Role of stem cells in treatment of age related macular degeneration and optic nerve atrophy

An essay Submitted for Partial Fulfillment
of Master Degree in Ophthalmology

By

Mohammed Mostafa Mohammed
M. B. B., CH.,

Under supervision of

PROF. DR. Fatma Mohamed El-Hennawi, MD

Professor Of ophthalmology
Faculty of Medicine
Ain-Shams University

DR. Mohamed Abd-Elhakim Zaki, MD

Ass. Professor Of ophthalmology
Faculty of Medicine
Ain-Shams University

Faculty of Medicine
Ain-Shams University
CAIRO
2009

List of Contents

List of Abbreviations

AMD	Age related macular degeneration
bFGF	Basic fibroblast growth factor
BLD	basal laminar deposits
BLinD	basal linear deposits
BM	Bruch's membrane
BMCs	Bone marrow–derived cells
BRB	blood-retinal barrier
CNS	central nervous system
CNV	Choroidal neovascularization
DA	disc areas
EBs	embryoid bodies
EGF	epidermal growth factor
ELM	External Limiting Membrane
ERG	electroretinography
ES	embryonic stem
ES	Embryonic Stem
FA	Fluorescein Angiography
FAZ	foveal avascular zone
FDA	Food and Drug Administration
GA	Geographic atrophy
GCL	Ganglion Cell Layer
hESC	Human embryonic stem cells
HSCs	hematopoietic stem cells
IGF-1	insulin-like growth factor1
ILM	Internal Limiting Membrane
INL	Inner Nuclear Layer

INP	Inner Plexiform Layer
IOP	intraocular pressure
mHAgs	minor histocompatibility antigens
MHC	major histocompatibility complex
MMP	matrix metalloproteinase
MSCs	mesenchymal stem cells
MTS	macular translocation surgery
NSCs	neural stem cells
NVL	Nerve Fiber Layer
OCT	optical coherence tomography
OD	optic disc
ONA	optic nerve atrophy
ONH	optic nerve hypoplasia
ONL	Outer Nuclear Layer
OPL	Outer Plexiform Layer
PDT	Photodynamic Therapy
PED	Pigment epithelial detachment
PVR	proliferative vitreoretinopathy
RGCs	Retinal ganglion cells
RPE	Retinal Pigment Epithelium
RSCs	retinal stem cells
rt-PA	recombinant tissue plasminogen activator
SC	Stem cells
SRNV	subretinal neovascularization
TGF-β	transforming growth factor-β
VA	visual acuity
VEGF	Vascular endothelial growth factor

List of figures

Introduction

Age-related macular degeneration (AMD) is defined as the loss of macular function from the degenerative changes that is related to aging. It occurs most frequently in adults over 60 years of age. The condition can be bilateral, but the onset is usually asymmetric. *(Fine et al, 2000)*

It is characterized in the early stages by drusen, pigmentary changes and degeneration of the retinal pigment epithelium (RPE). In the later stages there is atrophy of the photoreceptors and RPE (geographic atrophy or dry form) and choroidal neovascularisation (CNV or wet form). *(Tezel et al, 2004)*

Thermal laser photocoagulation for CNV has been the standard of care for many years. Photodynamic therapy (PDT) with verteporfin represents a major advance in the treatment of subfoveal choroidal neovascularisation. . *(Mittra and Singerman, 2002)*

Surgical treatments by removing CNV membranes by submacular membranectomy. Other surgical treatments include intraoperative lysis of feeder vessels, pneumatic displacement of submacular blood and translocation of the macula to an extramacular and healthier area of the RPE. *(Thomas et al, 1992)*

Pegaptanib Sodium (Macugen) is a highly selective anti--vascular endothelial growth factor agent with both anti-angiogenic and anti-permeability properties. Both intravitreal ranibizumab (Lucentis) and bevacizumab (Avastin) have successfully been used to treat CNV. *(Avery et al, 2006)*

Regenerative treatment using stem cell trying to restore lost vision in degenerative retinal diseases. Stem Cells are defined as cells with the

capacity to self-renew and to generate differentiated cells that compose an organ, including the retina. Extensive efforts expended on the transplantation of neural and retinal stem cells have shown that this method is promising as a strategy for therapy of diseased retinae. *(Klassen et al. 2007)*

Stem cells can form the progeny that can differentiate, i.e. develop into one of the different types of cells that comprise the living organism. There are three sources of stem cells (embryonic, adult, and fetal). *(Kim SY, et al 2005)*

Retinal progenitor (stem) cells have been isolated from ciliary margin of adult retina of different ages or from fetal retina then after transplantation, stem cells integrate and migrate into retina depending on the age, disease, or injury status of the recipient retina. *(Coles et al., 2004)*

There are two methods for implanting stem cells in the retina. Stem cells injected into the vitreous do not generally integrate well with the retina unless an outer nuclear layer (ONL) lesion or discontinuity of the photoreceptors is present. This is because retinal injury stimulates signaling mechanisms and the release of growth factors that guide the migration, proliferation and differentiation of stem cells into damaged areas. *(Zhang et al., 2003)*

Stem cells implanted sub-retinally form a protective sheet over photoreceptors. They readily integrate with and thicken the ONL. The thickness of the ONL is one measurement of photoreceptor density. Increased ONL density combined with the appearance of new cells expressing photoreceptor specific markers such as recoverin, rhodopsin and cone opsin have been presented as evidence that stem cells can differentiate into retinal photoreceptors. *(Meyer et al., 2005)*

In AMD photoreceptor degeneration initially leaves the inner retinal circuity intact, and newly transplanted stem cells (if they differentiate into new photoreceptors) need only make single short synaptic connections to contribute to the retinal network. *(MacLaren et al. 2006)*

Optic nerve atrophy is a blinding disorder that results from pathologic processes within the optic nerve itself. Direct damage by ischemia , inflammation , traction as the nerve fibers pass through the lamina cribrosa, gliosis of the lamina cribrosa, or trauma . In some cases, no obvious cause is identified. Indirect damage to the optic nerve occurs when the retinal ganglion cells degenerate during the course of intraocular diseases, especially glaucoma. *(Fischer D et al. 2004)*

In optic nerve atrophy adult neural progenitor cells (stem cells) grafted to diseased hosts can express mature neuronal markers and send processes to the appropriate plexiform layer, and extend neurites into the host optic nerve and can also reach up to brain and integrate with higher levels. *(Young MJ et al. 2007)*

ANATOMY

1. Structure and Function of the Human Retina

The retina (Fig. 1) is the innermost of three successive layers of the globe. It consists of two parts:

● **A photoreceptive part** (pars optica retinae), consisting of the first nine of the ten layers.

● **A non-receptive part** (pars ceca retinae) consisting of the retinal pigment epithelium (*Hamilton, 1998*).

The retina has two functions. The first, performed by the rod and cone photoreceptors, is to transduce information from an optical image into electrical signals. The second, performed by the neural circuits of the retina, is to process certain features of the visual world from the photoreceptor signals and relay this information to the brain via the optic nerve (*Penfold et al. 2001*).

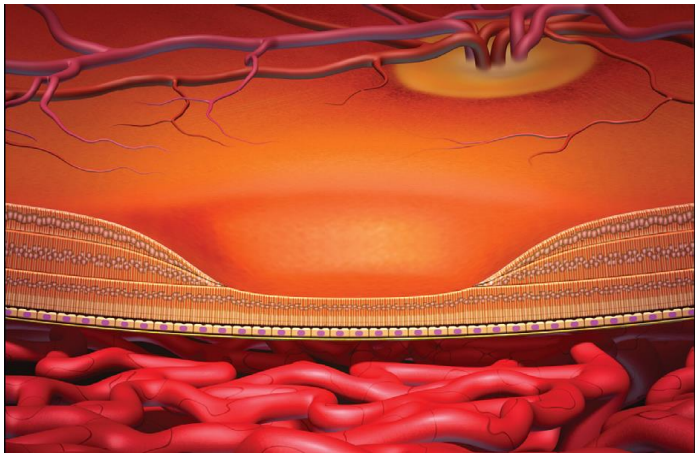

Figure 1 *Cross-section of a healthy retina* (*Penfold et al. 2001*)

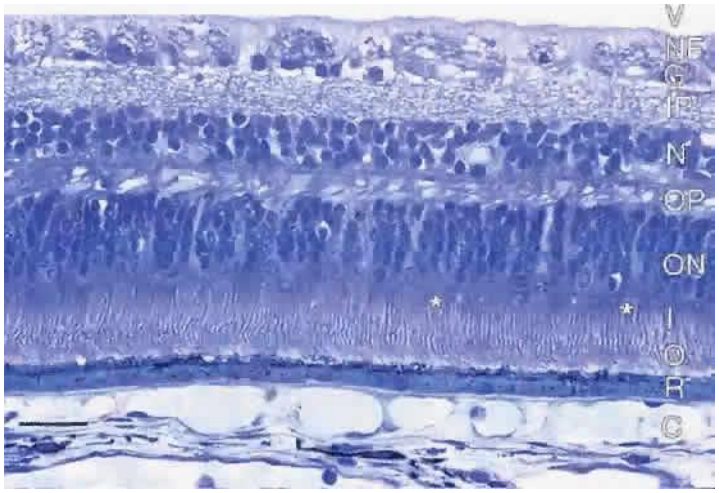

Figure 2 *Light micrograph of meridional section of human retina showing the characteristic cell layers C, choroid; R, retinal pigment epithelium; O, photoreceptor outer segments; I, photoreceptor inner segments; *, cone inner segments; ON, outer nuclear layer; OP, outer plexiform layer; N, inner nuclear layer; IP, inner plexiform layer; G, ganglion cell layer; NF, nerve fiber layer; V, vitreous chamber. Bar = 30 μm* (*Hamilton, 1998*).

Retinal Pigment Epithelium

The retinal pigment epithelium (RPE) is a monolayer of hexagonal cells extending from the margin of the optic disc to the ora serrata, where it is continuous with the pigmented epithelium of the pars plana of the ciliary body. The RPE cells are cuboidal in cross section and joined near their apical margins by junctional complexes that include maculae and zonulae adherentes (intermediate spot and belt junctions, respectively), as well as zonulae occludentes (tight belt junctions). In the zonulae occludentes between adjacent RPE cells, the plasma membranes are fused and are impermeable to water and ions. These continuous belts of tight junctions constitute the outer blood—retinal barrier, which blocks extracellular movement of water, ions, and macromolecules between the choriocapillaris and the subretinal space (*Tasman et al. 2006*).

The RPE cells are essential for photoreceptor health and have several other functions, including vitamin A metabolism and regeneration in the visual cycle, phagocytosis and degradation of shed outer segment tips, light absorption by melanin granules, heat exchange, secretion of the matrix surrounding the photoreceptor inner and outer segments, and active transport of materials between the choriocapillaris and the subretinal space. Photoreceptor density varies across the retina, but a relatively constant ratio of 45 photoreceptors per RPE cell is found in all retinal regions (*Marmor, 1998*).

Photoreceptors

Human retina contains two types of photoreceptor cells, rods and cones (Fig. 3). Rod cells mediate dim light vision, while cones function in bright light and are responsible for color vision. The names of these cells are derived from the shapes of their outer segments, which contain the visual pigments. Additional differences are present in the size and shape of the rod and cone inner segments, the location of the cell bodies, and the size and location of their synapses (Tasman et al. 2006).

The normal human retina contains approximately 4.6 million cones, with a peak foveal cone density of approximately 199,000 cones/mm2. There are approximately 92 million rods, with highest rod density (more than 150,000 rods/mm2) in a ring at the eccentricity of the optic disc. Humans have a single type of rod with a long, thin outer segment containing the visual pigment, rhodopsin, which absorbs maximally at 500 nm. The cone outer segments are shorter, wider, and somewhat conical. Each of the three cone types contains a different cone opsin that absorbs maximally in the red (564 nm; L cones), green (533 nm; M cones), or blue (437 nm; S cones) region of the spectrum (*Curcio et al. 1996*).

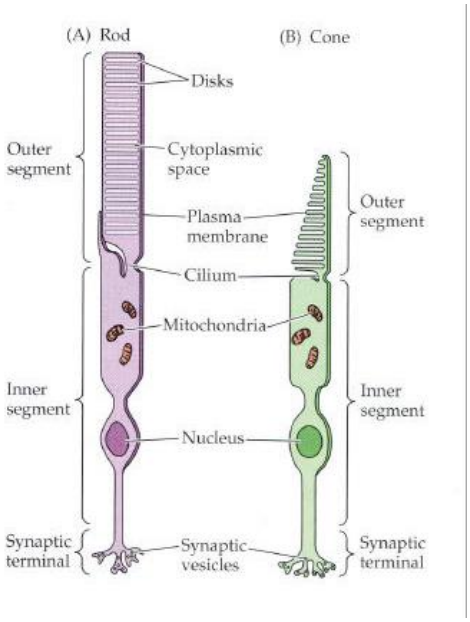

Figure 3 *Photoreceptors* (*Curcio et al. 1996*)

The photoreceptor inner segments contain a cluster of mitochondria forming the ellipsoid, and organelles for protein synthesis (ribosomes, rough endoplasmic reticulum, and Golgi apparatus) in the myoid between the ellipsoid and cell nucleus. The rod and cone outer segments are formed by stacks of membranes containing the visual pigments, rhodopsin and the cone opsins, respectively. In rods, the membranes pinch off at the base of the outer segment to form multiple discs resembling a stack of coins, surrounded by the plasma membrane. There is ongoing addition of newly synthesized rhodopsin into these membrane discs as they form at the base of the rod outer segment. The process of disc addition is balanced by periodic shedding of the outer segment tip, which is phagocytosed and degraded by RPE lysosomal activity. In cones, the outer segment membranes remain continuous with the surface membrane, such that newly synthesized photopigment is inserted diffusely into the outer segment, rather than as a band as found in rods (*Curcio et al. 1996*).

The photoreceptor outer and inner segments are joined by a thin stalk, the connecting cilium. This cilium originates in a basal body (centriole) in the inner segment and contains a ring of fine microtubule doublets typical of a sensory

cilium. The cilium is the only connection between the outer and inner segments and is thought to function as a conduit for transport of newly synthesized proteins such as visual pigments from the inner to the outer segment (*Eldred, 1998*).

External Limiting Membrane

A thin membrane is apparent at the level of the photoreceptor inner segments. This so-called membrane is actually a series of intermediate junctions (zonulae adherentes) between the rod and cone inner segments and the apical processes of the Müller cells (*Bunt-Milam et al. 1985*).

Outer Nuclear Layer

The outer nuclear layer contains the cell bodies and nuclei of the rods and cones. In the parafovea, the outer nuclear layer contains 8 to 10 rows of nuclei that belong mainly to cones. In the remainder of the retina, the outer nuclear layer contains five rows of nuclei, with a single, outermost row of cone nuclei and four rows of rod nuclei internal to them. The rod nuclei are smaller and contain denser chromatin than those of the cones (*Hogan et al. 1971*).

Outer Plexiform Layer

The outer plexiform layer contains the synapses of the rod and cone photoreceptors onto dendrites of the horizontal and bipolar cells. The rod synapses are oval and termed spherules. The cone terminals are broader and referred to as pedicles (*Bunt-Milam et al. 1985*).

Inner Nuclear Layer

The cell bodies of five cell types are found in the inner nuclear layer: horizontal cells, bipolar cells, interplexiform cells, Müller cells, and amacrine cells. The horizontal cells form the outermost row in the inner nuclear layer and