A Review On

"Neovascularization: Its molecular basis and therapeutic approaches for cardiovascular diseases"

A dissertation submitted to the Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University. In the partial fulfilment of the requirements for the degree of Bachelor of Pharmacy (B. Pharm.)

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APPROVAL

This review article, "Neovascularization: Its Molecular Basis and Therapeutic Approaches for Cardiovascular Diseases," has been recognized and approved by the Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, for partial completion of the criteria for the Bachelor of Pharmacy (B. Pharm.) degree in terms of style and quality.

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In accordance with the Bachelor of Pharmacy (B. Pharm) Degree Requirement, I thus declare that I'm conducting this thesis work under the guidance of Mr. Md. A.K. Azad, Assistant Professor, Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University. I, therefore, state that this project is entirely my original work. I further declare that neither this thesis nor any portion of it has been submitted for the bachelor's award or any other degree outside of the university.

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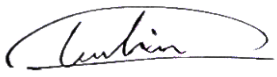

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Abstract

A disorder that affects the heart or blood vessels is referred to as cardiovascular disease (CVD). The development of fatty deposition within the arteries (atherosclerosis) and a higher chance of blood clots are typically connected with it. It may also be linked to artery damage in several organs, including the kidneys, eyes, brain, and heart. With high rates of morbidity and death, CVD is a significant healthcare and financial issue on a global scale.

Mainly, the importance of the neovascularization and its therapeutic approach for different cardiovascular diseases (CVDs) has been focused in this review. CVDs include coronary artery disease, peripheral arterial disease, stroke, and aortic (thoracic or abdominal) atherosclerosis.

There are over 60 published papers (from anytime) were described in which possible findings for, Integrated biological concept of neovascularization, vasculogenesis, angiogenesis, arteriogenesis, molecular mechanisms of neovascularization, VEGF, PDGF, FGF, MicroRNAs, Progress in vasculogenesis/angiogenesis/arteriogenesis as natural bypass therapy of cardiovascular disease.

Despite improvements in surgical and percutaneous revascularization methods, close to one-third of patients with ischemic coronary artery disease are either ineligible for revascularization because of their unfavorable anatomical conditions or obtain unfavorable revascularization from these basic methods. Besides the fact that neovascularization of the myocardium a physiological reaction to ischemia, but it may also be the focus of novel therapeutic approaches. Further research into the vascular environment and endothelial dysfunction are nonetheless motivated by the failure to convert these improvements into people. We have to reconsider our treatment strategy in light of the knowledge that conditions like hypertension, diabetes, and hyperlipidemia not only put individuals at risk for coronary artery disease but also thwarted our endeavors at neovascularization therapy. Prospects for therapeutic neovascularization involve treatments that combine enhancement of the vascular environment, enhanced endothelial activity, and other vascular formation and development-related factors.

This article will explore emerging approaches for cardiovascular regeneration and therapeutic angiogenesis in addition to the cellular and molecular processes of neovascularization.

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Chapter-1

Introduction

1. Introduction

All tissues, with the exception of the cornea and cartilage, contain the blood vessel system, which is the body's biggest organ. The vascular system is susceptible to various disease conditions because it is so widespread. In reality, the most prevalent and fatal illnesses in industrialized nations are brought on by the blockage of large or medium-sized arteries in the brain and heart due to atherosclerosis. However, blood vessels that already exist can regenerate to take the place of damaged arteries and function properly again [1, 2]. Moreover, experimental and preliminary clinical investigations demonstrate that therapeutic neovascularization, or the encouragement of blood vessel formation, is advantageous to the perfusion and functionality of specific organs [3]. Neovascularization, a complicated process that involves the coordinated interaction of several types of cells and is essential for the development of the embryonic heart, is the mechanism by which the vascular system is established. It is necessary for the cardiovascular system to operate correctly, and its impairment causes a range of cardiovascular diseases. The establishment of efficient medicines to manage and prevent cardiac dysfunctions including coronary artery disease (CAD) and ischemic heart illness may benefit from the knowledge of the molecular processes of vessel creation and growth. The embryo grows rudimentary vascular systems to transport nutrients and oxygen to the tissues and remove harmful waste products of metabolism in response to partly hypoxic conditions and mechanical stressors. Vasculogenesis is the term used to describe this process, which is based on the development of endothelial precursor cells. Angiogenic factors, such as vascular endothelial growth factor (VEGF), are expressed when the hypoxia-inducible factor I (HIF-1) is activated, which is the basis for hypoxia-driven vascular development. Angiogenesis, the process by which a primary vascular plexus becomes a fully developed and efficient circulatory system, entailed the production of new microvessels by endothelial migration and proliferation, whether by trying to split a vessel in two (intussusception) or by sprouting to formulate different sections. Arteriogenesis, the process of developing mature arteries, is brought on by the modification and migration of accompanying smooth muscle cells (SMCs) and pericytes from the epicardium (at formation) or previously existing interconnected arterioles (ischemic areas in adult myocardium). The principal regulators of the development of the vascular system include transcription regulators (Notch and HIF), growth regulators (VEGF, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), neurotrophin (NT), and transforming growth factor β (TGFβ)), as well as microRNAs (miRNAs) [4, 5].

1.1. Vasculogenesis

Vasculogenesis is the process through which early vascular systems develop during embryogenesis from endothelial precursor cells. It is well known that this process takes place effectively within the adult heart as well as during prenatal development. The vascular system develops during ontogenesis when the first rudimentary heart tube grows larger; the multilayered organ needs capillaries to supply enough oxygen and nutrients. The mouse's proepicardium (PE), a temporary extracardiac population of mesothelial cells, is found on the surface of the cardiac septum transversum and marks the beginning of coronary vasculogenesis **(See Figure 1)** [6]. PE emerges in the embryonic stage in mouse models. There are several transcription factors that may contribute to PE development. Zinc finger transcription factor Gata4 would be one of them [7, 8]. Epicardium, coronary vascular system, cardiac fibroblasts, and the myocardium may all be produced by PE cells.

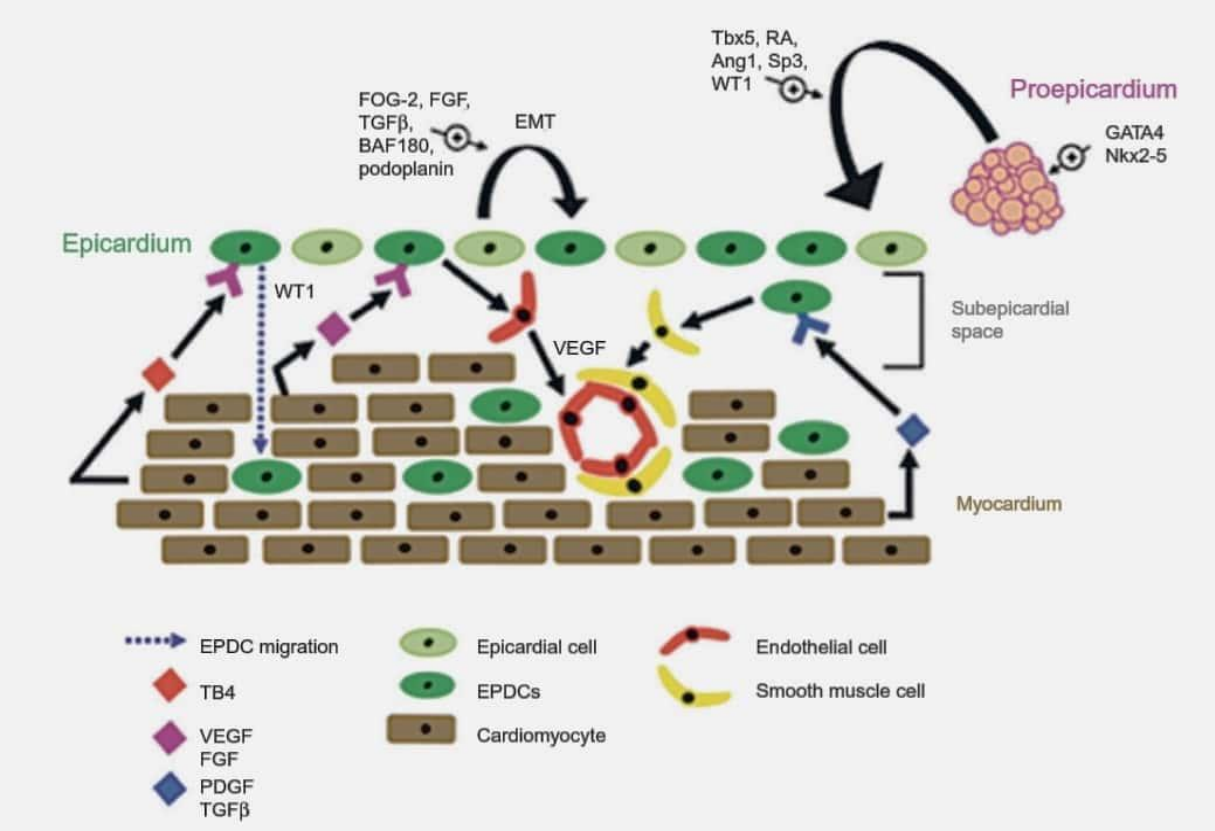

Figure-1: Mechanisms for the growth of coronary vessels. Nkx2-5 and GATA4 are needed for the creation of the proepicardium (PE), which is the first step in the development of the coronary vasculogenesis. Angiopoietin-1, retinoic acid, Tbx5, Sp3, and WT1 are among the factors that govern the migration of epicardial precursors from

the PE to the myocardium. With response to FGF and TGF signals from the myocardium, epithelial to mesenchyme transition (EMT) results in the formation of epicardium-derived cells (EPDCs). FOG-2, BAF180, and subepicardial matrix molecules, particularly podoplanin, also control epicardial EMT at the stage of gene expression. Tβ4 released by the myocardium causes EPDCs to move into the myocardium, where they react to angiogenic (VEGF/FGF2) and arteriogenic (PDGF/TGFβ) stimuli to generate coronary arteries [4, 9].

Initially, it was believed that this occurs during embryogenesis, during which embryonic mesenchymal cells (such as endothelial precursor cells or angioblasts) differentiate into endothelial cells and blood vessels created from scratch. However, it is now well known that this also contributes to adult neovascularization [10]. The angiogenic factors FGF, VEGF, and other angiogenic factors as well as tissue ischemia, are thought to trigger this mechanism. The capillary plexus, is the term for the fresh blood vessels that are created as a result of this process and are mostly made of endothelial cells. Various cell types, including pericytes, smooth muscle cells, and fibroblasts, migrate and proliferate to form the smooth muscle surface that fuses with the plexus [11, 12].

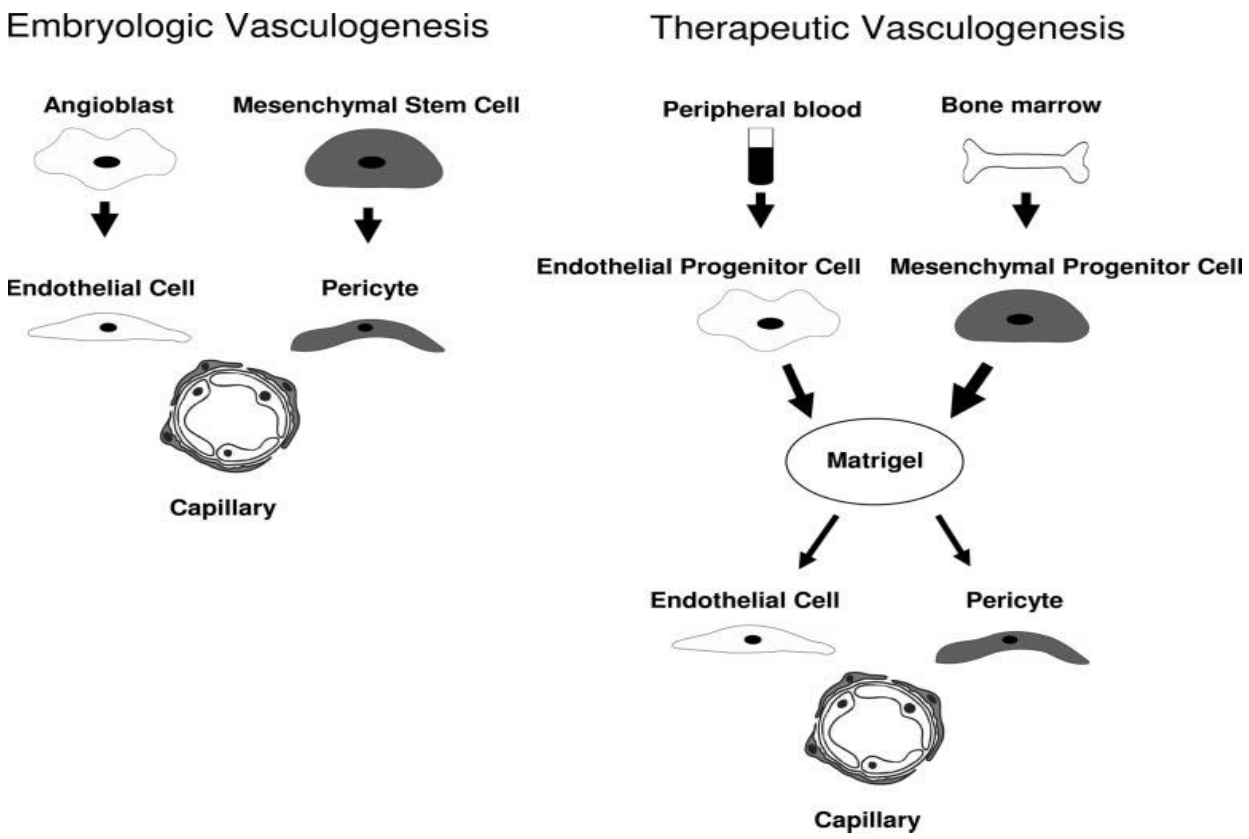

Figure-2: A tissue construction becomes vascularized in vivo when human mesenchymal progenitor cells and endothelial progenitor cells are implanted together. During embryogenesis, angioblasts transform into endothelial cells and group together to create structures that resemble primitive capillaries. In the freshly created vessels, mesenchymal stem cells develop into pericytes, which take up places around the endothelial cells. The development and stability of the vascular network are controlled by pericytes. Melero-Martin and associates used Matrigel as the framework to replicate vasculogenesis in a tissue construction. When implanted in vivo, bone marrow-derived or cord blood-derived mesenchymal progenitor cells produced a stable and developed vasculature when combined with either mature blood endothelial progenitor cells or cord blood endothelial progenitor cells. Human endothelial progenitor cells accompanied by smooth muscle actin-positive mesenchymal cells anastomosed with host arteries made up of the vascular systems [13].

1.2. Angiogenesis

Angiogenesis is the process by which old blood vessels divide into two (intussusception) or create new branches to produce new microvessels via EC proliferation and migration (sprouting). Angiogenesis begins as a modification of the first primitive vascular plexus created by varied EPDCs and other vascular cell precursors in the growing heart. A channel mimicking the full grown coronary artery tree is created via EC restructuring and patterning. Vascular growth occurs at the foundation of the heart, where it eventually joins the aorta. When ECs in established vasculature are activated during sprouting angiogenesis, they produce proteases that break down the basement membrane (BM) and enable ECs to release from the parent vessel walls. Migration-related ECs multiply in the extracellular matrix (ECM) and create solid sprouts that join nearby vessels. Sprouts spread outward in the direction of the angiogenic stimulation and form loops before fully developing into a vessel. The membrane of the vessel expands into the lumen to divide single vessel in two during intussusceptive angiogenesis. Pericytes and myofibroblasts are present at the region of contact between two nascent vessels, where they produce collagen fibers to function as an ECM for the expansion of the vessel lumen [14, 15].

The two forms of angiogenesis are physiological and pathological, and the latter always results in a variety of illnesses like heart disease, tumors, and inflammation. One of the most promising treatments for cardiovascular illness is therapeutic angiogenesis, which can increase blood flow, revascularization, and myocardial functioning. Additionally, the primary application of therapeutic angiogenesis has been the management of ischemic disorders (such as ischemic heart disease). Previous medication or surgical treatments cannot satisfy the needs of patients and physicians anymore due to the disadvantages of invasiveness, restricted drug diffusion, or shortage of selectivity towards target organs. A new method called UTMD has been put out for an angiogenesis treatment for cardiovascular disease that is noninvasive and targeted specifically. Microbubbles exposed to ultrasound (US) are referred to as UTMD; under specific circumstances, they may progressively activate or suddenly collapse. It may have a number of biological consequences, such as temporary increases in membrane permeability, extravasation, and localized tissue injury, which will make it easier for the targeted genes or medications to penetrate the tissue or cell of concern [16–19]. The main advantages of the UTMD methodology over other gene delivery techniques are its (1) high protection (less toxicity and immunogenicity especially in comparison with viral vectors and eliminating potentially dangerous ionizing radiation), (2) high cost efficiency, and wide availability (It is more appropriate to be employed in clinical applications because to its great cost effectiveness when compared to other imaging systems), (3)

Repeatability and non-intrusiveness (Since microbubbles are always delivered intramuscularly, many administrations are feasible), (4) high tissue selectivity [20, 21] (delivering medications or directed genes to the region of interest alone, as opposed to non-targeted positions). In a nutshell, UTMD is an excellent substitute for angiogenesis treatment for the cardiovascular disease since it has so many benefits.

1.3. Arteriogenesis

The method of constructing mature arteries is called arteriogenesis. It is followed by the migration of SMCs and pericytes from the epicardium throughout growth. The epicardium is where the majority of the SMCs in coronary arteries begin, however the neural crest is where the SMCs in proximal coronary arteries come from [22–24]. The immediate precursor of vascular SMCs (VSMCs) throughout mammalian vascular growth is a native culture of immature ECs that express tyrosine kinase with immunoglobulin-like and EGF-like domains 1 (Tie1+ cells), which is found in arterial beds throughout the body, along with those that have been originally defined as neural crest- or somite-derived [25]. Tie1+ cells need Notch signaling to differentiate into VSMCs in both the embryo and adulthood. Older arteries can emerge from already-existing, interconnected arterioles as a specialized kind of arteriogenesis (for instance, when coronary artery occlusion happens). An increase in the diameter of pre-existing arterial arteries is a crucial component in arteriogenesis. The mechanical stresses placed on the arteries are a crucial stimulant of arteriogenesis. The synthesis of monocyte chemoattractant protein 1 (MCP-1) by ECs is upregulated in response to mechanical stressors. The vessel membrane is attracted to by this protein by monocytes. Tumor necrosis factor α (TNF-α) is regionally released by monocytes to promote an inflammatory milieu in which they develop into macrophages, generating VEGF and inducing the release of FGF-2 and MMP by ECs. Recently, a brand-new feature of the molecular processes underpinning stress-induced arteriogenesis was discovered [26].

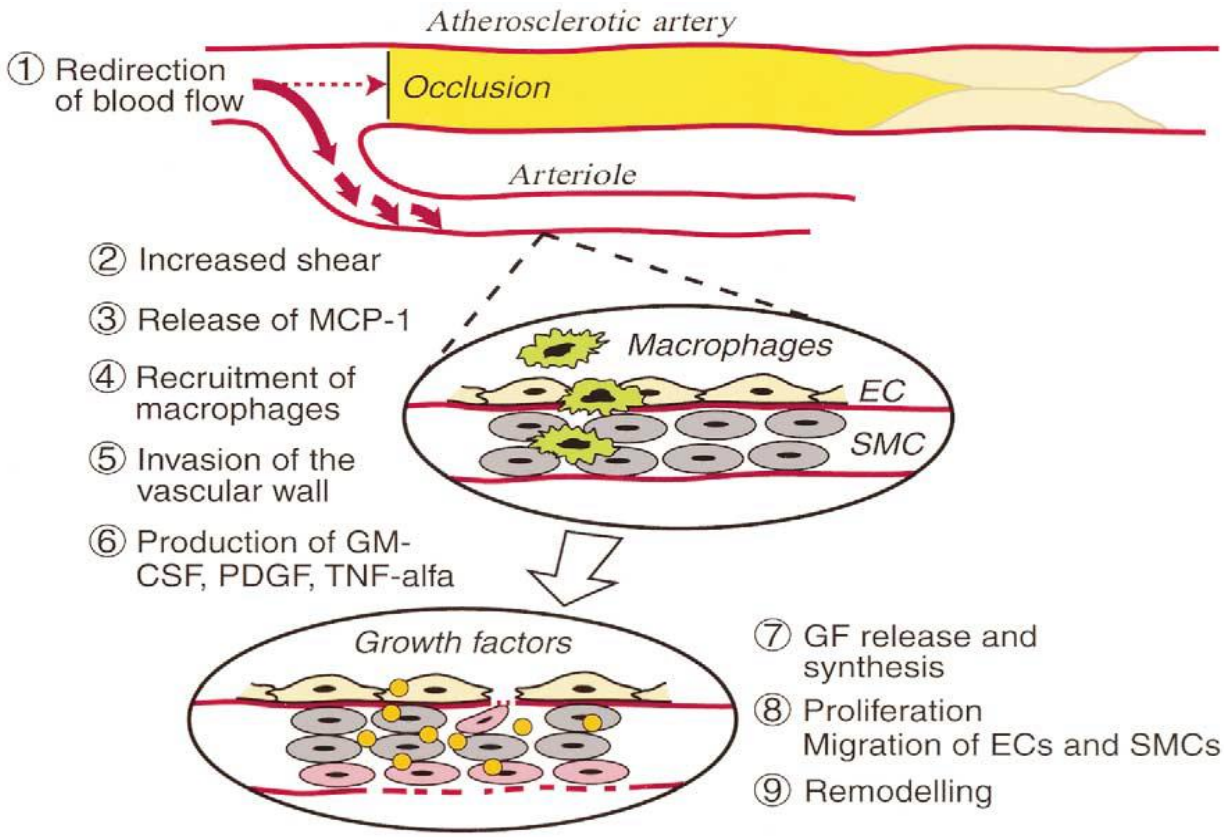

Figure-3: Diagram of potential arteriogenic process events. EC, Endothelial cell; SMC, smooth muscle cells; MCP-1, monocyte chemoattractant protein-1; GM-CSF, granulocyte-macrophage colony-stimulating factor; PDGF, plateletderived growth factor; TNF- α, tumor necrosis factor- alpha [27].

In both human patients with occlusive atherosclerotic vascular disorders and many animal models of arteriogenesis, the endothelium is crucial to the development of collateral arteries that avoid sites of arterial blockage. Following arterial occlusions, blood flow rises, which was before arteriolar interconnections, and FSS (fluid shear stress) distorts ECs [28]. Gene transcription is started by deformation, which causes endothelium activation. Cell migration and mobilization are controlled by a variety of substances that are increased in activated ECs. During ischemia-induced arteriogenesis, both FAK (focal-adhesion kinase) levels and FAK-activating tyrosine kinase (bone marrow tyrosine kinase, Bmx) are elevated in ECs, which is one of FAK's downstream modulators and affects cell migration.

Chapter-2

Purpose of the study

2. Purpose of the study

There are four main purpose of this study, which has been tried to discuss throughout the article. Mainly, the importance of the neovascularization and its therapeutic approach for different cardiovascular diseases has been focused. However, the four objectives are-

i. To know about molecular mechanisms of neovascularization.
ii. To know the progress in therapy of neovascularization in cardiovascular diseases.
iii. To give an idea about natural bypass a new life-saving treatment for cardiac patients.
iv. To spread how a healthy lifestyle lower heart disease risk.

Chapter-3

Methodology

3. Literature Search and Data collection

Fundamentally, I wanted to perform this sort of literature review to satisfy the prerequisites for the Bachelor of Pharmacy degree (B. Pharm.). That's why I started writing this literature. A literature review is a piece of academic writing that demonstrates knowledge and awareness of the available information in a certain subject area. An objective analysis of the sources is also a part of a literature review. Literature reviews must, like the majority of academic papers, include at least three fundamental components: an introduction or background data part; the review's body, which discusses the sources; and, lastly, a conclusion and/or suggestions section to finish up the study. As you can see, this work is divided into three sections: the introductory part, the result and discussion part, and the conclusion part.

There are over 60 published papers (from anytime) were described in which possible findings for, Integrated biological concept of neovascularization, vasculogenesis, angiogenesis, arteriogenesis, molecular mechanisms of neovascularization, VEGF, PDGF, FGF, MicroRNAs, Progress in vasculogenesis/angiogenesis/arteriogenesis therapy of cardiovascular disease. A succinct search method was initiated in order to find articles written with care in well-known publications.

Data sources such as Elsevier, Lancet, Scopus, Springer, PubMed, Cochrane, Crossref, Google Scholar, CAS, EBSCO, and Science Direct are used to collect information.

I evaluated the reference patterns of around 40 of the chosen literature using more accurate, appropriate, and accurate data.

Education, reading, knowing the purpose of the research, maintaining a laser-like focus on the topic, and knowing the boundaries of the material at the disposal in addition to talents are all important. In addition to that, I utilized a few more regular tools and programs. As- Microsoft Word, Sci-hub, Quillbot, Grammarly, Biorender, Mendeley, Turnitin.

a. Microsoft Word: It is used to create a better bibliography and serve as a basic text editor.
b. Sci-hub: In essence, Sci-Hub offers free access to more than 50 million publications, and researchers may easily do searches using a paper's headline or DOI to receive quick, free access, making it a convenient search engine for discovering academic papers.
c. Quillbot: Its main function is to paraphrase any text using artificial intelligence in a variety of distinct ways.
d. Grammarly: It ensures that everything we write is not only clear, captivating, and simple to read but also adheres to proper spelling, punctuation, and grammar.
e. Biorender: Making schematics, graphical abstractions, and figures using it is appropriate.
f. Mendeley: It is a web-based desktop tool created to assist in compiling, organizing, and citing all of the references.
g. Turnitin: It is a tool that students and researchers used to spot possible plagiarism.

Chapter-4

Results & Discussion

4.1. Molecular mechanisms that lead to neovascularization

Neovascularization, a complicated process that involves the coordinated interaction of several cell types, is crucial for the growth of the embryonic heart. The cardiovascular system must operate correctly, and its impairment causes a range of cardiovascular diseases. The development of efficient medicines to prevent and treat cardiac dysfunctions including coronary artery disease (CAD) or ischemic heart illness may benefit from the knowledge of the molecular processes of vessel creation and growth.

4.1.1. Platelet-derived Growth Factors

A key element of developmental vasculogenesis is PDGF signaling. This signaling regulates epithelial-mesenchymal transformation and differentiation and/or recruitment of SMCs and pericytes to the coronary vascular bed. This signaling controls SMC and pericyte differentiation and recruitment to the coronary vascular bed as well as epithelial-mesenchymal transformation. PDGF peptides are encoded by four different genes: PDGF-A, PDGF-B, PDGF-C, and PDGF-D. They are combined to form dimers. Among them, PDGF-AA, AB, BB, CC, and DD are the five active ligands that interact with PDGF receptors (PDGFRs). Transmembrane tyrosine kinases, or PDGFRs, are PDGF ligand-binding proteins. PDGFR has two known isoforms: PDGFR-alpha and PDGFR-beta. All the PDGF dimers except for PDGF-DD attaches with PDGFR-alpha. PDGFR-beta only attaches with PDGF-BB and PDGF-DD homodimers [29]. Cell elongation and migration are the early stages of EMT that are stimulated when embryonic epicardial cells are treated with PDGF. Because the PDGF-BB homodimer is far more effective than PDGF-AB or PDGF-AA, the EMT-inducing impact of PDGF is mediated by PDGFR-beta. The PI3K pathway is a downstream effector involved in PDGF-dependent EMT [30]. In studies using Pdgfrb/+ mice, the requirement of PDGF signaling for effective EMT has been demonstrated: The subepicardial area and myocardium have significantly less epicardial-derived cells (followed by a particular transgenic reporter) when PDGFR-beta is lost [31]. In explanted proepicardium, PDGF-BB is a powerful stimulator of SMC differentiation. Because neither PDGF-AA nor PDGF-AB induces SMC differentiation, this impact of PDGF is likely mediated by PDGFR-beta. Animals that lack or have insufficient VSMCs around the subepicardial and intramyocardial coronary arteries are known as pdgfrb/animals [32]. A critical function for PDGF in the control of mural cell migration during developmental neovascularization is shown by the targeted knockout of Pdgfb in the epicardium, which causes a comparable shortage of SMCs and pericytes in coronary vascular bed [31]. A C-terminal motif found in PDGF

ensures that the secreted factor is kept in the pericellular region. The appropriate recruitment and organization of pericytes within the adult organism's vasculature depend on the retention of PDGF-B released by ECs. Pericytes are improperly integrated into the micro-vessel wall [33] when the PDGF-B retention motif is removed via gene targeting in mice. By steering mesenchymal stem cells toward vascular cells, the PDGF receptor also plays a crucial part in postnatal neovascularization [4, 5].

4.1.2. Vascular endothelial growth factors

In a rabbit model of chronic hind limb ischemia, early investigations suggested that intra-arterial & intramuscular delivery of VEGF165 may greatly increase perfusion & formation of collateral arteries [34]. In the same rabbit model, arterial gene transfers using cDNA expressing VEGF also resulted in revascularization to a degree equivalent to that attained with the recombinant protein [35]. Other investigations have demonstrated that the treatment of VEGF also causes the damaged endothelium to regain its normal endothelial responsiveness. Furthermore, in a rat model of previous limb ischemia, VEGF165 given by an adenovirus induced an angiogenic response that prevented acute arterial occlusion. In a rabbit model, it was also discovered that VEGF gene transfer prevented the ischemic peripheral neuropathy linked to lower limb vascular insufficiency [36]. In a pig model of chronic myocardial ischemia, extra luminal injection of as little as 2g of recombinant human VEGF was shown to cause a considerable enhance the coronary blood flow. In a pig model, it has also been discovered that VEGF121 gene transfer using an adenoviral induces the formation and improvement of collateral vessels [37].

Isner et al. [38] used a gene therapy strategy to conduct the earliest investigation into the possibility that VEGF can induce therapeutically meaningful human’s angiogenesis. A patient with chronic limb ischemia, arterial gene transfers of naked plasmid DNA expressing VEGF165 was reported to cause in angiographic & histological indications of angiogenesis in the knee mid tibial and ankle levels four weeks after the transfer. In a second trial, the VEGF165 cDNA was administered intravenously (IV) into 10 limbs of 9 patients who had ischemic ulcers that had not healed and/or rest discomfort from peripheral artery disease. It was observed that numerous patients' distal blood flow had improved as a result [39]. The previous group also noted that local injection of VEGF165-encoding naked plasmid DNA had a therapeutic effect on myocardial ischemia patients [40]. None of these research, though, used a placebo. Recombinant human VEGF165 was administered as a single dose intra-coronary infusion pursued by 3

intravenous injections in a sizable (patients=174) placebo-controlled phase II research, but no clinical effect was seen. At least at a 60-day evaluation, the medication was not more effective than a placebo in terms of treadmill time and pain reduction, while a later time point showed modest improvement in angina class [41]. This study demonstrated that the placebo effect is far larger than previously thought and that even patients with severely impaired cardiac function can first respond to a placebo with a considerable improvement. The ability of young, healthy animals to generate an efficient endogenous angiogenic response that may be enhanced by an extra stimulus given by recombinant protein and/or gene therapy, as opposed to patients who has extensive atherosclerotic disease, who may have impaired responses to both endogenous & exogenous factors, may be a key distinction between human patients & animal models. However, a controlled research including limb ischemia patients and VEGF165 administration through adenovirus recently demonstrated a rise in vascularity [42].

Several laboratories are currently investigating if longer-lasting exposure than what was achieved in the initial experiments could provide superior outcomes. Recent research employing a conditional VEGF switch has demonstrated that newly created capillaries in the heart or liver regress when the VEGF stimulation is stopped too soon. The vasculature did, however, continue for months after VEGF elimination after a crucial period of exposure, which improved perfusion of organ [43]. A blend of growth factors also has the potential to theoretically recapitulate at least a portion of the processes that result in the proper construction of the vessel wall. It has been hypothesized that co-administering VEGF and angiopoietin-1 will produce vasculature that is more normal and far low leaky than those caused by VEGF alone. In a model of limb ischemia, administration of both bFGF and/or PDGF-BB has been shown to induce the development of durable vascular networks [44].

4.1.3. Fibroblast Growth Factors

Heparin-binding growth factors that are highly angiogenic and powerful are known as FGFs. They interact with a variety of cell-surface receptors, such as integrins, heparan-sulfate proteoglycans, and tyrosine kinase receptors, to carry out their proangiogenic function. One of the most adaptable mammalian growth factor families is the FGF system, which has 22 FGF ligands and 4 tyrosine kinase receptors (FGFRs) [45]. Despite the substantial amount of information on FDF-dependent control of angiogenesis that has been gathered, their specific function in this process is still unknown. Transgenic animal models provide very limited and ambiguous information. Even though FGF1 and FGF2

are known to stimulate angiogenesis in vivo, there is no difference in vascular healing after mechanical injury in Fgf1/, Fgf2/, and Fgf1-Fgf2 double knockout mice [46]. While Fgfr1- and Fgfr2-null mouse embryos die at early stages of development, making it hard to assess their function in vascular development, neither Fgfr3 nor Fgfr4 knockout mice exhibit vascular abnormalities [4]. Recent research indicates that the interaction of FGFs with other growth factors may be the mechanism by which they influence neovascularization. The control of the VEGF system is connected to one role of FGF in the angiogenic process. For example, VEGF production in ECs and stromal cells is stimulated by FGF2, and this stimulation is necessary for the FGF to produce an angiogenic response [47]. In light of this, inhibition of VEGF signaling results in the reduction of FGF-induced angiogenesis (neutralization of VEGF-A with an antibody, blockage of VEGFR by an antibody, etc.) [48]. On the other hand, VEGF administration restores vascular development in Fgfr1/ embryos, which shows diminished VEGF expression and vascular formation [49]. Recently, Tomanek et al. provided evidence that each of the six FGF ligands requires VEGF signaling and documented involvement of various FGF isoforms (i.e., FGF-1, -2, -4, -8, -9, and -18) in coronary tubulogenesis. Therefore, it appears that VEGF signaling is necessary for FGF signaling to have angiogenic effects in vivo. Recent research suggests that during vascular development, FGF2 signaling interacts with the PDGF cascade on mural cells (VSMCs and pericytes). Although PDGF is a strong recruiter and activator of VSMCs, it is insufficient to mature vessels on its own [50]. Additionally, it appears that the action of FGF2 on VSMC is mediated through the overexpression of PDGFR [51].

4.1.4. MicroRNAs

It has been shown that several miRNAs exhibit proangiogenic properties. In vitro sprout production is inhibited by particular anti-miRNA 2'-O-methyl oligonucleotides that target miR-27b and the miRNA precursor let-7f. Several miRNAs' proangiogenecity is depends on the inhibition of VEGF-dependent signaling cascades in ECs by proteins that are negatively regulated. For instance, the proteins Sprouty-2 & Spred-1 that block Raf activation are downregulated by miR-23 and miR-126. Semaphorin6A (Sema6A) protein is repressed by the overexpression of miR-23b & -27b in HUVECs [52]. This presents another way of angiogenesis improvement by targeting Sema6A, which is considered to be a potent inhibitor of VEGF-dependent angiogenesis. MiR126 has more targets than Spred-1. For instance, the p85 regulatory component of PI3K is increased in miR-126 knockout mice [53]. This subunit is required for the proangiogenic PI3K-Akt pathway to be activated as well as for the stability & recruitment of PI3catalytic K's subunit.

Currently, the observed arterial rupture & hemorrhaging of miR-126 knockout mice cannot be explained by overexpression of p85 and has to be further investigated.

Ephrin A3 is a target of miR-210, which when activated during hypoxia reduces Ephrin A3 and promotes the creation of capillary-like formations. A growth-arrest homeobox (GAX) and HoxA5 are transcription factors that inhibit angiogenesis when miR-130a is present (homeobox protein A5). The fetal bovine serum causes an increase in miR-130a in HUVECs, which counteracts the inhibitory activity of homeobox proteins upon EC migration, tube formation & proliferation [54]. Additionally, antiangiogenic miRNAs have been discovered. In vitro wound healing, migration, and tube formation of ECs are inhibited by miR-221 and miR222 transfection [55]. The stem cell factor (SCF) receptor, c-kit, is downregulated in the molecular process underlying this activity. C-kit is a cytokine receptor tyrosine kinase that aids in the vital EC activities of capillary tube creation, migration, & angiogenesis. The endothelial nitric oxide synthase (eNOS), which is a crucial regulator of EC development, migration, and angiogenesis, is also indirectly downregulated by overexpression of miR-221 and miR-222 [56]. Recent experimental findings link miR-24 to the inhibition of endothelial spheroid formation, proliferation, and tube formation under hypoxic circumstances. This miRNA inhibits the transcription factors GATA2 and PAK4 and is increased in response to myocardial ischemia and hypoxic culture conditions. GATA2 and PAK4 have been linked to vascular biology in earlier studies. Heme-oxygenase-1 (HMOX1), SIRT1, the histone-coding gene H2AFX187, RAS p21 protein activator RASA1, and other endothelium targets were also shown to be downregulated in HUVECs overexpressing miR-24. By preventing endothelial apoptosis and promoting vascularity, inhibiting endothelial miR-24 in the mouse model reduced the extent of MI, preserving heart function and prolonging life [57].

4.2. Innovative approaches to therapeutic angiogenesis and arteriogenesis in Peripheral Arterial Disease (PAD)

Peripheral arterial disease (PAD) affects more than 10 million individuals in the United States, and it is a major global reason of death and morbidity. Being a blood vessel disease, PAD is a kind of cardiovascular disease (CVD). It is typically brought on by an accumulation of fatty plaques in the walls of the arteries in the legs. Cholesterol as well as other waste products make up the fatty deposits, or atheroma. The most prevalent clinical effects of PAD include claudication discomfort (pain when walking), decreased functional ability, pain while at relaxation, and loss of tissue integrity in the distal limbs, which may result in lower extremity amputation. Additionally, the incidence of myocardial infarction, stroke, and cardiovascular mortality are greater than usual in patients with PAD. Although improvements in surgical and endovascular techniques, some patients with PAD cannot be treated due to concomitant diseases, and revascularization methods may be as effective in symptom relief [58]. Because of extensive microvascular disease, in certain conditions, alleviating obstructive disease in the big conduit arteries may not guarantee total limb salvage. Medical interventions to increase perfusion to the distal limb are of poor efficacy despite years of research efforts. While PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibitors and anticoagulants like rivaroxaban have been shown to minimize severe cardiovascular and limb events in PAD patients, chronic limb ischemia is still mostly resistant to medical treatment. Cell treatment and the delivery of angiogenic cytokines (either as recombinant proteins or as gene therapy) have both been used experimentally to enhance limb results. Early research on angiogenesis and cell treatment were encouraging, but they lacked adequate controls, and bigger randomized clinical trials have not yet shown a meaningful improvement.

4.2.1. Vasculogenesis, Angiogenesis and Arteriogenesis, and PAD

The main function of the vascularization in an individual is to control blood flow to the organs, which includes the transport of oxygen, the carrying of nutrients, and the removal of waste products, in order to satisfy their metabolic needs. The primary concern with PAD is the restricted perfusion to the leg's soft tissue and muscle. Consequently, it would make sense to try to increase vascular regeneration and function in PAD to enhance perfusion. The term "adult vasculogenesis" refers to the process by which factors like "granulocyte macrophage colony stimulating factor" (GM-CSF) and "granulocyte colony stimulating factor" (G-CSF) move cells with angiogenic potential from the bone marrow

into the circulation. Although a small percentage of bone marrow-derived angiogenic cells may develop into mature endothelium in vivo, the majority increase perfusion by secreting cytokines and metalloproteinases [59–61]. It's also likely that angiogenic cytokines lead to the mobilization of mature ECs (endothelial cells) from other non-ischemic organs into the bloodstream, where they provide ischemic tissue with a home and aid in angiogenesis [62]. Endogenous arteriogenesis occurs to some extent in PAD patients, but even when it does, the mechanisms have a limited capability to return limb perfusion to normal [63]. Capillary density is frequently decreased and is associated with functional ability in claudication patients [64]. Exercise program, which continues to be the cornerstone of treatment for PAD patients, is notable for causing an increase in capillary thickness in the limb skeletal muscles of affected individuals [65].

The development and multiplication of blood vessels from pre-existing vascular structures is known as angiogenesis [66]. In general, angiogenesis is important for embryonic development. Adult tissue reorganization or healing, as well as reestablishing tissue perfusion, need angiogenesis [67]. Angiogenesis can respond to physical activity as it is physiologically controlled. The emerging of endothelial cells (ECs) from established capillaries under the control of angiogenic factors like VEGF (vascular endothelial growth factor) produced by ischemic tissue is a significant step in angiogenesis. The germinative ECs are migrating, multiplying, and forming lumens, while other procedures that could aid in the development of an operational microvasculature include the intussusception of preexisting capillaries [66, 67], the inclusion of circulating endothelial progenitors [59, 68], and the production of cytokines by transmitting angiogenic cells [69]. When the angiogenic activity to ischemia is insufficient to fulfill tissue needs, pathological angiogenesis results. This most frequently happens in the context of coronary artery disease and PAD. Here, the hypoxia-inducible factor-1 (HIF-1) transcription factor is activated as a result of inadequate tissue perfusion, causing hypoxia in the heart and leg muscles. HIF-1's component HIF-1α possesses an oxygen tension-dependent degradation domain [70]. Low oxygen concentrations cause HIF-1α to become stabilized, move to the nucleus, and stimulate target genes to promote angiogenesis. These genes include VEGF, PDGF (platelet-derived growth factor), HGF (hepatocyte growth factor), FGF (fibroblast growth factor), angiopoietins, Del-1 (developmental endothelial locus-1), and matrix metalloproteinases. Remarkably, it has been demonstrated that exogenous delivery of each of these genes or the proteins they express increases limb blood flow in experimental animals of PAD. Arteriogenesis is the reorganization of pre-existing collateral pathways. These collateral routes have limited blood flow to their distal tissue bed in their natural state because they are small, high-resistance arteries. Blood flow is instead rerouted into the collateral channels when a

primary conduit is blocked, changing the shear stress on the vascular wall. With vascular cell proliferation and vascular matrix turnover, this hemodynamic stimulation causes an increase in the width and layer thickness of the collateral routes [71, 72]. Chemokines and adhesion substances like ICAM (intercellular adhesion molecule), Delta-like 1, CCR2 (C-C chemokine receptor 2), and SDF-1 (stromal-derived factor 1), which connect in monocytes, help the favorable modification by developing metalloproteinases and cytokines, are other contributing factors in arteriogenesis.

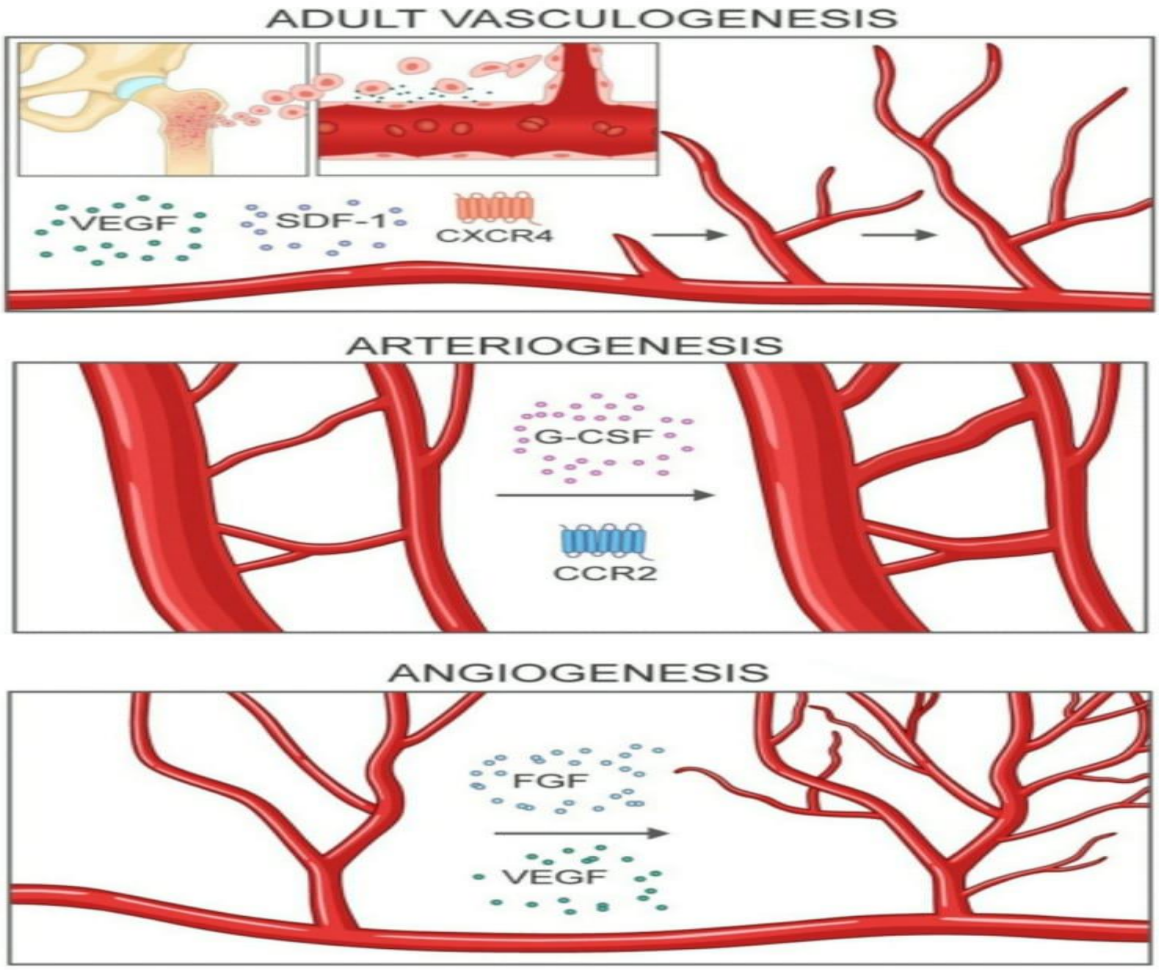

Figure-5: Procedures to reestablish perfusion in peripheral arterial disease (PAD) [58].

4.2.2. Development in potential therapeutical directions for PAD

VEGF-independent angiogenesis:

The well-known, canonical, VEGF-VEGFR2/Akt-1/eNOS (endothelial nitric oxide synthase) pathway has been the focus of the vast majority of human therapeutic angiogenesis trials that have been completed up to this point, where an important mediator is an increase in bioavailable nitric oxide [73–75]. Humans with PAD commonly have concomitant illnesses (including diabetes, which frequently induces and changes PAD) that might impede the synthesis or bioavailability of nitric oxide, therefore encouraging angiogenesis independent of this channel may be crucial [76]. For evaluating the potential effectiveness and bioactivity of suspected treatment drugs as well as to discover new therapies, preclinical models of PAD continue to be a useful resource.

Noncoding RNAs' (miR and long noncoding RNA's) function:

15 to 23 nucleotide (noncoding) RNAs known as micro-RNAs have become important regulators of the body's response to hemodynamic pressures, vascular damage, and hypoxia [77, 78]. The main mechanism by which a miR is assumed to act is by joining the RNA-induced suppression complex, which subsequently binds to a target mRNA, often in the 3′ UTR of that target mRNA, to produce its desired effects [79, 80]. A particular gene target may be suppressed by the miR's binding to the mRNA, or the miR may be able to control numerous genes along a single pathway at once by encouraging mRNA breakdown or preventing mRNA translation to protein [81]. MiR-92a and miR-100 were the first miRs to be reported as potential candidates for modulating neovascularization in PAD [82, 83]. Based on how the EC reacts to damage, these two miRs were found. The function of each in PAD-related angiogenesis was next examined in preclinical models, where perfusion recovery was improved by the suppression of the miR by its antagomirs [82, 83]. In addition to having a minimal effect in cells or tissues where the target miR is not expressed, antagomirs (antisense miRs) have been found to exhibit a high degree of specificity; even in a target tissue, antagomirs can maintain expression levels of closely similar miRs. There have been studies on the capacity of miRs to control angiogenesis in PAD situations [84–89]. Based on the existence of an injury, it is reasonable to predict that various cells or target tissues will have different responses—that is, distinct genes or gene pathways—modulated by a miR. Focused on the miR(s) that controlled arteriogenesis after switching from angiogenesis to arteriogenesis [90]. Following femoral artery ligation, techniques that targeted miR-199a

and miR-146a in mouse PAD models showed enhanced perfusion recovery. In vitro experiments under shear stress revealed potential miRs that may modulate arteriogenesis [91, 92].

Improved autologous cell therapies generation:

Large, meticulously designed clinical studies of cell treatments for PAD, like the PACE research financed by the National Heart, Lung, and Blood Institute, have not demonstrated any positive effects of adult stem cell therapy. The quantity and quality of autologous stem cells derived from people who have the cardiovascular disease may be a factor in the gap between clinical trials and preclinical models (where many cell therapies are effective) [93]. In this sense, attaining a repeatable and meaningful effect is likely to depend on the capacity to supply a sufficient quantity of high-quality therapeutic cells. In every case, several strategies are required. Using autologous skin fibroblasts to produce therapeutic cells is one cutting-edge method of cell therapy for PAD [94]. In this instance, a group of fibroblasts is subjected to retroviral vectors expressing the main controllers of pluripotency (for example, Sox2 [SRY (sex-determining region Y)-box 2], Oct 4 [octamerbinding transcription factor 4], cMyc [cellular myelocytomatosis]), and KLF4 [Kruppel-like factor 4]. Over the course of many weeks, many of the fibroblasts will develop into induced pluripotent stem cells (iPSCs) as a result of these transcriptional factors activating a cascade of genes necessary for pluripotency. Utilizing growth factors and tiny chemicals that aid in the differentiation into a specific lineage, like ECs, the iPSCs may then be differentiated into any of the three germ layers and then further distinguished into the desired lineage. In addition to displaying endothelial surface indicators like VE-cadherin, producing nitric oxide and angiogenic cytokines, and creating vascular networks in Matrigel, iPSC-derived ECs exhibit all of the anticipated traits and activities. Additionally, perfusion was enhanced when administered into the underperfused limb of the murine hindlimb-ischemia condition. Intriguingly, bioluminescence and laser doppler spectroscopy measurements show that ECs produced from pluripotent stem cells may move to the ischemic hindlimb and enhance perfusion. According to these outcomes, autologous iPSC-derived ECs may be helpful as a cell treatment for PAD patients. The removal of pluripotent cells from the therapeutic agent and the integrity of reprogramming must both be extensively evaluated for iPSC derivatives [95].

4.3. The development and prospects of therapeutic neovascularization for coronary artery disease (CAD)

Despite improvements in surgical and percutaneous revascularization methods, close to one-third of patients with ischemic coronary artery disease are either ineligible for revascularization because of their unfavorable anatomical conditions or receive unfavorable results from these common procedures. Not only is neovascularization of the myocardium a physiological reaction to ischemia, but it may also be the focus of novel therapeutic approaches. Initial studies employing otherwise normal animal models demonstrated the potential of inducing angiogenesis via protein, gene, and cell-based treatments. Further research into the vascular environment and endothelial dysfunction were nonetheless motivated by the failure to convert these improvements into people. It has had to reconsider treatment strategy in light of the knowledge that conditions like hypertension, diabetes, and hyperlipidemia not only put individuals at risk for coronary artery disease but also thwarted the efforts at neovascularization therapy.

4.3.1. Neovascularization: the development of endogenous and artificial blood vessels

Vasculogenesis, angiogenesis, and arteriogenesis are the three processes that makeup neovascularization, the development of blood vessels from scratch, which may occur both physiologically and pathologically. All of these processes may be the focus of innovative neovascular therapeutics [96]. Vasculogenesis is the process through which endothelial or pluripotent stem cell precursors develop into early vascular architecture. The cells that make up the coronary vasculature during the developing fetus are generated from the epicardium and have passed epithelial to mesenchymal transition [9]. The process by which endothelial cells proliferate and migrate in response to the vasodilation of venules that results in the formation of microvessels from the existing vasculature is referred to as angiogenesis. The expanded venules then divide into new capillaries via intussusception or bridging. This process occurs physiologically during wound healing and at the border of myocardial infarctions, as well as in diabetic retinopathy and tumor growth [97]. The mechanism of revascularization in response to arterial obstruction into genuine, functioning collateral arteries was the definition of arteriogenesis. Arteriogenesis is the process by which mature arteries with fully developed tunica media are created through the migration and proliferation of endothelial and smooth muscle

cells. This process is capable of adequately perfusing ischemic areas, and it is in mainly influenced by the shear stress produced by pathologic pressure gradients [98]. Arteriogenesis would lead to collateral expansion, which would be observed on a coronary angiography in an individual with persistent myocardial ischemia due to CAD. Arteriogenesis can be distinguished from angiogenesis by the presence of smooth muscle cells, even though endothelial remodeling is important in both processes [99]. The process of collaterogenesis—the de novo generation of collaterals observed during embryonic development—involves the joining of arteries by endothelial tubes.

4.3.2. Development of epicardial coronary vascular

The myocardium receives oxygen and nutrients through simple diffusion in the initial stages of fetal growth. The myocardium must be vascularized when the limitations of diffusion are approached as the heart matures and the ventricular walls widen. An embryonic epicardial endothelium grows and migrates across the heart to produce the proepicardium. Because the epicardium serves as a source of vascular smooth muscle cells, endothelium, and perivascular fibroblasts, the epithelial to mesenchymal transition of the epicardium is essential for cardiac vascularization. From neural crest cells, the bigger proximal coronary arteries are produced. Throughout the heart, endothelial cell precursors move and create a plexus of vascular tubes from scratch (vasculogenesis). Over the epicardium, these circulatory tubes multiply and extend toward the heart's foundation [100, 101]. The primitive vasculature is transformed into bigger arteries after this plexus connects to the aorta and gets perfused. The established epicardial coronary vasculature then develops from the perfused vascular plexus. Research in the lab and on animals has demonstrated that vascular endothelial growth factor (VEGF), transforming growth factor (TGF), placental growth factor (PlGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), along with other modulatory proteins like angiopoietin and erythropoietin, have been shown to regulate this epicardial to mesenchymal transition and the later growth of epicardial and myocardial vascularization [102]. As demonstrated by the pathologic overexpression of these channels found in cancer and other diseases, as well as the initial development of these structures, the necessary genetic code and molecular machinery to build arteries de novo should be available, or at least representable.

4.3.3. Angiogenesis signaling and molecular processes

In addition to upregulating angiogenic growth factors and mobilizing and activating circulatory cells and components that promote neovascularization, the endogenous physiological response to cardiac ischemia, hypoxia, and vascular damage also includes these factors. Historically, research on VEGF, TGF, and FGF has focused the most. Heparin-binding glycoproteins like VEGF and FGF that connect with their corresponding tyrosine kinase receptors trigger the generation of endothelial cell progenitors from the bone marrow and also function as mitogens for vascular endothelial cells [103–106]. Cardiomyocytes and vascular smooth muscle cells generate VEGF, which functions not only in redox signaling but also in stimulating neovascular mechanisms [107, 108]. The cycle of Akt phosphorylation, which has several downstream consequences, is started off when VEGF binds to its tyrosine kinase receptor. Endothelial nitric oxide synthase (eNOS) is phosphorylated and activated by Akt following VEGF-induced phosphorylation in the context of ischemia [109, 110]. Nitric oxide (NO), formerly known as endothelium-derived relaxation factor (EDRF), is a substance generated by eNOS in the vascular endothelium that causes smooth muscle in the arterial wall to relax. Proliferating endothelial cells were found to have six times more eNOS protein and mRNA expression than growth-attained endothelial cells in cultured bovine endothelial cells. In the context of ischemia, vascular progenitor cell proliferation, enhanced endothelial cell growth and survival, and enhanced arterial permeability are further downstream consequences of VEGF and eNOS activation. Additionally, it has been demonstrated that the powerful anti-angiogenic drug thalidomide administration inhibits VEGF production in the coronary artery wall [111]. In addition to the pathways through which VEGF functions, fibroblast growth factor is a nitric oxide release stimulator that partially functions through this signaling pathway. FGF, like VEFG, is a protein that binds heparin sulfate. It activates the FGFR tyrosine kinase receptor, which in turn activates the MAPK, PKC, and Akt pathways. FGF is found in normal cardiac tissue and becomes active when there is hemodynamic pressure. In a manner related to VEGF, downstream consequences of FGF activation include mobilization and stimulation of endothelial cell precursors, cell differentiation, enhanced cell longevity, higher vascular permeability, and endothelial NO generation. FGF stimulates and mobilizes fibroblasts, macrophages, and smooth muscle cells in addition, as its name suggests. Additionally, through activating matrix metaloproteinases (MMPs) and other proteases in the vascular endothelium, it has been suggested that this process plays a significant role in angiogenesis [112–114].

4.3.4. Gene therapy

Protein and gene treatments frequently target similar growth factors, but gene therapy has the benefit of having effects that last longer. Gene therapy can provide a longer therapeutic window than exogenous protein injections, which frequently have a limited effect on the vasculature and a short half-life. The challenge of providing sufficient amounts of genetic material to the target tissue with clinically meaningful genetic integration, or transduction, and subsequent expression, has substantially hampered the effectiveness of gene treatments in both investigational and clinical studies. For instance, although having a favorable safety profile, intravenous injection of bare plasmid DNA has a relatively poor transduction efficiency. However, due to their immunogenic and pro-inflammatory properties along with their propensity to be broken down in the target cells, liposome and synthetic polymer vectors have exhibited restrictions in transduction. Although intended to provide more site-specific delivery, viral vectors like retroviruses and adenoviruses raise questions about the possibility of malignant transformation, insertion of the desired gene into the incorrect DNA portion, and the inability to control or shut off gene expression after transduction. Since the appropriate vector has not yet been discovered, other, more creative methods of delivering genes have been developed, such as covering coronary stents with genes and vectors or delivering bare DNA hydrogel polymers using catheters [115, 116]. These methods allow for dosage and exposure duration management as well as site-specific distribution. In a double-blinded RCT (randomized control trial) conducted in 2002, patients with chronic myocardial ischemia unresponsive to traditional revascularization were randomly assigned to either a placebo injection or a catheter-directed injection of bare plasmid DNA encoding VEGF into the left ventricular myocardium. Patients who got the VEGF plasmid showed a notable improvement in their Canadian Cardiovascular Society angina class and a powerful trend to effectiveness in an improved activity period at the 12-week mark, but there were no appreciable improvements in myocardial viability in ischemic segments or myocardial perfusion. The viability of this strategy has been shown in further RCTs using catheter-directed cardiac injections and infusions with adenovirus vectors, bare plasmids, and liposome vectors expressing VEGF in patients with CAD.

4.3.5. Protein therapy

Exogenous angiogenic growth factor delivery, such as the use of synthetic and isolated proteins, is a feasible strategy for therapeutic neovascularization. While protein therapy has several benefits, such as control over the timing and dosage of the administered substance, it faces difficulties in delivering proteins to the intended tissues over an extended period. Protein has been administered using a variety of techniques in several animal experiments and clinical trials, including intravenous and oral infusions, sustained-release osmotic pumps connected to the heart, sustained-release polymers, and sustained-release microparticle carriers. Research on animals that were otherwise healthy has shown that protein treatment is effective in a number of ways. In normal pigs exposed to chronic myocardial ischemia, both FGF-2 and VEGF produced potent angiogenic activity [117, 118]. Additionally, activation of growth factors and induction of pro-angiogenic processes were seen after the neurotransmitter neuropeptide Y was administered to the heart using an osmotic pump. A slow release microparticle injection of VEGF in a rat model of acute ischemia and reperfusion not only showed that VEGF microparticles were present in the myocardium for more than a month after administering, but also that there was a considerable rise in LV wall thickness, noting positive modification of the myocardium [119]. With the injection of either FGF-2 or VEGF-B, lower limb perfusion was improved in a rabbit model of bilateral hind limb ischemia, and combined treatment with both proteins expanded the quantity of collateral arteries. However, the majority of animal investigations using hypercholesterolemic or diabetic models have not been able to significantly increase collateral-dependent perfusion to either VEGF or FGF-2 or generate substantial collateral vascular development. Clinical trials were then conducted in an effort to replicate the advantages in people after consistent increases were seen in animal tests involving the infusion of exogenous proteins. During the FIRST experiment, which took place in 2002, 337 individuals with CAD who were deemed to be less than ideal candidates for traditional surgical or catheter-based revascularization participated in a double-blind, randomized control trial (RCT) utilizing recombinant FGF-2. Only a little tendency toward symptomatic betterment found at 90 days persisted through 180 days. Exercise tolerance and myocardial perfusion did not improve.

4.4. Stroke-related neovascularization and regeneration of new blood vessels

One of the most intriguing avenues for potential treatments in the developing field of stroke therapy is the development of new blood vessels following acute ischemic stroke. The processes that cause new blood vessels to develop include angiogenesis and postnatal vasculogenesis. Endothelial progenitor cells (EPCs) produced from bone marrow are regarded to be crucial for neovascularization, regenerative mechanisms following vascular damage, and endothelial integrity management. Finding techniques to heal injury in the adult nervous system after irreversible neuronal cell loss, such as in neurodegenerative illnesses, stroke, and traumatic brain injury, is one of the biggest issues facing neurology today. The majority of biological tissues are capable of regeneration, however, the nervous system is thought to be non-regenerative. Recent advancements in neuroscience have shed light on potential strategies for encouraging neuronal regeneration in the central nervous system, though. The upcoming advancement of novel therapeutic strategies may be greatly aided by new theories regarding angiogenesis and postnatal vascularization of the penumbra area after an ischemic stroke, including the potential contribution of endothelial precursor cells and the significance of vascularization in poststroke neurogenesis [120].

4.4.1. Endothelial progenitor cells may play a role in angiogenesis and vasculogenesis development.

The two primary processes in the development of new blood vessels after vascular damage, such as during an ischemic stroke, are angiogenesis and vasculogenesis. Vasculogenesis is the generation of new arteries by the "de novo" synthesis of endothelial cells, such as the distinctiveness of precursor cells (angioblasts) into endothelial cells. Angiogenesis is the development of fresh vessels from existing arteries [121]. Endothelial progenitor cells (EPCs) generated from bone marrow are angioblasts that are regarded to be crucial in endogenous vascular healing following vascular damage and the preservation of endothelial stability. EPCs are undeveloped endothelial cells that circulate in peripheral circulation and share features with hematopoietic stem/progenitor cells. After vascular damage, for instance, they are recruited from the bone marrow and transported to the site of neovascularization, where they undergo endothelial cell differentiation. This is consistent with the widely accepted idea of "vasculogenesis" in the development of embryonic vascular structures. EPCs are believed to have both the structural and functional traits of matured endothelial cells and stem cells. The modern understanding that is most frequently recognized is the finding of co-expression of the

surface markers CD34, CD133, and VEGF receptor-2, also known as kinase insert domain receptor (KDR). A certain progenitor cell subunit at a specific maturation stage may be identified by the co-expression of particular surface indicators including CD34, CD133, and VEGF receptor-2. Different functional traits may exist among EPC subgroups. Therefore, when EPCs develop into endothelial cells, the expression of CD34, the initial marker identified to characterize EPCs, declines with time [122].

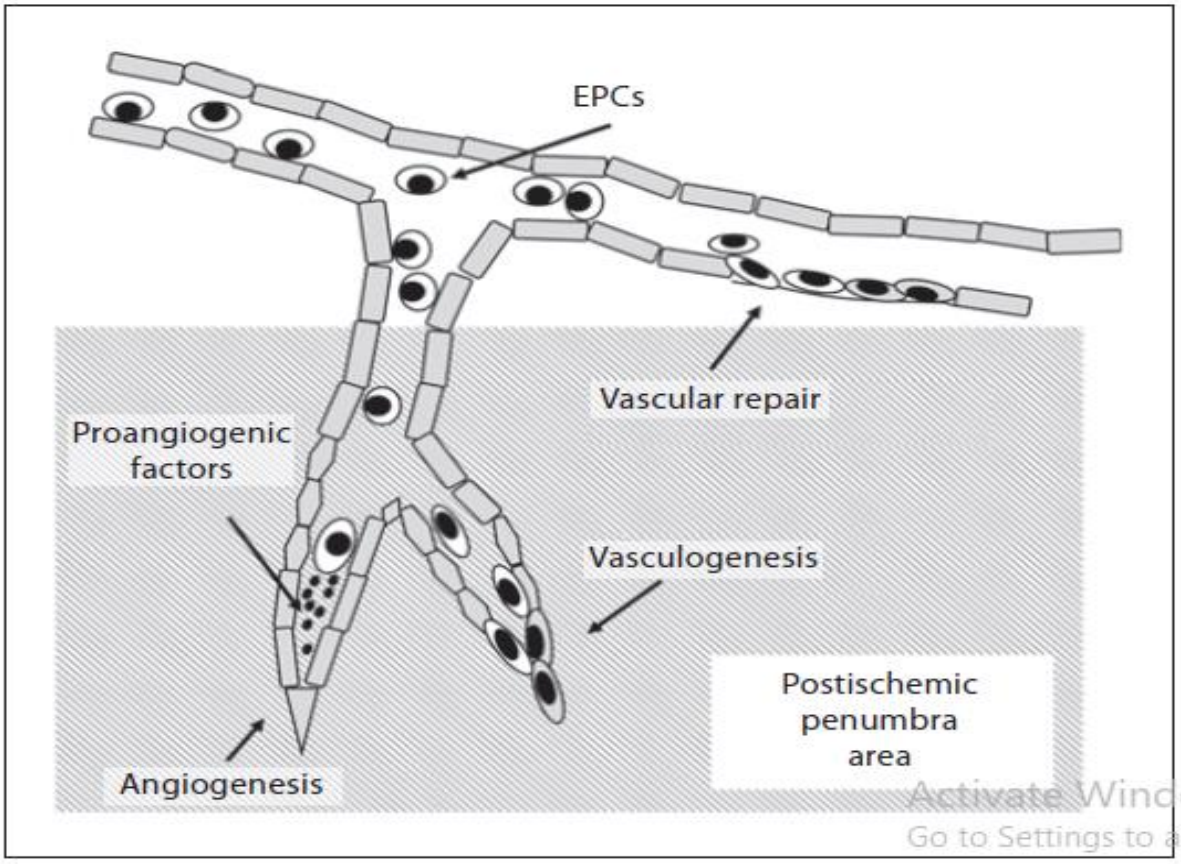

Figure-6: EPCs' possible operational significance in angiogenesis, vasculogenesis, and vascular healing following cerebral ischemia is depicted schematically.

4.4.2. Targeting endothelial progenitor cells (EPCs) as a therapy

Endothelial nitric oxide synthase's (eNOS) critical function in neovascularization has previously been reviewed. It has been proven that the eNOS protein, which is produced by bone marrow stromal cells, is crucial for the movement and activity of stem and progenitor cells [123]. Therefore, decreased endothelial NO bioavailability may be the

cause of poor function and inadequate EPC recruitment following vascular damage in individuals with cardiovascular risk, which may exacerbate the vascular injury and inhibit repair within those individuals. When rosuvastatin is given to rats that have had their carotid arteries damaged, the favorable rise in EPC levels that results promotes bone marrow-dependent re-endothelialization and slows the growth of vascular lesions. It is found that physical exercise has an impact on the quantity and quality of EPCs in mice via a NO-dependent pathway [124]. Individuals with elevated vascular danger, or those with coronary artery disease, who were asked to a 4-week training session had their outcomes validated. Physical activity improves EPC numbers in both mice and men (CD34+KDR+), as well as makes neovascularization development and neointima loss following carotid damage in mice. Overweight, hyperlipidemia, cigarettes, and pressure are other lifestyle-related variables that have been connected to lower EPC numbers and activity. Additionally, after 4 weeks of quitting, EPC levels returned quickly, mirroring the benefits of quitting smoking on endothelial function. EPC injection or therapy has lately been demonstrated to enhance behavioral testing results and infarct volume in mice following temporary 24-hour blockage of the mean cerebral artery. It's interesting to note that inhibiting CXCR-4 on EPCs reversed the neuroprotective effects of EPCs, supporting the idea that SDF-1/CXCR-4 is essential for EPC-mediated brain protection. Information that EPC therapies may optimize vascular/cardiac function and the actual result after acute myocardial infarction is arising from clinical pilot studies in cardiovascular patients, such as the Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in the Acute Myocardial Infarction research. EPCs might be a pharmaceutical target in addition to stem cell transplantation. Future treatments for cardiovascular illnesses may heavily rely on medications that mobilize and functionally alter EPCs in vivo. Angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and β-blockers, in addition to statins, have shown a direct positive impact on the recruitment and function of circulating EPCs [125]. But a deeper comprehension of the elements governing EPC biology is crucial.

4.4.3. Stroke and Angiogenesis

In ischemic tissue, angiogenesis is a critical component of the regeneration procedure. Higher blood flow, a reduction in infarct size, and support for the healing and rebuilding of neurovascular networks following ischemia are all possible outcomes of proangiogenic therapy in stroke. Intensive restoration and vigorous angiogenesis are occurring in the ischemic penumbra. Several growth factors are known to promote angiogenesis and endothelial cell proliferation, including VEGF and hypoxia-inducible factor-1. This may

begin as soon as 12 to 24 hours after a stroke and last for up to a few weeks after ischemia. It was enabled to show the protective effects of workouts by inducing angiogenesis in a mouse model of moderate cerebral ischemia [126]. It was proven that moderate exercise grew the number of developing endothelial cells, activated eNOS in the blood vessels, and raised the concentrations of EPCs in the blood. Additionally, when mice were given a NOS inhibitor or an antiangiogenic substance, this was totally eliminated and was linked to a superior functional result at 4 weeks. A superior cerebrovascular responsiveness and higher absolute blood flow levels were demonstrated to be caused by jogging, which also caused considerably larger density of perfused microvessels. Finally, bone marrow chimeric mice were used to show how physical activity increases bone marrow-derived cells, which are involved in new vascular creation. Additionally, there is proof that angioblasts and brain precursors multiply along in groups near the microvasculature, supporting the idea that adult neurogenesis takes place in an angiogenic environment, or the "vascular niche" concept. The majority of precursor cells in the subgranular zone multiply in this situation, where tightly clustered neuronal, glial, and endothelial precursors multiply. Small capillary branches or termini may include these clusters, indicating an active angiogenesis activity.

4.4.4. Stroke and Arteriogenesis

Immediately following a stroke, cerebral blood flow is reduced. Acute reconstruction of tissue perfusion by mobilization of blood from nearby or distant sites can lower the level of infarction and confer safety, based on the scale of native collaterals and anastomotic vessels (circle of Willis, artery-artery interrelations, ophthalmic, and leptomeningeal arteries). After a stroke, both pre-existing collaterals and effective or fresh collateralization increase the advantages of thrombolytic and endovascular therapy and improve the therapeutic outcomes [127, 128]. A key trigger for arteriogenesis is fluid shear stress, which is preceded by the nitric oxide pathway activating endothelial and vascular smooth muscle cells. Increased growth factor and cytokine release caused by monocyte invasion and induction of inflammatory mechanisms result in the formation of collaterals and favorable external modification of the vasculature. In animal experiments, collateral development, higher cerebral blood flow, artery lengthening, and external vascular remodeling have all been seen promptly and up to one month following stroke. Vasodilation and the pharmacological stimulation of blood pressure have both been found to increase pre-existing collateral circulation, with beneficial functional effects and

a decrease in stroke volume. Granulocyte macrophage colony-stimulating factor been shown to lessen functional impairments following stroke by promoting the development of new collaterals. There are so many studies discussing the value of collateralization following stroke, but none have specifically addressed the arteriogenesis pathways following traumatic brain injury (TBI). However, studies show that between 7 and 28 days following a TBI, vascular density gradually increased. Four days after TBI, the average diameter of both microvessels and big vasculature greatly increased, which was accompanied by an uptick in the expression of Ang-1, stromal cell-derived factor-1, vascular endothelial growth factor (VEGF), and endothelial nitric oxide synthase [129, 130]. Arteriogenesis exhibits this favorable external reshaping of the vasculature, which raises the possibility that collateralization may be a form of neovascularization following TBI. Then comes a vascular density growth brought on by angiogenesis. To further understand the two mechanisms in TBI at the early and later time periods, more research is required.

4.4.5. Stroke and Vasculogenesis

Endothelial precursor cells are essential to the mechanism of neovascularization and have been researched for their therapeutic potential to increase vascular density. A group of circulating progenitor cells known as "endothelial progenitor cells" develops into an endothelial lineage with vasculogenic capabilities and develops into new blood vessels. By combining pre-existing capillaries and excreting growth factors and cytokines, endothelial progenitor cells (EPCs) aid in the restructuring of the brain's blood vasculature and neural healing following stroke. The co-expression of CD34+, CD133, and VEGFR-2 on the cell surface is a well-known marker of EPCs [131–133]. According to reports, after a stroke, EPCs play a role in the movement of neuronal progenitors that cluster around the peri-infarct area. Both ischemic stroke and TBI damage models have been shown to mobilize EPCs from the bone marrow and peripheral circulation in the injured area. EPCs are seen in the peripheral system and close to the injured brain tissue as fast as 24 hours after TBI and reach their peak at 48 hours. After TBI, angiogenesis is correlated favorably with the number of CD34+ progenitor cells. The ability to induce an increased neovascularization response by therapeutic delivery of EPCs and conditioned media produced from EPCs makes them prospective instruments for promoting vascular healing after stroke and TBI [134, 135].

Chapter-5

Reference

5. References.

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Chapter-6

Conclusion

6. Conclusion

Neovascularization, the process by which the vascular system is formed, is a complicated one requiring the coordinated action of several cell types. Although the formation of fresh blood vessels, or neovascularization, is crucial for embryonic progress and improvement in addition to a variety of human diseases, the function of therapeutic angiogenesis in cardiovascular diseases (CVDs), such as ischemia and atherosclerosis, is still not fully recognized. The objective of modifying neovascularization in cardiovascular diseases is very appealing. The decision to use protein or gene therapy is largely influenced by technological advancements in vector modeling and manufacturing on the one side, and the creation of proteins in delayed matrix formulations on the other. Pairings of growth factors, for example, to stabilize arteries, merit further study, but will complicate preclinical and clinical study approaches. According to the latest studies, just one type of growth factor therapy can produce solid and functioning vasculature with the right dosage and treatment plan. In order to recognize possible treatment targets, such as vascular regeneration and stem cell enrollment, the idea of three distinct mechanisms of neovascularization has been useful. However, it is anticipated that as the pleiotropic nature of the angiogenic cytokines is properly known, this idea will become less relevant. Measurements of angiogenesis, arteriogenesis and vasculogenesis need to be linked with the evaluation of efficacy when evaluating results in clinical research, especially basic investigations. Methodologies that are more successful than those that have been tried in clinical trials up to this point will emerge as a result of the huge and quickly expanding amount of evidence that has been gathered on growth factors and pro-angiogenic techniques. Therefore, it is still crucial that these methods be logically founded on basic and preclinical evidence.