Vascularization of Tissue

Single Cell Source
• Limited proliferation potential

Using stem cells to create tissue

Using only mesenchymal stem cells, bone can be made and vascularized

Differentiation can be evaluated by two methods: Immunofluorescence or angiogenic response on Matrigel™

The Problem

What is the difference between these three images?

Objective

Our objective was to develop two techniques that can be used to quantify both immunofluorescent and Matrigel™ images

• Criteria for quantifying
  • Total length
  • Number of branch points.
  • Using software to design a non-bias way of quantifying

Immunofluorescent Analysis

The original, unaltered image is difficult to see and it is hard to quantify differences between images

Thresholding the pixel value intensifies all pixels within the specified range, making those pixels bright green. This method makes the picture much clearer; however, also it also enhances background noise

To remove background noise, we restricted the area to a higher pixel connectivity value. By doing so, areas that have a large number of connected pixels remain green while smaller areas turn blue. The remaining green areas are considered positively-marked cells.

To find the total number of cells, we decrease the threshold value, which allows more pixels to be counted. More background noise is included, so the area restrictions must also be adjusted. The remaining green areas denote the total number of cells in the image.

Matrigel™ Angiogenesis Analysis

As referred to in “The Problem” looking at the angiogenic response on Matrigel™ is not an accurate way of comparing images. So we must quantify the images by using the criteria mentioned earlier

The length for each segment was individually measured and added to determine the total length.

The number of branch points was manually determined and was defined as places where three or more line segments were connected

Results

Quantification of Immunofluorescent Images

Conclusions

• The technique provides a non-biased way of quantifying images

• For all three markers, the fraction of positively-stained cells trends upwards over time. This trend is most pronounced for the VEGF Receptor 2.

• On matrigel™ all three markers increased from week 1 to week 2 in number of branch points & network length. The QK peptide appeared to form a better network.

Future Directions

Studies will be done to compare the cues that guide differentiation

• Substrate compliance

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