Developing an *in vitro* model to predict the impact of the foreign body response on controlled protein release from injectable depot formulations

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Background: It is well-established that the foreign body response (FBR) can eventually lead to fibrotic encapsulation of implanted biomaterials. Whilst the fibrotic capsule is reported to act as a barrier around encapsulated materials, there is limited understanding as to how the progression of the FBR may influence sustained drug release profiles from depot formulations. This is in part due to the lack of an established tool to study the FBR during formulation development. Thus, we developed an *in vitro* co-culture model to study how the dynamic cellular events of the FBR may impact controlled protein release from injectable hydrogel depot formulations.

Methods: We initially identified optimal conditions to induce macrophage fusion for generating foreign body giant cells, which are a key feature of the FBR and stimulate fibroblasts to produce collagen during fibrotic encapsulation. Human primary monocyte-derived macrophages (n=4 donors) were cultured on either tissue-culture polystyrene (TCPS) or polyethylene terephthalate (PET) surfaces for up to 28 days with various cytokine combinations added to induce macrophage fusion. FBGCs were characterised by comprehensive image analysis at multiple time points and quantification of secreted cytokines and growth factors by ELISA. To establish a co-culture model of macrophages and human foreskin fibroblasts, we first performed a series of indirect co-cultures using conditioned media and Transwell inserts to evaluate the effects of paracrine signaling between the cell populations. In addition to analyses of FBGC as described above, profibrotic fibroblast activity was evaluated by quantifying COL1A1, COL3A1, TGF-β1 and α-SMA gene expression and visualising myofibroblast differentiation and collagen deposition with fluorescence microscopy. Finally, we incorporated a 3D matrix into our model to provide a more representative environment and are currently optimising conditions to produce a 3D direct co-culture model to study the FBR to injectable hydrogel depots.

Results: Less than 20% macrophage fusion occurred on TCPS, regardless of cytokine combinations. Changing the material surface to PET resulted in at least 30% of macrophage populations fusing without any additional cytokine stimulation. The combination of PET in the presence of the interleukin(IL)-4 cytokine stimulated up to 97% of macrophages to fuse into FBGC, the largest of which contained up to 48 nuclei. High levels of fusion were achieved ≥3 weeks of culture. However, after 7 days of culture, cells on PET with IL-4 displayed filopodia-like protrusions and were observed to clump together in large cell clusters. This observation was also correlated with an increase of the chemokine IL-8 when compared to the control cells on TCPS (993.8 ± 567.6 (SEM) pg/mL vs. 56.9 ± 11.9 (SEM) pg/mL) and may facilitate cells to develop the necessary motility and contact to enable fusion to occur. We also detected a significant increase in PDGF ($p<0.01$) at day 14 when cells were cultured on PET with IL-4 compared to TCPS (682.2 ± 398.1 (SEM) pg/mL vs. 10.0 ± 17.4 (SEM) pg/mL). The increased PDGF secretion correlates with early stages of macrophage fusion and suggests a polarisation bias towards tissue repair activities, which may also stimulate collagen deposition from fibroblasts in our co-culture model.

Conclusions: We successfully identified conditions to induce high levels of macrophage fusion and large FBGCs. We observed a synergistic effect of biomaterial surface and cytokine stimulation on macrophage fusion and conditions associated with high macrophage fusion were correlated with an increased secretion of mediators that influence cell migration and collagen production. We later aim to introduce hydrogel formulations into our optimised 3D co-culture model to predict the impact of the FBR on protein release.