

Cell viability assay in corneal endothelium

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Introduction

Endothelium is the inner layer of the cornea, which must be viable for transplanting. The limited availability of corneas makes necessary the developing of preservation techniques that allow a long storage without losing endothelial viability.

Objectives

Determination of the presence of live cells in cryopreserved corneas after thawing.

Methods

Two corneas were cryopreserved by classical cryopreservation while in other two corneas, vitrification was realised. Extracted endothelia from thawed corneas and on endothelia from a cornea which was storage in hypothermic conditions (control) were stained with LIVE/DEAD imaging kit and Hoechst. Corneal endothelia were imaged using a fluorescence microscope.

Results

Viable cells were found in corneal endothelia of cryopreserved corneas by classical cryopreservation (Figure 1, A and B) and in corneal endothelium of cornea which was storage in hypothermic conditions (Figure 2).

Endothelia from vitrified corneas showed cells with low esterase activity, and any viable cell (Figure 1, C and D).

Non viable cells, intermediate cells, and cells with positivity for Hoechst and for the two components of the kit was observed, but in a small number (Figure 2 and 3).

Conclusions

Classical cryopreservation offers viable endothelial cells in thawed corneas, while vitrification cause the lost of viability of endothelial cells.

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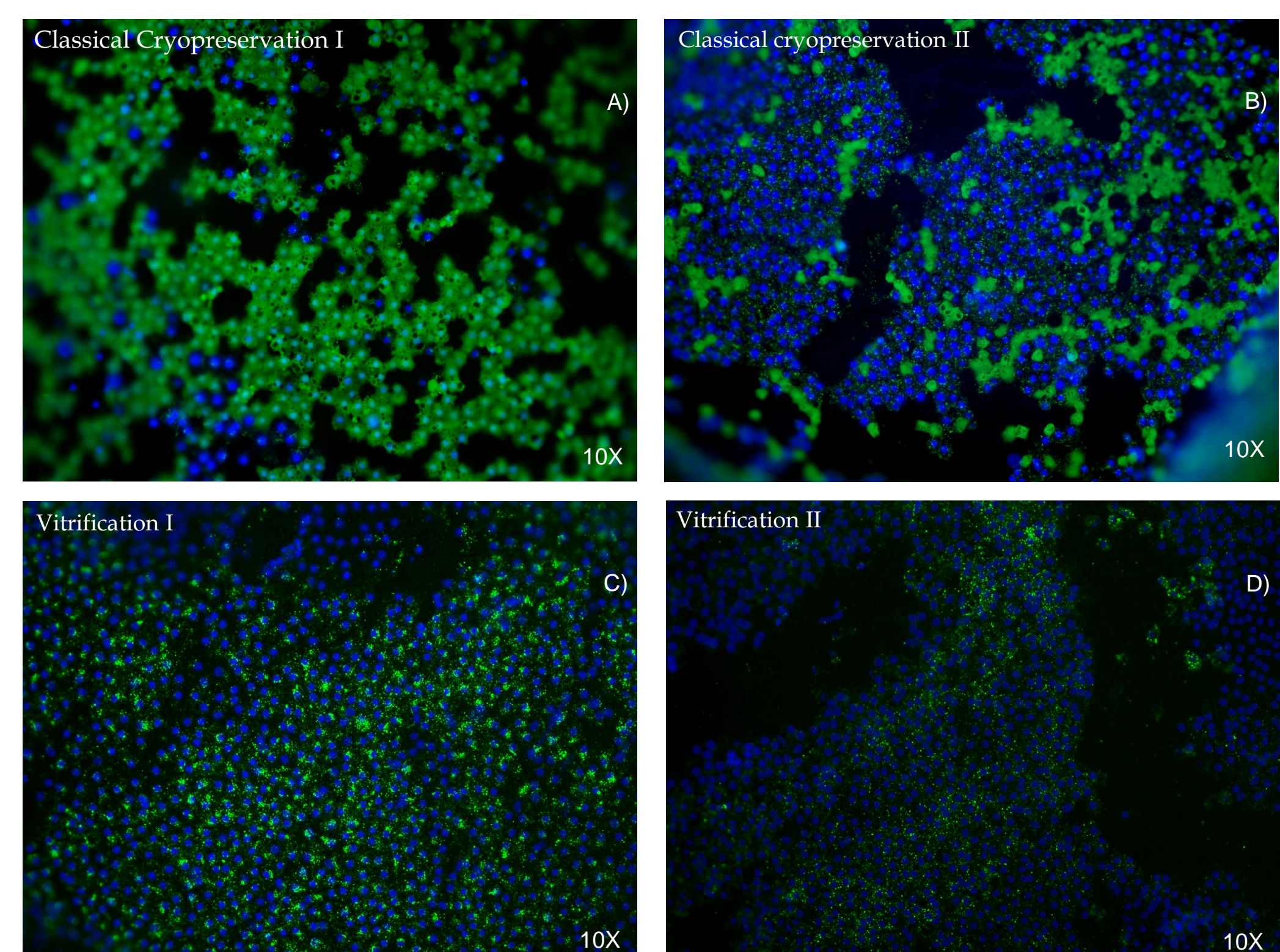


Figure 1. Endothelia of four thawed corneas after they were stained. Both endothelia of corneas cryopreserved by a classical way show viable cells with green cytoplasm and blue nuclei (A,B). Endothelia of vitrified corneas do not show any viable cell although cells with Hoechst stain and a low esterase activity are present (C,D), like in the corneal endothelium of the image B.

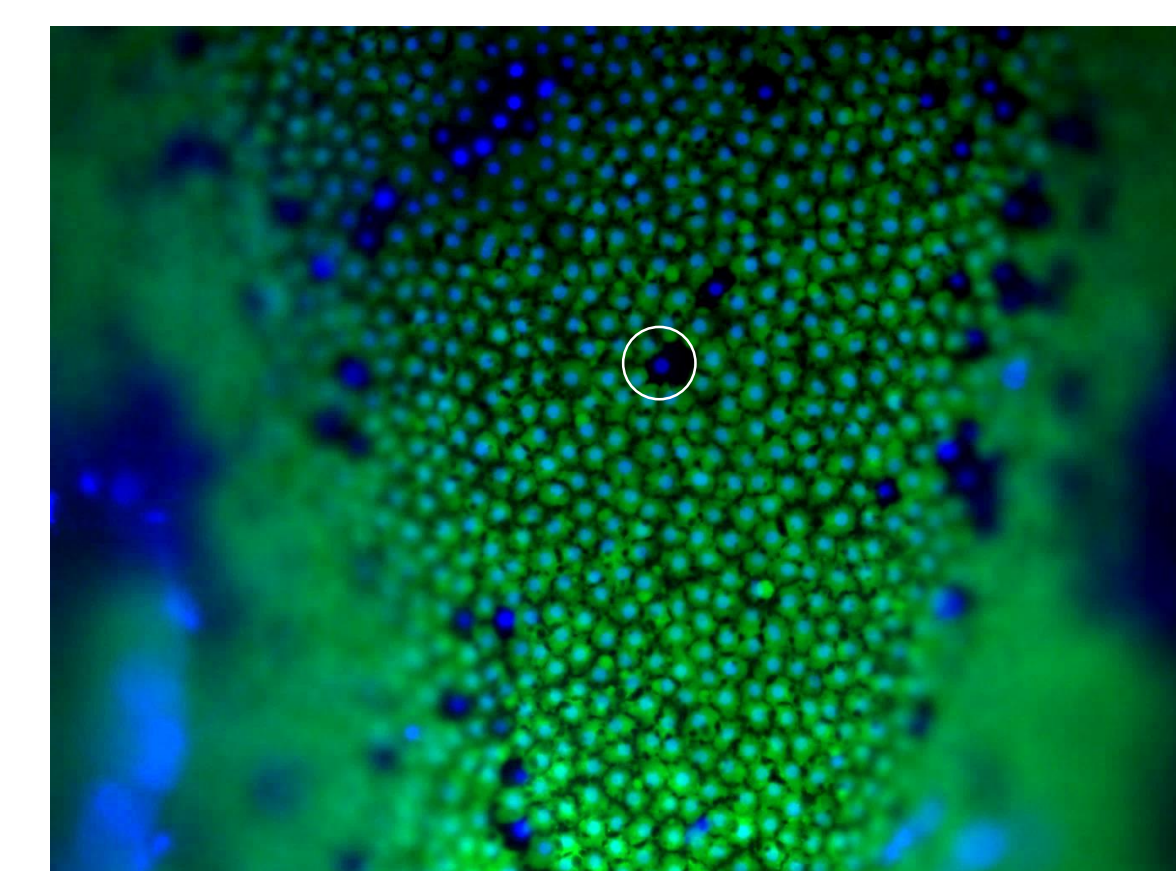


Figure 2. Endothelium of a cornea stored at 4°C. Viable cells and cells with only Hoechst stain are present (white circle).

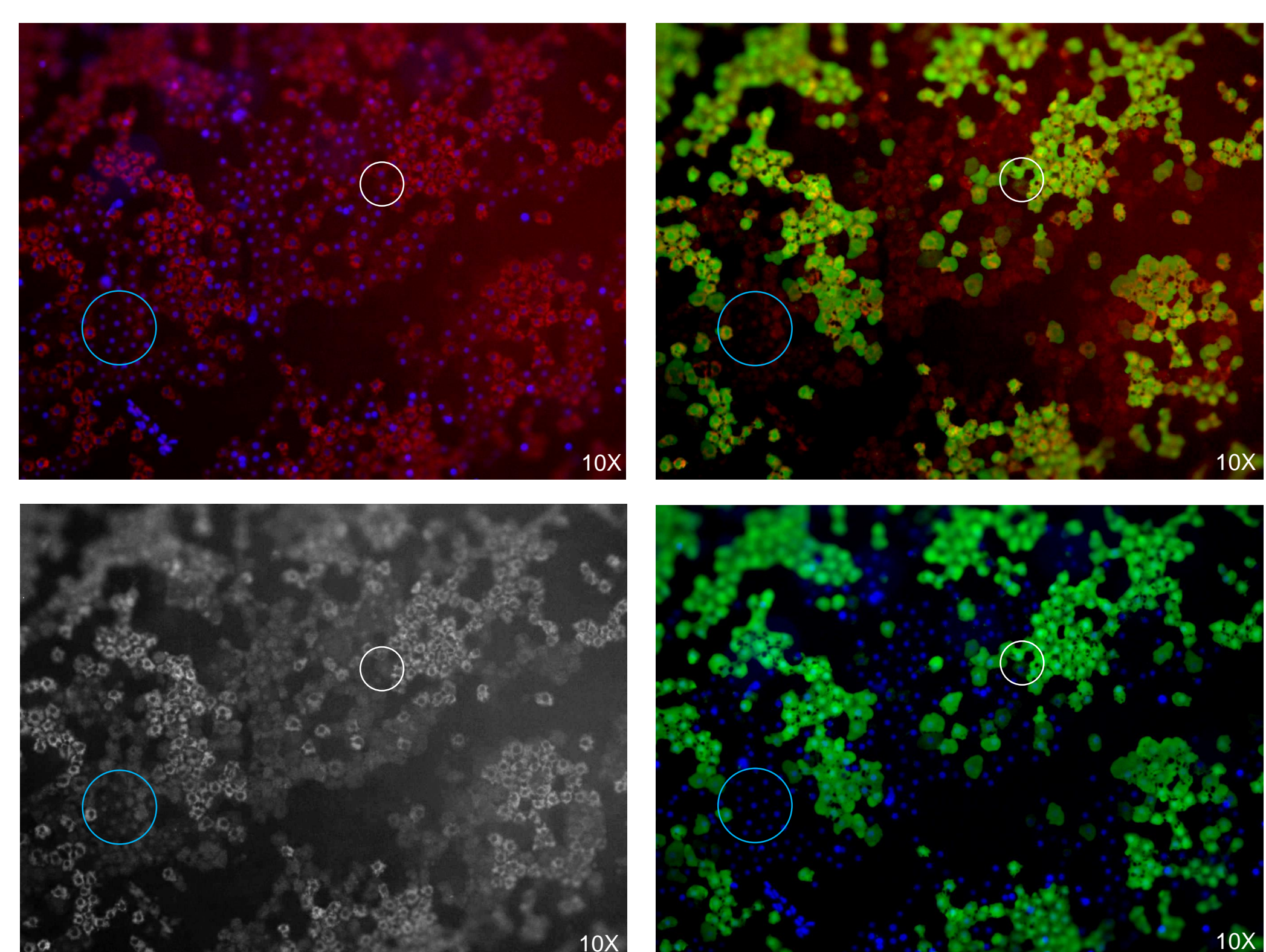


Figure 3. Example of an endothelium with cells that show positivity to the three components of the viability assay: Hoechst, Red Component, and Calcein AM, that exhibit a low esterase activity (white circle). A group of non-viable cells are showed (blue circle). These cells are stained by Hoechst and by Red Component. Grey image corresponds to that which shows positivity to Red Component.