1.1 Introduction to Cancer:

Cancer can be defined as “a cellular malignancy whose unique trait is loss of normal controls resulting in an unregulated growth, a lack of differentiation, and an ability to invade local tissues and metastasize”. These three characteristics: abnormal growth, lack of appropriate differentiation and capacity to invade other tissues or organs are common to different types of cancers (Gutman, et al. 2011). The approach to treating cancer depends on where in the body it occurs, the types of cells making up the cancer, how advanced is the cancer.

Under normal circumstances the number and growth of all our cells is a highly controlled mechanism. But when the control signals in one of these cells goes wrong, and its life cycle becomes disturbed, it divides and divides. It continues multiplying uncontrollably, and the result of this accumulation of abnormal cells is a mass of cells called a "tumor". A tumor can be either benign or malignant (Gutman, et al. 2011).

**Benign tumors** are non-cancerous and are rarely life-threatening. They do not spread (metastasize) to other parts of the body. Many breast lumps, for example, are benign tumors. **Malignant tumors** are cancerous and can spread to other parts of the body. When a malignant tumor spreads, the malignant cells break off and travel through the blood lymph system to other places in the body to settle and multiply; or metastasize, resulting in a new tumor called a secondary tumor, or metastasis.

1.1.1 Classification of cancer:

Cancers are classified by the type of cell that the tumor resembles and is therefore presumed to be the origin of the tumor. These types include: Carcinoma, Sarcoma, Lymphoma and leukemia, Germ cell tumor and Blastoma.

1.1.2 Pathophysiology of Cancer:

Cancer is fundamentally a disease of failure of regulation of tissue growth. In order for a normal cell to transform into a cancer cell, the genes which regulate cell growth and differentiation must be altered. (Croce 2008)
The affected genes are divided into two broad categories. Oncogenes are genes which promote cell growth and reproduction. Tumor suppressor genes are genes which inhibit cell division and survival. Malignant transformation can occur through the formation of novel oncogenes, the inappropriate over-expression of normal oncogenes, or by the under-expression or disabling of tumor suppressor genes. Typically, changes in *many* genes are required to transform a normal cell into a cancer cell (Knudson 2001).

Genetic changes can occur at different levels and by different mechanisms. The gain or loss of an entire chromosome can occur through errors in mitosis. More common are mutations, which are changes in the nucleotide sequence of genomic DNA.

![Diagram showing how cancer cells keep on reproducing to form a tumour](https://cancerhelp.org.uk/images/pathophysiology_p1.jpg)

**Figure 1.1: Pathophysiology of cancer**

### 1.1.3 Causes of Cancer:

Cancers are primarily an environmental disease with 90-95% of cases attributed to environmental factors and 5-10% due to genetics (Anand *et al.*, 2008). *Environmental*, as used by cancer researchers, means any cause that is not genetic, not merely pollution. Common environmental factors that contribute to cancer death include tobacco (25-30%), diet and obesity (30-35%), infections (15-20%), radiation (both ionizing and non-ionizing, up to 10%), stress, lack of physical activity, and environmental pollutants (Anand *et al.*, 2008).
Chapter 1. Introduction

Design and Evaluation of Surface Modified Nanocarriers for Tumors Targeting

Figure 1.2: Statistics of cancer [American cancer society, 2012]

1.1.4 Cancer Current Status

Cancer is the second leading cause of death in the western world after heart disease and affects millions of people worldwide (Figure 1.2). Cancer rates in India are lower than western countries, but are rising with increasing migration of rural population to the cities, increase in life expectancy and changes in lifestyles.

1.2 Colorectal Cancer

Colorectal cancer (also known as colon cancer, rectal cancer, or bowel cancer) is the development of cancer in the colon or rectum (parts of the large intestine) (NCI. Accessed 14-12-6). It is due to the abnormal growth of cells that have the ability to invade or spread to other parts of the body (NCI. Accessed 14-10-6). Colorectal cancer is the third most common type of cancer and the third most common cause of cancer related mortality. Although incidence has been steadily decreasing, the NIH estimated colorectal cancer to cause 49,960 deaths in 2008 (NCI. Accessed 12-15-8). Colorectal cancer is an uncontrolled proliferation of cells in the largest part of the
large intestine, the colon or rectum. Most colorectal cancers are adenocarcinomas, meaning they originate from the glandular cells that line the intestine (NCI. Accessed 10-23-8).

Figure 1.3: Colorectal region and colorectal cancer

1.2.1 Colorectal Cancer Stages

Staging is the categorization of cancer according to the extent that it spreads. It is used for diagnostic, therapeutic, and prognostic purposes. There are several staging systems, but the most common system is the American Joint Committee on Cancer (AJCC) system, also called the TNM system. This system uses Roman Numerals I-IV to describe the extent of the primary Tumor (T), the absence or presence of metastasis to nearby lymph Nodes (N), and the absence or presence of distant Metastasis (M) (American Cancer Society, Accessed 1-11-7).
Table 1.1: TNM staging system of Colorectal Cancer

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Node</th>
<th>Metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1: Tumor invades submucosa</td>
<td>N0: No regional lymph node invasion</td>
<td>M0: No distant metastasis</td>
</tr>
<tr>
<td>T2: Tumor invades muscularis</td>
<td>N1: Metastasis in 1-3 regional lymph nodes</td>
<td>M1: Distant metastasis present</td>
</tr>
<tr>
<td>T3: Tumor invades serosa</td>
<td>N2: Metastasis in 4 or more regional nodes</td>
<td></td>
</tr>
<tr>
<td>T4: Tumor invades other organs/structures</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1.2: TNM System: (American Cancer Society. Detailed Guide: Colon and Rectum Cancer, How is Colorectal Cancer Staged).

<table>
<thead>
<tr>
<th>Stage I: T1 N0 M0; T2 N0 M0.</th>
<th>Stage II: T3 N0 M0; T4 N0 M0.</th>
<th>Stage III: any T, N1-2, M0.</th>
<th>Stage IV: any T, any N, M1.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer is contained to inner lining.</td>
<td>Cancer has spread to other nearby organs, but not reached lymph nodes.</td>
<td>Cancer has spread to lymph nodes, but has not been carried to distant parts of the body</td>
<td>Cancer has been carried through the lymph system to distant parts of the body. The most likely organs are the lungs and liver</td>
</tr>
</tbody>
</table>
1.2.2 Treatment for Colorectal Cancer

There are three types of treatment available for patients with colorectal cancer. They are Primary Surgical Therapy, Adjuvant Chemotherapy, and Adjuvant Radiation Therapy (NCI, Accessed 10-23-8). The route of therapy chosen is selected according to the stage of the disease.

1.2.2.1 Primary Surgical Therapy

Surgery is often the main treatment for colorectal cancer. It is often the best choice, when the cancer has not metastasized. Surgery may be used alone, or it may be employed with other options, such as chemotherapy or radiation therapy. Advanced techniques have greatly improved cure rates and reduced the level of damage to normal tissue (American Cancer Society. Accessed 11-4-10).

1.2.2.1.1 Radiation Therapy

Radiation therapy is one of the most common forms of treatment for cancer. It uses high-energy particles to attack the cancer cells. It may be used by itself, or in conjunction with another form of treatment (American Cancer Society. Accessed 11-4-10). Unlike Chemotherapy, radiation is localized to the area of the tumor.
1.2.2.2 Chemotherapy

Chemotherapy is a common method of cancer treatment. It employs the use of chemical/biological compounds to destroy cancer cells. These drugs may be used alone, or in combination with other drugs. And unlike surgery and radiation therapy, chemotherapy is a primarily systemic treatment (American Cancer Society. Accessed 11-4-10). This means the drugs are not localized to a specific region. Rather, they are administered to the patient in such a way that would allow them to travel throughout the body, reaching the cancer cells wherever they may have spread.

1.2.2.2.1 Chemotherapy Options for Colorectal Cancer

The drugs most often used for colorectal cancer include:

- 5-Fluorouracil (5-FU), which is often given with the vitamin-like drug leucovorin (also called folinic acid) or a similar drug called levo-leucovorin, which helps it work better.
- Capecitabine (Xeloda), which is in pill form. Once in the body, it is changed to 5-FU when it gets to the tumor site.
- Irinotecan (Camptosar)
- Oxaliplatin (Eloxatin)
- Trifluridine and tipiracil (Lonsurf), a combination drug in pill form

Often, 2 or more of these drugs are combined to try to make them more effective. Sometimes, chemo drugs are given along with a targeted therapy drug.

1.3 Prostate Cancer:

Prostate cancer is the most common type of cancer among men, second only to nonmelanoma skin cancer in both men and women. Prostate cancer is a slowly progressing disease, and many men die of old age without ever knowing they have it. Prostate cancer is often found when an autopsy is performed, and studies indicate that perhaps 80% of all men in their eighties may have undiagnosed prostate cancer when they die.

Prostate cancer, also known as carcinoma of the prostate, is the development of cancer in the prostate, a gland in the male reproductive system (NCI. 14-12-10). Most prostate
cancers are slow growing; however, some grow relatively quickly (World Cancer Report 2014). The cancer cells may spread from the prostate to other parts of the body, particularly the bones and lymph nodes. It may initially cause no symptoms. In later stages it can lead to difficulty urinating, blood in the urine, or pain in the pelvis, back or when urinating. A disease known as benign prostatic hyperplasia may produce similar symptoms. Other late symptoms may include feeling tired due to low levels of red blood cells (NCI. 2014-04-11).

1.3.1 Epidemiology:

The National Cancer Institute estimates that there will be 220,800 new cases of prostate cancer diagnosed in the year 2015, and there are an estimated 2,796,000 men living with prostate cancer in the United States. Statistical models show that rates for new prostate cancer cases have been falling on average 4.3% each year over the last 10 years. NCI researchers are studying this fall in incidence, which may be related to changing PSA screening patterns.

Prostate cancer is most often diagnosed among men ages 65 to 74 (6 in 10) and is rarely diagnosed before age 40. It is estimated that 14% of men (1 in 7) will be diagnosed with prostate cancer at some point during their lifetime, with a median age of 66 at time of diagnosis (NCI, 2015).

![Prostate Gland](image)

**Figure 1.5:** Male reproductive system and Prostate gland anatomy.
1.3.2 Risk Factors

Factors that increase the risk of prostate cancer include: older age, a family history of the disease, and race. About 99% of cases occur in those over the age of 50. Having a first degree relative with the disease increases the risk 2 to 3 fold. In the United States it is more common in the African American population than the White American population. Other factors that may be involved include a diet high in processed meat, red meat, or milk products or low in certain vegetables. An association with gonorrhea has been found, but a reason for this relationship has not been identified. (Caini et al, 2014). Prostate cancer is diagnosed by biopsy. Medical imaging may then be done to determine if the cancer has spread to other parts of the body. (NCI, 2014-04-08.)

Prostate cancer screening is controversial. Prostate-specific antigen (PSA) testing increases cancer detection but does not decrease mortality (Djulbegovic M., et.al, 2010). The United States Preventive Services Task Force recommends against screening using the PSA testing, due to the risk of over-diagnosis and over-treatment as most cancer diagnosed would remain asymptomatic. The USPSTF concludes that the potential benefits of testing do not outweigh the expected harms (NCI, 2012-07-02). While 5α-reductase inhibitors appear to decrease low grade cancer risk they do not affect high grade cancer risk and thus are not recommended for prevention. Supplementation with vitamins or minerals does not appear to affect the risk. (NCI. 2014-04-08)

Many cases can be safely followed with active surveillance or watchful waiting. Other treatments may include a combination of surgery, radiation therapy, hormone therapy or chemotherapy. When it only occurs inside the prostate it may be curable (NCI. 2014-04-08). In those in whom the disease has spread to the bones, pain medications, bisphosphonates and targeted therapy, among others, may be useful. Outcomes depend on a person’s age and other health problems as well as how aggressive and extensive the cancer is. Most people with prostate cancer do not end up dying from the disease. (NCI. 2014-04-08). The five year survival rate in the United States is 99% (NCI. 2014-06-18). Globally it is the second most common type of cancer and the fifth leading cause of cancer-related death in men. In 2012 it occurred in 1.1 million men and caused 307,000 deaths (Bade et al., 2009). It was the most common cancer in males in
84 countries, occurring more commonly in the developed world. Rates have been increasing in the developing world. Detection increased significantly in the 1980s and 1990s in many areas due to increased PSA testing. Studies of males who died from unrelated causes have found prostate cancer in 30% to 70% of those over age 60 (NCI. 2014-04-08).

1.3.3 Pathophysiology of Prostate Cancer:

Prostate cancer is classified as an adenocarcinoma, or glandular cancer, that begins when normal semen-secreting prostate gland cells mutate into cancer cells. The region of prostate gland where the adenocarcinoma is most common is the peripheral zone. Initially, small clumps of cancer cells remain confined to otherwise normal prostate glands, a condition known as carcinoma in situ or prostatic intraepithelial neoplasia (PIN). Although there is no proof that PIN is a cancer precursor, it is closely associated with cancer. Over time, these cancer cells begin to multiply and spread to the surrounding prostate tissue (the stroma) forming a tumor. Eventually, the tumor may grow large enough to invade nearby organs such as the seminal vesicles or the rectum, or the tumor cells may develop the ability to travel in the bloodstream and lymphatic system. Prostate cancer is considered a malignant tumor because it is a mass of cells that can invade other parts of the body. This invasion of other organs is called metastasis. Prostate cancer most commonly metastasizes to the bones, lymph nodes, and may invade rectum, bladder and lower ureter after local progression. The route of metastasis to bone is thought to be venous as the prostatic venous plexus draining the prostate connects with the vertebral veins (NCI. 2011-04-28).

1.3.4 Stages of Prostate Cancer: An Overview

A prostate cancer diagnosis is made by looking at prostate tissue under a microscope. A pathologist (a doctor who identifies diseases by studying tissues under a microscope) will look at the biopsied tissue for cancer cells. Once the disease has been identified, the doctor must determine both the stage and grade of the prostate cancer.
To plan for prostate cancer treatment, depends on the extent (stage) of the disease. This is based on:

- The size of the tumor
- Whether the cancer has spread outside the prostate
- If the cancer has spread, where it has spread.

**Specific Stages**

The stages of prostate cancer are as follows:

**Stage I:** The cancer cannot be felt during a digital rectal exam (DRE). It is found by chance when surgery is done for another reason, usually for benign prostatic hyperplasia (BPH). The cancer is only in the prostate.

**Stage II:** The cancer is more advanced, but it has not spread outside the prostate.

**Stage III:** The cancer has spread outside the prostate. It may be in the seminal vesicles. It has not spread to the lymph nodes.

**Stage IV:** The cancer may be in nearby muscles and organs (beyond the seminal vesicles). It may have spread to the lymph nodes. It may have spread to other parts of the body.

**Recurrent:** The cancer has come back (recurred) after a time when it could not be detected. It may recur in or near the prostate. Or it may recur in any other part of the body, such as the bones.
Table 1.3 TNM staging of Prostate cancer:

<table>
<thead>
<tr>
<th>Stage</th>
<th>T</th>
<th>N</th>
<th>M</th>
<th>PSA*</th>
<th>Gleason</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>T1a-c</td>
<td>N0</td>
<td>M0</td>
<td>PSA &lt; 10</td>
<td>Gleason ≤6</td>
</tr>
<tr>
<td>I</td>
<td>T2a</td>
<td>N0</td>
<td>M0</td>
<td>PSA &lt; 10</td>
<td>Gleason ≤6</td>
</tr>
<tr>
<td>I</td>
<td>T1-T2a</td>
<td>N0</td>
<td>M0</td>
<td>PSA X</td>
<td>Gleason X</td>
</tr>
<tr>
<td>IIA</td>
<td>T1a-c</td>
<td>N0</td>
<td>M0</td>
<td>PSA &lt; 20</td>
<td>Gleason 7</td>
</tr>
<tr>
<td>IIA</td>
<td>T1a-c</td>
<td>N0</td>
<td>M0</td>
<td>PSA ≥10 but &lt; 20</td>
<td>Gleason ≤6</td>
</tr>
<tr>
<td>IIA</td>
<td>T2a</td>
<td>N0</td>
<td>M0</td>
<td>PSA &lt; 20</td>
<td>Gleason ≤7</td>
</tr>
<tr>
<td>IIA</td>
<td>T2b</td>
<td>N0</td>
<td>M0</td>
<td>PSA &lt; 20</td>
<td>Gleason ≤7</td>
</tr>
<tr>
<td>IIA</td>
<td>T2b</td>
<td>N0</td>
<td>M0</td>
<td>PSA X</td>
<td>Gleason X</td>
</tr>
<tr>
<td>IIIB</td>
<td>T2c</td>
<td>N0</td>
<td>M0</td>
<td>Any PSA</td>
<td>Any Gleason</td>
</tr>
<tr>
<td>IIIB</td>
<td>T1-2</td>
<td>N0</td>
<td>M0</td>
<td>PSA ≥20</td>
<td>Any Gleason</td>
</tr>
<tr>
<td>IIIB</td>
<td>T1-2</td>
<td>N0</td>
<td>M0</td>
<td>Any PSA</td>
<td>Gleason ≥8</td>
</tr>
<tr>
<td>III</td>
<td>T3a-b</td>
<td>N0</td>
<td>M0</td>
<td>Any PSA</td>
<td>Any Gleason</td>
</tr>
<tr>
<td>IV</td>
<td>T4</td>
<td>N0</td>
<td>M0</td>
<td>Any PSA</td>
<td>Any Gleason</td>
</tr>
<tr>
<td>IV</td>
<td>Any T</td>
<td>N1</td>
<td>M0</td>
<td>Any PSA</td>
<td>Any Gleason</td>
</tr>
<tr>
<td>IV</td>
<td>Any T</td>
<td>Any N</td>
<td>M1</td>
<td>Any PSA</td>
<td>Any Gleason</td>
</tr>
</tbody>
</table>

*If PSA or Gleason is not available, grouping should be determined by T stage and/or either PSA or Gleason, as available.

The **Gleason grading system** is used to help evaluate the prognosis of men with prostate cancer using samples from a prostate biopsy. Together with other parameters, it is incorporated into a strategy of prostate cancer staging which predicts prognosis and helps guide therapy. A Gleason score is given to prostate cancer based upon its microscopic appearance (NCI, 2009-05-13). Cancers with a higher Gleason score are more aggressive and have a worse prognosis (Epstein *et al.*, 2005). The most important
distinction made by any staging system is whether or not the cancer is still confined to the prostate. In the TNM system, clinical T1 and T2 cancers are found only in the prostate, while T3 and T4 cancers have spread elsewhere. Several tests can be used to look for evidence of spread. Medical specialty professional organizations recommend against the use of PET scans, CT scans, or bone scans when a physician stages early prostate cancer with low risk for metastasis (American Society of Clinical Oncology 2013).

Figure 1.6: TNM staging of Prostate cancer

1.3.5 Management of Prostate cancer:

The first decision to be made in managing prostate cancer is whether treatment is needed. Prostate cancer, especially low-grade forms found in elderly men, often grows so slowly that no treatment is required (Kolata et al., 2011). Treatment may also be inappropriate if a person has other serious health problems or is not expected to live long enough for symptoms to appear.
Which option is best depends on the stage of the disease, the Gleason score, and the PSA level. Other important factors are age, general health, and a person’s views about potential treatments and their possible side effects. Because most treatments can have significant side effects, such as erectile dysfunction and urinary incontinence, treatment discussions often focus on balancing the goals of therapy with the risks of lifestyle alterations. A combination of the treatment options is often recommended for managing prostate cancer (Lu-Yao et al., 2009; Mongiat-Artus et al., 2009, Mongiat-Artus et al., 2016).

Guidelines for treatment for specific clinical situations require a good estimation of a person’s long-term life expectancy. People can also use an 18-item questionnaire to learn whether they have good knowledge and understanding about their treatment options before they choose. Most of those who are newly diagnosed and made a treatment choice cannot correctly answer over half of the questions (Mohan et al., 2011). If radiation therapy is done first, and fails, then radical prostatectomy becomes a very technically challenging surgery and may not be feasible. On the other hand, radiation therapy done after surgical failure may have many complications (Mouraviev et al., 2006). It is associated with a small increase in bladder and colon cancer (Wallis et al., 2016).

In localized disease, it is unknown if radical prostatectomy is better or worse than watchful waiting (Hegarty et al., 2010). A meta-analysis on the effects of voiding position during urination in males with prostate enlargement showed that sitting was superior to standing. Bladder emptying was significantly improved, while there was a trend towards a higher urinary flow and shorter voiding time (De Jong et al., 2014).

1.3.5.1 Surveillance

Many men diagnosed with low-risk prostate cancer are eligible for active surveillance. This term implies careful observation of the tumor over time, with the intention of treatment for cure if there are signs of cancer progression. Active surveillance is not synonymous with watchful waiting, an older term which implies no treatment or specific program of monitoring, with the assumption that palliative, not curative, treatment would be used if advanced, symptomatic disease develops.
Active surveillance involves monitoring the tumor for signs of growth or the appearance of symptoms. The monitoring process may involve serial PSA, physical examination of the prostate, and/or repeated biopsies. The goal of surveillance is to avoid overtreatment and the sometimes serious, permanent side effects of treatment for a slow-growing or self-limited tumor that would never cause any problems for the person. This approach is not used for aggressive cancers, but it may cause anxiety for people who wrongly believe that all cancer is deadly or themselves to have a life-threatening cancer. For 50% to 75% of people with prostate cancer it will cause no harm before a person dies from other causes (Cancer.gov. 2011-04-19).

1.3.5.2 Management for Aggressive cancer

Treatment of aggressive prostate cancers may involve surgery (i.e. radical prostatectomy), radiation therapy including brachytherapy (prostate brachytherapy) and external beam radiation therapy, high-intensity focused ultrasound (HIFU), chemotherapy, oral chemotherapeutic drugs (Temozolomide/TMZ), cryosurgery, hormonal therapy, or some combination (Hong et al., 2009; Peyromaure et al., 2009). Although the widespread use of prostate specific antigen (PSA) screening in the USA has resulted in diagnosis at earlier age and cancer stage, the vast majority of cases are still diagnosed in men older than 65 years, and approximately 25% of cases are diagnosed in men older than 75 years (Fitzpatrick JM. 2008). Though US National Comprehensive Cancer Network guidelines recommend using life expectancy greater than or less than 10 years to help make treatment decisions, in practice, many elderly patients are not offered curative treatment options such as radical prostatectomy or radiation therapy and are instead treated with hormonal therapy or watchful waiting. This pattern can be attributed to factors such as medical co-morbidity and patient preferences regarding quality of life in addition to prostate cancer specific risk factors such as pretreatment PSA, Gleason score and clinical stage. As the average life expectancy increases due to advances in treatment of cardiovascular, pulmonary and other chronic disease, it is likely that more elderly patients will be living long enough to suffer the consequences of their prostate cancer. Therefore, there is currently much interest in the role of aggressive prostate
cancer treatment modalities such as with surgery or radiation in the elderly population who have localized disease.

If the cancer has spread beyond the prostate, treatment options significantly change, so most doctors that treat prostate cancer use a variety of nomograms to predict the probability of spread. Treatment by watchful waiting/active surveillance, external beam radiation therapy, brachytherapy, cryosurgery, HIFU, and surgery are, in general, offered to men whose cancer remains within the prostate. Hormonal therapy and chemotherapy are often reserved for disease that has spread beyond the prostate. However, there are exceptions: radiation therapy may be used for some advanced tumors, and hormonal therapy is used for some early stage tumors. Cryotherapy (the process of freezing the tumor), hormonal therapy, and chemotherapy may also be offered if initial treatment fails and the cancer progresses.

![Figure 1.7: Treatment options for Prostate cancer.](image)

### 1.3.6 Prostate Cancer models

Scientists have established a few prostate cancer cell lines to investigate the mechanism involved in the progression of prostate cancer. LNCaP, PC-3 (PC3), and DU-145 (DU145) are commonly used prostate cancer cell lines. The LNCaP cancer cell line was established from a human lymph node metastatic lesion of prostatic adenocarcinoma. PC-3 and DU-145 cells were established from human prostatic adenocarcinoma metastatic to
bone and to brain, respectively. LNCaP cells express androgen receptor (AR); however, PC-3 and DU-145 cells express very little or no AR. AR, an androgen-activated transcription factor, belongs to the steroid nuclear receptor family. Development of the prostate is dependent on androgen signaling mediated through AR, and AR is also important during the development of prostate cancer. The proliferation of LNCaP cells is androgen-dependent but the proliferation of PC-3 and DU-145 cells is androgen-insensitive. Elevation of AR expression is often observed in advanced prostate tumors in patients. Some androgen-independent LNCaP cell lines have been developed from the ATCC androgen-dependent LNCaP cells after androgen deprivation for study of prostate cancer progression. These androgen independent LNCaP cells have elevated AR expression and express prostate specific antigen upon androgen treatment. The paradox is that androgens inhibit the proliferation of these androgen-independent prostate cancer cells. (Kokontis et al., 1994; Umekita et al., 1996; Kokontis et al., 2005).

1.4 Outcomes of Chemotherapy:

The outcome of chemotherapy is determined by many factors, including the drug(s) used and dosage, timing of chemotherapy, treatment schedule, and the extent of cancer cell penetration through the bowel wall and other tissues, and the health condition of the patient. In conventional treatment, chemotherapeutic agents are usually administered at maximum tolerated dose, aiming to kill as many cancerous cells as possible. However, the chemotherapy efficiency is far from satisfactory due to the limited drug concentration at tumour sites. Moreover, anti-cancer drugs cause damage to healthy cells and tissues and patients suffer from severe side effects including nausea, easy bruising, hair loss and susceptibility to infections. To balance efficacy and toxicity, chemotherapy is often followed by 3-4 weeks without drug administration to enable the suppressed bone marrow cells to recover. The duration of chemotherapy influences the outcome of treatment and the quality of life of patients. In general, chemotherapy should be maintained to achieve the best survival but because of the severe toxicity of the drugs, different strategies have been developed to minimize the cumulative toxicity, including treatment breaks and restarts, shifts between less intensive therapy and more intensive therapy and minimizing toxicity. Metronomic chemotherapy involves the administration
of relatively low and nontoxic doses of drugs without a long-term drug-free break, but few clinical studies have evaluated the feasibility and efficacy of this approach. (Lin et al., 2007; Ogata et al., 2009).

Most side effects were mild, but its ability to prolong patient survival time remains to be proven. Recently, the sequential use of active single drugs instead of combination regimens has been evaluated in clinical trials and shown to reduce the overall toxicity of therapy without compromising survival benefits (Andrea et al., 2014). Overall, the strategy of chemotherapy should be based on therapeutic performance, toxicity parameters, patient condition and preferences to achieve the balance between therapy efficacy and toxicity. Clearly, there still exists significant scope for improving the mechanisms by which chemotherapeutics are delivered and internalized by tumour cells, including optimization of methodologies to maximize localization of the administered dose to the site of the tumour.

1.5 Future Perspective for Chemotherapy in Cancer Patients

Despite improvements in chemotherapeutic drugs, the efficacy of conventional delivery systems for cancer is limited by non-specific toxic effects on normal non-cancerous tissue. Hence there is a need to develop delivery systems capable of delivering high drug concentrations to the tumour site, while minimizing damage to surrounding normal tissue. (Choi et al., 2012; Glavas-Dodov et al., 2013; Rai et al., 2014).

Of the numerous strategies being investigated, drug-loaded nanoparticles (NPs) and site-specific drug delivery systems show particular promise. Although mostly designed for intravenous injection, NPs have been shown in animal models to deliver anticancer drugs more effectively to tumour cells than straight injection of neat drug. By protecting drugs from release in the upper gastrointestinal tract, colon-specific or prostate specific drug delivery systems, such as microparticles, pellets, capsules and tablets, have the potential to deliver drug at higher concentrations to the solid tumor site following intravenous administration. Recent advances in site-specific delivery systems include the encapsulation of NPs in a microcapsule or pellet matrix and these ‘NP-in-particle’ systems have been shown to enhance delivery of anticancer drugs or gene-based therapeutics to the colon. These studies used biodegradable NPs for a complete release of
their cargo in the intestinal tract and focused on the overall therapeutic effect relating to the tissue distribution of the drug or gene. However, the fate of those NPs that were encapsulated in the microcapsules was not well elucidated in their results.

1.6 Nanoparticles Drug Delivery

Since the 1950s, when biopharmaceutics and pharmacokinetics were developed, to pursue advanced drug delivery systems (DDS), for retarded and controlled release of drugs has been a major focus of attention for pharmaceutical scientists (Kreuter 2007). Later on, with the rapid progress of biomedical science, drug targeting to specific organs and tissues has also become one of the critical endeavours in the pharmaceutical field. Nowadays, the strategy of DDS has been regarded far more beyond a fairly simple approach to deliver therapeutic agents into the body with consistency and uniformity, but rather a powerful tool to improve the pharmacological and therapeutic properties of the “free” drugs by means of ameliorating some of their intrinsic properties such as solubility, in vivo stability, pharmacokinetics (PK), and bio distribution (BD) (Allen & Cullis., 2004)

Particularly in recent years, a great deal of novel biologically active substances with high molecular weight have been emerging continuously, including vaccines, plasmid DNA, and RNAi etc., most of which are still suffering from their inherent drawbacks such as low membrane penetration ability, low selectivity of targeting aimed organelles or/and tissues, sensitivity to physiological environments and so on. Thus, to develop a desired DDS to overcome those drawbacks is imperative. Nanoparticle-based drug carriers, being a kind of “small” DDS have attracted considerable attention from pharmaceutical scientists in the last decades. For example, the first submicronic lipid vesicle so-called liposome was reported in 1960s, (Bangham et al., 1965; Gregoriadis 1976)) followed by an increasing series of tiny drug carriers with size scale in nano meter range. These novel carriers were termed as “nanocarriers” or “nanoparticles”, possessing many crucial advantages over conventional DDS. (Kreuter 2007; Gelperina et al., 2005)

For instance, by virtue of their tiny dimensions (1-1000 nm, defined for pharmaceutical purpose), nanocarriers own excellent cell or tissue penetration ability rather than traditional DDS in large scale. Further, within this dimension some types of particles
such as quantum dots and gold nanoparticles display unique optical properties, leading to multiple applications of these nanoparticles with respect to not only drug delivery but also imaging and labelling. (Hutter and Maysinger., 2011) As a result of their small sizes, nanoparticles have far higher surface to mass/volume ratio relative to that of bulk materials, which enables them to adsorb, bind and carry plenty of therapeutic compounds, thus leading to high carrier capacity of drugs. Besides, capability of incorporation of both hydrophilic and hydrophobic substances and feasibility of variable routes of administration, ranging from oral administration, inhalation, to transdermal and injection are also two main advantages of nanoparticles as drug carriers. In addition, nanoparticles can be modified to allow controlled (sustained) drug release from the matrix for various therapeutic purposes. Overall, these properties enable nanoparticles being a promising tool with considerable potential to be developed as efficient, safe, and elegant pharmaceutical carrier system to fulfil those expectations mentioned above.

Table 1.4: Overview of the types of nanoparticles and their application in pharmaceutical sciences

<table>
<thead>
<tr>
<th>Particle class</th>
<th>Materials</th>
<th>Application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metal based</td>
<td>Gold</td>
<td>Drug/gene delivery, imaging</td>
<td>(Schneider et al., 2010; Polavarapu et al., 2011; Kneipp et al., 2006; Le Guevel et al., 2012; Jung et al., 2011)</td>
</tr>
<tr>
<td>Semiconductor</td>
<td>Ag</td>
<td>Antimicrobial, sensing</td>
<td>(Lee et al., 2006; Aslan et al., 2004; Tang et al., 2012)</td>
</tr>
<tr>
<td>Based</td>
<td>Silica</td>
<td>Drug delivery</td>
<td>(Zhao et al., 2009; Yuan et al., 2010)</td>
</tr>
<tr>
<td></td>
<td>ZnO</td>
<td>Drug/gene delivery, imaging</td>
<td>(Rasmussen et al., 2010; Nie et al., 2006; Shen et.al, 2008)</td>
</tr>
<tr>
<td>Magnetic</td>
<td>Drug delivery</td>
<td></td>
<td>(Sun et al., 2008; Jain et al., 2005; Gupta et al., 2005)</td>
</tr>
<tr>
<td>TiO2</td>
<td>Drug delivery</td>
<td></td>
<td>(Qin et al., 2011)</td>
</tr>
</tbody>
</table>
Chapter 1. Introduction

1.6.1 Strategies in Designing Nanoparticles for Appropriate Safety and Efficiency

Safety and efficiency are two crucial concerns in terms of nanoparticles used as drug carrier systems. The primary aims for designing nanocarriers for pharmaceutical applications can be summarized as following:

- Efficient and specific drug delivery and targeting
- Suitable/tuneable drug release rate at the target site,
- Reduction in toxicity and side effects from not only the drug but also the nanoparticles themselves

To realize all these aims is extremely attractive but not an easy task. Because the drug delivery efficiency and biological safety are mainly determined by a series of complex factors such as the intrinsic properties of the nanomaterials, bio physicochemical interactions at the nano-bio interface and the fate of the nanocarriers after penetration into the cells/organelles or tissues. The knowledge of those factors is basic prerequisite for the design of novel nanocarriers and is not fully understood so far. Actually, none of the existing nanocarriers could achieve all the aspects mentioned above to the full extent. Thanks to the rapid progress in nanotechnology and some related techniques, for instance

<table>
<thead>
<tr>
<th>Lipid based</th>
<th>SLN</th>
<th>Drug delivery</th>
<th>(Panyam et al., 2002)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NLC</td>
<td>Drug delivery</td>
<td>(Feng et al., 2013)</td>
</tr>
<tr>
<td>Polymer based</td>
<td>PLGA</td>
<td>Drug/gene delivery</td>
<td>(Bhaw-Luximon et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>Polycarolactone</td>
<td>Drug/gene delivery</td>
<td>(Guo et al., 2011; Damascelli et al., 2003)</td>
</tr>
<tr>
<td>Biological materials</td>
<td>Albumin</td>
<td>Drug delivery</td>
<td>(Dyer. et al., 2002)</td>
</tr>
<tr>
<td></td>
<td>Chitosan</td>
<td>Drug delivery</td>
<td>(Huang et al., 2000; Kaul et al., 2002)</td>
</tr>
<tr>
<td></td>
<td>Gelatin</td>
<td>Drug/gene delivery</td>
<td>(Cascone., et al., 2002; Sarmento., et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Alginates</td>
<td>Drug/gene delivery</td>
<td>(You., et al., 2006; Ahmad., et al., 2006; Nel., et al., 2009)</td>
</tr>
</tbody>
</table>
polymer-chemistry and single particle probing techniques, more and more parameters of the particles had been revealed influencing the delivery efficiency and safety with certain probability by theoretical simulation or practical experiments. Until now, enormous endeavour in design of nanocarriers with appropriate safety and efficiency had been tried, and in general they can be classified into three types: surface coating, size controlling and shape tailoring, because these three parameters dramatically impact the nanoparticles’ cellular uptake behaviours that mainly determine the safety and efficiency of nanoparticles.

1.6.2 Cellular Uptake of Nanoparticles

To make use of nanoparticles’ potential for drug delivery applications, their cellular uptake behaviour must be addressed because most commonly used drugs, for instance certain antimicrobial and antitumor drugs have to pass through the cell membrane before acting their functions on the sub-cellular sites. A series of interactions occurred at the interfaces between nanoparticles and certain biomaterials such as proteins, cells and DNA were found playing a great role in dominating the cellular uptake behaviour of nanoparticles. Thus during the last decade, there has been an increasing interest in these bio-physicochemical interactions at the nano-bio interface and will be described in detail later.

Figure 1.8: Nanoparticulate drug delivery and cellular uptake across the biological membrane.
1.6.3 Factors Influencing Interactions of Nano-Bio Interface

Typically, the ‘nano-bio interface’ is defined as the interface between nanomaterial surfaces and the surfaces of biological components such as cell membranes, organelles membranes, phospholipids, proteins and biological fluids. Numerous dynamic physicochemical interactions, kinetics and thermodynamic exchanges occur at this interface, leading to unprecedented types of complex reactions that can hardly be fully predicted by theoretical manners so far. However, investigation in the interactions at the nano-bio interface has been the most attractive aspect of nanotechnology in recent years. This knowledge is essential for us to deeply understand the cellular uptake process of nanoparticles and to design suitable nanocarriers for the aims of drug delivery. An assembly of conceptual factors influencing interactions of nano-bio interface is summarized in Table 1.5

Table 1.5: Main bio-physicochemical influences on the interface between nanomaterials and biological systems. Adopted from (Nel et al., 2009)

<table>
<thead>
<tr>
<th>Sources of factors</th>
<th>Factor contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanoparticles</td>
<td>Size, shape and surface area; Surface charge, energy, roughness and porosity; Valence and conductance states; Functional groups; Ligands; Crystallinity and defects; Hydrophobicity and Hydrophilicity.</td>
</tr>
<tr>
<td>Suspending media</td>
<td>Water molecules; Acids and bases; Salts and multivalent ions; Natural organic matter (proteins, lipids); Surfactants; Polymers; Polyelectrolytes</td>
</tr>
<tr>
<td>Solid-liquid interface</td>
<td>Surface hydration and dehydration; Ion adsorption and charge neutralization; Electrical double-layer formation, zeta potential, isoelectric point; Sorption of steric molecules and toxins; Electrostatic, steric and electrosteric interactions; Aggregation, dispersion and dissolution; Hydrophilic and hydrophobic interactions.</td>
</tr>
</tbody>
</table>
| Nano-bio interface     | Membrane interactions: specific and nonspecific forces; Receptor-
ligand binding interactions; Membrane wrapping: resistive and promotive forces; Biomolecule interactions (lipids, proteins, DNA) leading to structural and functional effects; Free energy transfer to biomolecules; Oxidant injury to biomolecules; Mitochondrial and lysosomal damage, decrease in ATP

As shown in Table 1.5, numerous characteristics of nanomaterials and biological systems have been demonstrated affecting the bio-physicochemical interaction at the nano-bio interface and as well further governing the cellular uptake process of nanoparticles. However, an accurate quantitative relationship between all those parameters and the cellular uptake behaviour of nanoparticles is still unclear because of the difficulties to simultaneously manage so many variations in one experiment. In addition, the interaction between each individual parameter also requires special consideration in most of the cases. Therefore, firstly to investigate the effect of individual parameters on nanoparticles cellular uptake behaviour and then collect all the assembled knowledge together could be an advisable strategy for this exploration.

1.6.4 Anticancer Drug Delivery with Nanoparticles:

Therapeutic and diagnostic agents can be encapsulated, covalently attached, or adsorbed into such nanocarriers while the nanoparticle surface can be functionalized with synthetic polymers and appropriate ligands. Such techniques enable researchers to modulate the pharmacokinetic profiles of injectable nanocrystals which may vary from rapidly soluble in the blood to slowly dissolving, making the drug release system controllable.

A variety of polymeric nanoparticle applications as drug delivery systems, have been presented in the literature. For instance, Lu et al. have developed a polymeric drug delivery system for Paclitaxel, synthesizing poly-lactic acid nanoparticles by ultrasonic emulsification and demonstrated how this system inhibits the growth of ovarian carcinoma xenografts (Lu Z et al., 2004).
1.6.4.1 Natural polymers nanoparticles:

Natural polymers can also be used to manufacture nanocarriers for drug delivery. Among them the most utilized polymers are gelatin, dextran and chitosan. In general these nanoparticles have high encapsulation efficiency.

Abraxis Oncology (Los Angeles, CA, USA) produces ABRAXANE® Injectable Suspension, which is an injectable suspension of paclitaxel protein-bound nanoparticles (albumin-bound) (Roy et al., 2003; Abraxis oncology, 2012). The advantage of using a protein based carrier is that albumin normally transports nutrients to cells and has been shown to accumulate in rapidly growing tumors. ABRAXANE® is solvent-free, so solvent related toxicities are eliminated, which enables higher doses of Paclitaxel to be administered.

1.6.4.2 Gold Nanoparticles, Quantum dots and Dendrimers:

Gold nanoparticles represent a novel technology in the field of particle-based tumor-targeted drug delivery. Paciotti et al. (Paciotti et al., 2004) have reported an application of these carriers for the targeted delivery of tumor necrosis factor-alfa (TNF-α) to solid tumors. Brown et al. has tethered the active component of the anticancer drug Oxaliplatin to a gold nanoparticle for improved drug delivery (Brown et al., 2010).

Quantum dots have the potential to dramatically improve clinical diagnostic tests for the early detection of cancer. These engineered semiconductor particles combine cadmium with selenide in a tightly packed atomic structure that emits light in a spectrum of six colour, plus four near-infrared colour, as the dots decrease in size. By finely tuning the size of the dots, thousands of subtle colour variations could be created. These tiny glowing particles, when conjugated with anti-bodies, peptides, proteins, or DNA, form bio-conjugated dots that can act as markers on cells and genes, giving scientists the ability to rapidly and differentially mark pathologic tissues.

Dendrimer-based drug delivery molecules have several potential advantages: dendrimers are comparable in size to proteins, being small enough (<5.0 nm in diameter) to escape the vasculature and target tumor cells, while also being below the threshold of renal filtration to allow urinary excretion. For instance, acetylated dendrimers have been
conjugated to folic acid, methotrexate, tritium, fluorescein and 6-carboxytetramethylrhodamine, in order to allow simultaneous treatment and drug uptake monitoring in tumors (Kukowska-Latallo et al., 2005). A biocompatible polyester dendrimer composed of the natural metabolites, glycerol and succinic acid, were used for the encapsulation of the antitumor camptothecins, 10-hydroxycamptothecin and 7-butyl-10-aminocamptothecin (Morgan et al., 2006).

1.6.4.3 Lipid Based nanoparticles

Lipoproteins are another interesting type of vector for lipophilic drugs that can be incorporated into the a polar core without affecting lipoprotein recognition. They could be recognized and taken up via specific receptors and mediate cellular uptake of the carried drug. In addition, they are biodegradable. Although only low density lipoproteins have been explored intensively as drug carriers for cancer chemotherapy, new investigations are focused on the use of high density lipoproteins (HDL). Bin Lou et al. (Lou et al., 2005) have shown that a recombinant complex of HDL and aclacinomycin, prepared by co-sonication, is able to deliver a drug to hepatoma cells. Loading anticancer drugs into HDL as well as LDL has little effect on properties of complexes and enhances cytotoxicity to human carcinoma cells (Kadar et al., 2002).

16.4.4 Magnetic and Ceramic-based nanoparticles

Magnetic-drug targeting can offer a unique opportunity to treat malignant tumors loco-regionally. Alexiou et al. (Alexiou et al., 2006) have treated squamous cell carcinoma in vivo with the injection of magnetic nanoparticles (ferrofluids) bound to mitoxantrone, as a chemotherapeutic agent, that was locally induced to concentrate by means of a magnetic field. The intra-tumoral accumulation of the particles can additionally be visualized by means of MRI. Lubbe et al. developed magnetic drug delivery for 4-Epidoxorubicin and evaluated for advanced solid tumor (Lubbe et al., 1996). These nanoparticles have been extensively investigated because of their enormous potential in the photodynamic cancer therapy (PCT) field. This is an emerging modality for the treatment of a variety of oncological, cardiovascular, dermatological and ophthalmic diseases. PCT is based on the concept that light-sensitive species or photo-
sensitizers can be preferentially localized in tumor tissues upon systemic administration. Roy et al. (Roy et al., 2003) have shown that ultra-fine organically modified silica-based nanoparticles, carrying a water-insoluble photosensitizing anticancer drug-dye, 2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide (HPPH), were efficiently taken up by tumor cells in vitro, and light irradiation of such impregnated cells resulted in significant cell death.

16.4.5 Polymeric nanoparticles: (Catarina et al., 2006)

Polymeric nanoparticles have been extensively studied as particulate carriers in the pharmaceutical and medical fields, because they show promise as drug delivery systems as a result of their controlled- and sustained-release properties, subcellular size, and biocompatibility with tissue and cells. Several methods to prepare nanoparticles have been developed during the last two decades, classified according to whether the particle formation involves a polymerization reaction or arises from a macromolecule or preformed polymer.

Nanocapsules are vesicular systems in which the drug is confined to a cavity consisting of an inner liquid core surrounded by a polymeric membrane. In this case the active substances are usually dissolved in the inner core but may also be adsorbed to the capsule surface.

1.7 Drug Delivery Systems Selected for the Study

1.7.1 Solid Lipid Nanoparticles:

SLNs consist of spherical solid lipid nanoparticles in the nano-meter range, which are dispersed in water or in aqueous surfactant solution. SLNs are produced from solid lipids producing a solid matrix with few cavities at room and body temperatures (figure 2). They are made of one or more lipids with melting points higher than body temperature, so this carrier remains in solid state after administration.
These carriers have a hydrophobic core being specially adapted to the transport of hydrophobic drugs such as resveratrol. These controlled-release systems are suitable for transporting and protecting this important bioactive compound against degradation, increasing its physical stability and have a special ability to penetrate cell membranes, allowing the increased cellular uptake of compounds they are loaded with SLNs are very promising since they combine a variety of advantages: (Kaur et al., 2008)

- They are composed of biodegradable and biocompatible excipients (“GRAS” Lipids - generally recognized as safe, FDA);
- This is a water based technology, avoiding organic solvents that may be a focus of toxicity;
- These systems have a high physical stability;
- SLNs in a range of 120-200 nm are not taken up readily by the cells of mononuclear phagocyte system (MPS) and thus bypass liver and spleen filtration;
- It can be achieved a controlled release of the incorporated drug for up to several weeks;
- It is possible to functionalize the surface directing the drug to its site of action (maximizing therapeutic effects and minimizing side effects);
- Depending on the composition and preparation they may be quite stable over time;
- It can be achieved a high entrapment efficiency, depending on the drug lipophilic properties;
- This is a delivery system economically more affordable than others because it is easy to scale-up due to its facility of preparation and reproducibility.
Therefore, SLNs have a lot of advantages as a system for drug delivery, namely for lipophilic compounds. However, our aim is that nanoparticles reach the tumor site, so we need to develop a specific target to colon and prostate cancer. In the late 90s SLNs were proposed for brain drug targeting application even though the first proof of lipid particle transport across the BBB had already been provided (Blasi et al., 2007). Polymeric nanoparticles have been characterized as a good vehicle to deliver drugs into the brain although there are various problems associated with the use of these nanoparticles like residual contamination from production process (organic solvents), polymerization initiation, large polymer aggregates, toxic monomers and toxic degradation products, expensive production methods, lack of large scale production method and suitable sterilization method (Kaur et al., 2008). Lipid-based nanocarriers hold strong promise to delivery of drugs to tumor site because these materials are biocompatible and biodegradable as already mentioned and because the lipophilic materials have the natural tendency to target through physiological cell membrane. Several studies have been made to direct drugs to cancer site and there are other important features which makes the SLNs as appealing:

- Due to its lipid nature (lipophilic) SLNs have the natural tendency to cross the physiological membrane.
- The SLNs appear suitable as a drug carrier system for potential intravenous use due to their very low cytotoxicity in vitro (SLNs cause less non-specific cell toxicity even compared to nanoparticles made of PLGA).

For the preparation of SLNs it is necessary the use of one or more surfactants that stabilize the lipids in aqueous solution and allow the formation of spherical nanoparticles. However the type of surfactant used can be crucial to the success of the formulation since it determines the type of plasma proteins that will adsorb in the surface of the nanoparticles when they are administered. The composition of the protein layer is regarded as the decisive factor, for example immunoglobulin G is known to be a specific activator of the complement system promoting recognition and phagocytic uptake of particulate carriers by MPS, whereas albumin creates a more hydrophilic surface which was found to reduce the phagocytic uptake in vivo (Torsten et al., 2005).
In the present study, the desired size of nanoparticle was achieved by first high speed homogenization and then passing through high pressure homogenization at various pressure and homogenization cycles.

1.7.2 Preparation of Solid Lipid Nanoparticles

High pressure homogenization: In high pressure homogenization fluid is passed under pressure through a narrow gap. During this process low pressure zones are produced as the result of a local increase in velocity brought about by the fluids passage through a narrow gap.

According to the Bernoulli’s law (Muller et al., 1995), if the pressure falls below the vapour pressure of the fluid, tiny bubbles filled with the steam or gas form. These bubbles begin to collapse when they are moved into regions where the local pressure is higher than the vapor pressure. When the bubbles implode the surrounding particles, such as the lipid bilayers are accelerated towards the center of the collapsing bubbles. This causes reduction in the size of particles, or the rupture of cells. This phenomenon called cavitation is believed to be the main cause of size reduction.

In the present research work, for homogenization High speed homogenizer Kinematica, High pressure homogenizer from GEA Niro Soavi, model PANDA (Fig no.1.3) were used.

![High speed homogenizer and High pressure homogenizer](image)

**Figure 1.10: High speed homogenizer and High pressure homogenizer**

This work intends to take advantage of nanotechnology developing solid lipid nanoparticles to increase the concentration of anticancer drug in the solid tumors (Colon
and Prostate tumors) which may exert its therapeutic effect. Thus main target is maximum drug reaching the tumor site. SLNs of a size below 200 nm in presence of hydrophilic surfactants i.e. Poloxamer have an increased blood circulation. Additionally the delivery process can be made more selective and efficient by tagging the nanocarrier surface with ligand molecules that match with a receptor type that is strongly and specifically expressed on the surface of the cells to be targeted (Wong et al., 2010). This is a highly regulated and energy-dependent process, but may allow the whole nanocarriers and the loaded drug to go the specific tumor site. Ideally nanoparticles for therapeutic usage should remain in circulation for an adequate time and be present at the site of action in adequate amounts to promote a controlled drug release and an efficient uptake by cells for improving the therapeutic efficacy.

1.7.3 **SLNs as a targeted carrier for anticancer drug to solid tumors:**

SLNs have been reported to be useful as drug carriers to treat neoplasms (Shenoy et al., 2005). Tamoxifen, an anticancer drug incorporated in SLN to prolong release of drug after i.v. administration in breast cancer and to enhance the permeability and retention effect (Murthy et al., 2005). Tumour targeting has been achieved with SLNs loaded with drugs like methotrexate (Ruckmani et al., 2005) and camptothecin (Yang et al., 1999).

1.7.4 **Future Perspectives of Lipid-Based Nanoparticles in Drug Delivery**

Various nanotechnology platforms are currently being developed with the aim of improving drug delivery, especially to combat cancer. Among these, lipid-based nanoparticles present one of the promising drug-delivery candidates and have been the longest-studied nanocarriers. Despite tremendous efforts, only a few formulations are approved for clinical use thus far. In addition, the clinical applications of targeted nanoparticles remain to be seen. Therefore, it is imperative to re-visit considerations of current approaches and strategies employed in the design and development of these anticancer nanocarriers. In our opinion, efforts could be focused primarily on two areas: i) technical aspects such as fabrication strategies, the development of techniques for reproducible nanocarriers, large-scale production, and the conjugation of targeting molecules such as scFvs and peptides to the nanoparticle surface; and ii) novel concepts
and approaches to accomplish on-demand release of drugs from the nanoparticles (based on the unique properties of the assembly components of lipid-based nanocarriers). SLN are very attractive drug delivery candidates, primarily due to their relatively stable constituents and probable ease of drug encapsulations. However, their future in the clinical setting is also subject to extensive research. Additionally, it is our viewpoint that two important aspects for clinically viable nanocarriers will facilitate their usefulness in the clinic: the development of triggering modalities that are amenable to human applications and the development of alternate strategies for in vivo stabilization of drug-delivery vehicles. Although the concept of PEGylation to increase half-life of nanoparticles revolutionized the nanoparticle-mediated drug-delivery field, significant improvements are warranted in this area.

1.8 CANCER AS A TARGET OF DRUG DELIVERY SYSTEM:

Progress in fundamental cancer biology has not yet been met by a comparable advancement in its clinical treatment. Fundamental reason for this discrepancy is the inability to selectively reach and eliminate tumor tissue with marginal damage to healthy organs rather than the availability of potent chemotherapeutics (Ferrari et al., 2005; Moses et al. 2003). Cancer cell targeting by DDS aims at increasing selectivity and overcoming biological barriers, while transporting higher drug amounts. Generally, targeting may be a result of (i) the unique tissue physiology of the target (passive targeting) (ii) a specific recognition of target cells by carrier-conjugated molecules (active targeting) (iii) a localized external energy activation or (iv) a synergistic combination of the above strategies. In tumor targeting all the above mentioned strategies are being investigated.

1.8.1 Enhanced Permeation and Retention (EPR) effect

Tumor angiogenesis is dysregulated as a consequence of rapid cancer growth and leads to a physiologically and structurally defective formation of vasculature (Ruoslahti et al., 2002). The architectural anarchy, combined with an overproduction of permeability enhancers and impairment of lymphatic drainage, results in the preferential extravagation and retention of high molecular weight (MW) macromolecules and colloids in developing tumors, a feature which has been termed ‘enhanced permeation and retention effect.
(Maeda et al., 2000). The EPR effect is the most widely used targeting method, with clinical products based on it.

![Diagram of EPR effect](https://via.placeholder.com/150)

**Figure1.11:** Schematic representation of the EPR effect; healthy endothelium prevents extravasations of high MW molecules and colloids, whereas low MW agents are drained by the lymphatics (left). Dysfunctional lymphatics and highly permeable vascular endothelium allow the preferential accumulation and retention of macromolecules and colloids, in solid tumors (right).

### 1.8.2 Active Targeting

Active targeting is accomplished by attachment of specific molecules on the carrier’s surface, which enhance the binding and interactions with antigens or receptors expressed on specific cell populations (Allen 2002). Targeting ligands explored for cancer therapy include, but are not limited to, antibodies and antibody fragments (Dinuuer et al., 2005), vitamins (Na K et al., 2003), peptides (Fahr et al., 2002), folate (Leamon et al., 2004) and transferrin (Sahoo et al., 2005). The choice of appropriate ligand is based on its specificity, stability, availability and selective display of its corresponding pair on the target cells, as well as its cost. In addition to the above considerations, conjugation chemistry (Nobs et al., 2004), density and accessibility of the ligand (Torchilin et al., 2001), need to be properly designed for efficient vector targeting.

Active targeting complements passive accumulation into tumors; selectivity and retention are improved as a result of specific interactions with target cells, at the expense of increased complexity, cost and risks (e.g. adverse biological reactions to ligand).
1.8.3 Intracellular trafficking

Once the drug delivery vehicle has reached the tumor tissue, subsequent drug release may occur in the extracellular space, or following internalization of the carrier. Drugs with intracellular action, incapable of crossing cell membranes, need to be assisted in reaching their target. Cellular uptake mechanisms vary according to cell type (e.g. phagocytic vs nonphagocytic cells), physicochemical properties of the internalized entity and mode of activation (e.g. receptor mediated endocytosis) (Torchilin V. P., et al., 2001).

Figure 1.12: Different mechanisms (or their combination) of colloidal drug delivery system. Ligand-targeted colloids (A) bind to epitopes on the cell surface (i, iii). Endocytosis might occur non-specifically (ii) or following binding to receptors which promote internalization (iii). Upon internalization, the carrier either escapes into the cytoplasm (v) or releases the cargo in vesicular organelles in response to environmental stimuli (enzymes, pH, and reductive conditions). Non-targeted colloids (B) which have reached their target through passive targeting, release the drug in the proximity of the cell (vii) or in contact with the cell membrane (vi).

Various strategies have been developed and successfully applied to attain desirable subcellular localization: lysosome degradable linkers (Jensen et al., 2003), nuclear localization signals (Jensen et al., 2003; Trensin et al., 2005) and acid- or reduction-responsive carriers exploiting the endosomal maturation transformations (Murthy et al., 2003).
2005; Gillies et al., 2005), are some examples. Moreover, intracellular targeting is feasible through the use of ligands that trigger receptor-mediated endocytosis. Active targeting still faces challenges, but it also holds immense potential; discrepancies are frequently observed between in vitro and in vivo situations. The use of diverse targeting moieties per carrier, the development of even more selective and efficient ligands (e.g. via phage display) may allow more precise control over the biological fate of colloidal drug delivery systems.