Experimental Study

Assessment of localized therapeutic effect for prolonged release biodegradable implants loaded with 5-Fluorouracil on rats with induced liver cancer

Ahmed Fathy Hanafy, PhD a,*, Mokhtar I. Yousef, PhD b, Sana A. Mortada, PhD c and Abdulla M. Molokhia, PhD d

a Department of Pharmaceutics and Pharmaceutical Technology, School of Pharmacy, Taibah University, Almadinah Almuhannwarah, Kingdom of Saudi Arabia
b Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University, Alexandria, Egypt
c Department of Industrial Pharmacy, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt
d European Egyptian Pharmaceutical Industries, Alexandria, Egypt

Received 19 March 2013; revised 29 August 2013; accepted 2 September 2013

Abstract

Objectives: The objective of this research was to assess ability of biodegradable implantable 5-Fluorouracil dosage forms, to maximize the regional exposure of the drug while limiting its systemic toxicity in rats with induced liver cancer.

* Corresponding address: Assistant Professor of Pharmaceutics and Pharmaceutical Technology, School of Pharmacy, Taibah University, AlMadina, Kingdom of Saudi Arabia.
E-mail: drafathy@gmail.com (A.F. Hanafy)

Peer review under responsibility of Taibah University.
Liver cancer is one of the major lethal malignancies worldwide. The main histological subtype is hepatocellular carcinoma (HCC), which is derived from hepatocytes, the predominant epithelial cell type in the liver. Surgery is the only curative modality for HCC, if the cancer is localized. Standard chemotherapy approaches and external beam radiation for treatment of HCC have little utility. The liver and its surrounding viscera are very sensitive to radiation. Thus, the dose required to achieve a decrease in tumor size will kill the host due to liver failure. Similarly, chemotherapy as a primary or adjuvant approach has had no benefit. A good reason for not using standard chemotherapy is that liver cancer patients often have marked decrease in their ability to metabolize drugs and thus often experience significant systemic toxicity.

The poor efficiency of conventional anticancer drugs can be explained by the special solid tumor structure like liver and brain tumors. This would prevent anticancer drugs from reaching the tumor area in required concentration, and the doses of anticancer drug have to be increased consequently. However, toxicity is a very limiting factor for most of the chemotherapy agents. Therefore, there is a need for a therapy, that would be able to concentrate drugs close to the tumor site, and avoid their too large distribution. Local administration of biodegradable polymeric drug delivery systems can present a promising method that gives clinicians the opportunity to deliver large dose of chemotherapeutic drugs close to the tumor site for a prolonged period of time. This would help to augment the therapeutic effect of chemotherapy together with minimizing the possible toxic side effect. 5-Fluorouracil (5-FU) was selected as a model chemotherapeutic drug as it is one of the widely used anticancer drugs for liver cancer treatment.

This research work aimed to assess ability of implantable dosage forms in liver cancer region, manufactured using biodegradable polymers like Polyactic acid (PLA) and loaded with 5-Fluorouracil, to concentrate 5-FU in liver cancer region, and decrease the systemic exposure and side effects in rats with induced liver cancer.

### Materials and Methods

#### Materials

PLA was prepared as previously described. Methyl nitroso Urea (MNU), Diethyl nitrosamine (N-Nitrosodiethylamine) (DEN) No: 7566 and N-(2-fluorenyl)acetamide (AAF) A7015 were bought from SIGMA, USA. Carbon Tetrachloride (CCl4) was bought from ADWIC Co. (Elnasr Pharmaceutical Chemical Company), Egypt. Ethanol 95%, Formalin 10%, Dimethyl Sulfoxide (DMSO) (high purity grade), corn oil, sterile sodium chloride solution 0.9%, heparin (5000 IU) ampoules (Nile Co.), Nylon sutures, Catalar anesthesia, Betadine surgical solution, Ciprofloxacin Hydrochloride powder (Cipla Co.), Cataflam drops, 5-Fluorouracil powder (Beckmann Chemikalien KG), trichloroacetic acid (TCA) (Analar grade), ethyl acetate (Analar grade), sodium hydroxide (high purity grade), Potassium dihydrogen phosphate (Analar grade).

#### Preparation of 5-FU biodegradable implants

PLA injection molded implant formulation containing an equivalent amount of 12 mg 5-FU per 120 mg implant and coated with 200 mg PLA and which produced in vitro prolonged release for approximately 45 days as has been previously described. PLA implants were sterilized using gamma radiation at 25 kGy under air prior to implantation.

#### Induction of liver cancer in Wistar rats

Two different protocols were used to induce liver cancer in Wistar rats weighing 100 g each. The first protocol included injecting rats with 60 mg/kg MNU dissolved in sodium citrate 0.01 M pH 6 intraperitonealy (ip injection), on day 26 the injected rats received CCl4 2 mL/kg dissolved in corn oil by gavage. While, the second protocol included injecting rats with 200 mg/kg DEN (Diethyl nitrosamine) dissolved in 0.9% sodium chloride intraperitoneally (ip injection), on days 18,19,20, and 22 a daily gavage dosage 20 mg/kg of 2-AAF dissolved in DMSO and corn oil, (1:29 V/V) was given to Wistar rats. On day 21, 2 mL/kg of CCl4 in corn oil was given to Wistar rats by gavage. On days 42 and 72, 6 rats were examined from control and treated group using the tests discussed below to decide if successful induction of liver cancer occurred. Rats were examined physically for any rat skin color change and for presence of rat fur sheds. Rats were then
sacrificed, liver sections were H&E (hematoxylin and eosin) stained and examined by a pathologist to confirm induction of cancer after the time specified in the protocol and another 3 rats were sacrificed after 1 month at the specified time in the protocol to confirm the previous results. The following tests were performed on the sacrificed rats: body weight to liver weight ratio, complete blood count (CBC) on pooled blood from 3 rats, and liver functions tests (ALT – alanine aminotransferase and AST – aspartate aminotransferase) on pooled blood from 3 rats.

Operating on Wistar rats for initiation of therapy using prolonged release implant loaded with 5-FU[10]

All instruments used during the operation were sterilized by autoclaving. All the areas of work and cages were disinfected using 70% ethyl alcohol. Each rat was given 0.3 mL of ketamine and within 2 min the rats were anaesthetised. Hair was removed from the area of operation; this area was then disinfected using Betadine surgical solution. A clean cut was performed using a sterile scalpel beginning from lower than the thorax, till the liver is clear while cutting both the skin and abdominal cavity.

Twelve rats with induced liver cancer were used during this experiment. Biodegradable implant containing 12 mg 5-FU per 120 mg PLA as previously described,[5] was inserted in the largest lobe of rat liver which is the left lateral lobe and after 2 days they were behaving normally as control group.

Tests performed on rats with induced liver cancer

Rats were examined and then sacrificed at selected time intervals to be in accordance with in-vitro studies performed previously at 5, 15, 30, 45 and 60 days.[6]

At each time interval, rats were examined for any physical changes especially rat skin color and rat hair. Each rat was weighed using digital weighing balance and the rat body weight was recorded (BW). After sacrificing each rat, the liver was dissected fully and separated from any other viscera then weighed and the liver weight was recorded (LW). Liver to body weight percentage (LB%) was then calculated using the following equation:

\[ LB\% = \frac{LW(g)}{BW(g)} \times 100 \]

Excised liver from the control and treated groups were fixed using 10% formalin then dehydrated and embedded in paraffin wax blocks. They were then cut into 4 μm thick sections followed by deparaffinization of the sections, staining by hematoxylin and eosin stain (H&E stain) and examined under light microscope. Pooled blood from 3 sacrificed rats from each group was collected in heparinized epindorph tubes then the following tests were performed: complete blood count (CBC), liver enzymes tests (ALT and AST), and measurement of the amount of 5-FU in blood at the different time intervals using HPLC.

Method for detecting and quantification of 5-Fluorouracil in rat blood after insertion of implantable device in rat liver

An HPLC method for quantification of 5-FU in rat blood was used,[11] after performing a calibration curve and after testing drug losses due to binding to protein and other blood components. Three millilitre from pooled blood was heparinized in polypropylene tubes. Heparinized blood was centrifuged at 10,000g for 10 min immediately after collection to separate plasma. Plasma proteins were then precipitated by addition of trichloroacetic acid 2 M (5 μl of TCA per 100 μl of plasma), and after centrifugation (10,000g, 5 min) plasma samples were then stored at −20 °C. 5-Fluorouracil was extracted from plasma samples by ethyl acetate: 100 μl of phosphate buffer (0.5 M, pH 8) and 6 mL of ethyl acetate were added to 500 μl of plasma, after vigorous shaking for 5 min and centrifugation (4000g, 3 min), the organic phase was collected. Organic phase was evaporated using nitrogen at 55 °C, and then samples were reconstituted with 100 μl of KH₂PO₄ 0.01 M, pH 4, and 5-Fluorouracil concentration in the sample determined by HPLC using UV–Vis absorbance detector and computing integrator. The stationary phase used is Spherisorb ODS2, C18, 5 μm (25 cm × 0.46 cm). The eluent was KH₂PO₄ 0.01 M, pH 4. The flow rate was set at 1 mL/min and the detector wavelength was 266 nm.

For calibration, standards of 0.1–100 μg/mL 5-FU in phosphate buffer (1 mM, pH 7.4), as well as drug-free plasma pooled with known amounts of 5-FU, to obtain a 5-FU concentration between 0.01 and 100 μg/mL, were used in a manner similar to the previously used procedure. For calculating the circulating blood volume of rats, the recommended mean value of 64 ml/kg of body weight was used.[12]

Non-compartmental methods can be used to determine certain pharmacokinetic parameters without deciding on a particular compartmental model.[13] The basic calculations are based on:

\[ LB\% = \frac{LW(g)}{BW(g)} \times 100 \]
on the area under the plasma concentration versus times curve (AUC). The AUC can be calculated by the trapezoidal rule.

**Scanning electron microscope pictures used to study degradation of the 5-FU loaded PLA implants at different time intervals**

Surface morphology for implants before and during in vivo release was evaluated by scanning electron microscopy (SEM). Retrieved implants were dried and sputter-coated with gold under vacuum using an electron beam (10 kV) to prepare the sample for Scanning Electron Microscope. The implant surface was viewed under low (10.6×) and high (342×) magnifications and representative photomicrographs were obtained. The pore morphology and pore size distribution of the samples were investigated by SEM at 1000× magnification. SEM pictures of the retrieved implants were compared with other retrieved implants at different time intervals of in vivo study.

**Results**

The tests performed after induction and during treatment of liver cancer at each testing time interval on Wistar rats are discussed as follows:

### Rat physical examination

Physical examination of control group rats and rats group after induction of liver cancer is presented in Table 1. Physical examination of rats showed no change in skin/fur color of rats and no fur shed in both groups. These results signify that induction of cancer protocol had no effect on rat’s physical examination.

After initiation of chemotherapy by placement of implant in rat liver slight yellow skin coloration in all treated rats from this group was noted after 15 days together with an increase in hair loss when rats were held by hand for physical examination but this diminished after 30 days and completely disappeared after 2 months as shown in Table 1.

### Liver weight to body weight percentage

Liver to body weight percentage for control group rats and rats with induced cancer group are presented in Table 2. The presented results show that there was slight increase in body weight percentage after performing induction of liver cancer protocol. These results are in agreement with the findings of Attarchi et al. The results of liver to body weight percentage for rats with induced liver cancer after placement of implant ranged from 3% to 4.2% which when compared with

---

**Table 1: Physical examination results for rats in control group and liver cancer induction group.**

<table>
<thead>
<tr>
<th>Description item</th>
<th>Control</th>
<th>After induction</th>
<th>After 5 days of implantation</th>
<th>After 15 days of implantation</th>
<th>After 30 days of implantation</th>
<th>After 2 months of implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin color</td>
<td>White</td>
<td>White</td>
<td>White</td>
<td>Slight yellow</td>
<td>Very slight yellow</td>
<td>White</td>
</tr>
<tr>
<td>Hair loss</td>
<td>–</td>
<td>–</td>
<td>White</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

(-) No hair loss, (+) Slight hair loss, (++) increased hair loss.

**Table 2: Liver weight to body weight percentage results for rats in control group and liver cancer induction group.**

<table>
<thead>
<tr>
<th>Description item</th>
<th>Control</th>
<th>After induction</th>
<th>After 5 days of implantation</th>
<th>After 15 days of implantation</th>
<th>After 30 days of implantation</th>
<th>After 2 months of implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>160</td>
<td>167</td>
<td>231</td>
<td>210</td>
<td>235</td>
<td>298</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>5.7</td>
<td>6.1</td>
<td>7</td>
<td>8.1</td>
<td>8.65</td>
<td>7.5</td>
</tr>
<tr>
<td>Liver/body weight%</td>
<td>3.56</td>
<td>3.7</td>
<td>3</td>
<td>3.9</td>
<td>3.7</td>
<td>2.5</td>
</tr>
</tbody>
</table>

**Table 3: Complete blood picture and liver enzyme results for control rats and rats after induction of liver cancer.**

<table>
<thead>
<tr>
<th>Blood test results</th>
<th>Limit</th>
<th>Control</th>
<th>After induction</th>
<th>After 5 days of implantation</th>
<th>After 15 days of implantation</th>
<th>After 30 days of implantation</th>
<th>After 2 months of implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>11.3–14.1</td>
<td>14.1</td>
<td>11.5</td>
<td>11.5</td>
<td>13.1</td>
<td>18.1</td>
<td>16.1</td>
</tr>
<tr>
<td>RBC (10⁶/µL)</td>
<td>3.5–9.5</td>
<td>4.8</td>
<td>3.8</td>
<td>3.8</td>
<td>4.2</td>
<td>5.3</td>
<td>5.2</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>33–47</td>
<td>43</td>
<td>34</td>
<td>34</td>
<td>39</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>Platelets (×1000/µL)</td>
<td>120–240</td>
<td>185</td>
<td>220</td>
<td>220</td>
<td>210</td>
<td>160</td>
<td>85</td>
</tr>
<tr>
<td>WBC (10³ cells/µL)</td>
<td>1.6–9.3</td>
<td>7.3</td>
<td>10.4</td>
<td>10.4</td>
<td>9.1</td>
<td>6.1</td>
<td>3.1</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>NLT 80%</td>
<td>94%</td>
<td>96%</td>
<td>96%</td>
<td>96%</td>
<td>90</td>
<td>94</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>13–37%</td>
<td>6%</td>
<td>4%</td>
<td>4%</td>
<td>4%</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>NMT 15%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AST (units/mL)</td>
<td>70–160</td>
<td>78</td>
<td>113</td>
<td>100</td>
<td>98</td>
<td>119</td>
<td>144</td>
</tr>
<tr>
<td>ALT (units/mL)</td>
<td>40–120</td>
<td>95</td>
<td>104</td>
<td>106</td>
<td>116</td>
<td>121</td>
<td>174</td>
</tr>
</tbody>
</table>
Liver to body weight percentage for control group was 3.56% and to rats group with induced liver cancer 3.7% showed that there were no clear variability between these groups.

*Complete blood picture and liver enzymes tests*

Complete blood picture (CBP) and liver function blood enzyme tests (AST and ALT) performed on control group and group with induced liver cancer are presented in Table 3. The results showed increase by about 40% in AST enzyme and very slight increase of about 10% in ALT enzyme in the group of induced liver cancer compared to control group. These results might signify that there was disturbance in liver functions caused by the induction of liver cancer.

Liver enzymes test results for rats after placement of implant in rat liver with induced liver cancer showed gradual increase in liver enzymes levels with time when compared to rats group with induced liver cancer before implantation, AST level showed approximately 27% increase and ALT level showed approximately 67% increase after two months of implantation.

*Liver tissue sections examination*

Liver biopsy examination was performed for hematoxylin and eosin (H&E) stained sections from rats of control group or group with induced liver cancer using light microscope. Examination of liver biopsy of control group in Figure 2a and b...
showed normal liver lobules with central vein and radiating hepatic columns. Examination of liver biopsy of rats group with induced liver cancer in Figure 3a and b using DEN protocol showed well differentiated hepatocellular carcinoma. The hepatocytes showed loss of lobular organization and the nuclei were hyperchromatic and some of them contained prominent nucleoli. Examination of liver biopsy after using MNU protocol which was also tried for induction of malignant changes, showed moderate increase in the diameter of nuclei. The lobular architecture was preserved. No frank malignant changes were seen. From the above results, the DEN protocol was found to be successful in achieving induction of malignant changes and accordingly this protocol was used throughout the research to induce cancer in Wistar rats used in this study.

Pathological reports on implants at the different time intervals revealed small areas of necrosis in Figure 4 which signify slight therapeutic effect in the area of implantation in liver after 5 days. Figure 5 for liver sections of rats sacrificed after 15 days showed increase in area of necrosis, while liver sections for rats sacrificed after 30 days in Figure 6 shows the largest area of necrosis signifying maximum therapeutic effect. Liver sections in Figure 7 for rats sacrificed after 60 days does not show increase in necrosis of liver tissue.

Detection and quantification of 5-Fluorouracil in rat blood after insertion of implantable dosage forms in rat liver with induced liver cancer

The amounts of 5-Fluorouracil detected in pooled rat blood samples taken at different time intervals after placement of implant in rat liver with induced liver cancer are presented in Table 4. AUC presents the proportionality to the total amount of drug eliminated from the body and hence absorbed. The total amount of 5-FU released in rat blood was calculated by comparing the total area under curve (AUC) which is 1250 μg h/mL for single 12 mg IV bolus injection for a 150 g rat containing an approximate amount of blood 9.6 mL, with the calculated total AUC from 5-FU for implants at the different time intervals. AUC for 5-FU calculated by trapezoidal method was 168.48 μg h/mL and the average total amount of drug released in rat blood was calculated to be approximately 1.6 mg for 2 months.

Studying implants degradation through scanning electron microscope pictures

SEM pictures for implants explanted from rat livers at different time intervals were used to study in vivo degradation with time. Figure 8 shows SEM pictures for the coated implants before placement in rat liver which showed smooth surface and the cut section showed smooth surface with slight protrusions which might represent 5-FU particles. Figure 9 shows SEM pictures for implants explanted after 15 days in liver rat, these pictures showed smooth external implant surface with small cracks together with liver tissues attached to the external surface. These tissues might indicate compatibility of the polymer implant matrix with the liver tissues, moreover, few perforations on the surface might indicate that 15 days of implantation did not cause strong polymer degradation. Cut section in Figure 9 shows the presence of few perforations in coat on implant surface and much more perforations in the inside of implant which might be attributed to drug dissolution, polymer hydrolysis and degradation due to water diffusion inside the implant.

Figure 10 shows SEM pictures for implants after 30 day implantation in rat liver. The surface view showed corrugated

<p>| Table 4: Area under the curve and amount of 5-FU detected in blood after placement of implant. |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Amount of 5-FU (μg/mL rat blood)</th>
<th>Average rat weight (g)</th>
<th>Area under curve for amount of drug in blood (μg h/mL)</th>
<th>Total amount of drug released in rat blood (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.56</td>
<td>120</td>
<td>168.48</td>
<td>1.6</td>
</tr>
<tr>
<td>15</td>
<td>0.12</td>
<td>145</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>158</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0.013</td>
<td>190</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 8: SEM picture for coated implant surface view on the left and cut section view on the right before in vivo experiment.

Figure 9: SEM picture for coated implant surface view on the left and cut section view on the right after 15 days implantation in rat liver.

Figure 10: SEM picture for coated implant surface view on the left and cut section view on the right after 30 days implantation in rat liver.

Figure 11: SEM picture for coated implant surface view on the left and cut section view on the right after 60 days implantation in rat liver.
The protocol used for induction of liver cancer was selected to be devoid of partial heptectomy (PH) step as operation on rats for two times, one time for induction of cancer and one time for implantation therapy, might increase the number of deaths of rats and might have a negative effect on therapeutic efficiency of the 5-FU loaded PLA implant.

During rat physical examination after placement of implant in rat liver with induced liver cancer, transient skin yellow coloration was noted which might be due to 5-FU localized chemotherapy on the liver causing transient chemical hepatitis that might cause disturbance in ALT, AST and bilirubin. Slight hair loss was noted, this signifies that some systemic release of 5-FU occurred which has caused this mild dermatologic toxicity. These mild side effects showed that this implant dosage form offered sustained release of drug because of appearance of side effects only after 15 days and persistence of these side effects for 30 days.

A. F. Hanafy et al. 19

Complete blood picture results for induction of liver cancer showed similar findings as reported by Attarchi et al. The CBP test results were within normal reported limits except for an increase of about 40% in WBCs’ in the induced liver cancer group compared to control group. These results might be caused by the inflammatory process initiated by the induction of liver cancer. The complete blood picture results for rats after placement of implant in rat liver with induced liver cancer showed increased mylosuppression with time. Mylosuppression is a condition in which bone marrow activity is decreased, resulting in fewer red blood cells, white blood cells, and platelets and it is usually a side effect of some cancer treatments like 5-FU. Mylosuppression due to the implant loaded with 5-FU had the greatest impact on WBC’s and platelets, while it had a minimal or almost no effect on RBC’s, Hb and Ht%. Though the WBCs were decreased, its count remained within acceptance limits. Platelets have undergone depression that was lower than recommended limits after 2 months only.

The mylosuppression caused by the implant seems to be minimal compared to the serious mylosuppression reported in references which can be attributed to the small amount of systemic release of 5-FU from the implant. Liver enzymes test results after placement of implant in rat liver with induced liver cancer are in conformance with researches reporting that 5-FU localized injection in the liver is usually accompanied with chemical hepatitis causing elevation of liver enzymes. But in our case the liver enzymes elevations were less than two-fold that of control which is considered as slight chemical hepatitis.

Liver tissue sections examination after placement of implant in rat liver with induced liver cancer showed increase in localized necrosis area of liver tissue around the implant with time due to 5-FU killing cancer cells. The above results may signify that implants present a promising dosage form for localized liver cancer therapy.

Quantification results of 5-Fluorouracil in rat blood after insertion of implantable dosage forms in rat liver with induced liver cancer show that a very low dose was released in rat blood when compared to the amount of drug loaded per implant which was 12 mg, and when compared to the recommended therapeutic dose of 5-FU injected by IV per day which was 1.5 mg for a 150 g rat with an average blood volume of 9.6 mL. These results signify the benefit of using implants in decreasing systemic release of drug and accordingly the associated side effects compared to IV injection. These results are conforming to in vivo study performed by He et al. that investigated the toxicities, biodistribution and anticancer effect of 5-Fluorouracil controlled release implant on Walker 256 carcinosarcoma cells in Wistar rat livers. Results of this study showed that 5-FU was able to improve effectiveness and minimize the systemic toxicity associated with current systemic therapy of 5-FU.

Studying implant degradation using scanning electron microscope pictures for implants explanted after 15 days, 30 and 60 days in rat liver as seen in Figures 9–11 respectively, show increased attachment of liver tissues to implant surface and extensive degradation with time. Attachment of liver tissues to implants might indicate compatibility of the polymer implant matrix with the liver tissues.

Conclusions

PLA coated injection molded implants loaded with 5-FU presented a promising dosage form for liver cancer treatment. It showed sustained drug release in rats for about two months as well as localized drug release in liver with a perimeter of 0.3 to 0.6 cm around the implants. Moreover, implants showed very low systemic drug release; only approximately 13% of drug dose reached the blood over 2 months. The localized necrotic areas seen in the rat liver biopsies after 5 days of implantation indicated localized 5-FU therapeutic effect.

Conflict of interest

None declared.

Acknowledgment

The authors would like to thank the European Egyptian Pharmaceutical Co. (Pharco Corporation) for their support and Prof. Dr. Mona Marie, head of the Tissue Engineering Department, Faculty of Dentistry, Alexandria University, for access to the scanning electron microscope at her facility.

References


