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Review of Nano-Chitosan Based Drug Delivery of Plant Extracts for the Treatment of Breast Cancer

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Breast cancer is the most commonly diagnosed cancer and the leading cause of death in females, worldwide. Many therapeutic strategies though available does not effectually reduce the cancer burden. Alternative system of medicine and an effective mode of drug delivery is a major part of ongoing cancer research. Traditional Siddha literature refers to cancer as "*Putru*" and elucidates the use of extracts from various plant parts for the treatment of cancer. For example, extracts of *Mimosa pudica, Plumbago indica, Vitex trifolia, Glycyrrhiza glabra, Alstonia scholaris, Withania somnifera, Aegle marmelos* have been studied and shown to possess anticancer property. It is shown to decrease the adverse side effects of chemo and radiotherapy due to the presence of antioxidants. To heighten the bioavailability of the extract and controlled release, it can be delivered along with or encapsulated within a biomaterial. Chitosan and their derivatives are well-known polycationic polymers in the field of biomaterials. Chitosan can be prepared as a colloidal system for delivery in the form of microsphere, hydrogel, nanoparticles and can be modified to improve adhesion by crosslinking, chemical modification and conjugation with macromolecules. They have the advantage of being able to penetrate tight junctions of the cell membrane, biodegradable and mucoadhesive. Glycol-chitosan nanoparticles exhibited tumour-homing property which is an advantage for its use in targeted delivery of anti-tumour agents. Drug loaded-glycol modified chitosan nanoparticles have tumour inhibitory property because of enhanced permeation and retention capacity. Chitosan as a delivery system enhances the controlled drug release and modulates sustained drug bioavailability thereby delivering effective therapy. The use of chitosan encapsulation of anticancer extracts of medicinal plants can be a promising avenue to explore for their potential in breast cancer therapy.

Introduction

Cancer is a large group of diseases and a major public health problem. It is one of the major non-communicable diseases and the second leading cause of deaths globally [1]. In women, breast cancer represents one in four diagnosed cancers and very recently have become the most commonly diagnosed cancer [2]. The survival rate for breast cancer patients have increased over the years but still there remain disparities because of various factors such as early screening and diagnosis and cost-effective therapy.

The various treatment options available include surgery for excising solid tumours, chemotherapy involving cytotoxic drugs, hormonal therapy, radiation therapy with high-energy waves and immunotherapy [3]. These treatment options hold good for breast cancer also. Based on the type of breast cancer and the extent to which it has spread (metastasized), the age and general health of the patient, the treatment regime will be decided by the physician. By far, chemotherapy is the most used form of treatment for breast cancer with or without surgery. The adverse effects of the

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drugs used in chemotherapy is of major concern. Since they do not target cancer cells specifically, they tend to destroy healthy cells and thereby leading to systemic ill effect on the patient.

There are many plant species that can hold answers for providing us with a better drug(s) having anticancer potential. At the same time, the efficacy of such drugs come with markedly reduced side effects on the healthy cells of the body. Ancient and traditional literatures can guide us in identifying such plants for extraction of anticancer agents. Also, if we are able to deliver these less-toxic, plant-derived agents, to the site of tumour, with the help of nano-delivery systems, it would open new avenues in breast cancer treatment.

Drug delivery systems make use of natural and synthetic biopolymers like chitosan, alginate, polyethylene glycol (PEG), poly (lactic-co-glycolic acid) (PLGA) to carry the drug and deliver it on site [4]. This is to reduce systemic exposure to the agent as well as to deliver maximum drug concentration at the required site. Biomaterials possessing excellent therapeutic efficacies which acts as a cancer drug delivery tool can prove to be a superior option when compared to chemotherapy. When considering natural material, chitosan can be a good choice for drug delivery system at the nano scale. Chitosan is obtained from abundantly sourced chitin and has been studied for their various properties for many years. Chitosan, a polycationic polysaccharide material, is one of the favourable choices of biomaterial since it is non-toxic, mucoadhesive and biodegradable [5]. Certain derivatives of chitosan have higher water-solubility, form polyelectrolyte complexes with anionic substances. Chitosan can be modified in such a way that pH-sensitive release of conjugated or encapsulated agents can be formulated.

Being the era of personalized medicine and targeted delivery, the study on chitosan, its derivatives and their modified products as a nano-carrier system for targeted delivery of plant extract in the treatment of breast cancer is called for. As a part of our ongoing research in the development of novel scaffolds [6,7,8] and based on our previous experience in the development of candidates from natural sources [9,10], in the search of anti-cancer molecules, we intend to write this review to exemplify the role chitosan and its derivatives and by conglomerating selective herbs being used in the traditional *Siddha* system of medicine, to develop chitosan encapsulated-nanoparticles based drug delivery systems.

Herbs in Cancer Treatment

Natural products containing bioactive agents or phytochemicals is a major part of traditional medicines. These are used by approximately 70% of the Indian population and about a quarter of the modern drugs in use are of plant origin. These phytochemicals are mostly the secondary metabolites such as alkaloids, phenols, tannins possessing varied medicinal activities. Many chemotherapeutic agents currently used like vinca alkaloids, anthracyclins and taxane are plant-derived materials [11]. Anticancer potential of whole plants or their parts are long known and are being scientifically established for the retro pharmacology

Many plants have been mentioned in traditional literature for their effectiveness against breast cancer which includes *Asparagus racemosus, Alstonia scholaris, Aegle marmelos, Mimosa pudica, Plumbago zeylanica, Plumbago auriculata, Plumbago rosea, Plumbago indica, Pterocarpus marsupium, Semecarpus anacardium, Taxus baccata, Withania somnifera, Plumeria alba, Plumeria rubra, Euphorbia umbulata, Vinca rosea* [12]. The parts of the plant used ranges from whole plant, leaves, roots, stem/bark, flower to fruits/seeds. The ethanolic fruit extract of *Aegle marmelos* possess anti-proliferative property along with being hepato-renal protective effect [13]. The chloroform/methanol leaf extract of *Asparagus racemosus* showed reduction in tumor incidence when female rats were subjected to a carcinogen [14]. *Mimosa pudica* has shown to exhibit high cytotoxic activity against human breast cancer cell lines. The extracts of *Plumbago indica* inhibits growth and spread of breast cancer due to their antiproliferative activity. The growth of MCF-7 cells was inhibited by the leaf extract of *Vitex trifolia* and root extract of *Glycyrrhiza glabra*. The alkaloid fraction of *Alstonia scholaris* exhibited time dependent antineoplastic effect. As an external application the root extract of *Withania somnifera* has shown to possess tumor inhibitory capacity. The pulp and seed extract of *Aegle marmelos* is said to affect the gene expression of MDA-MB-231 cells thereby inhibiting cell proliferation.

These phytoconstituents impose their effect through antiproliferative property, apoptotic activity, anti-neoplastic activity, protection from oxidative damage of healthy cells [15,16]. The molecular basis of the action of isolated compounds or crude plant extract are being assessed to better understand their mode of action. Through extensive research on some of the plants, using *in vitro* and *in vivo* assays, their *modus operandi* at the molecular level has been established. The natural source such as the plant kingdom still remains as an invaluable source for identifying and obtaining new bioactive molecules as well as to develop novel Phytopharmaceuticals.

Nanodelivery System

The advent of nanotechnology in biomedical application has created a vast impact. The drug delivery system is one major avenue wherein nanotechnology is conglomerated with the phytochemicals for developing novel drugs. The nanoparticulate system as a carrier for drug in cancer treatment has many benefits. It helps in targeting the drug precisely to the site providing high drug concentration, formulating its druggability properties such as solubility and stability, and sparing non-target organs to the maximum extent possible. Various vehicles through which nanostructures can be used as drug carriers includes polymeric nanoparticles, micelles, liposomes among others [17].

The therapeutic efficacy of nano formulations is enhanced due to increased circulation time of the drug loaded formulation and increased drug exposure of the tumour by preferential drug accumulation. The synthesis of drug encapsulated polymeric nanoparticles has been studied vastly. The polymers used for the purpose varies from natural polymers like chitosan, collagen to synthetic polymers like Poly (lactic co-glycolic acid) (PLGA), Polyethylene glycol (PEG) or a combination thereof. Vincristine and Verapamil loaded PLGA nanoparticles have been studied, where the synergistic activity showed effective anti-tumour activity in a multidrug resistant human breast cancer xenograft model [18]. Paclitaxel and Etoposide co-loaded PEG modified PLGA nanoparticles were synthesized and studied. It showed sustained release profile thereby enhancing the chemotherapeutic effect of the drugs. The improvement in pharmacokinetic profile, enhanced blood circulation time and accumulation of the drug in tumour could be due to the enhanced permeation effect [19]. To circumvent the poor solubility of hydrophobic agents, encapsulated polymeric nanoparticle can be used as a drug delivery system. The solubility of curcumin was shown to be enhanced when delivered as an encapsulated polymeric NPs, using micellar aggregates [20]. Liposome mediated drug delivery is a well-known system but having a major drawback of rapid uptake by mononuclear phagocyte system. Surface modifications of liposome can help to overcome this hurdle and enhance blood circulation time. For example, pH-sensitive liposomes of urosilic acid had better anticancer activity against MDA-MB-231 cells when compared to urosilic acid [21].

Vincristine encapsulation as a liposome, enhanced the drug accumulation at the target site thereby increasing the lethality dose more than 2-fold in mice model [22].

Chitosan as a Drug Carrier

Chitosan is a cationic polymer and is derived from deacetylation of chitin obtained from shrimp, fish and crab exoskeleton. As a biomaterial it has gained a lot of attention for its low toxicity, biodegradability, muco-adhesiveness and biocompatibility. The physicochemical properties such as degree of deacetylation (DDA), water content, viscosity, molecular mass, among others, forms the basis of its varied uses. Chitosan exhibits anticancer activity and low toxicity on non-cancerous cells, which is attributed to the molecular mass and DDA [23]. Better uptake of nanosized chitosan by fibroblasts cells increases with increase in DDA. With such advantages, chitosan can prove to be an effective carrier vehicle for anti-cancer agents.

Chitosan nanoparticles (CSNPs) can be prepared predominantly by three methods such as, ionic-gelation, emulsification and spray drying.The method of ionic-gelation is an organic solvent free approach wherein there is interaction between macromolecule and a multivalent material. The major advantage in this method of CSNP preparation is high loading capacity. Emulsification process involves dispersing one liquid in another in the presence of an emulsifier and a surfactant to stabilize the synthesized structure. Spray-drying is a simple and cost-effective method of CSNP preparation. The bioactive compound has to be homogenized with the matrix. Capsules are collected when evaporated water comes in contact with atomized material [24]. Apart from CSNPs chitosan can be used for the synthesis of nanofibers, nanogels, nanocomposites and nano-coatings. All forms of chitosan delivery system can be used to encapsulate anti-cancer drugs. The chitosan polymer can be modified chemically or enzymatically to improve solubility and to modify release profiles of the encapsulated drugs.

Modified chitosan-alginate nanoparticles encapsulating alkaloid extract of *Sphaeranthus amaranthoides* showed anticancer activity against Non-Small Cell Lung Cancer (NSCLC) A549 cell line [25]. Chitosan nanoparticles containing hydroalcoholic extract of *Posidonia oceanica (L.) Delile* possessed better encapsulation efficiency and release profile with inhibition of cell migration in neuroblastoma cells [26].

Curcumin-loaded Solid Lipid Nanoparticles (SLNPs) coated with chitosan was found to lower doxorubicin resistance in triple negative breast cancer [27]. Curcumin-loaded chitosan NPs showed better activity against colorectal cancer than curcumin. This may be attributed to the mucoadhesive property of chitosan and sustained release of the encapsulated drug [28]. Epigallocatechin-3-gallate obtained from *Camelia sinensis* encapsulated in water-soluble chitosan inhibited the growth of prostate cancer cells [29].

One of limitations of chitosan is its low solubility in water and polar solvents which can be overcome by modifications in chitosan and its derivatives. Glucosamine chitosan derivative has enhanced solubility [30]. Arginine-modified chitosan co-encapsulating curcumin and BSA showed enhanced intestinal permeability [31]. Chitosan coated magnetic alginate nanoparticles were used to encapsulate curcumin and studied against breast cancer cells. The uptake of curcumin by MDA-MB-231 cells were 3 to 6 times higher and also the uptake by noncancer cells were significantly low [32].

Route of Delivery

Chitosan coated polymeric drug carrier system has advantages like enhanced muco-adhesiveness, tissue penetration, controlled and prolonged drug release and improved drug bioavailability. Chitosan coating gives a positive charge thereby increasing cellular internalization due to ionic interaction with cell membranes [33]. The nasal route of delivery for compounds like resveratrol is enhanced by surface modification of chitosan nanoparticles. Chitosan coating might overcome the blood-brain barrier in delivery of many drugs [34].

The chitosan nanoparticle can be prepared and administered for different routes of delivery. The oral route of administered is the most convenient route at the same time with several short falls like hepatic first-pass, digestive enzymes, pH variation and intestinal barrier for the absorption of drugs [35]. Chitosan as a nano carrier in oral drug delivery can overcome the above limitations thereby improving oral bioavailability. Lecithin-chitosan nanoparticle carrying tamoxifen improves the permeation of the drug through intestinal epithelium [36]. The doxorubicin drug containing chitosan and carboxymethyl chitosan showed enhanced intestinal absorption in the small intestine [37].

Compared to other routes, pulmonary route of drug delivery has the advantage of having high efficacy, sustained release and no hepatic first-pass. The use of chitosan NPs for delivering drugs through pulmonary modality has the advantage due to the positive charge of chitosan. Since this helps in the interaction of chitosan and mucosal layer which opens the tight junctions of the epithelial cells of the lungs [38]. In addition to the advantages, the safety and toxicity studies on chitosan-coated PLGA showed lower toxicity and better bioavailability when compared to non-coated Nps [39].

Mucoadhesion is a natural property of chitosan and this can be utilized to produce mucoadhesive nanoparticle for drug delivery. Many of our system like respiratory, gastrointestinal, urogenital have mucous lining and hence chitosan based mucoadhesive films can be prepared to deliver drugs [40]. Curcumin loaded nanoparticles which was coated with chitosan was used to prepare a mucoadhesive film to test buccal curcumin release. The results showed that chitosan based mucoadhesion can be a good candidate for buccal route of drug delivery [41]. Flavonoid fractions from *Cecropia glaziovii* was loaded into PLGA nanoparticle coated with chitosan for buccal delivery and it showed no signs of cytotoxicity and improved bioavailability [42]. The toxicity study of chitosan-based buccal NPs showed that chitosan was comparatively less toxic compared to alginate and pectin NPs [43]. The preparation method of chitosan for drug formulation and the choice of delivery route should be addressed with care to achieve maximum efficacy and minimal toxicity. Human toxicity profile for chitosan has to be focussed.

Conclusion

The role of Chitosan and its derivatives in line with the potential herbs being used in the traditional *Siddha* system of medicine has been reviewed. The effective utilization of nanoparticles in Novel Drug Delivery Systems (NDDS), opens the new avenue in the treatment of personalized health care concepts as well as scientific validation of traditional systems of medicine especially in breast cancer treatments. The application of flora and fauna with biodegradable nano-Chitosan derivatives will substantially reduce the toxicity in humans. However, the exact molecular mechanisms pertaining to the efficacy and toxicity will be derived based on scientific and statistical analyses.

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