OPEN ACCESS
Freely Available Online

JOURNAL OF EVOLVING STEM CELL RESEARCH

ISSN NO: 2574-4372

REVIEW ARTICLE

DOI : 10.14302/issn.2574-4372.jesr-16-1055

Biocompatible Scaffolds for Human-Induced Pluripotent Stem Cell Transplantation and Modeling Post-Stroke Recovery in Three-Dimensional Neural Cell Culture

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Abstract:

Human-induced pluripotent stem cells (HiPSCs) demonstrate promise in their ability to differentiate into neural cells and ultimately replace the cell types and thereby brain tissue damaged by stroke. This may diminish cognitive impairment due to stroke. Prior to transplantation, an appropriate scaffold must be determined to allow for heightened accuracy by facilitating proper adhesion, differentiation, and proliferation, increasing the likelihood of success, as will be defined in this review, *in vivo*. This paper aims to provide a review of available biocompatible scaffolds and their efficacy, to provide insight for future research utilizing clinical trials to study stem cell therapy as a form of post-stroke recovery. A systematic review of scaffolds outlined in full-text, peer-reviewed articles with unique experimental data, available on PubMed, will be conducted to determine an ideal scaffold, based on article and scaffold selection criteria best suited for the transplantation of human-induced pluripotent stem cells.

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Keywords: Biocompatible, scaffold, stem cell, stroke, three-dimensional neural cell culture

Received Apr 28, 2016; **Accepted** Jul 05, 2016; **Published** Jul 13, 2016;

Introduction:

Stroke is a leading cause of morbidity and mortality worldwide, costing the United States in particular, tens of billions of dollars annually in reduced productivity and hospitalizations.[1] Currently, scientists are researching novel treatments for post-stroke recovery, integrating the fields of tissue engineering and stem cell research. Potential exists in the ability of stem cells to differentiate into neural cells and regenerate brain tissue damaged by stroke, thereby reducing cognitive impairment. Scaffold-mediated delivery of stem cells has been proven to result in greater control and success of transplantation.[2] Scaffolds serve as a medium for delivering seeded cells into a bodily cavity, providing structural support, promoting cell-biomaterial interaction, facilitating cell adhesion, allowing for adequate exchange of gases, nutrients, and growth factors to ensure cell survival, proliferation, and differentiation, and limiting the immune response, thereby increasing the prospect of success *in vivo*.[3] Therefore, research on the efficacy of available, biocompatible scaffolds is necessary to provide insight on which scaffold is best suited for use in seeding and transplanting stem cells, in order to evaluate the efficacy of stem cell therapy as a treatment for post-stroke recovery in clinical trials in the near future. This paper intends to compose and analyze the methods, and experimental results, if applicable, of research studies involving scaffolds that have met the selection criteria, and provide commentary for future directions.

Methods

We searched PubMed in December 2014 with the following search terms: (Neur* AND "Stem Cell") AND (Scaffold* OR Hydrogel OR "Tissue Engineering" OR Sheath*). The inclusion of the terms "Tissue Engineering" and "Sheath" expanded the search results, presumably because emerging laboratories in the field of tissue engineering are developing noncommercial scaffolds or adapting existing scaffolds, and on occasion use the broader term "Sheath" in lieu of "Scaffold". We included full-text articles in English published prior to and in December 2014 of unique experimental data that analyzed the efficacy of scaffolds exhibiting a degree of biocompatibility and the potential to be transplanted, in facilitating adhesion, differentiation, and/or proliferation, in addition to other criteria listed. Titles and abstracts were reviewed to determine if each search result matched our selection criteria.

Results

The search returned 605 results, of which 11 matched our selection criteria. Articles were selected if the title and/or abstract referenced or suggested biocompatible, potentially transplantable scaffolds, and if the scaffold proved to be unresponsive to light and not pH-altering, since such characteristics would be detrimental *in vivo*. We excluded abstracts and articles that cited methods described in prior publications if no modifications were mentioned. We observed variability in the cell source, type, and culture used, scaffold material base and added growth factors or homing motifs, gelling method, porosity, duration the seeded cells were grown in the scaffold, and degree of biocompatibility, biodegradability, and bioactivity.

It is understood that scaffold-mediated delivery of stem cells generally results in greater control and success of transplantation; hydrogels in particular are notable for their similarity to the natural extracellular matrix and easily manipulated properties, which can ease assembly, transplantation, and seeding of the hydrogel scaffold.

There are multiple design criteria to consider in scaffold engineering. The rate and extent of biodegradation influence proliferation rates. Biocompatibility must be assessed so as not to produce an immune response *in vivo* or cause leakage of

substances used in hydrogel preparation into seeded cells or nearby tissues. Porosity and pore interconnectedness influence the transfer of gases and nutrients, which facilitates cell growth and interaction. Mechanical and surface (physicochemical and topographical) characteristics also need to be assessed since they impact adhesion and proliferation. Adhesion, differentiation, and proliferation, and the extent of biocompatibility, bioactivity, and biodegradation may be further enhanced by the incorporation of growth factors or homing motifs, so they should also be noted in reviewing available biocompatible scaffolds. Moreover, preparation should be as efficient and economical as possible, and transplantation minimally invasive.

Ideally, a scaffold should contain a well durable enough to hold the contents to be transplanted, allow for gradual degradation in order for the tissue to develop around and ultimately replace the scaffold, have large enough pores to allow for cell-cell and cell-biomaterial interactions in addition to adequate exchange of gases and nutrients, and have modifiable mechanical properties. Furthermore, the gelling method should avoid extreme temperatures and changes in osmotic pressure or pH, and heat or free radical production, which jeopardize the scaffold's efficacy. Such qualities are best achieved utilizing three-dimensional neural cell cultures and hydrogels, which complementarily promote success of transplantation *in vivo* and best replicate the extracellular matrix of the brain.

In 2014, Yuan et al. constructed a double-layer collagen scaffold with a loose, inner layer of 100 μm diameter pores and a compact, outer layer of 10 μm diameter pores.[4] After preparing the scaffold from porcine tendons via chemical crosslinking, it was seeded with neural stem cells (NSCs) and transplanted into the spinal cord injury site of Sprague-Dawley rats. Adhesion of NSCs began at day 1 and was nearly complete by day 3. The interfiber space developed neuronal tissue, which fused with the spinal cord at the injury site. Nestin, a neural stem/progenitor cell marker, was tested positive for at day 6, and experimental results reported improved differentiation and motor function. Following behavioral testing, the test group recovered in the left lower extremities, and demonstrated more activity in the right lower extremities.

In 2014, Hwang et al. constructed a nanofibrous poly-L-lactic acid (PLLA) polymer scaffold via electro/wet spinning.[5] Human neural stem cells derived from embryonic brains were seeded into pores ranging in size from 50-200 μm. The material proved biodegradable and non-toxic. Four markers for proteins involved in stem cell self-renewal were expressed (>90%) in transfected cells. Cells were implanted with F3 cells prior to transplantation, and Luciferase expression was not detected 2 weeks post-implantation, indicating that F3-effLuc cells within the scaffold differentiated into the neuronal lineage. Cell adhesion was observed, and proliferation occurred gradually for the first 2 days, then exponentially thereafter. Bioluminescence activity was detected for 2 weeks, suggesting increased survival of the implanted neural stem cells in the corticectomized rat. However, both the corticectomized and motor-cortex -ablated rats scored high for abnormalities.

In 2014, Wang et al. constructed a scaffold from porcine urinary bladder matrix (UBM), seeded with neural stem cells derived from mice.[6] Rapid biodegradation was observed. After 1 week, NSC spheres flattened and adhered onto the substratum. Phenotypic markers for neurons were present, indicative of the differentiation of NSCs to neuronal, astrocytic, and oligodendrocytic lineages. Proliferation of NSCs was supported at over 80% viability at 7 days. Compared to Matrigel, UBM provided a more stable environment for NSCs to remain undifferentiated. Comparable levels of Tuj1 and GFAP suggested equivalent support from UBM and Matrigel for NSC proliferation and differentiation. Following behavioral testing, it was concluded that there was no

significant difference in performance for rats receiving UBM alone and UBM seeded with NSCs for the hind limb test. However, rats receiving UBM seeded with NSCs performed better than rats receiving UBM alone for the forelimb test, and exhibited a decrease in memory and cognitive impairments long-term (14 days). In summation, seeding and transplantation resulted in reduced neuron/tissue loss and white matter injury, in addition to a significant recovery of memory, and motor and cognitive function.

In 2014, Havasi et al. constructed a poly-caprolactone (PCL) nanofibrous scaffold via electrospinning, seeded with neural progenitors derived from human induced pluripotent stem cells (HiPSCs).[7] The average fiber diameter was estimated to be 369.42 nm with a 100-1000 nm diameter range. Embryoid bodies were found suspended after 7 days, suggesting differentiation capability. Nestin was expressed, and differentiated cells tested positive for β-tubulin and Map2. Adhesion of seeded cells was observed. PCL scaffold demonstrated biocompatibility for attachment of neural progenitors *in vitro*. Scanning electron microscopy (SEM) detected arrangement of cell bodies along nanofibers of the scaffold, and cell attachment on the scaffold itself.

In 2013, Wang et al. constructed a poly(D,L-lactide-co-glycolic acid) (PLGA) scaffold seeded with Nogo-66 receptor gene-silenced bone marrow mesenchymal cells and Schwann cells.[8] Combined transplantation of bone marrow mesenchymal stem and Schwann cells, paired with Nogo-66 receptor gene silencing reduced glial scar formation, promoted axonal growth of nerve cells, and rapidly repaired injured nerves. Good histocompatibility was demonstrated. Neuron-like morphological changes were observed. Following behavioral testing, it was observed that the test group had better lower extremity motor function.

In 2013, Li constructed a grapheme foam-based scaffold with laminin coating via chemical vapor deposition, seeded with NSCs in pores ranging in size from 100-300 μm.[9] Nearly no free-floating cell was found in the culture medium 10 hours after seeding. Extensive spreading of cells and cell-biomaterial interaction was observed. Structural integrity remained after 2 weeks. Nearly 90% of cells were viable after 5 days in culture, and all were immunopositive for nestin. The scaffold's macroporous structure and high surface area facilitated proliferation, as indicated by the fact that nearly 80% of cells stained positive for Ki-67 protein. Healthy neurite outgrowth and confluent network covering of the biomaterial surface was exhibited after 5 days of differentiation. Immunofluorescence staining detected Tuj-1+ (neuron marker), O4+ (oligodendrocyte marker), and GFAP+ (astrocyte marker), indicating that the NSCs maintained pluripotency, the ability to differentiate into all 3 neural subtypes.

In 2011, Cunha et al. constructed a self-assembling peptide scaffold with specifically designed functional motifs: RGD (Arg-Gly-Asp), BMHP1 (bone marrow homing peptide 1), and BMHP2, for the culture of NSCs, prepared using the Fmoc solid-phase method, using a Liberty Microwave Peptide Synthesizer and seeding with adult neural stem cells extracted from the subventricular zone of mice.[10] Pores ranged in size from 5-200 nm in width. Differentiation within the scaffold was difficult to quantify due to possible binding of antibodies to the scaffold and the thickness of the sample. The morphology of cells that had proliferated could not be identified specifically as neurons, astrocytes, or oligodendrocytes.

In October 2010, Johnson et al. constructed a fibrin-based scaffold via polymerization, seeded with 70% nestin-positive neural progenitor cells derived from genetically modified CE3 mouse embryonic stem cells.[11] Over-proliferation was observed. At 8 weeks post-transplantation, an enhanced number of ESNPCs in the treated spinal cords and ESNPC-derived NeuN positive

neurons were present. Spontaneous function recovery was observed over the time course of the study. Test groups receiving ESNPCs improved in the grid walk test, though a significant difference in test score compared to the control was not observed at 8 weeks.

In January 2010, Johnson et al. constructed a fibrin-based scaffold with growth factors and a heparin-binding delivery system via polymerization, seeded with embryonic stem cells derived from neural progenitor cells.[12] Quantification using stereological counts suggested increased survival and proliferation post-implantation. The cell count increased ten-fold, demonstrating high proliferation ability. Staining for neuronal markers showed that there was an increase in the number of ESNPC-derived NeuN-positive mature neurons.

In 2009, Olson et al. constructed a poly-lactic-co-glycolic acid-based scaffold via injection molding-solvent evaporation, seeded with neural stem cells and Schwann cells.[13] There were 68,000 cells per channel and 476,000 cells per scaffold, 2 mm in length and 3 mm in diameter with 7 internal channels, each measuring 660 μm in diameter. Structural integrity was maintained for over 8 weeks. Significant degradation started by 24 weeks. Over a period of 3 days in culture, NSCs and SCs were distributed evenly within the channels, and continued to proliferate. Axons were found in distinct channels and pores. A significant difference in the axonal counts of NSCs and SCs versus the control group was observed, though this was found to have no impact on functional recovery.

In 2009, Banergee et al. constructed an alginate-based scaffold seeded with NSCs, isolated from the hippocampi of Fisher rats, in 40 mm diameter pores.[14] After 7 days in culture, the number of NSCs increased fourteen-fold, demonstrating high proliferation capability. Proliferation increased significantly with a decrease in the hydrogel modulus. Maximum intensity of β-tubulin III staining was observed in cells grown at the lowest modulus.

Discussion

Variability in multiple characteristics was observed across the scaffolds mentioned in the articles that matched our selection criteria, and were the basis for determining the best biocompatible scaffold for three-dimensional neural cell cultures. This literature review aims to highlight scaffolds that demonstrated potential, or were determined to be biocompatible, rather than those utilized in *in vivo* over *in vitro* studies. Consequently, more articles fit the selection criteria, since biocompatibility may be implied by an array of indicators, such as producing no immune response, assessment of histocompatibility, positive cell-biomaterial and scaffold-surrounding tissue interaction, etc. However, this review is limited by the assumption that any indication of biocompatibility is suggestive of success in transplantation. *In vitro* studies were included, though biocompatibility does not infer transplantability.

Furthermore, a major obstacle to making comparisons across studies was the lack of operational variables. Protein markers were the most common means for evaluating bioactivity. The best scaffold is one that meets the specifications of the study being conducted and also suits the needs of the laboratory, and therefore is highly subjective and difficult to determine, since multiple aspects of a scaffold may be manipulated while some are inherently unpredictable. Of similar note, success of transplantation can be defined in various ways, whether that be by the percentage or absolute number of cells that test positive for a particular marker or those that adhere to the scaffold, at what point in time do cells begin to differentiate, to what day do cells continue to differentiate or proliferate, etc.

Multiple studies utilized the same protein markers, though they adhered to different protocols in staining and testing for cells positive for the particular

protein marker, checked at different times in the course of the study, measured either the number of differentiated cells or than percentage of positive-testing cells, or reported results relative to a control group, which oftentimes was the scaffold or stem cells alone. Therefore, not all studies measure the same variables in the same metrics, and discrepancies in methodology and therefore data collection complicate the ability to make comparisons across studies.

No gold standard has been established; some studies had a control group that received the scaffold or stem cells alone, to be compared to the experimental group, which received the scaffold seeded with stem cells. Wang, et al. made broad comparisons to Matrigel in regards to NSC proliferation and differentiation, but to our knowledge, no other comparisons have been made between scaffolds, though some exist between scaffold and scaffold plus growth factors. It is understood that scaffolds increase the rate of success of transplantation *in vivo*, so there is little basis for comparison across studies utilizing scaffold or stem cells alone as a control group. Analyses may be conducted between assigned control and experimental group(s) within a particular study, but not necessarily amongst studies.

Conclusion

Scientists will continue to research novel treatments for post-stroke recovery, advancing the field of tissue engineering. Since scaffold-mediated delivery of stem cells has been proven to result in greater control of stem cell migration and success of transplantation, research on the efficacy of biocompatible scaffolds will provided insight on which is suitable for seeding and transplantation. This will then allow for the evaluation of the efficacy of stem cell therapy as a treatment for post-stroke recovery in future clinical trials, with the intent to reduce cognitive function loss and the financial burden of stroke.

This paper serves to summarize and assess the current methods used in scaffold preparation, highlighting the need for studies involving the direct comparison of scaffolds, to help the scientific community navigate the broad spectrum of methods used in the referenced studies and advance their research in the near future. In providing an overview of the methods, and experimental results, if applicable, available, biocompatible scaffolds, this paper aims to provide insight for future directions with the ultimate goal of assessing the plausibility of stem cell therapy as a form of post-stroke recovery. Additional methods are likely to exist, that have not been included as a result of the search terms and/or selection criteria. However, the search strategy is replicable, and can be furthered in the future to incorporate novel methods as new and modified scaffolds are engineered and tested.

Acknowledgements

We appreciate the editorial advice of Claire-Marie Canda, Megan Gilbertson, Crystal Hall, Cassandra Heilingoetter, Susanna Kwok, Jin Kwon, Jacob Ritchie, and Morgan Suhre.

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