Tamoxifen Loaded Beads for Concurrent Breast Cancer Therapy and Tissue Engineering

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Statement of Purpose: Breast cancer ranks second among cancer deaths in women. Lumpectomy procedures to remove cancerous tissue are followed by plastic and reconstructive surgery to repair the soft tissue defects resulting from the tumor resection. Since lumpectomy may not remove all cancerous cells, neoadjuvant chemotherapy is often prescribed for these patients. The objective of this study was to evaluate select components of an injectable tissue engineered composite [1] that would be potentially useful for both breast tissue reconstruction and for neoadjuvant chemotherapeutic applications.

Methods: Injectable sized polylactide (PL) beads loaded with tamoxifen (TAM) were manufactured using an emulsion-solvent evaporation technique. Briefly, TAM and PL were dissolved in dichloromethane. The solution (TPL) was then added to deionized water containing 0.5% (w/v) polyvinyl alcohol (PVA), and continuously stirred at a rate of 400 rpm with a mechanical stirrer. The TPL/water ratio was 1:5. Agitation was continued to evaporate the organic solvent. The resulting beads were collected using membrane filtration, washed with deionized water, and dried under reduced pressure. The bead surfaces were aminolyzed with a hexanediamine /isopropanol solution; the beads were then washed with water and dried. The aminolyzed beads were moistened in 1% glutaraldehyde solution to transfer the amino groups to CHO groups. After washing, the beads were surfaced coated with collagen. The excess collagen was filtered and the beads were washed again. PL beads without TAM were also manufactured using the same procedure and used in subsequent studies as control samples.

TAM-loaded PL beads were moistened in PBS before use in cell culture experiments. Beads were transferred to wells of a 12-well plate; hMSCs were seeded at 2.1×10^4cells/cm^2 into each well and co-cultured with the beads for 8 days. To identify the effect of the TAM-loaded PL beads on human breast cancer cells (MCF-7), beads were either cultured in a well of a 12-well plate directly with cells, or placed in transwell inserts above and without contact of the cultured cells. MCF-7 cells were seeded at 2.1×10^4cells/cm^2 and cultured for 8 days. Cell viability was assayed on Days 4 and 8 using an alamarBlue® assay. The cells grown on the bead surfaces were observed with LIVE/DEAD® assay.

Post-confluent hMSCs were differentiated to adipocytes according to the manufacturer's protocol. The cells were treated with various concentrations of TAM for 3 days beginning at the first induction cycle. Samples of the cells were also cultured with TAM-loaded PL beads placed in transwell inserts or with PL beads used as control samples. At the end of the 25 day culture period, cell samples were assayed to measure total triglyceride content.

The hMSCs were co-cultured with beads with and without MCF-7 cells. First, hMSCs were cultured to confluence. MCF-7 cells were seeded in transwell inserts and allowed to attach overnight. TAM-loaded PL beads or PL beads were added to the inserts and placed into each well. All inserts were renewed at the end of each cycle of induction/maintenance of hMSCs. At the end of the 25 day culture period, cell samples were assayed to determine the fatty acid composition of the cells.

Results: The viability of hMSCs cultured directly with TAM-loaded PL beads was not negatively affected when evaluated at Day 4 and Day 8. MCF-7 cell viability, however, when cultured either directly with TAM-loaded PL beads in the well or indirectly with beads in a transwell insert, was significantly inhibited ($p<0.05$).

Triglyceride levels showed that TAM alone at various concentrations (1~ 4 μM) and TAM-loaded PL beads had no effect on hMSCs adipogenic differentiation.

When differentiated hMSCs were co-cultured with TAM-loaded PL beads with or without MCF-7 cells within the insert, no difference in the fatty acid composition of the mature adipocytes, when compared to that of control samples (no MCF-7 cells and no beads within insert), was observed (Table 1). Results for hMSCs co-cultured with PL beads with or without MCF-7 cells are not shown here.

Table 1. Fatty acid composition (%) of the hMSCs co-cultured with TAM-loaded PL beads with or without MCF-7 cells in transwell inserts.

Insert / *Fatty acid*	*Control*	*TAM-loaded PL beads*	*MCF-7 + TAM-loaded PL beads*
Palmitic	34.39±3.97	33.80±4.58	34.75±2.97
Stearic	1.84±1.44	2.27±2.25	3.45±3.90
Oleic	11.29±1.50	11.61±2.39	12.00±4.16
Linoleic	0.62±0.70	0.64±0.68	0.65±0.59
Total	56.30±1.01	55.57±1.11	59.73±4.33

Conclusions: TAM-loaded PL beads had no effect on hMSCs adipogenic differentiation. TAM-loaded PL beads may be useful as a cell microcarrier for injectable tissue engineered composites, while providing an anti-cancer therapeutic for neoadjuvant chemotherapy.

References:

[1] Burg et al., "A Novel Approach to Tissue Engineering: Injectable Composites", Trans 2000 World Biomaterials Congress, Kona, HI, 5/2000.

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