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Biological performances of collagen-based scaffolds for vascular tissue engineering

F. Boccafoschi[a,b], J. Habermehl[a], S. Vesentini[c], D. Mantovani[a,*]

[a]*Laboratory for Biomaterials and Bioengineering, Laval University, Québec City, G1K 7P4, Canada*
[b]*Human Anatomy Laboratory, Research Center for Biocompatibility, University of Eastern Piedmont "A. Avogadro", Novara, Italy*
[c]*Department of Bioengineering, Politecnico of Milan, Italy*

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Abstract

Collagen is widely used for biomedical applications and it could represent a valid alternative scaffold material for vascular tissue engineering. In this work, reconstituted collagen films were prepared from neutralized acid-soluble solutions for subsequent haemocompatibility and cell viability performance assays. First, haemoglobin-free, thrombelastography and platelet adhesion tests were performed in order to investigate the blood contact performance. Secondly, specimens were seeded with endothelial cells and smooth muscle cells, and cell viability tests were carried out by MTT and SEM. Results show that neutralized acid-soluble type I collagen films do not enhance blood coagulation, do not alter normal viscoelastic properties of blood and slightly activate platelet adhesion and aggregation. Cell culture shows that the samples are adequate substrates to support the adhesion and proliferation of endothelial and smooth muscle cells.

Keywords: Collagen; Blood compatibility; Endothelial cells; Smooth muscle cells; Platelet adhesion

1. Introduction

According to the World Health Report (2003), 16.7 million people around the globe die of cardiovascular diseases each year, nearly one-third of total global deaths [1]. Cardiovascular diseases are often caused by atherosclerosis, which could lead to evolutive arterial diseases like thrombosis and aneurysms [2]. When the substitution of a diseased vessel is necessary, artificial prostheses, made of natural or synthetic material, are therefore required. Different materials are currently used as synthetic vascular substitutes; for instance, Teflon is used for prostheses of medium diameter (around 6 mm) vessels [3]. However, no biomaterial has yet shown satisfactory performances when in contact with blood for long time periods. This leads to clinical complications, such as aneurysms, thrombosis or restenosis [4,5]. Moreover, no appropriate biomaterial has yet been developed for small diameter vessel replacement.

Tissue engineering has already shown a strong potential for regenerating and repairing chronic wounds [6], burns [7] and, at the experimental level, cartilage defects [8] and represents a promising field in vascular reconstruction. The overall goal of vascular tissue engineering is to obtain the same mechanical and biological properties as a native vessel. Different approaches have been taken using collagen as a main scaffold constituent. In 1986, Weinberg and Bell [9] performed their groundbreaking study using a scaffold based on collagen for blood vessel reconstruction. They used an in vitro model generated using multiple layers of collagen integrated with a Dacron mesh to provide the necessary tensile strength. Smooth muscle cells were cultured in the graft and endothelial cells were used to line the inner lumen. Various polymeric materials have

*Corresponding author. Tel.: +418 656 2131 #6270; fax: +418 656 5343.
E-mail address: diego.mantovani@gmn.ulaval.ca (D. Mantovani).

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also been used for mechanical support in addition to collagen such as PGA and PLGA. Moreover, cells have been grown under dynamic conditions in particular bioreactors to closely mimic in vivo conditions [10]. Research has also been done using decellularized vein as a potential scaffold for vascular tissue engineering. This process preserves the extracellular matrix, the basement membrane structure and sufficient strength for vascular grafting [11]. It is clear that collagen plays a crucial role in all these studies, and that it could represent a suitable candidate to scaffold tissue regeneration.

Finally, collagen is one of the main proteins forming the vascular extracellular matrix. It is primarily produced by the smooth muscle cells of the media and the fibroblasts of the adventitia. Functionally, collagen fibres impose constraints on the elongation of large vessels under pressure, limiting the distension of the vessel. It also provides attachment to smooth muscle cells, transmitting force around the circumference of the vessel [12]. Collagen has low antigenicity, low inflammatory and cytotoxic responses [13] and is also biodegradable [14]. However, there exists a lack of knowledge about the biological performance of collagen-based scaffolds when in contact with blood and cells. Therefore, the aim of this work was to investigate neutralized collagens' impact when in contact with blood and cells.

2. Materials and method

2.1. Sample preparation

Specimens consist of flat sheets of collagen type I, which was extracted from rat tail collagen. Briefly, rat tail tendons were dissolved in acetic acid for 48 h; then the gel-like mass was mixed in a blender, frozen and lyophilized. The lyophilized sponge was mixed again with 0.02 N acetic acid at a dry weight to a solution ratio of 4 mg/ml. The resulting solution was centrifuged, degassed in vacuum and sterilized. Sterilization was performed in dialysis bags (Spectra/Por 1, MWCO:6–8000, Spectrum Laboratories Inc., California, USA) and soaked in 0.02 N acetic acid for 1 h followed by 1 h in 1% chloroform in water. Dialysis was continued in 0.02 N sterile acetic acid for 4–5 days. The resulting sterile solution was neutralized to a pH of 6.8 with 1% NaOH immediately prior to use. Samples were prepared in the form of thin films by solvent evaporation from neutral collagen solutions poured onto glass coverslips.

2.2. Blood performances

2.2.1. Reference materials

Teflon, which is a commonly employed material for vascular prostheses [15], was used as a reference material for clotting time measurement and thrombelastography. Glass, although not used for surgical purposes but due to its negative reaction when in contact with blood [16], was considered as a negative control.

2.3. Blood

Native whole blood was collected from 11 healthy male donors having taken no medication for at least 10 days prior to donation. 40 ml of blood, collected in tubes containing sodium citrate, was used for thrombelastograph assays and platelet extraction. 10 ml of blood without anticoagulants was used for clotting time assessment.

2.4. Clotting time

The haemoglobin free method for clotting time measurement is described elsewhere [17]. Briefly, 0.1 ml of untreated blood was immediately dropped onto the specimens. After 10, 20, 30, 40 and 50 min, 5 ml of distilled water was added to each specimen and incubated for 5 min. Red blood cells not entrapped in a thrombus were haemolysed and free haemoglobin molecules in the water were colorimetrically measured by monitoring the absorbance at 570 nm using a spectrophotometer ELISA reader (BioRad mod.450, Mississauga, Ontario, Canada). Absorbance values were converted into a percentage of the maximum amount of free haemoglobin present in the water. Statistical significance was determined by the Student's *t*-test.

2.5. Thrombelastograph (TEG)

Blood (400 μl) was exposed to the surface of collagen and controls for 30 min at 37 °C. Then, 300 μl of this blood was placed in TEG stainless-steel cups and 60 μl of 1.29% isotonic $CaCl_2$ solution were added for recalcification. TEG traces were obtained with a Thrombelastograph D (Hellige GMBH, Germany) and analyses were conducted following manufacturer's instructions. Briefly, the cylindrical cup containing blood oscillates through an angle of 4°45′ and is heated to a temperature of 37 °C. Each rotation cycle of the cup lasts 10 s. A stainless-steel cylindrical piston is suspended from a torsion wire and immersed in the cup. The torque of the cup is transmitted to the piston through the fibrin fibres that gradually form between the piston and the wall of the cup; the rotation of the piston becomes increasingly stronger as the clot becomes more solid.

Thrombelastography measures the elastic properties of blood clots as they form. The strength of the clot is graphically represented over time as a characteristic cigar-shaped figure. There are three main parameters of the TEG trace which are used in this study: r, k and MA. The time for the initial fibrin formation is given by r. The time from the beginning of clot formation until 20 mm of amplitude (k) is characteristic of the dynamics of clot formation. The maximum amplitude (MA) represents the strength of the clot, which is dependent on the number and function of platelets and their interaction with fibrin [18]. Combining k and MA leads to the thrombogenicity index. Statistical significance was determined by the Student's *t*-test.

2.6. Platelet adhesion

In vitro testing was performed to investigate the morphology, aggregation and pseudopodium of the adherent platelets.