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EDITORIAL

Hiding in plain sight: an encapsulated approach to cardiac cell therapy

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This editorial refers to 'Sustained subcutaneous delivery of secretome of human cardiac stem cells promotes cardiac repair following myocardial infarction' by A.R. Kompa *et al.*, pp. 918–929.

Cell-based therapies for treating myocardial injury were initially expected to enhance structure and function of damaged tissues via direct cell replacement, but relatively few injected cells engrafted at sites of injury.[1] Over a decade of research has led to the currently prevailing dogma that cellular therapies affect tissue repair largely via secreted paracrine factors and stimulation of host cells, rather than by cell replacement. For example, medium conditioned by human embryonic stem cell-derived mesenchymal stem cells (MSCs) significantly reduced infarct size in both mouse and pig models of myocardial ischaemia/reperfusion injury.[2] An important parameter of the Timmers *et al.*[3] study in 2007 was inclusion of size fractionation experiments that narrowed the active component in media to >1000 kDa (50–200) nm size range, later to be identified as exosomes. Exosomes are classified as extracellular vesicles (EVs) or cell-derived lipid bound membranous structures shed from most cells under normal as well as pathological conditions.[4] Developing a much needed therapeutic approach for sustained delivery of paracrine-mediated benefits for cell therapy upon damaged myocardium has been the main focus of both basic research and clinical testing.

Kompa *et al.*[5] demonstrate subcutaneous implantation of TheraCyte devices as a safe and feasible technique for cell transplantation into damaged myocardium (*Figure 1*). The TheraCyte medical device is a thin membrane-based polymeric chamber, well established for cell encapsulation studies *in vivo* since 1996 when pancreatic islets were first transplanted into nude mice[6] and appears in over 80 scientific publications to treat insulin resistance and diabetes,[7] deliver molecules such as C-X-C motif chemokine 12 (CXCL12),[8] and to provide for continuous delivery of therapeutic peptides.[9] In this present study by Kompa *et al.*, cardioprotective effects in a rat model of myocardial infarction (MI) were mediated by W8B2$^+$ human cardiac stem cells (CSCs) encapsulated in TheraCyte devices subcutaneously implanted on the dorsal side of the animals. Specifically, rats receiving CSCs at time of infarction showed preserved left ventricular ejection fraction and attenuated maladaptive cardiac remodelling in post-MI hearts via sustained release of EVs. Encapsulated CSCs avoid immune rejection protected within the semi-permeable inner membrane bilayer of the TheraCyte implant. The two membrane layers are further specialized for functional effects with the inner membrane allowing soluble factors and EVs to freely pass, while the outer membrane promotes blood vessel formation to facilitate exchange of oxygen and nutrients. Cellular encapsulation allows trafficking of CSC-derived EVs intended to provide the reported cardioprotective outcome mediated by CSC secretome and associated paracrine factors. Cells cultured in the TheraCyte device three-dimensional (3D) environment secreted more EVs with smaller size and more homogenous shape compared to two-dimensional culture condition *in vitro*, consistent with 3D culture influences.[10]

Distribution of EVs produced by W8B2$^+$ CSCs following implantation was tracked using a multimodal membrane reporter construct[11] prior to delivery. Unexpectedly, cells transduced with the biotinylated EV-GlucB reporter (GlucB + sshBirA) secreted a higher number of EVs and exhibited enrichment in protein composition mainly associated with membrane fusion, ubiquitination, and membrane trafficking. The spectrum of potential consequences associated with use of the reporter construct in the present study remains undetermined and is significant since tracking of donated CSCs and EV by-products will be irrelevant for clinical utilization. If the reporter system contributes to elevation of cardioprotective effects, then mechanism(s) of action will need to be delineated to assess impact of GlucB + sshBirA transduction. Subcutaneous implantation of TheraCyte devices leads to systemic rather than cardiac-restricted impact in the host. In addition to heart, EVs spread to multiple tissues including skeletal muscle, lung, liver, spleen, and kidney. Thus, possible impact of non-cardiac effects that influence cardiac reparative outcome cannot be excluded. For example, in addition to EVs localized within damaged myocardium, alteration of systematic inflammatory responses could also be involved. In particular, cardiac hypertrophy and angiogenesis were improved in the damaged myocardium following TheraCyte implantation. Multiple cell types including cardiomyocytes, pericytes, endothelial cells, and inflammatory cells provide molecular mediators to regulate angiogenesis and hypertrophy under physiological and pathological conditions. Potential off-target effects upon various tissues that may influence cardioprotection were not examined and need to be considered in future investigation. Bioinformatic analysis of EVs derived from cultured W8B2$^+$ CSCs demonstrated up-regulation of proteome constituents consistent with inflammation, immunoregulation, tissue

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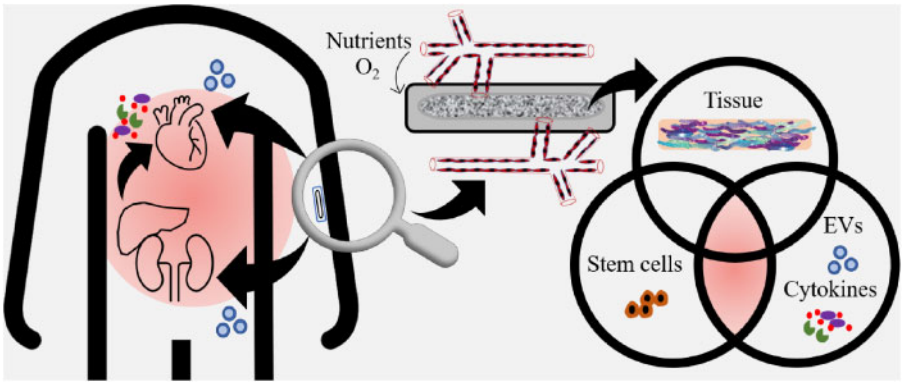

Figure 1 Subcutaneous implantation of a TheraCyte device (gray box, centre) supported by host vasculature allows various cell types to be implanted in recipient hosts allowing for long-term tissue survival as well as extended release of extracellular vesicles (Evs), cytokines and growth factors (circles on right). Injured tissue such as infarcted myocardium benefits from systemic delivery of secretome and paracrine factors (pink shaded regions).[5]

remodelling, cell survival, and angiogenesis. Identification of candidate paracrine factors provides valuable insights towards potential therapeutic targets, but the study provides only a descriptive catalogue based on correlative data rather than testing with cause-effect experiments. As such the present study stops short of delving into cellular mechanism(s) for the observed cardioprotective phenomenon of the W8B2$^+$ CSC's secretome, with future preclinical research and clinical testing needed to determine proteomic differences that occur once the CSCs are implanted *in vivo* within the TheraCyte devices.

Examination of the encapsulated cells 4 weeks post-implantation revealed vimentin expression, lack of CD31endothelial marker and limited smooth muscle actin (SMA) expression by W8B2$^+$ cells. These findings indicate partial change of cell properties as a consequence of *in vivo* implantation, consistent with an early study using TheraCyte devices reporting maturation of primary β cells into functional insulin-producing cells.[12] Such phenotypic drift of implanted cells following long-term *in vivo* transplantation raises questions for future assessment including: (i) what extent the W8B2$^+$ cells resemble their original characteristics over time, (ii) what phenotypic characteristics are changed, and (iii) influence of altered cell phenotype upon the secretory EV profile. By Week 4 following implantation, subdermal implants were encircled with host vasculature. Surrounding vessels not only circulate cell-secreted factors throughout the host, including the damaged heart, but also diffuse oxygen and nutrients into the implant to maintain survival of the encapsulated cells. However, as the cells expand inside TheraCyte devices,[5] cells residing within the central core away from host microcirculation surrounding the implant may undergo hypoxic stress and cell death. Clinical application of TheraCyte device as a long-term cell delivery approach would require cells within the device to promote their own self-sustaining angiogenic and vasculogenic activity. One benefit of TheraCyte encapsulation is that unlike injection of exosomes,[13] naked DNA,[14] or specific growth factors and cytokines,[15] the CSCs are able to sense their environment and adapt to secrete both soluble proteins and EVs relative to environmental cues. The TheraCyte device protects cells within, allowing the W8B2$^+$ CSCs to survive *in vivo* unlike delivery approaches directly into the harsh cardiac ischaemic zone typified by high levels of free radicals and oxidative stress. Intravenous delivery of traditional cell suspensions is hampered by inefficient trafficking to the injury site due to trapping in capillary beds of organs such as the lungs. In comparison, encapsulation protects cells within a permselective membrane. Cell encapsulation devices also could facilitate allogenic and xenogeneic cardiac cell therapy, but such immunologically incompatible transfers require extensive testing in non-immunocompromised animals and heart disease patients subsequent to the immunodeficient models as utilized by Kompa *et al.* in the present study.

Encapsulation in permselective membrane has been employed for diverse applications including drug and standard cell-based therapy as well as immunosuppressive-free cell-based therapy. The TheraCyte device is one of many immunoisolation devices being explored to harness sustained delivery of a cell's secretome. Further validation, such as the study by Kompa *et al.*, is required for both safety and efficacy before a cell encapsulation device will be approved to treat cardiac injury.

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