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## Physical Energy for Drug Delivery; Poration, Concentration and Activation

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### INTRODUCTION

The purpose of a drug delivery system (DDS) [1, 2] is to devise a method that enables delivery of a therapeutic agent that may have sub-optimal physicochemical properties in biological tissue, thus enhancing efficacy and safety by controlling the rate, time, and release of the agent [3]. Targeted DDS (TDDS) includes the administration of the therapeutic substance, the release of the active ingredients from the DDS, and the subsequent transport of the active ingredients across the biological membranes to the specific site of action, often using what is so called “smart drug carriers” or “smart nanoparticles (NP)”. Smart NP can improve drug delivery mechanisms based on their particular formulations [4, 5]. NP used in such biomedical applications include lipid-based NP (liposomes) [6, 7], polymeric micelles [8, 9], block ionomer complexes [10, 11], water-soluble synthetic polymers (dendrimers) [12, 13], inorganic [14] and polymeric NP [15], nanorods [16, 17], quantum dots [18, 19], carbon nanotubes [20, 21], silica-based NP [22], metal and semiconductor NP, upconversion NP [23, 24], self-illuminating NP [25, 26] and polymer-drug conjugates [27]. However the main limitations of employing NP for drug delivery are the following: 1) the relatively small amount of drug that can be linked to each NP; 2) the possibility of drug deactivation once it is chemically bound to the NP; 3) the possibility of immediate uncontrollable passive release (burst effect); and 4) NP agglomeration producing quick elimination from bloodstream by macrophages before reaching the target cells [28]. Most of these factors in turn require injection of unnecessarily high concentrations of NP, with potential systemic toxic effects. In order to overcome some of these hurdles, physical energy methods have been explored to enhance not only NP but also drug and gene delivery.

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The enhancement of effective drug delivery to the target achieved by the addition of physical energy to the traditional TDDS systems has been widely explored in recent years [29–36]. These techniques have the potential to be used in different biological and medical applications as an advanced TDDS that can minimize the current limitations and unwanted effects. Biophysical energy such as electric fields, magnetic fields, ultrasound and mechanical forces, light and temperature gradients can act as enhancers for drug delivery. Technique-specific terms have been associated with each form of physical energy such as electroporation [37, 38] for electric current, iontophoresis for electric potential, magnetoporation [39, 40] for magnetic field, sonoporation [41, 42], mechanoporation [43] and phonophoresis [44, 45] for ultrasound, optoporation [46, 47] for pulsed light and thermoporation [48, 49] for temperature, respectively (Figure 1). Table 1 summarizes all of the different types of physical energy used in this review for porating the cells. The advantages and disadvantages of the techniques are analyzed in the table and the corresponding gene transfection names are also included. Synergistic combinations of two or more physical energies such as magnetic-electroporation [50] are also of special interest and have been explored as a potential system for effective drug delivery. The formation of temporary pores in the cell membrane achieved through the application of various physical energies is the common phenomenon involved in such systems. Once the pores have been formed, drug-carrying NP or smart drug carriers (SDC) [51] can easily penetrate through these pores in the membrane and enter the cells where the drug/gene is further released and activated. Thereby, some of the above-mentioned constraints inherently limiting the efficacy of NP can be overcome by use of physical energies.

Smart NP along with the application of physical energy comprise a new arena of novel nanotechnology that enables electrical engineers, physicists, chemists, biologists and pharmaceutical engineers to explore and develop interdisciplinary research. For instance, physicists and engineers can come up with new devices by understanding the concept of “poration” using different modalities. Although, there are several factors that will affect the efficiency of drug delivery while using such physical energies, the key is the possible optimization techniques for individual “poration” methods. The aim of this review is to cover comprehensive up-to-date information about the usage of various such types of physical energy applications called “poration”

Though many different types of physical energy have been used for poration and some for concentration, activation is the final step that completes TDDS after the drug has reached its target. Photodynamic therapy (PDT) [52, 53], is an emerging therapeutic modality. It is a two-component system that uses light to activate the photosensitizer (PS) drug after delivery by physical enhancement has been applied and reaches its target. Special emphasis will be given in the review to applications in the PDT arena (when relevant) partly due to the experience of the authors' laboratory in PDT; nevertheless other types of drugs, DNA vectors and transfection procedures will also be covered in this review. In a similar manner, photothermal therapy [54] is also a light-mediated activation process, and will be covered as well. Cancer therapy will be emphasized due to the prevailing demand in this area of biomedicine. The efficacy of each physical energy system and the limitations and possible optimizations techniques will be given special importance in the review. Theranostics [55,

56], an emerging multifunctional modality, will be discussed in context with the various techniques of biophysical action.

## Photodynamic Therapy

PDT involves the use of photosensitizers (PS) in combination with visible light of the correct wavelength to excite the PS. In the presence of molecular ground (triplet) state oxygen ( $^3\text{O}_2$ ), the excited state PS transfers energy or electrons to produce reactive oxygen species (ROS) that are able to kill cells. PDT has mostly been developed as a cancer therapy [57], but recently has been proposed as an antimicrobial therapy.

The PS molecule has a stable electronic configuration in the singlet state with the most energetic electrons in the highest occupied molecular orbital (HOMO) [58]. Following absorption of a photon of light of the specific wavelength according to its absorption spectrum (Figure 2 shows a Jablonski diagram illustrating the process), an electron in the HOMO is excited to the lowest unoccupied molecular orbital (LUMO), causing the PS to reach the unstable and short-lived excited singlet state. In this state, several processes may rapidly occur such as fluorescence and internal conversion to heat, but the most critical of these to PDT, is the reversal of the spin of the excited electron (known as intersystem crossing) to the triplet state of the PS. This excited triplet state is less energetic than the excited singlet state, but has a considerably longer lifetime, as the excited electron, now with a spin parallel to its former paired electron, may not immediately fall back down (as it would then have identical quantum numbers to that of its paired electron, thus violating the Pauli Exclusion Principle). This much longer lifetime (many  $\mu\text{s}$  as compared to a few ns) means the triplet PS can survive long enough to carry out chemical reactions which would not have been possible with the excited singlet PS. Dyes without a significant triplet yield may be highly absorbent or fluorescent but are not good PS.

The photochemical reactions of the triplet state can be divided into two different pathways, either the Type I mechanism involving  $\text{e}^-$  or hydrogen atom transfer from one molecule to another, or the Type II mechanisms involving energy transfer to molecular oxygen. It should be noted that both these mechanisms can occur at the same time but the relative proportions may depend on the PS structure and also on the microenvironment.

The type I pathway can involve an electron-transfer reaction from the PS to  $\text{O}_2$  in the triplet state which results in the formation of toxic oxygen species such as  $\text{O}_2^{\bullet-}$  that can further transfer to form ROS, such as  $\text{H}_2\text{O}_2$  and  $\bullet\text{OH}$ , which are formed by the Fenton reaction in the presence of divalent metal ions such as  $\text{Fe}^{2+}$  [59] (Eq. 7–9). Another possible mechanism has been proposed that may operate in cases where the triplet state PS is a good  $\text{e}^-$  donor. Here  $\text{H}_2\text{O}_2$  (formed from  $\text{O}_2^{\bullet-}$ ) can undergo a one  $\text{e}^-$  reduction to form  $\bullet\text{OH} + ^-\text{OH}$  (the redox potential is only +0.32V, see Table 1).

The two most prevalent damaging ROS ( $\bullet\text{OH}$  and  $^1\text{O}_2$ ) are able to react with many biomolecules in cells. The exact targets and reaction mechanisms involved depend on the following considerations. Firstly the localization of the PS generation is critical because most of the ROS are highly reactive and cannot travel far from their site of production before disappearing (these distances are the order of nm to  $\mu\text{m}$ ). Secondly the relative

abundance of the target biomolecule is important. Thirdly we have the question of whether Type 1 or Type 2 mechanisms produce the ROS in question.

PS are usually organic delocalized aromatic molecules consisting of a central chromophore with auxiliary branches (auxochromes) that are responsible for further electron delocalization of the PS, thus altering the absorption spectra of the PS. Due to extensive electron delocalization, PS tend to be deeply colored. This means that the energy required to excite the  $e^-$  in the HOMO to the LUMO is low compared with less delocalized molecules and therefore, the absorption bands are in the longer wavelength (red) spectral region and are large, reflecting the high probability of excitation.

## Magnetic Drug and Gene Targeting: Magnetoporation

Magnetic fields have promising applications in drug delivery due to their relatively non-invasive nature compared to their counterpart of electrical fields. Magnetic energy is an important technique for delivering drugs to specific locations within the body [60]; this phenomenon of application of magnetic field for drug delivery has been called magnetic drug targeting (MDT) [61, 62]. In principle, MDT consists of the following steps (Figure 3) [63]: 1) coupling the drug to the magnetic NP (MNP); 2) applying an external magnetic field to attract the MNP to the desired location such as a tumor; and 3) the release of the drugs from the NP under the influence of external alternating magnetic field once it reaches the target. Furthermore applied magnetic fields can also be used to release drugs from nanocarriers such as liposomes or nanoemulsions. These nanoparticles can contain superparamagnetic iron oxide nanoparticles (SPION) that heat up to temperatures of the order of 45C under the influence of alternating magnetic fields [64].

MNP have been tested pre-clinically or clinically as targeted drug and gene delivery agents and also to enhance diagnostic imaging such as magnetic resonance imaging (MRI) [4]. The primary attractive features of MNP for drug delivery include their simplicity of synthesis, ability to be controlled remotely via magnetic fields, the fact that magnetic fields easily penetrate through biological tissue and their biocompatibility and low toxicity [65]. One of the unique properties of MNP, exclusively available on the nanoscale level, is the switchable magnetic properties of superparamagnetic NP, which may pave the way for applications in the biomedical field which include advanced medical imaging and cell tracking. The primary step in MDT is that therapeutic agents are attached to or encapsulated within these MNP which are exclusively used as magnetic delivery vehicles. These MNP can be constructed from magnetic cores with a polymer or metal coating that can be functionalized (Figure 4). The polymer (that can be modified) acts as the most important material for modification of MNP for drug delivery. Sometimes the units may consist of porous polymers that contain MNP precipitated within the pores. Through functionalization of the polymer or metal coating it is possible to attach, for example, cytotoxic drugs for chemotherapy or DNA for gene delivery [66]. Different kinds of functionalization have been described in detail by Chomoucka et al. [67]. Once functionalized, the MNP/therapeutic agent complexes are injected into the bloodstream, often using a catheter to position the injection site near to the target. When solid (rare-earth) external magnets, typically with high-field and high-gradient, are focused over the target site, the magnetic fields drive the

MNP to be accumulated at the target site. This technique is advantageous over simple direct injection of the drug into the diseased area as the natural route of drug absorption into the tissue is via the capillaries rather than a direct bolus of drug into the tissue that may not be distributed into all the cells. Thus, trajectories of such MNP are almost fully controlled by the externally applied magnetic field though other smaller forces may also coexist. While this may be effective for targets close to the body surface, due to the fact that the magnetic field strength attenuates rapidly as a function of distance, deeper areas within the body become more difficult to be targeted [66]. Sometimes complicated trajectories are possible in the presence of an applied field; drugs attached to MNP can be slowed down by drag or even captured by other cells that are non-targeted. The drag force experienced by the MNP is mostly due to the blood flow inside the blood vessels. In order to effectively overcome the drag, the magnetic force due to the external field must be greater than the drag force [68].

$$F=6\pi\eta r_h v$$

Where,  $\eta$  is the viscosity of blood,  $r_h$  is the hydrodynamic radius of the particle and  $v$  is the velocity of the particles. It is important to note that the hydrodynamic radius of the particles could be much larger than the magnetic radius of the particles, this is especially the case if the particles are coated with polymers and proteins (typical values:  $r_m = 5\text{nm}$ ,  $r_h > 20\text{nm}$ ) [4].

In general, the force experienced by the material (cells/physical body) when placed in an external magnetic field is given by [68]:

$$F_m=\mu_0 VM.\nabla H$$

Where,  $V$  is the volume of the material,  $\mu_0 = 4\pi \cdot 10^{-7}\text{N/A}^2$  is the magnetic permeability of vacuum,  $M$  is the magnetization (magnetic dipole moment per unit volume) and  $H$  is the applied (auxiliary) magnetic field. The magnetization of the cells/NP can be taken approximately proportional to the applied magnetic field up to a certain value called the saturation magnetization  $M_{\text{sat}}$ . Beyond this level all the constituent dipoles are aligned with the field and no further increase in magnetization is possible. It is critical that these conditions have to be clearly taken into account during drug delivery; be it the external magnetic field or the particle that carries the magnetic field, effective delivery may be successfully accomplished only when a comprehensive understanding of the behavior of fields is considered.

Recently multi wall carbon nanotubes (MWCNT) have been used as magnetically driven nanotools for effective cell transfection without noticeable cell damage [69]. The paramagnetic MWCNT interact with cells when exposed to magnetic fields, driving cell migration toward the magnetic source. Decreased viability of the tumor cells caused by cell membrane damage accompanied by cell component leakage paves way for the possibility of an image-guided in situ ablation of tumors. An increased uptake of cytotoxic agents and magneto-cell lysis producing mechanical tumor ablation is speculated. For instance, the

effect of TiO<sub>2</sub> and Fe-doped TiO<sub>2</sub> MNP on the activity of HL60 leukemic cells was studied by observing the influence on the activity of cells exposed to light compared to un-exposure [70]. It was observed that the PS (TiO<sub>2</sub>) completely eliminated tumor cells using illumination under an applied magnetic field..

When using magnetic fields for gene transfection the phenomenon is called magnetofection. For magnetofection to take place *in vitro*, a high-field, high-gradient magnetic force is focused onto a multi-well culture plate in which the cells are growing [66]. Next, the gene/DNA is attached to magnetic NP and the external magnetic field is turned on; the external field apart from controlling trajectory of the MNP also enhances sedimentation rates, particle internalization and gene expression. Although the mechanism of increased internalization is not completely understood it is hypothesized that the oscillating fields introduce extra energy to the system which produces a non-linear motion of the particles as they move along the field gradient that may aid cell and tissue penetration. For *in vitro* controlled transfection, three important points of magnetofection needs to be considered, they are: 1) very low vector dose; 2) reduced incubation time for achieving high magnetofection/transduction efficiency; and 3) the possibility of gene delivery to otherwise non-permissive cells (cells that do not easily internalize DNA material). It is presently uncertain if this magnetofection technique could be usefully applied *in vivo*.

Non-viral gene vectors are considered safer than viruses, but owing to their inherent low efficiencies, limitations apply to their utility in gene therapy. Therefore, gene vectors with superparamagnetic NP in conjunction with TDDS using magnetic fields have been explored [71]. The use of super paramagnetic iron oxide nanoparticles (SPION) under oscillating magnetic fields (OMF) have been shown to improve diffusive transport of drugs across biological barriers by increasing the local temperature by inductive heating [72]. SPION often tend to remain in circulation unaffected by the liver or the immune system. The two functionalities associated with SPION for drug delivery are: 1) increased delivery into cells and tissues due to heating and motion induced in the SPION by the oscillating field that affect the potential for MNP to be used as targeted drug delivery agents under low magnetic field strengths; and 2) activation for achieving triggered release of drug attached to the surfaces of the particles using OMF with minimal damage caused due to heating of the surrounding tissues. In essence, such biocompatible magnetic field conditions avoid significant disruption that can be caused by the use of synthetic biopolymers-based NP for drug or gene delivery [65]. Combined thermosensitive and magnetic NP have been utilized in controlled TDDS with added advantages [73]. In superparamagnetism thermal fluctuations take place such that they can spontaneously demagnetize a NP that had previously been magnetically saturated leaving these particles with no hysteresis [67].

Multi-functional SPION consisting of tetra-ethyl-ortho-silicate (TEOS) encapsulating monodisperse Fe<sub>3</sub>O<sub>4</sub> NP (which serve as a core) and further grafted by poly (N-isopropylacrylamide) (PNIPAM) are of special significance. These SPION could be introduced to the desired location by external magnetic field by controlling the magnetite core [73]. There is tremendous potential to further explore MNP as facilitators of drug delivery provided all factors that affect its efficiency are controlled.



### Parameters that affect Magnetic Drug Delivery efficiency

In MDT the magnetic field can be modulated externally in order to control the drug-release with the characteristics of driven magnetic accuracy, targeting, and high drug-carrying capacity cells. This property of MDT can be used to lower toxic effects and to enhance the therapeutic effect. The efficiency of the MDT for targeted drug delivery depends on at least a dozen of different factors [4] that includes: 1) the size of the MNP; 2) magnetic property of the MNP; 3) the biocompatibility of MNP; 4) physicochemical properties of the drug-loaded in the MNP; 5) applied magnetic field strength and geometry of the system; 6) penetration depth of the target tissue; 7) resistivity caused by blood flow; 8) vascular supply; 9) NP aggregation due to large surface/volume ratio; 10) the short half-life of the particles in blood circulation; 11) the low efficiency of the intracellular uptake of NP; and 12) nonspecific targeting. Although small MNP have much better drug delivery properties than larger ones due to their ability to pass through biological barriers, their small size may make them insufficiently responsive to the applied magnetic field.

### Limitations and possible solutions

There are several limitations that hinder full clinical therapeutic usage of MDT. The limitations include inappropriate gradient modulations by applied magnetic fields which causes non-specific targeting [4], the potential for embolization (blocking blood flow) when the particles accumulate within the bloodstream, and the development of cytotoxicity as an unwelcome side-effect with the increased concentration of MNP in the liver. However, some of these problems may be potentially turned to advantage and could increase targeting, for example, in the case of tumors in the liver the blood supply to the tumor mass gets blocked [63]. Some of the other problems of efficient drug delivery can be avoided if SPION are used as MNP as described earlier, however, further investigations need to be carried out for a comprehensive understanding of the field.

### Electric Field Drug and Gene Delivery: Electroporation and Iontophoresis

Depending on the size and the shape of the cell, when a pulse of intense external electric field is applied, polarization [74, 75] takes place; the anode facing side becomes hyperpolarized and the cathode facing side becomes depolarized [76]. This leads to a phenomenon called “cell poration” or “cell fusion” that involves the fundamental behavior of cell membranes defined by the term “electroporation” (EP). EP can introduce dipolar molecules into the cell by varying membrane permeability of the cell or tissue [75]. EP is inexpensive and simple to perform in the laboratory, and therefore has been widely used in biomedical research. Since traditional methods of increasing cell uptake can involve biological and chemical side effects due to over dosage, EP can be used as enhancer for TTDS with higher spatial resolution. Though the fundamental principle by which the electric field induces cell poration or cell fusion is not yet thoroughly understood, it is widely assumed that electrical breakdown of the cell membrane could be a key explanation of the phenomenon. Assuming a spherical cell the cell membrane potential is given by

$$V_{\max} = (3/2) rE \cos \theta$$

Where  $\theta$  is the angle between the electric field and the normal vector of the cell membrane,  $r$  is the radius of the cell when placed in an external electric field of strength  $E$ ,  $V_{\max}$  is the potential maximum near the cell poles where  $\theta = 0^\circ$  and  $180^\circ$ . For very large  $E$ ,  $V_{\max}$  reaches a critical value  $V$  (on the order of 1 V), the field within the cell membrane is high enough to cause an electrical breakdown of the lipid bilayer [77]. Membrane pores could be created as a result of such breakdown [76] as shown in Figure 5. Apart from the primary electrical breakdown of the membrane a second breakdown mechanism of the cell membrane is possible caused by a phenomena called “electro-mechanical coupling effect” [78].

EP has a wide range of applications, including drug delivery [79–83] and gene transfection (electrofection) [84–87]. EP has been incorporated for drug and gene delivery in various areas of the human body that include lung [88–90], skin [91–93], cornea [94, 95], spinal cord [96, 97], heart [98, 99], brain [100–102] and dental tissues [103, 104]. Owing to its high gene transfection efficiency, safety, low toxicity and reproducibility, EP also has the potential for introducing genes into mammalian cells [105]. DNA, (pSV2-CAT gene) when introduced into a mammalian cell in the presence of electric field in vivo the transfection efficiency was found to multiply by 100–1000 times compared to the one without field [106].

Similarly, experiments have demonstrated that when EP was introduced in PDT malignant cells were eradicated in a much shorter time compared to PDT without field. To demonstrate such capabilities of EP, in a study, a Chinese hamster fibroblast cell line (DC-3F) was targeted using two different PS; chlorin(e6) (Ce6) and aluminum phthalocyanine tetrasulfonate (ALPeS4) with different dose levels followed by EP with 8 electric pulses at 1200 V/cm intensity, 0.1 ms duration and 1 Hz frequency. After 20 min of incubation the cells were irradiated using a visible light source (660 nm). The malignant cells were found to be dead in a significantly shorter time span proving enhanced efficacy contributed by EP [107]. Another group applied EP on human histiocytic lymphoma U937 and *Saccharomyces cerevisiae* cells incorporating some of the reliable PDT agents, such as, thiopyronine, protoporphyrin, zinc phthalocyanine, copper phthalocyanine sulfonate, adriamycin and daunomycin and achieved remarkable results from their experiments. The dye diffused rapidly into the porated cells and the membranes resealed over a period of 6–10 min. Faster destruction of all resealed cells compared to control cells (without EP treatment) has been observed in these experiments demonstrating the advantages of the applied electric field.

Field strength is an important parameter in EP that needs to be controlled for better efficacy of drug/gene delivery. Recently high-intensity direct current (DC) electric field has been applied for electroporation in cancer therapy. In an experiment, macromolecular chromophore dextrans (cibacron-dextran) acting as PS were transported into cancer cells (U-937 cells) by electroporation of their membrane using short pulses in between two electrodes dipped in culture medium. After electroporation by electric pulses the penetration of cibacron blue dextran and the resealing of membrane pores, during 20 min in the dark, took place and the following irradiation by 66 J/cm<sup>2</sup> light produced a distinct PDT effect on cancer cells [108]. However, in the control measurements, PDT alone using cibacron-dextran produced negligible effect. Likewise, Xu et al [109] observed that a combination of



EP and TiO<sub>2</sub>-conjugated NP with monoclonal antibody showed significant photokilling after photoexcitation. While EP accelerated the delivery speed of the TiO<sub>2</sub> NP to cancer cells, use of monoclonal antibody increased the photokilling selectivity of TiO<sub>2</sub> NP to cancer cells with less damage to healthy cells.

There are several ongoing clinical trials that are associated with EP-mediated gene delivery [110]. However, efficient use of EP for drug/gene delivery is still in a preliminary stage due to possible side-effects of electric fields and currents, and further research is underway.

### **Parameters that affect electric field drug/gene delivery efficiency**

Gene/drug delivery efficacy during EP relies on cell membrane permeability and electrophoresis [111]. Formation of pores such that membrane reseals when field is turned off is a prerequisite for drug/gene entry. The size of pores and number of pores created are important parameters to be considered for drug/gene delivery efficacy. The pore size is directly proportional to the applied electrophoretic force. The pore size is also affected by the presence of anionic lipids during EP [112]. Peng et al investigated the number of pores created and the dependence on the interaction of the DNA plasmid fragments with the electroporated membranes [110]. It was found that a relatively low number of electropores was necessary to avoid toxicity and that polymeric forms of the DNA vectors enhanced the transgene expression to give efficient delivery. Electrofection efficiency depends on a number of optimizing parameters including: 1) electric pulse parameters; 2) electrode design; 3) DNA buffer composition; and, 4) pretreatments of the target tissue. The overall goal is to produce the optimum number of temporary pores to allow DNA penetration into the cell without allowing the pores to remain open long enough to adversely affect critical cellular physiology leading to cell death.

### **Limitations and possible solutions**

Strong electrical fields can produce permanent cell permeabilization and thereby consequent loss of cell homeostasis eventually lead to cell death, a process known as irreversible EP [113]. Intense electric field impulses produce a mechanical shock (similar to forced oscillation using ultrasound) disrupting the structure of the membrane locally. For the thickness of the membrane ( $6 \times 10^{-7}$  cm) when the membrane potential is on the order of 1 V, the electric field within the cell membrane will exceed 10(6) V/cm and the following two processes can take place [113]: 1) compression of the lipid bilayer normal to the surface leading to electrical breakdown of the membrane; 2) the charged groups of the membrane are forced to move in the direction of the field leading to mechanical stress in the membrane eventually disrupting it. In order to avoid breakdown it would be more effective to apply an oscillating electric field to induce poration than to apply a DC field. EP has been widely applied for gene delivery in experimental animals to improve cancer treatment, vaccination and wound healing [114–116] and has been tested in a clinical trial of interleukin-12 plasmid delivery for melanoma [117].

## Iontophoresis

Iontophoresis which also uses an electric field is an emerging modality of drug delivery through the skin by using a potential producing only a gentle electrical current [118, 119]. Unlike formation of pores in EP, in iontophoresis the ionized form of the drug penetrates through sweat ducts, sebaceous glands, hair follicles and imperfection sites in the skin layer. The iontophoresis unit carries an anode drug reservoir that holds and releases the drug and a cathode that collects the opposite ions when a potential is applied across the two polarities as shown in Figure 6 [120, 121]. When a potential difference is applied the cationic molecules that are placed under the cathode are repelled due to the same charge and are transported through the dermal layers into deeper structures underlying the skin and absorbed into the bloodstream. Similarly, anions originating from tissues below the surface of the skin are extracted into the counter electrode which is the “anode”. Iontophoresis has been proposed for numerous uses, including the delivery of local anesthetics before skin procedures in children thus avoiding injections, local drug delivery for agents such as non-steroidal anti-inflammatory drugs (NSAID) or corticosteroids for musculoskeletal inflammatory disorders [122]. Singh et al. [123] investigated modulated iontophoresis that involves microneedles in transdermal permeation of ropinirole hydrochloride in Parkinson’s disease therapy and succeeded in delivering therapeutic amounts over a suitable patch area and time.

In PDT for skin cancer (actinic keratosis (AK)) [124], when traditional method is used, the PS 5-aminolevulinic acid (ALA) is absorbed percutaneously through passive diffusion which requires 4–6 h waiting time before performing the procedure. In order to find an alternative method to accelerate the PS delivery, ALA was iontophored using direct-current pulsed iontophoresis. Protoporphyrin IX (PpIX) production following iontophoresis were comparable to the conventional occlusive dressing technique (ODT) and biopsies from the treated lesion demonstrated disappearance of AK.

### Parameters affecting the efficiency of Iontophoresis

Several parameters affect the transdermal absorption of drugs through iontophoresis including: 1) concentration of drug; 2) polarity and ionizable property of drugs; 3) pH of donor solution; 4) co-ions availability; 5) ionic strength of solution; and 6) electrode polarity.

## Light Induced Gene and Drug Delivery: Optoporation

Owing to the ready availability of light sources and the good biocompatibility of light in biological systems, light has been evaluated for its use as an effective drug delivery enhancer. When light is used as an enhancer in drug/gene delivery then the phenomenon is called as “optoporation”. From fundamental experimental research to clinical practice, successful optoporation techniques have been investigated. Since light spans a wide electromagnetic spectrum (Figure 7) and the fact that some of the wavelengths can penetrate deep into the body, optoporation takes advantage of this special property. Optoporation has the potential to facilitate the permeation of topically applied drugs into deep tissue layers, mainly by means of light or lasers (light amplification by stimulated emission of radiation). Particularly in the skin, ablative fractional resurfacing procedures using CO<sub>2</sub> (10,600 nm)

and erbium-doped: yttrium-aluminum-garnet (Er: YAG – 2,940 nm) lasers have gained popularity due to their capability to disrupt the major cutaneous barrier - the stratum corneum - by creating vertical micro channels (Figure 8) which serve as pathways for the deep penetration of drug into skin layers [63].

The ablative fractional laser technique (Fraxel) was found to be an effective clinical procedure to enhance targeted drug delivery. Using this technique Togsverd-Bo et al. [125] conducted a randomized clinical trial comparing the fractional laser-assisted PDT with ordinary PDT for actinic keratosis (AK) in a field-cancerized face and scalp using CO<sub>2</sub> Fraxel laser on fifteen patients. Patients had two symmetrical sides treated one with ablative fractional laser prior to the PDT and the other with PDT only. When the drug methyl aminolevulinate (MAL), a precursor of PpIX, was topically occluded followed by PDT illumination with red light, a better therapeutic effect on the Fraxel-treated site compared with the non-treated site was observed. During such ablative techniques it is necessary to understand that the depth of drug entry into the skin depends on the energy of the light transmitted and also the pulse duration. In order to understand depth as a function of the energy of the light a study was conducted on ablative fraction laser technique [126]. In this study MAL was applied on Yorkshire swine normal skin treated with CO<sub>2</sub> laser with pulse durations of 3 ms and delivering energy of 37, 190, and 380 mJ per laser channel. This procedure created three different hole-depths, which reached down to 2,100 µm into deep dermis/subcutaneous layer. It was found that different intradermal laser channel depths enhance the drug permeation throughout the skin layers but it did not affect the PpIX accumulation.

CO<sub>2</sub> fractional lasers are now popularly used for such studies owing to their inherent capabilities. In order to achieve similar results, recently YAG laser was also suggested as an alternative method. For instance, using the Er: YAG (2,940 nm) laser in an experimental ex vivo study, Forster et al.[127] observed the penetration enhancement of topical 5-aminolevulinic acid (ALA) 20% - a precursor PpIX, on a porcine skin model. It was observed that the PpIX fluorescence was observed in deeper skin layers compared with non-ablated skin. Lee et al. compared the ability of Er:YAG and CO<sub>2</sub> lasers and microdermabrasion in terms of enhancement and control of in vitro skin permeation and deposition of vitamin C [128]. The flux and skin deposition of vitamin C across microdermabrasion-treated skin was found to be almost 20-fold higher than that across intact skin. Among the modalities tested Er:YAG laser demonstrated the greatest enhancement of skin permeation of vitamin C. It was further emphasized that the laser fluence and spot size played important roles in controlling drug absorption.

There are also other kinds of lasers used for optoporation. Using an argon ion laser (488 nm) and phenol red as a light absorbing dye the plasma membrane of Chinese hamster ovary cells was made permeable when the beam was focused [129]. With irradiation with laser, gene transfection has also been studied. Optical transfection is sometimes called “optofection”. Optofection effects were studied using a green fluorescent protein (GFP) encoding plasmid [129]. The optofection rates after laser irradiation were around 30% for younger cells and less than 10% for aged cells.

## Limitations of Light

Although optoporation has shown promising results, some reports raise concerns about being used with PDT owing to the phototoxic effects of PDT, such as inflammatory response and exacerbation of pain [130–132] attributed to the use of high PS concentration and high light fluences [133–135]. Another remarkable side effect is prolonged skin pigmentation. A recent study on *ex vivo* full-thickness porcine skin model has reported damage to the epidermis by thermal effects and other toxic outcomes at higher fluence rates which can lead to ultrastructural changes in epidermis [136]. However, these drawbacks can be avoided by decreasing the amount of light energy delivered to the tissue. There have been a few clinical studies with very small sample size conducted in order to understand such effects. However, controlled clinical studies that include histopathological outcomes need to be conducted in order to establish safe and effective parameters required for this therapy.

Nanobiophotonics is also an emerging field that uses light for molecular diagnostics that involves specific sequence characterization of nucleic acids for gene delivery systems of relevance for therapeutic strategies [137]. Though light can enhance the drug delivery into deeper layers of the tissue by opening microchannels that allows the molecule uptake, further randomized clinical trials are required to ensure standards in optoporation efficacy.

## Temperature Induced Drug and Gene Delivery: Thermoporation and Photothermal therapy

Thermoporation (TP) also alternatively called ‘microporation’ makes use of heat to produce tiny openings in the stratum corneum [138]. Heat is generated by an array of metallic filaments held temporarily against the skin. An activation electric pulse induces heating of the filaments. A vaccine or drug is applied over the created pores via an adhesive patch for treatment. In a study Ivanov et al. studied the structural changes in erythrocyte membrane during thermoporation involved in thermohaemolysis [138]. When intense electric fields ( $1.5\text{kVcm}^{-1}$  for 5s) were applied on cell suspension medium, heat variations were observed. However, corresponding classical thermal treatments at equivalent temperatures had no effect on the viability of cells [139]. It is commonly assumed that electrical conductivity variations of the intra- and extra-cellular matrix caused by ions and solute transfer across the membrane were shown to be involved in the observed heating. Though thermoporation is a less common technique that is used for poration and concentration of drug or gene, the most popular technique that combines both heat and light for activation is called “photothermal therapy” is rapidly growing recently.

## Photothermal Therapy for activation

Photothermal therapy (PTT) is a model with some similarities to PDT. Unlike PDT, where the excited PS releases singlet oxygen or free radicals, PTT takes advantage of the excited PS releasing vibrational energy in the form of heat. PTT uses near infrared radiation (NIR) and many different NP as agents to induce killing of the unwanted cells or tissues. When lasers are used as the activation source several factors need to be considered that for efficacy: 1) tissue absorption coefficient, 2) thermal relaxation time; 3) the sensitization of the tissue; 4) time duration of the laser exposure; and 5) tissue thickness [140]. Photothermal

sensitizers such as metallo derivatives of porphyrins and porphyrinoid compounds, azo dyes and triphenylmethane derivatives are used in this case for PTT [140]. Photothermal damage of tissues may be induced by pulsed irradiation of either endogenous chromophores added or dyes. Dyes have the advantage of significant absorbance at wavelengths longer than 600 nm that can penetrate deep into biological tissues. Spatial confinement of the photothermal process depends on the absorption coefficient of the photoexcited chromophore and its thermal relaxation time. In addition, the use of lasers in PTT has shown promising results for cancer therapy, since the start of the decade. In the study conducted by Chen et al., murine mammary tumors were treated using 808nm laser with indocyanine as the PTT agent [141]. Tumor regression was clearly observed when the treatment involved 3–5min time duration with 5–10W power; however the efficacy of the PTT against cancer was of concern. In a clinical study, feasibility and safety of image guided targeted photothermal therapy for localized prostate cancer was tested. PTT was employed to low risk prostate patients using optical fibres and treated with minimal adverse effects. Successful results from such *in vitro* and *in vivo* studies for cancer application through various agents have helped in moving forward with further exploration towards translational studies [142]. PTT has proved effective against breast tumors [143], Lewis lung carcinoma in mice [144], localized prostate cancer [142], murine glioma model [145] and Ehrlich carcinoma tumors [146] and gene therapy. For instance, Au NP covalently linked to a viral vector was used in gene cancer therapy [147]. When gene transfection takes place using thermal vibrations the phenomenon is known as “thermofection”. PTT killing can be broadly classified according to photosensitizer-induced [148] and hyperthermia-induced [149] based on the type of molecule is encapsulated by the NP.

Advancements in synthesis of NP provide a wide choice of materials for encapsulating PTT drugs which include but not limited to gold nanoshell, gold nanocages, gold nano particles, carbon nanotubes, graphene, copper sulfide (CuS) and Single Wall Carbon Nanotube (SWCNT). The unique optical properties of such NP makes them strong optical absorbers suitable for PTT [150]; The NIR spectral range of 750–1300 nm falls in the optical therapeutic window which allows maximum penetration depth into the biological tissue. Among the NP, gold nanorods are widely preferred for PTT due to their antenna-like structure that has two absorption bands due to the surface plasmon resonance oscillation. These physical properties improve the absorption of the light used to excite the nanorods. Gold- and CNT- conjugates show high selectivity towards cancer cells with destructive effects of laser irradiation. One of the upcoming gold nanostructures is the hollow gold nano cages for anticancer applications. They are usually of 30–50 nm in size and highly tunable to the NIR region. Likewise polyethylene glycol (PEGylated) nanogels (Figure 9) containing gold nanoparticles are of great interest [151]

Another carbon nanomaterial known for NIR absorption is single wall carbon nanotubes SWCNT with low cytotoxicity. For instance, Cobalt Molybdenum Catalyst (CoMoCAT) SWCNT [152] with a narrow absorption peak at 980 nm, were conjugated with folate receptor for tumor targeting. Laser irradiation (fluence 300 J/cm<sup>2</sup>) was directed onto the tumor site 8 days after the injection of folate-SWCNT directly into the center of the tumor

(EMT6 cells) in a Balb/c female mouse. The experiment was reported to produce significant tumor destruction.

Though both *in vivo* and *in vitro* experimental trials show promising potential for PTT, further research is necessary to improve the efficacy of PTT

### **Limitations of Thermoporation and Photothermal therapy**

Excess heat can produce burns (thermal or photothermal damage) on normal cells [153]. Only applied topically and deeper penetration into tissue is a challenge with this technique. Since the heat filaments are connected electrically some of the limitations of using electric field also apply here.

### **Acoustic-Mediated Drug Delivery: Sonoporation (Mechanoporation), Phonophoresis, Sonodynamic therapy**

Acoustic targeted drug delivery (ATDD) has been explored as a promising method to enhance the delivery of therapeutic agents to the target tissues. The underlying mechanism for ATDD is the use of ultrasound energy mediated transport of molecules into the target tissues. Ultrasound is defined as the transmission of sound pressure waves with a frequency greater than 20,000 Hz, the upper limit of human hearing. The technique of using ultrasound for drug/gene delivery is called “sonoporation”. Ultrasound waves can be refracted or reflected similar to light waves. However, in ultrasound waves the actual movement of molecules with compression or expansion of medium takes place. Thus, ultrasound waves being very physical in nature they can dynamically interact with biomolecules or cells. Another unique property of ultrasound waves that there is relatively little absorption of these waves by body fluids and tissues. Therefore, ultrasound can be used for non-invasive, painless transmission of energy at specific body locations, which is the foundation for ATDD. Thus, ultrasound enhances drug delivery by various mechanisms including induction of tissue hyperthermia [154, 155] and the recently discovered remarkable ability of these waves to produce cavitation activity [156–159]. The exposure of a medium to ultrasound causes formation and/or activity of gas-filled microbubbles called cavitation bubbles [160]. The use of ultrasound as an enhancer is referred to as phonophoresis or sonophoresis [44, 161]. “Sonoporation” can be defined as a “physical” method, in which an ultrasound is applied, in the presence of microbubbles that acts as cavitation nuclei, to porate the plasma membrane in order to allow direct transfer of molecules into the cytoplasm [162] of the cell. Sonoporation, involves ultrasound contrast agents (UCA) [42], to permit the transfer of drug and genes into cells. However, it is a transient and reversible phenomenon that ensures relatively good cell viability. A major challenge to the application of sonoporation in case of gene delivery is to optimize the ultrasound parameters both to obtain high gene transfection and to maintain good cell viability [163]. Another requirement is to avoid premature release of the cargo after ultrasound activation (dose-dumping) to produce predictable controlled release kinetics.

Cavitation bubbles expand and shrink in response to low-and high-pressure portion of the ultrasound wave. If the resultant oscillation in the bubble size is stable (repeatable over



many cycles), it is called 'stable' or 'non-inertial' cavitation. A circulating fluid flow (microstreaming) [164, 165] is created by such oscillations around the bubble with velocities and shear rates proportional to the oscillation amplitude. The associated shearing forces generated at high amplitudes have the capacity to shear open synthetic vesicles such as liposomes [164]. Therefore, ultrasound-mediated delivery of drugs and genes is a complex mechanism with interplay among target tissue, therapeutic agent, nature of ultrasound energy, and characteristics of microbubbles.

While using ultrasound, the microbubble formation reduces the peak negative pressure required for enhanced drug delivery by acting as nuclei for cavitation, thereby decrease the threshold of ultrasound energy required for this phenomenon. The transport of drug is significantly enhanced over diffusion alone by the presence of these oscillating bubbles. Stable oscillating bubbles create convection by evolution of circulating eddies around the oscillating bubble (microstreaming) and produce a force acting on other suspended particles in the vicinity of an oscillating bubble (acoustic pressure). The particle is pushed towards the bubble if it is denser than the surrounding liquid and vice versa [166]. Most of the drug carriers, such as micelles and liposomes, being denser than water are convected towards the bubble, and thus augment the dispersive transport of the drug carrier. The dispersive transport of the drug is further increased if the vesicle is drawn into the microstreaming field around the bubble and can be ruptured by high shear rate, thus releasing the drug. Another key mechanism contributing to ultrasound enhanced drug or gene delivery is related to the stress inflicted by cavitation events on tissue and cells. The ultrasound-induced microbubble cavitation induces pore formation in the cell membranes, aiding the drug/gene deposition. In a recent study Deng et al. [167] demonstrated ultrasound-induced increased transmembrane current as a direct result of membrane resistance due to pore formation. Microbubbles have another unique characteristic of increased adherence to the damaged vascular endothelium. This characteristic enable selectively concentrated delivery of drug or genes bound to albumin-coated microbubbles at the vascular injury site [168].

One of the most promising clinical applications of sonoporation technologies is efficient delivery of genetic material, such as plasmids and antisense oligonucleotides, for gene therapy. The process of gene transfection using ultrasound is known as "sonofection". The delivery of genetic material to the target tissue is tricky because it has to be tissue specific and at same time it has to be protected from degradation by DNase. Since the ultrasound beam can be focused on a particular tissue, ultrasound-mediated gene delivery holds tremendous potential. The first use of surface ultrasound in targeted DNA delivery was done in 1996, using intravenously delivered microbubbles carrying antisense oligonucleotide [169]. Since then, many studies, both in vitro and in vivo, have described the efficient use of ultrasound-mediated microbubble destruction by cavitation and subsequent cargo release for gene delivery [156, 170–177].

Another potential application of this technology is ultrasound-assisted delivery of large protein molecules, such as regulatory hormones. The delivery of large protein molecules is very complex compared to small molecular weight drugs because proteins are poorly diffusible through solids and gels. Therefore, the lipid bilayer of cell membrane prevents the transport of protein. Current research is primarily focused on transdermal delivery of insulin

for diabetes and some efforts for birth control hormones. Recent studies have reported increased permeability of the skin by ultrasound with collapse cavitation as a causative mechanism [178–180]. The ultrasound-induced cavitation events cause opening of reversible channels in the lipid bilayer of stratum corneum and thus easing the transport of proteins into the skin [181, 182]. The ultrasound-induced delivery has been used for other protein, such as thrombolytic enzymes [183–185] and ultrasonic nebulization by the pulmonary route [186–188]. Use of ultrasound for the potentiation of chemotherapeutic agent delivery has been recently explored. Studies have suggested that ultrasound has synergistic effects with drugs [159, 189, 190] due to increased delivery of the drugs to the tissues. Further advancement has been made in ultrasound-induced chemotherapeutic agent delivery by developing molecular vehicles that can deliver the drug locally using ultrasound stimulus at tumor site and thus reducing systemic side effects. Two such carrier molecules for delivery of chemotherapeutic agents are liposomes [191–193] and micelles [194, 195].

Use of ultrasound-assisted targeted drug delivery technology is ever-increasing in clinical medicine and research due to its use in delivery of various therapeutic agents including genetic materials, chemotherapy drugs, and proteins. In future, this technique is believed to further advance with improved efficacy and reduced side effects.

### Sonodynamic Therapy

Sonodynamic Therapy (SDT) uses ultrasound and PDT agents in synchronization for activation. SDT is a non-invasive and repeatable approach using low-intensity ultrasound with a sonosensitizer for cancer therapy [196]. For instance, PPIX produced from 5-aminolevulinic acid (5-ALA) is reported to be useful with low-intensity but focused ultrasound in deep-seated glioma models [197]. Also, 5-ALA-induced PPIX fluorescence has been used in malignant glioma in order to render more complete resection in surgical operations [198]. Further, various novel PS have been used for SDT such as a homogeneous complex of oligomers of hematoporphyrin, called photofrin II, a gallium porphyrin complex, ATX-70, a hydrophilic chlorin derivative, A7X-S10, and a novel porphyrin derivative devoid of photosensitivity and DCPH-P-Na (I) [199]. The mechanisms of action of SDT are still under investigation but are believed to be based on either the action of PS nanoaggregates as nuclei for formation of cavitation microbubbles that mechanically disrupt the cells, or on the emission of small “flashes” of light caused by collapse of cavitation microbubbles that are absorbed by the PS producing singlet oxygen that kills the cells as in PDT.

The applications and properties of various resonances from magnetic, fluorescence, nuclear and surface plasmon plays a significant role in the enhancement and activation of SDT. [200]. Recently, a triple-functional core-shell structured upconversion luminescent nanoparticles, NaGdF(4):Yb,Er@CaF(2)@SiO(2)-PS (hematoporphyrin and silicon phthalocyanine dihydroxide) which possess a multifunctionality in the fields of PDT, MRI and fluorescence/luminescence imaging was developed to investigate the effect of PDT in vitro in HeLa cells using a fluorescent probe. It has low dark toxicity and could be a promising modality for PDT [201]. Also, another study showed caspase-3 activation in single cells with FRET that induces apoptosis through PDT [202] and complexes like

Fe<sub>3</sub>O<sub>4</sub>/SiO<sub>2</sub> core/shell NP functionalized by phosphorescent iridium have been designed and synthesized that makes it well-suited for phosphorescent labeling and simultaneous singlet oxygen generation to induce apoptosis through PDT [203]. Thus, the effective use of various resonating techniques could provide a promising methodology for enhancing SDT.

SDT opens a new arena of medical-activated sensitizer therapy [204]. SDT makes use of sonosensitizers that are similar to photosensitizers but sensitive to ultrasound instead of light. Recently, Dai et al. applied the use of sonosensitizers in combination with liposomes to target chemotherapy drugs directly to tumor cells. SDT with low-intensity ultrasound combined with a sonosensitizer proved to be a promising approach to cancer therapy [205]. The effects of combined sonodynamic and photodynamic therapies with photolon on a glioma C6 tumor model were studied and found that the maximal tumor necrosis area that underwent sonication followed by PDT at a dose of 100 J/cm<sup>2</sup> was 100% in rats [206].

### Limitations

Being a very active process, the consequences of ultrasound-mediated targeted drug delivery could be detrimental for the tissues [160, 166, 207]. The ultrasonic cavitation bubble formation, a requisite for helping the transport of drugs into the cells and promoting the physical or chemical release of drugs from the carrier, is clearly not a healthy environment for the cells. A certain intensity of cavitational activity is essential for effective delivery of the drug. Thus, intensity of this cavitational activity should be at a level sufficient enough to permeabilize the cells without destroying them or a level that creates adequate microstreaming to break open drug carriers, such as liposomes, without damaging the host cells. However, these shear forces and liquid micro-jets could potentially damage the cell membrane. The essential cellular biochemical processes could be inhibited by free radical formation. On the other hand, microbubbles with stable cavitation can potentially be beneficial for the cells by increasing the convection of nutrients and oxygen [208]. Therefore, there is an urgent need to further understand ultrasound-microbubbles interaction and cavitation physics. Another pressing need is to develop better protected genetic material that is not degraded by the action of enzymes or shear forces before it gets delivered to the target cells. To bring ultrasound-assisted transdermal protein delivery to the clinic, further studies are required to conclusively understand the effects of ultrasound on protein conformation and reveal the underlying mechanism of enhanced transdermal protein delivery.

Moreover acoustic cavitation increases cell permeability and facilitates molecular internalization; however, it may also damage or even kill the cells if the pores induced by cavitation are too large such that the cell membrane cannot reseal quickly [162]. The problem is magnified for the effective delivery of large molecules, such as plasmid DNA, which requires that sufficiently large pores are created on the cell surface, but which risks that the disrupted cell membrane will lose vital cytoplasmic compounds, resulting in cell death. Moreover, it appears that the cells return almost immediately to an impermeable state after ultrasound exposure (Figure 10), suggesting that sonoporation is a transient phenomenon, with pore opening lasting on the order of milliseconds to seconds.

## Theranostics

Drug standardization protocols and the prevailing concept of universal or generalized medicine have caused a lot of problems in the past for the drug companies as well as consumers. Research and common-sense ideas raise a lot of questions on the credibility of the current medical system as a whole. Questions like “why use common medicines for everyone when individual genotypes are different?” are raised. Scientists and physicians are increasingly orienting their research towards the use of personalized medicine. Theranostics aims to develop such an idea of personalized medicine intended for a specific individual’s illness by understanding the cellular phenotype(s) and heterogeneity of the target [209, 210] in the individual’s body and designing an individualized treatment. Coined by Funkhouser [211], theranostics describes a multimodal functionalized system that functions at the molecular level [212] as shown in Figure 11. Any of the physical methods described above, can be used for porating and concentrating the drugs in therapeutic strategies such as drug, gene, nucleic acid delivery and chemotherapy and activated by hyperthermia/photothermal ablation or PDT which are combined with one or more imaging functionalities for both in vitro and in vivo studies.

For instance, recently, PDT has been used as a theranostics modality for various cancers and diseases [213]. With the use of highly tumor-specific albumin NP as efficient therapeutic agents and photodynamic imaging (PDI) reagents in cancer treatment, hydrophobic photosensitizer chlorin(e6) (Ce6) was first chemically conjugated to human serum albumin (HSA)[214]. Next, upon illumination with the appropriate wavelength of light the conjugate produced singlet oxygen and damaged target tumor cells in a cell culture system.

The progress of novel theranostic approaches based on carbon nanostructure is attractive in the field of nanomedicine. The inherent optical properties such as fluorescence make carbon nanostructures useful contrast agents in optical imaging and sensing [215, 216]. Functionalization of SWCNT with different moieties enables targeting and imaging therapy. One of the most interesting examples of such multimodal SWCNT constructs is carrying a fluorescein probe together with the antifungal drug amphotericin B or fluorescein and the antitumor agent methotrexate [217]. Fullerenes have shown potential applications in several different cancer therapeutic approaches such as PDT, photothermal treatment, radiotherapy and chemotherapy which can also serve as novel contrast agents in MRI [218]. The authors performed extensive research on PDT and emphasized on the application of fullerenes in various studies [219–221]. Similarly, graphene monolayers have also aroused widespread interest in the applications of biomedicine [222]. Unique physical and surface features of carbon-based nanomaterials make them a key player in the field of nanomedicine. Besides all its wide application in theranostics, still more research needs to be carried out on these materials to explore the interaction of such multifunctional and combinational therapy in both in vitro and in vivo applications.

## Conclusions

The use of physical energy methods for enhancing drug and gene delivery is a good example of the type of multi-disciplinary collaborations that have become increasingly common in

the modern era of personalized medicine and nanomedicine. Many of the interactions between physical energy and cells or tissues take place on the nano-scale, and need to be studied and optimized using nanotechnology techniques. A common unifying motif in many (if not most) of these applications is the creation of temporary pores or channels in the plasma membranes of cells or in the stratum corneum of skin. The desired therapeutic molecules (drugs or DNA) pass through these pores or channels, sometimes driven by gradients produced by the applied energy differential. These pores and channels should be self-sealing, in other words they should stay open long enough to allow the therapeutic cargo to pass through, but not stay open long enough to cause cell death or tissue injury. Many of the nanocarriers used for drug and gene delivery are considerably more efficient if they can be disrupted at their desired site of action thus releasing their drug cargo in a predictable controlled manner, rather than relying on the natural release mechanisms that may increase non-targeted drug distribution. Since many physical energy modalities can be spatially focused onto the tumor or other target tissue, they have been extensively used for drug release or drug activation. Magnetic and thermal energy can be further used to concentrate systemically administered drugs or drug carriers in the desired anatomical area by external application of the energy. Potential clinical applications of these technologies will however be complicated by their multidisciplinary nature. One other limitation is that unless and until location of the affected region is known with a high spatial resolution the use of physical energy may not be as applicable. By definition these technologies will be drug-device combinations which historically have been much harder to progress to regulatory approval than drugs or devices alone. With the introduction of nanocarriers into the picture, the path to regulatory approval becomes even more complicated. Nevertheless the relentless growth in biomedical research based on nanomedicine will undoubtedly continue and physical energy modalities are expected to be increasingly involved in these efforts.

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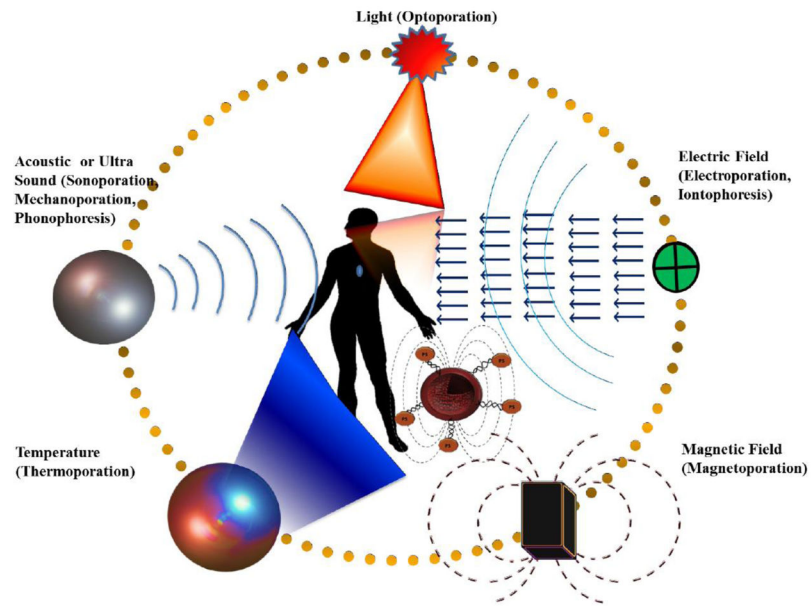
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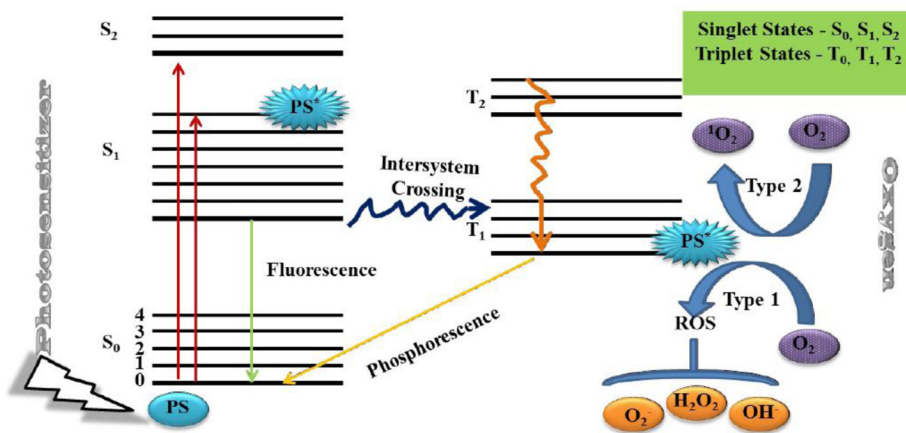
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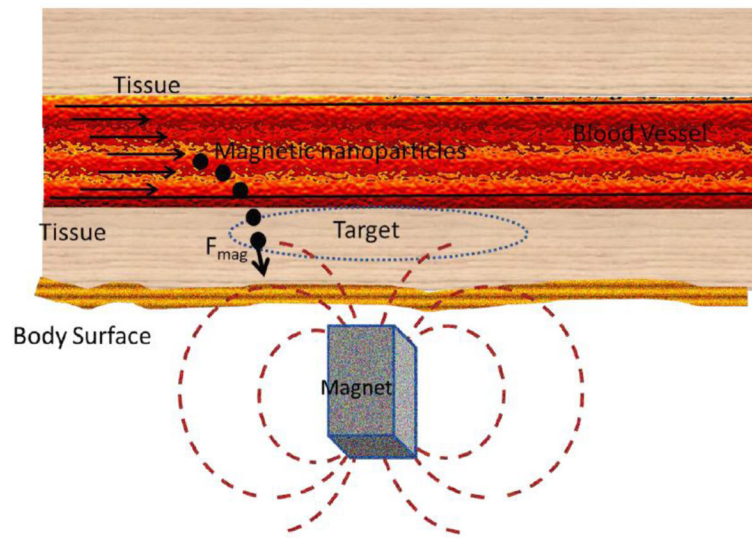
**Figure 1. Different physical energy modules used for drug delivery**  
Drug delivery enhancers such as electroporation, magnetoporation, thermoporation, sonoporation and optoporation are illustrated.



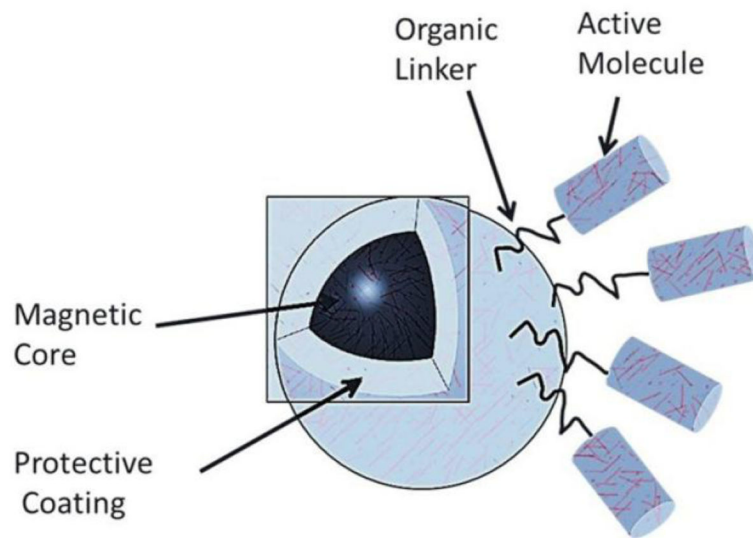


**Figure 2. Jablonski Diagram**

In the presence of molecular ground (triplet) state oxygen (<sup>3</sup>O<sub>2</sub>), the excited state PS transfers energy or electrons to produce reactive oxygen species (ROS).

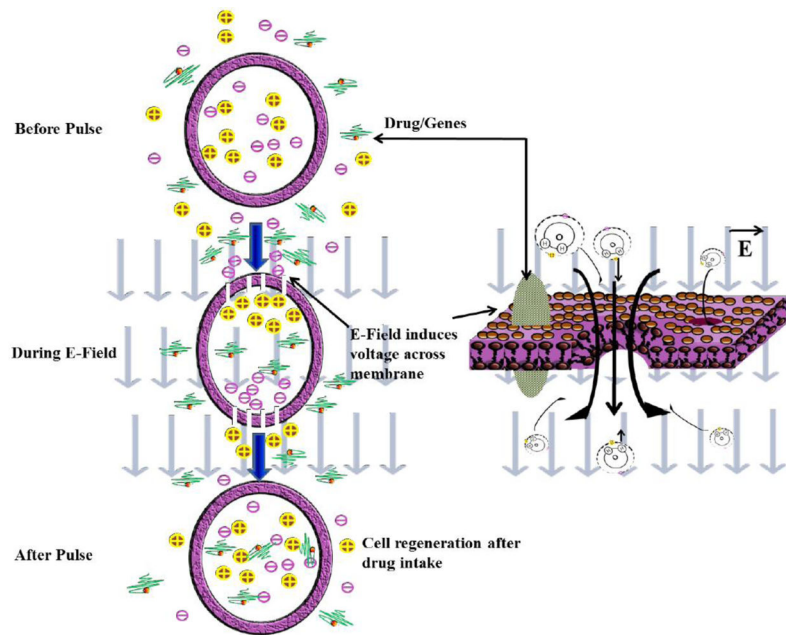


**Figure 3. Schematic representation of a magnetic nanoparticle-based drug delivery system**  
Steps for magnetic nanoparticle-based drug delivery are; coupling the drug to the magnetic NP (MNP), applying an external magnetic field to attract the MNP to the desired location such as a tumor, and the release of the drugs from the NP under the influence of external alternating magnetic field once it reaches the target.



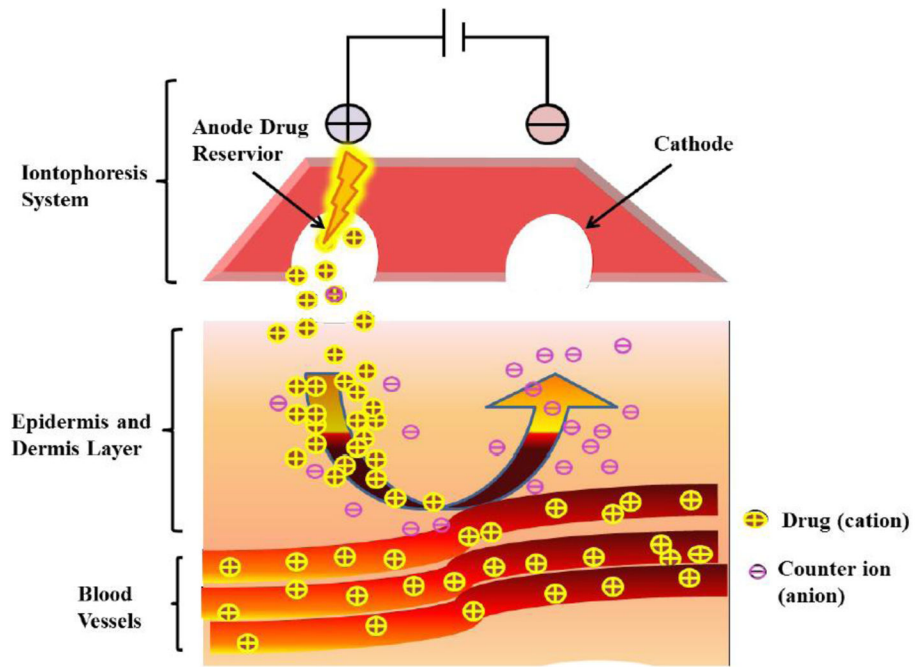
**Figure 4. A typical magnetic nanoparticle**

Magnetic nanoparticle consists of a magnetic core, a protective coating, an organic linker and an active molecule



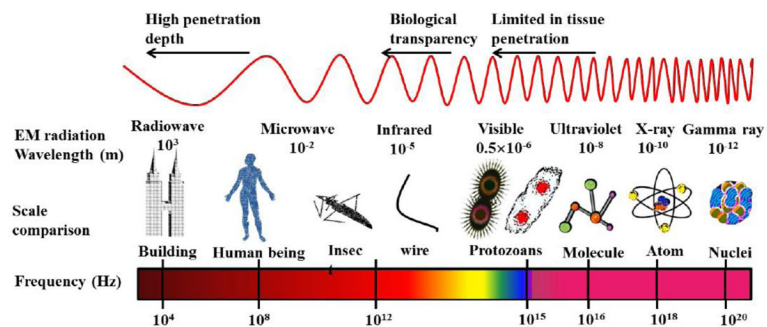
**Figure 5. Electroporation phenomenon**

Electric field disrupts cell membrane allowing drug/genes poration. The status of drug/genes before pulse, during the electric field (E-field) and after pulse is shown.



**Figure 6. Iontophoresis method**

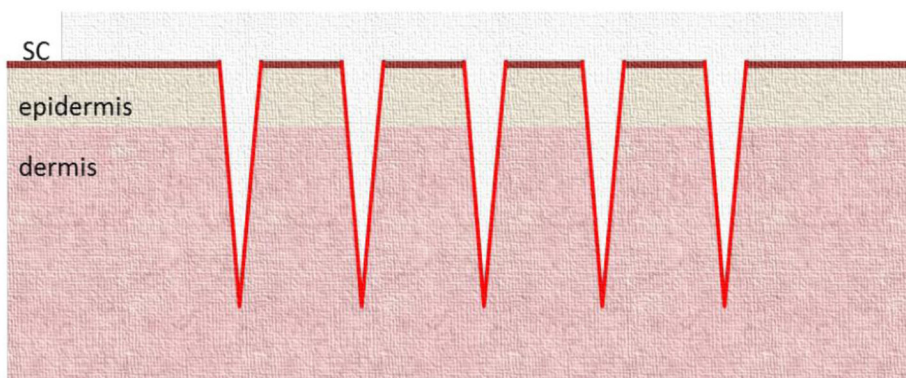
The iontophoresis unit carries an anode drug reservoir that holds and releases the drug and a cathode that collects the opposite ions when a potential is applied across the two polarities



**Figure 7. Light penetration as a function of wavelength**

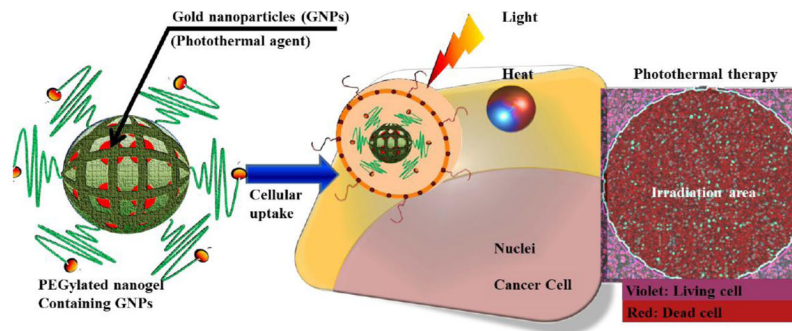
There exists a ‘window of transparency’ for human tissues in the far-red spectrum light is still limited in its penetration depth, or how far in can penetrate into the human body.





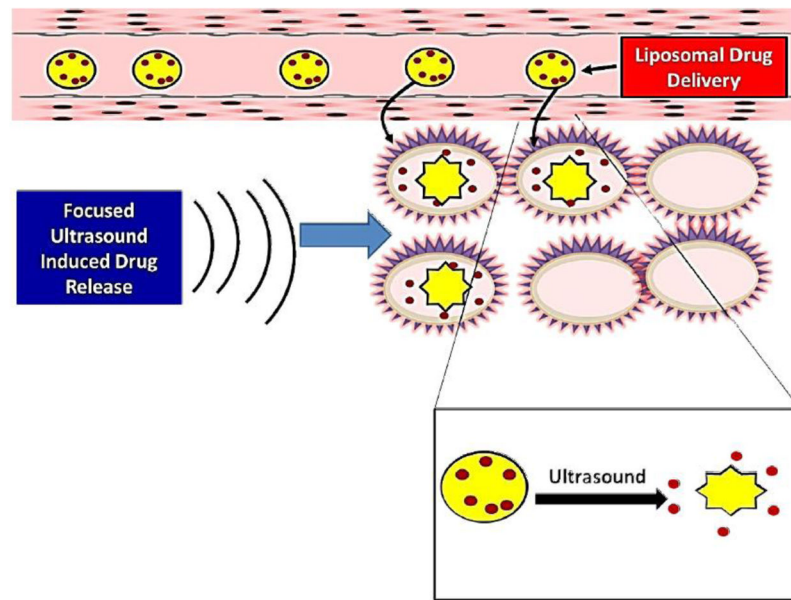
**Figure 8. Optoporation**

Schematic representation of micro-channels created by ablative fractional lasers in skin. The light beam disrupts the major skin barrier, the stratum corneum (SC), and reaches deeper layers (dermis).

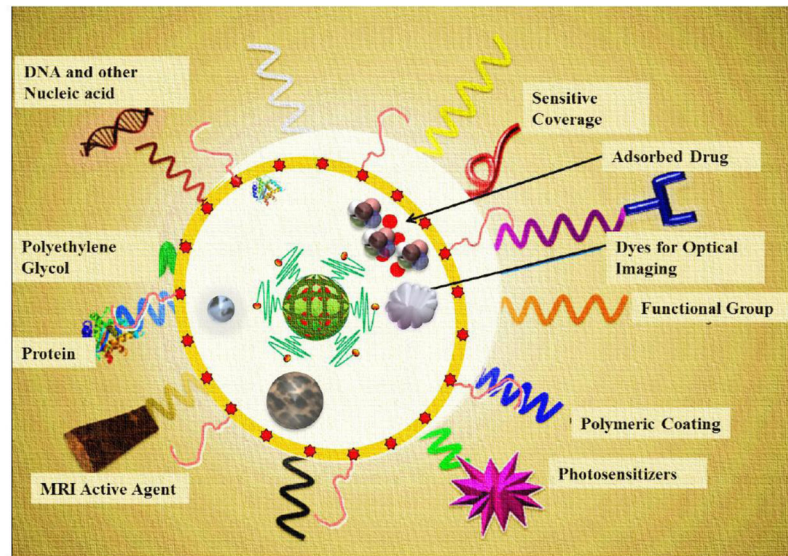


**Figure 9. Photothermal therapy**

PEGylated nanogels containing gold nanoparticles are uptaken by the cells. Irradiation (both heat and light) kills the cancer cells.

**Figure 10. Sonoporation**

Liposome bound drug is delivered to desired site. Ultrasound-sensitive liposomes burst open upon exposure to ultrasound leading to effective local delivery of the drug.



**Figure 11. Nanoplatforms with theranostic functionalities**

Fe<sub>2</sub>O<sub>3</sub>, magnetic NP, photosensitizer, appropriate polymeric coating, surface-charge tuning groups, optical imaging dyes, targeting agents, adsorbed/bonded drugs, sensitive coverage, DNA and other nucleic acids, polyethylene glycol, proteins and MRI active agents.

Table 1

Poration		Electroporation	Magnetoporation	Thermoporation	Sonoporation	Optoporation
Attributes						
Physical energy		Electric Field	Magnetic Field	Temperature	Ultrasound	Light
<b>Limitations</b>		<p>1 Narrow range of clinically safe electric field parameter (refer to current standards for safety levels)</p>	<p>1 Limited drug carrying capacity of MNP due to their biodistribution</p> <p>2 Narrow range of magnetic field[223]</p>	<p>Low Penetration depth,(since only applied topically so far)</p>	<p>1 Sonoporation devices have poor calibration in terms of the amount of ultrasound energy emitted[224]</p>	<p>1 Limited time duration between optoporation and drug delivery[225]</p>
<b>Disadvantages</b>		<p>1 Irreversible electroporation, Cell death with high fields. [226, 227]</p> <p>2 Electro-mechanical coupling effect</p> <p>3 Electrodes are in contact with the subject that may result in electrocution</p>	<p>1 Aggregation of MNP can cause embolization[228]</p> <p>2 Cytotoxicity at increased concentrations of MNP [229].</p>	<p>1 Excess heat can induce thermohaemolysis. [230]</p> <p>2 Relies on electric field to heat up the filaments, therefore the disadvantages of electric field use applies</p>	<p>1 Shear forces may induce rupture of cells.[231]</p> <p>2 Temperature increase as a function of frequency eventually rupturing cells[232]</p>	<p>1 Excessive inflammation and risk worsening (postinflammatory hyperpigmentation) [233]</p> <p>2 Laser usage and ultrastructural changes in epidermis.</p>
<b>Advantages</b>		<p>1 Inexpensive and simple to perform.</p> <p>2 Drugs overcome the cell membrane barrier field[234]</p>	<p>1 Non-invasive nature of the magnetic field [235].</p> <p>2 Field modulated externally without electrode contacts unlike EP [235].</p> <p>3 Efficiency of drug delivery is high compared to one without field</p>	<p>1 Non-invasive nature of low heat compared to EP</p> <p>3 Selective irreparable cellular damage</p>	<p>1 Less invasive compared to EP [236]</p> <p>2 Instant impermeabilization after ultrasound exposure[231]</p>	<p>1 Remote operation[237], less cellular damage</p> <p>2 Enhanced optofection efficiency compared to regular gene delivery[238]</p> <p>3 Deep penetration; key nanosurgical tool to the microscopist[239]</p>