**Towards cardiac stem cell therapy: characterisation and cryopreservation of canine cardiosphere-derived cells**

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The use of stem cells to treat cardiac disease has gained increasing interest in recent years. Cardiosphere-derived cells (CDCs), an adult cardiac progenitor cell population, are the most promising candidates for cellular therapy. Their application in rodent models and phase 1 human trials of ischaemic myocardial disease showed promise measured by increased left ventricular function. However, much remains unknown about their basic biology, especially in dogs. To expand this treatment to treat canine dilated cardiomyopathy requires the creation of cryopreserved allogeneic cell banks since this allows timely access to large cell numbers and avoids obtaining diseased autologous myocardial tissue from potentially unstable dogs. However, CDCs in culture conditions are subject to plasticity similar to other adult stem cell populations. We therefore investigated how passage number or cryopreservation may affect cellular potency of CDCs obtained from canine atrial explants.

CDCs were isolated and characterised from five cadavers with consent. Mesenchymal stem cells (MSCs) were isolated for comparison. CDCs demonstrated a population doubling time that was slower than MSCs (P < 0.05) but importantly was unchanged by cryopreservation (P = 0.71). Cryopreserved CDCs also demonstrated the same multi-lineage potential as fresh cells by showing commitment to myocardial, endothelial and smooth muscle lineages and maintained the ability to form clonal colonies. Flow cytometry analysis revealed fresh CDCs had a high proportion of cells expressing CD105 (89.0% ± 4.98) and CD44 (99.68% ± 0.13) with varying proportions of CD90+ (23.36% ± 9.78), CD34+ (7.18% ± 4.03) and c-Kit+ (13.17% ± 8.67) cells. CD45+ (0.015% ± 0.005) and CD29+ (2.92% ± 2.46) populations were negligible. Increasing passage number correlated with an increase in the proportion of CD34+ cells and a decrease in CD90+ cells (P = 0.003 and 0.03 respectively). Cryopreserved populations displayed increased positive populations for CD34 (P < 0.001) and fewer CD90+ cells (P = 0.042).

Our data revealed the impact of different canine donors on cell phenotype, as there was significant inter-donor variability on cellular morphology and marker expression. Overall, this study shows that despite these differences in the CD marker population, cryopreservation of canine CDCs is feasible without altering their differentiation potential. Importantly the CDCs we obtained from atrial tissue conformed to the generally accepted profile of CDCs obtained from other species and canine ventricular tissue. Further studies are required to investigate the fundamental biology and further characterise the phenotype of this heterogeneous stem cell population prior to clinical application.