CHARACTERIZING THE SEEDING DISTRIBUTION OF CELLS AND MICROSPHERES WITHIN TISSUE ENGINEERED BLOOD VESSELS

Alexander D. Josowitz¹, Jeffrey T. Krawiec¹,4, Justin S. Weinbaum¹,4
David A. Vorp¹,2,3,4,5
University of Pittsburgh

INTRODUCTION
Cardiovascular disease (CVD) is one of the leading causes of death in the United States, contributing to approximately a third of all deaths annually [1]. A variety of cardiovascular diseases exist, each of which displays different symptoms, though many common forms often involve damage to blood vessels and impeded blood flow. Atherosclerosis, as a common example, involves the build up of plaque along the arterial wall, causing wall thickening, blocking blood flow and leading to downstream tissue death. These effects can be especially damaging in the coronary arteries, where heart muscle can lose its blood supply – often contributing to the mortality of CVD.

Because of the variable nature of CVDs, a multitude of treatments exist that aim to assuage the different causes and symptoms. One such treatment, pursued by the Vorp Lab Group has been directed toward developing an implantable graft, called a tissue-engineered blood vessel (TEBV) [2]. The TEBV, in its current manifestation, is constructed of two components: a scaffold and adipose-derived stem cells (ADSCs). The scaffold, made of polyester urethane urea (PEUU), acts as a biodegradable, porous supporting structure that allows for cells to invade and begin reconstruction of the damaged artery [3]. ADSCs serve a regenerative function, releasing soluble factors to the surrounding cells to promote cell growth and migration in the nearby native vessel [4],[5]. If implemented, such a graft would allow for personalized treatment and minimal tissue rejection for patients suffering from CVD.

Unfortunately, the use of human cells introduces a variety of complications for TEBV development. Diabetic and elderly ADSCs have been shown to have reduced regenerative function in comparison to ADSCs from young, healthy donors, which could prevent effective implementation of a cell-based TEBV in two high-risk patient populations [6]. Therefore, a new paradigm is necessary to allow for at-risk groups to be treated.

A new technology, coined “Artificial Stem Cells”, could be a promising alternative to cell-based therapies. Artificial stem cells would incorporate polyactic glycolic acid (PLGA) microspheres, which have been shown to be capable of encapsulating and releasing drugs for tissue regeneration [7]. These microspheres could be used to encapsulate the same soluble factors released by ADSCs and replace their function within a TEBV. This would allow for a mass-producible and more universal treatment for CVDs.

There are multiple preliminary factors that must be taken into account prior to development of this new paradigm for TEBVs. One such factor is whether the process used to infuse ADSCs into scaffolds, cell seeding, can be used effectively with microspheres to ensure uniform scaffold distribution. Cell seeding is defined in this case as infusing cells through a scaffold while rotating and maintaining vacuum pressure to ensure uniform cell permeation in the longitudinal, circumferential, and radial dimensions of the scaffold [8]. Because microspheres’ composition and flexibility differs from those of cells, it is conceivable that such a method would not function properly. Concurrently, in order to overcome the possibility of manually analyzing cell and microsphere distributions, an automated image-processing tool would be required.

OBJECTIVE
The objective of this study is twofold. First, the proposed study aims to determine whether the aforementioned cell seeding process functions as effectively with microspheres as it has been shown to function with cells by comparing seeding percentages of scaffold sections in the three cylindrical dimensions. Secondly, this study aims to develop an automated cell counting tool for use in analyzing TEBV cross-sections.

SUCCESS CRITERIA
The cell seeding process should work similarly with both ADSCs and microspheres, exhibiting no significant differences (p<0.05) between cell and microspheres distributions in the three cylindrical dimensions. The image processing tool should be able to identify and count both cells and microspheres and be consistently able to approximate manual cell counts.

METHODS
ADSCs from three young, healthy female donors were cultured in ADSC growth media (DMEM+1:1 DMEM/F12 containing 10% FBS and antibiotics), replaced every two days until confluence. PLGA Microspheres (5-15 µm diameter) were obtained and resuspended in PBS. The ADSCs and microspheres were each seeded into six PEUU scaffolds, producing three TEBVs for each group. The cell-based TEBVs were dynamically cultured in spinner flasks for 48 hours to allow for cell attachment and then fixed in paraformaldehyde. The TEBVs were cut into three longitudinal sections for circular cross-sectioning using a cryomicrotome. The cells were
then stained with DAPI to identify cell nuclei. Multi-colored images at 10x magnification were obtained using an epifluorescent microscope with NIS Elements software. The ImageJ stitching toolbox was used to produce mosaic cross-sectional images for each scaffold. These longitudinal images were segmented to produce three radial sections and four circumferential quadrants. An ImageJ tool was developed, using built-in functions, in order to extract the desired color channel, filter noise, and count the number of particles in each image.

Cell seeding percentages were calculated for each image section and averaged for cells and microspheres. Two-sample t-tests were used to compare the seeding percentages of cells and microspheres (p<0.05). Three images were analyzed using the automated analysis tool and manually by three lab members. Cell counts for the manual analyses were averaged.

RESULTS

The analysis of cell percentages demonstrated no significant differences between cell and microsphere distribution in the longitudinal, circumferential, and radial directions (radial seedling percentage depicted in figure 1). P-values for each image section were greater than 0.2.

For the ImageJ automated analysis tool, average manual cell counts and automated cell counts appeared to be very similar. Statistical analysis could not be performed, however, because the analysis tool outputs the same cell count for a specific image.

![Figure 1](image)

**Figure 1**: Average seeding percentages for inner, middle, and outer radial sections are compared for cells and microspheres. P-values are 0.42, 0.53, and 0.46 respectively. Similar results were observed for circumferential and longitudinal data (not shown).

DISCUSSION

The objectives and success criteria outlined by this study were met. No significant differences between cell and microsphere distributions were observed in any of the three cylindrical directions, indicating that the next phase of research into the use of Artificial Stem Cells in TEBVs may begin. The automated analysis tool functioned well, allowing for a speedy and consistent analysis of scaffold cross-sections.

Despite the success of this preliminary study, a variety of limitations impede full certainty as to the applicability of the cell seeding process on microspheres. Though they remained contained within the scaffold throughout this process, the microspheres may become dislodged during native-like flow conditions. It is also difficult to truly claim uniformity of distribution for the microspheres, as the statistical methods used are designed to detect differences, not similarities. It is important that caution be taken when resuspending the microspheres, as low-resuspension time could lead to microsphere aggregation. Further study is necessary to optimize the resuspension process.

Regardless of the limitations, however, this study opens the door for the next stage of research into artificial stem cells. understanding their ability to encapsulate important regenerative factors and their behavior under flow conditions. Despite the many hurdles left, however, this new treatment presents a promising paradigm for regenerating arteries damaged during CVD in a robust, easily producible manner.

ACKNOWLEDGMENTS

We would like to acknowledge Steven Little and Morgan Fedorchak for providing the microspheres, Peter Rubin for providing the ADSCs, and William Wagner and Antonio D’Amore for providing the scaffolds. We would also like to acknowledge our funding sources, the Swanson School of Engineering, the National Institutes of Health, and the American Heart Association.

REFERENCES


