Folate Receptor Targeted Delivery Systems: A Novel Micellar Drug Delivery Approach

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Abstract
Cancer is a pathological condition characterized by uncontrolled proliferation of cells that invade surrounding tissue and metastasize to new sites in the body. This disease is difficult to treat since cancer cells, unlike bacteria or virus, do not contain molecular targets completely foreign to the body. The goal of any therapy is to treat the affected tissue with minimal damage to normal tissues. With the current cancer therapies, this is often difficult to achieve as the drugs are cytotoxic in nature and often causes widespread damage to normal tissues as well. Thus, a need for targeted therapy for cancer has evolved in recent times. This review details a novel targeted micellar drug delivery approach that involves targeting of drugs and drug delivery systems to cancer cells that specifically express folate receptors on the cell surface. This review describes the synthetic design approach, and the ability of folate labeled amphiphilic system to form micelles which can be used as targeted drug carriers to cancer tissues.

Key words: Cancer, Chemotherapy, Radiation Phagocytosis

Introduction
The goal of a treatment regime against cancer is to eradicate all cancer cells from the body or at least bring them down to such a number that the patient might outlive the time required for a relapse of the disease. This can be accomplished in a number of ways. An obvious strategy is to surgically remove the cancer that is only possible when the tumor is localized, the tumor has not invaded the neighboring tissues and the mass of tissue to be removed can be partially replaced by the body to maintain homeostasis. Surgery is often complicated by the fact that tumors may grow at certain anatomically critical or inaccessible sites and the tumor cells may be extensively intermingled with healthy tissue. Radiation therapy is another alternative to treat tumors. Proliferating cells in the G2/M phase are highly susceptible to damage by radiation since they do not have enough time for DNA repair (1). Thus healthy tissues with a rapidly dividing population such as bone marrow, hair follicles, gastrointestinal tract and oral mucosa also get affected during radiation therapy and show various symptoms of acute toxicity. Apart from this, healthy organs that fall in the path of radiation but do not have a rapidly dividing cell population also get affected over time and may cause reduction in the dose of radiation to be given to patients over their lifetime. This is due to the fact that these organs require a longer time to recover. Yet another approach to tumor mitigation is chemotherapy. Similar to radiation therapy proliferating cells are susceptible to cytotoxic drugs and conventional chemotherapeutic agents kill cells by disrupting the cell division or by DNA damage. Their action is non-specific and may
cause serious damage to healthy cells. Thus, in both these paradigms, the therapeutic window is narrow and the dose given to a patient relies heavily on the dose limiting toxicity experienced by the patient that arises due to non-specific cell kill from the treatments. This essentially forms the desired features a delivery system that is designed to target specifically cancer cells while doing minimal harm to normal tissues. Targeted delivery was originally proposed in the early 20th century by a German scientist Paul Ehrlich. This idea, called magic bullet, was developed from his desire to create compounds that selectively target the disease causing organism while sparing the normal tissues.

**Mechanism of Tumor Targeting**

The physical basis for tumor targeting lies in the fact that the tumor vasculature is more leaky than in normal tissues (2). Thus macromolecular drug conjugates get into the tumor by diffusion, convection and transcytosis in an exchange vessel. Among these routes of entry, diffusion is considered to be the major route of transvascular transport as the interstitial fluid pressure of the tumor is high due to high vascular permeability and low lymphatic drainage (3, 4). The drug conjugates targeted to tumors in this fashion are classified under passive tumor targeting. The submicron size range of drug delivery systems is often used to target tumor tissues passively by enhanced permeation and retention effect. Since tumor tissues have leaky vasculature, the delivery system escapes from the circulation into the tissue yet cannot drain back into the circulation due to high hydrostatic pressure in the vessel. The delivery system needs to be in circulation for a considerable amount of time is needed for both active or ligand dependent targeting as well as passive targeting. At present, Doxil®, pegylated liposomal formulation of doxorubicin and Abraxane®, nanoparticles formulation of paclitaxel, are examples of passively targeted chemotherapeutic agents. On the other hand, tumor cells not only differ in physical aspects from normal tissues but they also express different levels of pro-survival proteins that promote growth (5-7). The different levels of these proteins serve as biomarkers of cancer and are targeted for therapeutic purposes and are more commonly referred to as active targeting. The common mode of uptake of any drug delivery device in active targeting is by receptor mediated endocytosis (8). It has also been observed that functional inhibition of certain biomarkers in cancer leads to tumor cell death (9-11). Thus the targeted tumor therapy currently encompasses both the fields of active tumor targeting and chemotherapeutics that specifically target one or more biomarkers to elicit tumor cell death. The scope of this article is limited to active targeting and further discussions will be limited to active targeting of chemotherapeutics.

**Design Principles of Targeted Delivery Systems**

A targeted delivery system consists of a homing device connected to a delivery system which carries a payload of the drug. The homing device is usually a small molecule ligand or an antibody for a receptor to which the delivery system is targeted. Since antibodies to a target protein are highly specific they make good homing devices. The nature of the delivery system depends on the physicochemical properties of the drug and the ligand, the regional constraints of the target and the time for which the delivery system needs to be available for action. In some cases, the drug may be directly attached to the homing device. Reactive functional groups on the drug are often utilized for making conjugates of drugs and the homing device. In such a system, after the drug reaches the target, it must be cleaved from the homing device to exert its action as conjugation to the homing device often results

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in loss of pharmacological activity of the parent molecule. Insertion of acid labile linkers or cleavable peptide sequences is often used to tag drugs to homing devices so that they can be released later in the cell. Labeling a delivery system with the homing device constitutes another method of targeted delivery. The drug is either physically entrapped in the delivery system or chemically conjugated with it. Examples of such systems include drugs encapsulated in targeted liposomes or nanoparticles and targeted drug polymer conjugates. Homing devices are conjugated directly or via spacers to the delivery systems. The spacers often provide with reactive endgroups that are used for conjugation reactions or they may act to reduce steric hindrance offered to the homing device and target interaction by other components of the delivery system. Common spacers used in delivery systems include polyethylene glycols, whose terminal hydroxyl group is substituted by an amino or a carboxylic acid group, ethylene diammine and short alkyl dicarboxylic acids. The common elimination pathways for macromolecular delivery system are elimination by phagocytosis by macrophages and by the reticulo endothelial system. Phagocytosis can be minimized by the use of polyethylene glycol coating on the delivery system. The coating makes the system more acceptable to biological systems and thus evades phagocytosis. Some common approaches to targeted delivery involving cell surface receptors are illustrated in Figure 1.

**Fig. 1:** A summary of strategies used for targeting chemotherapeutics to tumors

A Novel Micellar Drug Delivery Approach
The Folate Receptor

The discovery of the folate binding protein in the human placenta provided clues for a method of site specific drug delivery (12-16). The folate binding protein also called the folate receptor is a 38.5 kD glycoprotein protein with a high affinity \([kD = 10^{-9} \text{ M}]\) for folic acid (12). The receptor may be lost from the cell surface by the activity of a metaloprotease and is found to be excreted in human or bovine milk (17). The receptors are also linked to the cellular growth kinetics and are found to be less expressed in slowly growing cells or when a colony reaches confluence (18). The genes encoding this receptor are located on chromosome 11q13 at the FGF3 locus (19). The folate receptors are diffusely distributed on the cell surface but multimerize by binding to secondary antibodies and are concentrated in the caveolae (20). After binding to folic acid, the receptor is internalized and is recycled back to the cell surface after dissociation from the substrate (21) (Fig. 2). This caveolar concentration of the receptors is also controlled by cholesterol and the internalization takes place by a non-endocytic process (22). The receptor mediated pathway of folate uptake is regulated by intracellular levels of folic acid (23). Although the folate receptor is found widely distributed in the body (24), the folate receptor-alpha is over-expressed consistently in non-mucinous ovarian carcinomas and tumors of epithelial lineage in endometrium, lung, breast, renal cells and brain metastases (25). Thus the therapeutic advantage of targeting the folate receptors is due to their over-expression, often twenty times more, in these types of malignancies than in epithelial cells or fibroblasts (24). Current approaches that utilized folate receptor in targeted delivery systems are listed in Table 1.

\[
\begin{align*}
\text{FR(α) occurs in caveolae as well as diffusely on the cell surface} \\
\text{Drug/delivery device diffuses into cytosol} \\
\text{Upon binding to folate the receptors are internalized in endosomes with v-type Na\text-/ATPase proton pump} \\
\text{Decrease in pH in the endosomes causes release of folate labeled conjugate due to change in conformation of the receptor} \\
\text{Receptors are recycled back to the plasma membrane} \\
\text{Folate receptor (α)} \\
\text{v-type Na/ATPase proton pump} \\
\text{Folate conjugates}
\end{align*}
\]

**Fig. 2:** Intracellular trafficking of folate receptors

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Table 1. Current approaches that utilized folate receptor in targeted delivery

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Drug</th>
<th>Delivery system</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA-TPGS-Dox+TPGS-Fol</td>
<td>Dox</td>
<td>Nanoparticle</td>
<td>(26)</td>
</tr>
<tr>
<td>Fol-peptide-imaging agent</td>
<td>Pyropheophorbide</td>
<td>Conjugate</td>
<td>(27)</td>
</tr>
<tr>
<td>Poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide-co-undecenoic acid)-Fol</td>
<td>Taxol</td>
<td>Polymeric micelle</td>
<td>(28)</td>
</tr>
<tr>
<td>Fol-PEG-PLGA</td>
<td>Dox</td>
<td>Polymeric micelle</td>
<td>(29)</td>
</tr>
<tr>
<td>Fol-PEG-OligoDN-GFP</td>
<td>Gene</td>
<td>Polymeric micelle</td>
<td>(30)</td>
</tr>
<tr>
<td>Fol-poly histidine-PLLA</td>
<td></td>
<td>Polymeric micelle</td>
<td>(31)</td>
</tr>
<tr>
<td>Fol-PEG-PANAM G3.5</td>
<td>Indomethacin</td>
<td>Dendrimer</td>
<td>(32)</td>
</tr>
<tr>
<td>Fol-PANAM G5</td>
<td></td>
<td>Dendrimer</td>
<td>(33)</td>
</tr>
<tr>
<td>Fol-PAMAM</td>
<td>Methotrexate</td>
<td>Dendrimer</td>
<td>(34-36)</td>
</tr>
<tr>
<td>Fol-PEG-DOX</td>
<td>Doxorubicin</td>
<td>Nanoparticle</td>
<td>(29)</td>
</tr>
<tr>
<td>Fol-PEG-chitosan</td>
<td>Gene</td>
<td>Nanoparticle</td>
<td>(37)</td>
</tr>
<tr>
<td>Fol-BSA</td>
<td>Protein</td>
<td>Nanoparticle</td>
<td>(38)</td>
</tr>
<tr>
<td>Fol-Chitosan</td>
<td>DNA</td>
<td>Nanoparticle</td>
<td>(39, 40)</td>
</tr>
<tr>
<td>Fol-PEO-PPO-PEO/PEG</td>
<td>Taxol</td>
<td>Nanoparticle</td>
<td>(41)</td>
</tr>
<tr>
<td>Fol-Penicillinn G amidase</td>
<td>Phenacetyl-Dox</td>
<td>FDEPT (ADEPT)</td>
<td>(42)</td>
</tr>
<tr>
<td>DPPC/DMPG/mPEG-DSPE/folate-PEG-DSPE</td>
<td>Taxol</td>
<td>Liposome</td>
<td>(43-48)</td>
</tr>
<tr>
<td>Desacetylvinblastine monohydrazide-Fol</td>
<td>Desacetylvinblastine</td>
<td>Conjugate</td>
<td>(49)</td>
</tr>
<tr>
<td>Polyether polyol-PEG-Fol</td>
<td>Tamoxifen</td>
<td>Dendrimer</td>
<td>(50)</td>
</tr>
<tr>
<td>Fe oxide-PEG-Fol</td>
<td></td>
<td>Nanoparticle</td>
<td>(42, 51, 52)</td>
</tr>
<tr>
<td>Thioctic acid-PEG-Fol on Au nanoparticles</td>
<td></td>
<td>Nanoparticle</td>
<td>(53)</td>
</tr>
<tr>
<td>Fol-Solid lipid nanoparticles</td>
<td>Hematoporphyrin, taxol</td>
<td>Solid lipid nanoparticle</td>
<td>(54)</td>
</tr>
<tr>
<td>Fol-PEG-Polycaprolactone</td>
<td>Paclitaxel</td>
<td>Nanoparticle</td>
<td>(55)</td>
</tr>
</tbody>
</table>
Micellar Drug Delivery System

The interaction of oil films on water surface has been well documented. But the interaction of hydrocarbon chains in the bulk of water was theorized principally by J. Traube in late nineteenth century. He noted that a long hydrocarbon chain attached to a polar group tends to migrate to the surface of water rather than stay in the bulk of the solution. Their presence at the surface of liquid can be measured by the decrease in surface tension which is linear at very low bulk concentration of the solute. At high concentrations of the solute the decrease in surface tension loses this linear inverse relationship and begins to saturate. It is observed that at low concentrations of an amphiphilic solute the ratio of the surface concentration of the solute to that of the concentration in bulk increases threefold for addition of one methylene group to the hydrocarbon chain. Such a relation also exists in homologues series of other amphiphilic molecules. Thus the cause of the observed effect is due to the lack of affinity of the water molecules for the hydrocarbon chains. Measurements of the free energy of attraction of water and hydrocarbons yield a value of -40erg/cm$^2$. The free energy of attraction of hydrocarbons for themselves is also about -40erg/cm$^2$ at the same temperature whereas, for water molecules the free energy of attraction is -144erg/cm$^2$. Thus it is the strong attraction between water molecules that supports the avoidance of water hydrocarbon interactions or the hydrophobic effect. The hydrophobic effect can be explained from a mechanistic point. Water itself is a highly structured liquid due to the presence of hydrogen bonds between water
molecules. For the dissolution of hydrocarbons in water some of these bonds must be broken in order to accommodate the hydrocarbon core. But at the same time the water molecules at the surface of the cavity formed by hydrocarbons in the bulk of the solution arrange themselves in order to regenerate the broken hydrogen bonds thereby, creating regions of higher degree of local order than present in pure water producing a decrease in entropy. An increase in the concentration of amphiphilic hydrocarbons in water will thus require the formation of hydrocarbon water interface resulting in a large decrease in entropy. It has been observed that the change in enthalpy ($H_{mic} - H_w$) for amphiphilic hydrocarbons is nearly zero for ionic and/or zwitterionic micelles and is positive for nonionic micelles hence the driving force for micelle formation, observed with an increase in the concentration of the amphiphile, solely arises from a positive entropy change. The hydrophobic effect drives micellization but the repulsion of headgroups limits its size. It is this balance of the two opposing forces that result in the formation of micelles as opposed to phase separation and are characterized by discrete aggregation number rather than a statistical size distribution (75). Some commonly used amphiphilic employed to construct micellar systems are listed in Figure 3.

One of the important applications of micellar systems is their solubilization capacity of poorly water soluble compounds. Solubilization of a poorly water soluble compound via micelles of an amphiphile is found to increase linearly after the critical Micellar concentration (cmc) has been reached (76). Micellar solubilization is analogous to partitioning of hydrophobic compounds between water and oil phases. It differs only in the fact that the micelles which compose the oil phase are dispersed in water resulting in clear homogeneous solution. The solutions are thermodynamically stable, but are sensitive to dilution if the concentration of the surfactant falls below the cmc (77). Thus the lower the cmc value of a surfactant, the more stable are its micelles towards dilution. This factor assumes importance in the formulation aspects of amphiphile drug blends used for parenteral administration as these undergo several folds of dilution in blood. As discussed previously, the micelles have a hydrophobic core and a hydrated hydrophilic shell, the loci of solubilization of drug molecules in the micelles thus varies with the degree of hydrophobicity of the solute (78). Compounds may be adsorbed at the micelle water interface or may be dissolved in the hydrocarbon core (Fig. 4). When adsorption takes place at the micelle water interface the solubility rises to a greater extent than when solubilization takes place at the hydrocarbon core (79). The shape factor of the micelle also influences the amount

![Fig. 3: Examples of different classes of amphiphiles classified according to their charge of the polar headgroup](image-url)
of drug it can solubilize. Depending on the balance of the head group repulsion and the hydrophobic effect from the tails, micelles tend to adopt a range of shapes from spherical to more ellipsoidal or disk like and in some cases rods and worm like shapes have also been observed. As the shape of the micelles deviate from the sphere to more disk like or rod like shape, the volume of the core region relative to that of the shell increases. Thus, solubility of drugs which tend to be dissolved at the core increases as the micellar shape deviates from sphere. In case of ionic amphiphiles the ionic strength plays an important role in determination of size and cmc in water. It is generally observed that an increase in the ionic species results in lower cmc and larger micelles. Solubilization of weakly ionic drugs by amphiphiles is often due to interaction of oppositely charged species. This is observed at a certain pH condition, the drug and the amphiphile acquire opposite charges and the drug is adsorbed onto the oppositely charged hydrophilic shell (80).

One of the well known applications of amphiphiles in amphiphile mediated drug delivery is that of Taxol®. Due to the poor solubility of paclitaxel in water which is 0.6 mM (81), it is formulated in cremophor EL and ethanol. Cremophor is a mixture of surfactants made from pegylated lipids derived from castor oil. Prior to administration it is diluted with water for injection and administered parenterally. The presence of the amphiphile, cremophorEL, prevents the drug from precipitation when diluted. The micellar solution results in altered pharmacokinetic profile of the drug than that observed for the free form. It has been suggested that the altered profile is due to formation of micellar carriers of the drug in systemic circulation (82). The use of amphiphiles in parenteral drug formulations suffers from a major drawback of toxic side effects which is partly due to the high levels of the amphiphiles that are used in the formulation to counter the effect of dilution of the solution in blood. CremophorEL is known to give rise to hypersensitivity reactions, abnormal lipoprotein patterns, aggregation of erythrocytes and peripheral neuropathy. Amphiphiles constructed from RGD-fatty acid conjugates were found to enhance the solubility of paclitaxel by 87% and their mixed micelles with commercially available Pluronics were found to increased the solubility to 2.12 (µg/mL) (83)

**Fig. 4:** Sites for drug solubilization in micelles

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Folate Labeled Micellar Drug Delivery System

In recent years, targeted drug delivery has become the method of choice in cancer chemotherapeutics due to their overwhelming non-specific tissue toxicity. One of the preferred targets for active targeting of chemotherapeutics is the folic acid receptor subtype α commonly referred to as FRα. This receptor is overexpressed in ovarian and endometrial cancers and has a high affinity for folic acid. Conjugation of drug moieties and drug delivery systems to folic acid offer a route to target cancer cells overexpressing FRα. The folic acid molecule bears a glutamic acid residue coupled via its amino group to pteroic acid. The carboxylic groups of the glutamic acid residue provide a site for conjugation of folic acid residue to a number of drug delivery platforms like polymeric micelles, nanoparticles, microparticles and bioconjugates. The regiospecific conjugation of the gamma carboxylic acid of the glutamyl moiety is preferred over either alpha conjugation or a mixed conjugated product of both alpha and gamma carboxylic acid groups as alpha conjugation reduces the affinity of the folate moiety towards its receptor (85). Conjugation of folic acid when performed with the usual amide coupling reagents like DCC, EDC and CDI usually result in a mixture of alpha and gamma products which are difficult to separate. Another synthetic scheme which offers regiospecific conjugation starts with the synthesis of a specifically gamma carboxylic acid derivatized folate acid analogs. The synthesis of various classes of folate labeled amphiphiles studied are summarized in Figure 5.

Micellar characteristics of Folate labeled amphiphiles

Micelle formation is regarded analogous to phase separation. But unlike phase separation, the formation of micelles occurs over a narrow critical range of concentration and it is customary to assign a single concentration in this transition.
zone as cmc. The cmc determination is based on a change in slope when an appropriate physical property that can distinguish between micellar and free amphiphile is plotted against total concentration. Various physical properties of amphiphilic solution such as osmotic pressure, solubilization of hydrophobic compounds, surface tension, light scattering intensity, turbidity and molecular conductivity change with increasing concentration of amphiphile as it approaches cmc (Fig. 6). To study the cmc of the synthesized amphiphiles pyrene fluorescence was used as a probe for microenvironment polarity. Pyrene is suited for this purpose as its monomer fluorescence has a long lifetime of 450 ns and it can efficiently form eximers. It is one of the few fused aromatic hydrocarbons that show significant vibronic bands in its monomer fluorescence spectra in solution phase. In the absence of any solvent interactions, the relative intensities of these vibronic bands in the spectrum are governed by relative potential energy levels of the excited singlet states relative to the ground state singlet and by Frank–Condon principle. The pyrene monomer fluorescence spectrum is considerably perturbed with the change of solvent from \( n \)-hexane (non-polar) to acetonitrile (polar). The major contribution to these perturbations is believed to be from specific solute-solvent dipole-dipole coupling. The pyrene monomer exhibits five distinct vibronic bands of which the third shows maximum variations in intensity relative to the first band and hence the ratio of intensity of the third to the first (I3/I1) is taken as a measure of perturbation (Fig. 7). This prominent solvent dependence of the vibronic fine structure is utilized in fluorescence probe studies of micellar systems. Pyrene is a hydrophobic probe with a logP of 6.0 and a solubility of 2-3 μM in water. In the presence of micelles and other macromolecular aggregates pyrene is solubilized in the hydrophobic domains of these systems. Below the cmc of the amphiphiles, pyrene exhibits a I3/I1 ratio of ~0.5, similar to that observed in water. As the amphiphile concentration is raised above the cmc, pyrene is solubilized in the hydrophobic interior and the I3/I1 ratio rises. The change in the microenvironment to non-polarity is also sensed by increase in the fluorescence life time of the pyrene monomer. Since both the lifetime and the I3/I1 ratio are a function of the microenvironment around the probe, both the parameters show sharp breaks of their slope with respect to total amphiphile concentration at the cmc and indicates the onset of micellization (Fig 7).

**CMC values for the folate labeled**

<table>
<thead>
<tr>
<th>Property</th>
<th>CMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmotic pressure</td>
<td></td>
</tr>
<tr>
<td>Solubilization of hydrophobic</td>
<td></td>
</tr>
<tr>
<td>compounds</td>
<td></td>
</tr>
<tr>
<td>Surface tension</td>
<td></td>
</tr>
<tr>
<td>Light scattering intensity and</td>
<td></td>
</tr>
<tr>
<td>turbidity</td>
<td></td>
</tr>
<tr>
<td>Molecular conductivity</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 6:** Changes in the magnitude of some observed physical properties of amphiphilic solutions below and above cmc values

**Fig. 7:** The stacked fluorescence spectra show a typical change in the vibronic pattern of pyrene fluorescence in the presence of amphiphiles. The arrows indicate spectral shifts with increase in the concentration of the amphiphile.

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amphiphiles were determined by pyrene fluorescence method. In surfactant solutions, above the CMC, inclusion of more than one pyrene molecule in the micellar core gives rise to an additional band at 480nm due to the formation of an excited dimer often referred to as eximer (Fig 7). Since the $\alpha$-carboxyl group of the glutamic acid was free in the final compounds, an alkaline pH of 8.4 was used to solubilize the amphiphile and the CMC of the molecules were determined at this pH. Increasing the pH further would make it unsuitable for biological studies and the amphiphile did not have sufficient solubility, neither for analytical studies nor for biological experiments, at any pH lower than this. It was observed that the cmc of the amphiphiles decreased with the increase in the hydrophobic chain length in a homologous series (Figs. 8-10 & table 2). The cmc of compounds bearing more than twelve carbon atoms in the first series FC(n) could not be measured due to very poor solubility of the compounds even at alkaline pH. The cmc(s) of all the other synthesized amphiphiles are listed in table 2.

**Table 2. CMC (s) of the synthesized amphiphiles and their yield**

<table>
<thead>
<tr>
<th>Amphiphile</th>
<th>Compound number</th>
<th>Critical Micellar Concentration (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC10</td>
<td>8a</td>
<td>37</td>
</tr>
<tr>
<td>FC13</td>
<td>8b</td>
<td>21</td>
</tr>
<tr>
<td>FDC10</td>
<td>9a</td>
<td>50</td>
</tr>
<tr>
<td>FDC13</td>
<td>9b</td>
<td>40</td>
</tr>
<tr>
<td>FDC15</td>
<td>9c</td>
<td>15</td>
</tr>
<tr>
<td>FPC10</td>
<td>10a</td>
<td>62</td>
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<tr>
<td>FPC12</td>
<td>10b</td>
<td>48</td>
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<tr>
<td>FPC14</td>
<td>10c</td>
<td>30</td>
</tr>
<tr>
<td>FPC16</td>
<td>10d</td>
<td>11</td>
</tr>
<tr>
<td>FPC18</td>
<td>10e</td>
<td>4</td>
</tr>
<tr>
<td>FPLB</td>
<td>10f</td>
<td>30</td>
</tr>
<tr>
<td>FDACC$^a$</td>
<td>-</td>
<td>35</td>
</tr>
<tr>
<td>Control</td>
<td>18</td>
<td>50</td>
</tr>
</tbody>
</table>

A Novel Micellar Drug Delivery Approach
Steady state fluorescence quenching of pyrene was used to measure aggregation number of micelles (86). In this method, it is assumed that the probe concentration is low when compared to micelles such that only one probe occupies a micelle and no emission takes place from micelles where both the probe and the quencher reside. Such a situation can be compared to distribution of $m$ random objects in $n$ boxes. Thus the distribution of the probe and the quencher among micelles follow Poisson statistics and the luminescence intensity of such a system is governed by

$$\frac{I}{I_0} = e^{-\frac{[Q]}{[M]}}$$

where $I_0$ = Fluorescent intensity in presence of quencher
I₀ = Fluorescent intensity in the absence of quencher

\[ [Q] = \text{Concentration of quencher} \]

\[ [M] = \text{Concentration of micelles} \]

Now the term [M] can be written as

\[ [M] = \frac{[C_{\text{total}}] - CMC}{n_{agg}} \]

where \( C_{\text{total}} = \text{Concentration of the amphiphile in solution} \)

\( \text{cmc} = \text{Critical micellization concentration} \)

\( n_{agg} = \text{Aggregation number} \)

Thus a plot of \( \ln \left( \frac{I}{I₀} \right) \) against the quencher concentration [Q] yields a straight line with slope as \([M]^{-1}\) where the amphiphile \([C_{\text{total}}]\) and probe concentrations are constant. Aggregation number for the FDC(n) series of amphiphiles could be measured by using pyrene as the fluorescent probe and cetylpyridiniumchloride as a quencher (Fig 3). From the equation above the aggregation number \( N_{agg} \) was calculated using the cmc(s) of the amphiphilic molecules, total concentration of the amphiphiles used and the micelle concentration (Table 3).

Solubility of a model lipophilic drug, paclitaxel, was determined in the presence of FDC15 and FPC18 above their cmc(s). The aqueous solubility of paclitaxel was found to be 0.25 µg/mL. FDC15 and FPC18 enhanced the solubility of paclitaxel by 85% and 62% respectively. Though the folate labeled amphiphiles did not increase the solubility to an extent that they can be considered as an alternative to Cremophor EL, but they can be used for the purpose of drug delivery in lieu of their targeting efficiency as cytotoxic activity elicited by a drug depends on its intracellular concentration.

**Table 3** Aggregation number for FDC(n) series of amphiphiles

<table>
<thead>
<tr>
<th>Amphiphile</th>
<th>Aggregation number ( N_{agg} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDC10</td>
<td>9</td>
</tr>
<tr>
<td>FDC13</td>
<td>18</td>
</tr>
<tr>
<td>FDC15</td>
<td>28</td>
</tr>
</tbody>
</table>

**Conclusion**

The understanding of tumor biology has come a long way in terms of its cause, therapy and chemoprevention. But the question of specificity of antitumor agents towards diseased tissues still remains to be addressed. Targeted therapies based on hindering cell signaling pathways have evolved and are specific to tumor cells. But they are usually used in addition to the standard chemotherapeutic agents. The dose limiting toxicity results from the nonspecific cytotoxicity of these chemotherapeutic agents. Thus it is of utmost importance that these agents be delivered by targeted delivery minimizing dose limiting toxic side effects. In this manuscript, folated ligand conjugated amphiphilic molecules as micellar drug delivery systems were reviewed. The feasibility of this folate receptor based targeted delivery system approach that deploys micelles created by amphiphilic surfactants has been established. A great advantage of targeting with amphiphiles is its versatility because of the diverse array of targeting ligands that can be attached to amphiphilic. These amphiphilic molecules may range from small molecule...
surfactants as reported here or may be large block copolymers such as pluronics that form polymeric micelles. Micelles from small molecule surfactants and amphiphiles are known to be unstable in biological systems due to extensive dilution in the body and interaction with plasma proteins. This can be overcome by the use of block copolymers which form stable polymeric micelles in biological systems.

The advent of these novel folate receptor based vitro methods coupled with deployment of block co polymers that are commercially available and have been in used in approved pharmaceutical products, give a compelling case for disciplined pre clinical evaluation for drug targeting in oncology space. Significant body of work needs to be completed, however, before such exciting opportunities can be advanced from academic laboratories into clinical evaluation to meet the unmet needs.

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