Controlled release of therapeutic agents: slow delivery and cell encapsulation

Abstract Some of the most promising systems for the controlled release of bioactive agents, i.e., peptides or hormones, involve the encapsulation or entrapment of hormones or peptides in biocompatible polymeric devices that enable their continuous release over prolonged periods. In urology, two major pathologic conditions, androgen deficiency and prostate cancer, currently benefit from treatments based on controlled delivery. Leuprolide acetate depot (Lupron-depot) was one of the first controlled-delivery systems used for the treatment of prostate cancer. Clinical studies indicate that patients with prostate cancer who undergo therapy with leuprolide acetate depot can benefit from this treatment. Currently available androgen-replacement therapies include the oral administration of testosterone tablets or capsules, depot injections, sublingual treatment, and skin patches. However, side effects such as metabolic inactivation of testosterone on oral administration; fluctuations in levels of the hormone; and burning, rash, and skin necrosis during the use of skin patches may occur. These side effects may be avoided through the application of encapsulated Leydig cells, which produce testosterone. Studies in our laboratory have shown that Leydig cells encapsulated in alginate/poly-L-lysine/alginate microspheres are capable of secreting testosterone in culture and in vivo. Microencapsulated Leydig cells delivered intraperitoneally into castrated rats maintained a testosterone level of 0.51 ng/ml for more than 3 months without any human chorionic gonadotropin stimulation. Similar studies are also being conducted in our laboratory on encapsulation of ovarian cells for the secretion of progesterone and estrogen in culture and in vivo using microencapsulation techniques.

For several decades the controlled delivery of bioactive agents, i.e., peptides or hormones, has attracted considerable attention as potential therapy for various diseases or conditions. Some of the most promising systems for the controlled release of these agents involve the encapsulation or entrapment of hormones or peptides in biocompatible polymeric devices that enable their continuous release over prolonged periods [12, 15]. These agents can be formulated in a variety of designs, including microparticles, osmotic minipumps, gels, and adhesive patches.

Controlled-delivery devices can also be designed as microspheres, which may enable the entrapment of living cells responsible for the production of the desired hormones or peptides. In such systems the living cells can secrete the desired agents continuously or in response to specific physiologic stimulation and requirements. The polymeric carriers may be designed to isolate the cells from the host's immune response while allowing oxygen and adequate nutrients to reach the encapsulated cells. In urology, two major pathologic conditions, androgen deficiency and prostate cancer, currently benefit from treatments based on controlled delivery.

Gonadotropin-releasing-hormone depot preparations

When given continuously, the luteinizing hormone-releasing hormone (LHRH) agonists act as potent inhibitors of gonadotropin secretion and result in the suppression of ovarian and testicular steroidogenesis, reducing testosterone values to castration levels and estrogen values to postmenopausal levels. The main indication for the clinical use of these agents is metastatic prostate cancer. These agents have also been used for the treatment of precocious puberty [7].

Leuprolide acetate depot (Lupron-depot), an LHRH analog, was the first controlled-delivery system used for
the treatment of prostate cancer. The formulation contains sterile lyophilized microspheres in which 22.5 mg of synthetic leuprolide is incorporated in a biodegradable polymer of polyactic acid. This preparation is mixed with 1.5 ml of an accompanying diluent (carboxymethylcellulose sodium, d-mannitol, polysorbate 80, and glacial acetic acid) before its intramuscular injection. Clinical studies indicate that patients with prostate cancer who are treated with the leuprolide acetate depot can benefit from this treatment [7, 8, 16]. Current formulations are designed to deliver the LHRH analog over a period of 3 months.

Goserelin acetate (Zoladex) is also a potent synthetic decapetide analog of LHRH. It has been formulated as a subcutaneous implant for continuous release over a 12-week period. The hormone is dispersed in a copolymer matrix consisting of α,β-lactic and glycolic acid (12.82–14.76 mg/dose), which are preloaded in a single-use syringe.

**Testosterone-delivery systems**

The main goal of androgen-replacement therapy is the maintenance of physiologic levels of both serum testosterone and its metabolites dihydrotestosterone and estradiol. The rationale for treatment in patients with hypogonadism is the restoration or enhancement of muscle strength, stabilization of bone density, improvement of osteoporosis, and restoration of secondary sexual characteristics, including libido and erectile function [3]. Hypogonadal states secondary to hypothalamic-pituitary disorders, gonadal abnormalities, and defects in androgen action or secretion may benefit from androgen replacement. Androgen replacement in women seems to have a role after menopause, contributing to the maintenance of sexual function [20].

Currently available androgen-replacement modalities include the oral administration of testosterone tablets or capsules [4, 9, 10], depot injections [11, 22, 24], sublingual treatment [25], and skin patches [2, 19, 28]. When taken orally, testosterone preparations are largely rendered metabolically inactive during the “first pass” through the liver. This metabolic inactivation necessitates the administration of high oral doses of testosterone, in excess of 200 mg/day, for the achievement of normal serum levels. Such large doses of testosterone may be toxic to the liver and may lead to hepatitis, hepatoma, or hepatocarcinoma [1, 13, 21].

Parental depot preparations include testosterone enanthate (Delatesty!l) and testosterone cypionate (Depot testosterone cypionate). These preparations are based on 17β-hydroxyl esters, which are given intramuscularly with slow-release oil-based injection vehicles every 10–21 days. During the administration of these preparations, testosterone values rise to suprathermal levels for 1 or 2 days, after which they gradually fall within the normal range for 10–12 days, reaching baseline levels after approximately 21 days. This fluctuation in testosterone levels may produce significant swings in mood, libido, and sexual function [3, 23].

Transdermal testosterone therapy includes both scrotal and nonscrotal patches. Testoderm and Androderm are multilayered skin patches that deliver measured doses of testosterone across the skin, with doses ranging from 4 to 6 mg/day. The scrotal skin is used as a delivery target because of the 5-alpha-reductase activity present within this site. Androderm patches deliver 2.5 mg of testosterone in an alcohol-based gel reservoir. The gel enhances drug penetration by contributing to the breakage of the epidermal barrier. When used on nonscrotal skin the patch has to be applied twice daily. Advantages of these systems include a reduced frequency of administration, resulting in increased patient compliance; avoidance of gastric and hepatic first-pass metabolism; and achievement of steady-state plasma concentrations of testosterone. However, despite these advantages the transdermal systems have been associated with adverse effects such as transient erythema, pruritis, induration, burning, rash, and skin necrosis [2, 14, 19].

**Cell encapsulation: urologic applications**

Cell transplantation has long been proposed as a treatment for several diseases involving hormone or protein deficiencies. However, this strategy has been limited due to cell rejection by the host’s immune system. Encapsulation of living cells in a protective, biocompatible, and semipermeable polymeric membrane has been proven to be an effective method for immunoprotection of the desired cells, regardless of the type of recipient involved (allograft, xenograft) [5]. A majority of the implantation work accomplished using microencapsulated cells as delivery vehicles has employed two polymers: sodium alginate and poly-l-lysine (PLL) [17]. Alginate microcapsules have been applied for various purposes [6], particularly for the encapsulation of pancreatic islet cells for insulin delivery [17, 27] and of recombinant cells for the delivery of therapeutic gene products [26].

Encapsulated Leydig cells may be useful for testosterone-replacement therapy. Leydig cells are responsible for the secretion of 95% of the total testosterone in the body. Thus, such a system may be capable of simulating the normal diurnal pattern of testosterone release by the testes, thereby avoiding side effects such as those associated with chemically modified testosterone administration.

Studies in our laboratory have shown that Leydig cells encapsulated in alginate-PLL-alginate microspheres (Fig. 1) are capable of secreting testosterone in culture and in vivo. Purified Leydig cells from rat testes were suspended in sodium alginate solution and extruded through an air-jet nozzle into a CaCl2 solution, where they gelled. The cell-Ca-alginate microspheres were further coated with PLL. The encapsulated cells were pulsed with human chorionic gonadotropin (hCG) every