Progress report — Three-dimensional *in vitro* Models of Prostate Cancer

Executive summary

The goal of our proposed research is to construct a three dimensional (3D) human prostate cancer tissue *in vitro* for the study of cancer biology and rapid screening of anti-cancer drugs. The specific aims of this research are:

1. Fabricate tissue engineering scaffolds for 3D culture of human prostate cancers
2. Build 3D models of human prostate cancer *in vitro*
3. Characterize the 3D prostate cancer models at cellular and molecular levels

In the first stage of the project, we have made significant progress in Aims 1 and 2 (see the first progress report). In the past year, we have made significant progress towards all 3 specific aims. We have 1. tested the constructs in a perfusion system; 2. evaluated the culture of endothelial cells, and 3. built tubular scaffolds to better mimic *in vivo* tumor development. From these results we concluded that 1. in order to mimic the *in vivo* cancer development and build a true 3D model *in vitro*, perfusion is needed; 2. prostate cancer cells will not withstand the shear stress of direct perfusion, thus co-culture with endothelial cells will be needed; and 3. a tubular scaffold with an endothelialized lumen and cancer cells grow in the pores around the endothelialized tube will best mimic the physiological conditions. We have submitted part of the results reported in the last and this progress report for publication in the ***Journal of Biomedical Materials Research*** in November 2006. The financial support of GCC was acknowledged in the manuscript. We plan to submit a R21 application to NCI in March 2007.

Report

I. 3D Culture of Human Prostate Cancer Cells under Perfusion

In order to increase the size of the 3D cancer constructs, we perfused the constructs with culture medium to increase the delivery of nutrients and removal of wastes. The perfusion did not alter the overall morphology of the LNCaP cells, however, no increase in cell cluster size was observed (Fig. 1 A). In fact, the cell number was lower than those of the static culture. this was likely caused by the detachment of LNCaP cells due to increase shear stress (Fig. 1 B). Indeed, floating cells were observed in the circulating medium. Thus we decided to use endothelial cells to shield the cancer cells from shear stress as is the case for *in vivo* systems.

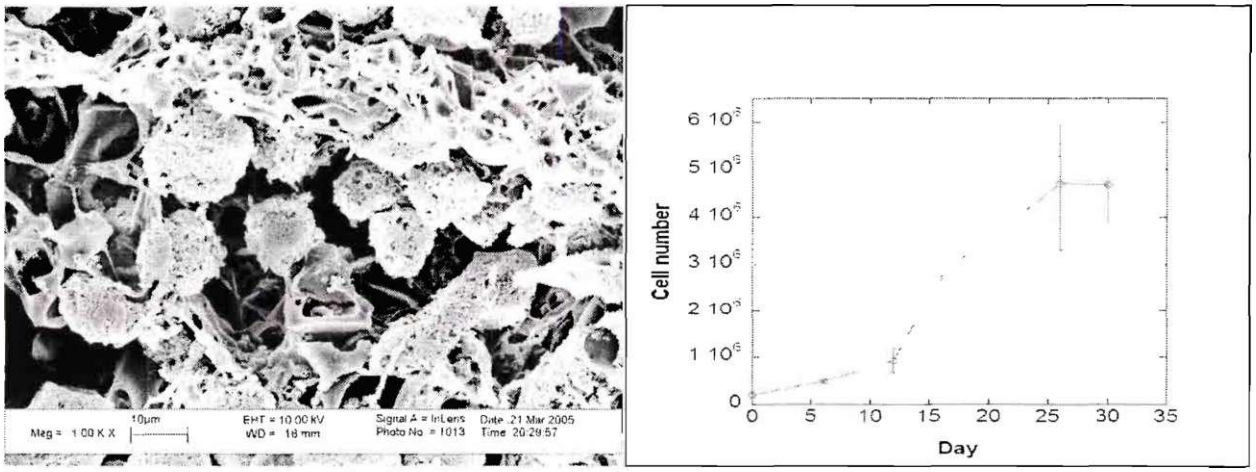
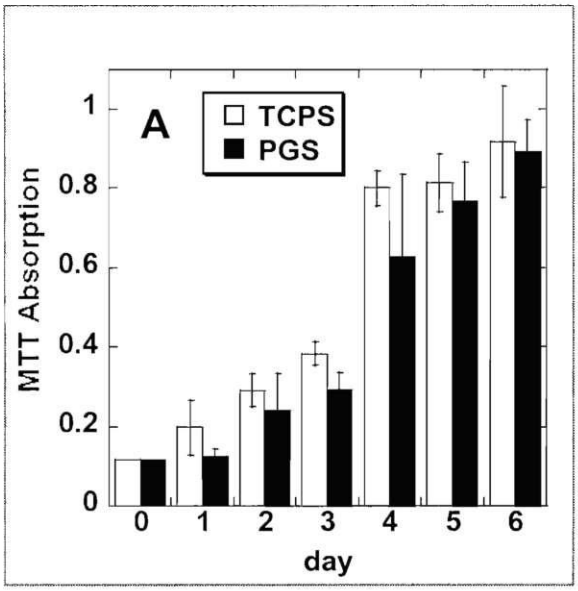


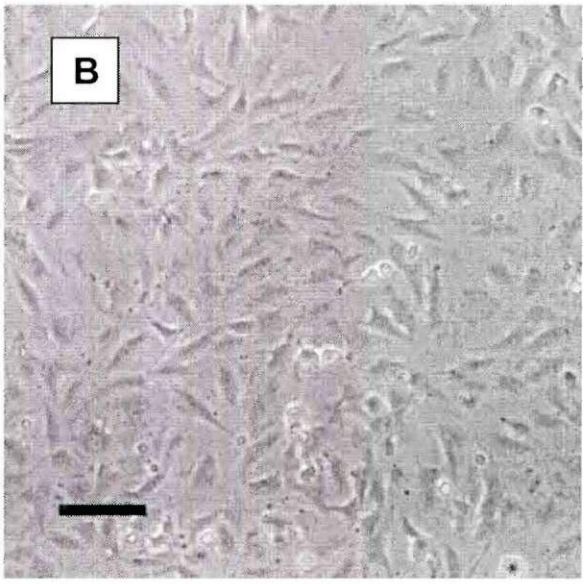
Fig. 1. (A) SEM micrograph of the cross-section of a perfused construct based on LNCaP Cells and PGS scaffolds. (B) MTT assay of LNCaP cells grown on scaffolds under constant perfusion. The cell number was lower than static culture conditions.

This new fabrication process allowed us to produce PGS scaffolds with controlled pore size, porosity, and well interconnected pores. The porosity and pore size of scaffolds can be easily controlled by the ratio of salt and PGS, and salt particle sizes. This process has three advantages. First, the scaffold has homogeneous distribution of pores and polymer, which is more suitable for culturing cells in three dimensions. Second, micropores in the walls of the micropores increases interconnectivity of the pores and are beneficial to cell-cell communication, cell interaction, and mass transport. Third, the scaffold can be directly fabricated into specific shape with mold.

II. Endothelialization of the PGS Scaffolds

To investigate the feasibility of endothelialize PGS scaffolds, we cultured primary endothelial cells (ECs) on PGS surfaces. The results indicated that EC can adhere and proliferate well on PGS, and the cell phenotype is well maintained as shown by the positive vWF-staining (Fig. 2).





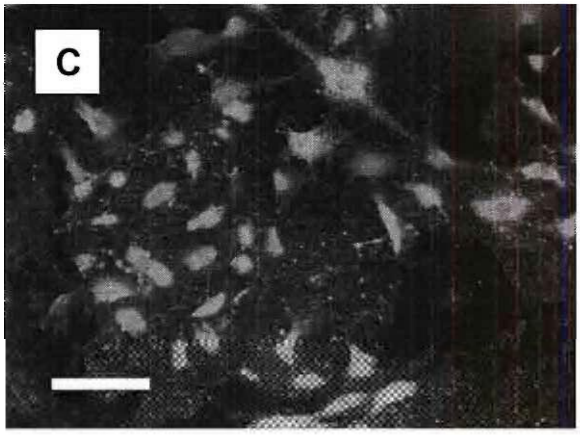


Fig. 2. The proliferation of primary endothelial cells on PGS was similar to that on TCPS. (A) The number of metabolically active cells was indicated by the absorption of MTT at 570 nm. (B) ECs (P14) cultured on PGS-coated cover slips exhibited typical cobblestone morphology. 160X; scale bar = 200 μm. (C) the ECs expressed vWF (red) in cytoplasmic granules on PGS surface, 200x, scale bar = 200 μm.

III. Fabrication of tubular PGS Scaffolds

To explore coculture of ECs and LNCaP cells, we redesigned our approach to use PGS tubular scaffolds. The plan is to first endothelialize the lumen of the tube, then LNCaP cells will be seeded in the pores of the wall. This shields the tumor cells from direct shear stress while enhancing the nutrient delivery and waste removal mimicking the *in vivo* tumor development. We have successfully fabricated PGS tubular scaffold using a strategy illustrated in Fig. 3. the use of heat-shrink tubing as a mandrel is necessary as we found the mandrel removal to be extremely challenging with conventional Teflon mandrel due to the large aspect ratio.

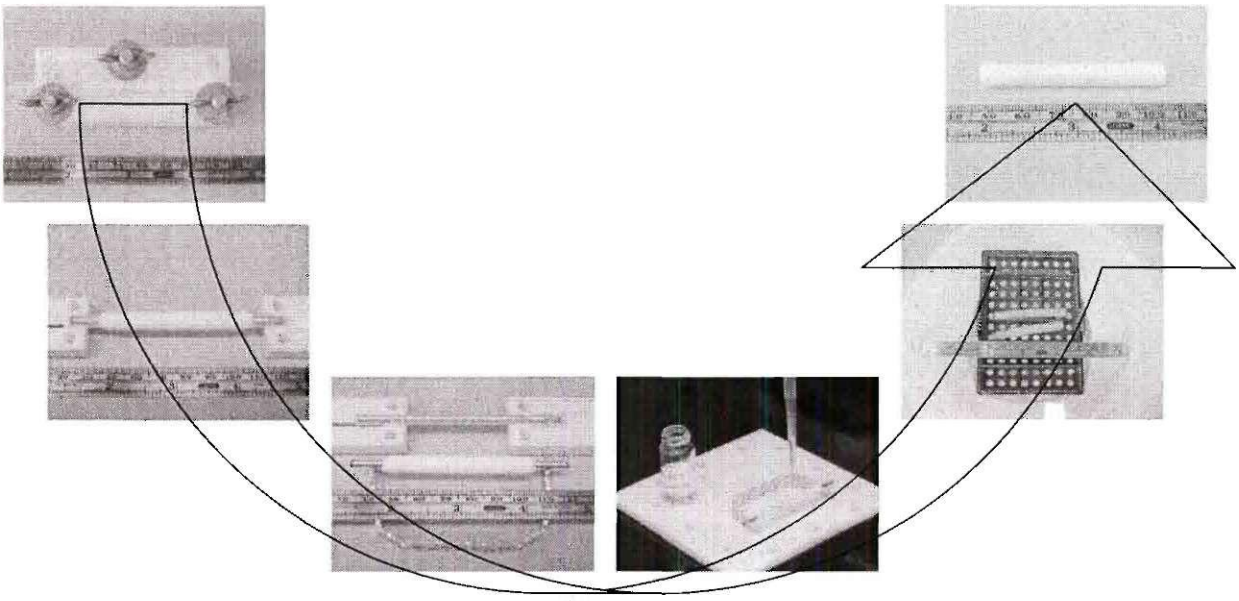


Fig. 3. The fabrication of the seamless tubular PGS scaffold succeeded after numerous iteration. The six sub-figures depicts 1. the Teflon mold with a heat-shrink tubing as the mandrel; 2. the salt template that is removed from the outer-mold; 3. the free hanging salt template after the mandrel was shrunk at 150 °C; 4. PGS solution was added to the salt template; 5. PGS was cured at 150 °C, and salt template was dissolved in water; and 6. the dry scaffold after water removal by lyophilization.

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Old Project Number: E-17-633
P/S Project Number: 1256633
Rev #: 4 Mod #: ADMIN
Current Revision Completion Date: 24-AUG-04

Doc ID #: 66780
Sponsor: WHITAKER FOUNDATION/
Award Document: GRANT
Contract #: GRANT DTD 001120 **Contract thru:** GTRC
Project Director(s):
PDPI LE DOUX, JOSEPH M
Unit: BME **Initiation Date:** 01-JAN-2001
Termination Perf Date: 30-JUN-2005 (Performance)
Termination Rpts Date: 30-JUN-2005
Project Title: QUANTITATIVE ANALYSIS OF TARGETED RETROVIRUS INFECTION

Rev. No (1)	Description of Deliver-able	Deliv Id No (2)	Period	Covered	Due Date to Sponsor (3)	Copies reqd	Date Mailed (4)	Sat*
3	FINAL RE-PORT	1	01-JAN-2001	30-JUN-2005	30-JUN-2005	1	05-JAN-2006	N

Total Count:1
* Satisfied

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E-17-633
#1

1/5/2006

FINAL REPORT on Whitaker Foundation sponsored research project (TF-03-0021)

Quantitative Analysis of Targeted Retrovirus Infection (PI: Joseph M. Le Doux, PhD)

I. ABSTRACT

Why the project was undertaken

Recombinant retroviruses are one of the most commonly used means to transfer genes in gene therapy clinical trials because they can permanently integrate a therapeutic gene into the chromosomal DNA of target cells, resulting, in principle, in a long-term cure. Unfortunately, the current generation of recombinant retroviruses often are not able to both efficiently and selectively transfer genes to cells. Given the large number of important clinical applications, there is significant motivation to improve the efficiency and selectivity of retroviral-mediated gene transfer. Successful gene transfer with retroviruses requires the completion of a complex series of steps that begins with binding of the virus to the cell, transport of the bound virus to a location where its cellular receptors are expressed, followed by an interaction between the envelope proteins of the virus and their cellular receptors that leads to fusion of the virus with the cell and its entry into the cytoplasm. The mechanisms by which these steps of infection occur are poorly understood. It is important that we understand the mechanism by which retroviruses accomplish these early steps of infection because such knowledge could lead to the development of more efficient and selective strategies for genetically modifying cells in human gene therapy protocols, or for blocking infection by wild-type, pathogenic retroviruses. Therefore we undertook the Whitaker project in order to make progress towards our *long-term goal*, which is to establish engineering-based strategies for improving the functionality of recombinant retroviruses and lentiviruses for the purposes of human gene therapy.

What research was performed

We had two major findings. First, we found that the route that retroviruses take within a cell is controlled by the receptors and co-receptors that they interact with when they bind to the cells they are infecting. In addition, the probability that they will successfully infect a cell is a function of the intracellular pathway they take. We showed this be developing a novel experimental system that was composed of several cell lines that expressed retrovirus receptors that were identical in the portion that interacts with viruses, but different in the portion that controlled how the receptors were trafficked within the cell. We engineered retroviruses to bind to these receptors, and studied the effect of receptor trafficking on virus trafficking and infection. Our second major finding was that retroviruses activate intracellular signals when they bind to cells. Specifically, they activate rac1, a molecule that coordinates the formation of actin filaments within the cells. We showed that viruses induce this signaling via proteoglycans on the surface of the virus and via integrins on the surfaces of the cells. We are currently investigating the role that rac1 activation has in retrovirus transduction.

Why the project was important

The project was important because it showed that the molecules that are on the surface of retroviruses dictate how the viruses will traffick within the cell, and whether or not they induce intracellular signaling. Our findings suggest that by manipulating the surface properties of

retroviruses, we should be able to control the signals they induce within cells, and how the viruses are transported within the cells, and thereby control the outcome of gene transfer, either making it more efficient, more selective, or both. The ability to do this will enable us to develop safer, more effective human gene therapy protocols.

II. PAPERS and POSTERS from project

A. Papers

Krishna, D., and Le Doux, JM, "Murine Leukemia Virus Particles Activate Rac1 in HeLa Cells", in preparation

Krishna, D., Raykin, J, and Le Doux, JM, "Targeted Receptor Trafficking Affects the Efficiency of Retrovirus Transduction", Biotechnology Progress, 21(1): 263-273 (2005)

B. Posters

Krishna, D., Le Doux, J.M. (2002). An Experimental System for the Analysis of Targeted Retrovirus Transduction. *The Second Joint Meeting of the IEEE Engineering in Medicine and Biology Society and the Biomedical Engineering Society, Houston, Texas.*

Krishna, D., Le Doux, J.M. (2003). An Experimental System for the Analysis of Targeted Retrovirus Transduction. *The 7th Annual Hilton Head Workshop, ET2003: Engineering Tissues, Hilton Head, S.C.*

Krishna, D., Coburn L., Sheng J., Rubin D.H., Hodge T.W., Le Doux, J. M. (2003) The development of an experimental system to investigate the role of host cell factors in retrovirus transduction. *The 6th Annual American Society for Gene Therapy Meeting, Washington D.C.*

Krishna, D., Coburn L., Sheng J., Rubin D.H., Le Doux, J. M. (2003) An experimental system to investigate the role of host cell factors in retrovirus transduction. *The Biomedical Engineering Society Annual Meeting, Nashville, TN*

Krishna, D, Le Doux, J.M. (2004). Receptor trafficking affects the efficiency of retrovirus transduction. *The 7th Annual American Society for Gene Therapy Meeting.*

Krishna, D, Le Doux, J.M. (2004). Receptor trafficking affects the efficiency of retrovirus transduction. *2004 Annual Meeting, American Institute of Chemical Engineers.*

Krishna, D, Le Doux, J.M. (2005). Targeted receptor trafficking affects the efficiency of retrovirus transduction. *The 8th Annual American Society for Gene Therapy Meeting.*

Krishna, D, Le Doux, J.M. (2005). Retrovirus particles activate cell signaling protein Rac1 upon binding to target cells. *Georgia Life Sciences Summit.*

III. GRANTS

Continuing support for Whitaker project
None, but I am currently preparing an R01 that is based on the Whitaker project data.

New projects funded by major grants
CAREER: Engineering Recombinant Lentiviruses for Cystic Fibrosis Gene Therapy
National Science Foundation, 2002-2007

Induction of Stem Cells to Adopt an Endocrine Fate
National Institutes of Health (R21), 2002-2004

Rapid Virus Concentration and Purification for Enhanced Throughput and Sensitivity of Molecular and Diagnostic Virus Assays
Coulter Foundation, 2005-2006