Physical and Numerical Design of a Fluidised Bed Bioreactor for Stem Cell Expansion

I. Benzeval[1], I.G. Turner[2], M.J. Ellis[1]
Departments of Chemical Engineering and Centre for Regenerative Medicine[1] and Mechanical Engineering[2], University of Bath, Claverton Down, Bath, BA2 7AY, UK
(email: i.benzeval@bath.ac.uk)

Introduction

Bone substitutes with enhanced biological activity are required for the replacement, repair and regeneration of skeletal tissue. This project aims to design a bioreactor for stem cell expansion on novel calcium phosphate based (HA/TCP) bone substitute materials designed for accelerated osseointegration of implants. The substitute material will be porous to allow cell expansion throughout. Mathematical modelling has been used alongside physical and biological experiments to define the bioreactor environment.

Bone Substitute Material

Porous bone substitute material has been produced from hydroxyapatite and tricalcium phosphate (HA/TCP)[1]. Figure 1 shows a photograph of a porous particle made from HA/TCP and some smaller, denser, medical grade calcium phosphate based granules. The aim of this project is to seed this material with bone cells and then grow them in a fluidised bed bioreactor; a method which allows both good mixing and transport of nutrients and also causes some controllable shear on the cells. Figures 2 and 3 show SEM images of the porous particles. The surface comprises individual grains of HA/TCP which can also be seen in Figure 4, an SEM image of a flat disk of the same HA/TCP material, produced in a press. These will be used as static controls and to evaluate cell-surface interactions. Figure 5 shows an SEM image of MG63 cells grown on a flat disk of HA/TCP.

Figure 1: A porous HA/TCP particle (left) and medical grade calcium phosphate granules (right). Porous particle dimensions are approximately 5x5x5 mm

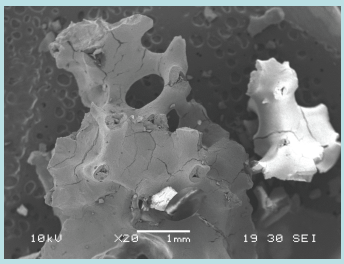

Figure 2: An SEM image of a porous HA/TCP particle (20x magnification)

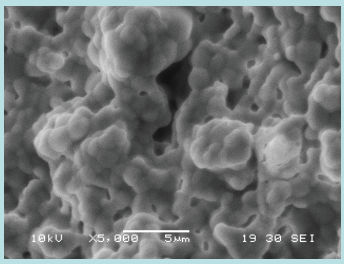

Figure 3: An SEM image of a porous HA/TCP particle (5000x magnification)

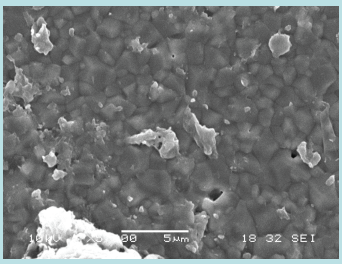

Figure 4: An SEM image of a flat disk of HA/TCP (5000x magnification)

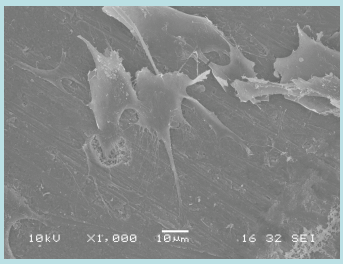

Figure 5: An SEM image of MG63 cells growing on a flat disk of HA/TCP (1000x magnification)

Modelling of Distributor and Fluidisation

A CFD model of the distributor and fluidisation is being developed to enable aspects of the design to be optimised outside of the laboratory. Figures 6 – 8 show a model of liquid flow through a distributor in a 25 mm diameter column. The 6 mm thick distributor is placed 10 mm above the narrow inlet nozzle to improve the distribution of the liquid across the column.

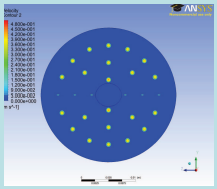

Figure 6: Top view of distributor, with velocity profiles

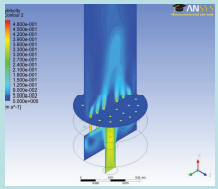

Figure 7: Isometric view of column with velocity profiles

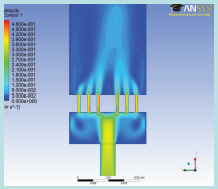

Figure 8: Side view of column with velocity profile

Figure 9 shows a model of just the 25 mm diameter column in 2D, without the distributor, but containing two phases. The solid phase volume fraction is shown in the images. The solid phase was modelled as an Eulerian continuum of 2 mm spherical particles. The superficial liquid velocities passing up through the column are, from left to right, 0.02, 0.03, 0.05, 0.07 and 0.08 ms^{-1}. It can be seen that as the velocity is increased the particles begin to fluidise within the column. The next stage of modelling will be to adjust the parameters of the model so that it closely mimics the actual fluidisation of the granules (see right).

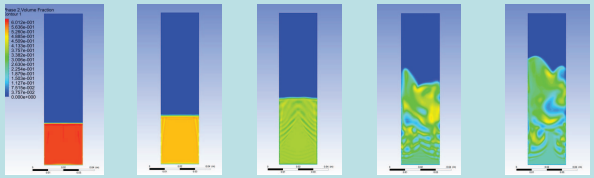

Figure 9: Fluidisation of 2 mm particles with similar physical properties to the calcium phosphate granules. Volume fraction of the solid phase is shown. Superficial velocities of liquid phase are, from left to right: 0.02, 0.03, 0.05, 0.07 and 0.08 ms^{-1}

Fluidisation of Calcium Phosphate Granules

Initial tests have been performed to fluidise the dense bone substitute material shown on the right hand side of Figure 1. Figure 10 shows images of the particles being fluidised in a 25 mm diameter column. The distributor comprised 1 mm holes in a non-optimised pattern. In the first image, the flow rate is insufficient to fluidise the particles. In the second image the flow rate has been increased and the bed has slightly expanded, the gaps between the particles can be seen to have increased in size. In the third image the flow rate has been increased again and has resulted in greater mixing of the particles. In the final image the flow rate has been increased to achieve full fluidisation, with a greatly expanded bed and significant mixing.

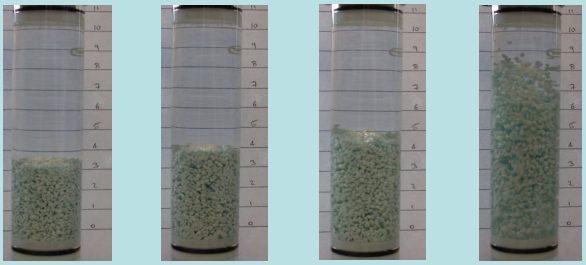

Figure 10: A bed of calcium phosphate granules at varying fluid velocities resulting in increasing degrees of fluidisation. Values for the superficial velocity through the bed are, from left to right: 0.030, 0.035, 0.048 and 0.071 ms^{-1}.

Fluidisation of these granules was facile and the flowrates relatively low. Attempts at fluidising the larger porous particles were hampered by their shape; a relatively smooth surfaced granule will readily brush past another, whereas the shape of the porous particles encourages interlocking. This resulted in the bed appearing to stop moving at times during the fluidisation, before an increase in the upward velocity of the liquid caused the particles to unlock and re-fluidise (images not shown).

The velocities required to achieve fluidisation of the granules were higher than those predicted by the modelling (see left). This is due to a combination of factors, but primarily the model is for spherical particles and the size distribution of the granules is not taken into account.

Modifications to the modelling and the experimental design will now be made to account for these findings.

Conclusions

- The SEM images show that cell expansion on this material is possible. Cell expansion on the particles and granules, as opposed to the flat disks, will also be dependent on the cells' interaction with the surface which shows greater undulation, but similar physico-chemical properties to the flat disks.
- Fluidisation of the granules was achieved, but fluidisation of the porous particles suffered problems due to their interlocking nature. This could be overcome with a larger diameter column, an optimised distributor or by applying mechanical force to the particles to cause any large protuberances to be removed, thus reducing interlocking.
- The modelling has shown that the velocity required to fluidise spherical particles of similar size and density to the granules underestimated the velocity required. The next stage of the modelling process will be to adapt the model to account for this difference.

References

1. Hsu, Y.H. *et al.* J. Materials Sci.: Mat. in Med. **18**, 1931-1937 (2007)