Development of a Vessel Organ Culture System: Characterisation of the Method and Implications for the Reduction of Animal Experiments

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Summary — In the field of cardiovascular research, the pig is considered to be an excellent animal model of human diseases. It is well-known that primary cultures of endothelial cells (ECs) are a powerful tool for the study of vascular physiology and pathology, and, according to the principles of the Three Rs, their use results in a substantial reduction in the numbers of experimental animals required. However, a limitation of EC culture is that the cells are not in their physiological context. Here, we describe and characterise a method for the culture of porcine vessels that overcomes the limitation of EC cultures, with the advantage of reducing the number of animals used for research purposes. The organ cultures were set-up by using an aortic cylinder obtained from the arteries of control pigs sacrificed for other experimental purposes. In order to characterise the method, vascular endothelial growth factor (VEGF) secretion, matrix metalloproteinase (MMP) activation and the vessel’s structural features were evaluated during organ culture. These analyses confirm that the culture of aortic cylinder lumen, in a medium specific for ECs, results in a stable system in terms of VEGF and MMP secretion. The ECs do not undergo cell division during the organ culture, which is also the case in vivo, if no stimulation occurs. Overall, we show that this novel system closely resembles the in vivo context. Importantly, porcine aortas can be collected from either veterinary surgeries or slaughterhouses, without having to sacrifice animals specifically for the purposes of this type of research.

Key words: aorta, endothelium, organ culture, reduction, vascular disease.

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Introduction

In the past 20 years, there has been an exponential increase in the number of scientific research studies in which pigs were used as the animal model for human pathologies. This is mainly due to the fact that the pig is biologically more similar (in terms of anatomy, physiology and genome sequence) to humans than are other non-primate animal species (1, 2). In particular, it has been demonstrated that the pig is an excellent model for research in the field of cardiovascular disease (3–7).

It is well-known that the development of several pathologies, such as atherosclerosis, hypertension and stenosis (i.e. neointimal hyperplasia; 8–10), is strongly linked to endothelial dysfunction (11). Endothelial cells (ECs) react to stress and injury with a pro-vasoconstriction, pro-coagulation and pro-inflammatory phenotype that might instigate certain vascular pathologies. Therefore, improvements to endothelial function can lead to earlier therapeutic intervention, and can contribute to the reduction of the risk of cardiovascular disease (12, 13).

In order to study the biology of the vascular endothelium, primary cultures of ECs from different species (e.g. murine, porcine, bovine and human) are commercially available, or can be derived from blood vessels. These are powerful tools that guarantee the reproducibility of the experiments, lower costs, and significantly reduce the use of experimental animals according to the principles of the Three Rs (14). However, primary cell cultures display some limitations — in particular, the cells are not in their physiological context (i.e. they are in a vessel), and they are in a proliferative state, as opposed to a non-proliferative state in vivo (where the average lifespan of an EC is more than one year; 15).

It has long been known that organ culture systems could overcome these limitations — vascular

These authors contributed equally to the work.