Cyclodextrins Application

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Cyclodextrins are a family of macrocyclic polysugars. Although a wide range of ring sizes have been synthesised, the most common variants of these moieties are α, β and χ-cyclodextrins, which contain 6, 7 and 8 α-1,4-d-glucopyranoside units, respectively, in the macrocyclic ring.

calixarene cyclodextrin cucurbituril

1. Sensing Applications

As with the calixarenes, it has been shown possible to utilise cyclodextrins for detecting cancer cells and biomarkers. Multiwall carbon nanotubes [1] could be used as a substrate for grafting β-cyclodextrin and, combined with an electrochemical probe, tetrathiafulvalene (TTF) carboxylate to construct electrodes capable of detecting cancer cells such as liver cancer cells SMMC-7721 and HepG2, leukemia K562/B.W K562/ADM cells, with a detection limit of 10^3 cells mL^{-1}.

In other work, β-cyclodextrin units could be immobilised onto electrode surfaces. The interaction between the macrocycle and ferrocene guests was shown to be controllable by electrochemically oxidising and reducing the ferrocene moiety [2]. This interaction combined with the use of a folic acid linker to bind to cancer cells enable the selective capture and release of these cells with a minimum detection limit of 10 cells. A similar cyclodextrin-ferrocene interaction was used in conjunction with ferrocene-labelled gold nanoparticles modified with aptamers for the breast cancer marker HER2 [3], thereby allowing the electrochemical detection of the target with a detection limit of 4.9 ng mL^{-1}.

A number of chemically modified cyclodextrins where the hydroxyl groups were selectively substituted with either amino, benzyl or mannose groups could be immobilised via non-covalent interactions to form a composite with the 2D form of carbon, i.e., graphene [4]. These composites could be sprayed onto interdigitated electrodes and served as electrochemical detector arrays for the determination of a range of organic compounds that are often found in the breath of cancer sufferers. The resultant “electronic nose” was capable of detecting cancer marker volatile organic compounds such as benzene at levels as low as 400 ppb. Nanocomposites could also be made of CuO_2/graphene oxide/β-cyclodextrin or ferrocene carboxylic acid/graphene oxide/β-cyclodextrin and used as substrates to immobilise antibodies to the cancer biomarkers alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) [5]. Graphene oxide/gold nanoparticle-modified electrodes could then be used to develop a sandwich-type electrochemical immunoassay for these cancer marker proteins. The resultant assay was capable

of detecting these moieties with linear ranges from 0.001 to 80 ng mL^{-1} for AFP and CEA with detection limits of 0.2 pg mL^{-1} and 0.1 pg mL^{-1} for AFP and CEA, respectively.

Cyclodextrins have also been utilised within the field of cancer imaging. The potential for combining the imaging properties of various nanoparticles with the sensing ability of cyclodextrins has been recently reviewed [6]. For example, spherical gadolinium oxide nanoparticles (75–95 nm in size) could be coated with a β-cyclodextrin copolymer and folic acid units [7]. The resultant composites displayed high blood compatibility and minimal cytotoxicity towards normal human breast cells and allowed magnetic resonance imaging (MRI) studies to be performed. This method allowed in vitro imaging of cancer cells and acted as an in vivo MRI contrast agent for imaging of tumours in mice. A similar method was used where superparamagnetic iron oxide nanoparticles coated with crosslinked curcumin were synthesised. When injected into mice these materials acted as excellent contrast imaging agents, allowing resolution of tumours 4–8 h after injection [8]. The use of cyclodextrins as scaffolds for the substitution and assembly of MRI contrast agents has been recently reviewed [9] and the modification of these agents by either covalent linkages or by forming inclusion compounds with cyclodextrins has been discussed.

Cyclodextrins can be used as imaging agents by modifying them with suitable dyes. This tends to be by forming inclusion complexes of dyes encapsulated in cyclodextrins, some of which will be discussed later since this procedure tends to be simpler than covalent modification. However, cyclodextrin/dye compounds can be synthesised, such as a β–cyclodextrin/cyanine dye compound [10] where “click” chemistry could be used to graft a single dye unit onto a β–cyclodextrin unit, the resultant compound being able to be imaged by confocal laser scanning microscopy in HeLa cells and also shown to act as a host for doxorubicin.

A highly fluorescent composite of β-cyclodextrin with gold nanoclusters could be synthesised and was shown to be highly biocompatible. The composite was selectively taken up by gastric cancer cells even in the presence of normal gastric cells [11]. The strong red fluorescence of the composite combined with dark-field imaging demonstrated that they were not only selectively taken up by the cancer cells but also had penetrated into the cells and were not just bound on the surface. Other workers utilised poly(p-phenylene-β-cyclodextrin)-graft-poly(ethylene glycol) modified gold nanoparticles to image U87 glioblastoma cells [12]. The resultant fluorescent complex was shown to image U87 cells with high selectivity over Vero cells. The poly(ethylene glycol) sidechains on the composite conferred high solubility and biocompatibility onto the composites, leading to minimal cytostatic and cytotoxic effects. Their high solubility allowed extensive uptake by the U87 cells, leading to high-quality imaging. Organic dyes could also be utilised; spherical nanogels of cyclodextrin/spiropyran/4-amino-7-nitro-1,2,3-benzoxadiazole composite (200 nm diameter) were formulated [13]. Depending on the illumination, these could fluoresce red or green. Selective imaging of Cal27 human tongue cancer cells was possible using these materials.

One major issue with cancers is that cells can break off tumours, so-called circulating tumour cells, and then be transported by the bloodstream to other parts of the body, giving rise to metastasis. Electroluminescent sensing could be utilised to detect these biomarkers [14], whereby a glassy carbon electrode could be modified with a gold nanoparticle/cyclodextrin/ruthenium complex/graphene composite. Ferrocene-labelled aptamers could be then immobilised on the surface of this composite. The ferrocene units interacted with the ruthenium complex,

quenching its electroluminescence. When exposed to tumour cells, binding occurs between the cells and the aptamers, and the resulting change in aptamer conformation causes the ferrocene to move away from the electrode, preventing its quenching effect and allowing electroluminescence to occur. This method allowed highly selective detection of the cancer cells with a detection limit of 40 cells per mL^{-1}.

2. Medical Applications

Cyclodextrins have been shown to have minimal oral toxicity since they are not adsorbed but instead simply “pass through” the gastrointestinal system and are excreted [15]. A number of cyclodextrin compounds have also been shown to be safe when administered by injection, although there are some that display adverse nephrotoxicity [16]. Reports have been produced that give suggested thresholds for the safe administration of cyclodextrins by various routes [17].

Their low toxicity has led to interest in the possibility of using cyclodextrins and substituted analogues as anti-cancer agents. The widest application is for use as transport agents for a variety of anti-cancer drugs due to their abilities to both solubilise these often poorly water-soluble drugs and stabilise them in conditions such as in the gastrointestinal tract.

Substituted cyclodextrins have been shown to have marked anti-cancer properties. Methylated cyclodextrins can display higher water solubility than their parent compounds [18] and interact strongly with cell membranes. For example, methylated cyclodextrin was shown to bind to and extract cholesterol from cell membranes [19]. A number of human cancer cell lines were treated with cyclodextrin, which led to decreases in transmembrane potential and DNA content in these cells. When applied by intratumoural injection to mice, there was a dramatic inhibition of tumour growth, indicating potential applications of this material as an anti-cancer agent. In other work [20], a number of substituted cyclodextrins were used and methylated derivatives were shown to induce apoptosis of cancer cells—again by extraction of cholesterol from their cell membranes. The cytotoxicity of the methylated compounds was shown to be higher than that exhibited by the parent cyclodextrins or their sulphonated derivatives. Methylated cyclodextrin was also shown to display good anti-cancer activity when utilised against MCF7 breast carcinoma and A2780 ovarian carcinoma cell lines [21]. When applied by injection to mice grafted with human tumours, the cyclodextrins were shown to accumulate within the tumour and reduce tumour growth by at least a factor of two compared to a control group.

The anti-tumour activity of the folate-derivative at a level of 30 mg kg^{-1} was shown to be higher than the same dose of methylated cyclodextrin or the common anti-cancer drug doxorubicin. All of the mice treated with methylated cyclodextrin or doxorubicin died within 70 days, whilst all the mice treated with the folate-derivative survived for at least 140 days.

In other work, the simpler methylated cyclodextrin was shown to remove cholesterol from plasma membranes, and, in conjunction with benzyl isothiocyanate, it demonstrated enhanced cytotoxicity against human colorectal cancer cells [22]. Similar effects could be obtained using methylated cyclodextrin along with the anticancer drug tamoxifen;

the cyclodextrin appeared to enhance the uptake of tamoxifen into melanoma cells and increased the cytotoxic effects of this drug [23].

There have also been a number of more recent papers published on the use of folate-appended β-cyclodextrins against a number of other cancers. Lhara cells, which are human melanoma cell lines that express folate receptors, could be treated with a folated methylated cyclodextrin [24]. The folated cyclodextrin entered the lhara cells and displayed cytotoxic effects as well as caused the formation of autophagosomes within the lhara cells, whereas a simple methylated cyclodextrin did not. Melanoma growth was suppressed in mouse models, suggesting the potential of this compound as a chemotherapy agent. In more recent work [25], folated-cyclodextrin was shown to be cytotoxic towards epithelial ovarian cancer cells, especially when combined with paxitaxel, and to reduce tumour growth in mouse models. The same cyclodextrin has been shown to be effective against folate receptor-carrying ES-2 (RFC+) ovarian cancer cells in mouse models [26], with cyclodextrin displaying high cytotoxic effects against the cancer cells.

Another cyclodextrin that has been used in medical applications is hydroxypropyl β-cyclodextrin, which has been approved for oral, buccal, rectal, ophthalmic and intravenous application [27]. This compound displays very low toxicity [28], with mice being able to tolerate intraperitoneal dosages of up to 10,000 mg kg^{-1}. Again, this compound displays a high affinity for cholesterol, which has led to its investigation as a possible anti-cancer agent [29]. Hydroxypropyl β-cyclodextrin was shown to inhibit the proliferation of a number of leukemia cell lines by removing cholesterol from the cell membranes leading to apoptosis and was also effective against cell lines containing the T315I BCR-ABL mutation, which makes them resistant to tyrosine kinase inhibitor drugs. Intraperitoneal injection of hydroxypropyl β-cyclodextrin to leukemia mouse models significantly improved their survival, whilst application to healthy mice controls showed no significant adverse effects. What makes this compound especially important is that it acts against a range of leukemias including acute myeloid leukemia, acute lymphoblastic leukemia and chronic myeloid leukemia as well as inhibiting hypoxia-adapted cells. The compound has also been approved for treating a lysosomal lipid storage disorder, Niemann-Pick Type C disease [30].

In recently published work, hydroxypropyl cyclodextrin has been shown again, probably by its cholesterol binding and depletion ability, to both impede breast cancer cell growth and cause cancer cell death [31]. Experiments using mouse xenografts have proved extremely promising, showing total eradication of early-stage tumours and what the authors describe as remarkable reductions in intermediate and late-stage tumours.

Substitution of hydroxypropyl cyclodextrin with a folate group as described for the materials above could be performed to provide a material with the capability to cause autophagic cell death in chronic myeloid leukemia cells, especially when combined with imatinib, an ABL tyrosine kinase inhibitor [32]. In mouse models, this combined therapy had a much stronger inhibitory effect on cancer progression than either component separately.

Folate-substituted cyclodextrins have also been very effectively used as drug carriers; again, this is outside of the scope of this research, but there are many examples in the literature, some of which are summarised here [33].

Cyclodextrins have also been used to construct “responsive” materials, which can undergo physical or chemical transformations in response to a stimulus or the nature of their environment. These materials will be discussed elsewhere in the review, but one example is where β-cyclodextrin was reacted with a difunctional crosslinking agent containing a disulphide linkage to give a crosslinked nanocage capable of hosting doxorubicin [34]. This composite showed good stability and biocompatibility in vivo and was selectively adsorbed into tumour cells where the high levels of glutathione in the tumour led to a reduction in the disulphide bond to thiols. This caused the nanocage to disintegrate, releasing the drug, and mouse models showed enhanced tumour suppression and improved survival rates compared to those treated with doxorubicin.

The results of the research above demonstrate the possibility of using substituted cyclodextrins as anti-cancer drugs. However, toxicological issues may need to be addressed before use since, unlike the compounds described above, many substituted cyclodextrins often display higher toxicology than their unsubstituted parent compounds.

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