Chapter 2

LITERATURE REVIEW

2.1 Anatomy and Physiology of Human Uterus

2.1.1 Structure of Human Uterus

The female reproductive system is made up of internal organs, including the vagina, uterus, ovaries and fallopian tubes, and the external genital organs (the parts that make up the vulva). All the internal organs are in the pelvis, which is the lower part of the abdomen between the hipbones. The uterus is a hollow, muscular, pear-shaped organ located posterior and superior to the urinary bladder. Connected to the two fallopian tubes on its superior end and to the vagina (via the cervix) on its inferior end, the uterus is also known as the womb, as it surrounds and supports the developing fetus during pregnancy. [1]. Figure 2.1 shows the structure of normal human uterus. The uterus can be divided anatomically into four segments: The fundus, corpus, cervix and the internal os. The lining of the uterine cavity is called the endometrium. It consists of the functional endometrium and the basal endometrium from which the former arises. Damage to the basal endometrium results in adhesion formation and/or fibrosis (Asherman's syndrome). [1]

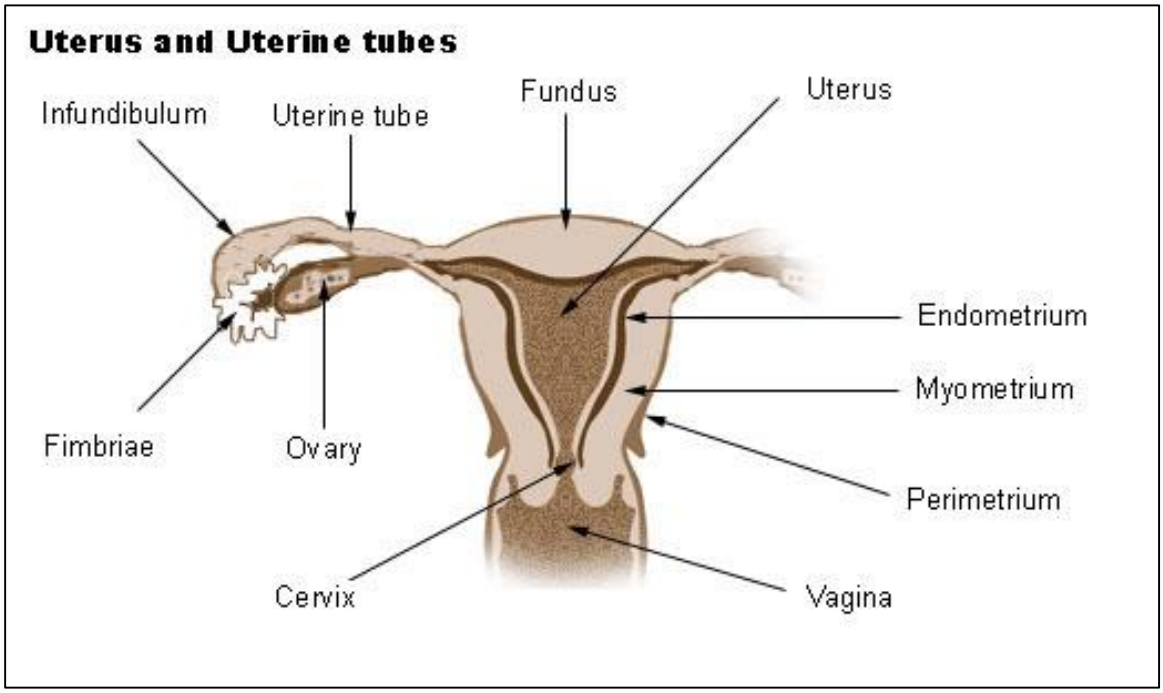


Figure 2.1 Structure of Human Uterus

The perimetrium is the outermost layer that forms the external skin of the uterus. It is a serous membrane continuous with the peritoneum that covers the major organs of the abdomino-pelvic cavity. The perimetrium protects the uterus from friction by forming a smooth layer of simple squamous epithelium along its surface and by secreting watery serous fluid to lubricate its surface. Deep to the perimetrium layer, the myometrium forms the middle layer of the uterus and contains many layers of visceral muscle tissue. During pregnancy the myometrium allows the uterus to expand and then contracts the uterus during childbirth. Inside the myometrium is the endometrium layer that borders the hollow lumen of the uterus. The endometrium is made of simple columnar epithelial tissue with many associated exocrine glands and a highly vascular connective tissue that provides support to the developing embryo and fetus during pregnancy. [2] Figure 2.2 shows the different layers of human uterus giving more insight of the different cell types.

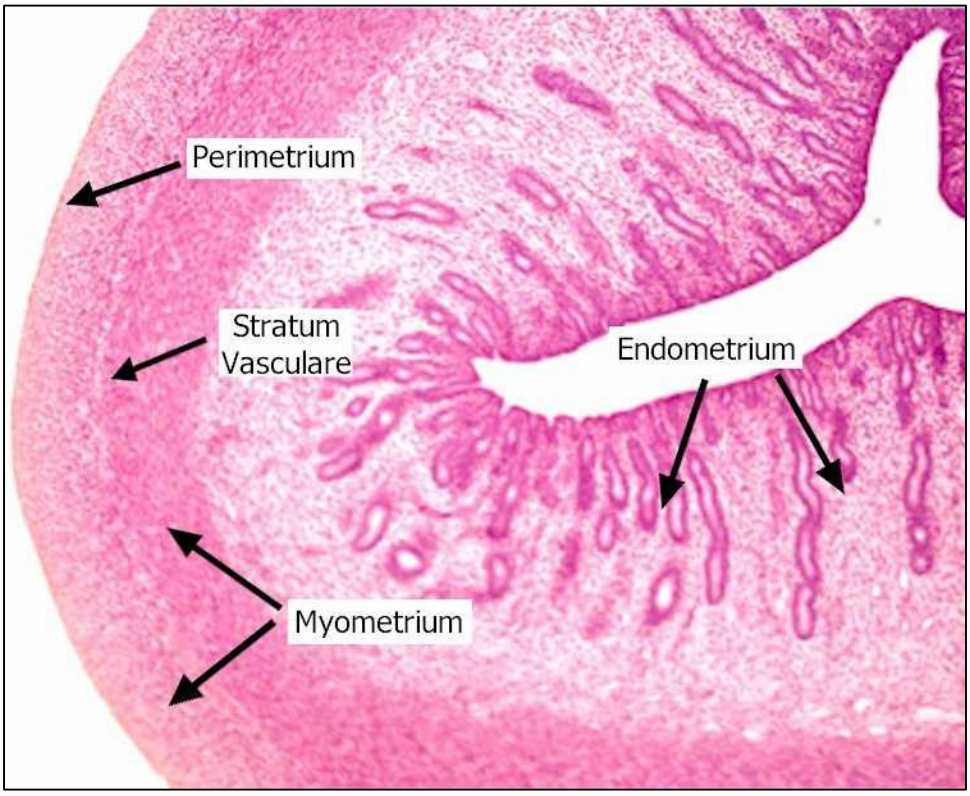


Figure 2.2 Histology of Human Uterus

In all placental mammals, including humans, the endometrium builds a lining periodically which is shed or reabsorbed if no pregnancy occurs. Shedding of the functional endometrial lining is responsible for menstrual bleeding (known as a menstrual period in humans, with a cycle of approximately 28 days, +/-7 days of flow and +/-21 days of progression) throughout the fertile years of a female. The uterus is primarily supported by the pelvic diaphragm, perineal body and the urogenital diaphragm. Secondarily, it is supported by ligaments and the peritoneum (broad ligament of uterus). [3]

The main purpose of the uterus is to nourish a fetus prior to birth. In menstruating females, the ovaries release eggs that travel via the fallopian tubes to the uterus. If fertilized, the eggs will bind themselves to the wall of the uterus and the fetus will develop. The uterus nourishes and protects the fetus until birth. The myometrium layer assists with labor in pushing the baby out of the uterus via the cervix and vagina. [3]

2.1.2 Uterine Vascular System

Anatomically, the uterus is mainly supplied with blood from the uterine arteries. The uterine artery originates bilaterally from the anterior division of the hypogastric artery and runs medial ward on the muscle “levator ani” toward the cervix uteri. It crosses approximately 2 cm from the cervix above the urethra, to which it supplies a small branch. Reaching the side of the uterus, it ascends in a tortuous manner between the two layers of the broad ligament to the junction of the uterine tube and uterus. Branches of the uterine artery form anastomoses with branches from the ovarian artery, creating an arterial arcade running in the upper part of the broad ligament. The uterine artery also supplies branches to the cervix uteri and vagina; the latter anastomose with branches of the vaginal arteries. [4]

Uterine veins parallel the arteries forming plexuses that end into the internal iliac vein; uterine veins merge with vaginal plexus (utero-vaginal venous plexus) downward and with the ovarian veins upward (utero-ovarian plexus). In recent years, interest has been focused on the close anatomical relationships between the venous and arterial wall in the female pelvic vessels. This makes possible the occurrence of a physiological exchange

mechanism known as “counter-current exchange”. A counter-current mode of exchange exists with an upward vagina-to-uterus transport which creates a functional portal system linking the vagina to uterus which is also known as the “First Uterine Pass Effect”. [5, 6]

2.2 First Uterine Pass Effect (FUPE)

In the past few years, researchers have demonstrated the effectiveness of the vaginal route for delivering hormones like progesterone preferentially to the uterus while their minimizing systemic levels and side effects with growing evidence supporting a preferential distribution to the uterus. This phenomenon is called as the First Uterine Pass Effect. [7, 8, 9] Among the different mechanisms that could explain the preferential distribution to the uterus after vaginal administration, the counter-current transfer from the vaginal veins to the uterine artery probably plays a pivotal role.

In 1994, Miles and co workers provided demonstration of selective distribution of progesterone from vagina to uterus. When comparing i.m. and vaginal progesterone, these researchers observed that vaginal administration led to lower serum progesterone concentrations and higher tissue concentration of progesterone in the endometrium as compared with measurements made after i.m. administration. [7]

Hence FUPE is a concept that proposes that vaginally administered drugs are preferentially delivered to the uterus through some form of direct transport mechanism. According to this principle, vaginally administered substances are targeted to the uterus where their tissue concentration is amplified and systemic absorption is minimized which limits the circulating level and side effects. [10] Several theoretical mechanisms have been proposed to account for the phenomenon:

i) Passive Diffusion through Tissues: Direct Vagina to Uterus diffusion through tissues. [11]

ii) Passage from cervical lumen from vagina to uterus (aspiration): passage through cervical canal has been proposed as possible mechanism explaining the direct diffusion from the vagina to the uterus. Evidence for this direct transport mechanism involving

aspiration through the cervical canal into the uterus, facilitated by uterine contractions has been provided by using vaginally administered sperm-sized ^{99m}Tc-labeled macroaggregates of human serum albumin. [11]

iii) The Venous or Lymphatic Circulatory Systems: drugs placed in vagina are absorbed as through other mucosae. Absorption is dependent upon transport in blood and/or lymph. Lymph originating from the mucous membrane and muscles in the cranial part of the vagina are collected by the small lymph vessels. The small vessels join to form two or three bigger vessels, which run on each side of the organ passing through the plica urogenatalis. Ultimately these vessels unite with lymph vessels draining the caudal part of the uterus. Therefore the lymphatic vessels of the upper part of the vagina being in direct communication with those of the uterus may represent a potential route for direct passage to the uterus of substances applied to the vagina. [12, 13]

iv) Countercurrent vascular transport with diffusion between utero-vaginal veins/ lymph vessels and arteries: it is widely considered that the countercurrent mechanism, whereby substances are transported from the vagina (through the vaginal vein) to the uterus (through the counterflowing uterine artery), is of primary importance in understanding the first uterine pass effect. The vein-to-artery transport is facilitated by the fact that in women the utero-ovarian veins are known to form a plexus on top of and in intimate contact with the ovarian artery, thus providing a large surface area favoring direct partitioning of substances between vessels and in accordance with the concentration gradient established by vaginal application [14].

Figure 2.3 shows the possible mechanisms for the First Uterine Pass Effect.

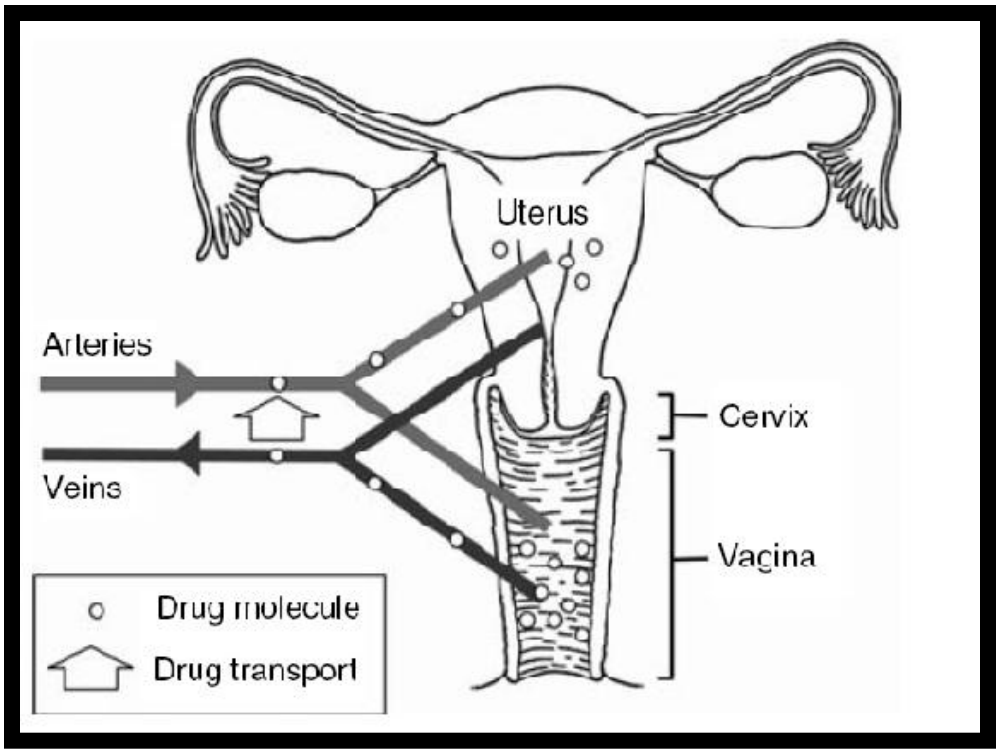


Figure 2.3 First Uterine Pass Effect

It has recently been demonstrated that the extent of the first uterine pass effect might be dependent on the exact location within the vagina of the administered formulation. A study performed showed preferential vaginal to uterine distribution of estradiol when a single estradiol tablet was placed in the upper third of the vagina, whereas no effect was observed for placement in the lower third [15]. Although there is evidence for the first uterine pass effect after vaginal drug application, the mechanism of this has not yet been elucidated. Whether this is due to absorption in to rich venous or lymphatic vaginal system and/or counter current transfer between utero vaginal lymph vessels or veins and arteries or due to direct diffusion through the tissues or through intraluminal transfer from the uterus to vagina. Nevertheless, the vaginal route might as a result of FUPE be a valuable route of drug delivery to the uterus. Targeted drug delivery to uterus through vaginal administration is particularly appealing for substances destined to exert their primary action on the uterus itself.

2.3 Uterine Fibroids and Endometriosis

2.3.1 Uterine Fibroids

Uterine leiomyomas, commonly known as fibroids, are well-circumscribed, non-cancerous tumors arising from the myometrium (smooth muscle layer) of the uterus. In addition to smooth muscle, leiomyomas are also composed of extracellular matrix (i.e., collagen, proteoglycan, fibronectin). Other names for these tumors include fibromyomas, fibromas, myofibromas, and myomas. Fibroids are the most common type of solid tumur in women of reproductive age, with an incidence of 20–25%. [16] Although they may be asymptomatic, fibroids often cause a variety of health problems for women, including menorrhagia, chronic pelvic pain, pressure symptoms on adjacent pelvic organs, recurrent miscarriages, obstructed labour, postpartum haemorrhage and sepsis. Leiomyomas are also associated with a range of reproductive dysfunction including premature labor, fetal malpresentations and infertility. [17] Leiomyomas are usually detected in women in their 30's and 40's and will shrink after menopause in the absence of post-menopausal estrogen replacement therapy indicating that ovarian hormones play a role in promoting growth. [18, 19]

Leiomyomas are classified by their location in the uterus. Subserosal leiomyomas are located just under the uterine serosa and may be Pedunculated (attached to the corpus by a narrow stalk) or sessile (broad-based). Intramural leiomyomas are found predominantly within the thick myometrium but may distort the uterine cavity or cause an irregular external uterine contour. Submucous leiomyomas are located just under the uterine mucosa (endometrium) and, like subserosal leiomyomas, may be either pedunculated or sessile. Figure 2.4 shows the different types of fibroids based on their origin. [20]

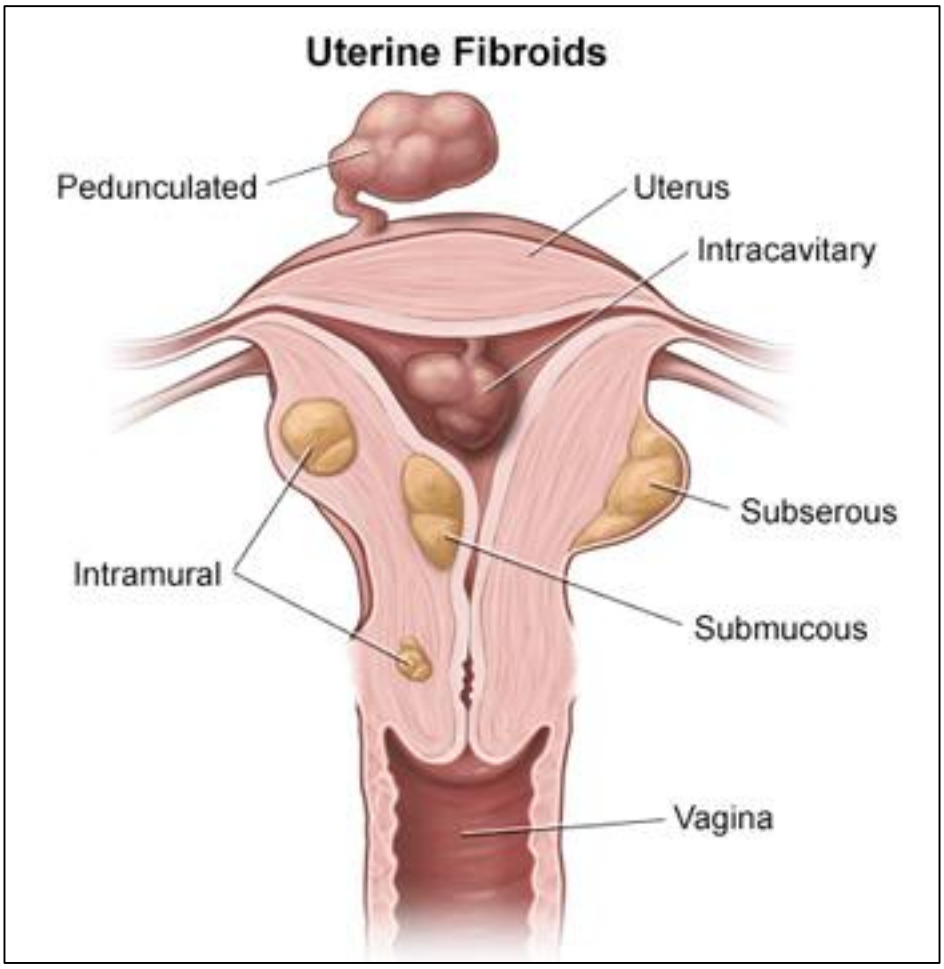


Figure 2.4 Types of Uterine Fibroids

The two most common symptoms of fibroids are abnormal uterine bleeding and pelvic pressure. The most common bleeding abnormality is menorrhagia (prolonged and/or profuse uterine bleeding, also called hypermenorrhea). Normal menstrual periods typically last four to five days, whereas women with fibroids often have periods lasting longer than seven days. Although abnormal bleeding can occur with any of the three classes of fibroids, women with submucous fibroids seem particularly prone to this complication. Pelvic pressure results from an increase in size of the uterus or from a particular fibroid. Most women with leiomyomas have an enlarged uterus. Pressure on these structures can result in difficulty with bowel movements and constipation or urinary frequency and incontinence. Rarely, fibroids can press on the ureters, which can lead to kidney dysfunction. [20]

Although the aetiology of uterine fibroids is unknown, there are some reports for understanding of the pathogenesis of this condition. [21] As with all tumors, there are two distinct stages in development: transformation from a normal to an abnormal cell; and growth and proliferation of the abnormal cells. Although fibroids may be multiple or single in a woman's uterus, studies have established that, irrespective of size, each fibroid develops from one single cell (the monoclonal development of fibroids concept). The different phases in a cell cycle are well known; a phase of DNA replication and synthesis (S-phase) and a phase of cell mitosis (M-phase), separated by gap or rest phases (G0, G1 and G2 phases). This results in orderly and identical replication of cells with the same phenotype. Transformation to a different cell phenotype appears to be a stepwise process determined by genomic alterations. Transformation is independent of cell proliferation, although unrestricted proliferation may be favored with repeated and progressing transformation. However, transformed cells often exhibit one of the following features: immortalization; change in cell morphology; decreased contact inhibition; altered dependence on growth factors; and tumourigenicity. Initiation of transformation may result from altered activity of any factor in the transformation signalling pathway or from reduced activity of transformation-inhibiting factors such as tumour suppressor genes and anti-oncogens. [22, 23]

Several factors appear to influence the growth of uterine fibroids. These include ovarian steroids, growth factors, and angiogenesis.

i) *Ovarian Steroids*: Uterine fibroids are oestrogen and progesterone dependent by a cellular and molecular mechanism. It has long been known that fibroid growth only occurs in women of reproductive age, and growth is diminished under hypo-oestrogenic states such as the menopause or during treatment with gonadotrophin-releasing hormone agonist (GnRH analogue) with a reversible reduction in fibroid size with GnRH analogue therapy. [24, 25, 26] There is experimental evidence of increased estrogen receptor gene expression in uterine fibroids compared with normal myometrium, and an enhanced response to estrogen stimulation by fibroid cells compared with normal myometrial cells. [27, 28, 29] Progesterone has been shown to up regulate, in fibroids, the level of some

proteins that influence cell proliferation, such as anti-apoptotic protein Bcl-2 and proliferating cellular nuclear antigen. Finally, the maintenance of a differentiated cellular state in fibroids appears to be regulated negatively by progesterone but positively by estrogen. Ovarian steroids therefore appear to stimulate fibroid growth but in a non-uniform manner, with both estrogen and progesterone stimulating growth. [30, 31, 32]

ii) *Role of growth factors and angiogenesis*: Ovarian steroids, to which fibroids are responsive, influence the secretion of growth factor peptides and the expression of their receptors, with both estrogens and progestogens inducing expression of vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), and epidermal growth factor (EGF). It has been shown that IGF-1 expression in human uterine fibroids may be up to three times greater than that found in normal myometrium. It appears that autocrine stimulation of IGF-1 receptor plays a role in the maintenance of normal myometrial growth, and that disordered IGF-1 signaling may contribute to uterine fibroid growth. [33, 34] Similarly, Fibroblast growth factor (FGF-2) increases the proliferation, migration and differentiation of endothelial cells, smooth muscle cells and fibroblasts, all of which express FGF receptors (FGF-R). [35, 36] Both estrogen and progestogen can induce VEGF expression in human cells. Estrogen has been shown to increase the expression of VEGF mRNA in the uterus through a primarily estrogen-receptor mediated effect. Both estrogen receptors a and b are involved in this regulation of VEGF mRNA. Progestogens have also been shown to induce VEGF expression in human myoma cells. [37, 38, 39] The progressive growth of solid tumors such as uterine fibroids is therefore dependent on their ability to stimulate formation of new blood vessels (angiogenesis) that will supply tumor cells with oxygen and essential nutrients.

iii) *Role of Apoptosis*: Apoptosis, or programmed cell death, is a physiological and highly regulated form of cell death that occurs in many normal and pathological tissues. Cells die in response to a variety of stimuli, and during apoptosis, they do so in a controlled, regulated fashion. This distinguishes apoptosis from necrosis; another form of cell death that is uncontrolled and associated with morbidity. A balance in favor of anti-apoptotic activity will lead to prolonged cell survival, and may conceivably contribute to growth of

fibroids. Both bcl-2 protein and TNF-α expression is higher in fibroid cells than normal myometrium. Because bcl-2 protein inhibits TNF-α-induced apoptosis it is believed that bcl-2 protein also has a role in fibroid growth by controlling TNF-α-induced apoptosis in fibroids. [40, 41, 42]

2.3.2 Endometriosis

Endometriosis is a complex, estrogen-dependent disease that is defined as the presence of endometrial glands and stroma outside the uterine cavity. [43] It is diagnosed mainly in women of reproductive age, and estimates show that up to 15% of all pre-menopausal women, and 35–50% of women with infertility and pelvic pain are affected. Ectopic endometrial tissue can be found in different parts of peritoneal cavity, thus leading to three different conditions: ovarian, peritoneal, and deep infiltrative endometriosis. [43] Its frequent occurrence seen at laparoscopy in asymptomatic women has led to the suggestion that this maybe a normal physiological process, which only becomes a disease when the patient is symptomatic. However, once symptomatic it can be an extremely debilitating condition causing chronic pelvic pain and infertility, dysmenorrhoea, deep dyspareunia, erratic pelvic pain, dyschezia (pain with defecation), and haematuria. [44] Figure 2.5 shows the various locations where endometriosis is commonly seen.

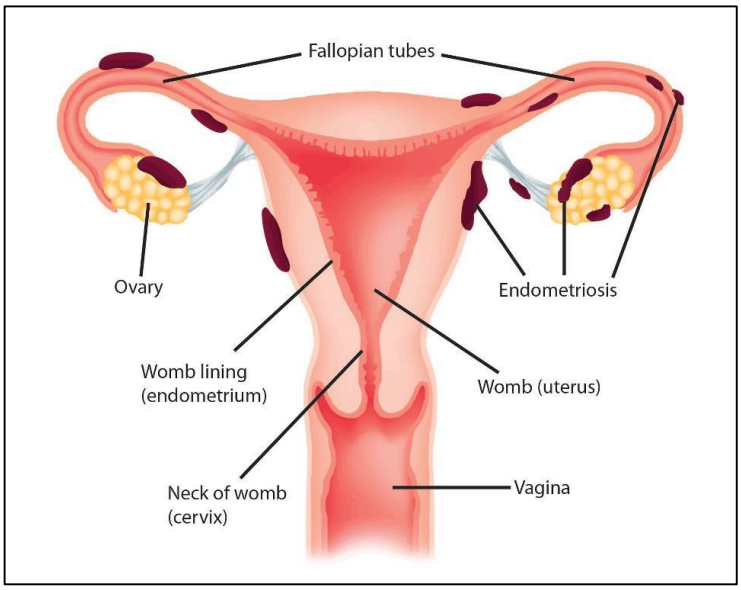


Figure 2.5 Endometriosis

The pathogenesis of endometriosis is very complex and remains not completely understood. The most widely accepted is the theory on retrograde menstruation and disturbed immune system. [45] However, the pathogenesis also involves changes in apoptosis, cell adhesion, degradation of the extracellular matrix, angiogenesis, cell communication, loss of differentiation capacity [46], as well as alterations in other biological pathways. Also environmental factors, increased local formation of estradiol, and diminished progesterone action affect the development of endometriosis. Enhanced inflammation has been seen in eutopic endometrium of endometriosis patients, and growth of the endometrium in ectopic sites leads to chronic pelvic inflammatory responses, as supported by the increased concentrations of PGE2 and PGF2α in the peritoneal fluid of endometriosis patients. [47, 48] PGE2 regulates proliferation of endometriotic cells, immune suppression, and angiogenesis. [49, 50]

Several theories have been postulated to explain the evolution of endometriosis, but no single theory can explain all types and sites of endometriosis. Different types of endometriosis may have different origins.

i) *Retrograde menstruation and implantation theory (Sampson's theory)*

Sampson based his theory on observations during pelvic surgery, such as menstrual blood exiting the tubal ostea in menstruating women. Endometrial fragments were also detected within the fallopian tubes removed at hysterectomy. Reflux endometrial tissue is thought to implant on the peritoneal and ovarian surfaces. Reflux menstruation occurs in about 80% of women, but does not result in endometriosis in most of the cases. Endometriosis develops only in women with a disordered immune system i.e. one that cannot identify and destroy menstrual endometrial cells reaching the peritoneal cavity. [51]

ii) *Coelomic metaplasia (Meyer's theory) and the induction theory*

Meyer's theory is based on the fact that cells from the peritoneum, the ovarian surface and endometrium arise from a common embryological precursor: the coelomic cell. At puberty, rising estrogen production induces these mature peritoneal or ovarian surface cells to undergo metaplasia into endometrial cells. This metaplasia may also be induced

by substances produced from endometrial cells reaching the peritoneal cavity by retrograde menstruation. [51]

iii) *Vascular and lymphatic metastasis (Halban's theory)*

Halban's theory suggests that distant endometriosis occurs via vascular or lymphatic spread of viable endometrial cells. This theory explains the rare endometriotic lesions occurring in extra- pelvic sites. [51]

Three distinct pathological types of endometriosis have been recognized: [51]

a) Superficial endometriosis (free endometriosis)

Peritoneal – two types of peritoneal endometrial implants can be distinguished: sub-mesothelial and intraepithelial lesions. Both types consist of glandular and stromal tissue, and respond to the hormonal changes associated with the menstrual cycle, showing cyclical changes similar (but not identical) to the normal endometrium This type of lesions is non- responsive to hormonal changes.

Ovarian – superficial ovarian endometriotic lesions are similar to these of peritoneal disease, and can occur on all sides of the ovary. Hemorrhagic lesions are commonly associated with peri-ovarian adhesion formation of varying severity, commonly found on the posterior aspect of the ovary.

b) Deep infiltrating (adenomatous) endometriosis (enclosed endometriosis) is characterized by proliferative fibromuscular tissue with sparse endometrial glandular and stromal tissue (similar to adenomyosis) with no surface epithelium. Unlike peritoneal endometriosis, deep endometriosis does not show significant changes during the menstrual cycle. These nodules are typically present in the rectovaginal space and can involve the uterosacral ligament, the posterior vaginal wall, and the anterior rectal wall. They can also extend laterally and affect the ureters.

c) Ovarian endometrioma: an endometrioma is an ovarian cyst lined by endometriotic tissue and containing dark brown or chocolate-colored fluid, which results from recurrent chronic bleeding from the endometriotic implants. In longstanding endo- metriomas, the

endometriotic tissue is gradually replaced by fibrotic tissue.

2.4 Management and Treatment of Uterine Fibroids and Endometriosis

The choice of treatment depends on the patient's age, the reason for treatment, the issue of fertility preservation, and the patient's preference. The treatment spectrum includes medical therapy, surgical intervention, uterine artery embolization or ablative techniques. Medical therapy is an option for women with symptomatic myoma who prefer non - surgical treatment, consider fertility preservation, and expect a less aggressive operation after shrinkage the uterine volume. [52, 53]

2.4.1 Surgical treatment

Surgery offers a more effective treatment with a longer-lasting effect for uterine fibroids and endometriosis compared with medical therapy. Surgical treatment options currently include abdominal myomectomy; laparoscopic myomectomy; hysteroscopic myomectomy; endometrial ablation; and abdominal, vaginal, and laparoscopic hysterectomy. [54]

Hysterectomy is the removal of uterus by surgical means. However, for women who desire pregnancy or who wish to avoid surgery, hysterectomy is not a viable therapeutic option. Morbidity after a hysterectomy can include bleeding, infection, injury to adjacent organs, and vaginal shortening. Hysterectomy with ovarian conservation is also associated with some loss of ovarian function and a decreased age of menopause. Ovarian failure chances also increases upon hysterectomy. Women who undergo hysterectomy for very large uterine myoma have been shown to have an increased risk of blood loss with increasing uterine size. [54]

Myomectomy is for patients who desire future childbearing or uterine preservation. It can be performed by laparotomy, laparoscopy, or hysteroscopy. However, myomectomy is associated with significant morbidity including hemorrhage, adhesion formation, leiomyoma recurrence, blood transfusion, and bowel injury. [55, 56]

2.4.2 Medical treatment

2.4.2.1 Non-Hormonal Therapy

Although non-hormonal medical treatment does not deal with the disease process itself, it is an important part of the management of fibroids and endometriosis. Non-steroidal anti-inflammatory drugs are a main part of non-hormonal therapy for leiomyomas. NSAIDs are given for the symptomatic relief from the pain and inflammation that these disorder causes. [57]

2.4.2.2 Hormonal Therapy

Steroid hormones are used to antagonize or modulate the effect of the endogenous estrogens. Medical hormonal treatments mimic situations in which fibroid and endometriosis is either rarely found or where symptoms are alleviated, creating a pseudo-pregnancy, pseudo-menopause or pseudo-male environment. By creating and maintaining amenorrhea, medical treatment is very efficient at reducing pelvic pain and may be considered an effective treatment in 80% of cases. Choice of drug is usually based on patient and clinician preference after discussion and consideration of factors such as:

- contraindications
- risks
- side-effects
- costs.

i) Combined estrogen and progestogen

One of the first medical treatments, which have been in use since the 1960s, is the combinations of estrogens and progestogens i.e. combined oral contraceptive pill. A low-dose monophasic oral contraceptive when used continuously or tricycled will render the endometrium hypoproliferative with hypotrophic glandular epithelium and atrophy of endometriotic tissue. Side-effects which may occur include: weight gain, headache and breast tenderness. However they lack the required efficacy for the management of the fibroids and endometriosis and the recurrence rate is high. [58]

ii) Progestogens create a hypo-estrogenic, hyper-progestogenic state causing decidualization and atrophy of the endometrium. Two main classes may be used:

• Derivatives of 19 nor-testosterone (e.g. norethisterone, norgetrel, norethynodrel and lynoestrenol)

• Those related to progesterone (e.g. medroxyprogesterone acetate and dydrogesterone).

Progestogens may be administered by a variety of routes: oral, depot injection and locally as the levonorgestrel-releasing intrauterine system (Mirena). When prescribed for the treatment of endometriosis, progestogens are usually given for six to nine months. The side-effects of progestogens are excessive bleeding, increased appetite, weight gain, fluid retention/oedema, bloating, acne, breast tenderness, mood changes and reduced libido. [59, 60]

iii) Danazol

Danazol was introduced in 1971 and was commonly used in the 1970s and 1980s. It is an isoxazole derivative of 17 alpha-ethinyl testosterone and has a complex mode of action with androgenic and anabolic effects. [61] Danazol produces a hypo-estrogenic and hyper-androgenic environment through several mechanisms:

• Directly by inhibiting ovarian enzymes of steroidogenesis

• Indirectly by decreasing GnRH pulse frequency and secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH)

• Androgenic effects of Danazol are due to its affinity for androgen receptors and its ability to decrease serum sex hormone- binding globulin (SHBG) levels and displace testosterone from SHBG

• immunosuppressive properties.

Danazol may induce insulin resistance, therefore, it is not ideal for those with diabetes. Danazol is usually given in a starting daily dose of 200 mg (p.o.) and is then gradually increased until amenorrhoea is achieved, not exceeding a maximum daily dose of 800 mg. Although highly effective in achieving symptomatic relief in ~90% of cases, Danazol has two main drawbacks limiting its use:

• Metabolic side effects causing dislipidaemia (elevation of LDL and reduction of HDL)

limit its use to six months

• High recurrence rate of endometriosis symptoms after the completion of the six-month course of Danazol makes it a short- to medium-term solution in many cases

Other side effects includes: Hot flushes, acne, reduced libido, oily hair/ skin, weight gain, nausea, headache, hirsutism, voice changes and muscle cramps. Danazol use has declined since due to severe side effects and increased utilization of the other safer therapeutics available. Danazol is now licensed only for second-line therapy. [61]

iv) Mifepristone (RU486)

Mifepristone is a synthetic steroid, with anti-progesterone and anti-glucocorticoid properties. It blocks progesterone receptors in the endometrium, leading to loss of functional integrity and eventual shedding. Preliminary data suggest that mifepristone at a dose of 50–100 mg/day results in anovulation, amenorrhea and symptomatic improvement in endometriosis. However, it has not become an established therapy. [61]

v) Gonadotrophin-releasing hormone (GnRH) agonists

Gonadotropin-releasing hormone (GnRH) is the neuropeptide that regulates pituitary function in women. GnRH acts on the pituitary to increase the release of FSH and LH (two hormones that lead to ovulation and estrogen production by the ovary). GnRH-analogs overemphasize the action of GnRH. They suppress the production of LH and FSH by the pituitary gland. This results in decreased levels of estrogen. It prevents ovulation and produces a pseudo or false menopause. [62] Also it has been reported that GnRH receptors besides being present in anterior pituitary gland, are also found to be expressed in ectopic endometrial cells and its proliferation can be directly inhibited by GnRH analogues like Leuprolide [62, 63]. Treatment with these compounds causes a significant shrinkage in the size of leiomyoma tumors within three to six months. Analogues include Leuprolide, Goserelin, Buserelin, Nafarelin and Triptorelin. Leuprolide, Goserelin, Buserelin and Triptorelin are given subcutaneously or intramuscularly. Nafarelin is given by nasal route. The frequency of administration is weekly or monthly. However, these compounds cannot be used as a long-term therapy because they cause a significant decrease in bone mineral density when used for more

than six months. Moreover, the therapy is too costly as these drugs are peptide based. Intramuscular or subcutaneous administration makes it a painful therapy lacking patient compliance. Add-back therapy equivalent to menopausal hormone replacement therapy (HRT) can be given either cyclically or continuously to prevent the decrease in bone mineral density. Of the all-available GnRH analogues Leuprolide acetate can be chosen over others due to its advantages like higher efficacy over other analogues, cost effectiveness and safety [64]. Although the effectiveness of both Goserelin and Leuprolide have been statistically proven similar, yet Leuprolide can be preferred primarily on the consideration of cost and its diverse clinical applications being well established drug for the treatment of uterine fibroids, endometriosis, and central precocious puberty [65]. Marketed formulation of Leuprolide includes Lupron depot® (microspheres), administered in a dose of 3.75 mg i.m. (weekly), or 11.25 mg i.m. (monthly) and Eligard® which is lyophilized powder for injection administered at doses, 7.5 mg s.c. (monthly) and 22.5 mg s.c. (three monthly). However, because of large volume of distribution (27 L), Leuprolide is not able to reach uterus in sufficient amount to act directly on GnRH receptors. This leads to various side effects like significant decrease in bone mineral density when used for more than six months, insomnia, decreased libido, headaches, mood swings, vaginal dryness, acne, muscle pains, dizziness, depression make it non patient acceptable. [66]

vi) Selective Estrogen Receptor Modulators (SERM)

SERMs are another class of compounds that have been investigated as potential treatment option for uterine leiomyomas and endometriosis. SERMs are molecules that bind to the estrogen receptor but exhibit tissue-specific agonist or antagonist activity. Ideally, a SERM would provide the positive, estrogenic effects on bone, brain and the cardiovascular system but would act as an antagonist in the breast and uterus. [67] Raloxifene is a benzothiophene derivative that does not show any agonist activity in the uterus. Raloxifene was specifically developed to maintain beneficial estrogenic activity on bone and lipids and antiestrogenic activity on endometrial and breast tissue. It is a USFDA approved drug for post-menopausal osteoporosis Many researchers are now

working upon Raloxifene to establish its potential use for treating uterine fibroids. It binds to the estrogen receptors over expressed on the leiomyoma cells, with high affinity and causes apoptosis of the cells. Oral administration has limited bioavailability of 2 %. Currently it is given by oral route as tablets (Evista ®) with the dose of 60 mg/day. Because of poor bioavailability it is not able to reach uterus in sufficient amount to elicit the direct action on estrogen receptors. The non-specific distribution throughout the body causes various systemic side effects like hot flashes, sweating, headache, dizziness, spinning sensation, leg cramps, joint pain, nausea, vomiting, and stomach pain [67] Raloxifene unlike Tamoxifene doesn't cause endometrial cancer. [68]

vii) Selective progesterone receptor modulator

Asoprisnil or J867 is a novel selective steroid receptor modulator that represents a new class of progesterone receptor ligands that exhibit partial agonist and antagonist activities. It has a high-binding affinity for progesterone receptors, low affinity for androgen receptors, and no binding affinity for estrogen. Data from animal studies have shown Asoprisnil to abolish menstrual cyclicity and induce endometrial atrophy. Early clinical studies of Asoprisnil showed a dose-dependent suppression of menstruation irrespective of the effects on ovulation. Unlike progestogens, Asoprisnil does not induce breakthrough bleeding. With favorable safety and tolerability profiles, Asoprisnil appears to be an attractive new treatment for endometriosis. [69]

viii) *Aromatase inhibitors*

Endometriotic tissue has an increased activity of aromatase enzymes in the stromal cells, which leads to an increase in the local production of 17-β-estradiol, which stimulates cyclooxgenase-2 to increase the production of PGE_2. PGE_2 in turn increases aromatase activity. The net effect of this cycle is an increase in the levels of local mediators of inflammation and pain. Aromatase inhibitors could break this cycle, resulting in improvement of endometriosis symptoms. Anastrozole and letrozole are non-steroidal aromatase inhibitors, and exemestane and formestane are steroidal aromatase inhibitors. [70] However, this class of drug is still under clinical trials for the therapy of fibroids and endometriosis.

2.4.3 Ablative Therapy

A variety of thermal ablation techniques have been applied to the treatment of uterine fibroids. This includes radiofrequency ablation, cryoablation, and laser ablation. The use of radiofrequency ablation (RFA) to achieve local control of a wide variety of tumors in many locations has become more accepted in recent years. However, the patient reviews revealed that outcomes were not as good as those seen after hysterectomy and there were no significant differences in quality of life before and after the radiofrequency ablation. [71]

Cryoablation destroys tissue by several mechanisms by cooling the target tissue to -20°C, which results in intracellular freezing, extracellular crystallization of interstitial water followed by cellular dehydration, thrombosis of small blood vessels, mechanical damage to cellular integrity by expansion of large ice crystals within the interstitial space. Complications associated with this therapy includes nausea, fever, nerve defect and laceration of a vessel coursing over the serosal surface of a fibroid that leads to bleeding requiring laparotomy and myomectomy. Multiple procedures are required to treat multiple fibroids, increasing the overall risk of utilizing this approach. In addition, if small fibroids are left untreated, then symptom recurrence may become a potential long-term issue. [71]

2.5 Targeted Drug Delivery Systems

Development of new drug molecule is expensive and time consuming. Improving safety efficacy ratio of “old” drugs has been attempted using different methods such as individualizing drug therapy, dose titration, and therapeutic drug monitoring. Delivering drug at controlled rate, slow delivery, targeted delivery are other very attractive methods and have been pursued vigorously. [72] Targeted drug delivery system is based on a method that delivers a certain amount of a therapeutic agent for a prolonged period of time to a targeted diseased area within the body. This helps maintain the required plasma and tissue drug levels in the body; therefore avoiding any damage to the healthy tissue via the drug. The drug delivery system is highly integrated and requires various disciplines, such as formulation scientist, pharmacologist and analyst, to join forces to

optimize this system. When implementing a targeted release system, the following design criteria for the system need to take into account: the drug properties, side effects of the drugs, the route taken for the delivery of the drug, the targeted site, and the disease. [73]

Products based on such a delivery system are prepared by considering the specific properties of target cells, nature of markers or transport carriers or vehicles, which convey drug to specific receptors and ligands. Ideally targeted drug delivery systems should be biochemically inert (non-toxic), should be non-immunogenic, should be physically and chemically stable in vivo and in vitro conditions, and should have restricted drug distribution to target cells or tissues or organs and should have uniform capillary distribution. It should have controllable and predictable rate of drug release. It should have minimal drug leakage during transit. [74]

2.5.1 Types of Targeted Drug Delivery

2.5.1.1 Passive targeting

It refers to the accumulation of drug or drug carrier system at a specific site such as anti-cancerous drug whose explanation may be attributed to physicochemical or pharmacological factors of the disease. For example in case of cancer treatment the size and surface properties of drug delivery carriers must be controlled specifically to avoid uptake by the reticulo-endothelial system (RES) to maximize circulation times and targeting ability. Passive targeting is simply the drug delivery system via blood circulation. Drug release or drug actions are limited to selective sites within the body such as a tumor but not the other healthy tissues. [75]

2.5.1.2 Active targeting

Active targeting means a specific ligand-receptor type interaction for intracellular localization, which occurs only after blood circulation and extravasations. This active targeting approach can be further classified into three different levels of targeting which are 1) First order targeting refers to restricted distribution of the drug carrier systems to the capillary bed of a predetermined target site, organ or tissue e.g. compartmental targeting in lymphatics, peritoneal cavity, plural cavity, cerebral ventricles and eyes, joints. 2) Second order targeting refers to selective delivery of drugs to specific cell types

such as tumor cells and not to the normal cells e.g. selective drug delivery to kupffer cells in the liver. 3) Third order targeting refers to drug delivery specifically to the intracellular site of targeted cells e.g. receptor based ligand mediated entry of a drug complex into a cell by endocytosis. [76]

2.5.2 Carriers for Targeted Drug Delivery Systems

Nanotechnology is increasingly considered to be the technology of the future. With nanotechnology, scientists are acquiring abilities to understand and manipulate materials at the scale of atoms and molecules. Nanotechnology has been utilized in medicine for therapeutic drug delivery and the development of formulations for a variety of diseases and disorders. Controlled drug delivery systems, which are aimed to deliver drugs at predetermined rates and predefined periods of time, have attracted increasing attention. [77, 78] The goal of this targeted delivery is to transport proper amounts of drugs to the desirable sites (such as tumors, diseased tissues, etc.) while minimizing unwanted side effects of the drugs on other tissues. Micro- and nano-scale intelligent drug delivery systems can maximize the efficacy of therapeutic treatments as they have the ability to rapidly detect and respond to disease states directly at the site, sparing physiologically healthy cells and tissues and thereby improving a patient's quality of life. Various types of nanocarriers used for targeted drug delivery includes:

2.5.2.1 Inorganic nanoparticles

Inorganic nanoparticles can be defined as particles of metal oxide or metallic composition possessing at least one length scale in the nanometer range. These nanostructures exhibit novel and distinct chemical, physical, and biological properties, and functionality due to their nanoscale size. The preparation of inorganic nanoparticles offers several challenges. There is not a one-fits-all type of production process for nanoparticles. [79] Figure 2.6 represents the types of inorganic nanoparticles.

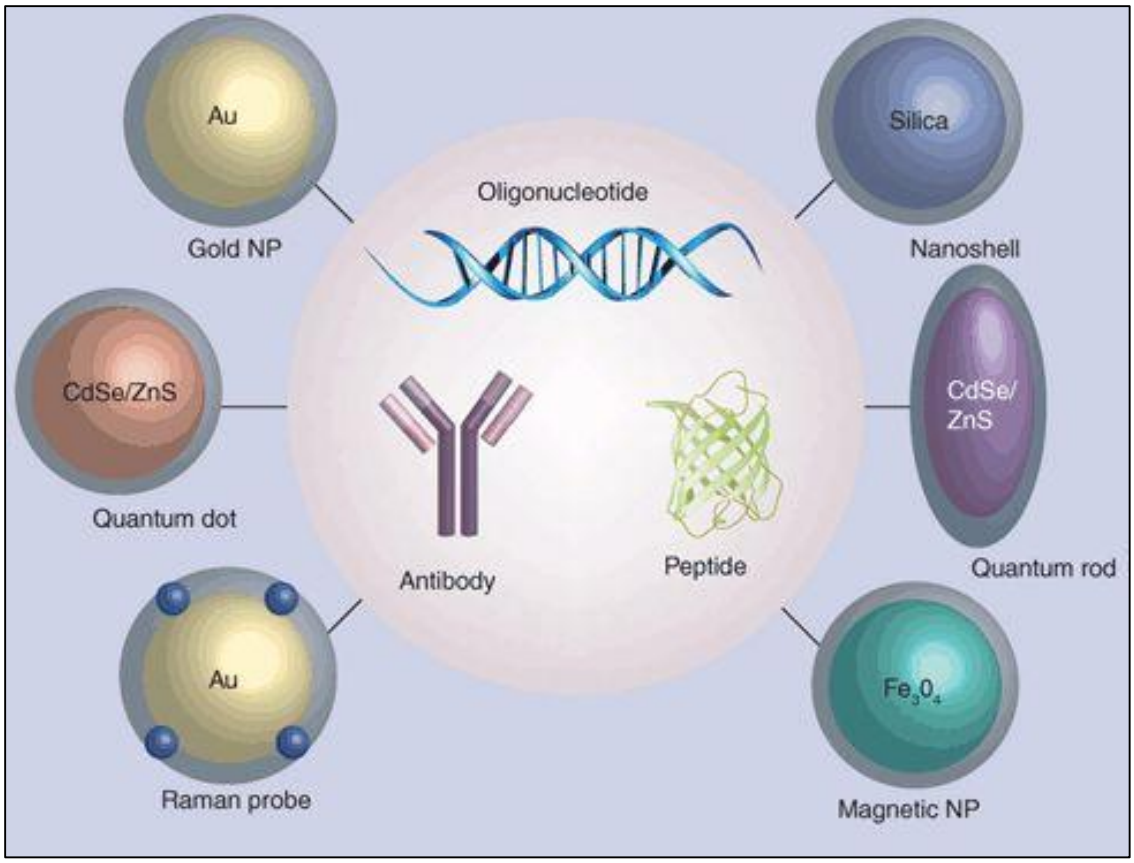


Figure 2.6 Types of Inorganic Nanoparticles

Although inorganic nanoparticles are attracting great interest in the field of nanomedicine the long-term, effects of these nanoparticles needs to be addressed. Concerns associated with long-term tissue damage, toxicity, immunogenicity, carcinogenesis, and inflammation need to be elucidated. It will be necessary to design inorganic nanoparticles whose stability, circulation times, and localization can be modulated without compromising theranostic efficacies in order to optimize the demands of short-term therapeutic and potential adverse effects due to long-term exposure. [80]

2.5.2.2 Polymeric Micelles

Polymeric micelles are nanosized amphiphilic block copolymers with a core–shell structure as seen in Figure 2.7. Polymeric micelles designate cores of biodegradable hydrophobic polymers protected by an amphiphilic block copolymer that stabilizes their dispersion in aqueous media. In addition, polymeric micelles display larger cores than

surfactant micelles, leading to higher solubilization capacity than the regular micelles. Among the polymers displaying micelle-formation ability, micelles with blocks made of poly(ethylene oxide) are sterically stabilized and undergo less opsonization and uptake by the macrophages of the reticuloendothelial system (RES), allowing the micelles to circulate longer in blood. [81] The unique characteristics of polymeric micelles, such as size in the nanometer range, relatively high stability due to low critical association concentrations (CMC), and core–shell arrangement, make them attractive for use in drug delivery systems, especially for hydrophobic drugs with very low solubility in water.

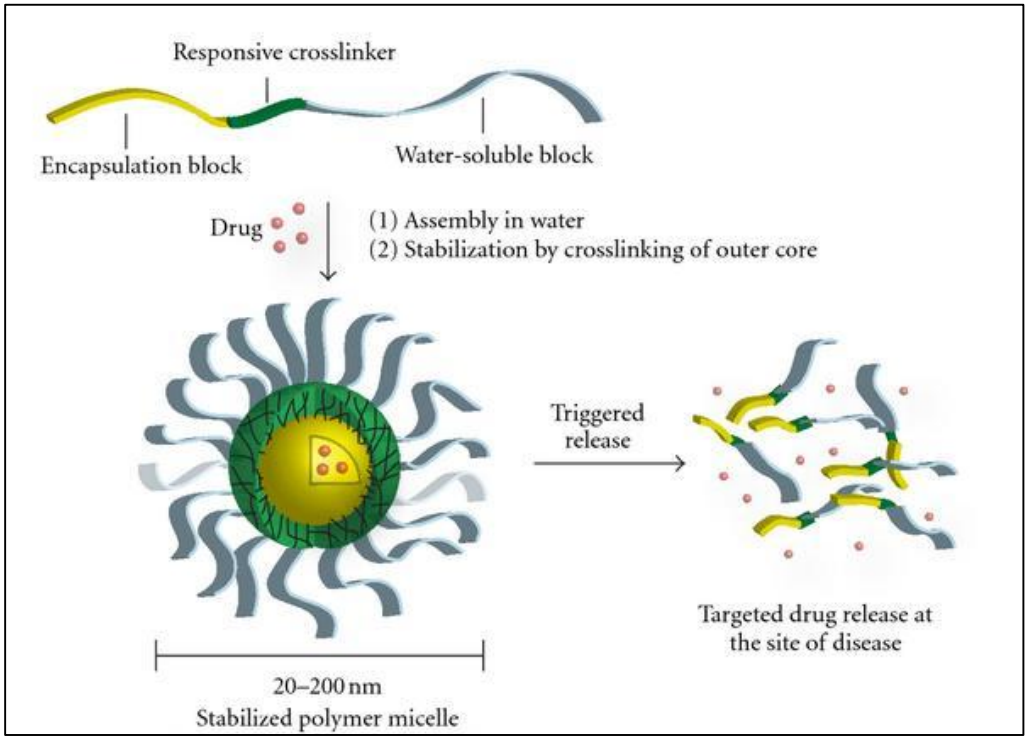


Figure 2.7 Polymeric Micelle for Targeted Drug Delivery

Amphiphilic block copolymers with having a large solubility difference between hydrophilic and hydrophobic segments, have a tendency to self-assemble into micelles in a selective solvent. In an aqueous solution, micelles with core– shell structures are formed through the segregation of insoluble hydrophobic blocks into the core, which is surrounded by a shell composed of hydrophilic blocks. This core–shell structure facilitates their utilization, where depending upon the polarity the drug molecule can be entrapped in the (i) core (non polar molecule), (ii) shell (polar molecule) and (iii) in-

between the core and shell (intermediate polarity). [81] For using polymers in drug delivery, a polymer must be biocompatible and biodegradable. They indeed contribute to the drug release as a result of their erosion/degradation, in addition to drug diffusion through the polymeric material.

2.5.2.3 Dendrimers

The term dendrimer, first proposed by Tomalia in 1985, was chosen due to its structural shape, with highly branched, three-dimensional features that resemble the architecture of a tree. [82] A typical dendrimer consists of three main structural components: a) a focal core, (b) building blocks with several interior layers composed of repeating units, and (c) multiple peripheral functional groups. The branched units are organized in layers called ‘‘generations’’, and represent the repeating monomer unit of these macromolecules. Two major synthetic strategies are used for the synthesis of dendrimers, namely, the divergent approach and convergent approach. Both synthetic strategies possess relative advantages and disadvantages and the appropriate route depends mainly on the kind of monomer employed and the target polymer structure. These macromolecules have a multi-branched, three-dimensional architecture with very low poly dispersity and high functionality. [82] Figure 2.8 shows the structure of a dendrimers.

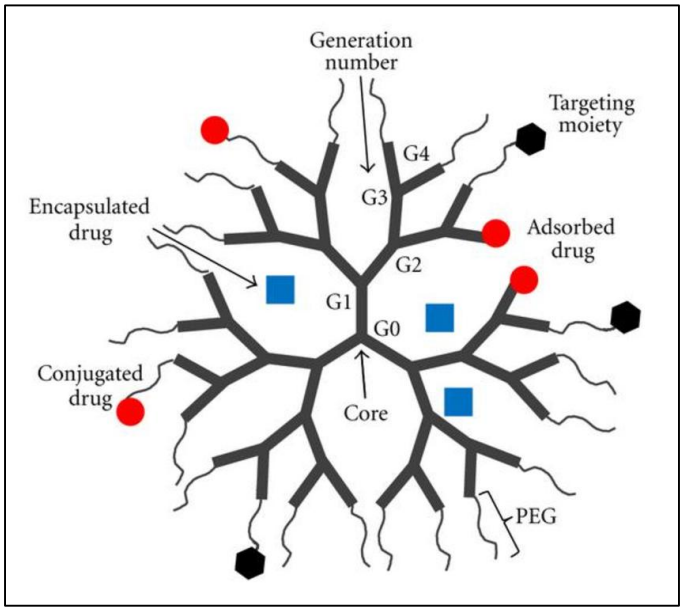


Figure 2.8 Structure of Dendrimers

Different types of dendrimers, include polyamidoamine (PAMAM), polypropylene imine (PPI), polylysine dendrimers have been used as host for both hydrophilic and hydrophobic drugs. An ideal dendritic drug-carrier must be non-toxic, non-immunogenic, preferably biodegradable; present an adequate biodistribution and allow tissue targeting.

2.5.2.4 Carbon Nanotubes (CNT)

CNTs have raised considerable attention due to their excellent mechanical, electrical and surface properties that have made them ideal candidates for a wide range of applications such as structural materials. [83] Recently, its potential application in biotechnology has attracted much interest, as CNTs have been reported to exhibit great advantages in biosensors, biomedical devices and drug delivery systems. [84] Figure 2.9 represents the structure of carbon nanotubes.

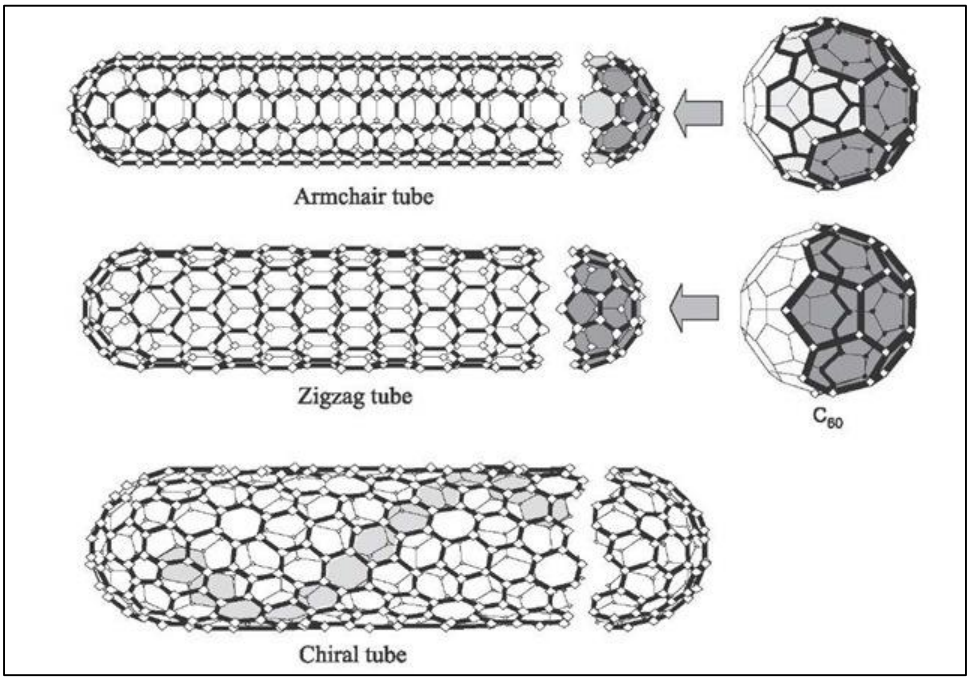


Figure 2.9 Structure of Carbon Nanotubes

CNTs tend to bundle up and are insoluble in most types of solvents making it difficult to use them in biological systems. Moreover, some CNTs without any functionalization have been shown to be cytotoxic. Therefore, to integrate CNTs into biological systems, CNTs need to be functionalized. Functionalization can make CNTs soluble and improve their biocompatibility properties. Moreover, through functionalization, bioactive agents can be conjugated to CNTs, which can serve as a carrier for drugs, antigens and gene delivery. [84]

2.5.2.5 Liposomes

Liposomes are among the most widely applicable and exciting of the new drug delivery system. Liposomes are microscopic vesicles (from 0.02 to 6.0 μm in diameter), composed of one or several lipid membranes surrounding discrete aqueous compartments. These vesicles can encapsulate water-soluble drugs in their aqueous spaces and lipid soluble drugs within the membrane. Depending on the gel-liquid crystalline transition temperature (Tc) of phospholipids (i.e, the temperature at which acyl chains melt), liposomal membrane can attain varying degree of fluidity at ambient temperature. This, infact, can be controlled quite accurately to achieve wide range of Tc values by using appropriate mixtures of two or more phospholipids. [85, 86] In addition, liposomal surfaces can be charged negatively or positively by the incorporation of charged amphiphiles, or enriched with reactive groups to which ligands can be covalently linked. It is also possible to adjust the average vesicle size by sonication, detergent dialyses, microfluidization, homogenization and other similar techniques. The nature of the liposome helps it to be distributed within the body in a pattern dramatically different from that of free drug. It is by taking advantage of this altered biodistribution that superior targeted therapies can be designed. Because of their biocompatibility, biodegradability, low toxicity, and aptitude to trap both hydrophilic and lipophilic drugs and simplify site-specific drug delivery to tumor tissues, liposomes have increased rate both as an investigational system and commercial as a drug- delivery system. Many studies have been conducted on liposomes with the goal of decreasing drug toxicity and/ or targeting specific cells [87, 88] Figure 2.10 shows the structure of Liposomes.

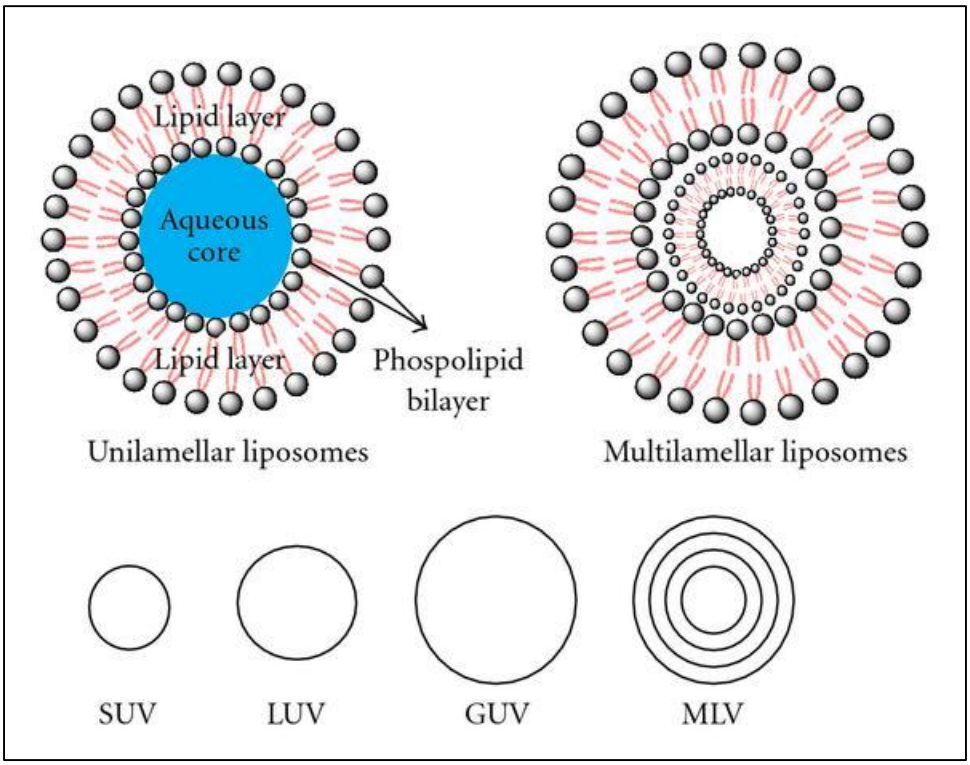


Figure 2.10 Structure of Liposomes

Classification of Liposomes

The liposome size can vary from very small (0.025 μm) to large (2.5 μm) vesicles. Moreover, liposomes may have one or bilayer membranes. The vesicle size is an acute parameter in determining the circulation half-life of liposomes, and both size and number of bilayers affect the amount of drug encapsulation in the liposomes. On the basis of their size and number of bilayers, liposomes can also be classified into one of two categories: (1) multilamellar vesicles (MLV) and (2) unilamellar vesicles. Unilamellar vesicles can also be classified into two categories: (1) large unilamellar vesicles (LUV) and (2) small unilamellar vesicles (SUV). In unilamellar liposomes, the vesicle has a single phospholipid bilayer sphere enclosing the aqueous solution. In multilamellar liposomes, vesicles have an onion structure. Classically, several unilamellar vesicles will form on the inside of the other with smaller size, making a multilamellar structure of concentric phospholipid spheres separated by layers of water. [89, 90]

Methods of Liposome Preparation

All the methods of preparing the liposomes involve four basic stages: 1. Drying down lipids from organic solvent. 2. Dispersing the lipid in aqueous media. 3. Purifying the resultant liposome and reducing its size as per requirement. 4. Analyzing the final product. Following are the methods of Liposome preparation:

i) Sonication: Sonication is perhaps the most extensively used method for the preparation of SUV. Here, MLVs are sonicated either with a bath type sonicator or a probe sonicator under a passive atmosphere. The main disadvantages of this method are very low internal volume/encapsulation efficacy, possible degradation of phospholipids and compounds to be encapsulated, elimination of large molecules, metal pollution from probe tip, and presence of MLV along with SUV. [91]

ii) French pressure cell extrusion: French pressure cell involves the extrusion of MLV through a small orifice [91]. The method involves gentle handling of unstable materials. The method has an advantage over sonication method that it gives better entrapment of drugs. The resulting liposomes are rather larger than sonicated SUVs. The drawbacks of the method are that the high temperature is difficult to attain, and the working volumes are comparatively small (about 50 mL as the maximum). [92]

iii) Freeze-Thaw: SUVs are rapidly frozen and thawed slowly. The short-lived sonication disperses aggregated materials to LUV. The creation of unilamellar vesicles is as a result of the fusion of SUV throughout the processes of freezing and thawing. This type of synthesis is strongly inhibited by increasing the phospholipid concentration and by increasing the ionic strength of the medium. The encapsulation efficacies vary from 20% to 30%. [93]

iv) Ether injection (solvent vaporization): A solution of lipids dissolved in diethyl ether or ether-methanol mixture is gradually injected to an aqueous solution of the material to be encapsulated at 55°C to 65°C or under reduced pressure. The consequent removal of ether under vacuum leads to the creation of liposomes. The main disadvantages of the technique are that the population is heterogeneous (70 to 200 nm) and the exposure of

compounds to be encapsulated to organic solvents at high temperature. [94]

v) Ethanol injection: A lipid solution of ethanol is rapidly injected to a huge excess of buffer. The MLVs are at once formed. The disadvantages of the method are that the population is heterogeneous (30 to 110 nm), liposomes are very dilute, the removal all ethanol is difficult because it forms into azeotrope with water, and the probability of the various biologically active macromolecules to inactivate in the presence of even low amounts of ethanol is high. [94]

vi) Reverse phase evaporation: This method provided a progress in liposome technology, since it allowed for the first time the preparation of liposomes with a high aqueous space-to-lipid ratio and a capability to entrap a large percentage of the aqueous material presented. Reverse-phase evaporation is based on the creation of inverted micelles. These inverted micelles are shaped upon sonication of a mixture of a buffered aqueous phase, which contains the water-soluble molecules to be encapsulated into the liposomes and an organic phase in which the amphiphilic molecules are solubilized. The slow elimination of the organic solvent leads to the conversion of these inverted micelles into viscous state and gel form. At a critical point in this process, the gel state collapses, and some of the inverted micelles were disturbed. The excess of phospholipids in the environment donates to the formation of a complete bilayer around the residual micelles, which results in the creation of liposomes. Liposomes made by reverse phase evaporation method can be made from numerous lipid formulations and have aqueous volume-to-lipid ratios that are four times higher than hand-shaken liposomes or multilamellar liposomes. [95]

vii) Dehydration-Rehydration: A novel method of liposome preparation, which is simple to use, employs mild condition, and is capable of efficient entrapment of high molecular weight hydrophilic drugs. This procedure is based on induction of fusion of preformed vesicles by means of dehydration and controlled rehydration. Liposomes obtained by this technique are oligo and multi lamellar. Labile materials like peptides which are destroyed by drastic conditions like sonication or ethanol/ether injections, can be encapsulated using this technique with high entrapment efficiencies. The only limitation of this method is that heterogenous population of liposomes is produced. Breifly, in this method, blank

SUVs are formed by using conventional thin film hydration method. Hydrophilic drug is then added to this SUV dispersion. The dispersion is then allowed to freeze at -80 ^{o}C for an hour and then kept in freeze dyer with shelf temperature of -20 ^{o}C for over night. The resulting lipid cake is then rehydrated using minimum amount of suitable hydration medium. Upon hydration, MLVs with high entrapment of hydrophilic drug are produced. [96]

viii) Dual Drug entrapped Liposomes: The closed vesicular structures consisting of one or more lipid bilayers surrounding an inner aqueous compartment allow both hydrophilic and lipophilic drugs to be effectively encapsulated. Water-soluble drugs can be encapsulated into the inner aqueous compartment, whereas lipid-soluble drugs can be embedded within the liposome bilayers. Dual Drug-loaded liposomes prepared using Ethanol injection is reported. [97] The hydrophilic drug is added to the aqueous phase while the lipophilic drug was added to the organic one. However, ethanol injection method is not suitable for the hydrophilic peptide based drug entrapment as it destroys the labile structure of the proteins.

Limitations of Liposomes

One of the drawbacks of the use of liposomes is the fast elimination from the blood and capture of the liposomal preparations by the cells of the Reticulo-Endothelial System, primarily in the liver. To increase liposomal drug accumulation in the desired tissues and organs, the use of targeted liposomes with surface-attached ligands capable of recognizing and binding to cells of interest has been suggested. Immunoglobulins (Ig) of the IgG class and their fragments are the most widely used targeting moieties for liposomes, which can be attached to liposomes, without affecting liposomal integrity or the antibody properties, by covalent binding to the liposome surface or by hydrophobic insertion into the liposomal membrane after modification with hydrophobic residues. Different methods have been suggested to achieve long circulation of liposomes in vivo, including coating the liposome surface with inert, biocompatible polymers, such as PEG, which form a protective layer over the liposome surface and slow down liposome recognition by opsonins and therefore subsequent clearance of liposome. [98]

Also the liposomes on long standing, tends to aggregate and fuse to form larger vesicles. Due to the oxidation and hydrolysis of the lipid chains, the membrane fluidity tends to increase, which leads to drug leakage during the storage. To avoid such stability issues, these products are lyophilized from simple aqueous solutions. Freeze-drying (lyophilization) involves the removal of water from products in the frozen state at tremendously low pressures. The process is normally used to dry products that are thermo-labile and would be demolished by heat-drying. Liposomes when freeze-dried in the presence of adequate amounts of trehalose (a carbohydrate commonly found at high concentrations in organism) retained as much as 100% of their original substances. It shows that trehalose is an excellent cryoprotectant (freeze-protectant) for liposomes. [98]

Hence, to summarize following are the benefits of drug-loaded liposomes: [98]

1. Improved solubility of lipophilic, hydrophilic and amphiphilic drugs and use of biodegradable, biocompatible phospholipids that are inert and non-toxic to the body

2. Passive targeting to the cells of the immune system, especially cells of the mononuclear phagocytic system

3. Sustained release system of systemically or locally administered liposomes

4. Site-avoidance mechanism

5. Site-specific targeting

6. Improved transfer of hydrophilic and/or charged molecules

7. Improved penetration into tissues

2.6 Vaginal Drug Delivery

The vagina is a muscular, tubular organ, which plays a major role in reproduction. It connects the cervix (the opening of the uterus) and the vulva (the external genitalia). It is positioned between the rectum, bladder and urethra. The vagina is a slightly S-shaped fibromuscular collasible tube and its dimensions range from 8.4 to 11.3 cm in length and 2.1 to 5.0 cm in diameter. The vaginal wall consists of three layers: the epithelial layer, the muscular coat and the tunica adventia. The main blood supply to the vagina is through the vaginal branch of the uterine artery. The vaginal mucosa has no goblet cells but it secrets a large amount of fluid containing enzymes, enzyme inhibitors, proteins, carbohydrates and amino acids. [99, 100] The enzymatic activity in the vagina is comparatively lower than in the gastrointestinal tract. The vaginal pH of healthy women of reproductive age is acidic. Lactic acid produced from glycogen by the Lactobacillus acidophilus present in the vagina acts as a buffer to maintain the vaginal pH between 3.8 and 4.2. [100]

The vagina has been studied as a favorable site for local and systemic delivery of drugs for female-related conditions [101]. Traditionally, the vaginal cavity has been used for the delivery of locally acting drugs such as antibacterial, antifungal, antiprotozoal, antiviral, labor-inducing and spermicidal agents, prostoglandins, steroids, etc. The vagina has also great potential for systemic delivery because of its large surface area, rich blood supply and permeability to a wide range of compounds including peptides and proteins. It offers a favorable alternative to the parenteral route for some drugs. By this delivery route the hepatic first-pass effect can be avoided. [102]

Despite the promising characteristics of the vagina for drug therapy, the development of a suitable vaginal delivery system remains an issue of concern. An optimal vaginal formulation should have (i) a long retention time to maximize drug release, (ii) a proper spreading over the vaginal epithelium to obtain fast absorption or to maximize the effect in case of local treatment and (iii) be easy to administer not causing discomfort to the patients. Traditionally available dosage forms include solutions, emulsions, suspensions, vaginal tablets, suppositories and semi-solid formulations such as creams and ointments.

[103] Liquid formulations are inappropriate for controlled drug release due to their short residence time in the vaginal cavity. Vaginal tablets and inserts are convenient, simple to manufacture and a sustained drug release can be achieved over several hours. [104] A large number of vaginal medications are available in form of suppositories. Although solid formulations are easy and inexpensive to manufacture and their application is simple, the vaginal residence time is still poor, making frequent application necessary. [105] The main advantages of semi-solid preparations are their acceptability, feasibility and low cost. However, they can be messy to apply, uncomfortable and sometimes embarrassing if they leak into the undergarment. Moreover they may not provide an exact dose because of non-uniform distribution. [106]

Several aesthetic and functional qualities must be incorporated into such intravaginal formulations which need to be designed for a specific prolongation of residence time and also for desirable distribution and delivery of the active substance for an extended period at a predictable rate, retention and release characteristics. Generally a prolonged vaginal residence time can be achieved by the use of mucoadhesive and/or in situ gelling polymers and by mechanical fixation. [107]

2.6.1 Mucoadhesive formulations

Mucoadhesive semi-solid formulations are able to facilitate intimate contact with the underlying absorption surface and improve the bioavailability of drugs. Rheological properties of these formulations are important for their retention on the vaginal surface. The remarkable elastic or swelling character and the improvement of the rheologic properties of the mucoadhesive gel or tablets or films prolong the residence time at the application site. For these kinds of formulations, selection of correct viscosity of the formulation is important in order to provide adequate retention and distribution in the vagina. [108]

Mucoadhesive polymers in formulations such as polycarbophil, hydroxypropylcellulose or polyacrylic acid are used to eliminate the effect of vaginal discharge, which shortens the residence time of vaginal formulations. Chitosan, Sodium alginate, Carbopol resins are well known polymers to formulate mucoadhesive tablets, gels, films, suppositories

and particulate formulations like microparticles and microcapsules. [109]

2.6.2 Mechanical Fixation

The mechanical fixation of drug delivery systems in the vagina can provide high patient compliance and can lower side effects by reducing the frequency of administration.

2.6.2.1 Tablets and Discs

Voorspoels et al. designed a specially shaped tablet to increase its adherence to the genital tract. The tablet has a diameter of 20 mm, a flat bottom and a concave upper surface, aiming to better fit the uterine cervix. [110] Vaginal Discs can be an alternative to tablets, prepared with ethylene-co- vinyl acetate (EVAc). However, More research is needed in relation to vaginal hydrodynamics and tablet/disc shape and the relation with tablet/disc residence time and erosion rate. [111]

2.6.2.2 Intravaginal Rings (IVR)

The intravaginal ring technology allows non-daily, low and continuous dosing, achieves lower side effects, makes drug administration easy and discrete for patients and improves patient compliance. Intravaginal rings are doughnut- shaped polymeric devices as illustrated in Figure 2.11 and are designed to provide a controlled release of drugs to the vagina for extended periods of time. [112]

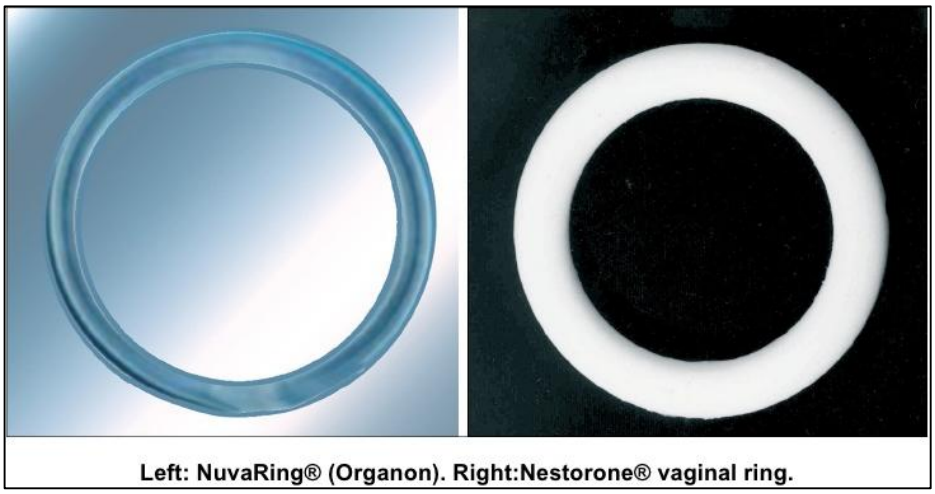


Figure 2.11 Intra Vaginal Rings

Vaginal rings are easily inserted and removed. The vaginal walls hold them in place. These rings commonly reside next to the cervix. Intravaginal rings deliver active substances, mostly hormones, at uniform concentrations and over a longer period of time; they allow lower doses to be used, and can still be user controlled. They also allow accurate, controlled administration of drugs from several weeks to a year and an earlier removal is possible. [113] Intravaginal ring delivery systems are usually based upon silicone elastomers with an inert inner ring, which is coated with another layer of elastomer containing drug. An outer rate controlling elastomer layer may be added as a third to prevent an initial burst release in various rings. The rings are 6 cm in diameter and 4-7 mm in cross-section. The rings are left for several days and can deliver drugs at a consistent ratio with approximately zero-order kinetics. The marketed rings loaded with hormones are Nuvaring ®, Nesterone ® and Femring ®. [114]

Nuvaring is a doughnut-shaped combined contraceptive (etonorgestrel/ ethinyl oestradiol) vaginal ring. It is worn vaginally for 3 weeks and removed for one week. Its contraceptive efficacy is 99.4% and the ring has been well tolerated with good compliance [114]. Nesterone-containing vaginal rings for contraception include Nesterone and ethinyl estradiol. The vaginal ring is made of dimethylsiloxane/ vinylmethylsiloxane copolymer and is supported with medical adhesives. The steroids are contained in cores within the ring body. The rings are designed for 1-year efficacy. While the Femring vaginal ring is an off-white, soft, flexible ring with a central core containing estradiol acetate used during menopause. [115]

Polymers used in IVR include thermoset silicones, thermoplastic EVA, and more recently a variety of thermoplastic polyurethanes. The drug is typically dispersed or dissolved in the elastomeric polymer matrix during formation of the IVR via injection molding or hot-melt extrusion. When the IVR is placed in the vaginal lumen, the drug concentration initially is homogeneous throughout the IVR, but immediately upon contact with vaginal tissue a spatial concentration gradient ensues. The drug present on the ring surface (at the polymer/ tissue interface) is the first to diffuse from the IVR into the contacting tissue, transiting through a thin conducting layer of vaginal fluid or directly into tissue. The rate of drug release is interdependent on a number of factors, including the solubility of the

drug in the elastomer, the diffusion coefficient of the drug in the elastomer, the solubility of the drug in vaginal fluid, the volume of the vaginal fluid, the partition coefficient of the drug between the IVR and the vaginal fluid and tissue, the rate of diffusion and elimination of the drug through the vaginal tissue, and the rate of anterior to posterior advection of the vaginal fluid. [116]

Reservoir device design avoids this time-dependent release rate by slowing the diffusion of the drug from the device with a rate-controlling membrane that is made from a different material than the core of the device, i.e. the reservoir. In this type of system, the drug concentration remains spatially uniform in the core to provide a driving force for diffusion across the rate-controlling membrane that is nearly constant with time, resulting in zero-order or near zero-order drug release. The drug release rate from reservoir-type devices can be nearly constant for several months, but they generally release less total drug in a given period of time than the matrix devices because of the rate-controlling membrane that impedes drug diffusion. [117]

Despite the rapid development and successful formulation of single drug IVR, concern over the development of multiple drugs loaded IVR is challenging. Also certain biological drugs like peptides and hormones often cannot withstand conventional IVR processing (injection molding and hot-melt extrusion) and/or have limited diffusivity in elastomeric polymers due to their size and limited solubility. Therefore, designing IVR capable of simultaneously attaining the target release rates for multiple drugs that possess a wide range of physicochemical properties for several days or more require new and innovative technology. A simple and elegant design developed by Loxley, et al. at Particle Sciences, Inc. [118] allows a drug to be microencapsulated in a secondary polymer (via spray drying, for example), which is then hot- melt extruded or injection molded along with a second drug in the primary polymer, resulting in microcapsules dispersed throughout a polymer matrix. Figure 2.12 shows the different types of intra vaginal rings.

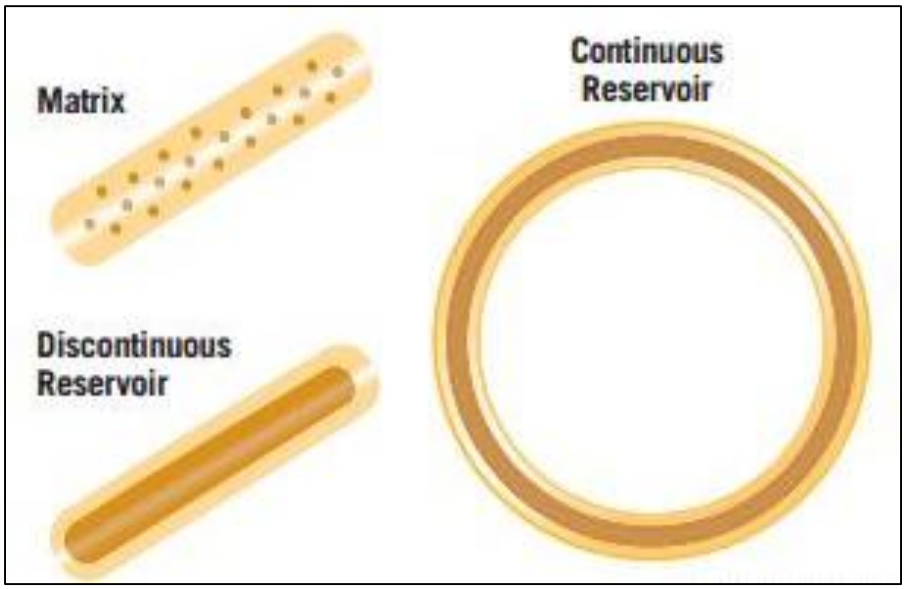


Figure 2.12 Matrix and Reservoir Type Intra Vaginal Rings.

Although this approach improves release rate modulation and is relatively simple to manufacture, its application is still restricted to hydrophobic drugs that are soluble in the primary polymer. Unfortunately, many hydrophilic drugs are not sufficiently soluble in conventional polymers and therefore cannot be delivered by simple diffusion at sufficient levels for a sustained duration. To adequately deliver two or more drugs with differing hydrophilicity from a single IVR, a multi-segment IVR has been designed where each drug is dissolved or dispersed in separate segments with different polymer composition. [119] This approach allows drug to be formulated separately in each segment so that release rates and chemical and physical stabilities may be independently optimized – for example, the drug loading, polymer composition, and surface area of each segment can be tailored for each specific compound. These segments can then be joined to create a ring using a variety of methods, including adhesive bonding, induction welding, or solvent welding. Drawbacks to this approach include a multistep manufacturing process and possible diffusion of drug from one segment to another during storage.

Biological molecule formulation in IVR requires additional design considerations since most macromolecules cannot diffuse through elastomers typically employed and are likely unable to survive hot- melt extrusion or injection molding temperatures. An

interesting approach to deliver macromolecules from IVR utilizes the hydrophobic co-polymer EVA. Inter-connected water-filled pores in the IVR are created once the macromolecule begins diffusing from the EVA. Although this approach can deliver a large amount of drug for sustained durations, solvent casting is currently used to create the IVR, which may leave residual toxic solvent or cause denaturation of the biological drug and is not a scalable pharmaceutical manufacturing method for IVR. [120]

Special attachments or inserts in IVR are being developed to place the macro- or bio-molecules in a cellulosic matrix or 100% compressed drug. Tablet presses at suitable room temperature processing are employed to fabricate and then manually insert or glue the tablet into or on the side of a prefabricated IVR. Pellet inserts have been shown to deliver proteins for several days in a nonlinear fashion. Lyophilized gel inserts can also be prepared to deliver the protein based high molecular weight drugs. The lyophilised gel inserts offer mild manufacturing conditions conducive to protein stability. Hence, the multi- compartment nature of the insert vaginal ring may ultimately prove useful in the administration of combination of drugs through the single IVR. [121] Figure 2.13 shows the type of inserts in the IVR.

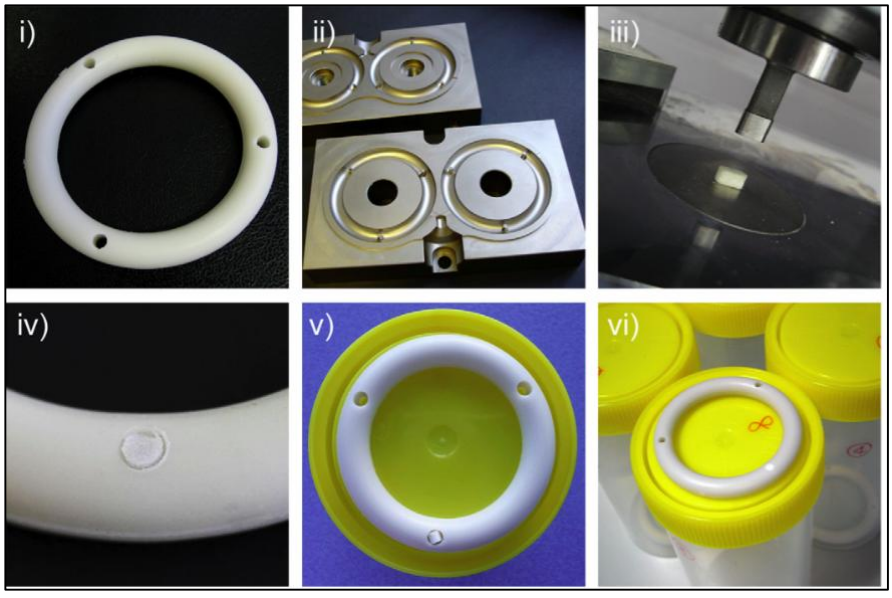


Figure 2.13 (i) Silicone insert vaginal ring, (ii) Injection moulds for IVR manufacturing, (iii) directly compressed insert, (iv) silicone insert, (v) directly compressed tablet insert, and (vi) lyophilised insert.

2.7 In Vivo Biodistribution Studies by Gamma Scintigraphy

In vivo biodistribution study is a method of tracking the compound as it travels through the body of animal or human subject. Improving drug efficacy and limiting potential side effects are cardinal steps in the development of new pharmaceutical compounds and/or formulations. In vivo biodistribution studies provide pivotal information about accumulation of compounds and/or formulations in various organs or tumors. This information helps to optimize drug formulations and improve biodistribution properties of compounds. Recent advances in nanotechnology have led to the development of nanocarriers for targeted therapy that can avoid the systemic side effects and help the drug to exert its effect directly at the site of action. [122] Many nanocarriers have undesirable tissue distribution due to several reasons like incorrect particle size, use of wrong polymer or coatings or ligands etc., uptake by RES so on and so forth. Biodistribution studies can thus help decide the correct particle size, coatings, or other features early in formulation development. In vivo fate of a molecule can be easily traced with these studies.

Biodistribution studies involve the estimation of drug in various tissues and organs at different time points post administration of dosage form. It can be done by sacrificing the animal, isolating the different organs and determining the concentration of drug in each organ/tissue. This method is invasive and requires sacrificing large number of animals for obtaining statistically pertinent results. Moreover, sophisticated instruments like high-performance liquid chromatography (HPLC), Enzyme-linked immunosorbent assay (ELISA) etc, which can measure very small tissue concentrations are also required. [123] Some methods involve the use of using near infrared (NIR) based technology. Near infrared fluorophore (NIRF) molecules are incorporated into nanocarriers to facilitate visualization of molecule's path towards targeted cells or tissues. [3] However, the most economic, rapid and non-invasive method of biodistribution study is Imaging Technology. Among the different imaging techniques like PET, MRI, CT-Scan etc., Gamma Scintigraphy is widely accepted method as it is inherently quantitative. In addition, Scintigraphy is a computer-based system attached to the software to perform a

variety of analysis options. The most common analysis tool is region-of-interest analysis, which determines the number of counts in a designated area. [123, 124] Scintigraphic imaging employs a gamma-emitting radionuclide label to track and quantitate the distribution of formulation in the body. It has ability to image the total organism in a single whole body scan that makes it superior to other techniques.

A radiopharmaceutical has two components: a radionuclide and a pharmaceutical. The usefulness of a radiopharmaceutical is dictated by the characteristics of these two components. [125] A suitable radionuclide is tagged onto the chosen pharmaceutical such that after administration of the radiopharmaceutical, radiations emitted from it are detected by a radiation detector. Thus, the morphologic structure or the physiologic function of the organ can be assessed. Radiations from the radionuclide of choice should be easily detected by nuclear instruments and the radiation dose to the patient should be minimal. As the radiopharmaceutical is bio-distributed entirely, the imaging of emitted radiations clearly shows the normal as well as the abnormality in the organs. Furthermore, they can assess the target specificity of cytotoxic drugs to the affected tissues. Also, radiolabeled receptor-binding peptides have emerged as a new class of radiopharmaceuticals for tumor scintigraphy and, more recently, to treat cancers by using peptide receptor radiation therapy (PRRT). [126]

A radionuclide is a nuclide that is radioactive. Also referred to as a radioisotope or radioactive isotope. It is an isotope with an unstable nucleus, characterized by excess energy available to be imparted either to a newly created radiation particle within the nucleus or via internal conversion. During this process, the radionuclide is said to undergo radioactive decay, resulting in the emission of gamma ray(s) and/or subatomic particles such as alpha or beta particles. The radioactive decay data determine the suitability of a radioisotope for in vivo tracer studies, both from the imaging and internal radiation dose considerations. These emissions constitute ionizing radiation. [127] It is important that the physical half-life of the radionuclide used matches the biologic turnover of the radiopharmaceutical in vivo. The clinical significance of half-life as a determinant of dose rate and time to response is important aspect. [128]

Among all the radioisotopes, "Technetium-99m" (^{99m}Tc) is most commonly used radionuclide for the biodistribution studies. Radioisotopes, with their half-lives, have been given in Table 2.1.

Table 2.1 Approximate half-life of various radioisotopes

Radioisotope	Approx. Half Life
^{81m}Kr	13 seconds
^{99m}Tc	**6 hours**
^{131}I	8 days
^{51}Cr	30 days
^{125}I	60 days
^{137}Cs	30 years
^{241}Am	462 years
^{226}Ra	1620 years
^{238}U	$4.59x10^9$ years

Radioactive Technetium has proved to be appropriate for labeling both large and small biomolecules, proteins and peptides as well as formulations. Technetium-99m is a short-lived metastable nuclear isomer produced from molybdenum 99. ^{99m}Tc has been widely used as a radiotracer in nuclear medicine and in biomedical research to label molecular and cellular structures as well as studying the biodistribution properties of the drug and/or delivery systems due to its many desirable characteristics: it emits a 140 -keV gamma ray with 89 % abundance, which is suitable for imaging with gamma cameras. Moreover, due to short physical as well as biological half-life, it causes less exposure to the animal body and the environment leading to its fast clearing from the body after an imaging process. [129]

Technetium is a transition metal of silvery grey color belonging to group VIIB (Mn, Tc and Re) and has the atomic number 43. No stable isotope of technetium exists in nature. The ground state ^{99}Tc has a half-life of 2.1×10^5 years. Technetium can exist in eight oxidation states namely, 1- to 7+, which result from the loss of a given number of electrons from the 4d and 5s orbitals or gain of an electron to the 4d orbital. The stability

of these oxidation states depends on the type of ligands and chemical environment. The 7+ and 4+ states are the most stable and are represented in oxides, sulphides, halides and pertechnetates. The lower oxidation states 1-, 1+, 2+ and 3+, are normally stabilized by complexation with ligands. [130]

The chemical form of ^{99m}Tc available from the Molybdenum generator is sodium pertechnetate (^{99m}Tc-$NaTcO_4$). The pertechnetate ion, $^{99m}TcO^-_4$, having the oxidation state 7+ for ^{99m}Tc, resembles the permanganate ion, MnO^-_4, and the perrhenate ion, ReO_4^- Chemically, $^{99m}TcO^-_4$ is a rather non-reactive species and does not label any compound by direct addition. In ^{99m}Tc- labeling of many compounds, prior reduction of ^{99m}Tc from 7+ state to a lower oxidation state is required. [131] Various reducing systems that have been used are stannous chloride ($SnCl_2.2H_2O$), stannous citrate, stannous tartrate, concentrated HCl, sodium borohydride (NaBFU), dithionite and ferrous sulphate. Among these, stannous chloride is the most commonly used reducing agent in acidic medium in most preparations of ^{99m}Tc-labelled compounds.

There is a possibility that reduced ^{99m}Tc may undergo hydrolysis in aqueous solution. In this case, the reduced ^{99m}Tc reacts with water to form various hydrolyzed species depending on the pH, duration of hydrolysis and presence of other agents. Some species of this category are $^{99m}TcO_2$, $^{99m}Tc^{2+}$ and $^{99m}TcOOH^+$. This hydrolysis competes with the chelation process of the desired compound and this reduces the yield of the ^{99m}Tc-chelate. The use of stannous chloride has a disadvantage in that it also readily undergoes hydrolysis in aqueous solution at approximately pH 6 to 7 and forms insoluble colloids. These colloids bind to reduced ^{99m}Tc and thus compromise the labeling yield. To prevent this colloid formation, an acid is added to prevent the hydrolysis of Sn^{2+} before the reduction of technetium. [132]

In recent years targeted drug delivery has become one of the prime objectives in the development of new dosage forms. Targeted delivery is designed to maximize the therapeutic effect while minimizing the potential for systemic side-effects. Traditionally the vaginal route was used either to provide women with therapy for local disorders or as an alternative route for systemic administration via the highly vascular tissue. During the

course of various research works, it has become interesting to know that drugs administered vaginally are preferentially uptaken in uterus where their tissue concentrations exceeds the systemic absorption. This may serve as a route of administration for substances used for the treatment of certain uterine disorders. Vaginal route of drug delivery offers the drug targeting to uterus through the First Uterine Pass Effect. To assess the drug targeting to uterus, via vaginal route, Gamma Scintigraphy plays a pivotal role. The distribution of vaginally administered radiolabelled drug loaded liposomes can be studied through the Gamma imaging.

2.8 Review of the past work done

2.8.1 Review of the past work done on Leuprolide acetate

Arul Sudar et al. developed a sustained injectable drug delivery system for Leuprolide acetate by formulating its sterically stabilized liposomes using Hydrogenated Soya Phosphatidylcholine and DSPG-mPEG 5000. The purpose of this study was to prolong the biological half-life of the drug, to reduce the uptake by reticuloendothelial system (RES), and to reduce the injection frequency of intravenously administered drug. The authors could attain long circulation of the sterically stabilized liposomes by decreased uptake through RES as well as increased tumor accumulation than as compared to the conventional liposomes. [133]

Byung H. Woo et al. Prepared and characterizaed poly (D,L-lactide) leuprolide microspheres. Leuprolide microspheres were prepared with PLA (m.w. 11 000 Da) by a dispersion/solvent extraction-evaporation method. Serum peptide and testosterone levels were analyzed after subcutaneous administration using a rat model. Spherical microspheres with a mean diameter of 52 μm containing 13.4% peptide, released 10% of the peptide within 24 hours, followed by a linear release for 150 days. Serum leuprolide levels increased immediately after administration of the microspheres to 45.6 ng/ml, but then fell to 4.3 ng/ml at 15 days and |2.0 ng/ml at 30 days where they remained for 120 days. A 120-day microsphere formulation of leuprolide was developed with excellent controlled peptide release characteristics and in vivo efficacy. [134]

Aliasgar Shahiwala et al., investigated the nasal route as a non-invasive alternative for delivery of leuprolide acetate to achieve an effective concentration of leuprolide acetate in blood after nasal administration for contraception in rats. Leuprolide loaded liposomes were prepared for nasal administration. The plain drug solution, physical mixture (plain drug along with constituents of liposomes), or drug encapsulated in either neutral or charged liposomes containing 5 μg leuprolide acetate were administered to rats through the nasal route. The plain drug solution was administered subcutaneously (s.c.). Simultaneous evaluation was performed on the influence of a mucoadhesive agent (chitosan) on nasal absorption of the plain drug and the liposome-encapsulated drug. Liposomal chitosan formulation administered nasally and leuprolide acetate solution subcutaneously achieved complete azoospermia. No implantation sites were observed after the mating of female rats with treated males. The findings of these investigations demonstrated that the bioavailability of the nasally-administered liposomal leuprolide acetate with chitosan formulation was comparable with that of the subcutaneously administered drug. Complete contraception was obtained in male and female rats that had been treated with either the nasally administered liposomal leuprolide acetate with chitosan or the subcutaneously administered drug. [135]

Mehdi Rahimi et al. developed In Situ Forming Poly(lactic acid-co-glycolic acid) Implants Containing Leuprolide Acetate/β-Cyclodextrin Complexes. The researchers improved the aqueous stability of leuprolide by complexation with cyclodextrins. Implants were prepared using solution of PLGA. Injecting the solution of PLGA containing drug, leads to precipitation of PLGA and in situ formation of implant entrapping the drug at injection site thus giving a sustained drug release. [136]

2.8.2 Review of the past work done on Raloxifene Hydrochloride

Dimitrios Bikiaris et al., prepared biodegradable nanoparticles loaded with Raloxifene Hydrochloride using a series of novel biodegradable poly(ethylene succinate-co-propylene adipate) P(ESu-co-PAd) polyesters to improve the bioavailability of the drug. The nanoparticles were prepared by a variation of the coprecipitation method. [137]

Hetal Thakkar et al., developed microemulsion and self-microemulfying formulations of Raloxifene Hydrochloride to improve its oral bioavailability by bypassing the hepatic first pass metabolism. Microemulsion was prepared using Capmul MCM C8, Tween 20 and Polyoxyhyrogenated 40 castor oil. Microemulsion formulations gave higher permeability than plain drug through intestinal membrane leading to enhanced bioavailability. [138]

Lakshmi Prasanthi Nori et al., prepared Fast Dissolving Tablet (FDT) of Raloxifene by direct-compression method by incorporating super disintegrants like crosscarmellose sodium and sodium starch glycolate to improve the dissolution rate of Raloxifene. [139]

Ram K. Jha et al., developed bioadhesive microspheres of Raloxifene Hydrochloride for its bioavailability enhancement. The present study describes two simultaneous approaches to improve its bioavailability, complexation of drug with cyclodextrin, and formulation of mucoadhesive microspheres of the complex using different proportions of carbopol and HPMC. Results of this study showed that mucoadhesive microspheres composed of inclusion complex of Raloxifene with Cyclodextrins could be a radical approach to improve the bioavailability of Raloxifene. [140]

Tran et al., worked upon Raloxifene loaded Solid Lipid Nanoparticles for enhancement of its oral bioavailability. [141] While, *Nirmal Shah et al.,* developed Nanostructured lipid carriers for oral bioavailability enhancement of Raloxifene. [142]

Syed Mahmood et al., developed Raloxifene hydrochloride loaded nanotransfersomes for transdermal application to enhance its bioavailability. [143]
N. Başaran Mutlu et al., studied the effectiveness of Raloxifene-Loaded Liposomes and in Breast Cancer Therapy. Highest antitumor activity was observed, and MMP-2 enzyme was also found to be inhibited with Raloxifene-loaded liposomes. [144]

No work has been yet reported on vaginal delivery of Leuprolide acetate and Raloxifene Hydrochloride for uterine targeting to treat fibroids and endometriosis. The present

investigation is a novel work based upon the liposomal formulations of Leuprolide and Raloxifene to be targeted to uterus via intravaginal delivery.

2.9 Drug Profile

2.9.1 Leuprolide Acetate

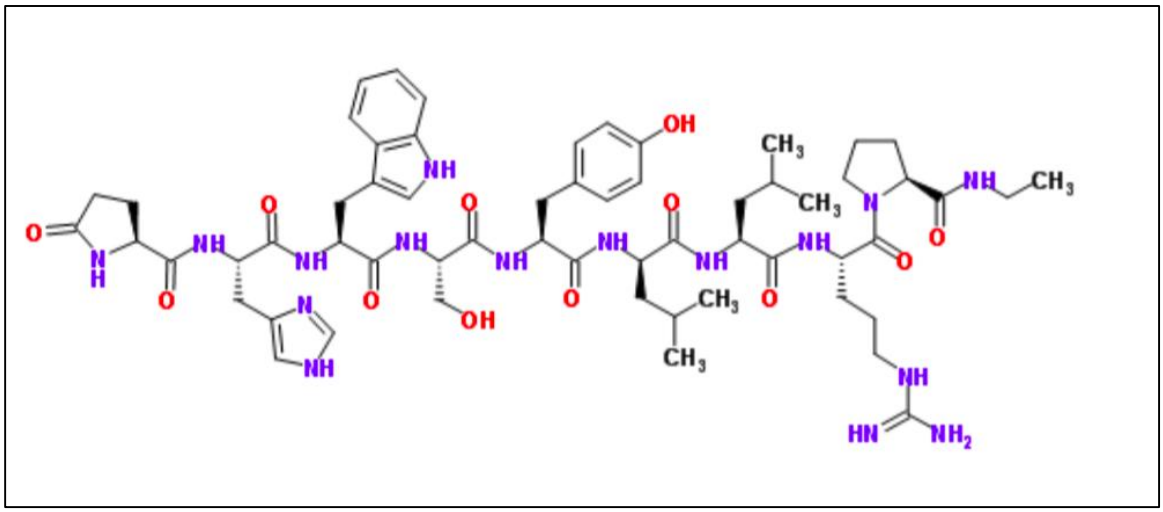
Category: GnRH analogue; hormone

CAS Number: 74381-53-6

Molecular formula: $C_{61}H_{88}N_{16}O_{14}$

Molecular Weight: 1,269.4 g/mol

Structural Formula:



Chemical Name: 5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-Lleucyl-L-arginyl-N-ethyl-L-prolinamide acetate

Physicochemical properties: Leuprolide acetate is a fine or fluffy, white to off-white powder. It is soluble in water, ethanol and propylene glycol with a pKa of 9.6. The melting point of Leuprolide acetate is 170 ^{o}C.

Mechanism of Action: Leuprolide binds to the gonadotropin releasing hormone receptor and acts as a potent inhibitor of gonadotropin secretion. Leuprolide acetate, a GnRH agonist, acts as a potent inhibitor of gonadotropin secretion. Animal studies indicate that

following an initial stimulation, chronic administration of leuprolide acetate results in suppression of ovarian and testicular steroidogenesis. This effect is reversible upon discontinuation of drug therapy. Administration of leuprolide acetate results in inhibition of the growth of certain hormone dependent tumors.

In humans, administration of leuprolide acetate results in an initial increase in circulating levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH), leading to a transient increase in levels of the gonadal steroids (testosterone and dihydrotestosterone in males, and estrone and estradiol in premenopausal females). However, continuous administration of leuprolide acetate results in decreased levels of LH and FSH. In males, testosterone is reduced to below castrate threshold. These decreases occur within two to four weeks after initiation of treatment. Long-term studies have shown that continuation of therapy with leuprolide acetate maintains testosterone below the castrate level for up to seven years. Leuprolide acetate is not active when given orally.

Pharmacokinetics:

Absorption

Following a single injection of LUPRON DEPOT 7.5 mg for 1-month administration to patients, mean plasma leuprolide concentration was almost 20 ng/ml at 4 hours and 0.36 ng/ml at 4 weeks. However, intact leuprolide and an inactive major metabolite could not be distinguished by the assay which was employed in the study. Nondetectable leuprolide plasma concentrations have been observed during chronic LUPRON DEPOT 7.5 mg administration, but testosterone levels appear to be maintained at castrate levels. Bioavailability after intramuscular injection of the depot formulation is estimated to be about 90%. The leuprolide acetate implant delivers 120 micrograms of leuprolide acetate per day over 12 months.

Distribution

The mean steady-state volume of distribution of leuprolide following intravenous bolus administration to healthy male volunteers was 27 L. In vitro binding to human plasma proteins ranged from 43% to 49%.

Metabolism

In healthy male volunteers, a 1 mg bolus of leuprolide administered intravenously revealed that the mean systemic clearance was 7.6 L/h, with a terminal elimination half-life of approximately 3 hours based on a two compartment model.

Excretion

Following administration of Lupron Depot 3.75 mg to 3 patients, less than 5% of the dose was recovered as parent compound in the urine.

Indications and Usage

Lupron Depot 7.5 mg for 1-month administration is indicated in the palliative treatment of advanced prostatic cancer. 3.75 mg depot for 1-month and 11.25 mg depot thrice a 3 month is indicated for Uterine fibroids and endometriosis and central precocious puberty.

Adverse reactions

Body as a Whole - Asthenia, Cellulitis, Fever, Headache, Injection site reaction, Neoplasm

Cardiovascular System - Angina, Congestive heart failure

Digestive System - Anorexia, Dysphagia, Eructation, Peptic ulcer

Hemic and Lymphatic System – Ecchymosis

Musculoskeletal System - Myalgia

Nervous System - Agitation, Insomnia/sleep disorders, Neuromuscular disorders

Respiratory System - Emphysema, Hemoptysis, Lung edema, Sputum increased

Skin and Appendages - Hair disorder, Skin reaction

Urogenital System - Balanitis, Breast enlargement, Urinary tract infection.

2.9.2 Raloxifene Hydrochloride

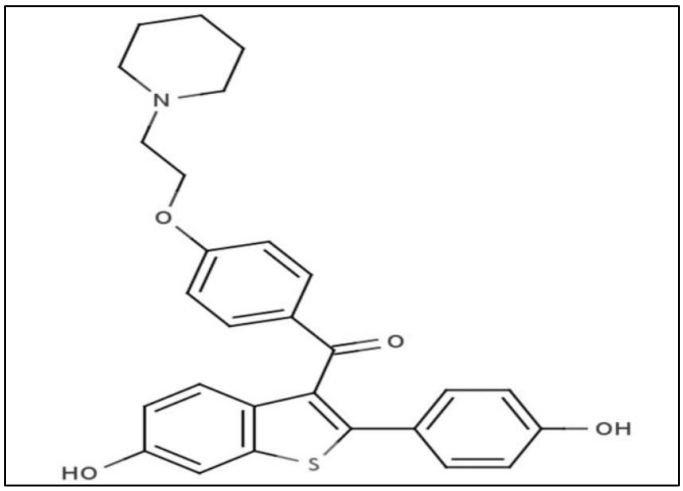
Category: A second generation selective estrogen receptor modulator (SERM); Estrogen antagonist

CAS Number: 82640-04-8

Molecular formula: $C_{28}H_{28}ClNO_4S$

Molecular Weight: 510.04 g/mol

Structural Formula:



Chemical Name: 6-hydroxy-2-(4-hydroxyphenyl)-1-benzothiophen-3-yl]-[4- (2-piperidin-1-ylethoxy) phenyl] methanone

Physicochemical properties: Raloxifene hydrochloride is off-white to pale yellow powder that is very slightly soluble in water. It is freely soluble in methanol and DMSO. Melting point range of drug is 263-267 ^{o}C, and has hydrophobicity of 5.2 on logarithmic scale. Raloxifene falls in class II of BCS classification system.

Mechanism of Action: Raloxifene binds to estrogen receptors, resulting in differential expression of multiple estrogen-regulated genes in different tissues. Raloxifene produces estrogen-like effects on bone, reducing resorption of bone and increasing bone mineral density in postmenopausal women, thus slowing the rate of bone loss. The maintenance of bone mass by raloxifene and estrogens is, in part, through the regulation of the gene-encoding transforming growth factor-β3 (TGF-β3), which is a bone matrix protein with antiosteoclastic properties. Raloxifene activates TGF-β3 through pathways that are estrogen receptor-mediated but involve DNA sequences distinct from the estrogen response element. The drug also binds to the estrogen receptor and acts as an estrogen agonist in preosteoclastic cells, which results in the inhibtion of their proliferative capacity. This inhibition is thought to contribute to the drug's effect on bone resorption. Other mechanisms include the suppression of activity of the bone-resorbing cytokine interleukin-6 promoter activity. Raloxifene also antagonizes the effects of estrogen on

mammary tissue and blocks uterotrophic responses to estrogen. By competing with estrogens for the estrogen receptors in reproductive tissue, Raloxifene prevents the transcriptional activation of genes containing the estrogen response element. As well, Raloxifene inhibits the estradiol-dependent proliferation of MCF-7 human mammary tumor cells in vitro.

Pharmacokinetics:

The disposition of Raloxifene has been evaluated in more than 3000 postmenopausal women in selected Raloxifene osteoporosis treatment and prevention clinical trials, using a population approach. Pharmacokinetic data also were obtained in conventional pharmacology studies in 292 postmenopausal women. Raloxifene exhibits high within-subject variability (approximately 30% coefficient of variation) of most pharmacokinetic parameters.

Absorption

Raloxifene is absorbed rapidly after oral administration. Approximately 60% of an oral dose is absorbed, but presystemic glucuronide conjugation is extensive. Absolute bioavailability of Raloxifene is 2%. The time to reach average maximum plasma concentration and bioavailability are functions of systemic interconversion and enterohepatic cycling of Raloxifene and its glucuronide metabolites.

Administration of Raloxifene HCl with a standardized, high-fat meal increases the absorption of Raloxifene (Cmax 28% and AUC 16%), but does not lead to clinically meaningful changes in systemic exposure. Raloxifene can be administered without regard to meals.

Distribution

Following oral administration of single doses ranging from 30 to 150 mg of Raloxifene HCl, The apparent volume of distribution is 2348 L/kg and is not dose dependent. Raloxifene and the monoglucuronide conjugates are highly (95%) bound to plasma proteins. Raloxifene binds to both albumin and α1-acid glycoprotein, but not to sex-steroid binding globulin.

Metabolism

Biotransformation and disposition of Raloxifene in humans have been determined following oral administration of 14C-labeled Raloxifene. Raloxifene undergoes extensive first pass metabolism to the glucuronide conjugates: Raloxifene-4- glucuronide, Raloxifene-6-glucuronide and Raloxifene-6, 4′-diglucuronide.

Excretion

Raloxifene is primarily excreted in feces, and less than 0.2% is excreted unchanged in urine. Less than 6% of the Raloxifene dose is eliminated in urine as glucuronide conjugates.

Indications and Usage

Raloxifene is supplied in a tablet dosage form for oral administration. Each tablet contains 60 mg of Raloxifene HCl, which is the molar equivalent of 55.71 mg of free base. It is indicated for osteoporosis and breast cancer prevention in post menopausal woman with osteoporosis.

Adverse reactions

Hot flashes, leg cramps, peripheral edema, flu syndrome, arthralgia, sweating.

2.10 Excipient Profile

2.10.1 DSPC: 1,2-Distearoyl-sn-glycero-3-phosphocholine

Category: Phospholicholine with stearic acid (lipid with high transition temperature: 56 ^{o}C)

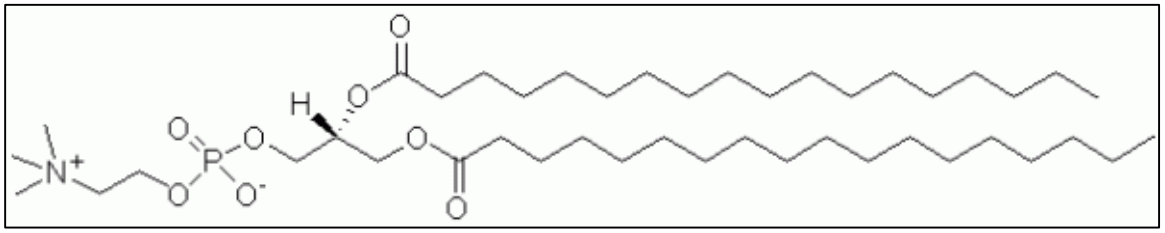
CAS Number: 816-94-4

Empirical Formula: $C_{44}H_{88}NO_8P$

Molecular Weight: 790.15 g/mol

Chemical Name: [(2R)-2,3-Di(octadecanoyloxy)propyl] 2-(trimethylazaniumyl)ethyl phosphate

Structure:



Storage instructions: Store at -20°C. Store under Desiccating conditions. The product can be stored for up to 12 months.

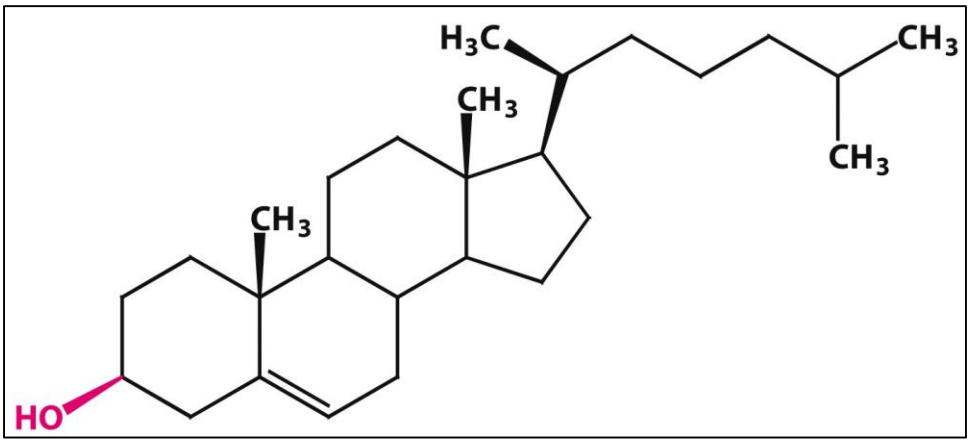
Handling: Wherever possible, prepare and use solutions on the same day. However, if needed to make up stock solutions in advance, store the solution as aliquots in tightly sealed vials at -20°C. Generally, these will be usable for up to one month. Before use, and prior to opening the vial allow product to equilibrate to room temperature for at least 1 hour.

It comes under Generally Regarded As Safe excipient. It approved by USFDA for in vivo use. It is white solid powder freely soluble in methylene chloride, methanol and Chloroform.

2.10.2 *Cholesterol*

Category: Organic alcohol present in body
CAS Number: 57-88-5
Empirical Formula: $C_{27}H_{46}O$
Molecular Weight: 386.65 g/mol
Chemical Name: (3β)-cholest-5-en-3-ol
Melting point: 148 °C

Structure:



It is a major component of all biological membranes; ~25% of total brain lipid is cholesterol. Cholesterol is a lipid that makes up about 20-25% of the structural components of the cell membranes. It determines the fluidity and permeability of the membrane, making it permeable to water but not to ions and protons. Cholesterol also regulates the functions of the transporters and signaling proteins present on the plasma membrane. The major sites of cholesterol synthesis are small intestine and liver.

Storage instructions: Store at -20°C. Store under Desiccating conditions. The product can be stored for up to 12 months. It should be stored in a well-closed container, protected from light.

Handling: Observe normal precautions appropriate to the circumstances and quantity of material handled. Rubber or plastic gloves, eye protection, and a respirator are recommended. May be harmful following inhalation or ingestion of large quantities, or over prolonged periods of time, owing to the possible involvement of cholesterol in atherosclerosis and gallstones. May be irritant to the eyes. When heated to decomposition, cholesterol emits acrid smoke and irritating fumes.

It comes under Generally Regarded As Safe excipient. It approved by USFDA for in vivo use. Cholesterol occurs as white or faintly yellow, almost odorless, pearly leaflets, needles, powder, or granules. On prolonged exposure to light and air, cholesterol acquires

a yellow to tan color. It is freely soluble in Isopropyl myristate, Ether, Methanol, Benzene, Acetone, Ethanol, Chloroform and Hexane.

2.11 References

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