

Cell Therapy for Diabetic Retinopathy—A Work in Progress

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Abstract

As the incidence of diabetes continues to rise worldwide, the challenge in preventing vision loss secondary to diabetic retinopathy (DR) remains a formidable one. Current treatments are only indicated in advanced disease when vision loss is imminent or has already occurred. In recent years, due to the discovery that post-natal vasculogenesis plays a role in vascular repair, there has been increasing optimism that cell therapy using endothelial progenitor cells (EPCs) can be harnessed therapeutically for conditions such as DR. Although autologous EPC therapy offers promise for DR, EPCs from patients with diabetes are themselves dysfunctional, while the diabetic milieu itself contributes further to this dysfunctionality. Additional research is required to unravel the complete science of vasculogenesis and the role of EPCs in repair, so that treatment can be optimized in terms of actual cell choice, pre-conditioning prior to transplantation, maximizing cell survival in the recipient, and preparation of the recipient tissue to ensure an adequate therapeutic response.

Keywords

Diabetic retinopathy, cell therapy, endothelial progenitor cell, angiogenesis, vasculogenesis, neovascularization, therapeutic angiogenesis, vascular repair

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In the developed world, diabetic retinopathy (DR) is the leading cause of vision loss in the working population.¹ Coupled with the fact that the prevalence of DR is expected to double by 2025, the impact on quality of life of affected individuals and the societal cost in general cannot be overstated.² The increasing burden on ophthalmic departments is similarly enormous, so the search for novel therapies has become imperative. Laser photocoagulation for DR has been the therapeutic gold standard for over a quarter of a century, while recently we have seen the introduction of intravitreally administered antiangiogenic therapy following the results of recently conducted clinical trials.^{3–8} Moreover, the recognition of the role of inflammation in DR has also focused the spotlight on the use of intravitreal steroids.^{9–11} These therapies, though variously effective, are late-stage interventions that are administered when the disease may have been present for many years and when vision loss is imminent or may already have occurred. Thus, in the absence of a cure for diabetes, a treatment that could be delivered at an earlier stage when the risk of vision loss is still remote, that is safe, and that directly addresses the underlying pathology of the disease would be a giant step forward. As cell therapy satisfies these criteria, it has potential as a future treatment for DR.

Terminology and Definitions

The terminology can be confusing when it comes to defining the various stem cell types. In the main, this article concentrates on what we currently understand to be endothelial progenitor cells (EPCs). These cells are derived from the bone marrow and were first isolated by Asahara

et al.¹² We now know that when we use this term we are probably not referring to a single cell entity, but instead to a group of cells that are capable of differentiating down an endothelial cell route or lineage.¹³ First, EPCs can be derived from hemangioblasts and express various cell markers, including vascular endothelial growth factor receptor 2 (VEGFR-2), CD133, and CD34.¹⁴ Second, EPCs are considered a subset of cells derived from bone marrow multipotent adult progenitor cells (MAPCs). While these also express VEGFR-2 and CD133, they lack CD34.¹³ Finally, bone marrow-derived myelocytic/monocytic cells can also differentiate into EPCs, express CD14, and form mature endothelial cells that express von Willebrand factor, VEGFR-2, and CD45 in culture.¹⁴ All EPCs, regardless of their origin, take up acetylated low-density lipoprotein (LDL) and bind *Ulex europaeus* lectin 1 (UEA1).^{15,16} Thus, *in vivo*, at least three groups of ‘EPCs’ can give rise to mature endothelial cells: hemangioblasts, MAPCs, and myelocytic/monocytic cells.¹⁴

In vitro EPCs can be isolated by using cell sorting, by exploiting immunotypic cell surface proteins, or by isolation of the mononuclear fraction of blood followed by cell culture using different substrates and media. Isolation of EPCs using cell sorting is challenging, mainly because there is still ambiguity as to what specific surface markers precisely define an EPC, because several markers can overlap between actual progenitors and subsequent cellular differentiation to cells other than EPCs, and because of the practical challenges involved in isolating the small numbers of relevant cells circulating in the peripheral blood.¹⁷ All these factors can give rise to heterogeneity of the resultant

populations.^{18–20} Isolation of the mononuclear fraction of blood and its subsequent culture on various substrates and/or media permits more cellular homogeneity. Using this technique, at least two types of EPCs have been identified in adult blood and umbilical cord blood.^{21,22} Early EPCs or circulating angiogenic cells are spindle-shaped, are found within one week of culture, take up acetylated LDL, bind UEA1, and stain for VEGFR-2. Late EPCs or outgrowth endothelial cells can be isolated from umbilical cord blood and adult peripheral blood, although those from cord blood have greater proliferation rates and form vascular networks that remain stable for longer than those from adult blood.^{23,24}

The foregoing demonstrates ambiguities and controversies surrounding the definition of an actual EPC, mainly due to the different isolation methods. However, regardless of whatever satisfies the precise definition of an EPC, there is fairly unanimous agreement that the unique properties of ‘EPCs’ should allow them to be exploited as a potential therapeutic vasoreparative tool.

Neovascularization—Angiogenesis Versus Vasculogenesis

Angiogenesis refers to the formation of new blood vessels from the pre-existing vasculature. It occurs by at least two mechanisms.²⁵ Endothelial cells within the vasculature migrate and proliferate and then it is the fusion of vacuoles within these cells that forms the vascular lumen. This is called sprouting angiogenesis and relies on the generation of hypoxic stimuli and the release of pro-angiogenic factors and proteases that result in the breakdown of the basement membrane to permit the migration and proliferation of the endothelial cells into this newly established permissive matrix. By contrast, non-sprouting angiogenesis is poorly understood and occurs when a pre-existing blood vessel splits into two.

Vasculogenesis, defined as the *de novo* generation of endothelial cells from vascular precursor cells (EPCs) that gives rise to a nascent vasculature, has long been recognized as a mechanism for vascular formation in the developing embryo. However, in recent years, it has been established that such a mechanism exists post-natally and it is thus referred to as post-natal vasculogenesis.¹⁶ EPCs appear to play at least two roles in this vasculogenic process. First, they can be engrafted directly into the developing vasculature. Second, they can release paracrine or autocrine factors that optimize the local environment to enhance the process of vascular repair.²⁶

Neovascularization, embracing both angiogenesis and vasculogenesis, plays a pivotal role throughout life in both health and disease. In health, physiological neovascularization maintains vascular integrity. It is also essential for normal wound healing and tissue repair. In disease, the whole neovascular mechanism can be hijacked for tumor growth, survival, and metastases.^{27,28} DR, particularly its neovascular form, can be considered a form of tissue repair, albeit with a potentially pathological outcome. If we can accept such an hypothesis, it provides a logical starting point for understanding DR, the role played by neovascularization and, by implication, post-natal vasculogenesis and how this vasculogenic component may be harnessed or exploited therapeutically to favorably modify disease outcome. We call this approach ‘cell therapy’.

Overall, cell therapy and its application can be considered as a novel approach to vascular repair. Indeed, vascular repair itself is but one component of an overall wound or tissue repair process.²⁹ Thus, repair of blood vessels using cell therapy cannot be considered in isolation, but instead its role must be interpreted in the overall context of tissue repair.³⁰ In general terms, repair is what is undertaken by the human body to ensure its survival. An insult to the body such as trauma, infection or the acquisition of a disease activates this reparative mechanism. Indeed, in many instances, it is the reparative response to the insult that defines disease morbidity. For example, the entity choroidal neovascularization (CNV) satisfies all the histological criteria of a tissue repair response and therefore can more correctly be termed submacular repair.^{31,32} Thus, an alternative aim of therapy for CNV could be to modify this healing response to preserve photoreceptor function and therefore vision. A similar approach using cell therapy could be considered in DR.

Diabetic Retinopathy

Clinically, we recognise DR by the constellation of intra-retinal hemorrhages, retinal edema and exudates, cotton wool spots, and pre-retinal neovascularization in the presence of underlying diabetes. Overall, these clinical changes are graded depending on their severity and this provides important information in regard to the risk of future vision loss, and by extension the optimum time to intervene with laser therapy with the aim of preventing this loss of vision.^{3–6} In broad terms, we can classify DR into non-proliferative or proliferative. Vision loss in DR is most commonly due to macular edema, but can also occur secondary to bleeding from pre-retinal neovascularization or, in advanced disease, from tractional retinal detachment. We can further define the extent of the disease by using fluorescein angiography and ocular coherence tomography, but to really understand DR we must delve into the retina at a cellular level. This demonstrates that chronic hyperglycemia causes dysfunction of the neurovascular unit and breakdown of the inner blood retinal barrier (BRB) due to injury and eventual death of key cells such as pericytes, vascular endothelial cells, and Müller cells.³³ Initially, inner BRB breakdown causes retinal edema and, when it involves the macula, vision loss can ensue. Further progression and cell loss lead to acellular or hypocellular capillaries and ischemia, a process often referred to as progressive vasodegeneration.^{1,34}

The Therapeutic Challenge of Diabetic Retinopathy

At its most fundamental level, DR is a disease at the capillary level, a microangiopathy or even an endotheliopathy where progressive disease eventually gives rise to retinal ischemia.^{11,34–36} The therapeutic challenge is quite straightforward in theory—neutralization of ischemia and preservation of vision. Our approach to ischemic DR is the destruction of these ischemic/hypoxic areas of retina with laser, thus removing the hypoxic stimulus and the pro-angiogenic tide that this promotes.^{1,37} Although by modern terms this may seem like quite a blunt instrument, it has its origins in the 1950s–1970s through anecdotal observations and the clinical trials that these first observations spawned. That such an approach prevented blindness is an astonishing breakthrough by any standards.^{3–6,38,39} These results, together with the macular photocoagulation trials for age-related macular degeneration, tailored our whole approach to the modern management of the acquired

vascular retinopathies, including DR—an antiangiogenesis approach.^{40–42} In general, our most up-to-date DR therapy combines laser (essentially thermo-destructive antiangiogenesis) with antiangiogenesis at the molecular level through the inhibition of VEGF.^{7,9} Of course, this approach works but at a pathobiological level it is a late-stage intervention. With an increased understanding of neovascularization, both physiological and pathological, gained over the past 10–15 years, there is now the realization that it may be possible to intervene earlier and for the first time consider ‘fixing’ damaged blood vessels, rather than collaborating with the disease itself to cause their destruction. In short, we must at least begin to embrace the concept of therapeutic angiogenesis rather than the current antiangiogenic approach.³⁵ The challenges that lie ahead in advocating and realising therapeutic angiogenesis are practical and ideological.

DR is a disease at the capillary level, so it is not amenable to approaches that can be used for large vessel disease such as vascular bypass or stenting, techniques routinely used by our vascular colleagues. Our approach, therefore, must by necessity be pitched at the cellular or molecular level to repair damaged but functioning capillaries or revascularize acellular non-functioning capillaries.

Endothelial Progenitor Cells from Patients with Diabetes Contribute to Retinopathy

Based on its success in several clinical trials, cell therapy should be a significant step forward in how we deal with the risk of vision loss associated with DR.^{43,44} However, there are important issues to consider in relation to diabetes before optimizing our therapeutic approach. In simple terms, we can consider DR occurring as a result of a mismatch between the rate of endothelial cell loss due to disease and the inability of EPCs to replace these cells. Thus it appears that diabetes can impair the vasoreparative ability of EPCs.⁴⁵ The evidence suggests that this impairment of EPC function is occurring on many levels on the journey from the bone marrow to the retina. First, the peripheral neuropathy associated with diabetes causes dysfunction of the circadian release of EPCs.⁴⁶ Second, underlying peripheral vascular disease can similarly cause decreased levels of circulating EPCs.⁴⁷ Third, even after mobilization from the bone marrow, migration to the site of injury appears dysfunctional due to EPC intracellular defects in nitric oxide metabolism.⁴⁸ Importantly, it appears that this reduced bioavailability of nitric oxide can be corrected pharmacologically.⁴⁹

Transforming growth factor β (TGF- β) is known to regulate the balance of multiple EPC functions such as proliferation, differentiation, and quiescence.^{50,51} However, the serum levels and perhaps even the intracellular levels of TGF- β in EPCs are elevated in diabetes, and it has been proposed that this could be one of the mechanisms whereby chronic hyperglycemia causes cellular injury by promoting cellular senescence and growth arrest.^{52,53} In general, then, it begs the question, do elevated TGF- β levels in diabetes contribute to EPC dysfunctionality, but also can these levels be reduced to more physiological levels and will this permit the return to EPC functional normality and consequently normal vascular repair in the retina? Bhatwadekar et al. transiently inhibited endogenous TGF- β in peripheral blood CD34+ cells by treating them *ex vivo* with phosphorodiamidate morpholino oligomers and tested cell function

in vitro and in two *in vivo* experimental models of vascular injury—the acute ischemia–reperfusion retina model and laser to Bruch’s membrane model.^{54–56} The results were striking in terms of correction of diabetic EPC dysfunctionality. Essentially, EPC survival, proliferation, migration, engraftment, and homing to the site of injury were all markedly enhanced compared with controls. It appears that transient inhibition of TGF- β leads to increased surface expression of CXCR4 with activation of these receptors with stromal-derived factor-1 (SDF-1), leading to increased nitric oxide production. This in turn enhances EPC migration, and this combined with increased EPC proliferation and survival generates a robust vascular reparative response.⁵⁴

Feasibility of Reparative Vasculogenesis

The above observations raise the possibility that patients could undergo autologous EPC transfusion once the dysfunctionality can be corrected *ex vivo*. This could also permit the pre-conditioning of cells with other growth factors, genetic modification, pharmacological manipulation, or subjecting them to physical treatments such as hypoxia.^{57–60} Even allowing for correctional strategies and pre-conditioning, there are still many other practical issues to consider. In reality, diabetes, once diagnosed, is a chronic disease lasting the lifetime of the patient, has a variable course from patient to patient, and can be present with other related and unrelated comorbidities. Cell therapy in such varying clinical scenarios may at the very least give hugely variable results. This certainly has been the experience when cell therapy has been subject to clinical trials in non-ophthalmic settings.^{43,61–64} There are also the issues of optimizing the most practical route of administration of cells, timing of administration, the likely need for re-treatments, how treatments will affect other aspects of diabetes, and of course complications associated with the treatment itself and the route of administration—not to mention how this may affect the bone marrow stem cell niche in the long term. In addition, the fate of the individual cells has to be considered. Clearly, further research is required to unravel these important therapeutic issues.^{65,66}

Factors limiting the efficacy of cell therapy include loss of homing receptors that may result from pre-conditioning *ex vivo* and massive loss of cells on the journey to, or upon arrival in, the potentially hostile environment of the diseased retina. Despite the fact that these practical obstacles can be overcome, how will the EPC itself perform in an environment that is potentially more hostile than the optimum conditions encountered in the experimental models? In general, many of these models are models of acute injury and therefore the conditions present are ideal to study the effects of a potential therapy in modifying ‘disease’ outcome. However, diabetes, often in conjunction with dyslipidemia and hypertension, is characterised by chronic hyperglycemia that has multiple adverse consequences for biological systems in the body, including the development of DR.^{46,47} Even DR most likely arises from several distinct but overlapping molecular pathways that give rise to the picture we recognise clinically. This setting is far from the ideal testing ground in experimental models, and in itself may pose an interesting therapeutic challenge in terms of whether reparative vasculogenesis will work in the diseased retinal habitat and whether this habitat can be favorably modified so that it becomes more conducive to cell therapy. For example, the most likely candidate for enhancing EPC

recruitment to the retina could be the manipulation of chemokine SDF-1 and/or its receptor CXCR4. We know it is significantly upregulated in various experimental models of retinal disease and in patients with vascular retinopathies.⁶⁷⁻⁷⁰ Several reports testify to the benefit of SDF-1-mediated mobilization of bone marrow-derived stem cells and their role in modifying experimental myocardial infarction through enhanced angiogenesis and improved cardiac function.⁷¹⁻⁷⁴ SDF-1 also appears to have other pertinent roles that add to its attraction as a therapeutic candidate to enhance stem cell behaviour. These include activation of cell survival signaling and cytoprotective pathways during repair.^{75,76} Ultimately, because DR is progressive, it raises the possibility that more advanced retinopathy may even be more refractory to intervention. The solution may therefore be to intervene at an early stage. Currently we intervene only when complications have arisen (diabetic macular edema) or when complications and their visual sequelae are likely (proliferative diabetic retinopathy). As we have seen, this process is based on clinical grading. Earlier intervention could render such an approach redundant, and if so we will require a new clinical or molecular signposting system to guide clinicians.

With respect to the mode of delivery, the last number of years has seen intravitreal injections become the standard method for delivering drugs to the retina. As this method is safe and easy to perform, it would seem logical to use it to administer stem cells for retinal disease. Certainly, in

animal models, EPCs have been shown to home to the retina when delivered to the vitreous by injection.^{48,54}

Conclusion

DR remains a significant and increasing cause of visual morbidity throughout the world. Current therapies, though effective, are delivered only when advanced disease is present and do not address the underlying pathobiology. Developments in cell therapy have now reached a stage where it can be considered as a potential therapy for DR. Moreover, cell therapy can be delivered at an early stage of the disease before vision loss occurs or is likely to occur. Realistically, though, many obstacles still need to be overcome before cell therapy becomes a therapy that is both practical and reproducible. First, there are still knowledge gaps concerning the EPCs themselves, including what actually constitutes an EPC. Second, we need to understand more about how best to pre-condition EPCs prior to transplantation. Third, we need to understand more about the fate of EPCs once they are within the recipient and that adequate numbers have reached the required treatment bed. Fourth, we need to understand more about the dysfunctionality of EPCs in patients with diabetes and how the diabetic environment itself needs to be modified to optimize EPC performance. Finally, we need to create the optimal permissive environment within the treatment field that permits EPCs to function correctly. This requires a greater understanding of both the cellular- and cytokine-driven processes that constitute DR. Only with this additional knowledge can cell therapy be realised in the future. ■

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