A COMPARATIVE STUDY ON PARENT AND RESISTANT MCF-7 CELLS ON MIGRATION CHARACTERISTICS WITH REGARDS TO BIOACTIVE GLASS TREATMENT

By

HARUNA S JALLOW

DISSERTATION IS SUBMITTED IN PARTIAL FULFILMENT

OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER OF SCIENCE

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DECLARATION

I hereby declare that this research was sent to Universiti Sains Malaysia for the degree Master of Science in Medical Research. It has not been sent to other universities. With that, this research can be used for consultation and photocopied as reference.

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LIST OF SYMBOLS

g	Gram
mL	Milliliter
mg	Milligram
ug	Microgram

LIST OF ABBREVIATIONS

BG	Bioactive glass
Ca^{2+}	Calcium oxide
$CaNO_3$	Calcium nitrate tetrahydrate
DMSO	Dimethyl sulfoxide
DMEM	Dulbecco's Modified Eagle Medium
ER+	Estrogen receptor positive
HA	Hydroxyapatite
HepG2	Hepatocellular carcinoma
HNO_3	Nitric acid
LINC	Linker of Nucleoskeleton and Cytoskeleton
LABC	Local Breast Cancer
MgO	Magnesium oxide
MCF-7	Michigan Cancer Foundation
Na_2O	Sodium oxide
K_2O	Potassium oxide
$NaNO_3$	Sodium nitrate

PBS	Phosphate buffer saline
PR+	Progesterone receptor positive
SEM	Scanning Electron Microscopy
SiO_2	Silicon dioxide
Si-OH	Silicon-hydroxyl bridge
TEOS	Tetraethyl orthosilicate
TEP	Triethyl phosphate
VB	Vinblastine

KAJIAN PERBANDINGAN KE AS SEL MCF-7 INDUK DAN RESISTAN TERAHADAP KARAKTERISTIK MIGRASI YANG BERKAIT DENGAN RAWATAN KACA BIOAKTIF

ABSTRAK

Pengenalan: Kanser payudara adalah salah satu punca utama kematian di seluruh dunia, terutamanya dalam kalangan wanita. Ia menyumbang satu pertiga daripada kematian dan merupakan kanser kedua yang paling umum di dunia. Kaca bioaktif (BG) 45S5 digunakan terutamanya dalam bidang ortopedik dan pergigian untuk pertumbuhan semula tulang dan tisu. Baru-baru ini, ia diuji sebagai kaedah penyampaian ubat terutama dalam terapi kanser. Objektif: Untuk melakukan kajian perbandingan terhadap migrasi titisan sel induk and titisan sel resistan kanser payudara (MCF-7) dengan rawatan 4555 kaca bioaktif. Kaedah: BG telah disintesis menggunakan kaedah *sol-gel*. Cerakinan penyembuhan luka telah dilakukan untuk menentukan penutupan kawasan luka pada masa berlainan. Setiap titisan sel ditanam dalam plat 24-ruang dan dirawat dengan kepekatan BG yang berbeza dan vinblastine ubat kemoterapi piawai. Data yang diperolehi dianalisa menggunakan Image J. Keputusan dan Perbincangan: Penemuan mencadangkan 2 mg/ml BG menggalakkan penghijrahan dan percambahan titisan sel induk dan sel resistan MCF-7 kanser payudara. Pada kepekatan yang lebih tinggi iaitu 4 mg/ml BG, 8 mg/ml BG dan vinblastine, ia menyebabkan perencatan titisan sel MCF- 7 induk dan resistan. Hasil kajian menunjukkan tiada perbezaan yang ketara kawasan penyembuhan luka antara kawalan dan 2 mg/ml BG. Namun perbezaan yang signifikan dikesan antara kawalan serta titisan sel induk dan resistan setelah dirawat dengan 4 mg/ml BG, 8 mg/ml BG dan vinblastine. Sementara itu, tiada perbezaan yang signit;ifikan antara ≥ 4 mg/ml BG sel dirawat dan vinblastine. Kesimpulan: Migrasi sel kanser payudara induk dan

resistan dapat dikawal dengan rawatan BG pada kepekatan ≥ 4 mg/ml atau diberi sebagai adjung dengan drug kemoterapi piawai

A COMPARATIVE STUDY ON PARENT AND RESISTANT MCF-7 CELLS ON MIGRATION CHARACTERISTICS WITH REGARDS TO BIOACTIVE GLASS TREATMENT

ABSTRACT

Introduction: Breast cancer is one of the leading causes of mortality worldwide, particularly among women. It accounts for one third of mortality and is the second most common cancer in the world. The 45S5 bioactive glass (BG) is predominantly used in the orthopaedic and dentistry field for bone and tissue regeneration. Recently, it is being tested as a mode of drug delivery particularly in cancer therapy. Objective: To perform a comparative study on parent and resistant breast cancer cell lines (MCF-7) migration with 4555 bioactive glass. Methods: BG was synthesised using sol-gel method. Wound healing assay was done to determine closure of wound area at different time-points. Each cell line was grown in 24-well plates and treated with different concentration of BG and standard chemotherapy drug vinblastine. Wound area was analysed using Image J and repeated measures ANOVA used to analyse the obtained data. Results and Discussion: Findings suggest 2 mg/ml BG promoted migration and migration of parent and resistant MCF- 7 breast cancer cells. While at higher concentration with 4 mg/ml BG, 8 mg/ml BG and vinblastine caused inhibition of parent and resistant MCF-7. There was no significant difference of closure area between the controls and 2 mg/ml BG treated cells. Significant difference was detected between the controls of parent and resistant MCF-7 cells with 4 mg/ml BG, 8 mg/ml BG and vinblastine treated breast cancer cells. Furthermore, there was no significant difference between ≥ 4 mg/ml BG treated cells and vinblastine.

Conclusion: Bioactive glass of ≥ 4 mg/ml can be useful as a prospective inhibitory agent in breast cancers or can be doped as an adjuvant to standard chemotherapy.

CHAPTER 1

INTRODUCTION

1.1 Background Information

The global prevalence of cancer has risen from 14.1 million diagnosed cases in 2012 to 18 million in 2018, out of which 2 million (11.6%) was breast cancer (Bray et al., 2018). Death rate worldwide was reported to be 9.6 million and breast cancer accounted for 6.6%. Cancer is a disease with uncontrolled manner of growth and migration of abnormal cells (Qi et al., 2017; Bai et al., 2018). The mutated oncogenes and tumour suppressor genes of cancer cause cells migration, avoid tumour suppression, withstand cell death, instigate angiogenesis, facilitate continuous replication and invasion and metastasis. These characteristics and phenomena give the scientific community a daunting challenge in treating cancer (Qi et al., 2017). Cancer have become the one of the highest causes of mortality after heart diseases (Qi et al., 2017).

It is forecasted that there will be 19.3 million new cases per annually by 2025 (Ferlay, J. et al., 2015). The prevalence of breast cancer is high in women globally. Around 1.7 million population is diagnosed annually and higher in developed countries as compared to developing countries, probably owing to growth and ageing (Ferlay et al., 2015; Soerjomataram et al., 2012). However, there is sudden increase in the number of cancer cases in developing countries, which could be attributed to the change in lifestyle or lack of proper screening strategies. Although the mortality rate of breast cancer is almost equal to that of developed countries, which may be due to lack of early and

poorer diagnoses that results to late treatment. Survival rate is reported to be high in developed nations with sophisticated health care than in developing nations (Andreeva and Pokhrel, 2013; Bray et al., 2018; Torre et al., 2016).

Breast cancer is the most common and is the leading cause of high mortality among women (Saha et al., 2015) responsible for roughly one third of all cancers (Wang et al., 2017). Globally, the magnitude of breast cancer continues to accelerate, resulting in the yearly reporting of new cases amounting to greater than a million, furthermore it is the second commonest cancers in the world and prevalent in all countries (Ghoncheh, Pournamdar and Salehiniya, 2016; Wang et al., 2017). The increase trend of breast cancer is foreseen achieving 3.2 million per annum by 2030 (Winters et al., 2017). One third of these cases are metastatic or locally advanced disease or recurrent cancer (Wang et al., 2017).

It is documented that breast cancer occurs 1 in every 8 women and accounts for 14% of newly diagnosed cancers in United State (US), mostly in the age group of between 55 to 64 years old (Winters et al., 2017). It was reported in US that about 40,450 deaths were due to breast cancer in 2016 (Winters et al., 2017).

In Europe, about 465,200 of breast cancer cases are newly diagnosed yearly. New female cancer cases amount to 29% (Ferlay et al., 2015). The mortality is about 131,200 annually and the cancer deaths of females is 17% (Ferlay et al., 2015).

Developing nations like Africa have registered about 168,690 new cancer cases and experience 74,000 deaths at an incidence rate of 8% (Bray et al., 2018). This could be due to improper health system, lack of diagnoses or higher percentage of younger population in this region.

In Asia approximately 1.7 million new cancer were reported. It has been gauged that 1.2 million cancers mainly in the South East Asia region. Breast cancer is among the most prevalent cancers among women in this region (Bray et al., 2018). This region has the highest breast cancer incidence with 43.6% compared to all the other continents. Global cancer observatory (Globocan) documented the continent has 911,014 new breast cancer cases with a mortality of 310,577 (Bray et al., 2018).

Malaysia has a population of about 29.2 million people. It was reported 15% mortality was due to cancers (Dahlui, M., Ramli, S. and Bulgiba, A.M., 2011). It is the third highest cause of death in Malaysia. The 2006 National Cancer Registry disclosed 3,525 female breast cancer cases and it is the most prevalent diagnosed cancer in females (Dahlui, M., Ramli, S. and Bulgiba, A.M., 2011). Breast cancer is the most common cancer among Malaysian women where 1 in 20 women is likely to develop breast cancer within her life time (Dahlui, M., Ramli, S. and Bulgiba, A.M., 2011). Risk of breast cancer increased with the age particularly between the age of 50-59 years. There are many risk factors of developing breast cancer such as a past history that lead to primary breast cancer, breast carcinoma *in situ*, a tissue biopsy revealing migrative cells, can develop cancer in a near future or benign breast disease with an unusual enlargement is at greater risk of developing cancer (Dahlui, M., Ramli, S. and Bulgiba, A.M., 2011).

Breast cancer is most common in women with a prevalence of 30% and is the leading contributor of cancer related death among women, although rarely seen in men (Bai et al., 2018). Breast cancer is a heterogenous disease that has many known biomarkers but still may not be limited to only these (Jézéquel *et al.*, 2015; Turashvili and Brogi, 2017). Even though, lymph node metastases, histologic grade, expression of steroid and growth factor receptors, estrogen-inducible genes like cathepsin D, protooncogenes like ERBB2, and mutations in the TP53 gene have been associated to the

disease (Sørlie *et al.*, 2001). Tumour can evolve and develop into malignant if not diagnose at the earlier stage (Ju, Zhu and Yuan, 2018). Breast cancer has been analysis by many platforms to determine the molecular markers of the disease not only limited to Gene expression analysis which has categories breast cancer into four distinct major molecular subtypes luminal A, luminal B, HER2-enriched, and basal-like (Curigliano *et al.*, 2017). The luminal A and luminal B subtypes are found within ER+ breast carcinomas but better in terms of treatment than HER2-enriched and basal-like subtypes (Martin, Smith and Tomlinson, 2014; Dai *et al.*, 2015; Jézéquel *et al.*, 2015; Schiff, 2015; Turashvili and Brogi, 2017). Despite all expressing ER+, luminal B tumours expressed more migration-associated genes and have substandard prognosis than luminal A tumours. However, there are four main subtypes of breast cancer, hormonal receptor positive; estrogen receptor positive (ER+), progesterone receptor positive (PR+), human epidermal growth receptor 2 and triple negative breast cancer (Ding et al., 2018; Gray et al., 2017; Israel et al., 2018). The subtypes relate to certain morphological features and different clinical outcomes, showing that breast cancer is not a single disease. Breast cancer is a diversified disease with different biomarkers (Ju, et al., 2018). Tumour cells slowly developed resistance against treatment regimens. Combine treatment will work better but many side effects have been reported. With the advance of technology, there are many promising hopes to come up with a cure to breast cancer, even though it is difficult to find a single treatment to suit all the subtypes (Ju, Zhu and Yuan, 2018).

To assess invasive breast cancer, molecular techniques are used. This will dictate the type of hormonal receptors or whether there is an overexpression of human epidermal growth factor receptor 2, estrogen or progesterone receptor positive. The HER 2- is the second most prevalent subtype, lethal and poor predictable treatment outcome with high

risk of recurrence than the others. The HER2- subtype is denoted with high expression of HER2 and migration genes and encompass ER-/ PR-/HER2+ and ER+/PR+/HER2+ tumours (Dai *et al.*, 2015; Turashvili and Brogi, 2017).

Approximately 12% to 20% of invasive breast cancers depict HER2 gene amplification and/or protein overexpression and are corelated with poor prognosis and predictive of response to anti-HER2 targeted therapy. On the other hand, 10% to 15% of IBCs are ER, PR, and HER2 negative (triple-negative breast cancer [TNBC]), and lack targeted therapy currently (Tang and Tse, 2016). Triple-negative (TN) tumour, is common in primary breast cancer, known to be the most aggressive and deadly breast cancer subtypes. It lacks estrogen, progesterone and HER2 receptors, making its therapeutic management challenging (Jézéquel *et al.*, 2015).

Triple-negative is a molecular subtype to claudin-low, denoted by low expression of cell-cell adhesion cluster containing claudin 3, 4, 7 and E-cadherin, luminal and migration-associated genes, strengthen in epithelial-to- mesenchymal transition (EMT) features, immune system responses, and stem cell-associated biological processes (Jézéquel *et al.*, 2015).

A cost effective and less time-consuming immunohistochemistry staining method have also been used as a molecular subtype panel tool for breast cancer comprising ER, PR, HER2, Ki-67, epidermal growth factor receptor (EGFR) and cytokeratin 5/6 (CK5/6) identifying the molecular subtypes of breast cancer. The routine immunohistochemical (IHC) analysis for ER, PR, and HER2 provides critical prognostic and predictive information for Invasive Breast Cancer (IBC). Nearly 70% of IBCs are ER positive. PR is hugely contained by estrogen, and PR negativity is linked to decreased response to tamoxifen therapy. Therefore, IHC and/or in situ hybridization assays are used to

determine the subtypes of breast cancer. Tumours are also distinguished by grade and proliferative fraction usually by Ki-67 immunostaining (Curigliano *et al.*, 2017). This knowledge is helpful in the diagnostic, prognostic, and therapeutic strategies for breast cancer. Regardless of this information, there is still unknown information in regard to tumour progression, as it is paramount knowing the driving factors for metastatic disease and treatment resistance. The use of these methods helps in projecting the response to chemotherapy and recurrence risk (Turashvili and Brogi, 2017).

The commonest breast tumour during menopause period in women are estrogen receptor (ER)-positive and/or progesterone receptor (PgR)-positive in which endocrine therapy will be the choice of treatment like tamoxifen, aromatase inhibitors to interrupt with ER signalling and/or block estrogen production (Wu et al., 2016; Tang and Tse, 2016; Curigliano *et al.*, 2017). Tamoxifen is the standard endocrine treatment for these patients for couple of years but currently the third-generation aromatase inhibitors (AIs) are also used because of its superior efficacy and tolerability profiles (Tang and Tse, 2016; Curigliano *et al.*, 2017).

Tamoxifen is still used, even though many clinicians use it further down the treatment sequence. Regardless of these changes in clinical practice, many patients with advanced breast cancer tumour encountered relapse or disease progression after endocrine treatment. Due to these there is necessity for, non– cross-resistant, well-tolerated agents that can be cooperated into the endocrine treatment sequence (Faslodex and Pharmaceuticals, 2007). Invasive breast cancers (IBCs) are multi-disparity disease, revealing molecular, pathologic features and biologic behaviour. Morphologic classification, histologic grade, status of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2), and the stage of the tumour, are useful in guiding clinical management (Tang and Tse, 2016). Estrogen receptor (ER),

progesterone receptor (PgR), Human Epidermal Growth Factor Receptor 2 (HER2) and ER and PgR are grouped together into the class of hormone receptors (HRs). ER expressed in roughly 75% of breast cancer patients. (Curigliano *et al.*, 2017).

ER+ is the most prevalent and accounts for about 70% of breast cancer tumour. ER and its ligand, estrogen, plays a vital role in the development and the continuity of breast cancer. Endocrine therapies, target ER activity, treating ER+ breast cancer in the early and the metastatic stages. However, resistance is still common, and it significantly influences overall morbidity and mortality in patients (Schiff, 2016).

Recent findings in ER provided the mechanisms contributing to alteration in ER signalling in endocrine- resistant tumours, inclusive of deregulated growth factor receptor signalling due to ligand- independent ER activation, unbalanced ER co-regulator activity, and genomic alterations in relation to the ER gene ESR1 (Schiff, 2015). The effects of estrogen/ER on cyclin D1 and c-Myc expression caused estrogen-stimulated cellular growth and migration (Mendes-pereira *et al.*, 2012).

This disease is a burden to the economy and the patients. It is the most prevalent cancer affecting women of all nations with high healthcare expenses. Additionally, death causes an annual productivity loss (Cun and Yang, 2018; Lucato et al., 2015). This will remain to be seen especially in developed nations with growing in ageing population (Bray et al., 2018).

1.2 Problem statement

The role of chemotherapeutic agents is to induce cytotoxic effects on cancers cells. However, these agents are accompanied with side effects on normal tissues and organs (Qi et al., 2017). They can also cause bone marrow haematopoietic dysfunction, hair loss, nausea, fatigue and vomiting. Some of these drugs have low bioavailability and are unable to reach their targets. These are some of the factors that contribute to their failure (He et al., 2016; Qi et al., 2017). Combination chemotherapy has been used to overcome some of these limitations and proven to be more effective than single agent chemotherapy treatment. Nano co-delivery system of drugs where drugs with different physicochemical and pharmacological qualities are submerged into the delivery system has been developed to improve the effectiveness of the anticancer drugs and reach their target (Qi et al., 2017).

Different anti-cancer chemotherapy drug have different mechanisms on tumour cells. The anti-microtubule alkaloid agents, for example vinblastine, prevent the formation of microtubules. Whereas taxanes like paclitaxel prevent the breaking up of the assemble microtubule (Qi et al., 2017; Varmaghani et al., 2014). Topoisomerase inhibitors (topotecan) are anti-cancer drugs that counter the activity of the enzyme by avoiding the over-winding or the under-winding of the DNA. Cisplatin, which is a platinum-based drug, blocks the multiplication of DNA. All chemotherapy drugs are used in many cancer treatments including breast cancer by preventing the complete mitosis of cancer cells or leading to DNA synthesis error facilitating the apoptosis of cancer cells. The current development of omics, antibodies and genes has improved anticancer therapy through their qualities in reaching targets in both pre-clinical and clinical application (Guo et al., 2017; Qi et al., 2017).

Despite all the challenges and problems with chemotherapy, it is still the best treatment option due to its less invasiveness, administered conveniently and effective in all ages. The poor uptake of these drugs and non-specific target distribution makes them ineffective (Qi et al., 2017). Furthermore, the dreadful effects such as the toxicity, resistance and restricted usage in clinics of single chemotherapeutic drug drive the needs to come up with a solution. Nanotechnology is used in the co-delivery of cancer drugs to yield better efficacy. Cancer cells are treated with this technology with less adverse effects in contrary to single chemotherapeutic treatment (Guo et al., 2017; He et al., 2016; Qi et al., 2017; Varmaghani et al, 2014). Nanotechnology has made it feasible for the delivery of anticancer drugs to the desired target. It disrupts the biological barrier, thereby penetrating cancer cells. Advancement of nanotechnology has assisted in the treatment efficacy against complicated cancer pathways. Efficient delivery to the targeted area with nano-drug co-delivery system (NDCDS) gives the possibility of drugs penetrating the blood circulation. With this technology, drugs are transported and released slowly to their targets (Guo et al., 2017; He et al., 2016; Hanauske et al., 1994; Qi et al., 2017; Wang et al., 2017). The treatment of malignant tumours with different regimens or ways had been explored but none of these strategies proves maximum achievement clinically. Bioactive glass has been investigated as result of its capabilities to deliver drugs effectively (Miola, M., Pakzad., 2018).

This research was intended to study the induced effects cause by bioactive glass on tamoxifen parent and resistance MCF-7 breast cancer cell lines. The study will compare the anti-migration and the morphology (apoptotic) effects of MCF-7 parent and resistant cancer cells treated with bioactive glass. Bioactive glass was chosen because of its potentials in not only orthopaedics and dentistry field but also in other medical cadres

and has proven effective in both *in vitro* and *in vivo* studies in bone and tissues regeneration. It is used clinically for its antibacterial effects.

1.3 Objectives

1.3.1 Main Objective

To compare the effect of bioactive glass (BG) on the migration and growth of parent and resistant breast cancer cells.

1.3.2 Specific objectives

I. To compare morphological changes before and after treatment using BG.

II. To determine effects of BG in parent and resistant cells of BG and Vinblastine on cancer cells.

III. To compare effects of BG with standard chemotherapy drug.

1.3.3 Hypothesis

Null hypothesis: Bioactive glass will not result in an anti-migration effect on Michigan Cancer Foundation 7 (MCF-7) cells and cause no morphological changes of the cells.

Alternate hypothesis: Bioactive glass will result in an anti-migration effect on Michigan Cancer Foundation 7 (MCF-7) cells and cause morphological changes of the cells.

CHAPTER 2

LITERATURE REVIEW

An urgent need in cancer management is to develop effective and affordable approaches for early detection, diagnosis, and treatment especially in developing countries. It is critical and crucial to bring morbidity and mortality in line with progress made in recent years particularly in developed countries (Tarver, T., 2012.; Ferlay et al., 2015).

Over the past decades, there has been a lot of efforts and progress to tackle breast cancer (Ju, Zhu and Yuan, 2018). Existing treatment approaches such as chemotherapy, surgery and radiotherapy are intended to improve the life span and the quality of life (Qi et al., 2017). Nevertheless, with all the treatment options, the mortality rate is inconsistent. The treatment relapses of these uncontrollable inconsistent cancer may be due to presence of resistant traits that encourage micro metastasis and have the capabilities to cause recurrence, thus, there is a need to improve therapeutics for resistant breast cancer (Bai et al., 2018; Ju, Zhu and Yuan, 2018).

However, these treatment options depend particularly on the subtype of cancer and need careful selection (Tarver, T., 2012; Ferlay et al., 2015). According to studies, breast cancer survival rate is more in the early stage of cancer but also depends on the stage, clinicopathological features and the subtypes of cancer (Bai et al., 2018; Winters et al., 2017). Additionally, chemotherapy relapse of breast cancer has been proven (Lucato et

al., 2015; Winters et al., 2017). Tamoxifen is a selective estrogen receptor modulator that has been indicated to be able to reduce the risk of breast cancer (Bai et al., 2018; Winters et al., 2017).

2.1 Tamoxifen

According to (the American Society of Clinical guidelines of Oncology) tamoxifen reduces the risk of invasive and metastasis breast cancer particularly in those with ER+, premenopausal or those above 35 years or *in situ* lobular carcinoma (Lucato et al., 2015; Winters et al., 2017). The MCF-7 resistant cells line used in the research obtained its resistance due to been continuously cultured with 1 µM 4-OH-tamoxifen for 8-12 months.

Chemotherapies that targets estrogen signalling yielded to reducing mortality from breast cancer. However, resistance to tamoxifen countered these achievements. Multiple genes are associated to resistance or sensitivity to tamoxifen. Combining whole-genome shRNA screening to RNA interference reagents targeting revealed genes on the cellular response to tamoxifen. Furthermore, silencing certain genes results to tamoxifen resistance (including BAP1, CLPP, GPRC5D, NAE1, NF1, NIPBL, NSD1, RAD21, RARG, SMC3, and UBA3) and equally the silencing of their genes contributes to the causes sensitivity to this endocrine agent (C10orf72, C15orf55/NUT, EDF1, ING5, KRAS, NOC3L, PPP1R15B, RRAS2, TMPRSS2, and TPM4). Genes like NF1, a regulator of RAS signalling, relating with clinical outcome after tamoxifen treatment (Mendes-pereira et al., 2012).

Endocrine therapy is used as a frontline treatment for hormone receptor-positive breast cancer, while trastuzumab for HER2-positive cancers. Traditional cytotoxic chemotherapeutics, like taxanes and anthracyclines are used in combination with specific

therapies as frontline therapies for triple-negative breast cancer. Th mechanisms behind the resistance to the various therapeutics are many and not fully comprehended. But, Phosphatidylinositide 3-kinase (PI3K)/Akt pathway, miRNAs, and epigenetic alterations in breast cancer are associated to multidrug-resistant phenotype (Mendes-pereira et al., 2012; Martin, Smith and Tomlinson, 2014). ER induces gene activation during transcription by nuclear translocation after ligand binding or can phosphorylation in the absence of ligand. ERs may also associate with the plasma membrane in the presence of SRC and other adaptor proteins. At this stage, ligand binding enhances nongenomic effects via activation of signalling pathways, including the Pi3K/Akt and the Ras/MAPK pathways. These pathways are also trigger by ligand binding to the GPR30 and by growth factor binding to receptor tyrosine kinases, including HER2, activating autophosphorylation and downstream signalling. The Pi3K/Akt pathway as predicted is a meeting point in the mechanisms implicated in drug resistance (Martin, Smith and Tomlinson, 2014).

It is stated that ERα phosphorylation has an impact in patients who have breast cancer. As higher level of ERα S167 and/or S118 phosphorylation is a signed of positive outcome in patients treated with tamoxifen therapy. In contrary, ERα S305 is associated to aggressiveness of tumours in which PKA and PAK1 are the possible candidates (Wei et al., 2014).

Resistant tumours causes changes in their intrinsic metabolic pathways over a long period of treatment to maintain survival and growth (Blundon and Dasgupta, 2019).

Estrogen G-protein coupled receptor (GPR30) signalling pathway up-regulation can elucidate the rise sensibility of TAM-R cells to GPR30-specific agonist G1 in MCF-

7 cells. The overexpression of GPR30 enhance the accelerated development of resistance if the natural ligand is presence (Ignatov et al., 2010).

Most breast malignancies are ER+. Tamoxifen is the standard drug treating such cases. Overall, outcome is promising but recurrence is still reported in patient with natural resistance, while some acquired resistance (S.S. Malik et al., 2018). It was reported that among patients with metastasis cancer, about 40% received tamoxifen as adjuvant therapy but encountered failure. Furthermore, patients with menopause who were treated earlier may have recurrent tumours and could become resistant to tamoxifen with time (Rondón-Lagos et al., 2016).

2.2 Vinblastine

Vinblastine (VB) is a vinca alkaloid with antineoplastic properties. It is divided into vindoline and catharanthine. They have been used as antitumour agents for decades (Amiri, B., et al., 2018). They are derived from Catharanthus roseus leaves. Besides anticancer, these compounds also exhibit an antileukaemic effects (Amiri, B. et al., 2018). Vinblastine causes cancer cell apoptosis and cell arrest which is enhanced by reactive oxygen species (ROS) (Lee et al., 2016; Varmaghani, et al., 2014). It obstructs the nucleic acid and protein production by preventing glutamic acid utilisation (Amiri, B., et al., 2018). Vinblastine targets the tubulins of cancer cells destabilising polymerisation and depolymerisation, thus influencing cell death (Amiri, B., et al., 2018; Lee et al., 2016). Microtubules are very important in cell functioning as they are involved in organising the cytoskeleton structure of eukaryotic cells, separation of cell division, internal cell trafficking, cell motility and the formation of flagella as well as cilia. Microtubules are a key treatment target in the development of drugs (Lee et al., 2016; Wang et al., 2019). The migration of cell is an important

physiological process including wound healing, tumour invasion, metastasis and neo-angiogenesis (Wang et al., 2019). In cell migration, loosen shape microtubules are first formed, which facilitate Rho protein to enhance an unbalanced actin shrinking and substrate adhesion. This results in difference and directional motility of cells (Wang et al., 2019). Vinblastine causes the deceleration of the microtubules, which causes their disability. Furthermore, there are other molecules that contribute to the binding of vinblastine to the tubulin. These molecules form and trigger the formation of a complex (Varmaghani, et al., 2014). The issues of multidrug resistance, neurotoxicity and risk of severe side effects have caused a setback in the positive impact of vinblastine clinically. Due to this, the use of a delivery system which will help in drugs reaching their target in a controlled manner is needed. It is postulated that drug formulation using nanotechnology will solve this issue. Nanoparticles are vehicles suitable to transport drugs due to their biocompatibility, biodegradable, non-immunogenic and stability (Amiri, B., et al., 2018).

2.3 Metastasis Breast Cancer

Metastatic disease leads to 90% of mortality in cancer. It is a challenge as there is no regimen that would permanently cure metastatic cancer (Meirson and Gil-Henn, 2018). Metastatic cancer spreads from the point of origin to other tissues via the bloodstream. Cancer cells form invadopodia and travel across these tissues (Meirson and Gil-Henn, 2018).

Metastasis is the process in which the primary tumour permeate the tissue around the tumour and colonise other sites of the body. Migration occurs because loss of cell to cell adhesive ability and disconnection from the primary tumour. Change in the matrix cohesion properties and release of substances that breaks the matrix support the process of tumour cell invasion. Tumour cells enter into the blood vessel or indirectly via the lymphatic system. They are distributed faster through lymphatic system rather than through the blood circulation (Nandy and Lakshmanaswamy, 2017). Cytokines were found to play a role in cancer progression and migration. They are involved in inflammatory responses, cell interaction, metastasis and angiogenesis (Nandy and Lakshmanaswamy, 2017).

Metastatic cells run into lot of physical forces along the whole process such as the one extracellular matrix pose on primary tumour cell and circulating tumour cells come across many haemodynamic forces. This pressure can lead to the Linker of Nucleoskeleton and cytoskeleton (LINC) complex being compromised and deforms the nucleus and weakens the cytoplasm.

Alteration of the Lamin A/C is seen in breast cancer which can result in the mutation of LINC-mediated linkage between the cytoplasm and the nucleus, causing

invasiveness of tumour cells due to the weakened cytoplasm and the nucleus (Nandy and Lakshmanaswamy, 2017).

Metastasis of cancer cells also depends on the microenvironment. Microenvironment supports and encourages the invasion and metastasis of cancer cells (Nandy and Lakshmanaswamy, 2017). Interaction between tumour cells and microenvironment is key in determining the speed of cancer progression, invasion and metastasis. It has been noticed that micro-environment frequently changes during tumour cell signalling. The active nature of micro-environment immensely affects the whole mechanism of metastasis (Nandy and Lakshmanaswamy, 2017).

Notwithstanding of all the improved technologies in the early diagnoses of breast cancer, locally advanced breast cancer (LABC) is prognose poor diagnosis, contributing to 30-60% of newly diagnosed breast cancer cases in underdeveloped nations. LABC is characterised by different diseases with advanced primary tumours, advanced nodal disease and inflammatory carcinomas (Saha et al., 2015). High percentage of treatment failures after initial responses to chemotherapy indicates drug resistance is an obstacle for managing patients with LABC.

Overexpression of apoptosis inhibitor survivin contributes to tamoxifen resistance. It has been seen that minimal level of survivin prolongs the overall survival time after treatment (Marcotte et al., 2017; Cheung, C.H.A., et al., 2010). It is also found in prostate cancer, where its predominance is related to metastasis (Hei et al., 2010; Cheung, C.H.A., et al., 2010).

2.4 Bioactive glass

Cytotoxic anticancer drugs have become ineffective due to drug resistance, that contributes to deaths of many women affected with breast cancer. High expression of adenosine triphosphate binding cassette (ABC)-transporters is shown to be link to resistant breast cancer (Wang et al., 2014). The promising avenue of tackling this menace problem is the use of nanocarriers by endosomal delivery of drugs. Doxorubicin (DOX) an anthracycline antibiotic used for breast treatment is been used with mesoporous silica nanoparticles on MCF-7 cells. It has showed the capability of overcoming of the resistance of MCF-7 breast cancer cells when DOX is embedded in mesoporous silica nanoparticles (Wang et al., 2014). It is been seen that bioactive glass of different powders have shown cytotoxicity at a concentration of greater than 5 mg/ml (Rismanchian, Khodaeian and Bahramian, 2013). Albumin nano-carrier for delivery of CuNPs have proven to be an effective anticancer treatment for invasive breast cancer cells due to its high toxicity and induced apoptosis cell death when compared to normal cells (Azizi et al., 2017). Nanocarriers are used as a targeted therapy on cancer cells and more useful than other methods (Burge, P., Rohr, C. and Daly, A. 2017).

Studies have demonstrated the idea of using nano-techniques of alleviating and replacing defects and damaged parts of patients' body. The invention of 45S5 Bioglass has changed the concept that implantable materials should be inert. Bioactive glass is predominantly used in tissue engineering field due to their abilities to bond to living tissues and induce tissue regeneration and growth as its dissolves with time (Baino, Novajra and Boccaccini., 2016). Bioglass precursors have been utilised in clinical settings since in the nineteen centuries like Perioglas and currently NovaBone useful for orthopaedic and dental application (Baino, Novajra and Boccaccini, 2016). Since its invention, bioactive glass has been used in a variety of field different from bone application. BG has been also

studied and applied in many medical fields, including wound dressings, drug delivery systems, and other pharmaceutical applications (Jebahi, S. et al., 2013). In justification, it has been applied in neuromuscular, artificial cornea, cardiac tissue engineering, treatment of gastric ulcers, non-osseous cancer therapy. It has been shown efficient in wound treatment and proven to be faster in wound healing bioactive glass treated wounds. Other BG-base products are used in wound healing and nerve regeneration and Ag- doped phosphate glasses with polymeric adhesive have been used for the treatment of wound (Baino, et al., 2018). Bioactive glass ointments show shortened wound healing time and, enhanced migration of fibroblasts and tissue growth (Baino, Novajra and Boccaccini, 2016). BG enhances and induces the growth and maturation of osteoblasts and nurtures the expression and preservation of the osteoblastic phenotype. Furthermore, BG promotes multipotent bone marrow stromal cell functions. Moreover, the osteoprogenitor cells proliferate and differentiate to matrix-producing osteoblasts (Baino, Novajra and Boccaccini, 2016; Jebahi., et al., 2013).

Bioactive glass has been used as a delivery system especially in the treatment of deadly inoperable type of malignant tumours (Lin, Mauro and Kaur, 2019). The treatment of cancers and many disease conditions today fail due to non-specific targeted treatment. Due to this, tumour treatment has been revolutionised by using targeted drug delivery system for cancer therapy, which has reduced the adverse effects of chemotherapy to healthy cells (Lin, Lin and Chan, 2013). Targeted delivery therapy will enhance the effectiveness of cancer treatment. Coating of drug carriers to specific binding factors or receptors can increase the efficacy of drugs as there will be high uptake of the drug. Currently, drug delivery systems such as mesoporous silica, polymers and liposomes to target cancer cells are used (Lin, Lin and Chan, 2013).

Folate conjugated mesoporous silica with doxorubicin drug is used as a targeted cancer therapy and improves cytotoxicity than when the drugs are used alone. Folate grafted mesoporous silica particles induce cancer cells to take more drug. Bioactive glass is used in the treatment of bone-tumour cancer therapy (Lin, Lin and Chan, 2013). Bioactive glass incorporated with lysozyme (LY) increases its function and forms hydroxyapatite in body fluid. LY bioactive glass acts as an antibiotic in treating gram positive *B. subtilis*. Synthesised Ag-loaded with mesoporous bioactive glass also have antibacterial effects by discharging ions from Ag-MBGs (Fan et al., 2015). LY bioactive glass equally proves cytotoxicity against hepatocellular carcinoma cell line. LY bioactive glass is useful for biomedical application especially for the treatment of bone tumours (Zheng et al., 2016).

Small amount of cancer drugs reach to their targets and the rest are distributed in the body which could affect normal healthy organs. Anticancer drug like 5-fluorouracil (5-FU) is used in the management and treatment of many cancers but has a short half-life due to being metabolised fast by the body and non-specific. Increased efficacy could be achieved if loaded with nanoparticles. The drug can be maintained and released gently, thus preventing the recurrence of tumours after surgery (El-Kady and Farag, 2015). Bioactive glass has been used in cancer cell removal (Kargozar et al., 2018a). Magnetic bioactive glasses (MBGs) are used in the treatment of deep bone cancer tumours (Lin, Mauro and Kaur, 2019). The qualities of MBG especially with DOX allow it to function well in cancer treatment and their applicable for bone cancer treatment (Wu, C., Fan, W. and Chang, J., 2013).

Radiation have been a conventional treatment for cancer which could bear some consequences to the patients. However, *in situ* radiation of high dose had been instrumental in the treatment of cancers for a minimal period without harming the patient.

Internal therapeutic irradiation of radioisotope in biocompatible glass beads injected to patients has been fruitful with high durability and are able to reach to the organs to be treated (Baino, Novajra and Boccaccini, 2016).

Recently, radioactive glass microspheres (TheraSphere®) are medically used in the treatment of hepatocellular carcinoma and metastatic liver cancer (Baino, Novajra and Boccaccini, 2016). Radioactive glass microspheres are medically used for the treatment of unresectable hepatocellular carcinoma, metastatic liver cancer and cholangiocarcinoma. The approach involves the submerging of radionuclide in protective insoluble microcapsules that are injected into the patient's body and targeting cancer cells like in the case of treating prostate cancer using 142Pr glass seeds (Baino, Novajra and Boccaccini, 2016., Baino et al.,2018). Biocompatible radioactive glasses has been applied for the treatment of liver cancer. Insoluble yttrium oxide (Y_2O_3– Al_2O_3–SiO_2) glass microspheres with diameter of 25 μm are injected into the patient and they are anchored in the capillary bed of the diseased liver. This will be discharged towards tumour cells that is tolerated by the patient. Radioactive glass has also been used in patients with metastatic colorectal carcinoma (Baino et al.,2018). This therapeutic approach leads to a significant improvement of survival times and quality of life for the patients. Iron oxide magnetic nanoparticles (Fe_3O_4, MNPs) are used in the treatment of cancer. Iron oxide nanocrystals simultaneously loaded with docetaxel, have been shown to have more efficacy of anticancer drug. The targeted nanoparticles showed an antimigrative effect in prostate cancer cells on cytotoxicity assay in a dose dependent manner (Namvar, F. et al., 2014). Mesoporous bioglass nanospheres incorporated with alendronate have the capability to control osteosarcoma cells and osteoclast activities (Boanini et al., 2016). Magnetic bioceramics and nanoparticles *in situ* have been utilised, especially calcium phosphate bone cement in the treatment of bone tumours by exciting hyperthermic and replacing

fracture bone. Evidence in clinical studies has proven *in situ* implant treatment of metastatic bone tumour (Cochis, Miola and Bretcanu, 2016; Miola et al., 2018). The positivity of the clinical application of this biomaterial is high but further research is still needed to fully comprehend the mechanism involved in the treatment of cancer. Furthermore, more *in vitro* and *in vivo* studies on the effects of bioglass and glass ceramics are needed (Miola et al., 2018).

2.4.1 Biomaterials

Biodegradable synthetic plastic and metal are made to replace natural bone recently but are weak as they cannot support the regeneration of load bearing bone. Metallic implant is non-self-repairable and cannot withstand the changing condition of the body due to the strong and stiffness of metal, breaking the normal process of bones and become less desirable at the long run. The used of calcium phosphate-based bio-ceramics and bioactive glass even though they are breakable but stronger than polymeric scaffolds, yield to the advance investigation and production of these biomaterials (Boccardi, et al., 2017; Fu, 2019; Hench et al., 2015; Jones, 2015a; Sepulveda et al., 2002)

Biomaterials are any natural or synthetic material (metal or polymer) that are suitable to be introduced into living tissue or body for medical benefits either to treat, repair or substitute a tissue function of the body. After all the recent breakthrough, autograft is still the preferable standard for bone defect treatment. Allograft which is the second option to autograft is expensive and can be risky. Biomaterials are grouped in class A and class B. Class A (Bioactive glass) materials can produce bones by triggering responses both intra and extracellular at the contact point (Dziadek et al., 2016; Jones, 2015b). Biomaterials have been used to replace autograft and allograft but are yet to meet the standard of a natural bone. Biomaterials used as templates for the repair and regeneration of bones and tissue should meet special criteria that are paramount in optimising tissue formation. Biomaterials should promote migration and pose no harm to both *in vivo* and *in vitro* tissues and should be degradable, neither be toxic on harmful to human body or should not trigger any reaction (Jones, 2015b). They can induce immature

cells into osteoblast. Biomaterials glass should be in three-dimensional structure with ability and potentials to triggering new bone growth and blood supply. The bone graft should be able to support the functional stresses of the substituted bone. Moreover, they should be able to converts the Hydroxyapatite (HA) at a quicker rate to a rate equal to the new bone ingrowth (Bi et al., 2014; Fu, 2019; Jones, 2015a).

2.4.2 Bioactive Glass

The BG was invented by Hench et al., 2015 and known as BG 45S5 has been widely used for tissue engineering (Hench et al., 2015). From the time of the discovery in the 70s, bioactive glass has been studied for its efficacy in bone tissue repair and substitution capability. This 3D structure accelerates the formation of hydroxycarbonate apatite layer after forming a bond with bones. This layer forms around implant facilitate their degradation and absorption (Lin et al., 2018). Inorganic oxide mixtures of various percentages have been used to produce divergent families of glasses. In addition to network-forming, network-modifying and intermediate oxide, BG production involves three types of inorganic oxides (Kargozar et al., 2018b). They are classified into silicate, borosilicate, borate and phosphate-based glasses. Apart from that, doped glasses and mesoporous BG are grouped as other classes of BG family. BG in modern medicine is used for repair and regenerative purposes (Kargozar et al., 2018b). It is not enclosed by tissues when embedded which making it useful in the clinical settings. Components of the bioactive glass chemically bind with host tissues, degrading as it is dissolving thereby releasing ions that will trigger or stimulate the formation of hydroxyapatite layer at its surface (Zain, N.S.M et al., 2016; Vichery and Nedelec, 2016). The treatment of bone defects is either through grafting which generates another wound to the bone tissue or implantation with metals which causes fibrous encapsulation. Since the invention of