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Cervical cancer treatment with a locally insertable controlled release delivery system

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Abstract

Local delivery of cancer chemotherapeutics enables sustained drug levels at the site of action thereby reducing systemic side effects. A novel insertable polymeric drug delivery system for cervical cancer treatment is presented. Cisplatin, the first line of therapy employed for cervical cancers, was incorporated in a poly(ethylene-co-vinyl acetate) (EVAc) device that is similar to those currently used for vaginal contraceptive delivery. Cisplatin crystals were uniformly dispersed in the polymeric system without undergoing significant dissolution in the polymer matrix. Cisplatin dissolution from the devices was biphasic, consistent with a matrix-type controlled-release system with an initial rapid release phase followed by a slower, linear release phase. Depending on the drug loading in the polymeric devices, the near-linear release phase varied in rate according both empirical, linear curve-fitting ($0.38 \pm 0.15 \mu\text{g/day}$ to $46.9 \pm 10.0 \mu\text{g/day}$) and diffusion analysis based upon diffusion through a porous structure (D_{app} from $1.3 \pm 0.5 \times 10^{-9} \text{ cm}^2/\text{s}$ to $5.8 \pm 0.3 \times 10^{-12} \text{ cm}^2/\text{s}$). The devices were tested for *in vitro* activity and found to be effective against both HPV positive and HPV negative cervical cancer cell lines. Preliminary studies indicate that this delivery system would be a good candidate for investigation as a choice of treatment in cervical cancers.

Keywords

controlled release; cancer; polymer; cisplatin; vaginal delivery; human papilloma virus (HPV)

Introduction

Cervical intraepithelial neoplasia (CIN), also known as cervical dysplasia, is characterized by the presence of abnormal cells in the cervix that can precede and develop into cervical cancer. The primary cause of such abnormalities is infection with human papillomavirus (HPV) types 16 and 18 [1]. In the US alone, approximately 50 million PAP smears are performed each year with approximately 1.2 million cases of low grade dysplasia (CIN1), between 200,000 and 300,000 cases of high grade dysplasia (CIN2) [2], and about 10,370 cases of cervical cancer being brought to light. In the United States, 10,370 cases of invasive cervical cancer among women were diagnosed in 2005 and approximately 3,700 women succumbed to this neoplasm [3]. Although this number appears small compared to other types of cancer, cervical cancer is the second most common form of cancer among women worldwide and the leading cause of death from cancer among women in developing countries. In a single year, cervical cancer accounted for an estimated 274,000 global deaths and at least 493,000 new cases are identified with 83 percent of these in developing countries [4].

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Regardless of global region, the management of cervical cancer is stage dependent. In the case of superficially invasive cervix carcinoma, surgical procedures are used. For locally advanced cervical cancers, a combination of therapeutic approaches is required to increase survival rates [5]. Women with locally advanced cervical cancers benefited from the combined use of radiation therapy and chemotherapy. In addition, clinical results have established the role of concurrent cisplatin (CDDP) chemotherapy as an adjuvant to radiation therapy [6–8]. Although the optimal concurrent chemotherapy regimen is controversial, randomized trials have shown weekly cisplatin as the ideal chemotherapy regimen [5]. With cisplatin, as well as with most other chemotherapeutic agents, the administration regimen is a balance between effectiveness and toxicity.

Chemotherapy with cisplatin is associated with varied toxicities, such as anemia, febrile neutropenia, gastrointestinal complications including nausea and vomiting, and neurologic complications which not only impair the patient's quality of life but can also be life threatening in preexisting conditions. At doses greater than 300 mg/m², cisplatin causes peripheral sensory neuropathy that is variable in recovery and sometimes worsens after cessation [9]. Due to these toxicities, alternative methods of administering toxic chemotherapeutics, specifically cisplatin, are needed.

The location of the cervix allows for easy accessibility and permits local delivery of chemotherapeutic drugs. Vaginal drug delivery systems for contraception and in the treatment of vaginal infections are utilized in clinical and research settings [10]. Vaginal delivery has become an area of great interest due to the accessibility of the site and also due to the permeability of the vaginal tissues [11,12]. Despite this, few delivery systems have been evaluated for local cancer chemotherapy delivery to the cervix, e.g. bioadhesive patches [13], cervical cap [14], and sponge and topical gels and ointments [15]. These methods have exceptional potential for only short term application. Bioadhesive patches are restricted by the turnover of the mucosal lining [13]. Cervical caps, sponges, and topical gels can only be retained in the vaginal canal for short periods due to blockage of natural canal openings. In addition to these short-term approaches, several polymeric controlled-release vaginal ring devices have become clinically available for contraception [16]. These devices are retained within the vaginal canal for extended periods up to a year in duration.

However, to our knowledge, no ring-based delivery system has been examined for local delivery of cisplatin, or any other cytotoxic chemotherapeutic, for treatment of cervical intraepithelial neoplasia or any other tumor type despite some suggestions in the recent patent literature [17,18]. Local chemotherapeutic drug delivery approaches for treatment of other forms of cancer have shown to ensure availability of the drug at the site of action, reduce the dose required for treatment, and thereby, reduce the toxicities normally associated with systemic administration. As an example, local cisplatin delivery via biodegradable, open cell poly(lactic acid) (pPLA-Pt) devices, in the treatment of intracranial glioma in rats was compared against intra-lesionally administered free cisplatin and systemic cisplatin. The pPLA-Pt devices delivered a cisplatin dose of 0.5 mg/m² which is 1/100th the standard systemic dose of 50 mg/m². These devices were considered highly effective in eradicating intracranial tumor with minimal histological evidence of toxicity [19]. Also, an intratumoral injectible cisplatin polymeric system, for the treatment of human head and neck squamous cell carcinoma, showed effectively reduced tumor volume while also reducing cisplatin related toxicities [20]. Several such local delivery systems have shown increased efficacy and significantly reduced systemic toxicity as compared to conventional cisplatin chemotherapy [21]. These devices are practical due to the localization of the drug at an adequate concentration for a sufficient time period within or near the tumor.

To augment the treatment of cervical cancer using the idea of localizing the release of a highly toxic chemotherapeutic agent, an insertable poly(ethylene-co-vinyl acetate) (EVAc) device impregnated with cisplatin has been preliminarily examined. A device modeled on the lines of currently available ring-based intra-vaginal devices was developed to allow for extended continuous release of the agent without refractory period for tumor growth. The location of cervical cancer makes it possible for the patient to self insert the ring-based device and replace the device when appropriate. The CDDP-EVAc devices developed were characterized chemically and physically to show that drug crystals were uniformly dispersed in the polymer matrix without undergoing any significant dissolution in the matrix. Depending on the drug loading in the device, extended periods of drug release ranging from weeks to months can be obtained. The release of the drug from the devices is biphasic with the released drug retaining activity *in vitro* by inhibiting proliferation of both HPV positive and HPV negative cervical cancer cell lines. The device may be of clinical significance in treating cervical intraepithelial neoplasia, is a potential substitute to systemic chemotherapy administration and can easily be used synergistically with radiation therapy and surgery.

Materials and Methods

All chemicals were purchased from Sigma-Aldrich Chemical Company as reagent grade and used without purification unless otherwise indicated.

Device Preparation

Matrix-type cisplatin-containing devices were prepared by the solvent evaporation technique [22]. Poly(ethylene-co-vinyl acetate) (EVAc; 28% vinyl acetate) was purified by soxhlet extraction with sequential acetone and water for approximately 48 hours each prior to dissolution in methylene chloride (10 w/v %). Cisplatin was dispersed in the methylene chloride solution of EVAc by vortexing to yield 1.6%, 3.3%, and 5.0% (w/v) solutions. This design achieved final devices containing 16% (CDDP16-EVAc), 33% (CDDP33-EVAc), and 50% (CDDP50-EVAc) cisplatin (w/w) since the total polymer concentration was ten percent of the total volume. The cisplatin-in-EVAc suspensions were poured into pre-chilled (−80°C) Teflon molds; molds were approximately 0.1 cm deep and 2.54 cm in diameter. The filled molds were placed in a −80°C freezer for half an hour followed by placement in −20°C freezer for at least 48 hours. Following this, the pellets were placed in vacuum at room temperature for at least 72 hours to remove residual methylene chloride. A 4-mm punch was used to obtain circular devices of approximately 50 mg (8 mg cisplatin) for CDDP16-EVAc, 60 mg (20 mg cisplatin) for CDDP33-EVAc, and 75 mg (37.5 mg cisplatin) for CDDP50-EVAc. The pieces remaining after creating circular devices were saved for chemical and structural analysis.

Chemical and Structural Analysis

EVAc devices were sectioned with a razor blade through the center creating either a single longitudinal section (through the equator of the disc) or a single lateral section (through the axis of the disc). In this way, the devices could be examined by scanning electron microscopy (SEM; Hitachi S-3000 N) and energy dispersive x-ray spectroscopy (EDS; Oxford Inca) to study the distribution and incorporation of cisplatin in the polymeric matrix. Uncoated samples were examined by EDS prior to coating with gold-palladium for SEM imaging. Sections were examined by attenuated total reflectance Fourier-transform infrared (ATR-FTIR) and Raman spectroscopy (Thermo Nicolet Nexus 870 with FT-IR and FT-Raman modules) to determine the chemical form of entrapped drug. Pure drug and polymer were used to identify the appropriate wavenumbers for analysis.

Cisplatin Release

A single device was placed within a thin stainless-steel coil to prevent settling of the device on the floor of the vial or floating on the surface of the solution. The device was then placed in 2 mL of phosphate buffered saline (PBS; pH 7.4) in a scintillation vial which was subsequently placed in a water bath maintained at 37°C while oscillating at 60 cycles/minute. At prescribed time intervals, the entire 2 mL sample was withdrawn for cisplatin analysis and replaced with fresh 2 mL of buffer, thus, maintaining sink conditions. The samples were analyzed spectrophotometrically by the *o*-phenylene diamine (oPDA) assay for cisplatin at a wavelength of 704 nm [23].

Cell culture

Ca Ski (CRL-1550), a HPV 16 and HPV 18 positive cervical cancer line, HeLa (CCL-2), a HPV 18 positive cervical cancer cell line, and C-33A (HTB-31), a HPV negative cervical cancer cell line, were obtained from American Type Culture Collection. HeLa and C-33A cell lines were grown and maintained in Eagles minimum essential media (EMEM) with 10% fetal bovine serum, 1% penicillin/streptomycin, sodium pyruvate (110 mg/mL) and non-essential amino acids. Ca Ski cell lines were grown and maintained in Dulbecco's minimum essential media (DMEM) with 10% fetal bovine serum, 1% penicillin/streptomycin, L-glutamine (584 mg/mL) and nonessential amino acids. Cells were cultured at 37°C in 5% CO₂. Cells were subcultured every 3–4 days with trypsin to retain cell densities below 80% confluence.

In vitro Bioactivity

To determine the activity (LC₅₀) of cisplatin against the three model cell lines, previously plated cells were treated with varying concentrations of cisplatin in media and incubated for pre determined time periods of 24 hr, 48 hr, and 72 hr. At the end of the incubation period, the viability was determined with modified MTS assay (Promga Cell Titer® 96) [24]. Once the LC₅₀ was calculated for a particular cell line, Table 1, that cell line was treated with this concentration to determine the actual survival at that dose. In addition, the amount of drug released from each of the devices *in vitro* was calculated based upon the *in vitro* release rate. From this, each cell line was treated with the calculated total amount of cisplatin released from that device to determine the expected activity of the devices at specific times. In this way, a comparison could be made between the actual activity of the device and the expected activity for the amount of drug released from the device in a particular time.

Based on cisplatin activity and the determined release kinetics, no significant cell death was expected in the first 24 hrs of incubation; therefore, activity was determined at 48 and 72 hours. Also, since there was expected to be a burst release from these devices followed by an extended zero-order release phase [25], two scenarios were examined to account for both initial placement and long-term effectiveness. In the first (burst) scenario, devices were used directly after production. For the second (near zero-order) scenario, devices were incubated in buffer for 7 days to attain devices that allowed us to examine the slower release phase. To accomplish this, devices were placed in phosphate buffered saline (PBS; pH 7.4) at room temperature for one week prior to beginning the activity study. These devices are referred to as “zero-order” devices.

One day prior to the beginning of the activity studies, cells were plated at a density of 5×10^4 cell/mL. After one day of culture, a transwell filter was placed with a single device into each well. Following the incubation period for either 48 hrs or 72 hrs, the transwell filters were removed and the viability of the culture was determined using a modified MTS assay. Just prior to the proliferation assay, morphology was documented using an inverted phase-contrast microscope (Olympus IX70) and digital images captured (QImaging Retiga 1300 C) and processed using available software (Scanalytics IP Lab).

Data Analysis

All data was expressed as a mean plus or minus the ($\pm$) standard deviation and were compared using two-way ANOVA with subsequent post-hoc test. Differences at p less than or equal to 0.05 were considered to be statistically significant. All samples were examined in triplicate at a minimum unless otherwise indicated. Finally, all error bars were presented as standard deviations.

Results

Chemical and Physical Characterization

The structural information obtained by ATR-FTIR and Raman spectroscopy, Figure 1, confirmed the presence and identity of cisplatin and EVAc in the devices. As expected, CDDP16-EVAc devices contained less cisplatin than CDDP33-EVAc devices, with the maximum incorporation being in CDDP50-EVAc devices. Characteristic IR peaks, Figure 1A, and Raman peaks, Figure 1B, for cisplatin and EVAc were observed between 3,300 and 3,200 and 2,800 and 3,000 cm^{-1} , respectively. Other peaks could all be attributed to either EVAc or cisplatin.

SEM micrographs of lateral sections of the devices, Figure 2, revealed two distinct phases were present throughout the section. Upon closer examination, Figure 2B, the minor phase seemed to contain regular shaped, non uniform-sized material. The second phase was heterogeneously distributed throughout the longitudinal sections. As expected, qualitatively more of the minor phase was observed in devices with greater incorporation of cisplatin (data not shown). EDS spectra of the major phase revealed that the regular, flat regions were composed of predominantly carbon and oxygen (100%) with no detected platinum or chlorine, Figure 2CI. The minor phase was composed of predominantly platinum (52%) and chlorine (48%) with little carbon and oxygen, Figure 2CII.

Cisplatin Release

A biphasic release profile was observed for each device. An initial burst release phase was observed for CDDP16-EVAc, CDDP33-EVAc and CDDP50-EVAc with less than 10% of loaded cisplatin released within the first two days, Figure 3. The release fit several possible models including a primary burst followed by a near-linear phase, Figure 3A. As the release rate *in vitro* is only an estimate for *in vivo* release, a zero-order release rate was calculated for initial comparison. Following the burst release phase, all devices released cisplatin at an almost constant rate for the 90 day period examined. A slower release rate was achieved with CDDP16-EVAc as compared to CDDP33-EVAc and CDDP50-EVAc. Following the initial rapid release phase, the amount of cisplatin being released per day was 0.38 ± 0.15 $\mu\text{g/day}$ for CDDP16-EVAc devices, 14.80 ± 4.0 $\mu\text{g/day}$ for CDDP33-EVAc devices, and 46.90 ± 10.0 $\mu\text{g/day}$ for CDDP50-EVAc devices ($R^2 = 0.93, 0.97, \text{ and } 0.98$, respectively).

In addition to the empirical zero-order release rate, a second model, Equation 1, was used to compare the three devices, Figure 3B [22,26]. In this model, the release was treated as being diffusion through a porous material of known geometry. The amount of drug release at a particular time, M_t , relative to the total drug delivered, M_∞ , was related to the thickness of the device, L , and the apparent diffusion coefficient, D_{app} . Similar to the empirical near-linear model, the diffusion coefficient for the CDDP16-EVAc devices was $5.8 \pm 0.3 \times 10^{-12}$ cm^2/s , the CDDP33-EVAc devices was $2.7 \pm 0.8 \times 10^{-10}$ cm^2/s , and the CDDP50-EVAc devices was $1.3 \pm 0.5 \times 10^{-9}$ cm^2/s ($R^2 = 0.89, 0.94, \text{ and } 0.97$, respectively).

$$\frac{M_t}{M_\infty} = 4\sqrt{\frac{D_{app}t}{L^2\pi}} \quad (1)$$

Unfortunately, the last data points of the release curve for CDDP33-EVAc and CDDP50-EVAc devices are an average of two points due to unexpected loss of samples when the isothermal bath malfunctioned.

***In vitro* Bioactivity**

When the activity of each device was assessed in the burst period, the released cisplatin actively inhibited proliferation of the cervical cancer lines, Figure 4A. During the zero order release phase (after one week of release), the activity was less for most cell lines than the activity of devices in the burst period at 48 hours, Figures 4B, and even following 72 hours of treatment, Figure 4C. Based on the delivery rate from the devices and cisplatin activity (LC_{50}) toward the cervical cancer cells, the amount of cisplatin estimated to be released during the zero order release phase over a period of 48 hours was determined. Cells were then incubated for 48 hours with free cisplatin at the concentration equivalent to the amount released from the devices in this period. There was excellent agreement between the expected cisplatin activity and actual activity observed for the cisplatin-loaded devices, Figure 5.

Morphological changes in each of the three cell lines on treatment with the devices and controls confirm the toxic effect observed in the proliferation results. Ca Ski cells, Figure 6, tended to circularize with cisplatin treatment and great decrease in cell number was apparent. HeLa cells, Figure 7, changed morphology from spindle-shaped to circular with cisplatin or device treatment in addition to clear, marked decrease in cell number. C-33A cells, Figure 8, did not greatly change in morphology upon cisplatin or device treatment, but clear decrease in cell number and subtle morphologic change was apparent with increased cisplatin treatment.

Discussion

The objective of this study was to develop an insertable polymeric system which would release cisplatin at a steady rate and be effective *in vitro* against cervical cancer. EVAc devices are known to release biologically active compounds, including small molecules and macromolecules, such as growth factors and antibodies, for prolonged periods at controlled rates [16,27,28]. EVAc matrices have been approved for human use in the uterus [16,29,30] and in the eye [31] and are considered biocompatible. In addition to commercially available products, EVAc implants have been widely researched in animal models for brain implants [32], cardiovascular disease [33], and diabetes [34] due to the sustained, long-term release and excellent compatibility.

To achieve the wide-ranging delivery spectrum, be it small molecule, protein or poly(nucleic acid) drug, both matrix-type and reservoir-type systems are utilized for controlled drug delivery from EVAc-based devices. Reservoir-type devices are widely recognized as exceptional delivery systems due to the reproducible-constant release rate that is obtained following an initial equilibration period that is predominantly determined by the concentration of the compound in the reservoir, [35]. Matrix-type devices also allow control of drug release for extended periods, although the kinetics of release are dependent upon dissolution of the compound, diffusion of the compound in the pores, relative compound composition, compound particle size, and other factors [22,36]. For compounds that do not partition readily into the polymer matrix, such as proteins, the particle size and density dictate the structure of the pore network and thus the release pattern [22]. For molecules that have low permeability into the polymer network and slow diffusion in the polymer, the release rate is expected to be dominated by the diffusion in the porous network at early time points. Regardless of matrix-type or reservoir-type device, drug release can be extended, reproducible, and useful in therapeutic modalities that allow local placement of the device.

Due to accessibility of the site of cervical cancer, local delivery of chemotherapeutics in polymeric implants can usher a new area in the treatment and management of cervical intraepithelial neoplasia. The CDDP-EVAc devices developed in this study have a relatively simple method of preparation. The methods used to produce the matrix-type systems in this study are different from those used in production of current ring-based devices which are extruded, i.e. the devices are formed by solvent evaporation; however, the final device maintains many similar properties and extrusion may be a possible mechanism of final device preparation.

Based on chemical and physical characterization, it was observed that the devices were matrix type controlled delivery systems with cisplatin crystals being heterogeneously dispersed throughout the polymer matrix. As confirmed by EDS, cisplatin was impregnated within the matrix without undergoing any significant dissolution in the polymer matrix itself. Since the polymer was inert and little dissolution of the drug can be observed in the polymer matrix, dissolution and diffusion through pores was expected to be the primary transport mechanism [22,36]. A well connected network of pores permits diffusion of cisplatin from the polymer matrix and the increase in release rate with incorporation supports this hypothesis. CDDP16-EVAc devices released less drug per time interval as compared to CDDP33-EVAc devices, and the CDDP33-EVAc devices released less drug per time interval compared to CDDP50-EVAc devices. This can be explained by a substantially less interconnected network of pockets (pores due to drug crystal) formed in CDDP16-EVAc devices than CDDP50-EVAc or CDDP33-EVAc devices. Based on the near-linear or diffusion-controlled release models observed over the first 90 days, the devices are expected to last for at least 90 days with controlled delivery in patients at rates similar to, but not necessary equivalent to, these values. In each case, only a fraction of the drug is released within this three-month period. It is expected that the lower incorporation of cisplatin results in a non-infinite pore structure that would poorly fit the diffusion-controlled model while the higher incorporation amounts produced infinite pore structures that are adequately modeled.

These extended periods of steady drug release are in concurrence with commercially available EVAc devices [37]. This timeframe is also in concurrence within the current device retention times being examined for contraceptive vaginal rings [38]. In trials, contraceptive rings are retained in the vaginal canal for up to a year without negative events. Within the year, the devices can be removed and replaced at will as long as the device is replaced within 2 hours. The perception is that these devices are safe, convenient and reliable [38,39]. It is hoped that the developed system will be examined to determine retention and adverse events, but the devices are still at the pre-clinical delivery stage showing delivery efficiency. However, delivering the drug is not sufficient for chemotherapy, the drug must be delivered in concentrations that are cytotoxic (active) to the cancer.

The drug released from the devices retained activity *in vitro* as was observed from the proliferation inhibition for three cervical cancer cell lines. Ca Ski, HeLa and C-33A cell lines were studied due to the differential response to cisplatin, Table 1, based on their HPV status [40]. Ca Ski cell lines contain evidence of HPV-16 as well HPV-18 sequences [41], HeLa cell lines contain HPV-18 sequences [41]. The C-33A cell line is a HPV negative cell line [42]. HPV-16 and HPV-18 are of interest because these viral strains are responsible for greater than 70 % of all cervical cancer cases [43]. As expected from published results, Ca Ski, HPV-16 and HPV-18 positive, cells were most resistant to the antiproliferative effects of cisplatin followed by HeLa, HPV-18 positive cells and C-33A, HPV negative cells.

Since all cells were sensitive to cisplatin at different levels, an initial *in vitro* assessment of the devices could be conducted that would be expected to be predictive of the outcome for different HPV status cancers. As expected, a 48 hour incubation of cells with the devices during the

'burst' phase release was more active than 48 hour incubation with devices during the zero order release phase (Figures 4A and 4B). Also, as expected, for all the sets of experiments performed, no significant toxicity was observed associated with EVAc devices containing no cisplatin and surprisingly an increase in cell growth was observed. In concurrence with cisplatin activity for a given cell line, the response of the cell line to the devices varied with the C-33A being the most sensitive followed by HeLa and Ca Ski (Figures 6B and 6C). Knowing the LC₅₀ values of cisplatin and the amount of drug being released per day at steady state, the response of the cell lines for a 48 hr incubation period with the devices could be predicted with fair amount of accuracy.

These results confirm that CDDP-EVAc devices can be used to incorporate an active therapeutic molecule and release the molecule for extended periods. Although only 90 days were examined for the release, EVAc-based devices continue to release drug at a predictable rates for extended periods that is predicted by the linear release in the early time points. These devices can be easily produced using conventional systems. The expense of production will be low, and therefore, the devices can be very applicable and affordable to the populations that most need treatment options for this devastating disease. In addition, the ease of administration and replacement are in agreement with the necessities of populations that are underserved by medical professionals, i.e. no medical professionals are needed to administer the treatment. These studies are very preliminary in the preclinical examination of the system; however, the results are very promising for the future application of the devices. Future studies are planned to examine the *in vivo* activity of these devices and to determine the local and systemic concentrations of the chemotherapeutic following placement.

Conclusion

Development of a locally insertable device for intra vaginal delivery of chemotherapeutic agents in cervical cancer can be a paradigm shift in the treatment of cervical cancer especially where recurrence of cancer is a main cause of death. The CDDP-EVAc delivery system developed in this manuscript has an advantage of being easy to use and administer. Devices can be easily and inexpensively manufactured since vaginal rings which are formulated on similar lines are commercially available for contraception. The inexpensive and stable nature of the devices is ideal for developing countries where cervical cancer is of most concern. CDDP-EVAc devices deliver an immediate high dose of cisplatin upon insertion that continues at a constant rate for extended periods. The released cisplatin is as active as expected for the amounts that are being released. From these results, it is expected that the CDDP-EVAc ring devices can be of clinical significance in the treatment of cervical intraepithelial neoplasia.

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Figure 1. Representative (A) attenuated total reflectance-Fourier transform infrared and (B) Raman spectra of poly(ethylene-co-vinyl acetate), cisplatin, and of the interior of CDDP16-EVAc, CDDP33-EVAc and CDDP50-EVAc devices.

Figure 2.

Representative scanning electron micrographs of lateral sections of a CDDP50-EVAc device at (A) low magnification (scale bar = 500 μm) and (B) higher magnification (scale bar = 25 μm) showing the two clear phases. (C) EDS spectra at the two indicated locations indicated in the inset scanning electron micrograph (not Au-Pd coated), polymer matrix (■, I) and CDDP crystal (■, II). The EDS spectra is the average of three scans on different single spots in the area indicated.

Figure 3.

In vitro cisplatin release (A) as a function of time and (B) as a function of the square root of time from (▲) CDDP16-EVAc, (◆) CDDP33-EVAc, and (■) CDDP50-EVAc devices. Data is presented as the mean $\pm$ standard deviation of three independent experiments.

Figure 4.

(■) CaSki, (■) C-33A, and (■) HeLa cervical cell survival after 48 hour treatment with devices showing (A) burst-phase release, (B) 48 hour treatment with devices showing zero-order release, and (C) 72 hour treatment with devices showing zero order release. Data is presented as the mean of the survival relative to the untreated control $\pm$ standard deviation of three independent experiments.

Figure 5.

(A) Ca Ski, (B) C-33A, and (C) HeLa cell survival following 48 hr treatment with the treatment indicated (actual) and treatment with cisplatin at the amount equivalent to that released from the device in 48 hours (expected). CDDP and EVAc are presented only as actual results for treatment with EVAc devices (no CDDP) and CDDP at the calculated LC50 for that cell type. Data is presented as the mean of the survival relative to the untreated control $\pm$ standard deviation of three independent experiments.

Figure 6. Morphology of Ca Ski cells following (A) no treatment and treatment with (B) EVAc, (C) CDDP at the LD50, (D) CDDP16-EVAc, (E) CDDP33-EVAc and (F) CDDP50-EVAc. (scale bar = 100 μ m).

Figure 7. Morphology of He La cells following (A) no treatment and treatment with (B) EVAc, (C) CDDP at the LD50, (D) CDDP16-EVAc, (E) CDDP33-EVAc and (F) CDDP50-EVAc (scale bar = 100 μ m).

Figure 8. Morphology of C-33A cells following (A) no treatment and treatment with (B) EVAc, (C) CDDP at the LD50, (D) CDDP16-EVAc, (E) CDDP33-EVAc and (F) CDDP50-EVAc (scale bar = 100 μ m).

Table 1

Cisplatin activity (LC_{50}) against the HeLa, Ca Ski and C-33A cervical cancer cell lines when administered for varying periods.

Time (hr)	Ca Ski	CDDP LC_{50} (μ M) C-33A	HeLa
24	186.0 $\pm$ 40.4	30.79 $\pm$ 5.39	32.05 $\pm$ 4.23
48	64.33 $\pm$ 4.74	4.26 $\pm$ 0.91	6.93 $\pm$ 0.02
72	37.84 $\pm$ 6.16	2.02 $\pm$ 1.01	5.43 $\pm$ 0.56