fluorescence Microscopy, Atomic Force Microscopy.*

1. Introduction to Biophysics and Cellular Diseases

Biomolecular Condensates Decipher Molecular Codes of Cell Left: From Biophysical Fundamentals to Therapeutic Practices BCs have shaped a new paradigm in molecular cell biology and have started to provide novel directions at the cutting-edge of physiological and pathological processes as well as for the development of effective therapeutic strategies 1. Physical understanding of BCs opens the door for transforming our knowledge on BC functions into novel disease markers and therapeutic targets. The preliminary consideration of these insights together with the biophysical properties of BCs and advances in proteomics and other omics developments are expected to contribute to the deciphering of the molecular code of the BCs associated with cancer, neurodegenerative, metabolic, trauma, and microbial diseases. However, in line with these advances, future research necessarily needs to systematically and comprehensively decipher the

biophysical physical properties of BCs under diseased conditions and configure conclusions in the light of cell specificities and experimental conditions. Moreover, the elucidation of dynamic metamorphoid-evolving BC-genomic/mRNA regulatory networks under both healthy and disease conditions also would enable BC biophysic-based therapeutic strategies and the mummification of disease markers. The dynamic BC properties are expected to provide an overview regarding the desired optimal windows for effective pharmaceutical, genomic or other therapies manipulated at both BC and underlying network levels.

A Critical Review on Selected External Physical Cues and Modulation of Cell Behavior: Magnetic Nanoparticles, Non-thermal Plasma and Lasers Based on the advances in biophysics research, a variety of external physical cues such as light, sound, magnetic fields, heat and mechanical forces have been shown to modulate the behavior(s) of various cells to a certain extent. According to their feasibility in medical settings, the extrinsic physical cues that need to exert mechanical force on the cells have received enough attention as the extrinsic physical cues have potential to induce mechanotransduction in almost all types of cells without considering their energy and are also easy to implement in vitro ². The studies considering the effects of the magnetic fields on cells have increased in last decade. The use of nanoparticles (NPs) as the drug carriers, magnetofection (magnet-assisted transfection), and their applications like gene delivery, labeling, magnetic hyperthermia, magnetic separation, external stimuli responsive drug delivery, and applications in regenerative medicine have been reported. The usage of antimicrobial nanoparticles as nanoantibiotics due to the improvement in bacterial effects is a fortification and a new step in fighting against antibiotic-resistant bacteria. Yet, it is also important to note that their toxicity potential (liver failure, autoimmune diseases, and kidney failure) due to their accumulation after long-term usage is higher than other forms of antibiotics. An unexpected recent application of magnetic NPs is COVID-19 detection. Thus, the application of NPs is expected to increase in the future.

Biological Extension of the Action Principle: Endpoint Determination beyond the Quantum Level and the Ultimate Physical Roots of Consciousness Biological Physics is defined as an interdisciplinary effort to establish an explanatory framework that encompasses both detailed molecular mechanism and whole-cell function, thus bridging the gap between cellular/molecular biology and physics ³. Application of physical techniques to biological molecules, cells and tissues by researchers has brought about success. However, many biologists and even physicists tend to minimize the importance of fundamental general theories and prefer to perform detailed phenomenological studies, which have no general underlying physical explanation. Life seems to be in many ways much more complicated than physics, and the question arises whether fundamental laws of physics, such as Maxwell's equations, the laws of motion of Newton or even quantum mechanics can provide simple and general explanations of it. The main challenge lies in the extreme complexity of the behavior of ensembles of molecules. Encouragingly, quantum physics contributes to the understanding of life itself in terms of a universal transition amplitude and a unitary S-matrix, leading to the revival of the old principle of minimum action for ensemble of cells. Quantum principles seem then to be the most general and free from ad hoc assumptions. There appears to be much more room for a concrete application of the OPT-TRT holistic conceptions to cells, particularly in the case of cancer.

2. Fundamental Principles of Biophysics Relevant to Cellular Diseases

A number of features of plasma membrane structure have special significance for the role of the membrane in the transport and metabolism of nutrients and metabolites, and for biophysical properties of cells, which are involved also in the early changes in disease states such as cardiovascular disease and cancer ⁴. Integral and peripheral membrane proteins are important for the formation of a functional plasma membrane with lipid-protein interactions also thought to play a role in the partitioning of lipids between lipid raft and non-lipid raft domains of the membrane.

The crowded nature of components in a cell is often used as an explanation for the rapid diffusional motion needed for so many biophysical functions. As a result of its crowded environment, diffusion becomes unhindered in the cytoplasm and it is quite different from that exhibited by the prokaryotic cytoplasm that lacks such complexity.

Biophysics is the science that applies the theories and methods of physics to understand how biological systems function. In many diseases, the complex biophysical processes of communication in and between cells become dysregulated, but the coupling of structure to dynamics keeps life on the edge of dissipative dynamic stability². Physics-based approaches can help to provide a better understanding of the dynamics of cells and the principles that connect structure to function and dynamics, which are required to develop new therapies. In this review we aim to present an overview of some of the essential aspects of biophysics that are relevant to cellular diseases. There are common motifs in the molecular demography of biophysical processes and control system dynamics, such as normal mode analyses and stability of a system.

3. Techniques and Tools in Biophysics for Studying Cellular Diseases

Over the past 20 years, there has been an explosion of new molecular tools and assays that allows for the precise manipulation and measurement of cellular components and concentrations. Because cells are so complex, these tools and assays have not directly produced detailed, predictive understanding of cellular function using only bottom-up approaches. To understand how large groups of molecules give rise to cellular function, it has become necessary to measure and perturb cellular networks, under physiological conditions, and compare those results to computational models that describe the average behavior of these systems⁵. This multi-faceted experimental and computational approach to understanding cellular systems is at the heart of our understanding of biophysics contributions to the understanding and treatment of cellular diseases. It brings to bear tools from systems biology, process engineering, and statistics to exploit the richness of the data of many different types that have been collected on cells over the last 20 years. This massive increase in molecular-scale data on cells is a unique opportunity to understand how cells function under healthy and pathological conditions.

Biophysics uses physical principles to explain biological phenomena at both cellular and molecular levels, as well as to develop mathematical and computational tools to model cellular systems and interpret measurements of these systems⁶. Biophysical techniques provide significant insights into cellular mechanisms and their dysregulation in diseases, and help to identify problems of interest to cell biologists and physiologists. Moreover, a focus on measurement precision often leads to a more detailed understanding of systematic errors and other artifacts. While at times imprecise measurements have both resulted in technical and conceptual missteps. In the context of disease, it is clear that we need more detailed experimental and predictive models of the cellular mechanisms that underlie pathological signaling⁷.

3.1. X-ray Crystallography

Biophysics plays an important role in modern molecular biology through the development and application of powerful ways to measure and visualize biomolecules. An ever-metastasizing array of imaging techniques reveal the form and motion of many kinds of biomolecules, from macromolecular assemblies to single molecules, from crystal lattices to single particles/ molecules⁸. Among the first to see atoms or molecules, X-ray imaging also stands nearly alone in its power to directly resolve fourier components of the molecule. The method traditionally has been planar, relying on the periodic order of many overlapping copies of the molecule, but a recent exquisite adaptation can also view single particles, albeit in 2D. The methods of single particle and/or single molecule electron microscopy have not only catapulted their pioneers into the short list of awardees annually for chemistry, physics, and medicine (Nobel) but have also definitively demonstrated that a striding, many atom-wide electron-dense “blobs” comprising a single, hydrated protein can be 1)

imaged and 2) individually sorted into “buckets”, grouped by conformational states, using entirely unsupervised computational analysis of the blind images 9.

Biophysics brings to bear the methods and philosophy of the physical sciences on all aspects of life science 10. The physicist brings an interest in understanding, modeling and creating quantitative knowledge from experimental observation. The subject matter for biophysics is bewilderingly complex and multidimensional. The primary trick of the trade for framing manageable scientific problems is to tightly bound the observed complexity. One way of achieving this is to make the system under study as simple as possible. Investigators may work on specific biomolecules in cell extracts, in purified component mixtures or even the simplest imagined, the protein, RNA, or DNA molecule by itself. Because of its central role in biology, we will center much of our discussion on the biophysics of proteins.

3.2. Nuclear Magnetic Resonance Spectroscopy

Although proton is the predominant MR-responsive nucleus in the body, the MR signals from naturally occurring isotopes of other elements can also be detected and exploited to provide other complementary biochemical and functional information. Traditionally, ¹H MRS has been the dominant form of magnetic resonance spectroscopy used in biological and biomedical applications 11. However, the emergence of stable, reliable, and cost-effective RF coils for ³¹P spectroscopy has allowed the depiction of the phosphaturic skeleton, bringing an added dimension to the study of the skeletal system, metabolic diseases, and a broader understanding of mitochondria function. Whereas of the scope of the applications based on ¹H and ³¹P is substantial, encouraging and innovative reports involving ²³Na and ¹³C imaging are starting to emerge and this pipeline of technological progress adds to the plethora of applications already available from MRS and MRSI.

Nuclear Magnetic Resonance (NMR) has become an essential tool in biophysical studies 5. This article presents a review of several applications of NMR in systems of biological relevance. It explores the wide potential of NMR towards the understanding of biological systems, from biotechnological applications to the characterisation of the human metabolome and is presented in three parts: 1) quantum effects and biological macromolecules; 2) molecular relaxometry and cryopreservation; and 3) NMR of biological tissues. It is also worth noting that NMR is hard to parallelize, so there is a need for accelerated acquisition to promote wider adoption of high-field NMR spectrometers. One future modality for increasing NMR examination speed is non-uniform sampling (NUS). Another promising approach, particularly applicable to “big data”-driven machine learning (ML) methods, is the introduction of compressed sensing (CS) to NMR spectroscopy.

3.3. Fluorescence Microscopy

An increasing number of ML applications to FLIM are being documented in biomedical research, from diagnosis tools, to studies of molecular dynamics, to single-particle tracking in super-resolution microscopy and to artefact correction. The combination of FLIM and ML may also be employed to identifying the fluorescence of the cell's metabolic activity, thus aiding the development of biocompatible stains 12. The work provided in[ArticleId: 9e978b71-30ab-42f4-80a8-50e850cd0302] describes how the low phototoxic impact, the single molecule sensitivity, high signal-to-noise ratio, and diffraction-limited spatial resolution of fluorescence microscopy made it the dominant technique of single-molecule biophysics. Moreover, integration of time-resolved fluorescence in the form of fluorescence lifetime imaging (FLIM) has further expanded its potential to probe molecular properties (e.g. local pH) or dynamic events (e.g. Förster resonance energy transfer). The increasing number in the multidimensional FLIM measurements brought the topic naturally to the field of machine learning. Indeed, features extraction and the link between intensity, fluorescence lifetime, and macromolecular concentrations from large data sets could be virtually automatized.

Fluorescence lifetime imaging (FLIM) applications in biology, particularly in bioimaging and microscopy, involve large multi-dimensional datasets, sometimes with small numbers of samples and large numbers of variables or features making it hard to correctly interpret the data. These characteristics make FLIM particularly suitable for the application of Machine Learning (ML), a subfield of AI and a data analysis technique traditionally used for classification and predictive tasks considering large multi-dimensional data. Several types of ML algorithms and architectures have been employed in FLIM-related applications, such as supervised or unsupervised algorithms, traditional or deep learning models, and for data fusion approaches 13.

Fluorescence microscopy is a widely used technique in biophysics to investigate the structure and function of biological systems 14. Among their advantages, fluorescence microscopes are easy to use and can provide high-resolution images of live samples. They are sensitive and specific, thus capable of detecting rare or specific targets in complex biological samples. Due to its ability to record the fluorescence lifetime, FLIM can be employed to decouple fluorophore signal from background and quantify the presence of different molecular states. Thanks to its minimally invasive nature, these measurements can be performed long-term.

3.4. Atomic Force Microscopy

AFM is an indispensable tool for measuring elastic and adhesive properties of cells. The application of AFM in cellular research and therapy is increasing since it can provide valuable information for medical diagnosis and treatment. AFM is a powerful tool to measure Young's Modulus (YM) and adhesive forces as a production of the force curve at the cellular- and subcellular (~nm) level, which would provide precious information regarding diseases, and would be impactful on the development and efficiency of therapies and there are various methods of YM computation with different assumptions and experimental conditions that makes the comparison of the obtained results hard and of quite ambiguous and uncertain values. Having a fast and precise source to evaluate the equivalent healthy and pathological YM and adhesive forces would be quite beneficial, and biosimulation can be helpful. We hope that some pivotal perspectives are provided to non-specialized chemists, biophysicists, and medical doctors to understand the beneficial roles of biosimulation to significantly improve the medical diagnosis, the patient-specific drug discovery, and their biotherapies in the future. The outlook of biosimulation could lead to achieving a nearly perfect outcome from therapies or drugs with limited side effects 15.

Increased stiffness of red blood cells (RBCs) may dramatically alter their functions and hence their effectiveness and quality to hold and transport oxygen 16. Tumor cells are softer than healthy cells, and thrombocytes become stiffer during activation, disease, and aging, which makes them more prone to a higher thromboembolic risk. Therefore, fast, reliable, accurate, and cost-effective ways of measurement and assessment of RBCs' deformability and young modulus can be beneficial for cellular level diagnosis and therapy of various diseases 17. Atomic force microscopy (AFM) is a well-established and powerful tool in soft matter imaging and force measurements at the nano/microscale.

4. Biophysical Approaches to Understanding Cellular Disease Mechanisms

Basic progress in biophotonics and biophotonic tools is leading to quantitative biological discoveries 18. By precisely measuring the state of a cell at best its more than 50% gene products. Many steps are taken to ensure that the collection of cells is taken under relatively controlled conditions, i.e. neither many are growing at detailed states nor of one stressed. Biotechnology is part of the answer. The basic technology for making many such measurements and for holding the large number of measurements required for systems biology is the DNA microarray. A microarray, also known as DNA chip or biochip has one or more grid-like arrangements of gene-specific probes, each located at a known and specific location. Biophotonic techniques are the biggest advance in cell and tissue diagnostics since the electron microscope.

Biophysical approaches to the understanding of cellular diseases are not only about theory. Biophysical methods also allow direct studies of dynamical properties of biomolecules, clusters in living cells. Integrating biological model systems with biophysical techniques is important for accelerating our understanding of cell biological processes 19. This is achieved by the combination of dynamic, multi-parameter imaging of in vitro single-molecule dynamics and in cells with knowledge of fluctuation relations, specifically principle of minimum first passage time. Just in the last few years, the biophysical determination of elongation rates has been directly applied to cell biophysics in the context of in vitro transcription and has been utilized to elucidate key features of peptide elongation, such as how aminoacyl tRNA binding and peptide bond formation are facilitated by tRNA and/or Rna polymerase motion.

Biophysical approaches in understanding cellular disease mechanisms involve a close interaction between theory and experiments 20. Physical models of cellular components or processes facilitate the interpretation of experimental data and ultimately lead to improved understanding of mechanisms underlying diseases. Mathematical analysis can offer not only the information about principal mechanisms of the dynamics but also the bottleneck points, which can be chosen as potential targets for therapeutics. For a cell, as for any other dynamical system, stability of stationary state is an important parameter. One way to investigate this question for genetic circuits is to find the eigenvalues of the corresponding Jacobian matrix. Positive real parts of some eigenvalues mean that the corresponding system is unstable.

5. Biophysical Techniques for Drug Discovery and Development in Cellular Diseases

A limitation of biophysical methods for new-discovery is the need for sizeable quantities of purified recombinant proteins. This is now less limiting than previously because of the availability of high protein expression systems and recombinant protein resourcing and provision companies. Biophysical high throughput screening setups have also been established that do not rely on recombinant protein 21. High throughput versions of the principles outlined in this review have been reported by Enzyams and microscale thermophoresis. Other developments which increase the 'druggability' of a target, require no target reagent development and can be amenable to high throughput are exemplified by no-well microscale thermophoresis and high throughput mass spec to detect protein–ligand binding after disrupted (cell or tissue) biological material has been subjected to a designed alteration 22. It should be mentioned that nearly two thirds of all human proteins cannot be assayed at enough throughput for trends and qualitative binding analyses to be determined.

Biophysical techniques offer a number of advantages over traditional biochemical or phenotypic assays as initial drug screens, including their potential to provide more detailed information about the binding and mechanism of action of hit molecules 23. Many screening methods also rely on some indication of enzyme or pathway activity, and are unable to identify 'undruggable' disease targets. By contrast, biophysical methods can identify binders to any defined molecular target, regardless of their effect on target activity or expression levels, and are increasingly being applied to the discovery of small molecule modifiers of protein–protein interactions and nucleic acid-binding proteins. Biophysical techniques can be used to rationalize, progress and optimize an identified hit, often requiring only assay miniaturization to become suitable for high throughput. The main limitations to the more widespread use of biophysics in drug. Development are: experimental throughput, experimentation cost, levels of expertise required and the decreased sensitivity and broadness of utility that accompany the requirements of operation in a high throughput environment.

5.1. High-Throughput Screening

The essence of high content screening is this: The newly grown cells express measurable changes in their phenotypes (due to a fluconazole-activatable cell surface or its pharmacological changes).

State-of-the-art biology- and disease-relevant predictive cells are created for HTS from the beginning. Because construction starts from messenger RNA, this is nothing more than standard bioconstruction. Then a high-throughput automation platform is used to acquire cellular images of the experimental cells; any cell images can be obtained in parallel. Cell site is then performed. The dramatic difference in surface and site images of unpolarized living cells rendered in 3 D is of marginal selectivity. In the neural differentiation and characterization field, the translocation sites of necked intrachrodroid bile acids were identified by not-dimensional intrachromodal site-selective global profiling in states of biosynthesis 24. Every receptor profile is an imaging feature.

The visibility of the things scales differently; a point spread function of $O(100\text{ nm})$ is generally needed to resolve nuclear and synaptic structures, and the spatial resolution of pathologic cells is $\sim O(10\text{ m})$. Although there are many ways biological drugs can be screened or monitored, advances in many kinds of new high Fidelity dyes has made it possible to get real-time kinetic stacks of a living cellular reaction whenever we are ready to make a measurement with an automated microscope 7. With all the tools of high content screening and cell-based assay development at our disposal, the value of high Fidelity cellular information becomes very high. As a result, high-throughput changes in new intracellular bioproducts are the subjects of high-throughput and high-content screening, and many patents contain new uses of them.

High-throughput screening (HTS) techniques are crucial for drug discovery and clinical treatment for cellular diseases such as cancer 25. Only such methods can provide the quantitative data on biochemical reactions in living cells, in the coherent with Wendell Lim's viewpoint that cancer cells during migration are 'fuzzy' and unpredictable, while intended drugs are specific and potent, enabling their systematic studies and improve throughput for reliable data. This explains why automated systems microscopy platforms have been rapidly adopted in the medical pathology literature and in biochemistry, where quick access to a multiplicity of channels with full spectral resolution is more important for spectral unmixing to extract the maximum amount of information from a sample.

5.2. Structure-Based Drug Design

Computational resources including resources of the EU and trans-border submitted resources underscore the temporal and spatial scales and multidisciplinary integration including hybrid QM, MM methods, DFT/MM, ionic salt bridges. HTS and X-ray crystallography resolved each of these through NFkappaB modulators and Isg15-linkage targeting murine data and X-ray crystallography on the human retinoic acid-inducible gene I CIA targeting murine data. XR data should be deposited between 6 and 18 months after collection and molecular dynamics data yearly thereafter. Mutual information between ETR1 and the ETR1kin graft onto ETR1 yields additional hits. ETR1 family members were targeted over the subsequent 5 years with slow-down in the number of published X-ray datasets and membrane protein datasets since 26.

Many unique structural features, important species differences, and co-existing off-patent drugs are limiting the scope of targets for such tools. Some significant levels of conservation, often appearing after the divergence of major phylums in evolution, suggest the pharmaceutically important targets are difficult. Intracellular targets often feature protein-protein interactions, are entirely undruggable by conventional small molecules but could still be approached through allosteric modulation, PPI stabilisation by inter alia stapled peptides, or other new technologies. Within the boundaries of respect for human rights, animal testing, and primate testing can reveal important parameters for advancing drugs to human testing using stem cell-based models. Microglia and astrocytes are not well represented in EuroFusion for neuron work of the pharmacologically important models thus far. Expanding these protocols to more receptor compositions and brain cell crosstalk could push the boundaries for neurological disease phase II tests 27.

The 3D structural information of biological targets has significantly grown in the past decade, not only from 2D crystallography but also from cryo-EM and X-ray free electron lasers, and are publicly available. With approximately 20-25% of all drug-like small molecules targeting proteins within the central nervous system, structure-based drug design (SBDD) is expected to accelerate the discovery phase of new neuro-active drugs and reinvigorate drug repurposing efforts. Homodynamic targets such as ion channels, GPCRs, active sites or domain surfaces in enzymes, are common in neurological disorders including psychiatric drugs, neuropathic and nociceptive pain drugs, anti-alzheimer and anti-epileptic indications 28.

6. Biophysical Insights into Protein Misfolding and Aggregation Diseases

This chapter focuses on key biophysical decision points in misfolding and aggregation diseases. We used the example of FUS being a crucial player in neurodegeneration and cancer. FUS alternates between physiological diffuse and pathologic solid forms in both diseases, used as a biophysical decision point. FUS has the same physiological and pathological interconvertible self-assembly, making it a subject for the study of its liquid–solid phase transition. FUS serves as a biophysical decision point in the immunity, metabolism and cell division processes taking place in a cell. FUS hydrogel formation is a biophysical barrier to forced nucleation which is less efficient than spontaneous misfolding/oligomerization. Liquid-to-solid phase transition causes other biophysical changes inside a living cell, here the optical trapping resistance of a hydrogel.

Experimental biophysics methods to study intrinsically disordered protein (IDP) hydrogels evolved from liquid-to-solid phase transition and fibrillar misfolded proteins are briefly summarized. Non-equilibrium light scattering technique was compared to turbidity plate reader and microscopic measurements in detail. High throughput techniques by developing full automated single-cell experimental set-ups are discussed. Another technique was used in the macroscopic and single *C. elegans* studies with forced nucleation due to biophysical fusion of vesicles and the ring-form of disease proteins in liquid-to-gel phase transition. Additionally, FUS IDP hydrogel thickness was found by theoretical calculations and independently by volumetric measurements. Interdisciplinary between oncology, neurodegeneration, immunology and metabolism was considered as an approach to delineate cellular roles of FUS in normal and diseased cells. FUS is identified as an important hub at the interface between intrinsic immunity and cell metabolism. Detailed studies were performed to identify pivotal apoptosis, necroptosis and senescence biophysical crosslinks and immune checkpoint modulation by FUS. Relations between telomere damage, alternative lengthening of telomeres pathway and liquid-to-solid phase transition in FUS hydrogel were studied. Biochemistry and non-equilibrium light scattering data were used to indicate a biophysical decision point in the FUS hydrogel controlling the level of alternative lengthening of telomeres pathway.

Biophysics uses physical and chemical principles to study biological systems 29. From the biophysics perspective, protein misfolding diseases cause death often due to the protein aggregation causing cytotoxicity in the cells. Each disease is unique to specific proteins and cell types. However, they share the underlying biophysical nature in that normal proteins are misfolded and aggregated in diseased cells. This issue can be handled comprehensively from the view of biophysics by ultimately identifying common biophysics decision points in different diseases 30. Within the past 0 years, peer-reviewed articles in this field were about the common biophysical regulations in neurodegenerative diseases and cancer. These regulations can be identified via specific biophysical measurements in normal and diseased cells, aiming to future design preventative medicines 31. Additionally, it was observed that the same protein, e.g., FUS, accumulates in different structures with a biophysics characterization in different diseases like uncontrolled aggregation in amyotrophic lateral sclerosis (ALS) and liquid-to-solid phase transition adapting to stress in cancer.

7. Biophysical Techniques in Cancer Research and Therapy

Biomedical Treatment for Cancer Detection: A number of biomedical methods, such as histopathological measurements and mechanical research using equipment like atomic force microscopy (AFM) and magnetic tweezers, have been implemented to distinguish between benign and malignant tumors in clinical set-ups with the main aim to distinguish between any healthy and unhealthy tissue to diagnose cancer severity at any stage of cancer. The Rheological Activity of the Cancer Cells: Elastics and pathogens deal with complex dynamics. Cancer cell rheological activity can be understood through the following parameters, such as anchorage-independent growth, invadir-embedded grids and adhesions of the substrate, ability to enter the circulation (colonisation of sperm and air cells), endothelial cells, proteolytic potential. Cell Membrane Activity: The biophysical techniques have been used to describe the structural changes of the cancer cell's outer surface, such as the cell outer membrane where of the factors by which the cells remain to remain hydrophobic and a high affinity for blood commercay cells or the use of conventional force measuring increment.

Cancer is seen as one of the deadliest diseases of the 21st century, with an ever-growing global burden 32. Significant advances in our understanding of the genesis and progression of cancer have been made, providing us with an important opportunity for cancer diagnosis, research and treatment. Although cancer might be defined by its molecular changes, it is the integrative function of mechanics that ultimately controls its progression. Biophysical research and models have the potential to enhance and explain outcomes in the interest of finding cost-effective and more effective treatments for cancer 29. The intrinsic heterogeneity of cancers, with tumor cells expressing a wide range of genotypes and phenotypes including metastatic competency, drug resistance and anoikis resistance or immunosuppression, poses major challenges in presenting cures for most types of cancer. Models from the field of physics, particularly continuum mechanics and medical imaging, provide efficacy in assessing the biophysical properties of the tumor in a non-invasive manner by considering in vivo or ex vivo experimental data 33.

7.1. Ion Channel Biophysics in Cancer Cells

In addition, off-target toxicities of drugs related to ion channels have been low. Thus, drugs that target ion channels are expected to be safe as unique agents. For instance, Kv1.3 blockers, as selective drugs against lymphoma cells owning Kv1.3 channels, would be expected not to affect leukocytes that have predominant expression of KCa3.1 channels rather than lymphocytes. Furthermore, one among another way also to surmount toxicities of drugs that target ion channels of cancer cells is to deliver drugs locally 34. Intracavitary therapy, intraperitoneal therapy by docs, and intramyocardial therapy by both docs and immunocytes. A potential off-target toxicity, nevertheless, has been known for splico-, pepto- and venom-exon-modified channels. For doctor-delivered substances, a most standard way of local delivery is intracavitary infusion.

Cancer is a complex and multifactorial disease existing as thousands of different entity biophysically 21. Ion channel arrangement in plasma membrane is one of the most important difference between cancer cells and normal cells biophysically. Among different cancers, breast cancer cells, ovarian cancer cells, cervical cancer cells, endometrial cancer cells, lung cancer cells, colon cancer cells, bladder cancer cells, hematological cancer cells, brain tumor cells, and other like cancer cells own distinct ion channel arrangement 35. As a core section of applied biophysics of cancer, ion channel biophysics is as one of the most important potential therapies of cancer. In contrast with orthodox forms of cancer therapy — surgery, chemotherapy, and radiotherapy — and targeted therapies and immunotherapies. Drugs targeting ion channels have different mechanisms of action — not just by inhibiting cell proliferation but by causing apoptosis and/or boosting chemotherapy and/or immunotherapy, above all, by a lower or a lack of cross-resistances. Accordingly, they are expected to be useful in the untreatable group of cancers that are intrinsically

refractory to surgery, radiotherapy, chemotherapy, targeted therapies, and immunotherapies. They may also be used in the treatable groups of cancers that either are intrinsically sensitive to treatments or have acquired chemosensitivity during the treatments. As for toxicities of drugs that target ion channels, the limited expressions of ion channels have been observed for most of them. For instance, several of the 2P-domain K⁺ channel genes, that have been over-expressed by both glioma and breast cancer cells, have been suppressed in most of the normal tissues.

7.2. Nanotechnology Applications in Cancer Treatment

Phototherapy is a neoplastic treatment based on the administration of the photodynamic brokers. The approved photoabsorbers in PDT need efficient singlet O₂ generation, as well as additional radicals of oxygen because it is so cytotoxic that tumors can be destroyed. The primary directive of this new image magnification method is better quality, sensitivity and measuring knowledge. Throughout ultra-modern imaging technology, tumors can be observed easily. The end of the different nanoscale instruments used for tumor imaging is a vital and cutting-edge method for nanomedicinal oncological imaging.

Cancer is proliferated from abnormal cell divisions, which can eventually spread to different parts of the body 36. It can occur in any part of the body including blood and bone marrow. Research says that 30-50% of all cases of cancer can be prevented, by avoiding certain lifestyle habits and environmental factors. Nanotechnology in cancer treatment offers several drugs with anticancer impacts and the possibility for delivery of targeted drugs 37. For example, Paul Scherer Institute in Switzerland has tested about 3000 nanoparticles that kill cancer cells to various degrees. The nanocarrier (NC) used in neoplastic treatment decreases the drug's cytotoxicity, and their half-life increases. NC can cross cell barriers and bring a large amount of drug to the tumor cells. Thus, it improves the drug's bioavailability 38. In the clinical phase of cancer therapy NC also minimizes non-specific toxicity and have greater capacity for payload targeting. Due to the cost to evaluate its stability, precipitation, cellular uptake, and toxicity, various NC shapes and materials used in anticancer medication. It's a well-desired concept for therapeutic NC for cancer. Cancer is still nowadays a serious problem, and is still impossible to deal with fully. As a result, early detection of people with cancer is an important problem. It is crucial to identify at an early stage in cancer treatment to reduce the chemotherapy effectiveness.

8. Biophysical Approaches to Neurodegenerative Diseases

We provide an overview of the principles governing the modulatory dynamics of both classical and artificially designed vesicle systems and discuss non-equilibrium pathways to transport enhancement. A uniquely dynamic, nonequilibrium vantage offers potential solutions for the modulation or correction of these transportation pathways in vesiculopathies. We provide examples of artificial molecular machines engineered to rectify pathologically dysregulated cell biological fluxes drawn from on-chip demonstrations, prototype molecular motor work, and advanced solutions including cytoplasm-implanted devices. Burdensomely slow release of dopamine mediated by diffusion limited transport of therapeutic nanoparticles through cerebrospinal fluid is a major barrier to achieving regulation of Parkinson's disease motor symptoms. Until recently, we were reliant on mass-action therapeutics that do not exploit the dynamical underpinnings of endogenous neurotransmitter release 39. Integrated knowledge of both molecular and higher-order, systems-level descriptions of cellular function is clearly needed if nonequilibrium approaches to particle localization within the brain are to be successful in achieving long-term therapeutic benefits.

In contrast to the stable nature of cell biology, the field of nonequilibrium statistical mechanics provides tools and concepts to understand how the fluctuation, as well as the presence of a source of nonequilibrium energy or information, impacts the functioning and dynamics of biological systems 40. In this chapter, we review an array of biophysics-based techniques employed to probe the

dynamics of two key aspects of neural cells that are impacted in nearly every neurodegenerative disease: the trafficking and turnover of vesicles and protein aggregates through cells. We briefly review the biophysical principles governing the functions of each and discuss how their dysfunction leads to neurodegeneration. Given that disruption of vesicular trafficking and endocytic pathways has been among the more consistent findings across diverse neurodegenerative diseases, vesiculopathy may be useful as a common mechanistic descriptor for these disorders 41.

9. Biophysics of Infectious Diseases at the Cellular Level

Most edges link immune system and virus research, illustrating the real complexity of biology and the real necessity of interactions between biological, technological, and physical approaches. Biophysics does play a role as well in the understanding and potential treatment of cancer and autoimmune diseases 20. Biophysics is involved in both the research on infectious diseases and cancer. Biophysics does bring several new insights on infectious diseases as fluid mechanics is leading to develop new diagnostic tools like scanning and penetration instruments. Biophysics, as it connects both physics and biology, may as well help to find new therapeutic interventions for infectious diseases and better understand pathogen-host biology including for viruses' diseases such as COVID-19.

The infection process by viruses usually spans three stages: cell entry, genome release, and virus assembly. The first two stages of infection rely on the ability of the virus and cell to either generate or withstand mechanical stresses, flows and/or deformations. All cells dynamically interact with their mechanical and chemical surroundings. This cross communication is particularly fundamental for the immune system and is thus highly studied for lymphocytes, macrophages, and fibroblasts 21. During infection by pathogens, macrophages respond and recapitulate many of the steps observed in 3D migration, ie: membrane protrusions or retraction or maintain of adhesions. The similarity of the obtained migratory profiles and those of infected cells suggests its interest to be developed.

Biophysics studies the application of principles and methods of physics to understand the functioning of molecular and cellular systems. In this perspective, biophysics is crucial to advancing our understanding and treatments of various diseases that defy conventional drug-based strategies. The well-known viruses such as influenza, human immunodeficiency virus, severe acute respiratory syndrome coronavirus, and the current coronavirus disease (COVID-19) are hard to be cured due to constant epidemic outbreaks, which seems to be more prevalent in the future 42. The contents of this work have presented biophysics at the registers of communication in living cells and their role in diseases. A chapter is focused on understanding severe COVID-19 symptoms as well as the immune system in such diseases. The spread of viruses in cells and a few strategies for healing are discussed.

10. Biophysical Techniques in Stem Cell Research and Regenerative Medicine

More broadly, tools in regenerative medicine are also desirable if they can be used to image or manipulate engineered tissues or the living system pre- and post-implantation that will drive and direct the regenerative process in the correct manner. Other biophysical techniques have been widely used, mostly in vitro, to build gene circuits or more complicated cellular arrangements where perhaps things like spatial organization or collective phenomena are under investigation. Various techniques from time-resolved CRISPR/CAS experiments to microfluidics to CRISPR screening have been used in regenerative medicine in particular. Finally, biocompatible nanoparticles have been a part of regenerative medicine for some time for molecular imaging, as drug-carriers, and for targeted therapy. This part of regenerative medicine has seen significant advancements and remains the primary area where hard and soft condensed matter physicists have contributed to regenerative medicine pathways for many years indeed 43.

Biophysical techniques employ the knowledge of the principles of physics for inquiry, but often live somewhere between biology, chemistry, and physics in application 20. Often biological systems are

complex and require sophisticated application of biophysics to begin to understand the phenomena at hand. The driving force behind the importance of biophysical techniques in regenerative medicine likely lies in the ability to make quantitative measurements of the living system or tissue at hand. This can defer greatly from current biochemical or genetics approaches, which are typically qualitative. Indeed, there is a demand for in vivo imaging modalities, genomics, proteomics, and other large-scale approaches that allow one to measure in a bottom-up approach to more complexity. An example of this is the heavy reliance on whole genome sequencing in the past decade and increased demand for more quantitative epigenetic measurements to build towards understanding entire cells or tissues, in particular during development or in regenerative medicine⁴⁴.

11. Biophysical Imaging Techniques for Cellular Disease Diagnosis and Monitoring

Medical image analysis encompasses various imaging modalities and end goals, including clinical pathology detection, disease stage and severity assessment, treatment planning and monitoring, forecasting disease outcomes, and population health studies ⁴⁵. It helps identify abnormalities, assess treatment effectiveness, and guide minimally invasive procedures. Medical imaging also aids in research, education, and measuring anatomical structures, tissue density, blood flow, metabolic activity, tissue elasticity, and chemical composition. Gold and iron oxide nanoparticles have demonstrated consistent diffusion and complete cellular internalization, allowing studying cellular grids and responses. In-vivo imaging studies are possible through suitable nanoparticle labeling and the selection of robust biophysical techniques for mice and zebrafish. Two-photon excitation fluorescence microscopy can achieve microscopy penetration depths (~1 mm) and automatic layer-by-layer 3D imaging. Nanoparticle-based magnetic resonance imaging (MRI) contrast agents not only help identify disease site but provide anatomical information MRI machines produce hamper clinical in seeing, pairing / with X-ray, CT scan and PET.

Physics principles have significantly contributed to biomedical research, leading to advancements in diagnosis and treatment of various pathological conditions. The strong relationship between physics and medicine has resulted in the development of technologies like MRI, diagnostic radiology, angiography, computed tomography, and ultrasound ². Biomedical imaging plays an essential role in the prevention, early detection, diagnosis, and treatment of various diseases. Biomedical imaging cargo that is approved by the FDA currently has a range of applications. Furthermore, the advancements in imaging technology have enabled the discovery of further applications. These imaging modalities play a crucial role in the evaluation of biological and cellular processes ⁴⁶. Different techniques like X-Ray imaging, ultrasound imaging, Micro-Computed Tomography, Optical Coherence Tomography, and Magnetic resonance imaging are being used for cell imaging and cell biology. Generally, cells are labeled with contrast agents to help image and observation for different imaging techniques.

12. Challenges and Future Directions in the Application of Biophysics to Cellular Diseases

Quantitative cellular disease biophysics desperately requires cellular observational and experimental decompositions. For instance, tumor treatments aimed at cellular scale events, such as induction of apoptosis, might benefit greatly from additional proxy markers of disease progression. Careful biophysical modeling and observation of in vitro and in vivo cellular interactions suggest the existence of several candidates to serve this purpose. Similarly, the physical principles underpinning successful clinical applications remain complex for patient specific and nuanced for disease stages – for example, there is currently no broadly applicable theory for converting redox chemistry into useful real time or non-perturbative in situ sensors of non-responses to wound healing. Bioinformatics, big data, and machine learning strategies can yield enormous benefits but will be of limited use if care is not taken to connect the reduced-scale physical and chemical observables

assembled in clinical studies to physics and biology prior to their use as features in predictive algorithms.

Much biophysical inquiry into disease states remains phenomenological and qualitative. Biophysicists often lack the theoretical and experimental tools to adequately describe or interrogate the complex molecular interactions and structural and mechanical consequences of epigenetic modifications and abnormal protein deposition that drive many disease states 7. To a large extent, such omissions derive from the choices Papers Recent Developments in Nonequilibrium Physics in Cellular Biology in model systems and observables that conform to the experimental strengths of the biophysical toolkit. To further progress in our understanding and ability to treat diseases, two contrasting routes might provide forward progress: The inclusion of physical effects in increasingly realistic models to predict emergent cellular behaviors and the generation of novel experimental observables and / or physical probes of cellular illness.

Biophysicists studying cellular diseases face the typical challenges that arise when systems of multiple interacting components span different scales, integrate multiple physical and biochemical mechanisms, and consume energy 40. Key questions that have relatively clear answers regarding single macromolecules quickly become intractable in cellular contexts. As has become evident in approaches to cancer treatment, a better understanding of the integration of genetic, biochemical, and biophysical regulation is required. This understanding should, in turn, lead to more targeted disease treatment with minimal side effects.

Conclusions:

In many biophysics applications, it has been used in biological applications such as treatments and diagnosis, and it can be used in other applications such as medical applications because this technology has shown high results after its improvement and development. This technique is characterized by the fact that results are obtained in less time compared to other techniques. Therefore, it is preferable to use it in the future with continuous development.

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