Implantation of muscle precursor cells grown as 3D structures improves muscle regeneration after myotoxic injury

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The implantation of muscle precursor cells offers exciting possibilities for enhancing the repair of skeletal muscle tissue after injury, however previous attempts at myoblast implantation have produced disappointing results. Recent evidence suggests that a number of cell types have far better rates of survival when cultured in 3-dimensional cell aggregates ('spheroids') prior to implantation (Yap *et al.*, 2013). To date, however, no studies have examined whether implanting spheroids made from muscle precursor cells can assist the repair of injured muscle. The aim of this study was to investigate whether growing muscle precursor cells into 3D spheroids before implantation into regenerating muscles would enhance muscle repair after injury.

All *in vivo* experiments were approved by the Animal Ethics Committee of La Trobe University and conducted in accordance with the codes of practice stipulated by the National Health and Medical Research Council (Australia). Immuno-deficient (SCID) mice (12 wks old) were anaesthetized (4% isoflurane in O_2, 2L/min *via* inhalation), and the *tibialis anterior* (TA) muscle of the right hindlimb was injected with cardiotoxin (CTX; 50µl of 10µM solution, *i.m.*) to cause complete muscle fibre degeneration. C2C12 myoblasts were either cultured in standard conditions or formed into 3D spheroid structures (25,000 cells/structure). Three days after the initial cardiotoxin injury, mice were re-anaesthetized (4% isoflurane in O_2, 2L/min) and the injured muscle was injected with either spheroids (20 × 25,000-cell spheroids in 40µl saline), an equivalent number of dissociated monolayer cells ($5x10^5$ cells in 40µl saline) or vehicle (40µl saline). Mice were allowed to recover for a further 4, 11 or 18 days (corresponding to 7, 14 or 21 days post-CTX). After the recovery period mice were anaesthetized (60 mg/kg, sodium pentobarbital, *i.p.*) and TA muscle function was assessed *in situ* as reported previously (Gehrig *et al.*, 2010). At the conclusion of the experiment mice were killed by cardiac excision while still anaesthetized deeply, and the TA muscles were removed for further analysis.

We found that spheroid implantation into regenerating muscle had no significant effect on twitch characteristics or absolute tetanic force (Po) at 7, 14 or 21 days post-injury when compared with mice who received monolayer cells, however tetanic force normalized to muscle size (sPo) was higher in mice who had received spheroids than those who had received monolayer cells. These results suggest that forming muscle precursor cells into 3D spheroid structures before implantation may enhance their ability to influence muscle regeneration.

Gehrig SM, Koopman R, Naim T, Tjoakarfa C & Lynch GS. (2010) *American Journal of Pathology* **176:** 29-33.
Yap KK, Dingle AM, Palmer JA, Dhillon RS, Lokmic Z, Penington AJ, Yeoh GC, Morrison WA & Mitchell GM. (2013) *Biomaterials* **34:** 3992-4001.