The Need for Tissue Engineered Models to Facilitate the Study of Oligodendrocyte Progenitor Cells in Traumatic Brain Injury and Repair

Rachel A. Mazur[1], Ryosuke Yokosawa[2], Pamela J. VandeVord[2], Kyle J. Lampe[1]

1 Department of Chemical Engineering, University of Virginia, Charlottesville, VA USA

2 Department of Biomedical Engineering and Mechanics, Virginia Polytechnic Institute and State University, Blacksburg, VA USA

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Correspondence to:

Prof. Kyle J. Lampe

lampe@virginia.edu

102 Engineers' Way

Charlottesville, VA 22903

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Abstract
Traumatic brain injury (TBI) is an important public health issue as these high-rate mechanical insults to brain tissue, even on a mild scale, progress to secondary injury cascades that prolong and augment the injury. More effective models are vital to study the injury mechanism and progression. Although oligodendrocyte progenitor cells (OPCs) and mature oligodendrocytes (OLs) have been shown to play a large role in TBI injury and recovery, research into OPC/OL effects post-injury has previously been ignored in favor of neuronal research. Optimizing a hydrogel-based *in vitro* model system to elucidate the unique contributions of OPC/OL to the injury cascade would elevate molecular knowledge in TBI research. In this manuscript, we identify several key parameters and potential next steps for consideration in the development of such models.

1. Introduction

TBI is a mechanical insult to the brain that can be caused by multiple mechanisms, such as rapid head acceleration, head collision with an object, and blast exposure. TBI has been described as a "silent epidemic", with recent estimates suggesting that 64-74 million individuals worldwide suffer a TBI each year [1]. Military service members are particularly at risk of suffering a TBI due to single or repeated blast exposures; within the US military alone, approximately 400,000 personnel have been diagnosed with TBI in the past 20 years [2, 3]. Literature supports that even mild TBIs (mTBI) can lead to long-term (>1 year) sequelae [4, 5]. Additionally, while the behavior and functional outcomes following TBI are well documented, cellular-level injury mechanisms and long-term anatomical effects, such as demyelination, are still poorly understood, leading to a lack of effective treatments for recovery [6]. In particular, the roles of OPCs and OLs in neurodegeneration following injury are understudied.

2. Current TBI Models and their Limitations

When reviewing TBI literature, the majority of experimental studies are conducted with *in vivo* models, particularly rodent models. These models have provided valuable insight into the aftermath of TBI, which includes microglia and macrophage recruitment, astrocyte activation, neurodegeneration, oligodendrocyte death and demyelination, and transient proliferation of OPCs [7-9]. While *in vivo* studies provide system level information on the aftereffects of TBI, it can be difficult to determine how individual molecular parameters, such as the extracellular matrix (ECM), influences the pathology due to the presence of multiple confounding variables. As a result, *in vivo* studies often fail to capture the molecular nuances of the cellular response to injury. Additionally, due to the differences between rodents and humans in terms of size, shape and composition, results generated through rodent models do not necessarily reflect the injury and recovery conditions of humans [10-12]. Development of a tunable *in vitro* platform that can accurately represent the human brain environment under varying injury conditions would be optimal for molecular-level discovery.

3. OPCs and OLs in Health and Disease

OPCs are present during early development, peaking between 15-20 weeks gestational age (GA) in humans [13]. OPCs are characterized by the expression of platelet-derived growth factor receptor α (PDGFR-α), a cell-surface receptor which plays a role in cell migration and proliferation. OPCs migrate through the developing brain to reach areas of unmyelinated axons. Upon reaching their target, they proliferate locally, followed by differentiation into pre-myelinating oligodendrocytes (pre-OLs) and ultimately into mature myelinating oligodendrocytes (OLs) [13]. Pre-OLs can be distinguished from OPCs as they begin to express oligodendrocyte markers including proteolipid protein (PLP), O1 and O4, while mature OLs express myelination markers such as myelin basic protein (MBP), myelin-associated glycoprotein (MAG) and myelin-oligodendrocyte glycoprotein (MOG) [14]. Myelination in humans begins between 20-30 weeks GA and peaks during the first year of life, but ultimately continues over the next several decades [13].

Although most myelination occurs in early development, OPCs persist in the fully developed adult brain, making up about 3% of the cell population in grey matter and 8% in white matter [15]. *In vivo* studies have shown that when remyelination is required following an injury, OPCs in the adult brain can migrate to the injury site, differentiate into adult OLs and remyelinate damaged axons [16, 17]. OPCs also act as regulators of neuroinflammation and are involved in phagocytosis of myelin debris [18]. Adult OLs adjacent to the injury site may also participate in remyelination to some degree [19]. Remyelinated axons can be identified by the presence of new myelin sheaths, which are shorter and thinner than myelin sheaths produced during early development. Multiple experimental models confirm the presence of thin myelin sheaths is a hallmark sign of remyelination [20].

Currently, the prevailing explanation for *de novo* axon ensheathment is the recapitulation hypothesis, which states that developmental myelination and remyelination should follow similar processes [21]. However, this is difficult to confirm as the details of OPC differentiation and myelination (such as the interplay of environmental cues, etc.) are not well understood. This is partially because OPCs and OLs are under-studied in comparison to their neuronal peers. As shown in Figure 1, historically, TBI models and pre-clinical intervention studies primarily focused on neuronal effects in grey matter and have not investigated the impact on OLs or the resulting demyelination which ensues in white matter following TBI [22]. The amount of white matter disruption plays a greater role than grey matter in predicting neurological outcomes [22]. A recent study demonstrated that white matter lesions in humans are correlated with cognitive impairment ($\Delta R^2 = 0.044$), while grey matter injury was less consistent ($\Delta R^2 = 0.034$) based on regression modeling [23]. The importance of OLs on neural regeneration and functional recovery has been overshadowed and research into how OLs respond to injury is warranted.

Neuroinflammation is a confirmed response following TBI. Both *in vitro* and *in vivo* models have demonstrated that OPCs and OLs are affected by the inflammatory response [24]. Following TBI, activated astrocytes and microglia initiate the inflammatory cascade through the release of inflammatory molecules, primarily chemokines and cytokines [9]. The resulting inflammation can both stimulate and inhibit OPC proliferation [9, 25-27]. Recent studies have shown that some of the inflammatory molecules such as proinflammatory chemokine CXCL10 and high mobility group box-1 (HMGB1) caused a reduction of OPCs proliferation and loss of OPCs and OLs [25, 27]. By contrast, leukemia inhibitory factor (LIF) and interleukin-1 beta (IL-1β) increase OPC proliferation at the expense of OLs [9, 26]. However, the exact mechanisms that influence the outcomes have not yet been established [9]. Thus, the incorporation of inflammatory molecules within an *in vitro* model, either via culture medium or hydrogel encapsulation, will allow for more relevant investigation into their individual effects on OPCs and OLs.

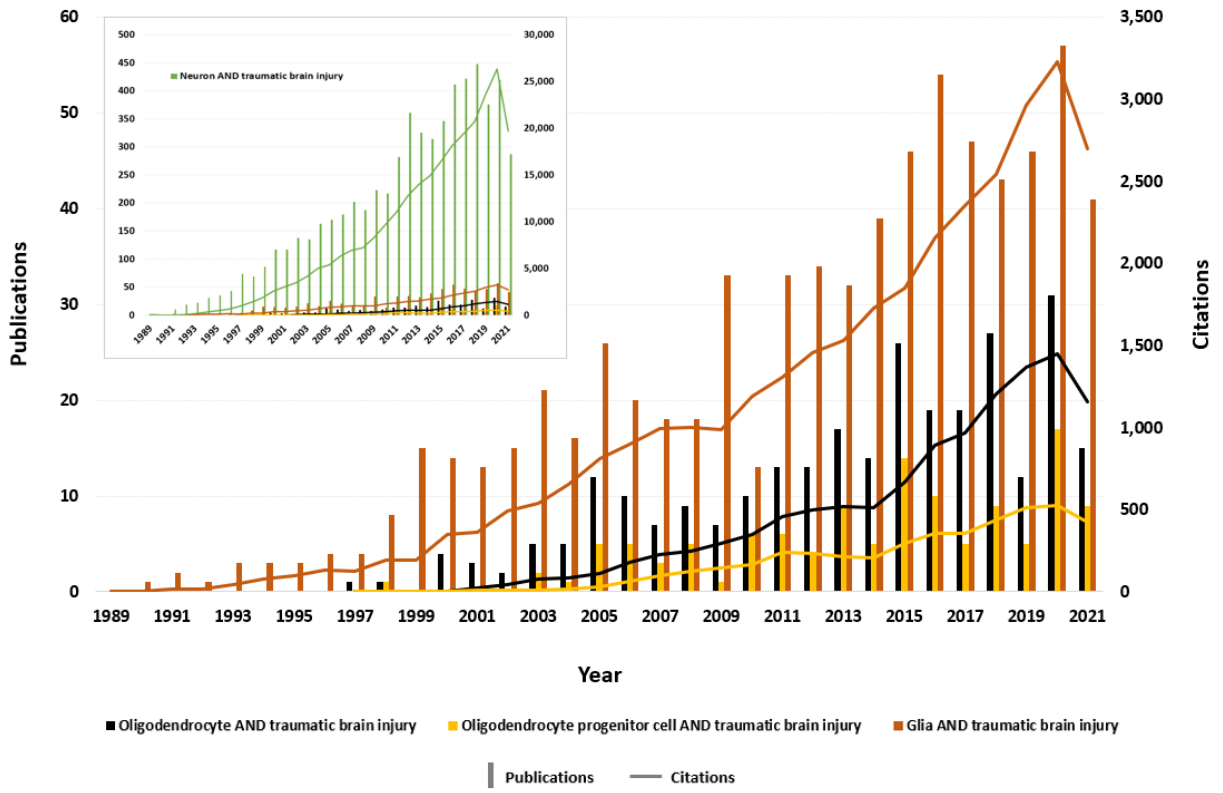

Figure 1. Trends comparing the number of neurons, glia and oligo publications and citations over the past two decades. Keywords included "oligodendrocyte AND traumatic brain injury", "oligodendrocyte progenitor cell AND traumatic brain injury", and "glia AND traumatic brain injury" between 1989 and 2021. Trends were generated using Web of Science's "Citation Report" tool. "Glia" returns the most results in conjunction with TBI, followed by "oligodendrocyte" and "oligodendrocyte progenitor cell". Note that the keyword results for "oligodendrocyte" trends also contain all results for "oligodendrocyte progenitor cell" trends. Inset: a comparison of oligodendrocyte, OPC and glia-TBI trends from the main graph to trends for "neuron AND traumatic brain injury". While all results are trending up, the results for "neuron" in conjunction with TBI far outnumber OPC, OL and glia-related search results, emphasizing the understudied nature of these important cellular contributors to understanding TBI and subsequent repair.

4. Proposed Hydrogel Research to Address Limitations of Current TBI Models

In vitro platforms offer a promising alternative approach to the challenges of working with *in vivo* models. In contrast to animal models, *in vitro* models require relatively low costs and preparation, while taking shorter periods of time to complete. Hydrogel properties can further enhance the benefits of *in vitro* systems, including the allowance of varying stiffness, topographical cues, and matrix degradation, that all impact cell growth and differentiation [28-30]. The flexibility can provide greater tunability allowing more precise modeling of the diverse range of TBI pathologies. *In vitro* modeling also gives researchers control over the inclusion or exclusion of individual parameters, allowing the elimination of potential confounding variables. Since the overwhelming majority of TBI studies have so far been conducted *in vivo*, there is a knowledge gap that persists at the cellular level.

The complex interactions that occur between glia and neurons has limited the number of research models proposed to study TBI [31]. *In vivo* studies on OPC/OL report white matter injury including axonal

damage, demyelination, and oligodendrocyte loss [32]. I*n vitro* model development is an emerging research area that studies the mechanics of white matter injury and mechanical properties of white matter. Studies include cell stretch devices to model axonal damage and demyelination, a 3D printed weight drop system, co-culture of glial cells and 3D cell culture in hydrogels [33-35]. However, few *in vitro* TBI models focus on OPC and OL, with only one study using *in vitro* methodology in the past five years [34]. This study, which used a cell stretching device to assess the impact of mechanical strain on mature OLs, found that mechanical stretch induced a transient loss of myelin protein [34]. Other studies continue to focus on axons, astrocytes, and microglia, but not the oligodendrocyte itself. Additionally, most *in vitro* models have been developed based on animal sources; in particular, 84% are based on rodent models [36]. This introduces a potential translational concern with the rodent *in vitro* models as well due to the differences between rodent and human brain physiology. Therefore, OPC and OL-focused models based on human design parameters are necessary to advance our understanding of OPC/OL injury mechanisms and contributions to functional recovery.

While *in vivo* models have attempted to investigate the cellular response to TBI, the inability to exclude confounding factors causes a lack of precise control over *in vivo* model parameters, making the effects of individual factors difficult to measure. In particular, the effects of TBI on OPC/OL remain under-studied. Creating tunable *in vitro* TBI models could be valuable platforms that would allow fundamental investigation of mechanical insult effects on OPCs, with precise and tunable control over both spatial and temporal parameters, as shown in Figure 2. Using a model system that encapsulates OPCs in a 3D hydrogel would allow the expansion into investigation of how specific hydrogel properties (stiffness, degradability, etc.) affect OPC growth and differentiation while eliminating confounding variables. Further, changes in these properties can be investigated in conjunction with a mechanical insult to simulate the effects of TBI on encapsulated OPCs leading to a fundamental understanding of the mechanostimulation within OPC.

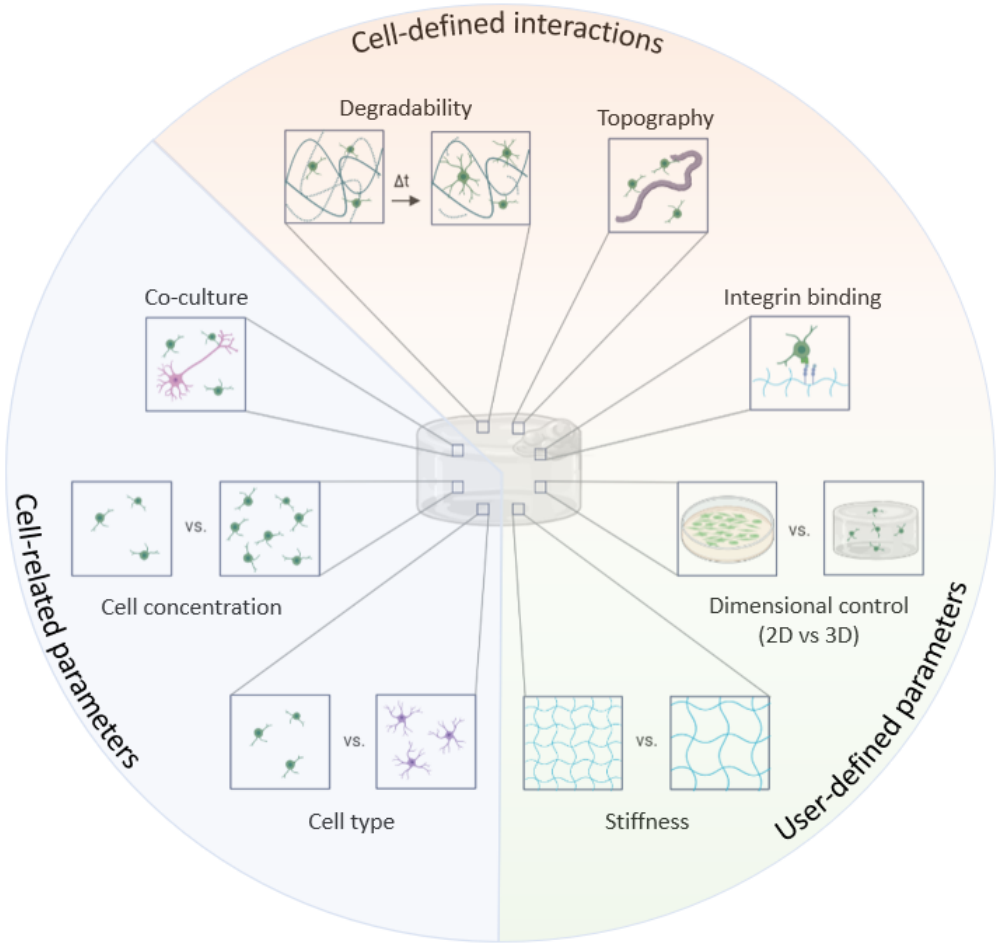

Figure 2. Potential injury model parameters for hydrogel biomaterial design. Parameters may be purely cell-based or involve cell-material interactions. Clockwise from top: hydrogels may be engineered for localized degradability by encapsulated cells; may incorporate topographical cues, e.g. electrospun fibers, to provoke cell response; may be designed to incorporate sites for integrin binding; may be designed in either two or three dimensions; may be constructed with specific material properties, e.g. stiffness; may include different cell types; may include different concentrations of cells; may include multiple cell types for co-culture.

Hydrogel properties are an emerging topic of investigation for *in vitro* model advancements with the goal of simulating the natural 3D tissue environment. Examples such as culturing OPCs in 3D gels and inducing TBI of varying mechanisms (stretch, compression) and severities would provide valuable data on the unique OPC responses to mechanical insult [28, 29, 37]. Current knowledge gaps regarding the role of OPCs and OLs in TBI injury response mechanisms can therefore be addressed by collaboration of multiple research groups with experience in hydrogel design, OPC culture, TBI models, and neuropathology to produce a 3D *in vitro* hydrogel model of TBI on encapsulated OPCs. Combination of these expertise would allow the development of a relevant model to examine outcomes such as cell survival, differentiation potential, myelin production, and how injury parameters impact hydrogel properties (see Figure 3). These interdisciplinary discussions can also be used to further optimize pre-existing *in vitro* models which investigate purely axonal effects of TBI, such as axon stretching [38]. Co-culture of both OPC and neurons within the same hydrogel would allow for further investigation into the impact of TBI on white matter specifically, which is more clinically relevant to neurological outcomes than grey matter [22]. Additionally, this would permit analysis of the interplay between neurons and OPC which cannot be obtained from models containing only one of these cell types individually.

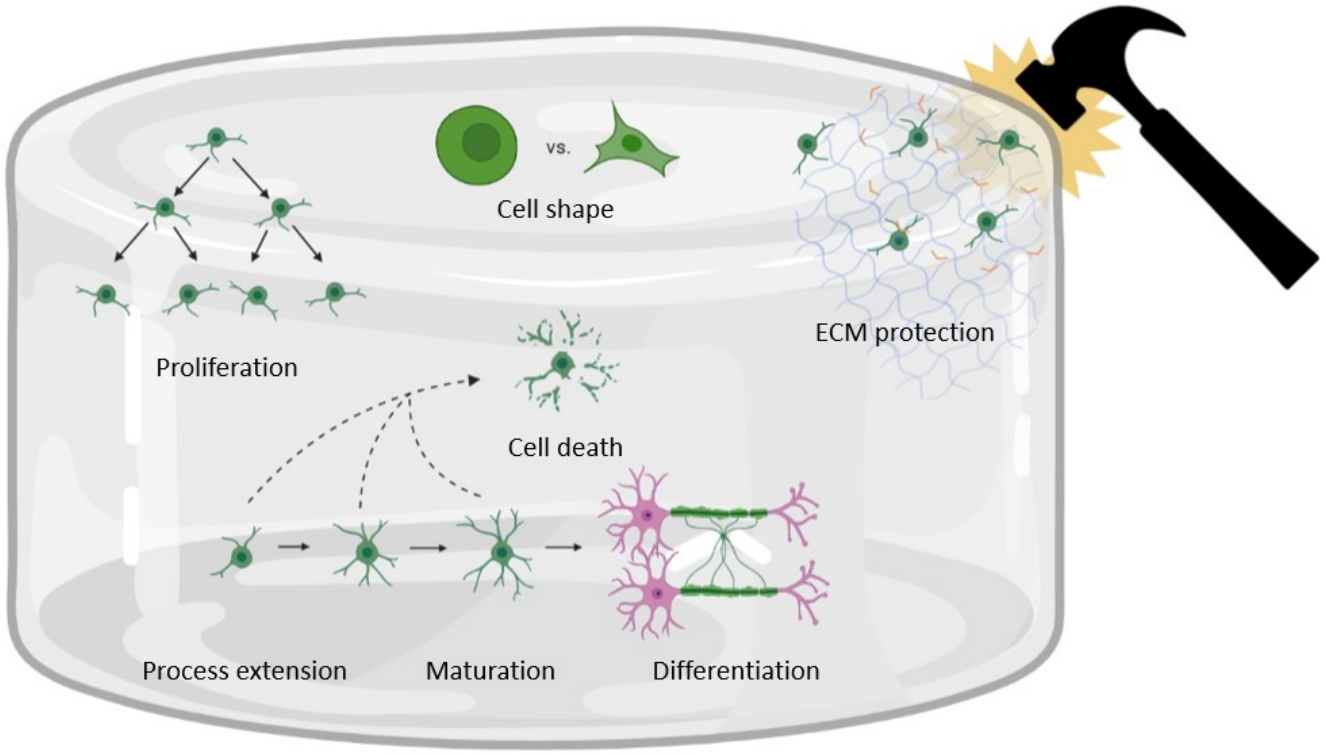

Figure 3. Potential impacts of hydrogel culture on encapsulated cells in an injury model. Hydrogel cultures may impact proliferation rates of encapsulated OPCs (top left) as well as cell shape (top center). The hydrogel matrix may also shield encapsulated OPC from mechanical insult (top right) by dissipating transmitted forces. Physical and biochemical cues from the hydrogel culture system may also impact the development of encapsulated OPCs such as the degree of process extension and the extent of maturation and differentiation (bottom). At any point in the maturation and differentiation process, the artificial ECM and developmental cell stage may have combinatorial effects on how mechanical forces transmitted through/by the hydrogel may differentially impact cell survival (center).

Preliminary investigations by our team have shown that OPCs can be cultured in both norbornene-functionalized hyaluronic acid (NorHA) and dimethacrylated polyethylene glycol (PEG-DM) hydrogels of tunable stiffness. Further advancement with the addition of electrospun methacrylated hyaluronic acid (MeHA) fibers that can be co-encapsulated with OPCs in order to provide topographical cues which mimic those of native axons has been conducted (see Figure 4). In these original experiments, we encapsulated MADM OPCs in 3D 1.5% (w/v) norbornene-modified hyaluronic acid hydrogels as previously published. However, here, we co-encapsulated OPCs either with, or without, methacrylate-modified hyaluronic acid fibers electrospun with diameters similar to mature axons. As before, cells encapsulated without fibers continued to proliferate and form OPC spheroids. However, in the presence of axon-mimicking fibers, OPCs slowed proliferation and instead developed processes indicating maturation. In future studies, both cell- and fiber-containing gels can be subjected to a mechanical insult, followed by confocal imaging, immunostaining, ATP/DNA analysis, and rheology to determine the impact of injury on OPCs or OLs in a tissue-like 3D environment. A 3D hydrogel that mimics native ECM mechanics could provide protective, force-dissipating characteristics that reduce damage to cells. Alternatively, an elastic matrix could propagate mechanical forces, transmitting them more directly to cells and thereby intensifying cell damage. We, among a limited number of other research groups, are attempting to elucidate this relationship [39, 40]. These researchers include the Kaplan lab, which uses an

in vitro contusion model to track the spread of neural network degeneration in 3D space following a TBI, and the Botvinick lab, which measures the displacement of fibers in a fibrin hydrogel to track the propagation of forces within fibrous ECM [39,40].

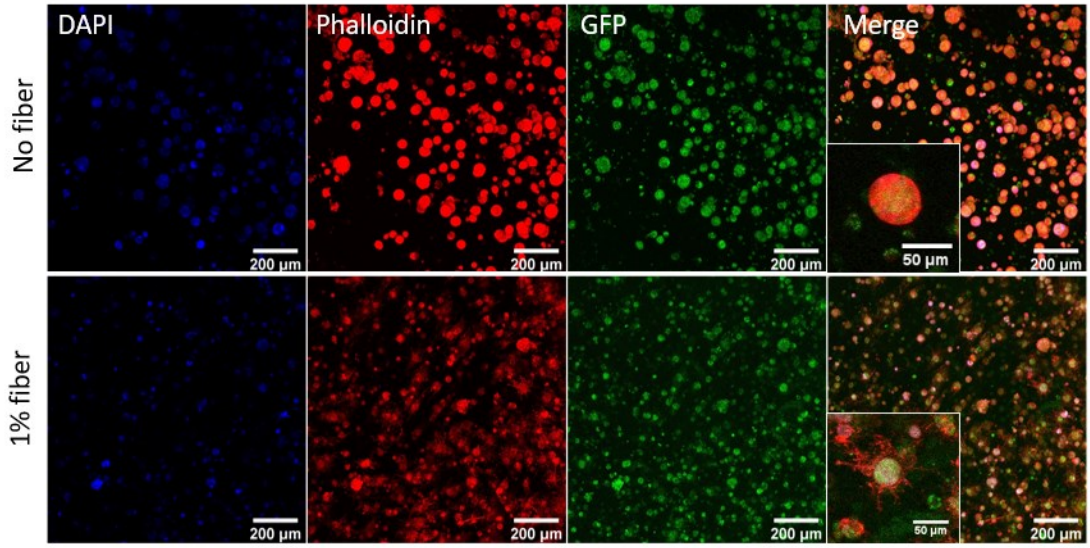

Figure 4. Representative Z-stack maximum projections (depth 400 µm) for MADM OPCs encapsulated in 1.5 % (w/v) NorHA gels with or without 1 % (w/v) MeHA fibers. Cell nuclei are stained with DAPI (blue), f-actin is stained with phalloidin (red) and living cells constitutively express GFP (green). Cells are co-encapsulated in the presence of 1% electrospun MeHA fiber (bottom row) or in control gels containing no fiber (top row). Cells encapsulated in the presence of electrospun fibers extend numerous processes, while those encapsulated in control gels retain a smooth, rounded morphology and form large multicellular spheroids.

Construction of a 3D *in vitro* OPC or co-culture model for TBI research is a vital step towards development of clinically relevant tools for identifying and testing therapeutics. Initial *in vitro* work with OPC in hydrogels will address the current knowledge gap by allowing precise, tunable control of hydrogel design parameters while simultaneously eliminating confounding variables. This permits the fundamental analysis of the influence of each parameter (e.g. topography, injury, severity) on the fate of post-TBI OPC as a function of time. Next steps towards more accurately recapitulating the native brain tissue environment may include neuronal co-culture with OPCs to study effects of TBI on white matter; further tuning matrix properties like stiffness, topography and integrin inclusion to better reflect native ECM conditions; and/or incorporation of inflammatory cytokines to determine the effects of inflammation on OPCs/OLs following TBI. Then, once the effects of TBI and ECM parameters on OPC and OL are better understood, the resulting knowledge can be adapted to develop an injectable therapeutic hydrogel for OPC delivery. This adaptation will depend on researchers with experience in hydrogel design, as injectable therapeutic delivery introduces new potential design considerations such as shear-thinning and self-healing [41-43].

5. Conclusions

In summary, OLs are an essential cell type within the CNS which maintains homeostasis of the axons and efficient neurotransmission. TBI results in damage to the tissue as a whole, but little research has been focused on how the OLs' response to injury is associated with clinical outcomes. There is a substantial knowledge gap that exists surrounding the impact of TBI on OPC/OL in white matter specifically. Effective therapeutics cannot be designed for axonal regeneration unless fundamental research on OL is

enhanced. Collaboration of researchers from various backgrounds spanning hydrogel design, OPC culture and brain injury models will be vital to comprehensively understand the role of OLs in TBI recovery. By combining these skill sets, *in vitro* platforms can be developed for studying cell development where OPCs grow, differentiate, and produce myelin similarly to *in vivo.* Such a model can then be perturbed by a mechanical insult to model the effects of TBI on growth and development of encapsulated OPCs. Through the development of a hydrogel-based TBI model, it may soon be possible to elucidate the impacts of brain injury on a molecular and cellular level. With some additional design considerations, the future looks bright for the development and screening of hydrogel-based injectable therapeutics that can positively influence the survival of OLs and ultimately enhance the functional recovery of neurons following injury.

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*Authors describe the shear-thinning and self-healing properties of a self-assembling peptide hydrogel which exhibits material properties similar to native brain tissue. These properties allow RAPID gels to be syringe injectable and protect cells, including OPCs, from mechanical and shear forces, thus increasing the viability of cells delivered via injection.