Physical and chemical targeting

This lecture elaborates on the other types of targeting concepts developed that include chemical targeting, sandwich targeting and magnetic targeting.

1 Chemical targeting or Pro-drug concept

A pro-drug is an inactive form of a drug that can be converted into an active form through chemical or enzymatic reactions. The pro-drug is not active until it is metabolized to its active form. Two key pro-drug concepts have been under development and have the potential to become Ehrlich’s proverbial ‘magic bullet’. The first concept is known as the ‘Antibody-directed enzyme pro-drug therapy (ADEPT) and the second concept is known as ‘Gene-directed enzyme pro-drug therapy (GDEPT). The principle of ADEPT revolves in using a specific antibody tagged non-endogenous enzyme that will selectively bind to the target cell surface. Then the pro-drug is introduced into the system. The pro-drug can be only metabolized by the enzyme under hypoxic conditions. Thus, when the pro-drug reaches the vicinity of the cancer cell, it will be converted to the active cytotoxic form that will kill the cancer cells. This is because the tumour tissues are more hypoxic (oxygen deficient) when compared with normal tissues. If the pro-drug is near normal tissues, it will not be converted to the active form due to aerobic (oxygen rich) environment and lack of the specific enzyme for the conversion. Figure 1 depicts the concept of chemical targeting.

![Concept of chemical targeting to cancer cells](image)

**Fig 1: Concept of chemical targeting to cancer cells. The prodrug is not activated in normal cells due to aerobic conditions**

It is important that the enzyme of choice should be non-endogenous i.e., it should not be produced in the system so that only those cells where the enzyme has been introduced will be able to metabolize the pro-drug.

In the case of GDEPT, a gene encoding for a specific enzyme is introduced into the target cell specifically. The pro-drug is then introduced into the system. It will be converted into the active
form only in the cells expressing the gene for the enzyme. Let us consider an example of a GDEPT concept. Ganciclovir is a structural analogue of the nucleoside deoxy guanosine. On phosphorylation of Ganciclovir it gets converted to a phosphate analogue of deoxy guanosine triphosphate (dGTP). This analogue will interfere with the DNA synthesis thereby initiating apoptosis of the cell. In GDEPT, a gene for Herpes Simplex Virus thymidine kinase (HSVtk) is delivered into cancer cells. Now, the ganciclovir is introduced into the system. The ganciclovir will get transformed into the corresponding monophosphate by the action of the HSVtk only in those cells that express the gene for HSVtk (in this case, the cancer cells). Normal cells will not express this enzyme and hence do not possess the ability to transform the ganciclovir. The monophosphate of ganciclovir will be converted by the cellular kinases to form diphosphate and triphosphate derivatives. Due to its structural similarity with the dGTP, the ganciclovir triphosphate will integrate into the newly synthesized DNA preventing further synthesis. As the cell cycle controllers will be unable to revert this integration and hence will initiate the apoptotic cascade. As the cell itself triggers the events leading to its programmed cell death, the gene encoding for the HSVtk enzyme is referred to as the ‘suicide gene’ and the therapeutic strategy is known as the ‘suicide gene therapy’. Figure 2 represents the principle of suicide gene therapy.

The apoptotic signals can also be transmitted to the neighbouring cells triggering their apoptosis. This effect is where the neighbouring cells are also killed is known as ‘bystander effect’ and can be very useful in improving the cell kill in a tumour tissue. The suicide gene therapy is in principle even better than the delivery of enzymes. However, the challenges in gene delivery to a specific cell (Refer Module 7 for detailed discussion on the challenges in gene delivery) limit the
applicability of GDEPT. As it is evident from the above discussion that the pro-drug targeting also involves active targeting, this strategy also forms part of combination targeting.

2 Pre-targeting or sandwich targeting

Yet another strategy that has been proposed to target tumour tissue specifically is the concept of sandwich targeting also known as **pre-targeting**. In this strategy, a tumour specific ligand is first introduced into the system. Then the drug along with a secondary ligand that exhibits specificity towards the primary ligand is introduced. This will enable efficient homing of the drug into the desired target. One of the trials that demonstrated this concept utilized the biotin-avidin binding for pre-targeting. Initially, biotinylated ligands were introduced into the target using a catheter. These bind to the specific receptors. Then the carrier/drug having avidin as the secondary ligand will be introduced. Avidin is a tetrameric protein that has extremely high binding affinity to biotin. In fact the biotin-avidin is one of the strongest known protein interactions to mankind. Due to this extremely high affinity to biotin, the avidin-modified carriers/drug will home into the target cell. Figure 3 depicts the principle of sandwich targeting.

![Fig 3: Principle of pre-targeting](image)

This technique can be used in such cases where the drug needs to be present for a long time as in the case of anti-angiogenic drugs (inhibits formation of new blood vessels) or intravascular drugs (that act inside the blood vessel) for cancer therapy. Though this strategy sounds promising, immunogenicity due to presence of foreign protein (avidin) is a setback. Further work in improving this targeting strategy, which is currently in its infancy, is required for widespread use of this method.
3 Physical targeting
In addition to using chemical modifications to enable the delivery system to preferentially home into the target, internal and external triggers have also been used to facilitate the carrier to reach the target site and release the drugs only after reaching the target. The internal triggers that have been used are pH and temperature. The pH variations in the region can be used to trigger the release of the drug at the desired location. For example, acid swelling chitosan nanoparticles tend to release their contents more in acidic pH and hence can be used in the stomach or endosomal compartments. Similarly polymers that display accelerated degradation in the acidic pH also can serve to release the drugs in specific compartments. Use of thermo-sensitive polymer components such as poly(N-isopropyl acrylamide) systems can enable the controlled release of the drug in different local temperatures. But, in the biological system, drastic changes in temperature are not expected and in most cases the difference in temperatures might be less than 0.5°C. Thus, it is evident that the use of internal triggers requires a system that can respond to even slight changes in the pH or temperature. However, this might not be always practically feasible. Hence use of external triggers is more preferred to control the localization as well as the release of the drugs from the nanocarrier.

The commonly employed external triggers are ultrasound, magnetic field, electric field and infrared radiation. The external triggers can be employed both with and without active targeting. In the case of only external triggers being used, then the targeting strategy is known as ‘physical targeting’. The use of both external triggers and active targeting is known as ‘combined targeting’. External triggers can also be used along with passive targeting and forms a subset of combined targeting.

4 Magnetic targeting
In magnetic targeting strategy, an external magnetic field is used to guide magnetic nanoparticles containing drug or without a drug to the desired location. The application of an external magnetic field can also cause an increase in the local temperature as the magnetic nanoparticles undergo different relaxation processes after removal of the field. The magnitude of this temperature change depends on the strength of the external magnetic field, the magnetization values of the magnetic nanoparticles as well as the concentration of the magnetic nanoparticles. The increase in temperature can be exploited favourably for release of drugs from a polymer coating surrounding the magnetic nanoparticles or for killing the cells due to a phenomenon known as ‘hyperthermia’ where increase in temperature results in denaturation of the enzymes and functional proteins thereby inducing cell death. The main advantages of magnetic targeting are:

- Highly efficient site-specific localization of the magnetic nanoparticles possible
- Minimum side-effects
- The magnetic nanoparticles can be diverted away from the immune system components thereby reducing immunogenicity
• Frequency of dosage can be minimized

However, there are several challenges in realizing the full potential of magnetic targeting. These are as follows:

• The application of an external magnetic field can cause aggregation of the magnetic nanoparticles that can cause blocking of the blood vessels
• The external magnetic field can also affect iron containing biomolecules such as hemoglobin resulting in alterations in the blood viscosity and rheology
• Treatment depth is limited as deep body targeting can lead to serious problems with blood flow
• The variations in the blood flow in different regions of the body makes it difficult to achieve homogenous distribution of the magnetic nanoparticles

5 Factors influencing magnetic targeting

One of the key factors that influence the efficiency of magnetic targeting is the nature of the magnetic nanoparticle. Materials with high magnetization values are preferred, as they will respond to even small magnitude of applied magnetic field. Ferromagnetic and ferrimagnetic materials including iron, cobalt, nickel, manganese and their alloys have high magnetization values. However, their biocompatibility is questionable. Hence iron oxide nanoparticles, which do not possess the same levels of magnetization but are more inert when compared to the metallic nanoparticles have been used for biological applications. Research is on in different parts of the world to develop a magnetic material that will possess high saturation magnetization as well as possess excellent biocompatibility. Table 1 lists a few ferromagnetic nanoparticles and their magnetic properties that have been explored for therapeutic applications.

<table>
<thead>
<tr>
<th>Magnetic material</th>
<th>Saturation magnetization at 25°C (emu/g)</th>
<th>Volume magnetization (emu/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe₃Co alloy</td>
<td>243</td>
<td>1993</td>
</tr>
<tr>
<td>Fe₅₀Co₅₀ alloy</td>
<td>233</td>
<td>1910</td>
</tr>
<tr>
<td>Fe metal</td>
<td>216-218</td>
<td>1717</td>
</tr>
<tr>
<td>Fe₃O₄ magnetite</td>
<td>93-97</td>
<td>484</td>
</tr>
<tr>
<td>γ-Fe₂O₃ haematite</td>
<td>75-77</td>
<td>404</td>
</tr>
<tr>
<td>Co metal</td>
<td>157-160</td>
<td>1424</td>
</tr>
<tr>
<td>Ni metal</td>
<td>43</td>
<td>484</td>
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It might be interesting to note that nanoparticles are more preferred for therapeutic applications when compared with larger sized particles. What is the significance of size of the magnetic material? When the size of the ferromagnetic particle becomes below a critical size in the nano-
dimensions, the magnetization occurs instantaneously on application of a magnetic field. Similarly, the demagnetization of the particle occurs instantaneously on removal of the external magnetic field. This property is known as ‘superparamagnetism’. Why does this phenomenon occur? When the size of a particle decreases, so does the number of its magnetic domains. Below a critical size, the number of domains reduces to one, which can instantaneously respond to the external magnetic field. The term paramagnetism is associated with a material that can be magnetized on application of an external magnetic field and demagnetized on removal of the magnetic field. As there is no time lag between the removal of the magnetic field and the demagnetization of the magnetic nanoparticle, this phenomenon is known as ‘superparamagnetism’! Commonly used iron oxide nanoparticles are therefore referred as SPION (Superparamagnetic iron oxide nanoparticles).

Okay! That explains the role of material and size. What about the shape of the magnetic nanoparticle? Any shape other than spherical can lead to magnetic anisotropic effects i.e., the magnetic property differs from one axis to another and thus orientation of the particle will be a key factor in deciding its response to an external magnetic field. Thus, spherical nanoparticles are preferred for biomedical applications.

The external magnetic field can be applied by means of a permanent magnet such as NdFeB or a superconducting magnet or through an electromagnet. The electromagnet offers the versatility of tuning the applied magnetic field, which is not possible with permanent magnets. Another important aspect in magnetic targeting is that the external magnetic field is usually applied in a pulsatile manner (alternating field) rather than a continuous field. The alternating field causes the localized heating effects that can be used for therapeutic applications.

6 Some applications of magnetic targeting
Magnetic targeting has generated considerable interest in recent years and many interesting strategies have been evolved using magnetic nanoparticles.

Magnetic Navigation System

A catheter probe containing magnetic nanoparticles at its tip has been developed to maneuver into intricate regions within the body guided by an external magnetic field. This path-breaking development can help in removing blocks as well in treatment of aneurysms and vascular complications!

6.1 Triggered release
Magnetic nanoparticles can be coated with a polymer layer containing drugs. When an external magnetic field is applied, then the magnetic nanoparticles can be guided to the desired location.
At the same time, the localized heat produced by the exposure of the magnetic nanoparticles to the applied magnetic field causes the polymer chains to become disentangled and hence more fluid causing the release of the entrapped drug. As this release can be programmed to occur in desired locations, adverse effects associated with conventional drug delivery are eliminated. Not only the location of release can be programmed, but the amount of drug released at a given instant can be programmed by regulating the magnitude of the applied magnetic field.

### 6.2 Magnetofection

Magnetic nanoparticles containing oligonucleotides can be not only targeted to specific cells but also can be guided in to their intracellular target such as the nucleus using an external magnetic field. This process facilitates integration of the oligonucleotide with the genetic material in the nucleus thereby causing its functional expression. This method of using a magnetic field to incorporate foreign genetic material into a cell is known as ‘magnetofection’. (*Transfection* is the term associated with incorporation of foreign genes into the cell and hence magnetofection means transfection using magnetic field!).

### 6.3 Embolotherapy

It is well known that application of an external magnetic field can cause the magnetic particles to agglomerate. The agglomeration depends on the size and shape of the magnetic particles with larger sized particles undergoing faster agglomeration. These agglomerates do not move away or disrupt even after removal of the magnetic field. Figure 4 shows the representation of the agglomeration of magnetic nanoparticles on application of an external magnetic field.

![Fig. 4: Agglomeration of magnetic nanoparticles in a blood vessel on application of external magnetic field](image)

This agglomeration can be directed to occur in blood capillaries that supply blood to tumours. As a result of the agglomeration, blood flow into the tumour is impaired resulting in death of the cancer cells. The agglomerates formed by the magnetic nanoparticles are known as a ‘**embolus**’.
and this method of killing cancer cells as ‘embolotherapy’. The key to the success of this therapy is to ensure that the embolus is formed in the desired location and not elsewhere.

6.4 Hyperthermia

When an alternating magnetic field is applied to the magnetic nanoparticles, they undergo various relaxation processes (Neel and Brownian relaxation) that result in increase in the local temperature. This phenomenon is known as ‘magnetic hyperthermia’. The magnitude of increase in temperature depends upon the nature of the nanoparticle as well as its concentration. Normally, an increase in temperature in the range of 41°C and 48°C is known as the hyperthermia range. In this range, the key enzymes and proteins in a cell start denaturing (change in conformation resulting in a loss in function) that in turn affects the metabolism of the cells leading to its death. What is interesting is that cancer cells are more susceptible to temperature changes than normal cells! This may be because the normal cells could have certain heat shock proteins that enables the proteins and enzymes to resist conformation change. This difference can be used to selectively kill the cancer cells. Figure 5 depicts the effectiveness of magnetic hyperthermia in killing cancer cells in an animal model.

<table>
<thead>
<tr>
<th>Without Hyperthermia</th>
<th>With Hyperthermia</th>
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<tbody>
<tr>
<td>Before Treatment</td>
<td><img src="image1" alt="Before Treatment" /></td>
</tr>
<tr>
<td>During Treatment</td>
<td><img src="image3" alt="During Treatment" /></td>
</tr>
<tr>
<td>After Treatment</td>
<td><img src="image5" alt="After Treatment" /></td>
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![Figure 5: Cartoon representing the efficiency of magnetic hyperthermia in annihilating cancer cells. The magnetic field is applied through the coils wrapped around the animal.](image7)

For inducing magnetic hyperthermia, the magnetic nanoparticles can be injected into a tumour or guided to the tumour using an external magnetic field to ensure sufficient concentration of the magnetic nanoparticle in the tumour tissue. Then an alternating magnetic field is applied. This
will cause an increase in temperature leading to cell death. The increase in temperature will also increase the tumour cell permeability, which can be exploited to deliver chemotherapeutic agents to ensure complete cancer cell kill. However, direct injection of the magnetic nanoparticles into the tumour tissue will not ensure homogenous distribution of the magnetic nanoparticles. It is essential that there is a uniform distribution of the magnetic nanoparticles for effective cell kill due to hyperthermia. Hence, in many cases, the magnetic nanoparticles are modified with specific targeting ligands that will localize in to the cancer cells i.e., a combination of active targeting and magnetic hyperthermia has been employed. Similarly, hyperthermia has been used in combination with embolotherapy and chemotherapy for more effective cancer cell kill.

Now, one may wonder why an upper limit (48°C) is prescribed for hyperthermia. Beyond this temperature, necrosis of the cell occurs instead of the programmed cell death or apoptosis. The necrosis is accompanied by coagulation of the proteins and carbonization of the tissues. This phenomenon is known as ‘thermo-ablation’. This carbonization can affect the neighbouring tissues also resulting in what is now known as ‘collateral damage’. Let us try to understand this using a modern day analogy. Let us suppose a group of anti-social elements are hiding in a building in a residential area. In order to eliminate them, a team of commandos can be sent in to the building and capture them dead or alive. On the other hand, the building can be bombed. In either case, the anti-social elements will be eliminated but what makes the key difference is in the former case, no civilian will be harmed while in the latter case, damage to neighbouring buildings and civilians cannot be avoided. While bombing the building is similar to thermo-ablation, the commando operation is akin to magnetic hyperthermia!

7 Reference

8 Additional Reading
Smart Nanoparticles in Nanomedicine (The MML series, Vol. 8), Editors: Reza Arshady & Kenji Kono, Kentus Books, 2006

Source:
http://nptel.ac.in/courses/118106019/30