ENDOTHELIAL CELL CULTURE AND SEEDING OF PROSTHETIC VASCULAR GRAFTS: AN EXPERIMENTAL STUDY

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ABSTRACT

Current synthetic vascular prostheses do not acquire lining of vascular endothelium in humans or dogs. Endothelial seeding of vascular grafts has been proposed as a means of reducing the thrombogenicity of these grafts. We examined feasibility of cultivating endothelial cells (EC) by tissue culture technique and their subsequent seeding onto small diameter polytetra fluoroethylene (PTFE) grafts. Twenty adult dogs underwent common carotid artery interposition with 4 mm PTFE grafts. Ten dogs received seeded and the remaining ten received unseeded grafts. Grafts were removed at 4 and 12 weeks and their gross/morphological features compared. Cumulative patency rates for seeded grafts were 70% as compared to unseeded ones 30%. Seeded grafts were completely surfaced with a mono-layer of endothelium by 4 weeks. Small graft patency appears to be related to the establishment of an endothelial surface, the development of which is clearly facilitated by seeding with autogenous endothelium.

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KEY WORDS: Endothelial cell seeding; Vascular grafts.

Introduction

Synthetic vascular prostheses are inherently thrombogenic. Although, this characteristic is of less importance in high flow situations through large grafts, in small calibre grafts with low flow, thrombogenicity negates most advantages of using these prefabricated conduits. It has been hypothesized that evolution of an endothelial lining might lessen the incidence of small graft occlusion. The first such study was carried out in the dog, an animal in which prosthetic graft maturation most closely approximates to man[1]. The methods by which EC can be introduced (seeded) on to prosthetic grafts vary in terms of simplicity and clinical applicability [2,3]. Two popular methods have been, the 'single-stage' technique as devised by Herring [2], and the 'two-stage' technique which involves extraction and cultivation of EC prior to the seeding on to the grafts, as described by Graham [3].

The study was designed to test the efficacy of endothelial cell seeding (ECS) of PTFE grafts. The objective was to produce a rapid endothelialization of the conduits so as to increase their patency rates and reduce pseudointimal fibrous proliferation.

Material and Methods

Twenty adult mongrel dogs weighing 15 to 25 kgs were stud-

ied. All dogs underwent common carotid artery interposition with PTFE grafts, of which 10 grafts were unseeded (controls) and 10 were seeded (experimental).

PTFE (Gore-tex) grafts 8 to 10 cm in length and 4 mm in internal diameter (ID) were used. All the operations were performed in a sterile environment, under general anaesthesia, in the Experimental Surgery Department of the Armed Forces Medical College (AFMC). Endothelial cells were harvested from 10 cm segments of external jugular veins. The harvesting and cultivation of EC was done at National Centre for Cell Studies (NCCS Pune). The cells were extracted enzymatically with 0.5 % collagenase in Hank's BSS (Table 1) and cultured in cold medium 199 (Table 2). The mean number of EC harvested from a 1 cm segment of vein were 6.4×10^5 cells. The cells were added to 1 ml of dogs own heparinised blood and forcibly flushed through the grafts. Approximately 7×10^4 EC per sq cm of luminal surface to be seeded were added to the preclot mixture used for flushing the grafts. Graft interposition was performed in an end to end fashion in the common carotid artery position with continuous 6-0 poly propylene/PTFE sutures. Systemic anti-coagulation was achieved with intravenous heparin (100 IU/kg). The grafts were explanted at 4 and 12 weeks after surgery. Graft patency was determined by use of portable Doppler (EMCO-Huntleigh, 3 Mhz probe) and palpation of a pulse in the distal carotid artery. The grafts were opened longitudinally and divided into 1 cm segments. They were immersed in 1% neutral buffered formalin solution, embedded in paraffin sectioned and stained with hematoxylin & eosin for light microscopy.

RESULTS

A total of 20 dogs underwent graft interpositions in this study. Mean operating time was 1 hour and 20 minutes $\pm$ 14 minutes.

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TABLE 1

Composition - Hank's solution - balanced salt solution

Substrate	Strength
Distilled water (DW)	500 ml
Sodium chloride	8 gms
Potassium chloride	0.5 gms
Potassium hypophosphate	0.5 gms
Sodium hypophosphate	0.04 gms
Phenol red	0.011 gms
Mixed with	
D Glucose	1 gm in 100 ml DW
Sodium bicarbonate	0.35 gm in 100 ml DW
Calcium chloride	0.180 gm in 100 ml DW
Magnesium sulphate (Anhydrous)	1.85 gm in 100 ml DW

TABLE 2

Composition - cold medium 199

Substrate	Strength
Medium 199 (M 199)	9 ml
Porcine heparin	10 µg/ml
Fetal bovine serum (FBS) 5%	1 ml
Endothelial cell growth Factor (ECGF)	50 µg/ml
Penicillin G	100 u/ml
Streptomycin	100 µg/ml
Amphotericin B	0.25 µg/ml

Cross clamp time was 24 minutes $\pm$ 6 minutes.

There were no per/post operative complications/deaths. However bacterial contamination of endothelial cells during harvesting lead to rejection of five veins segments. Gross morphology and histology of experimental and control grafts was compared at 4 and 12 weeks after surgery.

Patency rates: At 4 weeks 2 out of 5 control and 4 out of 5 experimental grafts were patent and at the end of 12 weeks 1 out of 5 control and 3 out of 5 seeded grafts were patent. Cumulative patency from 4 to 12 weeks among experimental grafts (70%) and control grafts (30%) was significantly different ($p=0.03$).

Clot free surface area: The fraction of the flow surface grossly free of red clot at 12 weeks was significantly more for seeded grafts than for unseeded grafts (Table 3) ($p=0.023$)

TABLE 3

Clot-free surface area

Duration	PTFE grafts	
	Seeded	Unseeded
28 days	58.2%	54.2%
(n)	(5)	(5)
90 days	74.4%	32.9%
(n)	(5)	(5)

Light microscopic features: The histologic differences in graft incorporation between the control and the experimental animals were apparent at 4 weeks and became more marked by 12 weeks. (Fig 1 & 2 and Table 4).

TABLE 4

Histological features of seeded vs control grafts

	Seeded	Unseeded
Surface	Monolayer of endothelium	Layers of fibrin with trapped blood cells
Inner capsule	Thin, cellular organised	Thick, acellular and unorganised
Interstitium	Filled with fluid (plasma) and platelet remnants	Amorphous material filled with scattered RBCs and platelets
Outer capsule	Well developed perigraft connective tissue	Well developed perigraft connective tissue

The thickness of inner capsule, in the seeded and control grafts, from 4 to 12 weeks remained more or less constant, averaging 22.23 microns and 52.46 microns respectively (Table 5) ($p=0.05$).

TABLE 5

Thickness of the inner capsule (Microns)

Duration	PTFE Grafts	
	Seeded	Unseeded
28 days	20.24	50.46
90 days	24.22	54.46
Average	22.23	52.46

TABLE 6

Graft patency rates (Cumulative)

Study (Year)	PTFE	
	Seeded	Unseeded
Stanley (1982) [6]	73%	27%
Herring (1984) [3]	77%	51%
Douville (1986) [8]	58.3%	27.3%
Allen (1990) [9]	96%	29%
Present study (1996)	70%	30(%)

Discussion

Inspite of the availability of balloon and laser angioplasty, vascular surgeons still have to carry out open bypass surgery below the inguinal ligament. A major constraint in achieving long term patency rates is the failure of the currently available prosthetic grafts to match that of autogenous vein. The disparity in patency of vein and prosthetic graft has been the subject of intense research. Since platelet deposition contributes to occlusion of small diameter prosthetic grafts, the concept of promoting prosthetic endothelialization in an effort to decrease platelet deposition was proposed. This was achieved by the technique of endothelial cell seeding [4], which involves inoculation of grafts with autogenous EC prior to implantation. In the present study EC was successfully harvested, cultured and seeded onto 4 mm PTFE grafts

Fig. 1 : Light microscopic view of the luminal surface of the seeded PTFE graft at 4 weeks showing a monolayer of endothelial cells over a thin organised inner capsule. (H and E, X 400).

Fig. 2 : Light microscopic view of the luminal surface of unseeded PTFE graft at 4 weeks showing a thick inner capsule with fibrin, and absence of endothelial cells. (H and E, X 200).

in a canine model using the 'two-stage' technique. Critical to our method of seeding were refinements in cell harvesting and culture techniques. Collagenase 0.5% used to extract EC is known to be superior to the mechanical method of scraping the intimal surface of veins with a steel wool pledget which is more traumatic and leads to smooth muscle cell contamination [4,5]. As compared to Eagle's medium as described earlier [3,6] we employed cold medium 199 [7] which yielded an adequate number of EC within 12 to 24 hours. An average of 6.4×10^5 EC were harvested from each vein segment which was sufficient to seed a 10 cm PTFE graft at an average of 7×10^4 EC per sq cm of the luminal surface. ECS has been conventionally assessed in terms of graft patency. We assessed cumulative patency rates from 4 to 12 weeks. Palpation of the distal carotid pulse, Doppler examination of

the graft in situ and gross inspection of the luminal surface was employed. Our patency rates of 70% of seeded and 30 % for control grafts compared favourably with other similar studies (Table 6). None of the animals in this study received antiplatelet therapy which has been otherwise routinely advocated.

In comparison few clinical trial have been undertaken and the results of most have been equivocal [10,11]. Evaluation has been indirect as the seeded grafts are unavailable for direct examination. In his preliminary report on seeded Dacron femoropopliteal reconstruction, Herring et al [12] observed favourable patency rates at one year, 81.6% for seeded and 30.8% for unseeded grafts.

Histologically also seeding appears to improve the microscopic appearance and accelerates maturation of the grafts. The thrombus free surface area was significantly larger for seeded grafts (74.4%) as compared to the control grafts (32.5%). At 4 weeks all patent seeded grafts exhibited complete endothelialization whereas only 20% of control grafts attained endothelial cover. In the seeded graft, the inner capsule averaged 20.2 microns, was more organised and the interstitium was devoid of smooth muscle cells, RBCs or platelets. The lining of unseeded grafts on the contrary represented a multilayered coagulum of platelets and RBCs. The endothelial cover was restricted to the pannus in growth at the anastomotic site. The inner capsule was thicker (50 microns) and largely organised. By 12 weeks the differences between the control and seeded grafts became more prominent though the thickness of inner capsule did not change significantly.

Douville et al [8] and Herring et al [12] also observed a confluent layer of endothelial like cells on the luminal surface of seeded grafts at 12 weeks. These were confirmed by immunohistochemical staining for factor VIII related antigen to be endothelial cells. Another noteworthy observation in the current study was that smooth muscle cells were not found in the inner capsule of the seeded PTFE grafts [13]. Their absence probably relates to the lower permeability of PTFE grafts to the perigraft tissue [14]. This significantly limited the ingrowth of fibroblasts and the thickness of inner capsule.

The current study hence supports the hypothesis that graft patency is related to the presence of an endothelial lining and that the development of such a surface is enhanced by seeding of autologous endothelium within vascular prostheses at the time of their implantation in the arterial circulation [15]. ECS is an exciting application of cell biology which could pro-

vide an early endothelial lining in small calibre PTFE grafts and may become an important means of lessening clinical failures so often attributed to these vascular prostheses.

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