Evaluation of Decellularization in Umbilical Cord Artery

P. Mallis[a], I. Gontika[a], T. Poulogiannopoulos[a], J. Zoidakis[b], A. Vlahou[a], E. Michalopoulos[a,*], T. Chatzistamatiou[a], A. Papassavas[a], and C. Stavropoulos-Giokas[a]

[a]Hellenic Cord Blood Bank (HCBB), Biomedical Research Foundation, Academy of Athens, Athens, Greece; and [b]Biotechnology Division, Biomedical Research Foundation, Academy of Athens, Athens, Greece

ABSTRACT

Major achievements in creating decellularized whole tissue scaffolds have drawn considerable attention to decellularization as a promising approach for tissue engineering. Developing a tissue-engineered small-diameter ($\leq$2 mm) vascular graft, using decellularized human umbilical arteries (hUAs), for reconstructive surgery is a challenging task. Polymers used in the past, proved unsuitable due to serious adverse effects and autologous vessels are available only in 40% of patients. In this study, histological and proteomic analysis was performed to evaluate the efficiency of two decellularization protocols. In decellularization protocol A, hUAs were incubated in 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS) and sodium dodecyl sulfate (SDS) followed by incubation in alpha minimal essential medium (α-MEM) with foetal bovine serum (FBS) while in decellularization protocol B the hUAs were incubated in Hypotonic Tris and SDS followed by incubation in nuclease solution. Histological analysis of decelullarised hUA with both protocols revealed good preservation of extracellular cell matrix (ECM) proteins and immunofluorescent staining detected collagen I and fibronectin. The DNA content within the hUAs after decellularization with protocol A was 6.2% and with protocol B 17.3%. Proteomic analysis identified cytoplasmic enzymes such as, dehydrogenase X, α-enolase and peptidyl-prolyl cis-trans isomerase A only in native samples, while, cytoskeletal proteins such as a-actin, filamin and ECM proteins like collagens were found both in native and decellularised hUA. In conclusion, both decellularization protocols effectively removed the cellular material while the ECM remained intact. Future studies are warranted to elucidate the specific effects of altered structure–function relationships on the overall fate of decellularized hUAs.

VESSEL development with tissue engineering techniques is a rapidly evolving field. Arterial bypass is the primary therapeutic strategy in patients with cardiovascular diseases. The use of synthetic materials such as Dacron and expanded polytetrafluoroethylene (ePTFE) as arterial grafts failed, due to very serious adverse reactions, such as clot development, rejection and chronic inflammation [1]. Each year, more than 570,000 arterial bypasses are performed, therefore there is a great need for arterial transplants [2]. In this study, the possibility of decellularizing hUAs in order to develop a potential alternative form of arterial transplant was investigated. The human umbilical cord reaches a length of over 50 cm, from which two artery segments of 20–30 cm each can be easily isolated. There are no branches along this vessel that is characterized by a uniform diameter throughout the whole length [3,4]. Native tissues, including human saphenous veins, have been decellularized and have shown potential use as small-diameter vascular grafts [5]. The human umbilical artery might represent a more attractive source because it is widely available and easily isolated. In this study, two decellularization processes were evaluated. All cellular populations were eliminated, from the umbilical artery, without affecting the (ECM). Proteomic analysis and histological evaluation in native and decellularized arteries were

P. Mallis and I. Gontika have contributed equally to this work.

*Address correspondence to Efstathios Michalopoulos, Soranou Efesiou 4, Papagou, Athens, Greece. E-mail: smichal@bioacademy.gr

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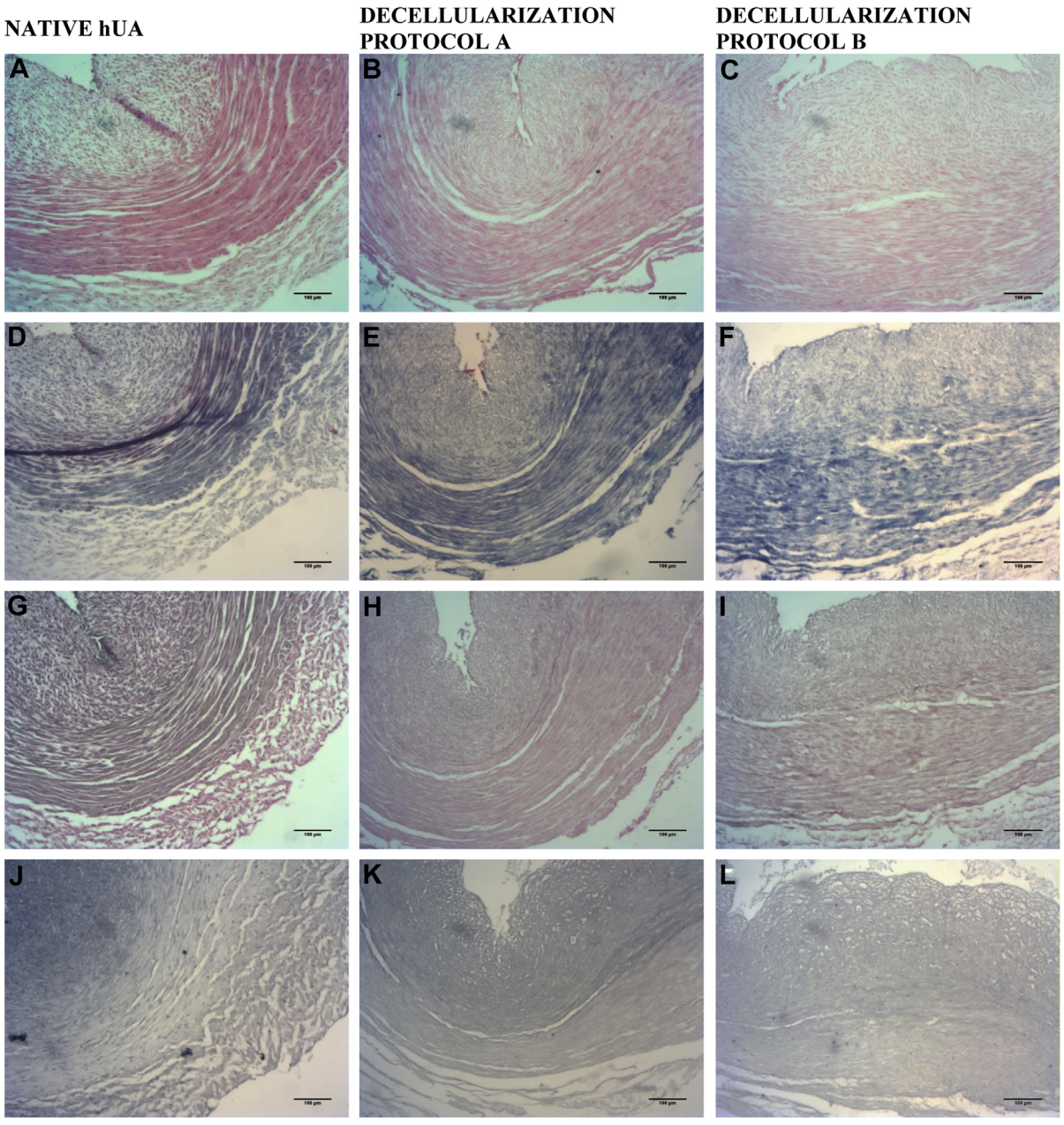

Fig 1. Native and Decellularized human umbilical artery with Protocol A and B stained with H&E, Masson's Trichrome, Elastic Van Gieson and Toluidine blue. Native hUA with H&E (**A**), Masson's Trichrome (**D**), Elastic Van Gieson (**G**), and Toluidine blue (**J**) stainings. Decellularized Arteries with protocol A and B, stained with H&E (**B**, **C**), Masson's Trichrome (**E**, **F**), Elastic Van Gieson (**H**, **I**), and Toluidine blue (**K**, **L**), respectively. Original magnification: 10x, scale bars 100 μm.

conducted, in order to assess the efficiency of the decellularization protocols.

MATERIALS AND METHODS

Preparation of Human Umbilical Arteries

Umbilical cords were collected after informed consent from healthy donors and stored at 4°C immediately after birth, and the overall storage time until processing for decellularization did not exceed 24 h. The arteries were dissected using sterile surgical tools from the entire human umbilical cord in a sterile fashion, followed by briefly rinses in phosphate buffer saline 1x (PBS 1x).

Decellularization Process

Decellularization of umbilical arteries was accomplished using methods that are similar to those described previously [5,6]. Briefly, in decellularization protocol A, hUAs (n = 20) were incubated in CHAPS buffer pH 7 (8 mM CHAPS, 1 M NaCl, and 25 mM EDTA in

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