PROJECT 4A. IS CELL DIFFERENTIATION ALTERED BY STRAIN STIFFENING

Bone tissue engineering with autologous or allogenic mesenchymal stem cells (MSCs) has been widely developed. MSCs can be isolated from bone marrow or other tissues such as adipose tissue or umbilical cord, and can be implanted in bone defects with or without prior amplification and stimulation. However, the outcome of most pre-clinical studies remains relatively disappointing. A better understanding of the successive steps and molecular mechanisms involved in MSC-osteoblastic differentiation appears to be crucial. Recently it was shown that encapsulated hMSCs can be induced to differentiate down osteogenic and adipogenic pathways by controlling their three-dimensional environment using tethered small-molecule chemical functional groups.

Based upon the observation that HUVEC vasculatisation was modified by polymer length, we initiated an investigation using our GRGDS PIC cell culture biomimetic hydrogels, to see if strain stiffening could dictate stem cell fate. This 3D system is based on physiologically soft (~0.2–0.4 kPa) polysiocyanate-peptide (PIC) gel, which was thought to be a close approximation of the adult stem cell niches present in the human body. Importantly this soft material shows biopolymer-like stress stiffening which can be altered without modifying the gel stiffness and porosity. Intriguingly we observed that osteogenic commitment and differentiation of mesenchymal stem cell, is modified by changing only the polymer length, not the matrix stiffness. The ability to tune the matrix, allows several questions to be asked:

- Is the strain stiffening really a key parameter in cell differentiation?
- How would differentiation change if strain stiffening was fixed, but the integrin binding density was altered?
- How would the differentiation alter if the material properties were fixed and an additional integrin modified polymer, not connected to the matrix, was added?
- What would occur if the strain stiffening properties were fixed and the material stiffness was altered?

PROJECT PLAN

1. Variation of the ligand density of the PIC polymers at constant polymer lengths (and hence σC). An especially important experiment will be to load approximately the same number of ligands (as present in the longest polymer) on the shortest polymer, and vice versa (small number of ligands in the long polymer). Study if the multivalency and focal adhesion assembly (integrin-ligand clustering) dictate osteogenesis. Investigate if the polymer length is the key important parameter to determine stem cell fate (since strain stiffening may only serves to stabilize the already assembling/assembled focal adhesion complex).

2. Doping of the polymer matrix with a small percentage of RGD-modified tetra-polymer (which are more flexible) This experiment may identify if flexible chains not attached to the gel matrix can achieve the same stem cell fate as observed in the present experiments. This will further serve to identify the importance of integrin-RGD clustering.
3. Embedding of single beads modified by RGD-interacting motif in the gel, and pulling of the beads by optical tweezers. Examination of whether the forces exerted by the cells (~10 nN) can actually take the gel into stress-stiffening regime.

4. Quantification of the integrin-RGD bond formation, and shedding light into the formation of integrin-RGD clustering gels and their correlation with stem cell fate using Confocal-Rheometry.

5. Probing the role of the protein associated with microtubule dynamics in the present set of polymer gels (stain for the proteins etc.); Check the expression of this protein in 3D stem cell culture in traditional stiff synthetic gels. In order to understand if indeed the expression of this protein reflects stiffness of the matrix helping unravel the mechanistic pathway of mechano-transduction.

THE CANDIDATE
Strong background in physical organic chemistry
Polymer characterization
Rheology, protein modification