# Tannic Acid Crosslinked Collagens and Potential for Breast Tissue Engineering

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**Statement of Purpose:** Current breast reconstruction options including artificial implants and autologous skin flaps are often associated with problems such as scarring, infection, and donor site morbidity. As a result, a current goal of tissue engineers is the development of an injectable tissue engineered device that can be employed following a lumpectomy or mastectomy to permanently restore breast tissue aesthetics.[1] However, it is important to recognize that there are several factors, both environmental and genetic, that initially led to tumor formation in the breast locale. It is possible that this "tumor microenvironment" persists even after removal of a tumor and that new cells introduced into the region could be susceptible to transformation.[2] Recent studies demonstrated that tannins allow proliferation of healthy cells while inducing a caspase-dependent apoptotic pathway in malignant cells.[3] Tannic acid, a plant derived polyphenol, has been suggested for use as a crosslinking agent in collagen-based injectable devices. The long-term goal of this study is to develop a biodegradable tissue engineering scaffold with anti-tumor capabilities.

**Methods:** The response of MCF-7 (human breast epithelial adenocarcinoma) and D1 (mouse bone marrow stromal stem cell) cell lines was first evaluated after incorporating tannic acid directly into the culture medium. Cells seeded in 24-well plates were allowed to attach for 48 hours. Tannic acid (Sigma) was introduced into the medium in concentrations of 1, 10, 25, and 50mg/L. An Alamar Blue (Biosource) assay conducted at 24, 48 and 72 hours after tannic acid incorporation established cell metabolic activity values. Using aseptic techniques, two-dimensional collagen membranes were constructed using a modified method described by R.B. Vernon and coworkers.[4] Dry membranes were crosslinked in a range of tannic acid solutions. Rehydrated membranes were characterized using scanning electron microscopy (Hitachi S-3400N) at 4000x magnification. MCF-7 and D1 cells were seeded on collagen membranes at 40% confluency. Cells were imaged on membranes using fluorescence microscopy.

**Results/Discussion:**

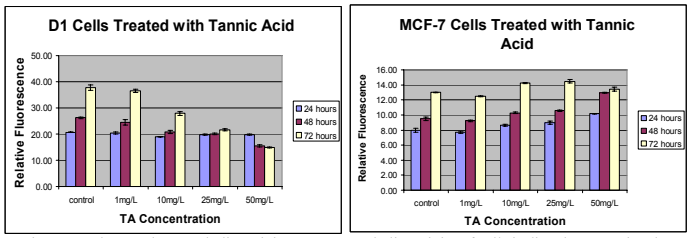


Figure 1. Alamar Blue metabolic activity assay. Metabolic activity of cells is directly proportional to relative fluorescence reading.

Metabolic activity of D1 cells increased over time in tannic acid solution concentrations up to 10mg/mL indicating that cells continued to proliferate. Decreased metabolic activity at higher tannic acid concentrations was likely due to cytotoxicity and cell necrosis. A trend in elevated metabolic activity of MCF-7 cells in higher tannic acid solution concentrations could be the result of apoptosis induction. Several other techniques including fluorescence staining and quantification of caspase activity will be required in order to fully understand the concentration-dependent effects of tannic acid on these cell lines.

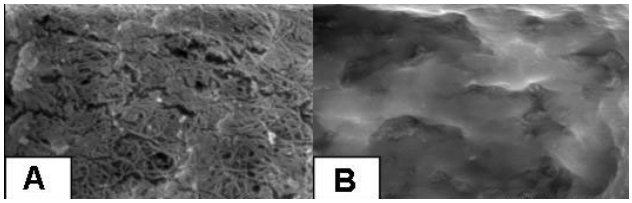


Figure 2. Scanning electron microscopy. 4000x magnification. (A) Collagen membrane crosslinked in 0.1% tannic acid solution. (B) 1.0% tannic acid solution.

Membrane surface was altered as a result of tannic acid crosslinking. The fibrillar collagen network was not apparent in the membrane crosslinked with a 1.0% tannic acid solution (right) when compared to the membrane crosslinked with a 0.1% solution (left).

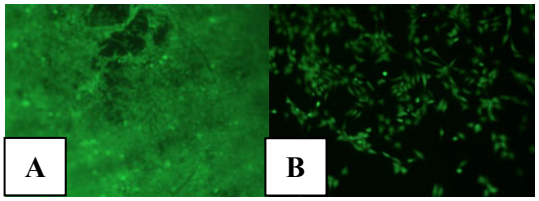


Figure 3. Healthy D1 cells seeded onto collagen membranes depicted by green fluorescence. 32x objective. (A) Control collagen membrane (uncrosslinked). (B) 0.02% tannic acid solution.

D1 viability indicated by green fluorescence was more apparent on control membranes (left) when compared to cell viability on a crosslinked membrane (right). It is likely that an optimal crosslinking solution exists that will allow proliferation of D1 cells while still inducing apoptosis in malignant MCF-7 cells. Following the determination of this concentration, the mechanisms controlling the anti-proliferative activity of tannic acid can be studied further.

**Conclusion:** Collagen-based scaffolds crosslinked in appropriate tannic acid solutions have potential in breast tissue engineering. Carefully produced, these scaffolds can provide a growing surface for healthy cells while triggering apoptotic events in malignant cells.

**References:**

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[4] Vernon, R.B. *et al*. *Biomaterials*. 2005. Apr;26(10): 1109-1117.

**Acknowledgements:** This work was supported by a Department of Defense Era of Hope Award and a National Science Foundation Presidential Early Career Award for Scientists and Engineers.