Tamoxifen Loaded Polylactide Beads for Breast Tissue Engineering Following Lumpectomy

Feng Xu, Katherine Neser, Karen JL Burg
Institute for Biological Interfaces of Engineering, Clemson University

Statement of Purpose: To minimize the chance for recurrence, a lumpectomy involves the removal of tumor plus a margin of surrounding healthy tissue. In 2000 we disclosed the concept of and successful *in vitro* testing of an injectable tissue engineered composite for use as a reconstructive option following mastectomy[1]. The composite is comprised of cellular and/or acellular beads housed in a gel delivery vehicle. We proposed that, unlike the classic, "continuous" tissue engineering scaffolds, the composite might be delivered in a minimally invasive manner and, because it was comprised of smaller units (i.e. beads), tissue infiltration (of both bulking and vascular tissue) was feasible. Although *in vivo* studies have confirmed efficacy of the design using "small" volumes of these composites, the efficacy of large volume composites remains in question. To support large volumes of tissue, the composite will likely need to release angiogenic factors; however, these factors, while promoting a vascular network and new tissue, can simultaneously induce further cancerous growth. Thus, we have focused on incorporating anti-cancer therapeutics into the tissue engineered composite, specifically for release from the beads. The purpose of this work was to determine the effect of tamoxifen, released from polylactide beads, on both human mesenchymal stem cells (hMSCs) and MCF-7 breast cancer cells.

Methods: Polylactide (weight average M_W = 212,000 Da; Dow Chemical) beads containing tamoxifen were manufactured. Briefly, two grams of polylactide (PL), with or without 100 milligrams of tamoxifen (Sigma-Aldrich) (TAM), were dissolved in 20 ml of methylene dichloride and beads were formed as previously described[2]. The beads were immersed in a 6% hexane-diamine/2-isopropanol solution, and maintained in a 60°C water bath for 10 minutes. Subsequently, the beads were washed extensively with de-ionized water and dried under reduced pressure at room temperature. The aminolyzed beads were immersed in 1% glutaraldehyde solution at room temperature for 3 hours. After a second extensive wash, the beads were immersed in a 0.3% collagen/3% acetic acid solution (1:1) at 4°C for 24 hours with occasional agitation. The beads were filtered and washed again, then separated using 300-450 μm sieves. Attenuated Total Reflection - Fourier Transform Infrared (ATR-FTIR) spectroscopy was used to examine the beads surface before and after collagen coating. ATR spectra were collected using a Thermo-Nicolet Magna 550 FTIR spectrometer equipped with a Thermo Spectra-Tech Foundation Series Diamond ATR. Samples and background collections were performed using 16 scans at a resolution of 4 cm^{-1}. Data was analyzed and ATR and baseline corrections were applied using the Thermo Ominc E.S.P. version 6.1a. software. The beads were sterilized with ethylene oxide and placed under house vacuum for a minimum of 24 hours before use.

MCF-7 and hMSCs (Poietics™), respectively, were seeded at $2.5\times10^4/cm^2$ in 24-well plates overnight, and then treated with TAM for 4 days. MCF-7 was also treated with beads for 8 days, either directly with beads or indirectly with beads in transwell inserts. The beads were immersed in Eagle's Minimum Essential Medium at 37°C for various times (0, 5, 15 and 30 days) before use in co-culture. The cell viability of MCF-7 was assayed on days 4 and 8 using an AlamarBlue assay.

Results:

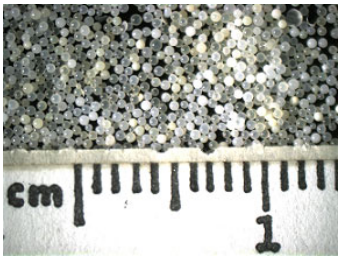

Figure 1. PL-TAM beads

ATR-FTIR analysis of the beads verified that collagen was successfully coated on the surface of the beads. TAM loading rate was approximately 3% and the encapsulation efficiency was approximately 7%. The cumulative percent of TAM released from beads was approximately 30%. The IC_{50} of TAM for MCF-7 cells was 6 μM, while for hMSCs was about 13 μM. The viability of MCF-7 cells, in both direct and indirect cultures, was also significantly inhibited by beads (Table 1).

Table 1. Effect on MCF-7 cell viability of beads (ratio of OD value in beads from different immersion days to OD value in beads of Day 0 immersion)

	Immersion Day	*Day 4*	*Day 8*
Direct	0	1±0	1±0
	5	0.90±0.09	0.93±0.02**
	15	0.84±0.03**	0.87±0.05**
	30	0.78±0.06**	0.83±0.04**
Indirect	0	1±0	1±0
	5	0.82±0.14*	0.93±0.05*
	15	0.60±0.23*	0.81±0.11*
	30	0.45±0.18**	0.74±0.12*

*P<0.05, **P<0.01 compared to Day 0 immersion

Conclusions: The results suggest that the PL-TAM beads may be useful as cell carriers in injectable tissue engineered composites, while providing an anti-cancer therapeutic.

References:

1. Burg KJL, Austin CE, Culberson CR, Greene KG, Halberstadt CR, Holder WD, Jr, Loebsack AB, Roland WD, *Trans 2000 World Biomater Congress*, Kona, HI, 5/2000.
2. Brown RQ, Mount A, KJL Burg, *J Biomed Mater Res*, 74(1):32-9, 2005.

Acknowledgements: This research was funded by DoD Era of Hope Scholar Award BC044778 and NSF EFRI 0736007.