

Tissue Construction and Development of Artificial Arteries of Optimized Graft

by

**Khaled Yousef
William Yang
Dr. Jag Mazumber (University of Adelaide)
Professor Yos Morsi,**

Abstract

This work is being undertaken at IRIS and is part of a SPIRT ARC grant received in 2000. In this paper a summary of the results obtained thus far is presented and discussed. The problem of atherosclerosis is highlighted from hemodynamic point of view. The work of the group so far is presented and future directions are given.

1. Introduction

The large arteries - the aorta and its branches - are commonly affected by atherosclerosis, a condition that causes them to become hard and narrow. Unfortunately there is no way of dissolving atheroma once it has formed, as can be done for a blood clot or thrombus. Solutions include atherectomy, angioplasty and coronary bypass surgery. Restenosis (i.e., recurrence of the disease is often a problem with such procedures).

Arterial bypass or replacement is a common treatment for vascular disease and includes several coronary bypass grafts performed annually. However, the main problem is that many patients do not have veins suitable for grafting use as grafts to pre-existing vascular disease or previous surgery. Hence the development of small diameter vascular grafts has been a rapidly growing area of research (Huynh *et al.*, 1999).

The surgical treatment for atherosclerosis is to bypass the seriously constricted artery section with an autogenous graft or artificial tube in an intrusive surgical procedure. The process of atheroma formation continues in the other coronary arteries. It progresses faster in the artery section that has been bypassed which may close off after 6 months. The essential blood supply now depends upon the graft and the graft-artery junction. Arterial bypass grafts tend to fail after some time, from months to a few years, due to myointimal hyperplasia or intimal thickening. Thickening occurs at the heel, the toe and along the suture line of the distal anastomoses. Thickening also occurs on the floor of the host artery (Bassiouny *et al.*, 1992, and Inzoli *et al.*, 1996).

Findings from literature indicate that the endothelial cells lining the artery wall have the ability to act as the fluid dynamic wall-shear biosensor. Over time, the arteries respond to low shear stress and remodel by intimal thickening in such a way as to maintain a more uniform shear stress level in the range 10 - 20 dyne/cm² (Giddens *et al.*, 1993).

The underlying hypothesis of restenosis is that of dysfunction of the endothelium leading to high cell turnover or individual bond rupture of endothelial cells as well as changes in gene expression that trigger excessive release of growth factors and subsequent smooth muscle cell proliferation and excessive platelet aggregation (Lei *et al.* 1997). Early atherosclerotic plaque localisation may be an unstable bio-chemical exaggeration of the otherwise physiologic response of arteries to remodel. If low-density lipoproteins are available in sufficient supply, the intimal thickening may become pathological and atherosclerotic plaque may develop (Giddens *et al.*, 1993). In regions of low shear stress blood elements have prolonged contact with the vessel wall thereby facilitating blood cell-EC adhesive interactions (Hinds *et al.*, 2001).

Background research indicates that in regard to intimal thickening and restenosis of anastomotic junctions:

- The effect of the material properties of the artery, graft and anastomoses in normal and stenotic conditions
- The *in vitro* simulation of hydrodynamic forces inside a bioreactor for cell adhesion, for bypass artery, has not been fully examined.

The complexities introduced into the study of coronary arteries due to the continually moving artery geometry, resulting from the motion of the heart walls, has precluded comprehensive studies of the hemodynamics of these vessels in comparison to the voluminous works on the carotid and femoral arteries and the aorta. Such studies would provide the necessary information for optimising the geometry of the artery with a view to achieving the best possible design of the artery that would overcome problems of intimal thickening and restenosis.

Hence our main objectives of this study are to:

- Optimize the best geometry for the coronary artery from a hemodynamic point of view.
- Engineer the best tissue artery for the above geometry and hemodynamic conditions.

The first part of the study (Study 1) will provide the optimum geometry and related hemodynamic parameters necessary for the design of biomaterials and scaffolding for the tissue-engineered coronary artery. In the second part (Study 2), this knowledge base will be used to design and engineer the tissue artery. To achieve these objectives we are planning to conduct a systematic study as outlined in the following sections.

The bypass artery can be made of natural tissues or synthetic (artificial) materials. The major drawback in using an artificial tube for bypass is that these are foreign materials that lack the ability to grow, repair, or remodel. In addition, in regions of low-flow or small diameter (less than 6 mm) such as coronary bypass, these grafts are potentially thrombogenic, which leads to stenosis (Stephen et al., 1977, and O'Donnell et al., 1984). An alternative approach is to use natural tissue grafts grown *in vitro* (tissue engineering).

Initial attempts in the area of tissue engineering of blood vessels involved the coculture of endothelial and muscle cells with natural and/or synthetic materials to create a cellular vessel *in vitro* (Weinberg and Bell 1986, Matsuda and Miwa, 1995, Ziegler et al., 1995, and Tranquillo et al., 1996). Although the mechanical strength aspects of the blood vessels were suitably addressed in later studies, the requirements of very long culture times together with the question of long-term *in vivo* efficacy, made this approach inadequate.

An alternative to tissue culture *in vitro* is to create a prostheses that will remodel *in vivo* using absorbable grafts composed of naturally occurring material (Huynh et al., 1999). Shin'Oka et al. (2000) reasoned, in the case of artificial heart valves, that the creation of an autologous tissue-engineered material would offer several theoretical advantages over the presently used substitutes (Shin'Oka et al., 1995, 1996, 1998). It is anticipated that an autologous bioprosthesis would be a living, viable structure and therefore should be able to demonstrate the normal biological mechanisms for growth, repair, and development, which would translate to a greater durability. In addition, a tissue-engineered autologous graft would be completely biocompatible, with minimal risk of infection and thromboembolic complications. Similar arguments hold good for our current work on arteries for bypass. The artificial artery would be constructed in a similar way, using biocompatible and biodegradable scaffolds and living cells.

However, one additional issue relates to the maintenance of the integrity of the composite polymer (scaffold material), under conditions of correct physiological and hemodynamic stress. All the experiments to date have been carried out in the low-pressure pulmonary circulation or venous circulation (Shin'Oka et al., 2000). This area of research would be a key issue addressed in our proposed study. Less is known about the response to biodegradable scaffolds in the systemic arterial circulation.

2. Methods and Materials

2.1 Computational Fluid Dynamics Studies

The mathematical description of the flow to be solved is the unsteady, three-dimensional Navier-Stokes equations for an incompressible, non-Newtonian fluid. These equations and their solution have been described and discussed widely in the literature on artery blood flows (e.g., Lei et al., 1997, Zhao et al., 2000, and Siaw et al., 2000). The background literature indicates that a laminar solution is appropriate. We

intend to use the commercial finite volume CFD code CFX 5 from AEA Technology Engineering Software, UK.

The research group members have experience with CFX-5 and have successfully used this software to model and simulate pulsatile flow in an end-to-side anastomoses in preliminary studies using data for a femoral graft from the work of Moore *et al.*, 1999. CFX 5 includes an integrated geometric modeller and CFD equation solver. Prof. Kleinstreuer is currently using this software for a similar work on arteriovenous access grafts, at North Carolina State University.

The construction of the initial models are being guided by the Vascular Surgeon (Prof. David Scott) and Cardiothoracic Surgeon (Assoc. Prof. Franklin Rosenfeldt) so that the range of geometric variables is representative of junction parameters existing in clinical practice. The variables to be studied are:

- The graft-to-artery diameter ratio,
- The acuteness of the angle of attachment,
- Out-of-plane asymmetry of the graft geometry and
- Geometry changes due to simulated restenosis.

Some preliminary results are shown in Figure1.

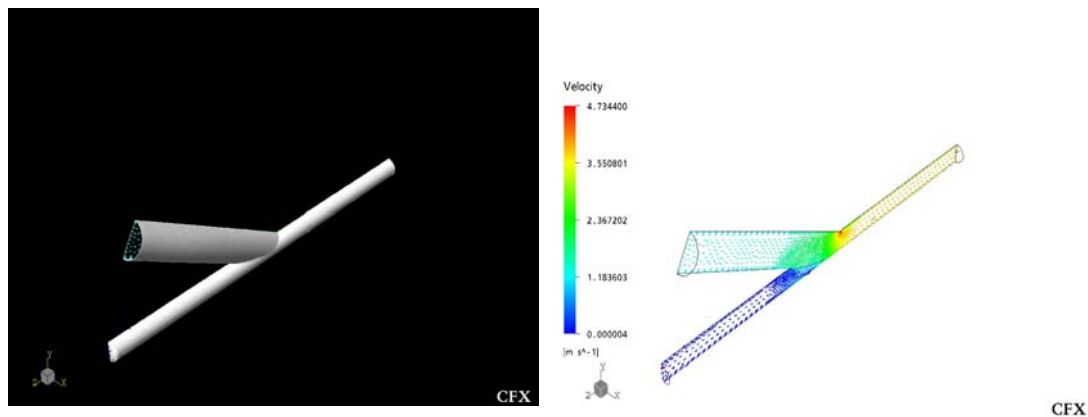


Figure 1 – Preliminary Results

2.2 Measurements of Flow Velocities and Wall Shear Stresses

The Aerometrics 2D LDA system (Figures 2 and 3), available at Swinburne, is being used for all velocity and shear stress measurements in the models. (e.g., see Naiki *et al.*, 1994, Sakhaeimanesh and Morsi, 1999). Full details of the technique are given in our earlier publication (Morsi 2000).

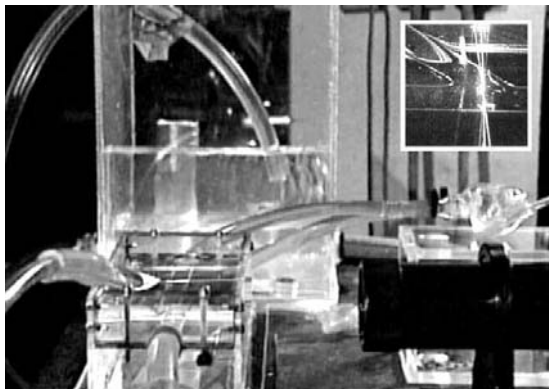


Figure 1 - Experimental Set-up

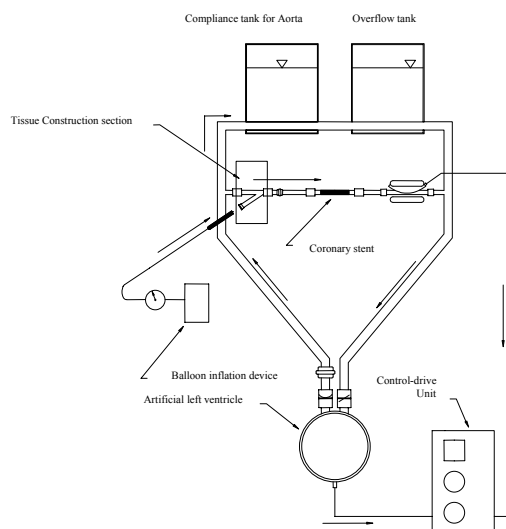


Figure 2 - Schematic drawing of in-vitro evaluation system

2.3 Cell culture, seeding and conditioning of coronary artery in vitro

Scaffolds produced by the FDM system will be seeded with primary cultures of cells established from artery. In a current study, cultures of cells have been established from surgical patients and preliminary trials are in progress to determine optimal seeding conditions required to establish cell growth on non-woven polyglycolic acid scaffold. These preliminary trials will establish techniques for the seeding of artery made by FDM from other polymers. A particular focus will be on obtaining a natural histology for the cell adhesion as there is no published data on the establishment of a complete endothelium. In addition, it is unclear whether a mixed population is sufficient for cell seeding or whether pure cultures are required in a two-step process (i.e., seeding of the myofibroblasts followed by endothelial cell seeding).

Therefore, a major aim of this part of the study is to develop a cell adhesion, which possesses an endothelial lining that best approximates a coronary artery. If required, separate cultures of endothelial cells from the primary cultures will be established and attempts made to form a functional endothelium on the developing tissue by a delayed seeding of these cells. Cardiovascular endothelial cells express a unique cell-adhesion molecule known as CD31 or PECAM-1, whereas myofibroblasts are characterized by the presence of smooth muscle actins (SMA). Currently some tissues culture training is being undertaken at the SES.

3. Conclusions

Laser Doppler Anemometry and CFD can be used to effectively analyse the problem of bypass. The long term objective of the research group is to work toward the development of totally functional coronary artery grown *in vitro*.

4. Acknowledgment

Various members of the modeling and process analysis group have contributed to the advancement of our knowledge in this topic. Their contributions are greatly appreciated.

5. References

AIHW and National Heart Foundation of Australia & National Stroke Foundation of Australia Heart, Stroke and Vascular Diseases Australian Facts 2001, ISBN 1-74024-105-3, 2001

Bassiouny H S. et al. Anastomotic Intimal Hyperplasia: Mechanical Injury or Flow Induced, *Journal Vascular Surgery*, Vol. 15, pp. 708-717, 1992

Inzoli F, et al. Numerical analysis of steady flow in Aorto-Coronary bypass 3-D model, *Trans ASME, Journal of Biomedical Engineering*, Vol. 118, pp. 173 - 179, May 1996

Huynh T et al., Remodelling of an acellular collagen graft into a physiologically responsive neovessel. *Anture Biotech*, Vol. 17, 1083-85, 1999

Lei M et al. Hemodynamic Simulations and Computer-Aided Designs of Graft-Artery Junctions, *Trans ASME, Journal Biomechanical Engineering*, Vol. 119, 343-348, 1997

Giddens, D.P. et al. The role of fluid mechanics in the localization and detection of Atherosclerosis, *Trans ASME, Journal of Bio-medical Engineering*, Vol. 115, pp. 588-593, Nov 1993

Hinds M T et al. Local Hemodynamics Affect Monocytic Cell Adhesion to a Three-Dimensional Flow Model Coated with E-selectin, *Journal of Biomechanics*, Vol. 34, pp. 95-103, 2001

Stephen M, Loewenthal J, Little J M, May J and Sheil A G, Autogenous veins and velour Dacron in femoropopliteal arterial bypass, *Surgery*, Vol. 81, pp. 314-418, 1977

O'Donnel T F et al. Correlation of operative findings with angiographic and non-invasive hemodynamic factors associated with failure of PTFE grafts, *J. Vas Surg*, Vol. 1, pp. 136-48, 1984

Birchall I E, Field P L and Ketharanathan, Adherence of human saphenous vein endothelial cell monolayers to tissue-engineered biomatrix vascular conduits, *J Biomed Mater Res*, Vol. 56, pp. 437-443, 2001

Werkmeister J A, Edwards G A, White J F, Casagrande F, Hunt J A, In vivo evaluation of modified mandrel-grown vascular prostheses, *J Biomed Mater Res*, Vol. 47, pp. 316-323, 1999