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# New method for the isolation of endothelial cells from large vessels

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**Abstract**

*Background aims.* Numerous protocols for the isolation of bovine aortic endothelial cells have been described in the previous literature. However, these protocols prevent researchers from obtaining the pure population of endothelial cells. Thus, this study aimed to develop a new and economical method for the isolation of pure endothelial cells by introducing a new strategy to the enzymatic digestion method proposed by previous researchers. *Methods.* With the use of this method, the lumen of a bovine aorta was filled with wash medium and the outer surface of the sample was washed with alcohol for 30 seconds. Under a laminar flow hood, the inner surface of the sample was covered with filter paper. Collagenase type II was dripped onto the filter paper as a digestion enzyme. The digestion fluid was seeded in T25 flasks and fed with complete medium every 3 days. *Results.* The isolated cells were characterized by markers such as CD31, von Willebrand factor, 1,1′-dioctadecyl-1,3,3,3′,3′-tetramethylindocarbocyanine perchlorate acetylated low-density lipoprotein and angiogenesis behavior. The purity of endothelial cells was detected by flow cytometry to be of nearly 90% purity; these results were confirmed by immunostaining. Moreover, endothelial cells formed blood vessel–like tubes in a three-dimensional environment, which is specific dynamic behavior for endothelial cells. *Conclusions.* The new strategy presented in the current report enables isolation of a highly pure population of endothelial cells that can survive long-term culture without inducing an overgrowth of fibroblast cells.

**Key Words:** *bovine aortic endothelial cells, characterization, fibroblast contamination, filter paper, new strategy, pure population*

## Introduction

Endothelial cells have historically been viewed as an inert membrane in the circulatory system (1). Harvey's description of the circulatory system and studies by Malphigi, Reckingausen and other researchers show the critical role of endothelial cells in physio-pathological processes (2,3). The entire vascular system is lined by a single layer of endothelial cells separated from outer tissues by connective tissue. Although the amount of connective tissue is generally dependent on the vessel diameter, endothelial cells are always present in all vessels (4). The vascular endothelium maintains the structure and integrity of vessels and acts as a barrier between the blood and parenchymal cells (2).

Physio-pathological events such as wound healing, inflammation and tumor generation can result in endothelial dysfunction, which is the primary cause of certain diseases such as sepsis (5), atherosclerosis (6), diabetes mellitus (7), vasculitis, hypertension and ischemic heart disease (8). Therefore, pure populations of endothelial cells provide opportunities to obtain specific and significant information about cell behavior in physiological and pathological situations *in vitro*. Significant progress in vascular biology has recently been achieved through the isolation of endothelial cells from vessel preparations (9–11).

Endothelial cells are commonly isolated from large elastic mammalian vessels such as rat (12), porcine (13), bovine aorta (14), bovine pulmonary artery and human umbilical cord (15). Several techniques have been reported in the literature for bovine aortic endothelial cell (BAEC) isolation such as physical removal, the outgrowth method, enzymatic digestion and the use of magnetic beads (6,14,16–18). Recently, these techniques are followed by methods such as manual weeding, the use of a specific growth medium, enzymatic detachment and size-defined filtration to improve the purity of endothelial cell populations. Although these methods are reportedly effective in endothelial cell purification, there are some drawbacks for these methods. Fibroblast contamination, particularly in

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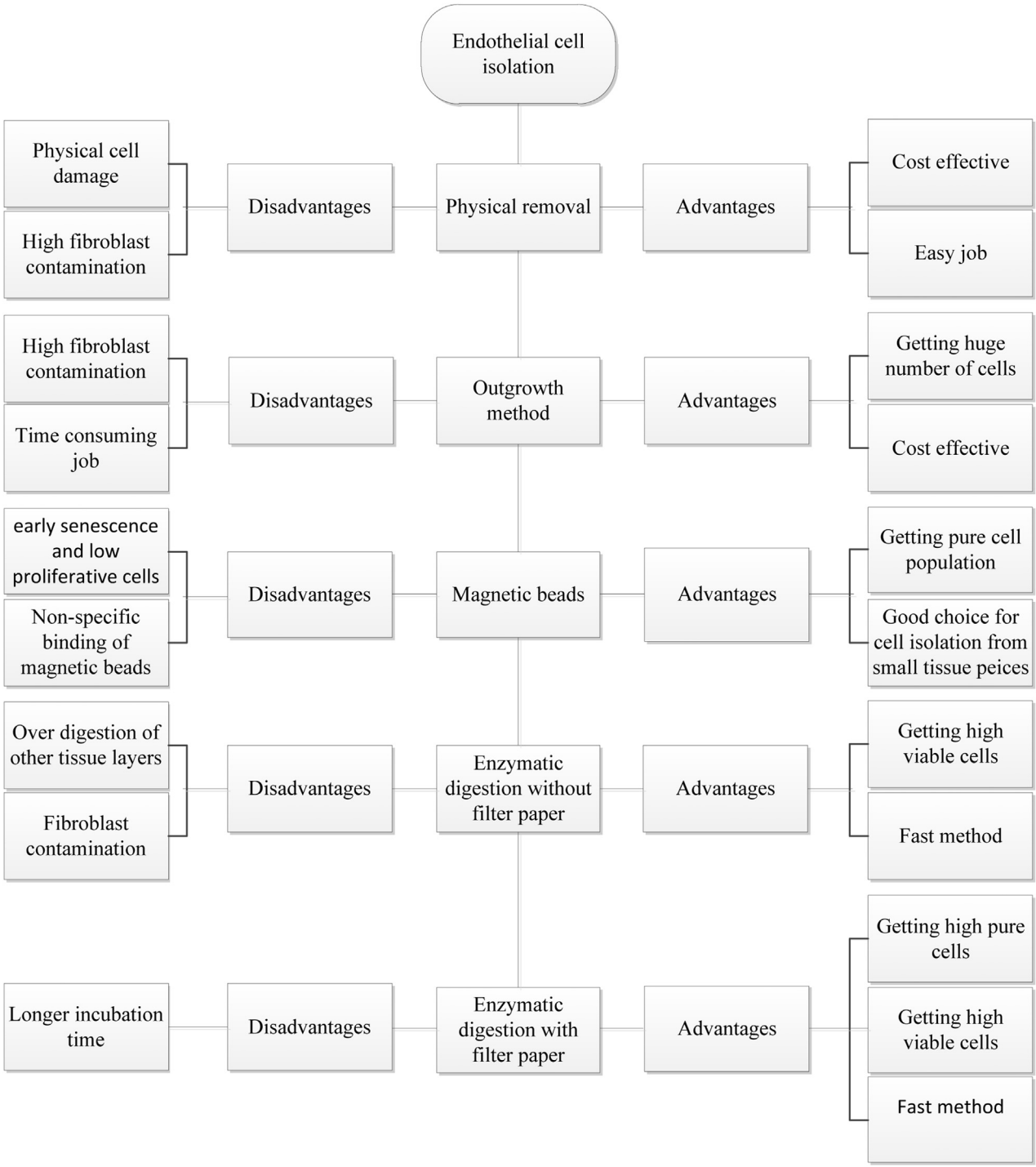


Figure 1. Summary of benefits and disadvantages of common methods in endothelial cell isolation.

the outgrowth and physical removal methods, is one issue that attracts researchers toward other methods (19,20). Low proliferative capacity, early senescence of endothelial cells and non-specific binding of magnetic beads to the other cell types may occur to a significant extent in the magnetic beads method (17,19). Thus, the current study proposes a new step in the enzymatic digestion to report this method toward isolation of non-sensitive and pure endothelial cells.

## Methods

Fresh bovine aorta was obtained from a local slaughterhouse. Both ends of the aorta were clamped with the use of sterilized plastic tie wraps immediately after