Controlled Release of Bioactive Doxorubicin from Microspheres Embedded within Gelatin Scaffolds

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Introduction

According the Worldwide Health Organization, more than 1.2 million people will be diagnosed with breast cancer this year worldwide. Numerous clinical trials have confirmed therapeutic equivalence for mastectomy and breast conserving surgery as well as adjuvant breast irradiation to decrease the local recurrence rate. Radiation therapy however, is expensive, time consuming, and increases cosmetic deformity of the surgery.

The objective of this study was to develop a construct that would be placed in the breast at the time of surgery (following lumpectomy or segmental mastectomy). The construct would locally deliver a controlled sustained release of doxorubicin for up to four weeks, temporarily maintain the structure of the native breast, and eventually promote tissue ingrowth. This approach could serve as alternative to adjuvant breast irradiation. As such, doxorubicin-encapsulated microspheres were fabricated and incorporated into gelatin scaffolds during gelation. The anti-tumor effect of doxorubicin and released doxorubicin from both the microspheres, and microspheres embedded within gelatin was assessed. The release profile was also examined under in vitro conditions.

Materials and Methods

Scaffold Preparation. The doxorubicin (Dox) was encapsulated in PLGA (75:25) using a double emulsion/solvent evaporation method. Gelatin scaffolds were prepared by crosslinking a 3% gelatin solution with 0.5% glutaraldehyde solution. Microspheres were added before complete gelation of the gelatin. The scaffolds were washed with a 0.1M glycine solution to react any residual glutaraldehyde. The microspheres and scaffolds were characterized using SEM.

Dox release studies. Dox microspheres and gelatin scaffolds containing dox microspheres were placed in microcentrifuge tubes with 1mL PBS and incubated at 37°C. At desired time points, the tubes were centrifuged, the supernatent was collected, and the dox concentration was analyzed spectrophotometrically.

In vitro studies. The effect of Dox on 4T1 tumor cells was determined by treating the cells with various concentrations of Dox from 0 to 0.017mg/mL. Viable cells were then quantified using the MTT assay. The effects of Dox were also determined qualitatively using a propidium iodide (PI) assay. The bioactivity of the released Dox was assessed using a Transwell basket assay. Viable cells were quantified using CyQuant®.

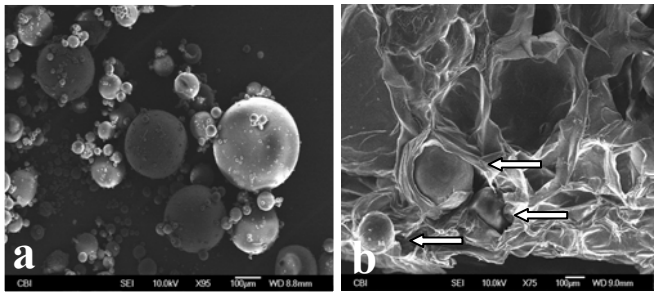

Figure 1. a) SEM of Dox-loaded microspheres, and b) microspheres embedded within a gelatin scaffold.

Results and Discussion

The microspheres and gelatin scaffolds were characterized using SEM. The microspheres exhibited an average diameter of 73.55 μm and the average porosity of the gelatin was 46.1% (Figure 1). The release of Dox was maintained over 30 days from both the microspheres and gelatin scaffolds (Figure 2a). Over days 5-16 the cumulative release was significantly higher from the Dox microspheres alone ($p<0.05$).

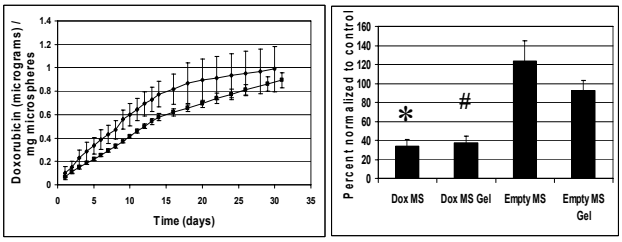

Figure 2. a) Cumulative release of Dox from microspheres (-♦-) and gelatin (-■-), and b) the viable cell number (normalized) after treatment.

The 4T1 cells responded in a dose-dependent manner to Dox. As the concentration of Dox increased, the number of viable cells decreased. This pattern was seen both quantitatively (MTT) and qualitatively (PI). The Dox released from microspheres and gelatin scaffolds both significantly reduced the number of viable cells ($p<0.001$) (Figure 2b).

Conclusions

We have designed a novel, controlled delivery system by incorporating PLGA microspheres within gelatin constructs. We demonstrated the bioactivity of the released Dox from the constructs. The implantable construct shows promise as an alternative solution to local recurrence of breast cancer and breast deformity due to tumor resection.