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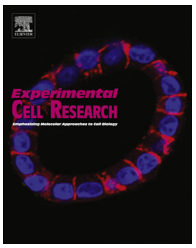

Research Article

Morphofunctional characterization of decellularized vena cava as tissue engineering scaffolds

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ABSTRACT

Clinical experience for peripheral arterial disease treatment shows poor results when synthetic grafts are used to approach infrapopliteal arterial segments. However, tissue engineering may be an option to yield surrogate biocompatible neovessels. Thus, biological decellularized scaffolds could provide natural tissue architecture to use in tissue engineering, when the absence of ideal autologous veins reduces surgical options. The goal of this study was to evaluate different chemical induced decellularization protocols of the inferior vena cava of rabbits. They were decellularized with Triton X100 (TX100), sodium dodecyl sulfate (SDS) or sodium deoxycholate (DS). Afterwards, we assessed the remaining extracellular matrix (ECM) integrity, residual toxicity and the biomechanical resistance of the scaffolds. Our results showed that TX100 was not effective to remove the cells, while protocols using SDS 1% for 2 h and DS 2% for 1 h, efficiently removed the cells and were better characterized. These scaffolds preserved the original organization of ECM. In addition, the residual toxicity assessment did not reveal statistically significant changes while decellularized scaffolds retained the equivalent biomechanical properties when compared with the control. Our results concluded

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that protocols using SDS and DS were effective at obtaining decellularized scaffolds, which may be useful for blood vessel tissue engineering.

Introduction

Cardiovascular disease is the leading cause of death in Western countries [1], associated with an increased incidence of peripheral arterial disease (PAD) [2]. Clinical experience in PAD treatments shows poor results when using synthetic grafts with a diameter less than 6 mm. This is especially true when it is intended to treat small caliber arteries such as those found in the lower limbs [3] and that have thus benefited from tissue engineering for the development of blood vessels using both synthetic and biological materials. The challenge of tissue engineering is to produce three dimensional (3D) biocompatible scaffolds: structures that provide seeded cells with a microenvironment mimicking natural in vivo tissue architecture [4]. Given that the development of tissues and organs using in vitro cellular engineering often suffers from a shortage of donor tissue, (which limits the potential of obtaining tissue engineered constructs), scaffolding technology is a promising approach to overcome these limitations [5,6].

Synthetic materials such as the commercially available polyglycolic acid (PLG) and polylactic acid (PLA) may be used to obtain 3D tubular structures, given that it presents some advantages; e.g., they do not induce immunogenic reactions [7]. These PLG/PLA based scaffolds were tested and proven efficient in providing mechanical support, good interaction with cells, and to have relative ease in handling. However, scaffolds from these synthetic materials merely provide three-dimensionality for cultured cells. They are artificially produced, and do not possess a natural 3D architecture [7]. An alternative is to use organs or tissues decellularized by different methods which does not promote the immunogenic reaction of allogeneic tissue transplants. Such methods have many advantages, such as the presence of extracellular matrix (ECM) proteins, bioactivity, and natural 3D tissue architecture [8].

A major concern around these bioengineered conduits for clinical application is whether these 3D tubular structures support the various mechanical forces resulting from blood flow, until the implanted cells reach a natural level of ECM deposition that allow for the development of natural tissue properties and, therefore, mechanical resistance [9].

Herein, we focus on the use of three different detergents in 12 decellularization protocols in order to obtain a decellularized blood vessel scaffold. Furthermore, the characterization of remaining ECM composition, its potential toxic residual effects, and its biomechanical properties were performed and analyzed.

Methods

Animal housing conditions and tissue harvesting

Fifty nonpregnant female adult rabbits (New Zealand) were used in all experiments. All procedures were conducted respecting the Ethical Guidelines for Animal Experimentation, after the approval by the Brazilian College for Animal Experimentation (COBEA Process no. 711). All experiments followed the US National Institutes of Health or European Commission guidelines. Rabbits were housed under controlled conditions and fed a standard pellet diet and water ad libitum, with the median age of animals being 6 months with weight ranging between 2.5 and 3 kg. Animals were anesthetized with tiletamine hydrochloride/zolazepam hydrochloride (20 mg/kg, i.m.) in association with 2% xylazine chloridate (4 mg/kg, i.m.). The areas of tissue harvesting were previously shaved and disinfected with water-soluble iodine polyvinyl pyrrolidone solution. All following procedures were conducted under aseptic conditions, and the animals were euthanized using high doses of pentobarbital.

The infrarenal inferior vena cava was surgically removed and their lumens washed off with sterile saline solution supplemented with unfractionated heparin (1000 UI/100 mL). These were then stored at a sterile RPMI-1640 cell culture medium (Invitrogen™, Carlsbad, USA) supplemented with 100 U/mL penicillin, 100 mg/mL streptomycin, and 25 mg/mL amphotericin B (Invitrogen™) at 4 °C. Adipose tissue samples were surgically removed from interscapular region, and immediately stored in RPMI cell culture medium supplemented as described above.

Adipose derived mesenchymal stem cell culture

Adipose derived mesenchymal stem cells (ADMSCs) were obtained as previously described through digestion with type I collagenase (Invitrogen™) [10]. Cell culture procedures were performed with an initial cell count of 2×10^4 cells/cm^2, obtained from five adipose tissue fragments (different donors). These cells were seeded and expanded in six-well culture plates (Techno Plastic Products™, Trasadingen, Switzerland) using Dulbecco's modified Eagle's medium nutrient F12 mixture medium, supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 mg/mL streptomycin, 25 mg/mL amphotericin B (2 mmol/L L-glutamine; Invitrogen™), 1% (v/v) minimum essential medium (MEM) essential amino acids solution (Invitrogen™), and 0.5% (v/v) of 10 mM MEM nonessential amino acids solution (Invitrogen™). Upon reaching 80% confluence, the monolayers were detached from culture wells with 0.25% trypsin/ethylenediaminetetraacetic acid (Invitrogen™) and seeded at 75 cm^2 culture flasks (Nunc™, Roskilde, Denmark). After two additional trypsin exposures, cells were cryopreserved with ice-cold FBS supplemented with 10% dimethyl sulfoxide and stored in liquid nitrogen [11].

Scaffold decellularization

Sodium dodecyl sulfate (SDS), sodium deoxycholate (DS), and Triton X100 (TX100) were chosen as the decellularizing agents, based on previous literature reports [12–17]. Working concentrations were 1% and 2%, both during 1 h and 2 h of incubation at 160 rpm at 37 °C in a shaker (News Brunswick Scientific™, Nijmegen, The Netherlands). Following the decellularization of