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ANTI-ANGIOGENIC PROPERTIES OF NANOPARTICLES:

A SYSTEMATIC REVIEW

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A SYSTEMATIC REVIEW

by

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DEDICATION

This thesis is dedicated to

My beloved mother and father

To

Brothers, sisters

To

My beloved wife, son and daughter

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Praise to Allah, the most merciful who gave me determination and strength to complete this work.

Deepest thanks go to the utmost important people in my life. My father, my mother, my wife and my kids, my brothers and sisters. Their prayers, blessings, love and care undoubtedly strengthen days in USM.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

SELECTED LIST OF ABBREVIATION

Ag	Silver
AgNPs	Silver nanoparticles
AMD	Age-related macular degeneration
Au	Gold
AuNPs	Gold nanoparticles
BBB	Blood-brain barrier
BRECs	Bovine retinal endothelial cells
C60	C60 fullerenes
CAM	Chorioallantoic membrane
Ce	Cerium
CNP	Chitosan nanoparticle
CNS	Central nervous system
CNV	Choroidal neovascularization
CONPs	Copper nanoparticles
DAPHP	Diaminopyridinyl heparin
DR	Diabetic retinopathy
ECs	Endothelial cells
EPCs	Endothelial progenitor cells
ERK	Extracellular signal-regulated kinase
FAK	Focal adhesion kinase
FDA	Food and Drug Administration
FGF	Fibroblast growth factor

FGFR	Fibroblast growth factor receptor
GBM	Glioblastoma multiforme
Ge-ZnO NPs	Gelatin-coated zinc oxide nanoparticles
GNPs	Gold nanoparticles
GNS	Graphene nanosheets
HANPs	Hydroxyapatite nanoparticles
HAP	Hydroxyapatite
Hb	Hemoglobin
HCC	Human hepatocellular carcinoma
HIF	Hypoxia-inducible factors
HP	Heparin
HRMECs	Human retinal microvascular endothelial cells
HUVECs	Human umbilical vein endothelial cells
IC50	Inhibitory Concentration 50
IGF-1	Insulin-like growth factor 1
KDR	kinase insert domain receptor
MAPK	Mitogen activated protein kinase
MeSH	Medical Subject Heading
MMPs	Matrix metalloproteinase
mRNA	Messenger ribonucleic acid
MW-RF	Microwave-radiofrequency
MWNT	Multi-wall nanotubes
NCe	Nano ceria
ND	Diamond nanoparticles
NG	Graphite nanoparticles

NO	Nitric oxide
NPs	Nanoparticles
OIR	Oxygen-induced retinopathy
PBS	Phosphate buffered saline
PEDF	Pigment epithelium-derived factor
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analysis
PTC	Presumptive therapeutic concentration
q-RT-PCR	Real-Time Polymerase Chain Reaction
ROP	Retinopathy of prematurity
RPE	Retinal pigmented epithelia
SiNPs	Silicate nanoparticles
SiO2NPs	Silica nanoparticles
TiO2	Titanium dioxide
TGF	Transforming growth factor
UDD	Ultradispersed detonation diamond
VBM	Vascular basement membrane
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
Zn	Zinc
ZnO	Zinc oxide
ZnO NPs	Zinc oxide nanoparticles

SELECTED LIST OF SYMBOLS

Symbol	Meaning
%	Percent
nM	Nano molar
µg	Microgram
ml	Milliliter
mg	Milligram
α	Alpha
pM	Picomolar
nM	Nanometer
µM	Micromolar

ABSTRAK

Pengenalan: Angiogenesis terhasil daripada pembentukan pembuluh darah yang baru dari pada pembuluh darah utama dan proses ini dikawal atur oleh keseimbangan faktor pro angiogenesis dan anti-angiogenesis. Dominasi bagi proses angiogenesis ini membawa kepada kondisi patologi angiogenesis yang mengakibatkan penyakit seperti retinopati diabetic dan kanser. Terapi anti-angiogenesis menyasarkan angiogenesis dalam mengubati penyakit-penyakit ini. Rentetan dari itu wujudnya nanopartikel sebagai salah satu agen terbaik yang dapat menghasilkan efikasi dalam terapi. Objektif kajian ini adalah untuk mengulas kajian secara sistematik bagi tajuk kesan nanopartikel ke atas angiogenesis.

Kaedah: Kajian literature (PubMed, ScienceDirect, Google Scholar, Medline, Scopus, and Springer) dikumpul bagi mengenalpasti sebanyak mungkin artikel yang berkaitan sebagai topik terpilih iaitu yang berkaitan dengan tajuk kesan nanopartikel keatas angiogenesis. Kriteria pilihan seperti artikel yang diterbitkan dalam bahasa Inggeris dan yang juga diterbitkan dalam jangkamasa Januari 2008 sehingga Januari 2018 juga antara kriteria yang ditetapkan. 'The Medical Subject Heading (MeSH)' bagi nanopartikel dan angiogenesis dan anti-angiogenesis atau angiogenesis digunakan bagi saringan dan pemilihan artikel dari PubMed dan Medline. Selain itu, pencarian terma dan kata kunci bagi tajuk telah diaplikasikan bagi mendapatkan lebih jurnal dan artikel dari pengkalan data yang lain. Tambahan, senarai rujukan yang dipilih dalam pencarian pertama, di saringkan lagi bagi pemilihan artikel-artikel yang lebih berkaitan. Artikel yang tidak mematuhi kriteria pemilihan tidak dimasukkan sama sekali.

Keputusan: Dalam 218 artikel yang diperolehi daripada pencarian literature, hanya 22 yang di pilih berdasarkan kriteria yang ditetapkan. Didapati, nanopartikel bentuk sfera merupakan bentuk yang paling banyak digunakan dalam terapi angiogenesis. Ukuran nanopartikel ynag paling tinggi memberikan kesan dalam terapi angiogenesis ialan nanopartikel daam ukuran 20 nm. Nanopartikel jenis emas menunjukkan kesan sebagai agen anti-angiogenesis yang baik. Nanopartikel juga memberi kesan yang baik dalam model *in vitro* dan *in vivo*. Didapati, nanopartikel dari biosintesis adalah selamat jika dibandingkan dengan nanopartikel dari kimia sintesis. Kesan ketoksikan bagi nanopartikel adalah bergantung pada dos yang digunakan.

Kesimpulan: Nanopartikel merupakan agen bagi terapi penyakit yang berkaitan dengan angiogenesis. Nanopartikel merencat angiogenesis dalam pelbagai cara. Ukuran dan bentuk memainkan peranan penting dalam memberikan kesan terhadap aktiviti angiogenesis oleh nanopartikel. Nanopartikel emas memberikan kesan perencatan yang tinggi keatas proses angiogenesis.

Kata kunci: Nanopartikel, angiogenesis, anti-angiogenesis, ukuran, bentuk, ketoksik

ABSTRACT

Introduction: Angiogenesis develops new blood vessels from the old ones, and this process is controlled by balancing the angiogenic and anti-angiogenic factors. The domination of angiogenic leads to pathological angiogenesis that causes diseases, such as diabetic retinopathy and cancer. Anti-angiogenesis therapy targets angiogenesis to treat these diseases. Hence, nanoparticles appear to be one of the most promising agents that offer efficacy in therapy. The objective of this research is to review systematically studies that have probed into the effect of nanoparticles on angiogenesis.

Methodology: The literature was searched electronically (PubMed, Science Direct, Google Scholar, Medline, Scopus, and Springer) to identify as many relevant articles as possible for the topic at hand, which is the effect of nanoparticles on angiogenic. The inclusion criteria, such as articles published in the English language during the period from January 2008 until January 2018, were strictly adhered to. The Medical Subject Heading (MeSH) (nanoparticles) and anti-angiogenesis or angiogenesis were used to extract articles from PubMed and Medline, while search terms or keywords of the title had been applied to retrieve more journals and articles from other databases. Additionally, references that were cited in the initial search were sought to identify more potential articles. Next, articles that did not meet the inclusion criteria and those duplicate were discarded.

Results: Out of the 218 articles obtained from the literature search, only 22 seemed to conform to the inclusion criteria. The spherical shape is the most common shape employed to investigate the role of nanoparticles in angiogenesis therapy. The size of

nanoparticles appears to play a crucial role for efficacy on angiogenesis, in which 20 nm emerged as the preferred size. Gold nanoparticle exhibit the most promising type as an anti-angiogenesis agent. The nanoparticles were active for both *in vitro* and *in vivo* models. The biosynthesis of nanoparticles is safer than chemical synthesis. The toxicity was adjustable based on the dosages applied.

Conclusion: Nanoparticles are a promising treatment for angiogenesis-related diseases. Nanoparticles inhibit angiogenesis in different pathways. Size and shape play a role in the anti-angiogenesis effects of nanoparticles. Gold nanoparticle exhibit higher anti-angiogenesis properties.

Keywords: Nanoparticles, angiogenesis, anti-angiogenesis, size, shape, toxicity

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Angiogenesis refers to the generation of new blood vessels from a pre-existing one (Gu et al., 2014, Lu et al., 2005, Yu et al., 2010). It plays an essential role in various physiological states like embryo growth, ovulation, and wound healing. Moreover, it is important in the progression of many diseases like diabetic retinopathy, arthritis, and metastasis (Kanwar et al., 2011). In the normal physiological state of the body, the censorious balance between the proangiogenic and anti-angiogenic keeps the angiogenesis under the critical control (Behl & Kotwani, 2015). The imbalance between these factors may eventually lead to the development of pathological conditions (Hsu et al., 2015).

First hypothesis pertaining to angiogenesis was developed almost four decades ago. It indicates that tumor growth depends on blood supply and prevention of this supply will treat the tumor. Hence, the term anti-angiogenesis means any measures that prevent the formation of new vessels and reaching of blood supply into the tumor (Kalishwaralal et al., 2010). The major mechanism of action of anti-angiogenic drugs is to attach with growth factors such as vascular endothelial growth factor (VEGF), therefore hinder any attachment between growth factors and their receptors like vascular endothelial growth factor receptor (VEGFR). The attachment between the growth factor and its receptor is the main method that induces the generation of new blood vessels (Mukherjee & Patra, 2016). Angiogenesis is considered as

an intricate process as it involves proliferation and migration of endothelial cell, permeability, and formation of blood vessels (Song et al., 2014). There have been many advances regard to angiogenesis in the past three decades accompanied with the elucidation of numerous anti-angiogenic agents that suppress angiogenesis (Banerjee et al., 2011).

The monoclonal antibody Bevacizumab (Avastin) is the first anti-angiogenic therapy that was licensed by U.S. Food and Drug Administration (FDA), as a drug to treat colorectal cancer cells by targeting overexpressed VEGF proteins and reducing blood supply of those cell (Banerjee et al., 2011). Nevertheless, the therapy seemed insignificant in some cases due to resistance and other limitations such as limiting pharmacokinetics. Therefore, the encapsulation of these drugs into nanocarriers is promising to overcome some of these drawbacks (Sousa et al., 2017). It is anticipated that unique properties of nanoparticles like anti-angiogenic and drug carrier can be applied as an alternative strategy in the treatment of serious angiogenic disease such as cancer (Mukherjee & Patra, 2016). Many studies are emerging to evaluate the influence of nanomaterial in inhibiting angiogenesis (Pan et al., 2014). Gold, silver, and silica are the most important inorganic nanoparticles that show anti-angiogenic effects (Jo et al., 2015).

The great funds are exploiting in research and development of anti-angiogenesis drugs. Notably, more than $4 billion has been attributed to angiogenesis research (Saghiri et al., 2015a). On that account, we systematically review the literature focusing on usage of nanoparticles as anti-angiogenic and the impact of nanoparticle's properties on anti-angiogenic activity.

1.2 Objectives

General objective

To review the efficacy of nanoparticles as an anti-angiogenic agent.

Specific objectives

1. To compare the anti-angiogenic activity of nanoparticles based on the studies from January 2008- January 2018.

2. To correlate nanoparticles size and morphology with the anti-angiogenic properties.

3. To determine the most promising nanoparticle as anti-angiogenic agent based on its properties.

CHAPTER 2

LITERATURE REVIEW

2.1 Angiogenesis

The term angiogenesis was first used to describe the formation of new blood vessels in the placenta 8 decades ago. It was then identified in wound healing and tumor growth. One study proposed that inhibition of angiogenesis may be a potential way of treating cancer as it can inhibit cancer progression (Folkman, 2002).

2.1.1 Types of Angiogenesis

The formation of the blood vessel during angiogenesis occurs in two types sprouting angiogenesis and intussusception (Ribatti & Crivellato, 2012). There are specific endothelial cells (ECs) within the capillary vessel wall that have the ability for sprouting (Figure 2.1a). These ECs are called the tip cells, which promotes the growing sprout (Adams & Alitalo, 2007). Sprouting angiogenesis takes place in several successive steps. It begins when specific growth factors react with its receptor on endothelial cells and activate the cells. Hence, the cells release activated proteases that degrade the extracellular matrix and basement membrane surrounding the endothelial cells. The endothelial cells become able to move and invade the surrounding matrix. Stalk cells behind the tip cell proliferate, elongate and form a lumen and sprouts fuse to establish a perfused neovessel. Proliferating stalk cells attract pericytes and deposit basement membranes to become stabilized. The strict balance between angiogenic and antiangiogenic modulators regulates sprouting angiogenesis and determines

the level of ongoing angiogenesis (Hillen & Griffioen, 2007). It should be known that angiogenesis generates blood vessels from preexisting one, dissimilar to vasculogenesis that is defined as the formation of blood vessels from angioblastic precursor cells (Figure 2.1b) (Djonov et al., 2000).

Intussusception is another type of angiogenesis. It is called splitting angiogenesis and occurs through the internal division of the preexisting capillary plexus without sprouting (Figure 2.1c). Intussusception is considered as a novel method of blood vessel formation and remodeling (Djonov et al., 2000). It may be directly involved in structural remodeling intend for optimization of vessel form and function (Kurz et al., 2003).

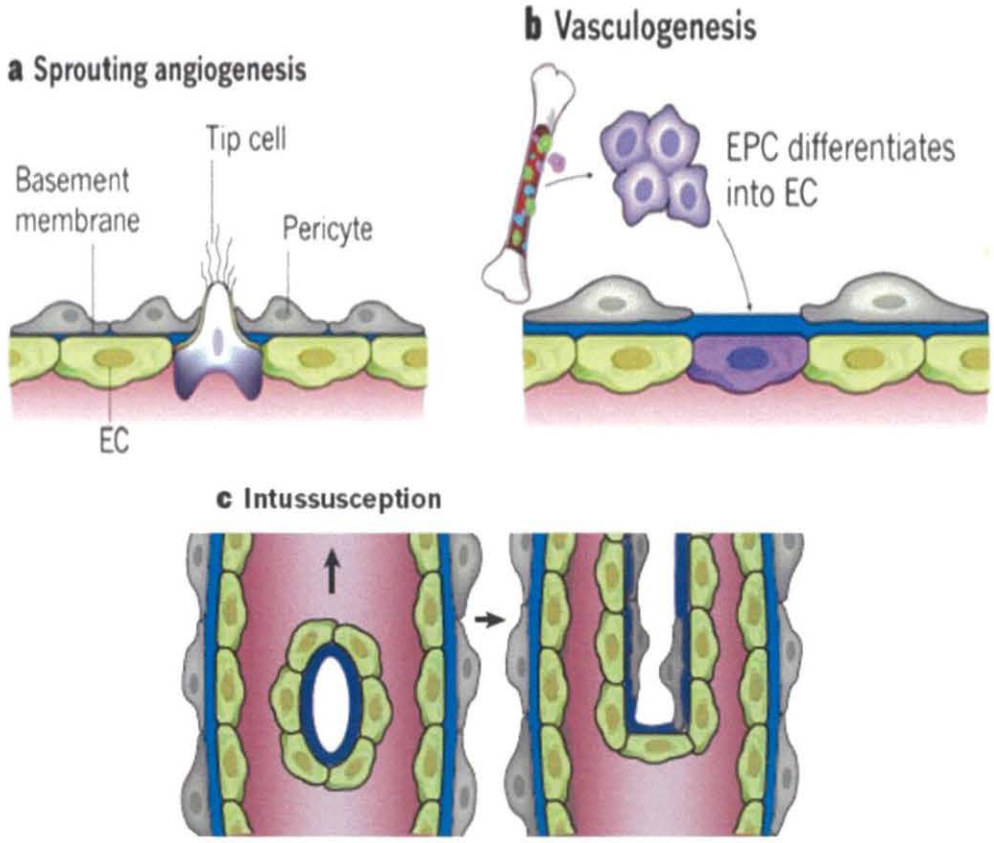

Figure 2.1: Vessel formation can occur via sprouting angiogenesis (a), the recruitment of bone-marrow-derived and/or vascular-wall-resident endothelial progenitor cells (EPCs) that differentiate into endothelial cells (b), and intussusception (c). Adapted from Carmeliet & Jain, 2011.

2.1.2 Physiological and Pathological of Angiogenesis

In normal conditions, the body allows the angiogenesis to take place only during physiological states like embryonic development, and wound healing (Behl et al., 2017). On another side, uncontrolled development of new blood vessels from preexisting vessels is considered as pathological status causing metastasis, blinding ocular illness and inflammatory diseases (Lu et al., 2005). Retinal neovascularization is responsible for various ocular diseases like advanced diabetic retinopathy (Gurunathan et al., 2009). Angiogenesis

plays an essential role in the formation of endometriosis as growing ectopic lesions require abundant blood (Wang et al., 2014).

The balance between angiogenic inhibitors and inducers keeps angiogenesis in the normal physiological conditions. On the other hand, disturbance the balance will cause pathological angiogenesis (Figure 2.2) (Xu et al., 2009). In fact, angiogenesis activities are rare in adult individuals who have stable circulation network vessels with exception in some cases like pregnancy (Shojaei, 2012). Growing evidence involving pathological angiogenesis in numerous serious illnesses, such as solid tumors, arthritis, and diabetic retinopathy has attracted interest many researchers (Hammady et al., 2009).

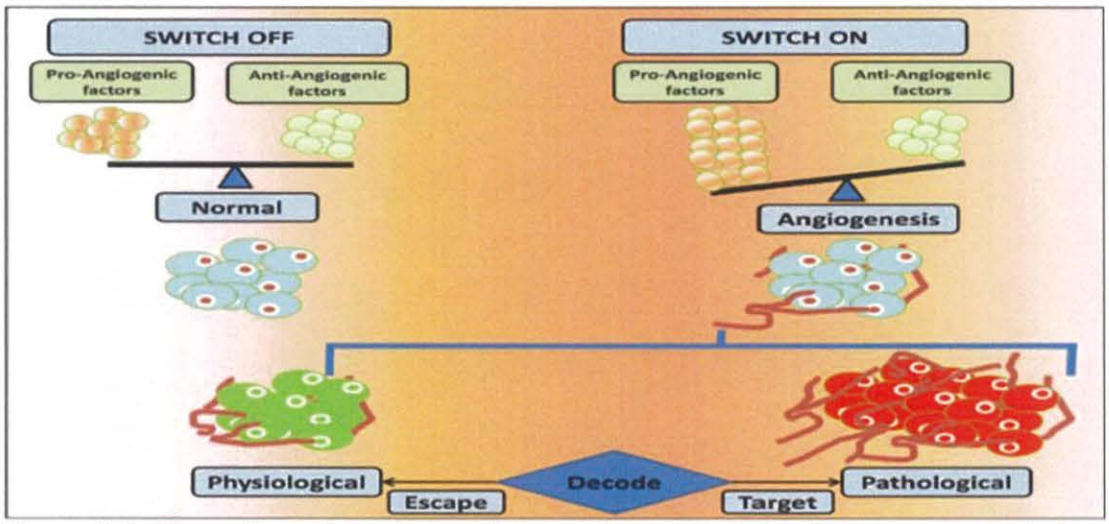

Figure 2.2: Illustrations of regulation of the angiogenic switch with respect to the balance between proangiogenic and anti-angiogenic factors (the upper portion). The lower portion describes two types of angiogenesis i.e. physiological (cells in green color) and pathological (cells in red color). Adapted from (Gacche & Meshram, 2014)

2.1.3 Regulation of Angiogenesis

Angiogenesis is strictly controlled by the balance between angiogenic and anti-angiogenic modulators that contribute either in stimulating or inhibiting angiogenesis. Regulation of angiogenesis is performed in three stages:

(1) Degradation of the extracellular matrix. (2) Adjustment of the angiogenic modulators like the growth factors, the cytokines, and enzymes. (3) The cell-cell and the cell-matrix interactions (Liekens et al., 2001).

In angiogenesis, the vascular basement membrane (VBM) is degraded by several matrix-degrading enzymes like the matrix metalloproteinase (MMPs). Hence, the endothelial cells can migrate and invade adjacent connective tissues (Raghu, 2003). The control on the activity of MMPs occurs in two levels either at their expression activation level by proteolytic enzymes or at their inhibitors level like the tissue inhibitor of metalloproteinase (Liekens et al., 2001). After degradation of the extracellular matrix, the vascular endothelial cells migrate through the degraded matrix, then proliferation is induced under the influence set of growth factors (Raghu, 2003). There are several modulators of angiogenesis have been discovered so far including inducers with a positive effect (angiogenic factors) like integrins, VEGF, angiopoietins, fibroblast growth factor (FGF), transforming growth factor (TGF) and CXC chemokines. On the other hand inhibitors with a negative effect (angiostatic factors) such as angiostatin, endostatin, and thrombospondin (Kim *et al.*, 2017).

Regulation of angiogenesis is maintained by a balance between angiogenic inhibitor and inducer. As such, when angiogenic agent dominate, then proliferation and migration of endothelial cells is growing, subsequently leads to the formation of new blood vessels. On

that account, angiogenesis can be prevented when anti-angiogenic factors predominate. The growth factors, cell adhesion molecules, and proteases enzymes play an essential role in the regulation of the angiogenesis consecutive events. Several types of adhesion molecules are known including members of the integrin, cadherin and immunoglobulin families. Integrins play an important role in lumen formation and the construction of functional capillary loops via facilitating many interactions that take place between cell-to-cell and cell-to-extracellular matrix, these interactions are necessary to angiogenesis (Duro-Castano et al., 2017).

2.1.4 Angiogenesis Consecutive Events

Angiogenesis takes place through many steps, one of these steps is interactions of the endothelial cell after migration out and facing with the extracellular matrix compounds (Figure 2.3). The normal endothelial cell in blood vessels (Figure 2.3a). The initiation starts when biological signals lead to increase of VEGF-165 expression and activate of the receptors on the endothelial cell by growth factors. Subsequently, the activated cells begin to release proteases enzymes (Matrix Metalloproteinase) causing detachment of the pericytes from the surface of blood vessels and degradation of the VBM and extracellular matrix (Figure 2.3b). Next, the underlying endothelial cells migrate from the blood vessel walls into the surrounding area of vascular under the effect of guidance signals (Figure 2.3c). The migrating cells proliferate and follow each other in line to form solid sprouts that link to the nearby vessels using integrins (Figure 2.3d). Next, basement membrane formation and pericytes attach around the newly formed vessels. Finally, the blood vessels sprouting fuse with other sprouts to form loops (Figure 2.3e) (Carmeliet & Jain, 2011).

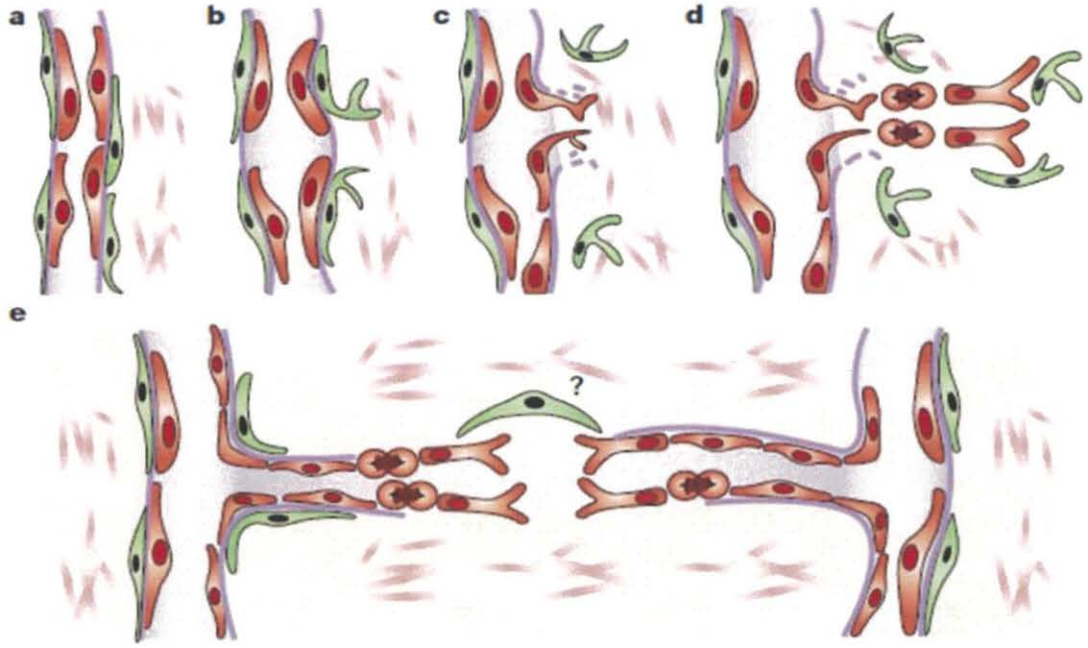

Figure 2.3: The angiogenesis process cascade. Adapted from Bergers & Benjamin, 2003.

2.1.5 Anti-angiogenic Targets

The process of growth of new blood vessels passes through many stages. Targeting these stages will lead to suppressing angiogenesis. Inhibition can be achieved by: (1) decreasing of endothelial cell activation, that could be done through the deactivation of growth factor signal production, suppression of receptors production or suppression of the binding between signals and receptors (2) targeting proliferation of endothelial cell (3) hindering of endothelial cell migration (3) suppressing of endothelial cell differentiation to form a tube-like structure (4) triggering of apoptosis in endothelial cells (Kang et al., 2016, Kalishwaralal et al., 2009, Song et al., 2014).

2.1.6 Anti-angiogenic Therapies

The angiogenesis nourishes diseases such as a tumor and contributes to metastasis. Hence, the suppression of angiogenesis has been considered as a successful strategy in the therapy of tumor growth and other angiogenesis-related diseases (Clément-Duchêne & Wakelee, 2010).

The anti-angiogenic medicines perform their function via attaching with VEGF and keep VEGF away from its receptor (VEGFR). The reaction between growth factors and their receptor is mainly responsible for stimulating the growth of new blood vessels. FDA approved bevacizumab which binds to VEGF and inhibits the interaction between VEGF and VEGFR (Sousa et al., 2017). Later on, FDA also approved small molecules like sorafenib, sunitinib, and pazopanib that classified as inhibitors of the VEGFR-2 tyrosine kinase, thus allowing control the growth of tumors and angiogenesis (Shojaei, 2012). Current strategies have been indicated for the inhibition of angiogenesis, categorized as follows: (a) monoclonal antibody inhibitors (b) VEGFR-2 tyrosine kinase inhibitors (c) inhibitors of endothelial cell proliferation (d) inhibitors of integrin's pro-angiogenic activity (e) MMP inhibitors (f) vascular targeting suppressors (Clarke & Sharma, 2006).

2.2 Nanotechnology

Nanotechnology is the science that concern with engineering materials on the nanoscale. It is an interdisciplinary arena, where the researchers from different backgrounds such as biology, chemistry, physics, and medicine can work together in an integrative research. The application of nanotechnology in medical sciences requires synthesis kind of nanoparticles that cause changes in biological processes. Rizvi and Saleh define nanoparticles as solid colloidal particles with a size between 10 nm and 1000 nm. However, it has been noticed that nanoparticles with a size less than 200 nm are the most suitable for nanomedical applications (Rizvi & Saleh, 2017).

2.2.1 Optimal nanoparticle characteristics for biomedical application

The scientist has made an intense effort in identifying the properties a nanoparticle should possess to be adequate for biomedical applications. The medicinal effects of nanoparticles are greatly determined by their physical and chemical characteristics such as shape, size and surface area. In general, the ability to manipulate the physical and chemical properties of nanoparticles increase their bioavailability as therapeutic agents that can be employed for both local and systemic therapy (Jo et al., 2015).

Several studies have reported that the size of nanoparticles is a major factor that influences the nanoparticle distribution into tumors loci which in turn affect their effectiveness. Basically, nanoparticles with sizes that are not larger than 100 nm are considered excellent for tumor targeting. Recently, some researchers investigated the impact of nanoparticle size on the nanoparticle accumulation in tumor cells. Their data revealed that

nanoparticles that are less than 20 nm are less accumulated in the cells because they are poorly retained even though they have fast permeation into the tumors, but nanoparticles that are larger than 100 nm have slow permeation into tumors (Wang et al., 2012). In the development of therapeutics against central nervous system (CNS) diseases, 20 nm-sized nanoparticles are adequate because they are passing through the blood-brain barrier (BBB) and large enough to escape the filtration of renal glomerular. Nanoparticles that are larger 20 nm are not adequate because they cannot pass through BBB (Jo et al., 2015). Yuan et al. had carried out an experiment to know the influence of size on nuclear localization and cellular uptake in human hepatoma HepG2 cells. They investigated Hydroxyapatite nanoparticles in size between 20 and 180 nm. The findings exhibited that size had an obvious effect on many activities such as apoptosis and anti-tumor activity, with the superiority effect of Hydroxyapatite Nanoparticles 45-nm (Yuan et al., 2010).

Both bottom-up and top-down are methods to manufacture various shapes of nanoparticles. Performance of nanoparticles in many biomedical applications as therapeutics and delivery of therapeutic agents depends on many physical and chemical properties, shape one of them that plays role in cellular uptake, transport, degradation and in ligand targeting of nanoparticles (Yuan et al., 2010). Furthermore, macrophage clearance, biodistribution and the adhesion pattern of nanoparticles can potentially affect by the change in shape. Another important property is a surface charge of nanoparticles, although positive-charged nanoparticles rapidly taken into a cancer cell, they also cause immune reactions. Thus, researchers prefer to use negative and neutral charge in their clinical applications (Wang et al., 2012)

2.2.2 Nanoparticles as anti-angiogenic agent

Lately, the researchers have synthesized a number of nanoparticles that inhibit angiogenesis. Henceforward, applied nanotechnology for medical purposes is considered as one of the promising fields that will play role in the treatment of angiogenic diseases like cancer. Some nanoparticles with potential influence on angiogenesis.

2.2.3 Gold Nanoparticles

Gold Nanoparticles (AuNPs) possess photothermal properties that can be exploited for localized heating in order to release drug, thus increasing their potential for therapeutic applications (Kemp et al., 2009). Low cytotoxicity, conjugation with enzymes, proteins, and making surface modifications with thiol-containing molecules are properties get AuNPs the preferable choice among nanoparticles in clinical applications (Kang et al., 2016).

Kang et al. investigated influence AuNPs on angiogenesis. They had induced choroidal neovascularization in C57BL/6 mice by laser. Choroidal neovascularization (CNV) is caused by the growth of abnormal blood vessels from choriocapillaries and susceptibility to leak blood and fluid that damage photoreceptor cells. They had injected AuNPs into the vitreous liquid of the mice on the following day of inducing choroidal neovascularization. On another hand, the control group had been injected the same volume of Phosphate-buffered saline (PBS). After two weeks of laser injury, the findings exhibited that 67.9% reduction in the extent of CNV lesions in AuNPs group compared to the control.They also evaluated the efficacy of AuNPs on endothelial cell tube formation and proliferation as well as the toxicity of AuNPs using human umbilical vein endothelial cells (HUVECs) or human retinal pigmented epithelial cells (RPE cells). Analysis activity of protein kinase B (Akt), and focal

adhesion kinase (FAK), extracellular signal-regulated kinase (ERK) 1/2 signaling pathways confirmed that AuNPs suppress VEGF induced HUVEC tube formation and proliferation but showed no RPE cell toxicity with the administered doses. The phosphorylation of ERK1/2, Akt, and FAK in HUVECs was also inhibited (Kang et al., 2016).

2.2.4 Silver Nanoparticles

Silver is one of the oldest metal that had used in ancient times. It was applied for medicinal purposes since the 8th century. Silver in nanoform has unique physical, chemical, and biological features. Silver Nanoparticles (Ag-NPs) is a therapeutic agent that treat many diseases such as retinal neovascularization, infectious diseases, angiogenesis and tumor growth (Baharara et al., 2014b). Silver nanoparticles can be prepared by chemical and biological methods. Currently, the organic solvents are the most of the synthetic methods, although it leads to serious effects on the environment. On another hand, green synthesis of silver nanoparticles offers many merits such as compatibility, environment-friendly, and safety for pharmaceutical and biomedical usage (Baharara et al., 2014a). From biosynthesis sources *Bacillus licheniformis,* Kalishwaralal et al. synthesized silver nanoparticles then investigated its anti-angiogenic effect. They found that AgNPs inhibited angiogenesis cascade events like cell proliferation, migration, and cell survival in bovine retinal endothelial cells (BRECs) via suppress the PI3K/Akt dependent pathway (Kalishwaralal et al., 2009).

Gurunathan and his colleagues reported that AgNPs synthesized from the plant can able to inhibit the growth of new blood microvessels induced by VEGF in the mouse Matrigel plug assay via suppression of the activation of PI3K/Akt pathways. *In vivo* Matrigel Plug assay in C57/BL6 mice, they removed Matrigel Plugs after 7 days and found plugs that contain VEGF alone appeared dark red with abundant vessels. On other side Matrigel plug

that contains Ag-NPs plus VEGF or Pigment epithelium-derived factor (PEDF) plus VEGF, in particular, group of Ag-NPs plus VEGF appeared pale that indicate fewer blood vessels formation (Figure 2.4) (Gurunathan et al., 2009).

A recent study was conducted by Baharara et al. that prepared AgNPs using green sources like *Salvia officinalis* plant extract distinguished with the safety of the environment. Investigations based on the measurement of hemoglobin (Hb), chorioallantoic membrane (CAM) assay revealed that AgNPs can work as anti-angiogenic agent (Baharara et al., 2014a).

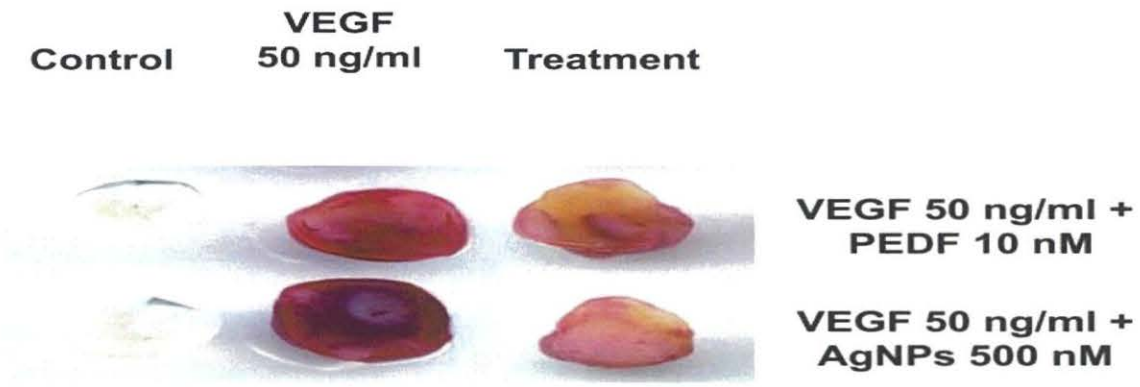

Figure 2.4: The Anti-angiogenic activity of Ag-NPs or PEDF using *in vivo* Matrigel plug assay. Five- to 6-week-old C57BL/6 mice were subcutaneously injected with Matrigel containing Ag-NPs (500 nM) or PEDF (10 nM) with VEGF or without VEGF. After 7 days mice were sacrificed and representative Matrigel plugs were removed and photographed. Adapted from Gurunathan et al., 2009.

2.2.5 Silica Nanoparticles

Silicate is a compound that includes a silicon-bearing anion, and silica, which is widespread in nature (Jo et al., 2012). Silica as nanoparticles (SiO2NPs) has been widely used in biomedical disciplines like drug delivery and gene therapy. Silica in nanoform also is used as additives for cosmetics and other industrial materials (Guarnieri et al., 2014).

Jo et al. showed that silicate nanoparticles (SiNPs) can able to treated retinal neovascularization without toxic effects. The anti-angiogenic effect of SiNPs was a result to inhibit VEGF-induced retinal neovascularization and suppress ERK 1/2 activation via suppression of VEGFR-2 phosphorylation (Jo et al., 2012).

2.2.6 Carbon Nanoparticles

Carbon is one of the most abundant elements in the biological system of a human. Carbon is widely used in nanomedicine research. There are many different types of carbon nanoparticles such as diamond nanoparticles (ND), multi-wall nanotubes nanoparticles (MWNT), graphene nanoparticles (GNS), graphite nanoparticles (NG), and fullerenes nanoparticles (C60) that possess influence on angiogenesis with low toxicity. Some carbon types showed profound anti-angiogenic activities (Wierzbicki et al., 2013).

The study investigated the role of microwave-radiofrequency (MW-RF) and ultra-dispersed detonation diamond (UDD) nanoparticles on angiogenesis using a glioblastoma multiforme (GBM) tumor model developed on a chorioallantoic membrane. The findings indicate that UDD nanoparticles showed a significant decline of FGF- and VEGF expression.

On the other hand, MW-RF nanoparticles only decreased VEGF expression, although there was a pattern of reduction in FGF expression (Grodzik et al., 2011).

Wierzbicki et al. elaborately demonstrated the effect of types of different carbon nanomaterials ND, NG, GNS, MWNT and C60 on angiogenesis. The study was conducted to evaluate blood vessel development *in an ovo* chick embryo chorioallantoic membrane model. The findings indicated that ND (anti-angiogenic) and MWNT (anti-angiogenic). Interestingly, C60 was (angiogenic). On another hand NG (no effect) and GNS (no effect). Diamond nanoparticles inhibit the expression of VEGF-R. ND and MWNT showed 2–3 fold reduced in the thickness of CAM confirming those nanoparticles inhibited effectively angiogenesis (Figure 2.5) (Wierzbicki et al., 2013).

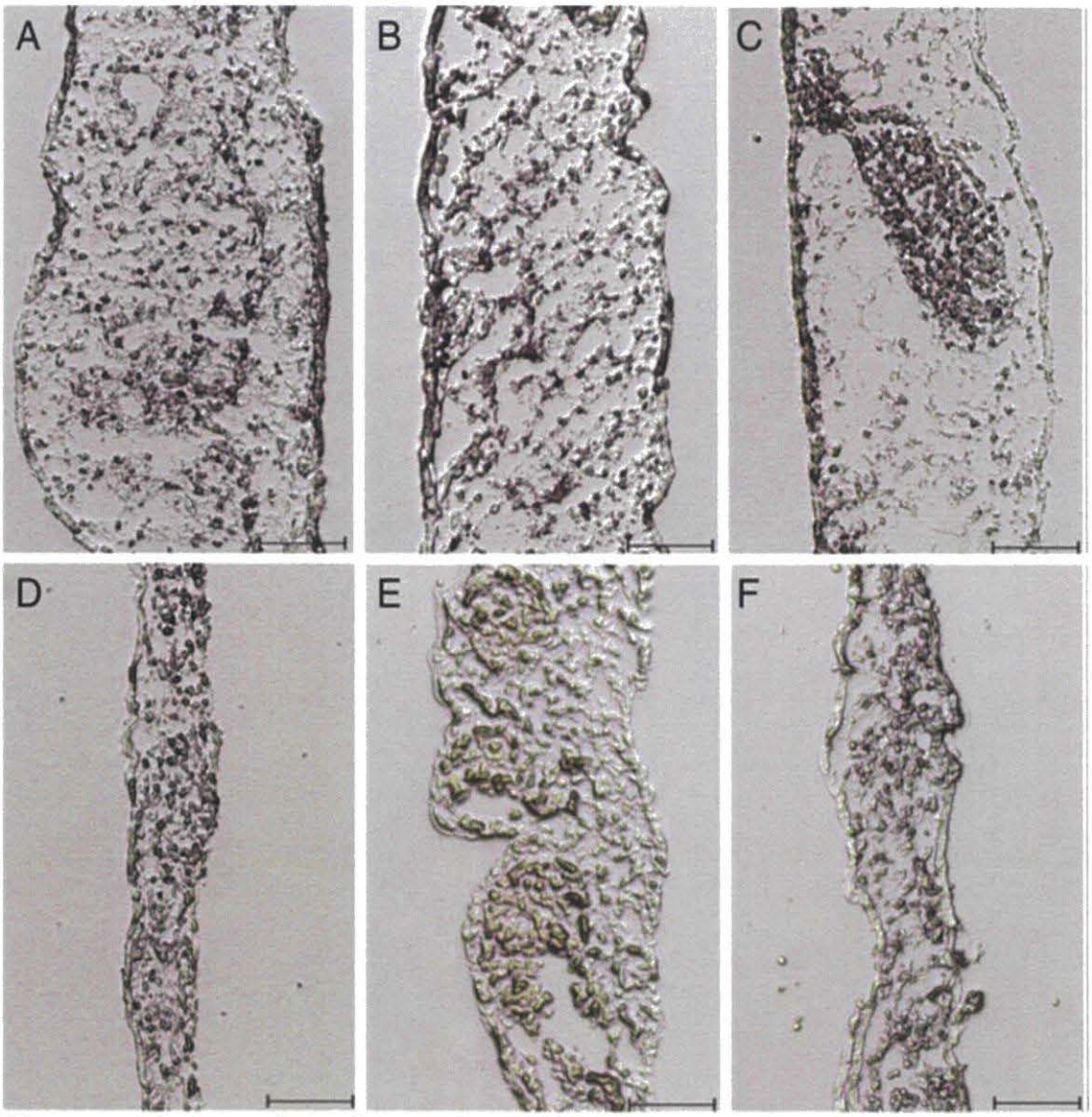

Figure 2.5: Cross sections of CAM tissue treated with carbon nanoparticles. (A) Control, (B) GNS, (C) NG, (D) ND, (E) C60 and (F) MWNT. Adapted from Wierzbicki et al., 2013.

2.2.7 Copper Nanoparticles

Copper Nanoparticles (CO-NPs) is widely used in many biomedical applications like an anti-microbial agent, tumor treatment, and drug delivery. Song et al. demonstrated the influence of cuprous oxide on angiogenesis *in vitro* and *in vivo* studies. Results have shown

that CONPs suppress angiogenesis and cell cycle (arrest in S-phase) in a manner dependent on time and a dose. Suppression of *in vivo* blood vessel formation noticed through CD31 staining confirming the angiogenic inhibitory of CONPs. The mechanism of action of CONPs was suppression of VEGFR2 expression at both the mRNA levels and protein (Figure 2.6) (Song et al., 2014).

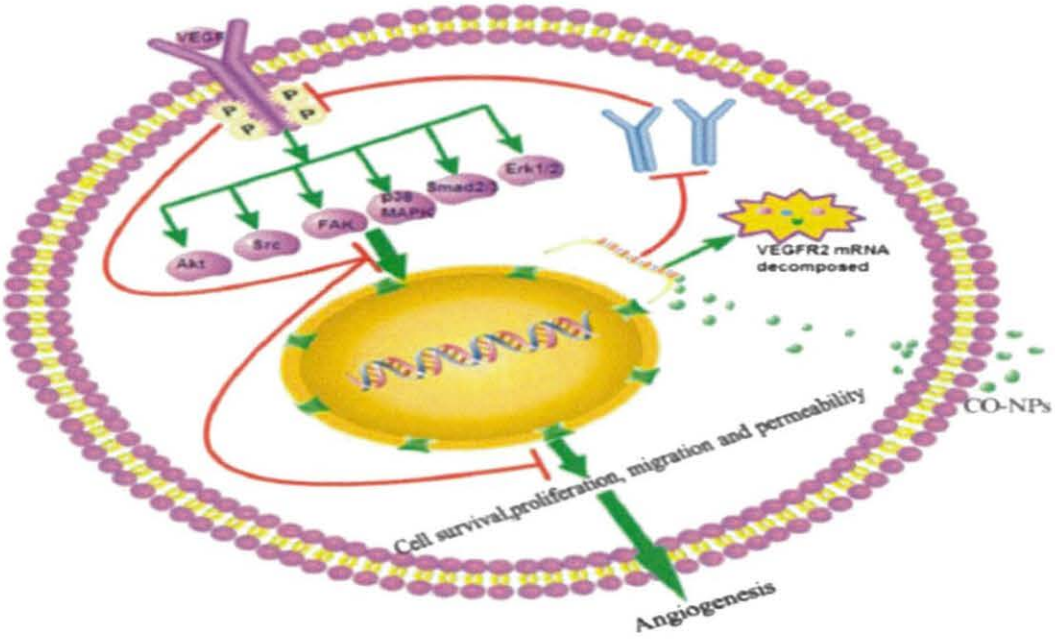

Figure 2.6: CO-NPs inhibited angiogenesis via down-regulation of VEGFR2 expression. VEGFR2 is a key regulator of angiogenesis by activating downstream pathways. CO-NPs were able to suppress VEGFR2 expression both at mRNA and protein levels, thus inhibiting VEGFR2 mediated angiogenesis. Adapted from Song et al., 2014.

2.2.8 Chitosan nanoparticles (CNPs)

Chitosan is a natural biopolymer with nontoxic effect. It prepares by the deacetylation of chitin. Chitosan exhibits antifungal and antimicrobial properties that attracted the concern of many researchers. Chitosan nanoparticles have been applied in drug delivery system. The intestinal absorption and pharmacological bioavailability of insulin increased when insulin-

loaded on chitosan nanoparticles. CNPs exhibit more activity due to a unique property of small size (Qi et al., 2004). Influence of chitosan nanoparticles in the suppression of angiogenesis was investigated by Xu et al. They xenografted human hepatocellular carcinoma cells (HCC) in nude mice as a model. The findings exhibited that CNPs suppressed tumor growth and trigger its necrosis in a manner depending on a dose and time. Application some of analysis method like Real-Time Polymerase Chain Reaction (q-RT-PCR) and immunohistochemistry exhibited that suppression of tumor occur via inhibiting angiogenesis by chitosan nanoparticles where contribute in impairing levels of VEGF and VEGFR-2. Hence, the blockage of VEGFR-2 leads to the suppression activity of VEGF. The results approved activity of low toxic CNPs in anti-angiogenesis therapy (Xu et al., 2009).

2.2.9 Cerium Nanoparticles

Cerium oxide considers as a rare earth metal that can be used in medicinal applications. In current time, it was synthesized in nanoparticles form and attracted considerable interest. The regenerative property of Cerium (Ce) nanoparticles as a nontoxic free radical scavenger protect from free radicals that induced due to chemical, biological and radiological insults is the most valuable properties of Cerium oxide nanoparticles (nanoceria) (Saghiri et al., 2016). Nanoceria also has the ability to induce anti-inflammatory effects (Jo et al., 2015).

Giri et al investigated the role of nanoceria (NCe) in ovarian cancer cells treated *in vivo* and *in vitro* conditions. Nanoceria had the ability to suppress the proliferation of EC, stimulate apoptosis of EC, and suppress metastasis, as an outcome, they minimize the microvessel density. Furthermore, phosphorylation of VEGFR2 and VEGF mediated downstream signaling were also decreased. It should be noted that nanoceria can able used as a suppressor of tumor growth *in vivo* by targeting angiogenesis (Giri et al., 2013).

2.2.10 Titanium Nanoparticles

Titanium dioxide nanoparticles (TiO2) have the ability to stimulate apoptosis and neural toxicity and trigger inflammatory responses in human bronchial EC (Saghiri et al., 2016). The mechanism of action of TiO2 nanoparticles emerges from their inhibition of angiogenesis not from their toxicity. Several results demonstrated that the inhibitory effect of TiO2 arises from suppressing the VEGF/VEGFR2/MAPK pathway independent of the PI3K/Akt pathway (Jo et al., 2014b).

2.2.11 Hydroxyapatite Nanoparticles

Hydroxyapatite (HAP) is the main inorganic element of teeth and human bones. Currently, it is extensively used in clinical applications as coatings on prostheses or implants. The researchers have widely studied synthetic HAP to exploit its biological properties like good biocompatibility, non-inflammatory effect, and non-immunogenicity and high osteoconductivity. Hydroxyapatite nanoparticles (HANPs) have attracted intensive attention, and great works have been made in the last ten year to know more about their synthesis, structure and other characteristics. HAPs in nanoform exhibit many benefits like high biocompatibility, and flexible structure, good bioactivity that play an essential role in their potential biomedical applications (Shi et al., 2009).

Shi et al. had conducted a series of evaluations to investigate the role of HANPs on angiogenesis *in virto* assay HUVECs.The findings exhibited that influence of HANPs on HUVEC did not exert by the modulation of VEGF expression. On another hand, HAPs inhibited phosphorylation of eNOS uncommonly in a manner depending on dose and the

inhibition of p-eNOS paralleled the down-regulation of p-Akt. In addition, the HAPs-inhibited NO production and cell viability of HUVECs could significantly be restored via treatment with Insulin-like growth factor 1 (IGF-1). Generally, the observations concluded collectively that HAPs might work as a regulator of EC function through the PI3K/Akt/eNOS signaling (Shi et al., 2017).

2.2.12 Zinc oxide Nanoparticles

Zinc oxide (ZnO) has valuable physical characteristics like conductive coating efficacy. ZnO nanoparticles as nontoxic and chemical stable can perform as UV-blocking and antibacterial agent. Additionally, its uses in a medicinal application like anti-oxidant activities (Becheri et al., 2008)

Bio-synthesis of ZnO nanoparticles may inhibit angiogenesis so can be used in many biomedical applications. (Divya et al., 2018). Zn might apply in cancer therapy due to its effect on angiogenesis. Endostatin is well known as a potent angiogenesis suppressor *in vitro* and *in vivo* assay. The researchers found that the ability of endostatin to bind Zn2+is essential for its anti-angiogenic activity (Saghiri et al., 2015b). Role of zinc oxide nanoparticles coated with biopolymer gelatin (Ge-ZnO NPs) as an inhibitor of angiogenesis was investigated in a study carried out on chick embryos. The results of CAM assay showed that 50 µg/ml zinc acetate and Ge- ZnO NPs initially resulted in the budding of blood vessels, however, blood vessel formation was completely inhibited after 24 h of incubation. On another hand, gelatin-treated chick embryos did not suppress blood vessels (Figure 2.7) (Divya et al., 2018).

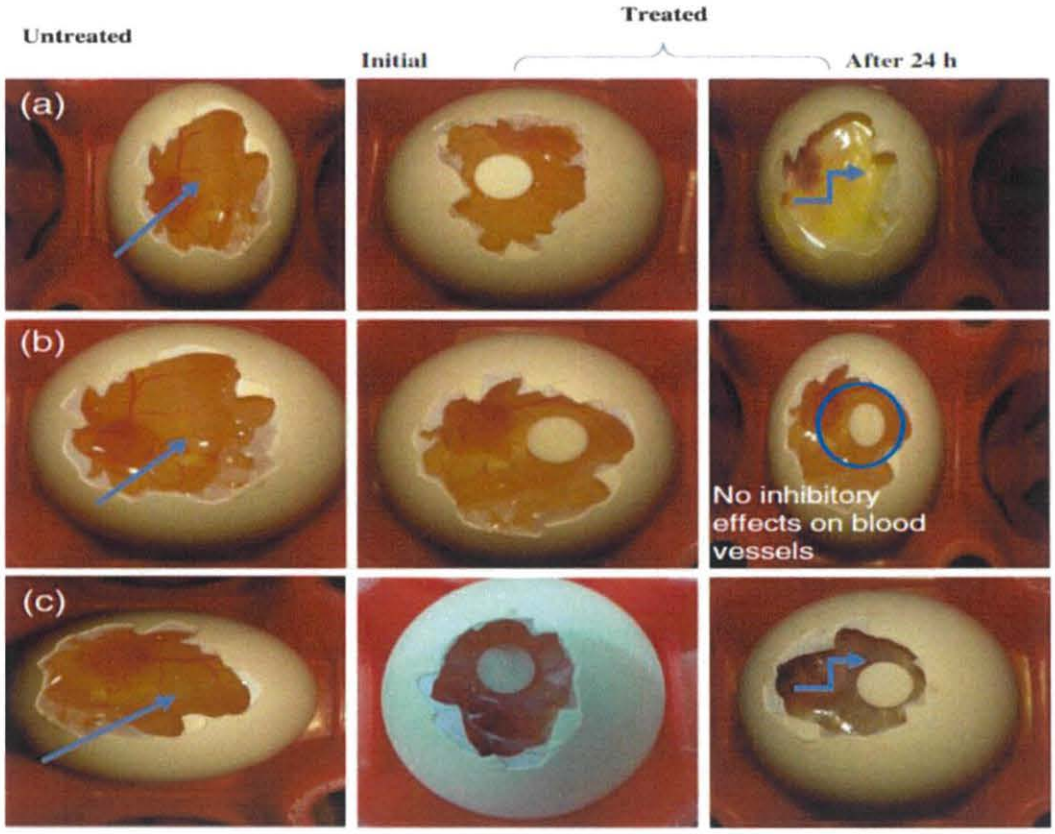

Figure 2.7: Sterile discs in the nervous region of the 7 or 8th day old chick embryo, treated with zinc acetate [a], gelatin [b], Ge-ZnO NPs [c] at 50 µg/ ml. Arrows on the left demonstrated the budding of blood vessels, arrows on the right outlined the inhibition of blood vessels. Adapted from Divya et al., 2018.