OPTIMIZING IMAGING OF A THREE-DIMENSIONAL SCAFFOLD-BASED ENDOMETRIAL MODEL USING CONFOCAL MICROSCOPY

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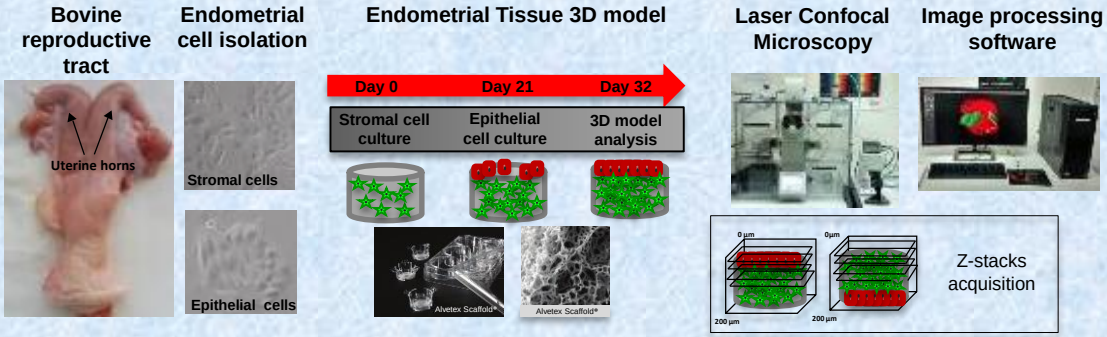
INTRODUCTION

Scaffold-based three-dimensional (3D) cell cultures are important tools in cell biology and tissue bioengineering because they closely resemble the microenvironment of natural tissues providing more physiologically relevant information and more predictive data for *in vivo* testing.

Nevertheless, characterization and visualization of 3D scaffold-based tissues models can still present considerable challenges due to the opaqueness and physical dimensions of thick tissue samples.

MATERIALS & METHODS



OBJECTIVES

To optimize the visualization and characterization of a 3D scaffold-based endometrial model combining classical immunofluorescence (IF) staining, conventional laser confocal microscopy and 3D visualization and image processing software.

RESULTS

Alvetex® technology allows endometrial stromal cells to produce their own endogenous extracellular matrix proteins within the scaffold (Fig. 1), which supports the growth and stratification of an epithelial cell layer (Fig. 2).

The use of compensation tools (Fig. 3B&D) enables to increase penetration depth of convectional confocal microscopy (Fig. 3A&C) which facilitates 3D model reconstruction. However the 3D reconstructions will vary according to cell density and the fluorophore used and immunofluorescence technique must be improved in order to rich greater depth (Fig. 4A&B).

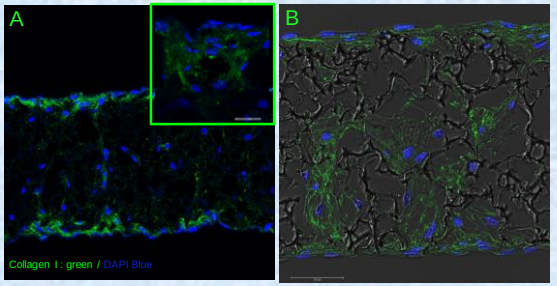


Figure 1: (A) Immunofluorescent staining of collagen I counterstained with DAPI. Images show protein expression in stromal cells cultured in Alvetex ® Scaffold. (B) Combination of fluorescence images and transmission image with confocal microscopy was used to visualized Collagen I expression on Alvetex® Scaffold.

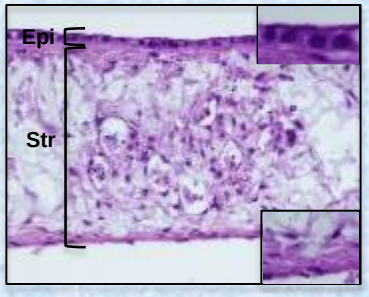


Figure 2: Hematoxylin-Eosin stained images of the resulting 3D co-culture model showing epithelial cells growing on top of Alvetex® Scaffold populated with stromal cells. Epi: epithelial layer; Str: stromal layer.

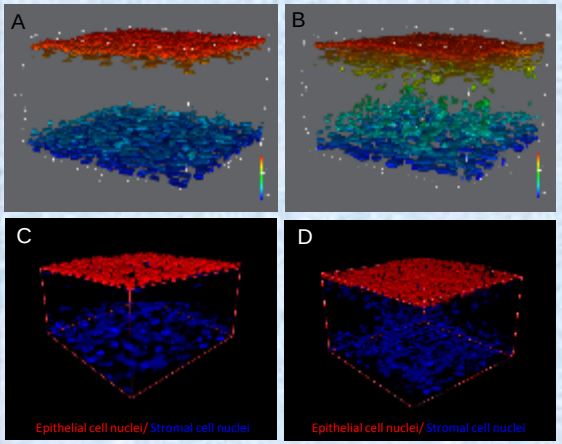


Figure 3: 3D reconstruction of confocal microscope images of epithelial and stromal cells co- cultivated on Alvetex® Sacaffold. Compensation tool enabled to visualize cell nuclei placed in deeper positions within the scaffold (B&D) in comparison to convectional visualization (A&C). Cell nuclei have been colour-coded based upon depth (A&B) or cell type (C&D).

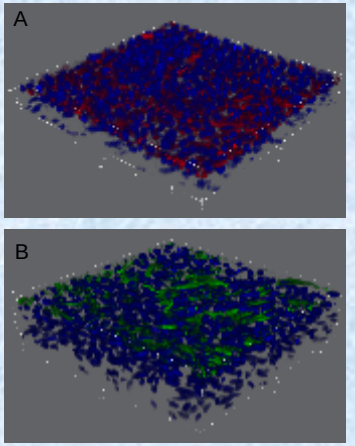


Figure 4: (A) Immunofluorescence of epithelial cell monolayer stained with cytokeratin (red) at the scaffold surface. (B) Vimentin expression (green) is detected in stromal cells to the outside lower surface of the scaffold. DAPI was used as a nuclear stain.

CONCLUSIONS

Laser confocal microscopy in combination with image processing analysis allows almost complete 3D visualization and reconstruction of 3D scaffold-based tissue models using readily available laboratory equipment.

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