SEM STEREO IMAGING, A MORPHOMETRIC APPLICATION FOR 3D VISUALIZATION AND VOLUME ANALYSIS OF CELLS IN TISSUE ENGINEERED CONSTRUCTS.

Vincent M.J.I. Cuijpers, X. Frank Walboomers, John A. Jansen.
Department of Biomaterials, Radboud University Nijmegen Medical Centre. P.O. Box 9101 6500 HB Nijmegen, The Netherlands
E-mail : v.cuijpers@dent.umcn.nl

KEY WORDS: SEM stereo imaging, micro-CT, SEM, 3D morphometry, cell analysis, tissue engineering.

In biomaterial and tissue engineering research, there are various techniques available to study the relation of cellular structures with the underlying biomaterial scaffold. Depending on the research question, each specific method will have individual pros and cons. When performing high resolution 3D morphometric analysis usually micro-computed tomography (CT) is applied, because of its non-destructive/ non-invasive character and the possibility to perform quantification. In addition, scanning electron microscopy (SEM) often is used because of the higher resolution and very high focus depth. Still, using SEM only the surface of samples with a pseudo-3D view can be assessed. In the current study we choose to combine SEM and 3D stereo imaging to fill the obvious void in analysis techniques between micro-CT and SEM for analyzing 3D cell morphology, and cell interaction with biomaterials. Alicona MeX™ software was used for 3D visualization and analysis. First the 3D SEM application was validated using standardized microspheres. Subsequently, cells were cultured on several biomaterials and tissue engineering scaffolds, to evaluated if the 3D SEM application could give additional quantitative 3D information. The results showed that reference and cutting plane adjustment are critical for final volume analysis. After adjusting and fine-tuning the iso-surfaces of the reference and cutting plane, the total cell volume was finally calculated accurately.

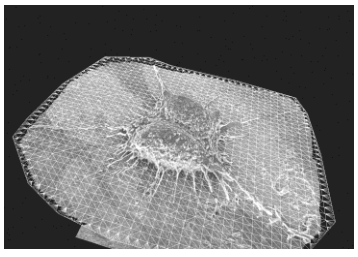
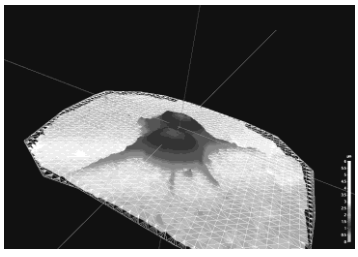
A.  B. 

Figure 1: Bi-nucleated MC-3T3 cell, (a) combined SEM and virtual reference plane image, (b) pseudo colored depth image.

CONCLUSION: 3D SEM stereo imaging is a useful tool to visualize and analyze quantitatively 3D single cells when conventional micro-CT and standard SEM techniques are not suitable.