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An insight on electrospun nanofibers inspired modern drug delivery system in the treatment of dreadful cancers

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In spite of ample researches and admirable achievements, still there is a reasonable amount of deaths happening every year due to cancer. Further, the number of new cases recorded are also not considerably reduced despite the advent of various preventive measures. Though current clinical approaches yield commendable results, it elicits dreadful systemic side-effects and also fails to avoid the recurrence of the disease. To address these issues, nanotechnology empowered modern drug delivery systems express fruitful properties for targeting and controlled delivery of biomolecules over a period of time. In the past decade, material based cancer research field has witnessed the exploration of several captivating drug delivery approaches for administration synthetic drug to genetic materials. Among those, the electrospinning based nanofibrous mesh has attracted several works on treating common dreadful cancers like lung, breast and colon respectively. The capability of nanofibers to enable increased drug loading, maintaining significant bioactivity, excellent drug encapsulation, controlled and targeted delivery has helped the researchers to achieve the successful administration of a variety of anti-cancer agents. This review gives an insight about the process of electrospinning, its essential parameters, types of drug incorporation and the works reported on common dreadful cancers. Moreover, the future direction of this effective alternative is also delineated, making electrospun nanofibers as a suitable vehicle for delivering drugs to the cancer sites.

1. Introduction

The concept of drug delivery system was formulated in the early 1970s and the first reliable system demonstrated was polymer (lactic acid) based. Even though it was introduced decades ago, extensive researches have been reported in the last five to ten years especially after the popularization of nanotechnology. Modern drug delivery systems are exploiting to carry drugs, growth factors, genes and biomolecules for treating cancer, promoting tissue regeneration and curing several clinical ailments [1]. However, the notion was solely intended to address cancer, a major health problem in all parts of the world. Typically, the functions and process of cells are controlled by biomolecules synthesized with the help of various DNA and RNA, the ultimate workhorses. When these prime-molecules undergo mutation it will generate faulty substances which in turn make the cells behave and proliferate anomalously, resulting in the development of cancerous tissue or neoplasm. By mimicking like normal cells, it escapes the attack of the immune system, starts invading neighbouring tissues and eventually spreads throughout the body. Factors responsible of this deadly mutation remain diversified, but it mostly includes occupational hazards, environmental pollution, genetic disorders, microbial attacks and deprived life style. There are over 200 types of cancer, which can befall at 60 different organs, insinuating that cancer can develop virtually in any part of our body. Unfortunately, irrespective of the age and sex, people are getting affected by cancer, though older population has higher probabilities [2, 3].

Recent statistics delineates that a total of 1,658,370 new cases and 589,430 deaths will be reported due to cancer by the end of 2015 in the United States alone. If it continues in this fashion, global deaths because of cancer will rise over 11 million annually within 2030 [4, 5]. Besides booming mortality, the economic loss owing to cancer is also increasing drastically. According to the American cancer society, the economic loss caused due to cancer is high among all types of non-communicable diseases, accounting for about 895 billion US dollars (excluding direct medical cost) globally in 2008 and it is expected to rise above $1500 billion within 2020 [6]. Among all types, the lung, breast and colorectal cancers are entitled as more deadly since they account for more than 40% of total cancer deaths. Further, the chances of developing metastasis is also relatively high in above three cases, whose targets include various important organs like bone, brain, liver,

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adrenal gland, etc., [7, 8]. Hence, in this review we have concentrated only on lung, breast and colon cancers.

But, an ideal therapeutic window to address this serious issue, particularly when it diagnosed in later stages, is still under research. Because of its fitful pattern, the method of treatment is generally chosen by physicians based on type, location, the extent of spread, patient age, health status, etc. Commonly, the selection will be from any one or combination of the following approaches like surgery, chemotherapy, radiation, hormone therapy, bone-marrow transplantation and immunotherapy. The main motive of these methods will be halting cancerous proliferation, spreading, sacking symptoms and promoting apoptosis or self-destruction. However, most of the therapeutic options currently available for clinical usage are hammered by series of systemic side-effects and recurrent problems. In general, treatments like chemotherapy will target rapidly growing cells (tumour cells) but it also destroys some normal rapidly growing cells in that process. This is mainly because of undirected or unspecified administration. Hence, during chemotherapy, the patient will experience abnormalities like heavy hair loss, unbearable body pain, accumulation of fluid, drastic changes in body weight, damages to kidney, nausea and metabolic imbalances. Meanwhile, options like surgery fail to avoid the recurrence of the disease, especially when it already spreads to nearby tissues [9]. Therefore, in most cases the drawbacks associated with available treatments have reported worsening the condition of the victim rather than curing.

Whilst, the modern drug delivery system serve as a compelling option to eliminate most of the complications exist in current clinical approaches for cancer. It comprises, a carrier for drugs, targeting agents and shielding materials which offer excellent drug loading efficiency, targeted delivery, controlled release and also high bioavailability. Ample researches have explored novel delivery systems, exclusively those inspired by nanotechnology and biodegradable polymers are of immense interest [10, 11]. Nanotechnology, popularly called as “Technology of future world”, has significantly influenced and updated several fields of research. As for the pharmaceutical area, scientists have already demonstrated its skills in controlling pharmacokinetic profiles of drug delivery systems and the possibility of intracellular delivery. Nanotechnology along with biodegradable polymers has given rise to smart or intelligent drug delivery systems which safely release the drug, only at tumours population and can maintain bioavailability for a long period. Moreover, with this sensible system, we can also reduce the drug quantity needed to achieve the appropriate therapeutic effect, treatment cost, can attain anticipated mode of delivery as well as sparing healthy cells [12]. Therefore by the end of 2015, total market value of nanotechnology-based drug delivery systems are expected to reach $220 billion, which is 37% more when compared to 2012 statistics. And within 2021, this sector is expected to cover more than 15% of nanotechnology market globally [13, 14].

Nanoparticles, micelles, liposomes, micro/nanofibers, nanotubes, microspheres, hydrogels, dendrimers, quantum dots [15, 16] are some of the popular delivery vehicles which can be administrated through oral, transdermal, topical, nasal, transvenous or implantable methods. With these systems, scientists have successfully reported safe delivery of spectrum of natural and synthetic biomolecules such as proteins [17], antibodies [18], antioxidants, polyphenols, anticancer drugs [19, 20], etc. In addition, recent researches have also reported the delivery of several genetic materials like RNA [21], miRNA, siRNA [22], DNA [23] to address cancer at the molecular level which is one of the future perspectives of modern drug delivery approaches. Moreover, advanced technologies available nowadays has simplified the synthesis of these intelligent structures and loading of desired substances. More interestingly, each structure has been illustrated to possess unique features. For instance, liposomes can carry both hydrophilic and hydrophobic drugs concurrently, dendrimers can hold multiple drugs whereas some system can invade cancer cells itself and deliver the drugs intracellularly [15, 25, 26].

However, all types of the modern system mostly exploit any one of the methods, namely active or passive targeting to reach and deliver drugs at the pre-determined site. Generally, the carcinogenic tissues are characterized by high vascular density and active angiogenesis to support its rapid proliferation and spreading, but with poor lymphatic drainage. It develops a condition called enhanced permeation and retention (EPR). In passive targeting, the delivery system reaches the tumour site by tracking this leaky vascular structure and accumulate drugs. In contrast, the active targeting system uses specific ligands attached at the surface to recognize pathological cells [27]. Though the modern drug delivery approach sounds like an ideal option, the complicated tumour microenvironment, presence of P-glycoprotein barriers, enzymolysis, endosomal or lysosomal degradation, hepatic and renal clearance remains as great challenges for researchers to achieve reliable *in vivo* outcomes [28, 29].

Nevertheless, drug delivery systems like liposomes, nanoparticles, micelles, microspheres etc., exhibits several advantages, most of them are nailed with problems of an instant burst of drugs, poor loading efficiency, renal clearance and fails to remain or maintain preferred drug concentration for long periods [30, 31]. For instance, in case of colon cancer treatment, the projected delivery system is supposed to remain constricted for a long period, but the problem of prompt burst may undesirably damage normal tissues in the gastrointestinal track before reaching the target. Furthermore, this undesired burst also disables the ability of the intended delivery system to accomplish the sustained release. Similarly, while treating solid tumours it is vital to maintain commendable drug concentration at the appropriate site. But, scientists successfully rectified above problems by utilizing the unique properties of non-woven electrospun micro/nanofibers. Further, because of its high surface area,

ability to enclose a variety of biomolecules, significant loading capacity, and excellent encapsulation efficiency researchers are also able to establish targeted-extensive drug release and achieved multiple therapeutic context as well [31]. This effective alternative can offer intensive protection to conjugated biomolecules meanwhile it also facilitates oral, transdermal and direct implantation of the drug delivery system at the tumour site itself. Despite, it can also be cut into required dimensions, which makes them as a reliable choice for several clinical applications.

Inspired by its capability, in this review the potential of electrospun micro/nanofibers, one of the most captivating and raising modern drug delivery system in fighting top three deadliest cancers (i.e) lung, breast and colon cancers as mentioned previously are summarised [4]. In the interim, *in vitro* and *in vivo* works conducted utilizing several natural and synthetic biomolecules along with the capability of electrospun nanofibers to safely deliver these active substances at the tumour site is discussed in detail. Moreover a brief overview about electrospinning process, essential parameters involved and techniques available to couple drug molecules into electrospun fibers is also presented.

2. Electrospun nanofibers

The technique of electrostatic spinning (electrospinning) is not a new notion, whose utility in textile industries can be tracked back to the 19th century. It uses high voltage to convert the liquid polymer solution into solid fibers of diameter ranging from micrometres down to tens of nanometres. A simple electrospinning unit comprises of a syringe provided with control pump, power supply and a grounded target to collect the fibers. Initially, the polymer solution loaded into desired spinneret will be subjected to high voltage, which makes the pendant droplet elongate due to repulsive forces between like charges in solution and attractive force exerted by the collector. Increasing the electric field at this point, will enable the electrostatic force to overcome the surface tension of the solution which forms Taylor cone. If the voltage increases beyond this point, it will lead to the formation of solid fibers followed by the evaporation of solvents before it reaches the grounded collector, as shown in figure 1. Through electrospinning technique, we can synthesize natural, synthetic, biodegradable or non-degradable fibrous mats or tubes and it also afford control over the geometry of fibers generated [31, 32]. But, the efficacy of this electrospun fibers is greatly influenced by the processing parameters, governing variables and the type of spinneret used. Hence, it is essential to know about these vital parameters to design a reliable drug delivery system with required fiber diameter, porosity, orientation, bulk properties etc.

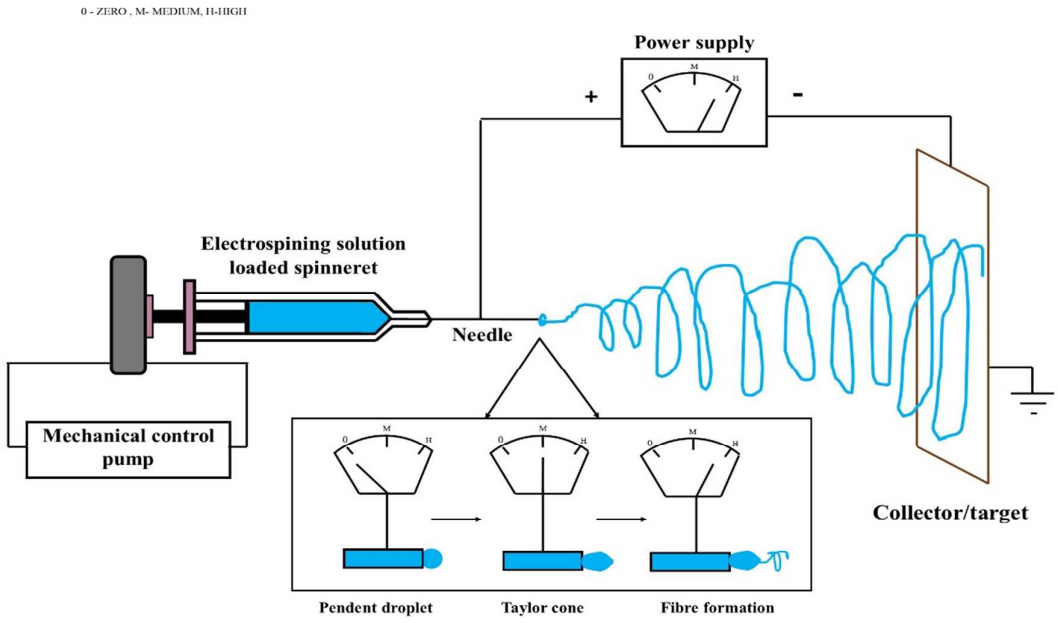

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Figure 1: Systematic representation of Electrospinning process

2.1 Essential parameters and its influence on nanofibers geometry

In order to produce fibers with better pharmacokinetic properties, notable care should be taken to study the parameters involved in electrospinning process. According to extensive researches and reports published, issues like solution concentration, viscosity, conductivity, solvent volatility, applied voltage, flow rate, environmental factors, collector shape and capillary distance are inferred to play a vital role in determining nanofibers geometry (figure 2). Depending on its range, nanofiber properties such as diameter, porosity, mechanical strength, structure, surface area, etc., are observed to differ significantly [33]. In general, most of the parameters are interconnected and its optimal range varies for different polymers. First and foremost, the concentration and viscosity of polymer solution determines spinnability of the preferred biomolecules (i.e) possibility of synthesizing fibers or not. To obtain beads less, smooth fibers with the minimum diameter and commendable mechanical strength, the concentration of the electrospun solution has to be low. But if the concentration and viscosity is too low it inferred to form broken fibers with poor structure and beads. In the interim, at a super high concentration it is difficult to form fibers and control the processing parameters [34, 35].

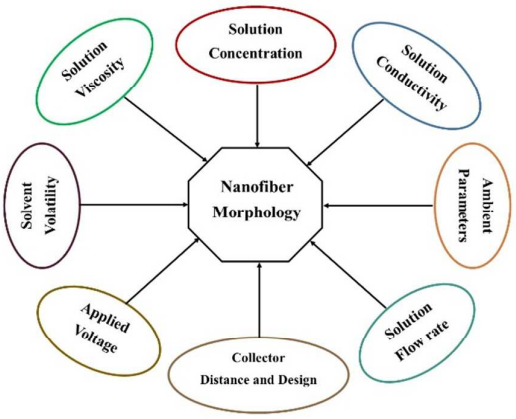
It is well known that electrospinning technique is largely depending on the achievement of electrostatic interactions; hence, the loaded solution should possess high conductivity to make the most of applied electric field. Solution with high charge carrying capacity will subject the fiber jet to greater tensile force, which yields non-beaded fibers with decreased diameter in contrast to resistive solutions [36]. The solvent volatility is one of the vital factors, especially when the fibers are proposed for drug delivery applications. In general, for preparing electrospinning solution a broad variety of solvents like chloroform, Teflon, water, etc., are used. However, the used solvent is expected to disappear during the travel of solid fibers to the collector. This will ensure bio-integrity of the nanofibers, furthermore high volatile nature of the solvent is greatly appreciated to produce a porous mesh with increased surface area as well. On the other hand, a solvent with poor volatility is reported to form nanofibers with increased pore size, which may affect the shielding of coupled active molecules from the attack of biological environment [37]. To add, the ambient factors like temperature and humidity is also noted to play a significant role in determining the fiber diameter and pore size respectively [38]. The polymers like poly polystyrene (PS), polycarbonate (PC) and poly-(methyl methacrylate) (PMMA) were found to attain interesting sub-micron features when it was electrospun in the presence of humid atmosphere [33, 37, 39]. For instance, the electrospinning of PS under the atmospheric humidity less than 25% is inferred to yield smooth defectless fibers. However, to gain the pore formation the humidity range of 30% is found to be ideal and further increase leads to increase in pore numbers and diameter respectively. On the other hand, at extreme humidity range it was difficult to obtain continuous fiber because it affects the rhythm of spinning unit [40]. Accordingly, the temperature range may also influence fiber morphology as beadless fibers with decreased diameter is reported to produce at relatively high temperatures. It is mainly due to lower viscosity of polymer solution with same concentration at high temperature than in room temperature [41]. Meanwhile, elevated temperature levels also increase the probability of electrospinning of concentrated solutions. As, Demir *et al* reported that the highest polymer concentration of polyurethane which could be electrospun at room temperature was only 12.8 wt. %. In contrast, at high temperatures, a stunning concentration of 21.2 wt. % could be electrospun into fine fibers with desired morphology [42]. Again, these ranges differ significantly based on polymer and the resultant electrospinning solution as described in the reviews [39, 41].

Besides solution parameters and ambient factors, applied voltage, flow rate collector distance and morphology also add some favourable characteristics. The strength of the electric field will chiefly decide the diameter of fibers. At optimal voltage, non-beaded smooth fibers with minimum size were formed meanwhile if the field strength is very high or too low, fibers with poor geometry and beads were recorded [31, 43]. On the other hand, the optimum flow rate serves as an essential factor to maintain continuous production of solid fibers without any breakage. However at high flow rates, undesired effects like beads, poor fibrous morphology and increased pore size were noted mainly because of partial drying [44]. In addition, the drying of fibers and solvent vaporization inferred to depend on the space between the

spinneret and collector unit. If they are separated by proper distance, we can able to obtain beadless, porous nanofibers with required diameter range. It also ensures complete drying of solvent before the solid fibers reach a collector of choice. Moreover, by changing the morphology of the collector like flat, rotating drum, parallel electrodes, rotating wire drum, rotating tube collector with knife-edge electrodes, disc collector, array of counter electrodes, water bath, conductive or non-conductive target, we can obtain randomly oriented or highly aligned porous nanofibers in both 2D and 3D forms [33, 45, 46]. To gain detailed information about processing parameters readers may refer following reviews [31, 32, 38, 39, 45-47].

Therefore, to the maintain integrity of the delivery system and to protect the coupled biomolecules against the dynamism of biological environment special efforts should be taken to explore the appropriate range of parameters for the prepared electrospun mixture.

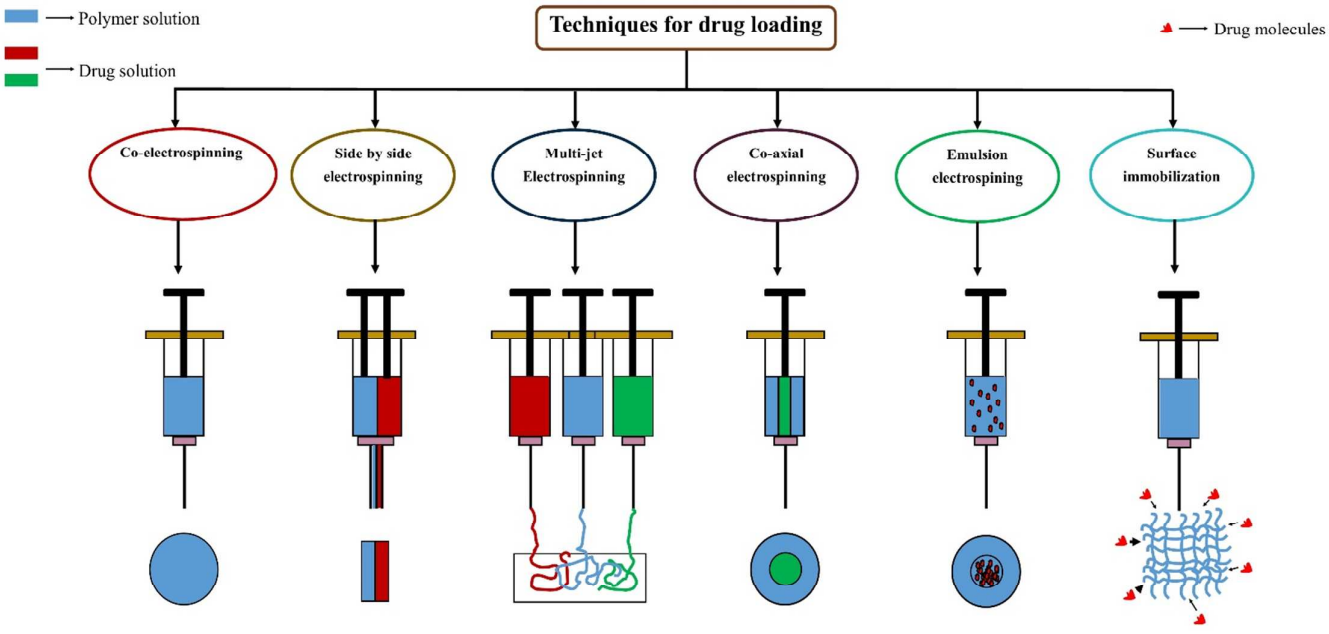
Figure 2: Essential parameters involves in electrospining process



2.2 Techniques employed for loading drugs into electrospun fibers

Electrospining not only offers control over the geometry and bulk properties of nanofibers but also afford various methods for loading drugs and biomolecules based on the requirement. By varying the nozzle configuration and exploiting surface modification technique, scientists were successfully reported the incorporation of active substances through co-electrospinning, side by side, multi-jet, multi-layer, co-axial, emulsion electrospinning and surface immobilization as shown in figure 3. And each method has reported exhibiting unique characteristics, whose selection depends on requirements like, high drug loading, increased initial burst, sustained release, prolong circulation, etc., [32, 33, 48]. The method of co-electrospinning is the simple and cost-effective technique in which the polymer solution and the drug/biomolecule to be incorporated is mixed prior to electrospinning. It offers high drug loading, homogenous spreading of drug molecules and increased initial burst. Though, the direct exposure of drugs to electric field involved in this method, may affect its bio-activity.

Since, the nanofiber surface is predominantly occupied by



loaded substances, the total drug release is reported within few days due to diffusion and polymer degradation. In a few cases, it fails to shield the conjugated biomolecules from attacks of bodily fluids, which may result in un-desired release [32]. If the drug/biomolecule of interest is not soluble in the common solvent, it still can be spun into nanofibers using side by side approach. In this method, the carrier/polymer solution and biomolecules are loaded in the separate spinneret. While applying the electric field, fibers with distinct upper and lower layer are deposited on a common target. This approach can be used to delay the initial burst of the drug molecules [33, 49].

Most of the cancer treatment involves in the exploitation of multiple medications especially in case of addressing solid tumours, this type of treatment method is popularly called as combination therapy. Through electrospinning, it is possible to load different biomolecules in a single system by using either multi-jet or multi-layer method. In the multi-jet method, the carrier and multiple drugs proposed will be loaded into different spinnerets and it will be drawn into nanofibers simultaneously [50, 51]. On the other hand, the multi-layer method involves in the sandwiching of drugs in between carrier and targeting layer. Further, the multi-layer nanofibers can be achieved using basic electrospinning unit and it will provide better protection for underlying biomolecule to deliver. Meanwhile the targeting layer can be replaced into another drug which intended to disperse instantly, followed with the sustained release of the second drug [52, 53]. By utilizing porous nature and high surface area of nanofiber system, the immobilization of drugs by physical and chemical methods also reported. The main motive of this mechanism is to preserve the bio-activity of laden molecules from the attack of electric field or solvent utilized. During physical immobilization, the drug molecules are reported to engage on the nanofiber surface by electrostatic, hydrophobic or van der Waals interactions. Whilst in chemical method, the nanofiber surface will be modified with amine, carboxyl, hydroxyl, or thiol groups to hold drug molecules. In the interim, the chemical immobilization reported allowing better control over the amount of drugs loaded when compared with the physical method [32, 54, 55]. Nonetheless, the co-axial and emulsion electrospinning techniques has been gaining great attention nowadays because of its excellent ability to shield biologically active substances and to reduce the complication of initial burst. In co-axial method, the drug particles will be sealed in the centre core surrounded by polymer/targeting shell. Here, the electrospinning solution will be loaded into specially designed spinneret that has separate compartments for loading polymer and drug solution respectively. Thus, the resultant nanofiber will exhibit core-shell structure and the drug molecules are completely isolated from the electric field since the outer surface of Taylor cone is formed by polymer solution. Moreover, in co-axial spinning the preferred carrier and drug will come in contact only at the point of synthesising nanofiber. It significantly avoids undesired reaction reported to occur between polymer and biomolecules prior to electrospinning [56-58]. Later, this core-shell structure was reported to generate using single nozzle electrospinning unit using emulsion input, popularly called as emulsion spinning. However, the formation of a perfect core-shell structure is not observed in most cases due to the effect of increased emulsion [59]. Apart from this, electrospun nanofibers based hybrid system powered by micro and nano- carriers have been extensively studied in the past five to six years. This technique allows the incorporation of other drug delivery vehicles like nanoparticles, nanospheres, microspheres, liposomes, micelles etc., into electrospun nanofibers. Through this novel strategy, we not only achieves better pharmacokinetics properties, but also ensure intracellular delivery of drugs [60-62].

3. Electrospun nanofibrous drug delivery system for treating lung cancer

In this section, the studies reported on lung cancer cells are alone discussed and for better understanding it is divided into subdivisions as dox loaded nanofibers, synthetic implantable nanofibers, stimuli-responsive and natural extracts loaded nanofibers (figure 4) respectively based on the works available. Before that, a brief introduction about lung cancer is also presented for quick recalling.

3.1 Lung cancer

Lung cancer is one of the best examples of clinical ailments, genesis due to dreadful habitude. Since the past decade, the lung cancer accounts for higher deaths in both men and women than any other cancers. By the end of 2015, a total of 221,200 new cases and 158,040 deaths expected to record because of lung cancer in the United States alone [4]. And it is reported to retain this position for the next 30 years by affecting twice as many peoples in 2040 than in 2010. Major risk factors associated with this mammoth issue are smoking (almost 90%), family history, radiation, diet, HIV infection, emphysema, scarring of the lungs and environmental pollution. This deadliest disease mostly arises in the cell linings of bronchi, bronchioles, alveoli or other parts of the lungs as a result of fatal mutations. However, this clinical condition will not generate tumour tissue all of the sudden, it will take months of time for the muted cells to develop into an outgrowth which is visible in imaging modalities like x-ray etc.

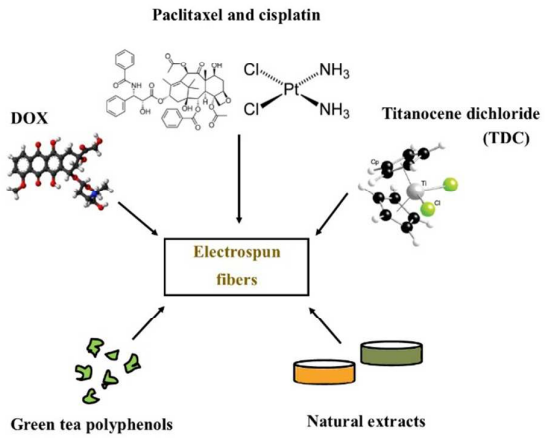
Therefore, the early prognosis of lung cancer is difficult and by the time of make out it would already in an advanced stage. Medical conditions such as persistent cough, severe chest pain while laughing or coughing, hoarseness, weight loss, shortness of breath and bloody phlegm are few possible indicators of lung cancer. In general, it is distinguished into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). The SCLC is least reported among two types, by accounting only 10% to 15% of total lung cancers. This small sized cancer growth mostly observed in the bronchi located nearby chest and likely to spread throughout the body in advance stages. In contrast, the NSCLC is diagnosed in 85% of lung cancer patients and it is

mainly caused due to smoking. Based on location and cell type, NSCLC is classified into adenocarcinoma, squamous cell carcinoma and large cell carcinoma. In spite of decades of research and exploration of advanced medical techniques, the one-year survival rate after diagnosis is 43%, whereas for five years it is only 17% percentage. This strongly represents the demand for reliable and promising mode of treatments [63].

3.2 DOX loaded nanofibers

Nevertheless, electrospun nanofibers loaded with robust natural and synthetic anticancer agents has expressed positive signs in the development of trustworthy therapeutic alternatives. Recently, Behnaz *et al* proposed doxorubicin (DOX) integrated polyethylene oxide (PEO), chitosan (CS) and graphene oxide (GO) based electrospun scaffolds to avoid the side-effects while using free DOX for lung cancer treatment [64].

DOX is one of the powerful anticancer drugs which has been in clinical use for more than 40 years by serving as an effective option to treat broad spectrum of cancers [65, 66]. Its mechanism involves in the formation of ternary complex with topoisomerase II (TOP-2) and DNA. The isoform Top2α, which is overexpressed in tumour cells is the primary target of DOX induced cell death. As mentioned, the undefined delivery of



DOX frequently reported to elicit onset of cardiomyopathy and other heart related ailments insinuating the need for proper delivery [67]. The chitosan exploited in this research, is one of the widely preferred natural polymers for applications ranging from tissue regeneration to drug delivery systems. Further, it also efficiently couple with common biodegradable polymers utilized for drug delivery purpose such as poly (vinyl alcohol) (PVA), cellulose, poly (lactic acid) (PLA), Poly (caprolactone) (PCL), polyethylene glycol (PEG) and various biomolecules of interest [68]. Likewise, GO is well known for its biocompatibility, degradable property and excellent stability among its fellow graphene's [69]. For providing a sustained release, DOX was initially coupled with GO and added in PEO/CS mixture to form electrospinning solution. Afterward, the prepared composite was spun into fine nanofibers using a typical electrospinning system. The successful loading of the drug into GO and establishment of chemical reaction between GO/CS was inferred from the FTIR fingerprints. Nanofibers with minimum diameter were obtained at the lower concentration of GO (0.5%) due to increase in the conductivity of prepared solution. In contrast, nanofibers with beaded morphology was noted in the higher concentration of GO due to increasing viscosity.

Figure 4: Schematic representation of electrospun fibers for lung cancer treatment

Meanwhile, TEM images confirmed, the fruitful cramming of drug loaded GO in nanofibrous system. Alike nanofiber morphology, the drug loading capacity of PEO/CS nanofiber was also affected when increasing GO concentration, due to the occurrence of π–π staking between DOX and GO. To add, the loading efficiency was also inferred to change expressively at different pH levels. As expected the extensive mechanical property of GO played a crucial role in delaying the drug release from PEO/CS/GO/DOX nanofibers when compared to controls. Further, the release kinetics were also found to depend on pH levels and maximum release was recorded at pH 5.3, which clearly exposed pH sensitivity of the proposed combination. At last the *in vitro* cytotoxicity of PEO/CS/GO/DOX nanofibers against A549 lung cancer cells was noted to be robust when compared with both GO/DOX and free DOX. More importantly, the prepared nanofibers were able to maintain anti-tumour activity for a long period, which decipher sustained release of DOX [64].

In a different study, Nadia and colleagues have reported another effective drug delivery system to safely deliver DOX at the site of cancerous tissue. Here, the drug was loaded into PLA/PEG/multi-walled carbon nanotube (MWCNT) composite and more importance was given to the selection of electrospinning parameters. Initially, the mixture to be electrospun was prepared through pre-designed protocol by using common solvents like acetic acid, chloroform and DMF. Then the electrospinning process was carried out by changing the concentration (C), applied voltage (AV), flow rate (FR) and collector distance (CD) to get an ideal model. As discussed, the processing parameters have an immense ability to influence the overall efficacy of projected nanofibers however the practical investigation involves in optimisation of each and every parameter is a time-consuming procedure. Hence they used Box–Behnken design (BBD) method, one of the statistical experiment design techniques available for gaining optimised values and confirmed that with practical values attained. A series of mathematical and statistical breakdown yields augmented values for four parameters C, AV, FR and CD as 8.15%, 0.2 ml/h, 18.50 KV, 13.0 cm respectively.

Meanwhile, the minimum fiber diameter (225 nm) inferred through SEM analysis was significantly corroborated with value (228 nm) prophesied by BBD analysis in optimised range. This meticulously expresses the precision of BBD analysis and also the importance of processing parameters. Further, the incorporated MWCNT's improved drug loading and retaining the efficiency of prepared nanofibers. In the interim, the *in vitro* drug release studies showed the degradation behaviour varies depend on the percentage of PEG, MWCNT and DOX used. From their report, it can be concluded that PLA/PEG 20%/MWCNT 5%/DOX 10% combination possess appropriate release behaviour with the minimal initial burst. Alike previous study, the *in vitro* cytotoxicity was expressed using A549 lung cancer cells, it portrays that the prepared delivery system possesses significant anti-tumour activity and killed 65-92% of cultured cancer cells. When compared with its counterpart (PLA/PEG/MWCNT), DOX-loaded PLA/PEG/MWCNT nanofibers maintained the cytotoxicity effect for an extended period and far better than free DOX which has a half-life of only 16–18 h [70].

3.3 Implantable synthetic drug loaded nanofibers

Chen and his research team projected a controlled drug delivery system for administrating Titanocene dichloride (TDC) to overcome the problem of solubility and short life period reported in its phase II clinical trials [71]. TDC is an effective anti-neoplastic agent, whose exploration was largely inspired from metal-containing complexes like cisplatin, etc. The main target of TDC is observed to be DNA, further the cytostatic and cytotoxic effects are depend on its ability to arrest the cell cycle in late S/early G2 phase by causing severe DNA damages. It reported to form DNA cross-linking, DNA adducts and accumulation of p53 which eventually leads to apoptosis of cell [72-74]. Here, poly-L-lactic acid (PLLA) polymer was used as the carrier system to offer targeted and controlled release of TDC. The drug was directly loaded into PLLA/ dichloromethane solution and fabricated into nanofibers. SEM micrographs and XRD results depict significant blending of the drug with PLLA nanofibers and formation of smooth bead-less morphology. However, beads were observed while increasing concentration of the drug more than 15%. Hence, it is concluded as the maximum TDC quantity which can be loaded without affecting nanofiber morphology. Likewise, the tensile strength also decreased with increasing TDC percentage, though it was reported to be inadequate level for an implantable drug delivery system. The drug release behaviour was recorded by dissolving nanofibers in three different solutions namely, PBS with 50 mg/L, PBS with 10 mg/L proteinase K and PBS without proteinase K. In all cases, the initial burst was observed only in the presence of the proteins K enzyme due to rapid degradation, but in virgin PBS the PLLA/TDC fibrous system exhibited excellent release kinetics. Furthermore, the cytotoxicity was scrutinized using human lung tumour spc-a-1 cells, MTT assay disclosed both free TDC and TDC coupled PLLA nanofibers possess equal inhibition rate at different concentrations. But the PLLA/TDC exhibited decreased IC50 values when compare with a free TDC to produce the same effect mainly because of sustained release. Therefore, they clinched that this nano-formulation can be possibly exploited as an implantable delivery system to administrate TDC for lung cancer treatment without reported complications [71].

Later, Yong *et al* fabricated electrospining inspired combination or multiple drug delivery system to manage the release of anti-cancer drugs paclitaxel and cisplatin [75]. Paclitaxel is also one of the highly reputed anticancer drugs in clinical usage, which is obtained from the barks of pacific yew tree [76]. It has a complex chemical structure formed by taxane ring with a four-membered oxetane ring and an ester side chain at C-13 position. It elicits a unique mechanism of action by targeting tubulin of the cells. Researchers have reported that paclitaxel treated cells have difficulty with the spindle assembly, cell division and also chromosome segregation, mainly due to its action against microtubules [77-79]. On the other hand, the drug cisplatin has been used for the treatment of several cancers which mainly includes bladder, head and neck, lung, ovarian and testicular tumours. The attacking nature of cisplatin against cancer cells is linked with its ability to crosslink with the purine base on the DNA, interfering DNA repair mechanisms, causing DNA damage, and subsequently inducing apoptosis [80]. So, the simultaneous administration of these two effective drugs will produce promising results. They used Poly propylene carbonate polymer to integrate both drugs and electrospining technique was followed to produce drug loaded microfibers. Through *in vitro* studies performed on A549 lung cancer cells, it was depicted that Poly propylene carbonate/ paclitaxel/ cisplatin microfibers showed synergism and excellent cytotoxicity than the controls, free drugs and fibers incorporated with single drug respectively [75].

3.4 Stimuli responsive nanofibers

In addition to the targeted and site-specific distribution, nowadays the drug delivery system also expected to act based on bodily environmental conditions virtually like a smart robot system. This artificial intelligent or stimuli-responsive nanofibers was first reported by Salehi *et al*, they used poly (N-isopropylacrylamide) (PNIPAAm) to offer predetermined delivery of DOX. PNIPAAm has the aptitude to behave differently in response to temperature change, but the problem higher water solubility hinders its application especially in the drug delivery system. To overcome this, PNIPAAm was cross-linked with hydrophilic polymers so as to yield water-insoluble fibers. After performing a series of procedures, a freeze-dried powder form of PNIPAAm was obtained and it dissolved along with DOX to form a homogenous electrospinning solution. Several characterization, release kinetics and *in vitro* cytotoxicity studies were performed to investigate the effects of electrospining parameters in influencing nanofiber competency was also established using Response surface

methodology (RSM) and BBD method. The mathematical minimum fiber diameter value (175.90 NM) obtained when using an optimised range of C (7.65 mg/L), AV (21.57 KV), FR (0.54 ml/h) and CD (9.75 cm) was confirmed by practical value (176.57 NM) inferred in SEM analysis. In the interim, SEM images also displayed the formation of smooth fibers which indicates efficient assimilation of drugs and the fiber diameter was noted to increase with DOX concentration.

Then, the retainment of DOX molecular structure after the electrospinning process was confirmed by UV–Vis investigation further FTIR spectrum reveals the presence of desired chemical substances by exposing characteristic peaks. The DOX loaded PNIPAAm nanofibers exhibited a linear relationship between the drug release percentage and time (approximately) at different concentration of the drug studied. It implies zero-order release kinetics of drug and also guarantee steady discharge of active molecules over an extended period of time. However, high initial burst was noted for a shorter period of time, later the discharge was levelled off indicating more amount of drug is encapsulated inside the nanofibers than on its surface. Interestingly, the stability of drug release was witnessed more than 30 days, which confirms excellent encapsulation and release kinetics profile of the proposed nanofiber system. And this behaviour is also recorded in, *in vitro* cytotoxicity study against A549 cells. The DOX integrated PNIPAAm nanofibers can retain the same level of toxicity for 72 hours due to sustained release and eradicated more number of cancer cells when compared to controls. Hence, they suggest that this intelligent stimuli-responsive nanofibers can be plausibly employed as an auspicious drug delivery system to treat lung cancer [81].

3.5 Natural molecules coupled nanofibers

Beyond synthetic drugs, various natural agents incorporated nanofibers also significantly fought against lung cancer cells. Sridhar and his colleagues synthesized PCL based nanofibrous drug eluting device to deliver extracts of curcumin (CU), aloe vera (AV) and neem (NE). The natural ingredients employed are a rich source of proven antioxidants, anti-cancer and anti-tumour biological molecules. To suggest a synergistic and efficient combination, different mixtures were electrospun to yield PCL, PCL/CU, PCL/AV, PCL/NE, PCL/CU/AV and PCL/CU/NE nanofibrous system. Initially, the presence of all active substances was confirmed through FTIR analysis. The ability of presented nanofiber mesh to maintain the integrity and stability was illustrated using the uniaxial testing machine. They observed that the addition of AV improved tensile strength, young's modulus and elasticity in contrast to CU and NE because of fiber producing substances in AV. Whilst, SEM investigation displayed that fiber diameter decreased with the addition of AV and NE when compared to CU. However, the incorporation of CU was inferred to advance both encapsulation and releasing behaviour of natural extracts. Lastly, PCL/AV, PCL/CU, PCL/CU/NE and PCL/CU/AV meshes expressed 70%, 65%, 23%, 18% cytotoxicity against A459 cells respectively. In particular, the 1% AV and 5% CU coupled PCL nanofibers observed to 15% more cytotoxic when compared with 1% commercial drug (cis-Platin) loaded PCL nanofiber [82].

Subsequently, in another work, Shao *et al* reported the anti-cancer property of PCL/MWCNT's/green tea polyphenols (GTP) electrospun nanofibers [83]. GTP is a natural storehouse of potential antioxidant, anti-inflammatory and cancer preventing active agents, whose capability in addressing lung, skin, oesophagus, duodenum, liver and stomach cancer is reported previously. However, the problem of instability and lack of site specificity hinders its utilization [84, 85]. To solve this, the GTP was loaded in MWCNT's to gain sustained release and PCL/ MWCNT's/GTP nanofibers were twirled with different concentration of GTP. Then, the occurrence of non-covalent interaction between GTP and MWCNT's was illustrated using UV–visible spectrophotometer further FTIR studies were also carried out to confirm the presence of desired components. The excellent conductive property of MWCNT's yields, fibers with minimum diameter however, it increases with the concentration of GTP due to the influence of elevated viscosity. Meanwhile, the well-oriented distribution of MWCNT's is expressed by TEM images and Laser scanning confocal microscope (LSCM) images disclosed, homogenous spreading of GTP along the nanofiber surface. Interestingly, the fluorescence intensity was higher in PCL/ MWCNT's/GTP nanofibers when compared to PCL which once again confirmed the non-covalent interaction between MWCNT's and GTP which leads to higher loading. As expected, the addition and appropriate orientation of MWCNT's significantly improved mechanical properties of PCL.

Nevertheless, with increasing concentration of GTP the young' modulus, maximum tensile strength and elongation at break of nanofibers were noted reduce gradually due to its plasticizing effects. The prepared meshes can sustain displayed fibrous structure, even after 4 weeks and the swelling was observed only after the 12th week. Moreover, sudden initial bursts of hydrophilic GTP was inferred in the absence MWCNT's, since the active components are dispersed on the fiber surface. This clarifies the role of MWCNTs and non-covalent interaction in delivering controlled and sustained release. So, the release behaviour can be tailored by adjusting MWCNTs content based on the requirement. In the interim, the Alamar blue assay was carried out using osteoblasts and human lung epithelial cells (A549) to present the toxicity of hypothesised delivery system against normal and cancer cell. Surprisingly, the cytotoxicity of PCL/GTP and PCL/ MWCNT's/GTP remained at similar range for first two days, but on the fifth day the cytotoxicity of projected nanofiber was significantly higher and list number of cultured malignant cells. This pattern was also reflected in fluorescence microscope images of lung cancer cells cultured on PCL/ MWCNT's/GTP fibers. Hence, they concluded that GTP incorporated PCL/ MWCNT's has commendable properties to destroy lung cancer cells in a controlled manner with only traceable cytotoxicity to normal cells [83].

From the above researches, it can be clearly inferred that resultant nanofiber geometry and property heavily depends on the selectivity of carrier (polymer), the concentration of drug molecules and processing parameters. In particular, the intended active substance is observed to affect fiber morphology, mechanical characters and release kinetics. So, extensive care should be taken while framing the quantity of drugs to be incorporated in favour of both nanofiber capabilities and its anti-cancer activity. While for optimising electrospinning parameters, it will be more appropriate to adapt available statistical experiment design techniques rather than going for time-consuming practical analysis. Successively, their significant precision with practical values are illustrated by several studies.

Overall, the works listed above evidently expose the aptitude of electrospun micro/nanofibers in achieving required pharmacokinetics and improved cytotoxicity effects against lung cancer cells when compared with free drugs.

4. Electrospun nanofibrous drug delivery system for breast cancer treatment

Several interesting works have been published on the utilization of electrospun fibers for treating as well as preventing the reappearance of breast cancer. This section will present the works carried out on micro/nanofibers based DNA silencing, combination therapy, implantable drug loaded scaffolds and bio-inspired delivery systems as illustrated in figure 5. Moreover, in all works the *in vitro* efficacy of the proposed prototype was demonstrated using breast cancer cell lines.

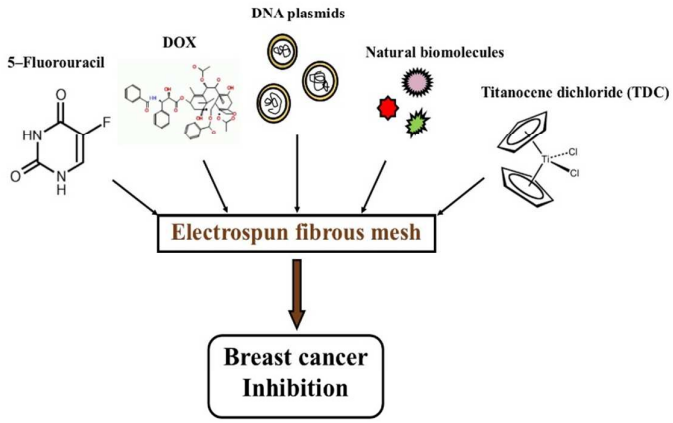
4.1 Breast cancer

Breast cancer is the second most noxious cancer reported globally, however it inferred to be the most frequently recorded cancers among the women population. This non-skin carcinoma is noted in nearly one out of every four diagnosed cancer cases, with more than 2.8 million survivors who have either past or current history are reported to alive in the US alone. The economic loss occurring due to breast cancer treatment is highest among other cancer and to settle this serious health issue National Cancer Institute (NSI) spending more than half (559.2 million dollars in 2013) of its total budget exclusively for breast cancer research [86]. This lethal malignant tumour arises due to troublesome molecular changes that occur at cells present in three vital parts of female breast namely lobules, ducts and stroma respectively. In breast cancer, the complication of metastasis (i.e) spreading of cancer cells to the bloodstream is essentially feasible because of the higher concentration of lymph nodes in the breast. Clinically, it is classified into non-invasive ductal carcinoma in situ (DCIS), invasive lobular carcinoma in situ (ILC) and invasive ductal carcinoma in situ (IDC).

IDC is frequently reported breast cancer type, accounts for about 50-80% of total breast cancer cases and one in eight women in the US is stated to develop IDC during their lifespan. This invasive carcinoma arises in the milk ducts, as the tumour tissue develops it will break through duct walls and reaches the fatty tissue. By the end of 2015, a total of 231,840 new cases of the IDC is expected to diagnose among US women. Further, people get affected by IDC have higher chances of developing metastasis. Unlike IDC, the non-invasive ductal carcinoma does not spread to surrounding breast tissue from its origin (i.e) ducts, therefore it is comparatively less lethal. Nevertheless, ILC is another invasive type of carcinoma develops in milk producing glands of the breast known as lobules. In addition, medullary carcinoma, mucinous/ colloid carcinoma, tubular carcinoma, inflammatory breast cancer, Paget's disease of the nipple, adenocystic carcinoma, and phyllodes tumour are some other rarely recorded breast cancer types. Though it sounds like a gender oriented disease, men population is also reported to develop breast cancer. Irrespective of age and sex, the five-year survival rate of victims with localized breast tumours is 98.3%, while those diagnosed with metastasis is stated to have only 23% existence [87].

4.2 Nanofibers assisted DNA delivery for silencing expressive breast cancer genes

At present, cancer research has attained significant evolution, it is looking for promising and persuasive treatment modalities beyond the horizon of chemotherapy. Treating cancer by using its merit (i.e) administrating chemical/genetic materials specifically to suppress the oncogenic expression, thereby disturbing it's cell cycle or inducing apoptosis is one of those advanced techniques. Regrettably, vital requirements like site-specific delivery and ability to maintain the bioactivity of



genetic materials have been a great challenge for scientists to

establish this notion. However, by making use of recent innovations and advancements, researchers have reported the successful delivery of DNA [23], RNAi, MicroRNA (miRNA), Small interfering RNA (siRNA) [21, 22], plasmids for tissue regeneration and cancer treatment. Favourable properties like high surface area, porous fiber morphology and pre-determined release made electrospun micro/nanofibers to appreciably maintain bioactivity of coupled molecules when compared to other nanostructures [88-91]. Inspired by this capability, Achille

Figure 5: Electrospining inspired fibrous mesh for breast cancer treatment

et al tailored PCL electrospun fibers for delivering DNA plasmid to suppress targeted oncogenic gene expression to halt cell division and eradicate breast cancer cells. Essentially the purified DNA plasmid (800 mg) was added directly in PCL solution and three different scaffolds were framed namely, pristine PCL, PCL/ pKD-Cdk2-v5 plasmid (Cdk2i) and PCL/pKD-EGFP-v1 plasmid (EGFPi). About 85% of the PCL / DNA solution was measured to electrospun with approximately 2.125 mg/cm^2 of DNA plasmid in each scaffold. From SEM, images it can be inferred that in pristine PCL, the fibers exhibited smooth and stacked morphology, whereas in both DNA plasmid loaded mats tangled fibers with grooved structure was noted. Meanwhile, the addition of plasmid increased the fiber diameter from few sub-microns in pristine PCL to 10 µm. Subsequently, EGFPi and Cdk2i loaded scaffolds expressed the similar pattern of degradation with sustained release of DNA plasmid for three weeks, followed by the maximum burst in first 24 hrs. These observations were confirmed by gel electrophoresis studies moreover both scaffolds were able to maintain the integrity of fibers for 21 days after that severe cracks were formed, indicating the degradation of the microfibrous system. Besides, to verify the achievement of this research objective (i.e) delivering DNA encodes for stimulating shRNA to disrupt the cell cycle of breast cancer cells by silencing Cdk2 genes, several test were done.

Firstly, the Quantitative Real Time PCR (Q-PCR) showed that the suppression of Cdk2 genes was higher in the cells while exploiting pKD-Cdk2-v5 plasmid than others. Similarly, the visual monitoring through the microscope exposed decreased cell viability in the presence of Cdk2 shRNA encoded plasmid. Interestingly, they were able to produce the same when culturing human breast cancer cells (MCF-7) on synthesised scaffolds at *in vitro* condition, it insinuates the fact that expression of Cdk2 genes were lower in cancer cells present on PCL/pKD-Cdk2-v5 plasmid scaffolds. And through LIVE/DEAD assay they also confirmed that the cell death observed is due to silencing of aspired oncogenic expression. This research serves as a "proof of concept" that electrospun fibers have the capability to administrate genetic materials with desired bioactivity [92].

4.3 Nanofibers based combination therapy for breast cancer

Often, the administration of single chemotherapeutic agent is not sufficient to address solid or advanced tumours. So, a blend of drugs will be preferred during this clinical condition. Milena *et al* designed a multiple drug delivery system to provide combination therapy for breast tumours. They used poly (L-lactide-*co*-D,L-lactide) (coPLA) as a carrier to deliver natural quaternized chitosan (QCh) and popular synthetic drug DOX [93]. QCh reported in this research, is one of the derivatives of chitosan which exhibits better antibacterial, antimycotic and *in vitro* anticancer capabilities [94-96]. To extend its application to breast cancer research, it was tested against MCF-7 cells by combining with a synthetic anticancer agent. Active molecules were directly added to coPLA solution then nanofibers with single and multiple anti-cancer agents were electrospun. The addition of QCh and DOX were reported to decrease fiber diameter by increasing the conductivity and decreasing the viscosity of electrospinning solution. Because of this, maximum drug loading percentage of 96 and 98 were able to achieve in couple/DOX and QCh/coPLA/DOX mesh respectively. MTT assay delineates excellent cytotoxicity of synthesized nanofiber mats QCh/coPLA, coPLA/DOX and QCh/coPLA/DOX against the cultured MCF-7 cancer cells. Further, the maximum reduction in cell viability was observed after 72hrs of incubation in coPLA/DOX (18.8 ± 1.0%), QCh/coPLA/DOX (18.8 ± 1.6%), and free DOX (19.1 ± 1.2%) respectively.

Meanwhile, the morphological changes occurred in MCF-7 tumour cells observed through SEM revealed better adherence of cancer cells on pristine coPLA nanofibers and expressed normal bilateral symmetric morphology with numerous microvilli on the surface. However, on QCh/coPLA and DOX/ coPLA nanofibers transformation of bilateral morphology to round, holes in the cell wall and cytoplasmic extrusions were inferred. While on the combined nanofiber system (i.e) QCh/coPLA/DOX, breast cancer cells were severely beaten by late apoptosis, cell shrinkage, surface blebs and formation of apoptotic bodies. This observation confirmed the higher viability of multiple drugs loaded nanofibers than individual drug delivery systems. And these effects were also confirmed through intravital staining of cells incubated on nanofibers for 24hrs. The colour changes expressed in fluorescence micrographs of the different nanofiber surface depict the appropriate state of breast cancer cells, such as normal morphology, the formation of apoptotic bodies, etc. Once again, the highest apoptotic percentage (100%) was reported on QCh/coPLA/DOX mats due to combined anti-tumour activity of biomolecules utilized. So, by using this integrated drug delivery system we not only avoid the side-effects of using free DOX such as cardiotoxicity, myelosuppression and cytotoxicity to normal mitotic cells but also deliver intense remedy [93].

4.4 Implantable drug loaded electrospun scaffold

Yuan and his research group developed an implantable electrospun scaffold for treating breast tumours exclusively in postoperative condition to prevent the disease rebound. DOX was used as the anti-cancer agent and it was dissolved along with PLLA to form the electrospun solution. However, the hydrophobic nature of PLLA may greatly hinder the release kinetics of DOX. In order to solve this, the electrospun fibers were surface modified with Polydopamine (pDA) to improve its hydrophilic properties [97]. Polydopamine is basically an adhesive protein, which can act as a feasible agent for surface functionalization of materials without affecting its geometry. pDA is reported to improve the wettability of materials by forming a thin nano - film on the surface, meanwhile it will encourage the integration of several biomolecules by serving as a coupling agent [98-100]. To make use of this excellent chemical, electrospun PLLA and DOX loaded PLLA fibers were immersed in pDA solution to form PLLA/pDA and PLLA-DOX/pDA fibers. The diameter of the electrospun fibers was measured to be in micrometres and the elevated nitrogen peak noted in XPS graph indicates possible grafting of pDA on microfiber surface.

After functionalization, the PLLA microfibrous mat exhibited excellent wettability but the drug encapsulation efficiency was slightly dropped from 98.4% in PLLA-DOX to 90.1% in PLLA-DOX/pDA fibers. Likewise, the percentage of DOX released from PLLA-DOX/pDA system (90.6%) was observed to higher than in un-functionalized PLLA (82.1%) over the course of 40 days. However, this minimal change is reported not to affect the efficacy of pDA grafted PLLA-DOX drug delivery system, these changes evidently depict successful embedding of pDA. In addition, extensive *in vitro* and *in vivo* pathological studies were also performed to prove the effectiveness of PLLA-DOX/pDA drug delivery system. The expected *in vitro* cytotoxicity of PLLA-DOX/pDA microfiber against human breast cancer cell line (MDA-MB-231) was illustrated by MTT assay. And the *in vivo* studies were carried out using 5-6 weeks old healthy Balb/c mice. Tumour growth was induced by subcutaneous injecting of MDA-MB-231 cells and the histopathological assay was performed after the tumour size reached 100 mm^3. The mice were divided into four groups then pristine PLLA, PLLA-DOX, PLLA-DOX/pDA fibers were implanted at the tumour site followed by removal of a small part. Biopsy performed on the third day after implantation showed, the tumour tissue has grown rigorously in control with no necrosis on both PLLA and PLLA-DOX group respectively. But in PLLA-DOX/pDA group, a small area of necrotic tissue inferred as the sign of the cytotoxicity activity of the projected drug delivery system. Likewise, on the 7^{th} day except PLLA-DOX/pDA group, all other expressed invasions of tumour tissue, hyperplasia and mesenchymal inflammation. Interestingly, in PLLA-DOX/pDA group massive necrosis of tumours were recorded. Moreover, TUNEL and Caspase-3 activity assay illustrated increased apoptotic activity and cancer cell dysfunction respectively on PLLA-DOX/pDA fiber implanted tumour. At last, the qRT-PCR analysis confirms increased mRNA level expression of Bax (induce apoptosis) and decreased Bcl-2 (anti-apoptotic) among PLLA-DOX/pDA implanted tumour cells. Therefore, from above inference it can be concluded that this pDA functionalized PLLA-DOX microfibers can be plausibly exploited as an implantable drug delivery system to fight against breast cancer [97].

In another study Laiva *et al* developed a drug delivery system for the safe delivery of TDC by rectifying the complications reported during clinical trials. Initially, pristine PCL, PCL/SF and PCL/SF/TDC nanofibers were fabricated from homogenous electrospinning solution. The Addition of SF and TDC measured to increase the conductivity and hence smooth fibers with decreased diameters were obtained. The SEM micrographs confirmed the formation of highly porous beadless nanofibers and those with minimum diameter was noted in PCL/SF/TDC system. Further, improved wettability after the addition of SF was confirmed by contact angle studies, meanwhile FTIR spectrum reveals the presence of appropriate chemical groups. Excitingly, the average fiber diameter reported to affect the stress-strain relationship and maximum tensile stress value was measured in PCL/SF nanofibers with the high percentage of TDC. Besides, the *in vitro* drug release delineates that proposed nanofibers maintained sustain release for a maximum of six days and PCL/SF system with higher concentration of TDC described to release more drug after incubation of 144 hrs. These results insinuate excellent stabilization of drug within nanofibers which offers organised release with minimum or negligible initial burst. On the other hand, the MTT assay exposed excellent cell viability of MCF-7 cells cultured on high concentrated TDC coupled PCL/SF nanofiber system when compared with both pristine and free drug at the end of the third day. It reported to increase significantly with respect to time, which reflects the outcomes of *in vitro* drug release kinetics. The optical microscopy and SEM images expressed adverse changes in morphology of breast cancer cells cultured on drug loaded nanofibers which evidently confirms the apoptotic capability of TDC [101].

Sundar and Sangeetha fabricated stimuli-responsive drug releasing scaffolds for providing post-operative chemotherapy for breast cancer patient. The electrospun scaffolds were drawn from the polymeric solution of collagen (CG) / PNIPAA/CS loaded with widely used anticancer drug 5-Fluorouracil (5-FU) and the concentration of CS was varied to yield different nanofiber mats. Collagen is the chief structural protein of all vertebrates which contributes over 90% of extracellular proteins of the tendon, bone, ECM and it is divided into several types. Further, the drug 5-FU used, is basically an antimetabolite agent which is been in clinical usage for over the past 20 years. Typically, it is an analogue of uracil with a fluorine atom at the C-5 position in place of hydrogen. It rapidly enters the cell and form several intracellular active metabolites like fluorodeoxyuridine, monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP), etc. These metabolites disrupt DNA formation, RNA synthesis and also inhibit thymidylate synthase (TS) which

eventually leads to cell death [102-104]. The nanofiber geometry was highly influenced by the concentration of chitosan but not by loaded drug. At higher concentration, nanofibers with uneven and beaded morphology were obtained mainly due to increase in viscosity of electrospinning solution. Further, the more percentage of the drug was deposited on nanofiber surface in the form of aggregates and only a few was assimilated inside. Because of this, the initial burst was observed in all mats, however increasing the concentration of CS observed to slow down the degradation and diffusion of 5-FU molecules. In addition, the release kinetics also varied in response to pH of buffer solution, having increased cumulative drug dispersion at low pH and vice-versa. This variation is inferred due to swelling and protonation of integrated natural polymers. As anticipated, the *in vitro* cell viability of cultured MCF-7 cells decreased with increasing the drug concentration. Meanwhile, normal L929 cells cultured on CG/ PNIPAA/CS/5 FU nanofibers exhibited typical cell morphology which proves retainment of bioactivity and oncogenic cell dependent anti-cancer activity of incorporated chemotherapeutic agent [105].

4.5 Natural extracts loaded nanofiber scaffolds

Polyphenols are bioactive substances which are abundantly found in common ingredients used to make our regular diet and popularly called as dietary polyphenols. It is highly essential for maintaining good cardiac health, normal cell viability, scavenging free radicals, etc. Exciting piece of researches, reported the capability of these familiar chemicals to induce cancer cell death mainly because of its rich antioxidant content. However, the same effect was difficult to reproduce at *in vivo*/clinical conditions mainly because of poor selectivity and less bioavailability at the tumour site [106, 107]. Though, with the modern drug delivery options available the above obscures can be easily rectified. Priya *et al* synthesized poly (D,L-lactide-co-glycolide) PLGA/PEO nanofiber carrier system for site-specific delivery of ferulic acid (FA) [108]. FA is a polyphenolic phytonutrient commonly present in rice, wheat, oats, coffee seeds, peanut, apple, artichoke and orange. It inferred to act severely against free radical and reactive oxygen species (ROS), the substances which associated with nasty DNA damage or mutation. Nevertheless, it documented to induce pro-apoptotic effects, especially in breast and liver cancer cells [109-111]. In this research, the FA was directly added to PLGA/PEO polymer blends, then basic electrospinning setup was used to fabricate free FA and PLGA/PEO/FA nanofibers. FESEM characterization studies show at lower concentrations of FA bead free, smooth and cylindrical nanofibers were formed. However, at higher concentration of FA, particularly between 6-8 % wt. electrospraying of polymer solution is reported rather than nanofiber formation. Therefore, to produce smooth fibers without crystallization, the maximum percentage of FA can be loaded was suggested as 2 wt%. Moreover, the fluorescent image revealed the presence of core-shell arrangement of FA inside PLGA/PEO polymer blend. On the other hand, XRD patterns exposed the amorphous state of encapsulated FA in composite nanofibers and TGA graphs presented the decomposition of FA loaded PLGA/PEO nanofibers at two different temperatures.

In addition, the drug encapsulation efficiency of the projected drug delivery system was measured as 66 ± 1.34 %. And the release kinetics studies exposed, more than 50% of FA was released within the first 24hrs which was followed with controlled release for next 10 days. Accordingly, MTT assay displayed significant cytotoxicity of PLGA/PEO/FA (2 wt %) nanofibers against MCF-7 cells when matched with controls, meanwhile it the achieved cell inhibition rate of 51.4 and 67 %. On the other hand, fluorescence microscopy images depict FA induced apoptosis signs of breast cancer cells, such as chromatin condensation, cell wrinkles and cytoplasmic remnants on PLGA/PEO/FA nanofibers. These observations clearly confirm the retainment of anti-cancer characteristics of loaded polyphenol even after the electrospinning process [108]. In another study, Sridhar *et al* synthesized PCL based nanofibrous drug eluting device to deliver extracts of curcumin (CU), aloe vera (AV) and neem (NE) for breast cancer treatment. The *in vitro* cell viability studies showed PCL/CU/AV/NE nanofibers was able to kill more number of MCF-7 cells cultured when compared to all other individual system PCL/CU, PCL/AV, PCL/NE/CU, PCL/AU/CU. This observed effect is inferred due to the synergistic action of exploited natural extracts [82].

4.6 Functionalization of hydrophobic polymers with natural materials

Though there are a variety of polymers available in the market, only a few of them can be electrospun into nanofibers. Because to get fabricated into nanofibers, the selected polymer should be dissolved in common volatile solvents to form a homogeneous solution. However, through melt spinning, an even non-degradable polymer can also devise into nanofibers by applying high temperature. Since it is difficult to control processing parameters, the melt spinning often yields fibers with poor morphology and porosity [112, 113]. In order to solve this problem and to cogently utilize poor or non-hydrophilic polymers for drug delivery applications, Thangaraju and colleagues reported an innovative solution. They demonstrated their notion with the help of poorly hydrophilic Poly L–Lactide (PLLA) polymer further the drug delivery application of modified PLLA nanofibers was also demonstrated using curcumin (CU). To make PLLA more hydrophilic, they initially used the water bath target to collect synthesized fibers. In another method, PLLA was coupled with natural hydrophilic polymers like cellulose acetate (CA) and silk fibroin (SF) respectively. Among described schemes, the water bath approach yields microfibers of range (2 to 2.5 µm) whereas nanofibers with different diameters was reported in the second method. SEM analysis insinuates that nanofiber diameter variation greatly depends on the concentration of CA, SF, C meanwhile the clear dispersion of curcumin in

synthesized fibers was displayed by TEM images. All three electrospun fibers presented diversified distribution of pores and the contact angle studies disclosed the super hydrophilic characters of PLLA/SF nanofibers than its counterparts. The *in vitro* drug release kinetics of C-PLLA microfibers and C-PLLA-CA, C-PLLA-SF nanofibers unveiled no significant changes among different systems and excitingly the initial burst release was absent in all cases. When comparing to microfiber system, C-PLLA-CA and C-PLLA-SF nanofibers exhibited better-sustained release of hydrophobic curcumin for 11 and 18 days respectively. Alike release behaviour, the proposed systems significantly reduce the cell viability of MCF 7 breast cancer cells through controlled the delivery of curcumin. The achieved effect was concluded due to curcumin induced cell cycle arrest by targeting highly expressive signalling molecules, increase in p53 levels and caspase-dependent apoptosis. In the interim, the modified curcumin loaded nanofibers expressed zero percent (approx.) cytotoxicity against normal NIH/3T3 fibroblast cells. Hence, they suggest that this modified super hydrophilic nanofibers can be preferred for delivering hydrophobic drugs intended for breast cancer treatment [114].

The successful cytotoxicity of various natural and synthetic nanofibers against different human breast cancer cell lines were reported in above discussed studies. More excitingly, the *in vitro* results were also reproduced in animal models which indicates excellent retainment of nanofibers potential under *in vivo* conditions as well. Each and every model possess unique features and in specific the usage of nanofibers to delivery gene therapy for cancer is one of the plausible therapeutic options under research. In contrast to influencing the nanofiber morphology, the addition of preferred biomolecules is reported to enhance the geometry of electrospun nanofibers in few cases. Therefore, by exploiting above appropriate drug delivery models we can offer combination therapy, natural therapy, gene therapy or site-specific chemotherapy to control and eradicate breast cancer cells.

5. Electrospun nanofibrous drug delivery system for colorectal cancer treatment

Besides lung and breast cancers, the ability of electrospun fibers to eradicate colon cancer is also reported in several studies. Researchers have proposed implantable scaffolds, drug-eluting stents, magnetic nanofibers and electrospinning empowered chemotherapy to successfully deliver drugs to hidden organ of the gastrointestinal tract (figure 6). Moreover, its cytotoxicity were illustrated using different colon cancer cells.

5.1 Colon cancer

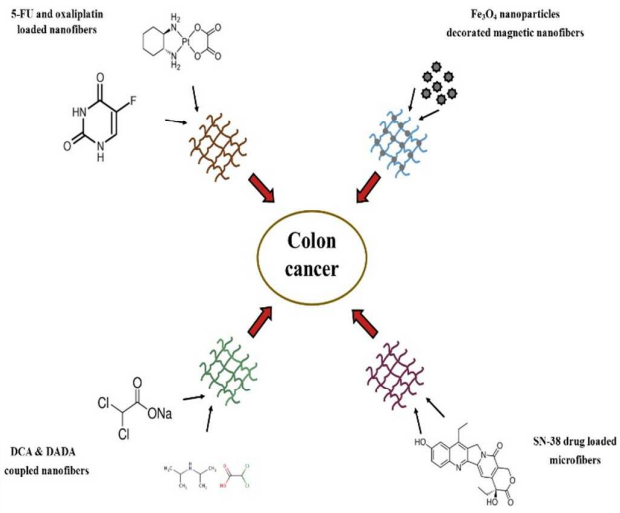
Colon is the largest part of the digestive system, it starts from the lower right abdomen and extends up to the rectum. The main function of the colon is to adsorb water, minerals, vitamins, electrolytes, maintain fluid balance, process fibers in indigestible food material and to propagate waste to the rectum which eventually expelled through the anus. The colon cancer is reported to develop in the caecum, the right lower part of the abdomen and it will progress through the right-ascending colon, transverse colon, left-descending colon and sigmoid colon. It is traditionally referred as colorectal cancer since cancer develops in the colon and rectum shares many common features. It usually arises in the form of non-cancerous polyp growth on the lining of the colon/rectum and it slowly develops over years to form a mass of cancerous tissue. Though all polyps are not advancing into cancer, of two main types the adenomatous polyp is alone observed to form oncogenic tissue. Since the colon is deep inside the abdominal cavity, it is difficult to detect the cancerous growth in early stages. The symptoms such as blood in the stool, change in bowel moments, constipation and persistent abdominal distress are chiefly the indication of maturity of disease.

Figure 6: Nanofibers for eradicating colon cancer cells

Frustratingly, the colorectal cancer is the third deadliest cancer in the United States among both sex and about 1 in 20 people is suspected to get affected by colorectal cancer in their lifetime. Besides, the American cancer society estimates that a total of 93,090 new cases and 49,700 deaths will be reported because of colon cancer by the end of this year. In colon cancer, the chance of developing metastasis is high, especially when it grows in the inner wall, where they have increased access to lymph nodes or blood vessels through which it spreads to distant organs. Among various types, the adenocarcinoma is reported in more than 95% of total colon cancer cases where the cancer starts in cells which gives lubrication to mucus. Furthermore, carcinoid tumours, gastrointestinal stromal tumours, lymphomas and sarcomas are some of the rarely recorded colon cancer types [115]. On the other hand the site-specific delivery of drugs to the colon is a complicated process since the preferred carrier system is suspected to tackle the attacks of gastrointestinal fluids, digestive enzymes, varying pH levels and long transit time. Hence, in pharmaceutical field, it is referred as “Black Box” of the gastrointestinal system.

5.2 Implantable super-hydrophobic fibrous mats

Alike other cancers, chemotherapy, radiation therapy and surgery are the most commonly preferred clinical treatments



for colon cancer as well. Among those surgery is exclusively adopted for managing localized tumours, though it is severely hammered by the problem of post-operative rebound which recorded in more than 40% of cases. To combat this scenario, chemotherapy and radiation are often pursued, but the problem of systemic side-effects discussed offsets the welfares of this treatment [116-118]. This creates a huge demand for a potential system to deliver site-specific therapeutics by tackling the attacks of gastrointestinal fluids. Recently, Stefan *et al* developed a super-hydrophobic drug loaded implantable fibrous mats to avert this problem of locoregional tumour regeneration. They used hydrophobic polymer dopant poly (glycerol monostearate-co-ε-caprolactone) (PGC-C18) to decrease the wettability of PCL and to enable controlled delivery of anti-cancer drugs (CPT-11 and SN-38) [119]. Among those, the drug SN-38 is an active metabolite of irinotecan, one of the effective topoisomerase I inhibitors available in the market. The Topo-I enzyme plays a pivotal role in DNA replication and transcription process, so disrupting the activity of Topo-I will cause DNA damage and transient S-phase arrest which eventually leads to the formation of double strand (ds) DNA breakages. This defective DNA strands are reported to activate the various apoptotic cascades of cells [120, 121]. The synthesized hydrophobic dopant and anti-cancer drugs were added to PCL solution, then four different delivery systems were electrospun namely pristine PCL, PCL doped with PGC-C18, PCL/ PGC-C18/CPT-11 and PCL /PGC-C18/SN-38 using a rotating drum target. The contact angle results interpret the super-hydrophobic nature of doped PCL and the formation of microfibers were inferred from SEM images. Meanwhile, they also varied electrospinning parameters to get optimised ranges and the resultant large fiber morphology indicates the possibility of offering controlled local delivery for an extended period of time. The mechanical tests exposed the appropriate tensile strength of synthesized hydrophobic fibrous meshes required to avoid the problem of leakage or instant burst of drug molecules. Further, the Differential scanning calorimetry confirms complete dissolution of CPT-11 and SN-38 drugs in microfibers and ultrasound studies proved the presence of entrapped air in hydrophobic meshes.

The utilization of super-hydrophobic surfaces will be a better choice to achieve a significant delay in degradation of electrospun fibers. Interestingly, in this study, they have designed a 3D super-hydrophobic meshes entrapped with air, which they depict to slow the degradation process more. Successfully, the PCL fibers doped with the higher concentration of PGC-C18 (10%) can able to keep the sustained release of SN-38 for 70 days and CPT-11 for 50 days respectively under *in vitro* conditions. They also proved the importance of entrapped air in influencing the release behaviour by showing in the absence of air the 10% PGC-C18 doped PCL mat released maximum dose of the SN-38 drug within 14 days and also had massive initial burst. Furthermore, the distribution of drugs inside the fiber was varied based on its concentration, at high levels it was concealed in the centre of fiber whereas at lower levels it found mostly on the surface which is clearly illustrated through confocal microscopy. The MTT assay performed using colorectal cancer cells (HT-29) exhibited the same trend of growth inhibition and 1% of SN-38 was enough to maintain the cytotoxicity for 90 days. In the interim, the CPT-11 loaded fibers bared, poor long-term effect in reducing the viability of colon cancer cells and higher doses was required to provoke the same effect elicit by 1% SN-38 drug loaded PCL. From the above inference, it can be concluded that the 3D super-hydrophobic PCL has potential to preserve the bioactivity of coupled substances and more active SN-38 drug can be used as a plausible replacement for prodrug CPT-11 to elicit intense anti-cancer effect against colon cancer cells [119].

5.3 Nanofibers inspired drug-eluting stent for colon cancer treatment

Li and co-researchers fabricated a drug-eluting stent to rectify the problem of restenosis associated with colorectal cancer patients [122]. Conventional stents available for clinical usage are nailed with the problem of tumour ingrowth and granulation of tissue inside lumen, moreover the treatment methods often practiced to restore this situation is allied with high mortality [123-125]. Hence, to address this clinical complication, the stent was coated with drug loaded nanofibers to repair lumen potency and avert intraluminal tumour growth. The drug 5-fluorouracil (5-FU) was used as an anti - cancer agent; it is in one of the oldest and eminent drugs introduced in 1957 for clinical use. Though the problem of poor selectivity and undesired dispersion reduce its cytotoxicity effects against colon cancer cells.

The electrospinning solution containing PLLA and different concentration of 5-FU was fabricated into nanofibers using optimized parameters and deposited on designated stent attached to the rotating drum target. At lower concentrations of 5-FU (1.6%), the fiber diameter of PLLA was decreased, in contrast fibers with uneven morphology was obtained at maximum concentration (i.e.) 12.8 % of 5-FU. It indicates uneven dispersion and increase in viscosity of the spinneret solution while adding high amounts of drug. Meanwhile, the establishment of chemical relations between 5-FU and PLLA polymer was confirmed by FTIR illustration. As expected the total percentage of drug released was depend on loaded concentration and the synthesized nanofibers were able to maintain sustained release for 240 to 400 h. Accordingly the cell viability test conducted against HCT-116 cells depicts that after 96 h, normal growth of cancer cells was observed on pristine PLLA whereas on 12.8 % 5-FU loaded nanofibers more number of cells were lysed even better than free 5-FU drug. Moreover, the MTT assay revealed that proposed nanofiber system can able to maintain the cytotoxicity effect for 120 h and it is more potent than IC50 of the free drug as well. This inference portrays controlled release of drug and decreased drug resistance of employed colon cancer cells [122].

5.4 Magnetic nanofibers for hyperthermic colon cancer treatment

Decades of cancer research have given rise to a different type of therapeutic systems, hyperthermia is one of the newly explored cancer curing treatment modality where elevated temperature is exploiting to attack tumour tissue. In this technique, the temperature of oncogenic tissue will be typically raised over 42 °C, simultaneously maintaining the normal tissue under harmless or tolerable temperature ranges. It works by utilizing high temperature sensitivity character of cancer cells when compared with its counterpart. Various techniques like ultrasound, microwave and radiofrequency have chiefly used as an energy source to destroy cancer cells by producing localized temperature elevation. And to increase the effectiveness, it is often coupled with available curative like chemotherapy or radiation therapy. Though, the clinical implication of this notion impedes by practical difficulties in providing spatiotemporal temperature distribution exclusively around tumour tissues [126-129]. Recently, Lin and colleagues successfully demonstrated hyperthermic eradication of colon cancer cells by using magnetic nanoparticles or thermoseeds loaded chitosan nanofibers [130]. The thermoseeds or ferrite magnetite are low toxic nanoparticles which possess superparamagnetism in nano-range. These captivating nanoparticles are approved by FDA and already find several applications in drug delivery, cancer tissue imaging, biomolecule separation, etc. While the required heat will be produced by applying strong electromagnetic field using the coils placed outside, followed by intravenous administration of magnetic nanoparticles [131, 132].

To overcome the hitches in using these nanoparticles such as RES clearance, *in vivo* instability and mass diffusion, here the ferrite nanoparticles are entrapped inside chitosan/PEO nanofibers to produce a plausible outcome. Initially, the chitosan was functionalized with Iminodiacetic acid (IDA) to clamp large number of magnetic nanoparticles and PEO/CS-IDA nanofibers were synthesized using a conventional electrospinning setup. Later, the prepared Fe ions are sealed on the nanofibers surface by immobilization method which consequently forms Fe_3O_4 nanoparticles by chemical co-precipitation and gives rise to magnetic nanofibrous composites. The electrospinning parameters were optimized to obtain smooth and beadless nanofibers meanwhile the magnetic nanoparticles were observed in large number on PEO/IDA-CS/Mag when compared to un-functionalized PEO/CS/Mag nanofibers. Meanwhile XRD patterns and TEM images confirmed the presence of desired crosslinking and successful assimilation of magnetic nanoparticles along the nanofibers surface. Finally, the LDH assay performed using mouse colon carcinoma (CT-26) revealed no significant changes in cytotoxicity of PEO/CS/Mag, PEO/IDA/Mag, PEO/CS-IDA/Mag nanofiber composite systems and free Fe_3O_4 nanoparticles respectively. And under applied magnetic field, the temperature of range 40-45 °C was created by magnetic nanofibers and also proved better cell viability in the absence of magnetic nanoparticles by reporting maximum cell deaths in all magnetic nanofibers under applied field. From the above observations, it can be concluded that this Fe_3O nanoparticles entrapped nanofibers can be preferred as a plausible treatment method without any systemic side-effects [130].

5.5 Nanofibers assisted chemotherapy for colon cancer

As mentioned, the site-specific delivery of drugs for colon cancer is highly intricate, especially in case of oral administration. The intended chemotherapeutic agent has to be intact during prolong transition time before it reaches the colon. It has to shield itself against the dynamism of the gastrointestinal track, otherwise it may end in undesired dispersion, which often end in damaging of normal cells. And the establishment of an effective shield is a difficult task for conventional pallets available in the market. But the electrospun fibers will be very handy in a situation like this, which not only retains bioactivity but also enable high loading. Motivated by this, Zhang *et al* demonstrated the anti-cancer activity of multiple drug loaded electrospun nanofibers under *in vitro* and *in vivo* conditions. They used PLA as the carrier polymer to safely deliver the drugs 5-FU and oxaliplatin, whose cytotoxicity against colon tumours are validated in several studies [133]. The drug oxaliplatin is the newest platinum based anticancer agent alongside cisplatin and carboplatin. Oxaliplatin exerts its cytotoxic effect through damaging DNA of cells. Briefly, the apoptosis of cancer cells is reported to cause by formation of DNA lesions, arrest of DNA synthesis, inhibition of RNA synthesis, and triggering of immunologic reactions. However, the underlying mechanisms of those effects are not yet revealed completely [134]. Typical electrospinning setup was used to fabricate anticipated nanofibers and the encapsulation efficiency of nanofibers was illustrated by various characterization studies. In the interim, the cell viability studies carried out on human colon cancer cells (CRC HCT8) expressed effective cytotoxicity of multiple drug loaded nanofibers which later confirmed by MTT and flow cytometry assays. Successively, they reproduced the inferred results also under *in vivo* condition using animal models. They implanted the synthesized nanofibers in CT26 tumour-bearing mice models then the anti-cancer activity was measured using histological and survival rate analysis. Alike cell studies, the multiple drug loaded nanofibers exhibits superior tumour inhibition when compared with intravenous injection of free drugs and commendably increased the life expectancy of tumour affected mice. It clearly proves the cogency of electrospinning based combination therapy in battling the progression of colon cancer [133].

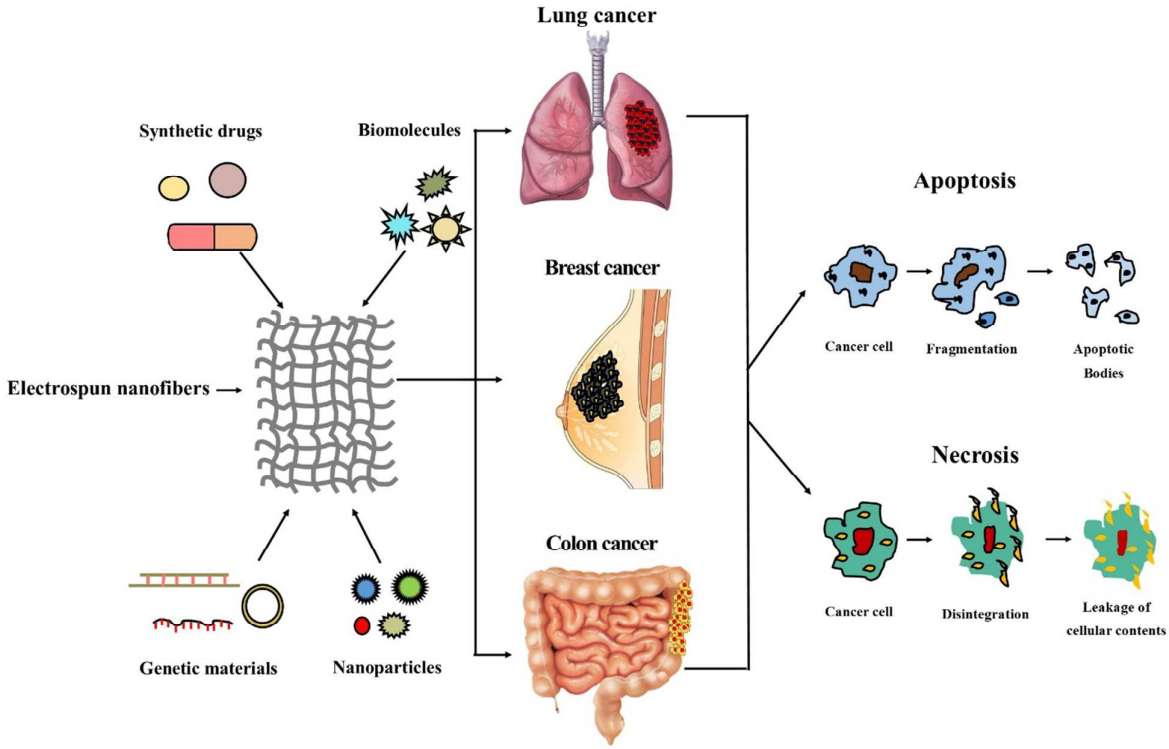
In another study, Liu *et al* developed a novel drug delivery system to fight against colon cancer by utilizing the concept of difference in energy metabolism between tumour and normal cells. They produced PCL nanofibers loaded with sodium dichloroacetate (DCA) and diisopropylamine dichloroacetate (DADA) respectively. And it was administrated through *in situ*

to C26 tumour-bearing mice separated into appropriate groups. The histological pathology performed after 12th d of conduct revealed that animals applied with PLA/DCA and PLA/DADA accomplished the suppression of tumour growth by 75 and 84 percentage respectively. Approximately, more than 95% of cancer reduction was achieved at the end of 15th d. Therefore, DCA or DADA coupled PCL nanofibers can be employed to avert systemic side-effects by discriminating cancer and normal cells [135].

6. Conclusion and future prospect

Establishment of modern drug delivery system is greatly essential to control current breach and future threats of cancer. In particular, the nanotechnology empowered delivery systems will play a pivotal role in full filling therapeutic goals such as increased drug absorption, distribution and minimum excretion. The current clinical modalities are badly hammered by the high affinity of systemic side effects and recurrence of the disease. Accordingly the booming death rates and economic loss, insinuates timely implementation of effective alternatives. Whilst the modern drug delivery systems like hydrogels, nanoparticles, nanocomposite, micro/nanofibers, liposomes, micelles, nanotubes, dendrimers, quantum dots etc., have reported to possibly avert the existing complications. The presence of devoted vehicle arrangement to carry drugs, shielding materials and targeting molecules are the main advantage of modern delivery systems which enable them to offer site specific medication. They precisely reach the cancerous site through passive or active targeting and can adjust the release of desired biomolecules in response to the bodily environment, hence they are popularly called as stimuli -responsive or intelligent drug delivery vehicles. Among various alternatives, the electrospun based micro/nanofibers are reported to be more emphatic because of the high surface to area, easy tailoring, cost-effective and able to retain the bioactivity of therapeutic agents. It helps to achieve pharmacokinetics viewpoints like high loading of drugs, targeted delivery, controlled extend release, maintaining required drug concentration in blood and avoiding drug resistance. In this review, the works reported on the exploitation of electrospun micro/nanofibers to address lung, breast and colorectal cancers were discussed in detail. As mentioned, more than 40% of total cancer deaths are reported due to the above types moreover the chance of developing metastasis is also high in these types.

Various promising ways of delivering biomolecules ranging from synthetic drugs to DNA plasmids were illustrated using electrospun micro/nanofibers. In all cases, electrospinning boosted drug delivery systems exhibited an excellent drug loading capacity, easy incorporation of desired agents, required encapsulation efficiency, pre-determined/controlled release thereby achieving advanced cytotoxicity as demonstrated in figure 7. Moreover, its pharmacokinetic properties can be productively modified based on the applications for instance, nanofibers can engineered to have an initial burst to maintain a high concentration of drugs in blood and vice-versa. It enables them to correspondingly treat developing tumours, advanced tumours and also post-operative tumours. Meanwhile, the importance of electrospinning parameters and loaded active substances in influencing the efficacy of nanofibers was also pinpointed. The poor selection of parameter ranges and the addition of less compatible anti-cancer agents were always reported to critically affect fiber diameter, surface area, degradation or drug releasing behaviour and cytotoxicity (as listed in table 1). However, these defects can be easily rectified by trying out different combinations of various parameters to achieve an ideal outcome. And they have also proposed the usage of statistical experiment designing methods for tailoring those essential factors to reduce the time consumed by the practical approaches. Though most of the work has been reported only chiefly fall within the horizons of chemotherapy. But to combat the future demand, the applications of nanofibers need to be expanded to emerging alternatives like gene therapy and immunotherapy. The notion of gene therapy aims to hammer oncogenic cells by suppressing or destroying its expressive genes so as to control its growth and induce apoptosis. This can be made possible by delivering DNA plasmids, miRNA, siRNA, RNAi and other genetic materials. But the clinical implementation was hindered by lack of site-specific delivery, unable to maintain bioactivity and meagre bioavailability. However, in recent years, scientists were able to get through these barriers commendably with the help of nanotechnology. Meantime, the capabilities of electrospun nanofibers have attracted several researches on the delivery of this genetic material through both viral and non-viral vectors. It has been reported to use for treating genetic disorders, infectious diseases and tissue engineering application as well [91]. In particular, the nanofibers based gene delivery or silencing is already proved to be a potential method for tissue engineering purpose like bone regeneration, vascular repair or regeneration, skin regeneration and also for neural restoration [136-140]. Furthermore, the work done by Achille *et al* meticulously showed the aptitude of electrospun fibers in succeeding at most bioactivity and also the significant eradication of breast cancer cells followed by successful silencing of expressive oncogenic genes [92].



on delivering synthetic or natural anti-cancer agents and

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Figure 7: Diagrammatic representation of cytotoxicity effects of various electrospun fibrous mesh

Likewise, the immune therapy is another exciting method which employs our own defence system to scavenge cancer cells without the usage of any toxic chemicals. Here, man-made antibodies will be utilized to train our immune mechanism to outsmart disguised cancer cells. Its Incorporation in nanofibers will help in both cancer prevention and treatment. In addition, the coupling of genetic materials or antibodies with chemotherapeutic agents may give rise to a new type of potential remedies typically referred as combination therapy. Therefore, future researches on electrospun micro/nanofibers have to be broadened beyond chemotherapy and more works need to be carried out on advanced concepts to bring out the best product for clinical usage. At the same time, probing process should not end with laboratory, it has to be encouraged to undergo clinical trials to enable timely establishment of this potential alternative.

Table 1: Effect of loaded drug/bioactive substances on surface morphology of electrospun fibers

Carrier Polymer	Drug/bioactive substances loaded	Key Changes in fibre morphology	Reference
Polyethylene oxide (PEO), chitosan (CS) and graphene oxide (GO)	Doxorubicin (DOX)	Nanofibers with minimum diameter was obtained in lower concentration of GO until 0.5% due to increase in the conductivity of prepared solution. At elevated GO concentration, nanofibers with beaded morphology were obtained due to increase in viscosity. Accordingly, the drug loading capacity also affected when increasing GO concentration because of π–π staking between DOX and GO.	64
Poly-L-lactic acid (PLLA)	Titanocene dichloride (TD)	Beaded nanofibers were observed while increasing the concentration of drug more than 15%. Further, increasing drug concentration also inferred to affect mechanical properties and release kinetics as well.	71
PCL	Curcumin (CU), aloe vera (AV) and neem (NE).	Excessive addition of CU and NE reduced tensile strength, young's modulus and elasticity in contrast to AV. Fibre diameter increased with increasing concentration of CU due to increase in viscosity. However, the incorporation of CU was inferred to advance encapsulation and releasing behaviour of natural extracts.	82
PCL/MWCNT's	Green tea polyphenols (GTP)	Animated levels of GTP increases fibre diameter due to increase in viscosity. The plasticizing effects of GTP reduced	83

		young' modulus, maximum tensile strength and elongation at break point of nanofibers.	
PCL	pKD-Cdk2-v5 plasmid (Cdk2i) and PCL/pKD-EGFP-v1 plasmid (EGFPi)	Tangled fibers with grooved morphology was noted while adding both type of plasmids. Drastic increase in fibre diameter from sub-micron level to micron was noted after plasmid incorporation.	92
Collagen (CG)/ PNIPAA/chitosan (CS)	5-Fluorouracil (5-FU)	The nanofiber geometry was highly influence by the concentration of chitosan but not by loaded drug. At higher concentration of chitosan, nanofibers with uneven and beaded morphology was obtained mainly due to increase in viscosity of electrospining solution. Because of this, more percentage of drug was deposited on nanofiber surface in the form of aggregates and only few was assimilated inside.	105
poly (D,L-lactide-co-glycolide) PLGA/PEO	Ferulic acid (FA)	Electrospraying was observed rather than nanofiber formation while increasing FA concentration more than 6%. Moreover, at higher concentration of FA series crystallization problem was raised which forms amorphous nanofibers.	108
Poly L–Lactide (PLLA), cellulose acetate (CA) and silk fibroin (SF)	Curcumin (CU)	Fibre diameter greatly varies depend on the concentration of CA, SF and CU respectively. All three electrospun fibers presented diversified distribution of pores based on loaded substances.	114
PLLA	5-FU	At lower concentration of 5-FU (1.6%), the fibre diameter of PLLA was decreased. Conversely, fibers with uneven morphology was obtained at maximum concentration (i.e) 12.8 % of 5-FU.	122

		Mainly due to uneven dispersion and increase in viscosity of spinneret solution while adding high amount of drug.	

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