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Review

Cell-based methods for benefits of neurology and cardiology: shared hurdles and achievements

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Abstract: From the first success in cultivation of cells in vitro, it became clear that developing cell and/or tissue specific cultures would widely open myriad of new opportunities for medical research. Expertise in various in vitro models has been developing over decades, so nowadays we benefit from highly specific in vitro system imitating every organ of human body. Moreover, obtaining sufficient amount of standardized cells allow for cell transplantation approach with the goal to improve regeneration of injured/disease affected tissue. However, various cell types bring various needs and place various types of hurdles. In this review, written by European experts gathered in Cost European action dedicated to neurology and cardiology - Bioneca, we present experience collected by sharing specific needs coming from focus on two rather different organs: brain and heart. When taken into account that diseases of those two organs, mostly ischemic in their nature (stroke and heart infarction) bring far the largest burden of medical systems around Europe, it is not surprising that in vitro cellular and tissue models of nervous system and heart muscle were in the focus of biomedical research in the last decades. Moreover, results of clinical trials in which cells are transplanted into brain and heart are waited and commented with uppermost interest. It is interesting to observe that some hurdles which still impair further progress are common to both fields, while some are rather specific. In this review, we discuss those elements and offer methodological solutions which can help each other fields to accelerate their development.

Keywords: stem cells; regenerative neuroscience; brain regeneration; neurology, cardiology, myocardial regeneration, clinical trials

1. Induced pluripotent stem cells – flying start boosting in vitro models of nervous system and heart

The pioneer studies of Yamanaka and his group yielded protocols for obtaining induced pluripotent stem cells (iPSCs), thus providing the opportunity to dedifferentiate any cell to pluripotent state and, equally important, to obtain autologous, patient-specific cells [1,2] . IPSCs are generated from somatic cells, which have been reprogrammed to acquire pluripotency and have the unique capabilities of self-renewal, proliferation, and differentiation [3]. Since iPSCs can give rise to virtually any cellular lineage, an important application of iPSC technology is the *in vitro* differentiation of specialized cells, like neurons and cardiomyocytes. This is then used for the investigation of a specific tissue, including both fully differentiated cells and their precursors. Such an approach paved the way for promising advances in patient-specific disease modelling, drug screening, and cell-based therapies without the risk of immune rejection[3,4,5,6].

2. iPSC-derived cardiomyocytes

The course of cardiomyocytes differentiation is validated using molecular markers for different stages of development, as well as investigating their beating capacity, electrophysiology and metabolism[7,8,9]. To date, there are different available methods to differentiate iPSC in cardiomyocytes, generating a mixed population of cardiac cells, as ventricular-, atrial- and pacemaker-like cardiomyocytes[10]. Currently, most of the existing protocols produce ventricular cardiomyocytes more efficiently than the other cardiac cell types [8,11,12,13]. Cardiomyocytes differentiation consists of two major steps: firstly, growth factors (e.g. activin A, BMP2) or small molecules (e.g. CHIR99021) activate the Wnt signalling, allowing the mesoderm (*Nkx2.5*$^+$, *Gata4*$^+$, *Mesp1*$^+$) induction[8,14,15,16]. Secondly, small molecules (e.g. dorsomorphin) or Wnt inhibitors (e.g. IWR-1, IWP-2) are used to enhance the cardiac lineage specification and differentiation (*cTnT*$^+$, *Myh6*$^+$, *Tnni3*$^+$)[14,16,17]. Based on the research aim, there are methods to purify and isolate only ventricular- (*Hey2*$^+$, *Mlc2v*$^+$) or atrial-like cell population (*Kcnj3*$^+$, *Kcnj5*$^+$, *Cacna1d*$^+$) [8,13,18,19,20,21]. On the other hand, the pacemaker-like cardiomyocytes are still difficult to obtain *in vitro*. So far, the inhibition of neuregulin1β/ErbB signalling seems the most efficient way to enrich the sinoatrial node cells population (*Hcn4*$^+$, *Tbx3*$^+$, *Tbx18*$^+$), and only recently it has been hypothesized that modulating the Wnt signalling through Nodal inhibition may promote the pacemaker cells fate[22,23,24].

3. Specific requirements for in vitro heart muscle model

Most methods are generating iPSC-derived cardiomyocytes, which correspond to the foetal-like state concerning their functional and physiological characteristics[25]. A very specific challenge is to obtain more mature cardiomyocytes, and several methods are

currently available[26]. Compared to immature counterparts, these adult-like cardiomyocytes metabolise fatty acids, display a high mitochondrial mass, well-arranged sarcomeres, and higher contraction force.

Another critical cell subtype of the cardiovascular system is those forming the organotypic vasculature[27]. The generation of these endothelial cells should also rely on organ-specific differentiation protocols, where functional readouts can validate the efficacy and quality of the production. Their specific function comprises barrier-forming continuous layers, a specific vasoactive and growth factor secretion profile and thrombogenic properties[27,28]. Most importantly, being more than a passive conduit, prevascularisation by these endothelial cells can support the long-term survival and instruct the contractility and other functions of adjacent cardiomyocytes within the in vitro generated multicellular constructs. To establish vascularisation, pluripotent stem cell-derived endothelial cells show a remarkable capacity to self-organise into functional microvasculature, like cardiac capillaries, thereby providing sufficient perfusion throughout the cell constructs with a substantial thickness[29].

When the current state of the art is taken in account, the most promising approach is cultivating cardiomyocytes in 3D form. It comes very close to heart's unique cytoarchitectural arrangement and to an even higher level of similarity to the original tissue, with the ultimate goal to establish a heart-on-a-chip model[11,30]. This scaffold-based approach can mimic the patient-specific anatomical microstructure and composition of the human heart and vessels and generate responsive constructs to study intact tissue-level cardiovascular physiology. Interaction between cells and the cardiovascular extracellular niche and matrix constituents leads to activation of physiological underlying mechanisms and responsiveness to mechanical, electrical and pharmacological cues. Thus, multicellular microtissue may prove useful for many cell-based applications, like cardiotoxicity assessment and modelling myocardial infarction in a dish[31]. However, comparing structural, mechanical, and biological properties of these structures head-to-head with perfused intact tissues like myocardial and vascular slices and wedges is still warranted. Interestingly, iPSCs obtained from cardiac sources suggest an improved differentiation capacity *in vitro* and possibly a higher degree of maturation of cardiomyocytes. In this regard, the epigenetic memory of somatic cell source may play a fundamental role[32,33].

4. iPSC-derived neurons

iPSCs can be differentiated into several specialized cellular subtypes with functional characteristics that are representative of those found in the brain, such as dopaminergic neurons, cortical neurons and neuroglia[34].

The *in vitro* neuroectodermal induction of iPSCs, initiated *via* the dual SMAD inhibition method, results in the efficient generation of neural rosettes comprised of neuronal stem cells (NSCs) (*Sox1*$^+$, *Nestin*$^+$) that represent a cross-section of the neural tube. These NSCs can then be passaged, producing neural progenitor cells (NPCs) which can be stably maintained in culture[35]. A neuronal differentiation medium can then be applied to NPCs, which can be plated and further differentiated into more mature neuronal

(*Map2*$^+$, *TH*$^+$, *SLC18A3*$^+$) and glial cultures, which can include astrocytes (*AQP4*$^+$, *s100β*$^+$) and oligodendrocytes (*NG2, Olig1/2, NBP)*[36,37].

The neuronal differentiation of iPSCs provides patient-specific cells of neural lineage, opening possibilities for developing therapeutics, analysing drugs, and studying the underlying mechanisms of neurological pathologies. It is possible to differentiate iPSCs into NSCs in a 2D setting, which can include primitive and neural rosette-type NSCs[38,39]. Neuronal differentiation can be undertaken in a 3D setting involving the generation of neurospheres, utilizing potentially artificial scaffolding or extra-cellular matrix (ECM) materials that are continuously under optimization to recapitulate the anatomical organisation of the brain[40,41]. Persistent advances in the methodology used to obtain *in vitro* brain tissue from iPSCs led to the development of 3D brain-organoids from embryoid bodies[42]. These organoids have been demonstrated to consist of several distinctive brain regions and intricate heterogenous tissues that can mimic the sophisticated architecture of the central nervous system[43,44]. It is worth noting, however, that as the complexity of these 3D cultures improves, so does their variability and heterogeneity. Therefore, improved methods of high content analysis will be required to determine the phenotypic characteristics of these cultures with multidimensional readouts[45].

There are many issues being investigated concerning the source, quality, stability, safety and scalability of human iPSC and derivative cell production for various purposes.

Concerning the somatic cell source, pre-existing mutations acquired during the lifetime of the donor are more frequent in skin samples than in bone marrow, and very early life stage sources, for example from umbilical cord blood banks, have much less of these potentially adverse events[46,47,48,49]. However, during the reprogramming, maintenance and scaling-up of iPSC cultures further mutations, including chromosomal rearrangements, can happen which need to be monitored, especially in case of further clinical use[50,51,52]. The process of adaptation to the *in vitro* culture conditions favours some chromosomal rearrangements occurring more frequently[53]. Development of culture conditions lessening this event and advanced quality control methods is an important direction of stem cell banking and a key towards clinical applicability. Major public and private investments created human pluripotent stem cell banks with many cell lines from different ethnic and patient groups, yet many of them have not been consented for industrial use and most of them have not been generated for clinical grade applications – these are potential hurdles to overcome if clinical applications are needed. The distribution of existing cell lines among ethnic groups is unbalanced, but more nations developing their own stem cell banks are gradually overcoming this ambiguity.

5. Specific requirements for in vitro nervous tissue model

While heart muscle is rather uniformly structured tissue not depending a lot on microanatomic region, nervous tissue brings inherent complexity stemming from various regions with various cell subtypes and various functions. Thus when cultivating cells of the nervous system in vitro, one can distuingish many types of cultures, existing in a range

from mixed spontaneously differentiated and heterogenous cultures to those ones in which selection of one subtype of cells is preferred (e.g. motoric neurons, cholinergic neurons, mixed glia-neuronal cultures, astroyctes, sensoric nerons, etc). Sometimes those experiments even include chimeric interspecies cultures[54]. Another important question bringing complexity to the next level is if nervous system can be at all investigated focusing on only one specific type of cells or a specific region of the system, e.g. brain cortex. Major function of the nervous system is to achieve well coordinated interaction between various regions and receiving and transmiting messages is crucial for the nervous system physiology.

Thus, rather different than in heart muscle, it is important to well define starting point with all advantages and limitations clearly accepted. Two dimensional cultures of nervous tissue brought numerous pioneering discoveries on cellular level, but their value in understanding higher order of cellular coordination is very limited. Thus, even more then in heart muscle, 3D cultures of nervous tissue are required for all the research aiming to elucidate physiological and pathological events occurring in interaction between cells.

6. Brain organoids

While stem cells platforms based on 2D culture are successfully used for modeling of human development and disease at the cellular and molecular levels, they lack the conditions imitating spatial and temporal signaling and the interactions of the cells in a natural niche. These limitations of *in vitro* culture might be resolved by the application of biomimetic 3D solutions, especially by combining microenvironmental bioengineering with the intrinsic capacity of pluripotent stem cells to build up 3D structures[55]. The intrinsic ability of pluripotent stem cells to self-organize under 3D *in vitro* culture conditions into highly structured tissue patterns, opened the era of "brain organoids"[43,56]. Yoshiki Sasai with colleagues were the first to obtain *in vitro* from human pluripotent stem cells, highly patterned neural structures resembling muti-layered brain cortex, using SFEBq (serum-free floating culture of EB-like aggregates with quick re-aggregation) protocol[56]. Further developments from Jourgen Knoblich group brought about advanced bran-like 3D *in vitro* structures with identified regions of cerebral cortex, retina, meninges and chordoid plexus. They were shown to recapitulate *in vitro* important stages of the prenatal human brain development with functional nervous tissue cell types and cortical layer architecture, thus offering an unprecedented model for investigating human neurodevelopmental and neurodegenerative diseases[57]. Multimodal Single-Cell Analysis (single cell RT-qPCR and functional-microfluidic linked single cell RT-qPCR) of cerebral organoids cultured for more than nine months revealed high level of neuronal and glial cells diversity and their functionality with identified cell-type specific responsiveness to neurotransmitters and spontaneous action potential activity[58].

Brain organoid system appeared feasible to model early human neurodevelopment and its pathology, however it has anatomical and functional limitations to study later developmental stages due to the lack of the correct neuronal network connectivity and

vascularization. Much work in the field has been addressed to overcome these limitations with two parallel, but interdependent, directions: the first is focused on developing new protocols to generate replicas of multiple brain regions (development of "directed", region specific organoids), the second is based on constricting regulatory control of the system through bioengineering approaches.

Apart from diseases modeling, brain organoid technology can be personalized for diagnostic or therapeutic purposes if patient-specific hiPSC are applied[59,60]. Whole brain (cerebral) patient-derived organoids were used to model microcephaly, macrocephaly (Sandhoff disease), periventricular heteroplasia, schizophrenia, Alzheimer Disease and other neural disorders[59,61,62]. Brain region specific organoids, e.g. forebrain to study autism spectrum disorders, or midbrain to study sporadic or idiopathic form of Parkinson's Disease have been already obtained[63,64]. The possible future therapeutic applications will require combining molecular and cellular treatment. In that line gene-editing approach was used to obtain "healthy/repaired" organoids by producing isogenic CRISPER/CAS9 engineered patient–derived iPSCs, as was shown for Sandhoff disease[65].

7. Sources of cells for transplantation into nervous and heart tissue

Cellular therapy refers to the use of cells as medical product to treat human disorders that have not alternative efficient pharmacological therapies. Stem cell therapy has thus a valuable potential in the treatment of brain and heart diseases through cell replacement and stimulation of endogenous repair systems. Stem cells of diverse origin (embryonic stem cells, mesenchymal stem cells, induced pluripotent etc.) are candidates with great potential for translation. Here we focus on two most often used stem cell type for diseases of brain an heart: neural and mesenchymal stem cells.

Neural stem cells are pluripotent cell population, expressing markers nestin and Nop2[66] already inclined towards differentiation into neurons and glia. Process of forming adult cells of the nervous system, neurogenesis is a process in which neurons are generated through the division of NPCs and their differentiation into neuron-specific progenitors. They give rise to immature neurons. They subsequently develop into fully functional and mature neurons which integrate into and modify existing neuronal networks. In gliogenesis, NPCs differentiate into glial progenitors, which differentiate into astrocytes, oligodendrocytes and ependymal cells.

Mesenchymal stem cells (MSC) are defined as a heterogeneous subset of stromal cells that can be easily isolated from many adult tissues and possess multilineage potential, i.e., ability to differentiate into cells of the mesodermal linage, such as adipocytes, osteocytes, chondrocytes, and myocytes[67]. Actually, the multilineage potential od MSCs hallow them to differentiate into neuron-like cells, which show molecular and cellular characteristics of neurons. Besides this transdifferentiate process that derives ectodermal from mesodermal derived cells, the possibility to isolate, culture and differentiate MSC derived

from dental ligament that are neural crest derived cells with progenitor molecular profile, opening de possibility to derive neurons from these progenitors that have natural neuronal potentiality[68–70].

7. Cell transplantation for heart ischemia

Heart failure and its direct consequences represent the leading cause of death worldwide[71]. Although heart transplantation developed substantially in the last decades, there are not enough donors which would satisfy all the needs. Moreover, heart transplantation is a very complex and expensive procedure that afterwards require lifelong immunosuppression. The mechanism by which transplantation of stem cells into infarcted heart leads to health improvement is not yet completely understood. The most straightforward expectation would be that transplanted stem cells form new myocardial cells with the capability to contract. However preclinical and clinical trials revealed at least two hurdles in this theoretically simple approach: first, transplanted cells survive very briefly, so differentiation into myocardial cells is not sufficient. Second, if maturation occurs, coupling with the host myocardium is not successful. As a result, arrhythmia is a very common side effect of such an approach[72].

Preclinical studies focusing on acute infarction reported beneficial effects[73], while those aiming to improve chronic ischemia were not so successful[74].

With that said, attention has shifted from the capacity of forming new cardiomyocytes towards secreting factors that improve the condition of damaged myocardium. Reported mechanisms include immune modulatory action which promotes endogenous cardiac repair. Also, it has been shown that stem cells transplanted into the heart secrete cytokines, with rather significant anti-apoptotic effect. One of the most positive effects observed after myocardial infarction is achieved by IL-10, which improves survival and function of myocardial muscle.

There are many clinical trials which assessed the efficiency of stem cells for acute myocardial infarction. However, their results are rather heterogenous. Those ones which focused on myocardial contractility and ventricular remodeling did not find statistically significant improvement**.** However, significant improvements were found when a longer follow-up was taken into account, ranging from one to three years (Cong et al., 2015; Henry et al., 2017). Most importantly, ejection fraction was regularly improved and even ventricular remodeling was shifted in a positive direction.

More detailed analyses of the heart muscle tissue after transplantation revealed that transplanted cells even after their disappearance boosted improvements. They reduced inflammation and stimulated vascularization for a long period, reaching up to few years[75].

Thus it became clear that unlike pharmacologic and surgical approaches, cell therapy can stimulate endogenous tissue regeneration to reverse worsening cardiac dysfunction.

Table 1.

Diagnoses	Requirements from cells
Ischemic heart disease	Reduces myocardial necrosis, promote myogenesis
Non-ischemic Cardiomyopathy	Cell survival improvement in idiopathic dilated cardiomyopathy
Diabetic Cardiomyopathy	Prevents apoptosis Reduces myocardial fibrosis Improve overall cardiac function
Stem Cells in Pediatric Heart Failure	Source of new viable cardiomyocytes originated from somatic stem cells
Cardiac Tissue Engineering	Stimulates cell attachment and migration Source of biochemical factors Regulates diffusion of nutrition and thus cellular physiology and metabolism

8. Specific requirements for further improvement of cell-based therapy of heart diseases

Future developments needed to boost cell-based therapy of heart diseases include nanotechnologies and bioengineered platforms, where stem cells are preconditioned to resist their implantation into a highly stressed myocardial tissue. Basically this approach consists of the development of bioactive membranes made of two integrated materials: (a) one nanofiber matrix made out of self-assembling peptides with molecule-release capacity (for growth factors such as VEGF and FGF), and (b) contained in a microscale elastomeric scaffold that provides the mechanical framework (elastic, loading) that will match the cardiac tissue mechanics. Both are essential to promote local angiogenesis in a necrotic affected tissue as well as its regeneration.

In many congenital heart diseases neonatal ventricles demonstrate a number of intrinsic pathologic modifications, including relative immaturity of the extra-cellular matrix, inappropriately low transcription factor expression and increased myocyte apoptosis, this should open the way for the evaluation of treatments associating tissue engineering with cells implants. It seems that the main mechanisms by which cell transplantation and tissue engineering can bring functional benefits in myocardial diseases is that this implanted material should provide a supporting 'band-aid' scaffolding effect, which can limit the spread of the pathologic area, preventing excessive remodeling and dilatation of the ventricle.

Emerging biomimetic technologies include 3D printing and additive manufacturing. For heart healing applications, 3D-printed porous poly-caprolactone (PCL) elastomeric scaffolds represent a promising material functionalized with bio-additives such as stem cells, exosomes and angiogenic growth factors. Cardiopatch and Cardiowrap ventricular support bioprostheses were able to integrate in the damaged myocardium and the adjacent healthy heart, becoming artificial extracellular matrix that offers adequate cell niches for the homing of stem cells. These approaches are expected to substantially contribute to the generation of Bioartificial Myocardium, deserving clinical translation for the treatment of ischemic heart disease and chronic heart failure, avoiding the indication for heart transplantation.

9. Cell transplantation for diseases of the nervous system

The limited neurogenesis capacity in the brain makes neurological conditions difficult to treat. That's why cell transplantation approach is intensively tested for neurological diseases.

Post-ischemic acute brain injury typically peaks within 24 h of the insult, and reaches the high point within 48 h. Due to the quick onset and short duration of acute brain injury, potential neuroprotective therapies need to be administered early, i.e. within 3–6 h of the onset. This has proven to be challenging in the clinical practice. Any treatment outside of the 48 h window will offer a limited neuroprotection, and could instead be mainly restorative, targeting angiogenesis, vasculogenesis, neurogenesis, and synaptogenesis[76,77]. Finding a therapeutic approach that would delay the progressive secondary neurodegeneration will also benefit stroke survivors. To date, most cell transplantation studies have been conducted on animals during acute phase of post-ischemic injury, leaving chronic time points understudied.

It has already been shown that in addition to anti-inflammatory, anti-oxidative and anti-apoptotic effects, transplanted cells also secrete various factors acting neurotrophically exhibiting neuroregenerative effects[78,79].

Upon optimized dose regime and the route of administration, the use of stem cells shows benefits in both the acute and subacute phase, as well as in the chronic phase of cerebral ischemia[80,81]. Similar has been observed in other diseases with neuroinflammatory componente, like amytrophic lateral sclerosis or multiple sclerosis. Since a higher degree of neuroinflammation is present in the acute and subacute phase of cerebral ischemia, in these phases it is necessary to use higher doses (10-1200 million cells) and to choose less invasive ways of stem cell application, such as intravenous, intra-arterial, intranasal and intraperitoneal[80,82,83]. In these phases, various stem cells have shown positive effects so far. In acute phase (1-3 days after stroke): mesenchymal stem cells (MSCs) and human mononuclear cells (MNCs), human embryonic stem cells (hESCs), human neural stem cells (hNSCs), and multipotent adult progenitor cells (MAPC) were used[80,84,85]. In subacute phase (7 days after stroke): autologous CD34+ stem/progenitor cells and BMSCs were used[80,86]. In the chronic phase (weeks, months, years) after stroke the smaller doses of stem cells were used (1-5 million cells), albeit with more invasive application methods (intracerebral and intraventricular) in order to allow greater bioavailability of injected cells near the affected brain region[77,87].

In the last two decades more than 70 clinical trials with stem cells for brain diseases have been successfully finished finished, but unfortunately no definitive efficacy trials have been concluded and currently there is still no approved cell therapy for neurological diseases. When talking about stroke, as the most common disease of the brain, various approaches were taken. Not entering into details of various type of stem cells and routes of cell delivery, all trials of Phase 1 and 2 reported safety and visibility. It is interesting to mention that one of the very first trials performed in 2005 in South Korea with 30 patients with cerebral infarct, who received IV infusion of autologous MSC reported a significant reduction in mortality within five years of stroke incidence compared to patients who did not receive MSC transplantation[88]. In clinical settings, the recipients of allogeneic MSCs demonstrated long-lasting or transient neurological improvement. Additionally, allogeneic MSC infusion was associated with a short term decrease in circulating T cells and inflammatory cytokines[89]. The implantation of SB623 to the sites surrounding the subcortical stroke region was safe and accompanied by improvements in neurological recovery in 12 patients in a 2-year study[90]. At this stage, clinically confirmed beneficial effects were shown by CTX0E03 cells (hNSCs) administered one month after cerebral ischemia (a single intracerebral dose of up to 20 million cells) and SB623 (allogeneic MSCs) administered several times with 2.5, 5, and 10 million cells for a period of 6–60 months after stroke[80,91]. As the systemic inflammatory response is a major pathological component in secondary post-ischemic cell death[92], including some specific types of death, like necroptosis[93], stem cell transplantation should to be the therapy of choice to reduce neuroinflammatory effects and help stroke outcomes. Considerable number of clinical trials with stem cell therapy for stroke is currently underway. Clinical trials should

include patient's co-morbidities which also can affect the efficacy and effectiveness of a cell therapy.

MSC are considered a harmless cell source for clinical therapy, as they can be safely harvested from and transplanted into patients, have low immunogenicity[94]. Additionally, MSC are capable to migrate towards lesioned areas upon attraction signals by certain chemokines, suggesting their potential use as vehicles for therapeutic agent's delivery[95]. Therefore, as therapeutic agents have multiple modes of action, including cell replacement, immunologic and metabolic properties; showing a pleiotropic activity that modify the tissues response to injuries and activate restorative mechanisms that improve organ function. Intense interchange of active cellular products between MSC and resident cells have been proved, demonstrating the potential of MSC secretome to active paracrine mechanisms of tissue trophism and immunomodulation[96]. Moreover, organelle interchange has been proved, including vesicular traffic (exosomes, microvesicles, etc), where in addition to the vesicular cargo, MSC inject membrane (carrying protein membrane complexes, receptors, ion channels, etc.) into host cells[97].

MScs from Bone Marrow had been widely used in clinical trials for neurological diseases. They demonstrated to be safe but their effects were not always consistent as preclinical studies suggested, which may be due to poor survival in disease environments and/or because inappropriate therapeutic dosage and route of delivery or inconsistent trial design[98–101]

In some studies, MSC treated ALS patients displayed a slight and transient decline in disease progression[102]. Interestingly, postmortem evaluation of ALS patients treated with MSCs showed that a more significant number of motor neurons were preserved at the height of the spinal cord area where the cells were administered, compared to other spinal sites[103].

10. Specific requirements for further improvement of cell based therapy of brain diseases

When one analyzes more than 300 publications reporting transplantation of cells in animal models and more than 70 clinical trials, some common breakthroughs and some common obstacles come to surface. First of all, dogma that transplanted cells need to integrate and survive for a longer period is not only seen as obsolete, but in some cases is even too much stressed. It became obvious that in the tissue with so strong mechanisms developed by evolution which recognize new cells within a nervous tissue as an alien object, one need to focus on cell products which are anyways secreted in large quantities by many cell

types. Secreted growth factors, short sequences of RNA in various forms and still yet to be discovered components, often packed in discrete form of extracellular vesicles obviously have a very strong and beneficial influence. So, it became clear that we need to focus on recognizing those beneficial products, to discover mechanism by which they improve regeneration and then on methods how to deliver them in sufficient quantities. Moreover many methodological gaps in clinical translation must be issued. Well-designed, biomarker oriented endpoints and comparative trials are needed to address specific issues such as type of cells, cell doses, responsive phenotypes and time window of efficacy.

Rather interestingly, transplantation of stem cells into brain tissue very rarely brings any significant side effects. Probably the most well defined are those ones linked to dyskinesia observed in transplantation to patients suffering from Parkinson disease. However, methods to predict which patients are more prone to those side effects have been already developed. It is interesting to notice that no serious effects coming from uncontrolled electrical activity of such cells have been reported. On the other hand, common obstacles observed is a limited period of activity of such cells, with very time limited secretion of needed molecules. Thus the main focus is in securing longer and more substantial effects of secretome.

11. Regenerative medicine for brain and heart – shared hurdles and achievements

In this review we gathered experience from the last few decades dealing with attempts to treat diseases of heart and brain (primarily ischemic in its nature) by using stem cells and their products. When we take a general overview what has been achieved with replacement strategy, ie approach in which transplanted cells will replace lost ones in the host tissue, results are very limited. With few exceptions: replacement therapy seems to be very promising in the case where a very specific subpopulation in the very small region need to be replaced and this is the case in Parkinson disease. In this case, results are very good. In all other cases, especially in brain ischemia (Stroke) and myocardial infarction, transplanted cells still hardly can replace what has been lost. It is very interesting to notice that we expected probably much more from this approach in the heart tissue, which is, in theory, much less complex, that neural one. However, cells which succeeded to survive there for a longer period hardly can coordinate their activity with rest of the healthy muscle and most interestingly, they often cause problematic arrhythmias. It is important to notice that arrhythmias in the heart muscle are much more common problem of stem cell transplantation than uncontrolled electric activity of the transplant in the brain.

When we take a look into humoral effects of transplanted cells, experience from both organs, heart and brain fully support this strategy as the right one. This has been even

more boosted by the discovery of several types of extracellular vesicles which carry short sequences of RNA, peptides, growth factors, etc. In both organs, products of transplanted cells clearly influence inflammation and in most of the cases, with measurable effects decrease damage. One of the probably most surprising effects, again seen in both heart and brain is that those effects are often more pronounced in chronic than in acute phase. Thus overall survival and improvement of major parameters often offers stasticial significance when patients are followed after 6, 12 or 48 months. Although this is is partly in discrepancy with the fact that majority of transplanted cells soon after transplantation disappear, it seems that remaining cells produce measurable benefits in the long run.

Another common point where brain and heart helped each other is a piece of knowledge about need of standardization of products secreted by stem cells. Standardization is not only needed in order to cause more comparable results but also to better define routes of delivery. When this will be achieved, and many efforts are currently being undertaken in that direction, one can imagine repetitive injection of solutions with extracellular vesicles, which will improve regeneration of either neural or cardiac muscle tissue. When this procedure enters a routine everyday practice, we will be witnessing the final confirmation of the value of regenerative medicine in the treatment of major human diseases.

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