Published in final edited form as:

Expert Rev Neurother. 2008 August ; 8(8): 1193–1201. doi:10.1586/14737175.8.8.1193.

Cell-based therapy in ischemic stroke

Abstract

Cell-based therapy for stroke represents a third wave of therapeutics for stroke and one focused on restorative processes with a longer time window of opportunity than neuroprotective therapies. An early time window, within the first week after stroke, is an opportunity for intravenously delivered bone-marrow and perinatally-derived cells that can home to areas of tissue injury and target brain remodeling. Allogeneic cells will likely be the most scalable and commercially viable product. Later time windows, months after stroke, may be opportunities for intracerebral transplantation of neuronally-differentiated cell types. An integrated approach of cell-based therapy with early phase clinical trials and continued pre-clinical work with focus on mechanisms of action is needed.

Keywords

Stem cell; stroke; restorative therapy; cell-based therapy; SDF-1; mesenchymal stem cell; marrow stromal cell; Phase I clinical trials

There is increasing scientific and public interest in the use of stem cells to treat a host of neurological conditions including stroke. A more precise term than “stem cell therapy” to describe this approach is “cell-based therapy”. Not all proposed cell types would meet the precise definition of a stem cell as a self-renewing cell that can give rise to all cells in an organ.

Cell-based therapy represents the “third wave” of stroke therapeutics. The first therapeutic approach was the “chemical” or small molecule in which drugs were designed to bind to specific targets such as the NMDA receptor. This “big pharma” approach has not yielded any Food and Drug Administration (FDA)-approved treatments in acute stroke and there is concern that the chemical approach is becoming exhausted as reflected in the decline of new drug approvals by the FDA. The next approach was the “protein therapeutic” in which proteins were genetically engineered to replace or supplement a normal human protein.[1] This approach has yielded one FDA approved treatment for stroke-tissue plasminogen activator (tPA). Cell-based therapy represents a third wave in the approach to stroke therapeutics. While this approach has been criticized for its lack of a specific target and its unclear mechanism of action, the combination of a dire clinical need, the advantage of a longer window of opportunity, and promising pre-clinical data has generated interest from patients, physicians, scientists and small biotechnology companies.

Cell-based therapy in stroke contrasts from the use of stem cells to treat diseases such as diabetes and Parkinson's disease where restricted populations are lost (beta cells in the pancreas or dopaminergic cells in the brain) and the focus is on cell replacement. In stroke, multiple cell types have been lost and it will be important to repair both blood vessels (endothelial cells, smooth muscle cells and pericytes) as well as astrocytes, neurons, and oligodendrocytes.

Another important distinction is that stroke is an acute injury and there is no ongoing degenerative process or major immunological attack. In diabetes, there is concern with ongoing immune destruction of transplanted cells and in the case of Parkinson' disease, the underlying pathological process (Lewy bodies) has been shown to appear in the graft.[2] In stroke, the injury process is acute and limited in time; the brain may be more hospitable to transplantation than in other diseases where there is an ongoing degenerative process or immunological attack.

Cell-based therapy of stroke has many striking parallels to cell-based therapy approaches to myocardial ischemia and infarction. The cardiovascular field got off to an earlier start with cell-based therapies and is more mature than the stroke field. As thrombolytic therapy of stroke learned many lessons from the use of thrombolytic in myocardial infarction, the restorative stroke field can learn much from the cell based therapy experience in cardiology.

In the cardiovascular field there was an ongoing debate whether the time had arrived for clinical trials of cell therapy in stroke. Concern was voiced of whether cell-based trials were premature and a moratorium on new clinical trials was proposed until the mechanism of action, the optimal cell type, delivery route, and dose could be defined from pre-clinical studies.[3] In response to this debate the National Heart Blood and Lung Institute (NHLBI) organized a Working Group that met August 5-6, 2004 in Bethesda, MD. This Working Group recommended an integrated approach with phase I clinical trials and continued basic research into the mechanisms of cell-based therapy. The Cardiovascular Cell Therapy Research Network was established and charged with designing and beginning phase I and II clinical trials of cell-based therapy to optimize ventricular function.[4] This strategy in cardiovascular diseases has resonated in the stroke community and been mirrored with the establishment of the Stem Cell Therapeutics as an Emerging Paradigm in Stroke (STEPS) group under the aegis of the Stroke Academic Industry Roundtable (STAIR). The first meeting of STEPS was on October 27-28, 2007 in Arlington, VA and a consensus paper is forthcoming recommending a similar integrated approach of starting phase I clinical trials and continued pre-clinical, basic work.

Cell-Based Clinical Trials to Date

There are two broad cell-based therapy approaches in stroke. The first relies on transplanting exogenous cells into the brain (the focus of this review) while the latter is more indirect and better termed “neurotrophic” therapy. Neurotrophic therapy aims to stimulate and mobilize endogenous stem cells in the brain or mobilize stem cells into the brain with growth factors. It is of interest that two leading candidates in this area are both hematopoietic growth factors—Erythropoietin (EPO) and G-CSF.[5,6]

It is unknown which cell type is optimal for transplantation. To date, three early phase cell-based therapy trials in stroke have been completed and reported. In the first reported clinical trial, human neuronal cells (LBS-Neurons) derived from a human teratocarcinoma line were stereotactically transplanted into the basal ganglia in 12 patients with basal ganglia infarct of 6 months to 6 year duration. [7] Six of 11 patients with follow-up PET scans at 6 months showed increased fluorodeoxyglucose at the transplant site and 6 patients showed improvement in the European Stroke Scale. This trial was followed a phase II randomized, observer-blinded trial of 18 patients with a basal ganglia stroke 1-6 years ago and a fixed, stable motor deficit. [8] Patients were randomized to two cell doses or a non-surgical group (n=4) and both the treated and the non-surgical control group received a stroke rehabilitation program. While the transplantation procedure was feasible and safe, there was no improvement in the primary outcome, the change in the European Stroke Scale at 6 months, in the transplanted group although there was significant improvement in some of the other functional scales (Action Arm Research Test and the Everyday Memory Test). This approach was abandoned when the sponsoring company encountered financial difficulties.

In a small Phase I trial of xenotransplantation, five patients with basal ganglia infarcts were transplanted intracerebrally with fetal porcine cells.[9] To reduce the risk of rejection, the cells were pre-treated with an anti-MHC1 antibody. Although two patients showed improvement that persisted at 4 years, the FDA terminated the trial after 5 patients; one patient had neurological worsening and another a seizure one week after transplantation. Finally, in a phase I study of autologous marrow stromal cells (MSC) in stroke, 5 patients were treated with a total of 1×10^8 intravenous autologous MSC in two divided doses at 4-5 and at 7-9 weeks after

stroke.[10] The treatment was feasible, safe and there was improvement in the MSC group in the Barthel index and modified Rankin scale compared to an untreated control group of 25 patients. While there was no apparent difference in the change in the infarct size at 12 months, there was less atrophy in the per-infarct area and less ventricular dilation in the MSC group.

Cell types: Pre-clinical work

Optimal design of cell-based clinical trials requires pre-clinical work where the mechanism of action, optimal cell type and dosing, route of administration, cell tracking, and engraftment and fate of cells can be defined. There is growing pre-clinical data of a number of cell types in animal models of stroke showing a beneficial effect on functional outcomes days to weeks after the stroke. (Table 1) These cell types (Figure 1) can be grouped into a number of categories: a.) bone marrow-derived such as marrow stromal cells (MSC) or mesenchymal stem cells, multipotent adult progenitor cells; b.) perinatally-derived- umbilical cord stem cells, placental stem cells, amniotic fluid; c.) neural stem cells d.) encapsulated porcine choroid plexus cells; and e.) embryonic stem cells. Marrow stromal cells, a type of mesenchymal stem cells, reside in the bone marrow and form a population distinct from hematopoietic stem cells. MSC develop into cartilage, fat, bone, and muscle and some studies indicate they differentiate into neuronal cells in culture. They are promising for their regenerative and tissue engineering potential. [11]

Chopp and colleagues have shown a beneficial effect of MSC on functional outcomes in rodent models of stroke delivered by intravenous, intraarterial, and intracerebral routes. [12-16]The IV route maintains efficacy even when treatment is delayed for 1 month.[17] The mechanism of this effect involves trophic effects of the MSC and increases in angiogenesis, neurogenesis, synaptogenesis with favorable remodeling of the brain.[13,18-20] Similarly, human umbilical cord blood stem cells (HUCBC) have improved functional outcome in rodent models of stroke. [21-23] [24]Intravenous delivery of HUCBC 48 hours after stroke reduced the expansion of infarct size and improved behavioral outcomes. [22]The IV route of HUCBC also seems to be more effective than delivering the cells directly into the striatum. [25] Entry of HUCBC into the brain parenchymal may not be necessary for the beneficial effect.[26] HUCBC reduce splenocyte activation and appear to work at least partially by an immunosuppressive effect on the immune and inflammatory reaction after stroke.[27] However, not all investigators have found a neuroprotective effect of HUCBC in animal stroke models.[28] Multipotent adult progenitor cells first isolated by Verfaillie and colleagues are multipotential cells isolated from human bone marrow that can differentiate into cells of the three germ layers.[29] They are effective at improving outcome when delivered intracerebrally into animals after stroke and also improved functional outcome in animal models of neonatal hypoxia-ischemia when delivered by an intravenous route days after injury.[30-32] Both MSC and multipotent adult progenitor cells are “immunoprivileged” and exert an immunosuppressive effect.[33] These cell types are being developed as an allogeneic, “off the shelf” therapy.

There are also approaches of combined cell and gene therapy where the stem cell has been differentiated down a “neural” pathway or the cell has been to express a gene product. A cloned neural stem cell line (ReN001) has been conditionally immortalized with the fusion transgene c-mycERTAM that permits controlled expansion in the presence of 4-hydroxytamoxifen.[34] In the absence of 4-OHT and growth factors the cells undergo growth arrest and differentiate into neurons and astrocytes. Transplanting of these cells in a “chronic model of stroke”, 3-4 weeks after ischemia in a rodent TMCAO suture occlusion model, improved sensorimotor function 6-12 weeks post transplantation.[35] There was no difference in lesion size. Dezawa and colleagues have transfected the Notch intracellular domain into marrow stromal cells and have efficiently induced functional post-mitotic neuronal cells from both rat and human MSC (so called marrow stromal cell-derived neuronal cell (MSNDC))[36] In a rat MCAO model,

intracerebral transplantation of these MSDNC in the peri-infarct forebrain on day 7 after stroke showed significant functional and behavioral improvement and the transplanted cells. Both the behavioral improvement and transplanted cell survival was significantly better than a comparison MSC group (not transfected with NCI). The cells migrated from the injection site into ischemic boundary area and more than 80% of the cells expressed the neuronal marker, MAP-2. [37] Both the ReN001 and the MSNDC intracerebral approach are being explored as a therapy for chronic stroke.

Although not as far advanced in pre-clinical studies, placental stem cells,[38] amniotic-fluid derived cells,[39] and stromal cells derived from adipose [40] all have attractive features that make them candidates for the pipeline of cell-based therapy in stroke. Embryonic stem cell-derived neuronal precursors therapy are effective in rodent models[41] although ethical concerns and issues of tumorigenicity limit its advance into the clinic. The embryonic stem cells will need to be differentiated into a neuronal or differentiated cell phenotype before transplantation.

The body of pre-clinical evidence suggests that the major mechanism of intravenous cell-based therapy in stroke is not by direct cell replacement. The most likely mechanism are “trophic” and anti-inflammatory and immunosuppressive effects. MSC and other cell types secrete a number of trophic factors such as VEGF, BDNF.[42] There also is cross-talk between MSC and endogenous tissue cells such as astrocytes and endothelial cells; MSC and other cell types may induce these endogenous cells to secrete trophic factors. Pre-clinical data suggest that cell long term persistence and engraftment in the brain is not necessary for a therapeutic effect. Finally, the immunomodulatory effect of these MSC may explain some of their neuroprotective effect. [33] MSC are being used to treat graft versus host disease. [43]

Timing and Route of Administration

The advantage of cell-based and other restorative therapies is the potential for a longer window of opportunity to intervene. While neuroprotective agents will require ultra-early administration, cell-based therapy may be applied days, weeks, and even months after the injury allowing many more patients to benefit.

There are likely multiple windows for intervention (Figure 2). An early time window of 24 hours out to a month may be ideal for intravascularly administered cells. Cells may be delivered by an intra-arterial catheter directly into the middle cerebral artery or by an intravenous route. Intravenously delivered cell will need to “home” and target to the injured tissue. After infarction, homing signals such as SDF-1 are expressed by the injured brain allowing cells that express CXCR4 and CXCR7 to home to the injured tissue.[44,45] In rodents, SDF1 is expressed for up to a month after injury; we know less about expression in human brain.

A later window of opportunity might be more ideal for intracerebrally transplanted cells. The approach here is to differentiate the cells down a neuronal or astrocyte lineage and transplant at later time points. These can be delivered at the site of injury and the lack of inflammation at later time points may allow better engraftment. Moreover, at later time points, after a cyst has developed, the use of NSC in combination with a biodegradable scaffold may enhance transplantation.[46]

Dosing

Doses tested in phase I clinical trials should be derived from pre-clinical data. Currently, best approach is to extrapolate the dose from rodents to humans based on weight or brain size. However, it is not clear that a cell dose in rodents can be extrapolated to humans. Rodents metabolize most pharmaceutical agents much faster than humans; it is not clear if the turnover

in cells differs between rodents and humans. There may also be a benefit in repeated dosing, particularly with intravenous administration. “Booster doses” may enhance the clinical effect.

Scalability

Scalability will be an important issue in bringing any cell-based therapy into clinical trial and its eventual approval by the FDA. The advantages of autologous cells are their lack of rejection and lack of any graft versus host disease. Moreover, minimally manipulated autologous cells can potentially escape regulatory burdens. However, under the governing statute, Public Health Safety Act, Section 361, the FDA regulates cells that are “highly processed, are used for other than their normal function, are combined with non-tissue components, or are used for metabolic purposes”. [47] The quantity of cells needed for IV or IA transplantation in stroke will likely require cell culture and expansion and thereby require FDA approval. Moreover, it can take weeks to grow enough cells to give back to the patient and this may extend beyond the therapeutic time window. In addition, mesenchymal cells from older stroke patients are senescent and may not be ideal for transplantation. [11,42] Allogeneic cells have the advantage of providing a scalable, off-the shelf, universal product.

Safety Issues

The specific safety concerns will be determined by the route of administration and cell type. With intravenously delivered cells, acute infusional toxicity will need to be monitored as cells can potentially plug and obstruct the pulmonary vasculature. Therefore, pulse oximetry and telemetry will be needed around the time of infusion. With intra-arterial delivery there can be plugging and obstruction of cerebral vasculature by the infused cells while with intracerebral routes, seizures and complications of the surgical procedure will need to be carefully monitored. With all routes, there will need to be long term monitoring for tumor formation.

Outcome Measures

One of the greatest challenges in designing clinical trials of cell-based and restorative therapies in stroke is the selection of the proper outcome measures. In cardiac trials, there are easily quantifiable measurements such as left ventricular ejection fraction and left ventricular end diastolic volume that serve as objective, quantifiable primary outcomes. [48-50] These quantifiable measurements are lacking in stroke, where the size, location, and mechanisms of stroke are more heterogeneous than myocardial infarction and where a quantifiable ‘brain function test’ analogous to echocardiographic or MRI measures of ventricular function is lacking

To date, neuroprotective trials in stroke have relied upon composite clinical outcome scales such as the Modified Rankin scale and the Barthel index measured at 90 days after stroke. However, these scales may miss clinically significant recovery of language in an aphasic patient or recovery of fine motor control in a patient with hand weakness. Cramer and colleagues have proposed that “modality specific outcome measures” are more appropriate in recovery trials. [51]. Thus the proper outcome measurement should be tailored to specific patients; in patients with upper extremity motor weakness, specific motor function tests such as the Fugl Meyer should be the primary outcome while and in aphasic patients, specific aphasia scales. It has been suggested that restorative therapies may even receive a “modality specific” FDA labeling, e.g. approval for motor recovery, or approval for language recovery. [51]

One of the advantages of restorative trials with late enrollment and treatment is the availability of a stable baseline deficit. This will be easier with studies enrolling patients 3-6 months after stroke when most recovery has ceased; a homogeneous patient population can be identified permitting smaller sample sizes. Enrolling patients in earlier, subacute phase trials will be more challenging in this regard; patients in the first week will still not have reached a plateau and

larger sample sizes or the inclusion criteria may be restrictive to only patients with specific stroke sizes and location by MRI.

It will be critically important to always combine cell-based therapy with stroke rehabilitation. Exposure of the animal with neurological injury and stroke to a stimulating and enriched environment improves functional outcomes.[52-56] Experience-dependent therapies also improve functional outcomes in stroke patients. [57] Every patient in a restorative clinical trial should receive standardized and comparable rehabilitation care. Cell-based and neurotrophic therapies can be viewed as “adjunctive” to experience-dependent rehabilitation therapies and part of “combination neurorestorative” therapies. While functional outcomes should be the primary outcomes in restorative trials, imaging techniques may serve as surrogate measures and measure of “proof of concept” and activity in early trials. Diffusion tensor imaging with tractography permits measurement of the integrity and recovery of white matter tracts [58-61] Moreover functional MRI allows the monitoring of the effects of therapies on cortical plasticity and may be used to select patients most likely to respond.[62] The recent findings of a technique employing magnetic resonance spectroscopy to image neurogenesis in humans may allow the monitoring of effects of cell therapies and neurotrophic factors on endogenous neurogenesis after stroke.[63]. Tagging and tracking of stem cells with superparamagnetic iron oxide (SPIO) labeled cells and MRI should be pursued to determine the fate of intravascular delivered cells and their migration in the brain and in other organs. [64,65]

Regulatory issues: the FDA

Any cell based therapy will follow under the regulatory review of the FDA Center for Biologics Evaluation and Research and will require submission of an investigational new drug application. The manufacturer must demonstrate that the cell product is “safe, pure, and potent”.[47] Some of these key issues include:

1. The donor of the stem cells must be screened for transmissible diseases.
2. Tissue processing must follow current good manufacturing processes to prevent transmission of communicable diseases.
3. The cells must have a stable karyotype. It will be important to show evidence of a stable karyotype with multiple passages in culture.
4. The types of individual cells and their relative proportions need to be determined by definitive expression pattern of identifying markers. While it may be advantageous to include multiple cell types in a cell-based therapy, the proportion of these cell types will need to be known.
5. The biological distribution, engraftment into tissue, and persistence of the cells in tissues needs to be known. This would include knowledge of their integration into the target tissue (brain) and other non-intended sites. This will be of particular importance with intravenous delivery where cells will often be trapped in the lung and spleen
6. There are concerns of tumorigenicity with all “stem cell” populations. This is a greater concern with cells derived from embryonic stem cells and less with MSC, although a recent report suggests that cultured MSC may also have malignant potential.[66] Long term safety studies in animals are needed as well as long term follow up of treated patients in registries.

Five Year View

Stroke remains the leading cause of disability in the United States and in the Western world. Many stroke patients and families have high hopes for “stem cell” treatments, often traveling to distant places to receive poorly regulated therapies. In response to the dire clinical need and

accumulating pre-clinical evidence of efficacy in animal models, we will see a number of clinical trials of intravenously delivered autologous and allogeneic bone marrow-derived and neonatal cells (umbilical cord, placental, amniotic) cells in stroke the next 5 years. These cells will be delivered in the subacute phase of stroke, starting at 24-48 hours and extending out for a week. These early phase studies will be sponsored by small biotechnology companies but later phase trials will need the support of larger pharmaceutical companies. Allogeneic cells will have more commercial potential as a “cell-based” product and represent a more scalable platform to be used as a “universal” off the shelf product available in many hospital settings. These cells may not require any immunosuppressive drugs to be used, an advantage in a patient population subject to pneumonia and infections.

There will also be cell-based trials at later time points in the more “chronic” phase of stroke. These will be more neuronally differentiated cells such as the MSDNC or the Re001. In order to gain FDA approval for an IND, it will be important for the sponsors to demonstrate the long term fate of these cells in the brain and their lack of tumorigenicity.

There will be continued research into the mechanism of how cell therapy works. This will provide new and improved treatments. In particular, further insights into homing may allow SDF-1 and other chemokines to be upregulated in ischemic tissue to allow greater homing or a longer time window of intravascularly delivered cells.[67] Cell-based therapy will likely be combined with a gene approach in which the cells will be modified to carry an expressed gene such as BDNF into the CNS for an enhanced neuroprotective effect.[68,69]

In five years, a patient may be given a thrombolytic and a neuroprotective in first 3-6 hours. Those with incomplete recovery could then be given an intravascular cell-based therapy or a neurotrophic factor such as EPO, carbamylated EPO or G-CSF in the first 24-48 hours. Patients with persistent deficits may be candidates for late (3 month) therapies that will include administered cells. These therapies will always be applied as “combination therapy” with experience-dependent rehabilitation therapies. The outlook in restorative stroke therapy is promising. However, it is important that we temper any overly optimistic predictions as many patients and families already have unrealistic hopes of what can be achieved with cell-based and ‘stem cell’ therapies.

Key issues

- An integrated approach to cell-based therapy in stroke is required with phase I clinical trials and parallel pre-clinical and basic work to better define the mechanisms and the optimal cell types, time windows, doses, and methods of administration.
- The time window of intravenously administered bone marrow-derived or perinatal-derived cells will be in the first week after stroke, starting at the edge of the neuroprotective window.
- Intracerebrally and stereotactically administered neural stem cells or neuronally differentiated cells will target chronic stroke with a time window of months after stroke.
- Due to scalability and greater ease of commercialization, allogeneic cell therapy of a bone marrow-derived product has the greatest of becoming an approved FDA cell product.
- Neurotrophic therapy with hematopoietic and other types of growth factors is also a promising avenue of restorative therapy after stroke. Combination cell-based and neurotrophic therapy may maximize recovery. Moreover, all these therapies will need to be given with stroke rehabilitation.

- While most early phase I studies will be sponsored and supported by small biotechnology companies, larger pharmaceutical companies (big pharma), hesitant to enter the field so far, will be needed to fund and execute large phase II-III studies of cell-based therapies.

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Figure 1.

The bone marrow is a rich source of stem and progenitor cells. Hematopoietic stem cells (HSC) give rise to CD133+, CD34+, and cells that are both CD34+ and CD133+. Marrow stromal cells (MSC) differentiate into multiple cells types. Rare cell types such as the multipotent adult progenitor cell and MIAMI cells can be expanded in culture and differentiate into cells of all three germ layers. The umbilical cord, the amniotic fluid and the placenta are sources of cell types with regenerative potential.

Figure 2.

Depiction of the three “time windows of opportunity” available for stroke. The neuroprotective window is short, lasting hours. The subacute window, is an area of opportunity for intravascularly administered cells by an intra-arterial or intravenous route. Neurotrophic factors will also play an important role in this window. “Chronic stroke” will likely require stereotactic surgical approaches with direct intracerebral delivery of cells differentiated down a neuronal lineage.

Table 1

List of some of the leading candidates for cell-based therapy trials in ischemic stroke. (This list is not exhaustive but representative). IC=intracerebral; IV=intravenous; IA=intra-arterial; MCA=middle cerebral artery. Phase 0=pre-clinical development

Origin	Cell Type	Delivery Route	Clinical Trial	Sponsor/Company
Bone Marrow-derived (adult)	Autologous marrow stromal cell	IV	Phase I autologous MSC completed	Korea Research Foundation
	Autologous immunoselected CD34	IA (MCA)	Phase I ongoing	Imperial College of London
	Autologous bone marrow	IA (MCA)	Phase I ongoing	Federal University of Rio de Janeiro
	Allogeneic marrow stromal cells	IV	Planned Phase I	Theradigm, Inc, MD
	Multipotent adult progenitor cells	IV	Phase 0; Planned Phase I of Multistem	Athersys, Inc, OH (Multistem)
	MSC transfected with Notch Intracellular domain (NICD)	IC	Phase 0; Planned Phase I	SanBio, Inc, CA
Perinatal Tissues	Umbilical Cord Blood	IV, IC	Phase 0	Saneron CCEL Therapeutics, Inc, FL
	Placenta (amniotic, chorionic)	IV	Phase 0	Celgene Cellular Therapeutics, NJ
	Placental expanded (PLX) derived from placental mesenchymal stromal cells	IV	Phase 0	Pluristem Therapeutics, Inc, Israel
Fetal Brain	Human neural stem cell line (REN001)	IC	Phase 0; Planned Phase I	ReNeuron, UK
Human Teratocarcinoma	Human neuronal cells	IC	Phase I, IIa completed	Layton Bioscience, CA
Porcine Choroid Plexus	Encapsulated porcine choroid plexus cells in an alginate-based gel coating	IC	Phase 0	Living Cell Technologies, Australia