# CONJUGATION OF BIOACTIVE GROUPS TO POLY(LACTIC ACID) MICROSPHERES FOR DRUG DELIVERY SYSTEMS

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**by**

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| | |
|---|---|
| AA | Antibiotic-Antimycotic |
| ALP | Alkaline phosphatase |
| API | Active pharmaceutical ingredients |
| ATCC | American Type Culture Collection |
| $BaCl_2$ | Barium chloride |
| $BaSO_4$ | Barium sulphate |
| bFGF | Basic fibroblast growth factor |
| BMP | Bone morphogenic protein |
| BMSC | Bone marrow stromal cells |
| $CO_2$ | Carbon dioxide |
| DAPI | 4',6-diamidino-2-phenylindole |
| DCM | Dichloromethane |
| DDS | Drug delivery system |
| DMEM | Dulbecco's modified eagle medium |
| DMSO | Dimethyl sulfoxide |
| DNA | Deoxyribonucleic acid |
| ECM | Extracellular matrix |
| EDAC | N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride |
| EDTA | Ethylenediaminetetraacetic acid |
| EE | Encapsulation efficiency |
| EGF | Epidermal growth factor |
| EGFR | Epidermal growth factor receptor |
| ELP | Elastin-like polypeptide |
| EPR | Enhanced permeation and retention |
| ERK | Extracellular signal-regulated kinase |
| ESE | Emulsion and solvent evaporation |
| *E. coli* | Escherichia coli |
| FBS | Fetal bovine serum |
| FDA | Food and Drug Administration |
| FTIR | Fourier transform infrared |

| | |
|---|---|
| GF | Growth factor |
| GIT | Gastrointestinal tract |
| GPC | Gel permeation chromatography |
| hBM-MSC | Human bone marrow-mesenchymal stem cell |
| hUCMSC | Human umbilical cord mesenchymal stem cell |
| HCl | Hydrochloric acid |
| HPLC | High performance liquid chromatography |
| HPMC | Hydroxypropyl methylcellulose |
| $H_2SO_4$ | Sulphuric acid |
| H&E | Haematoxylin and eosin |
| IC | Intracardiac |
| ICC | Immunocytochemistry |
| IM | Intramuscular |
| IP | Intraperitoneal |
| IV | Intravenous |
| KBr | Potassium bromide |
| LG-DMEM | Low glucose-Dulbecco's modified eagle medium |
| MAPK | Mitogen-activated protein kinase |
| MHA | Muller-Hinton agar |
| MHB | Muller-Hinton broth |
| MIC | Minimum inhibition concentration |
| MRI | Magnetic resonance imaging |
| MS | Microspheres |
| MSC | Mesenchymal stem cell |
| MSDS | Material Safety Data Sheet |
| $M_w$ | Molecular weight |
| NaOH | Sodium hydroxide |
| NP | Nanoparticles |
| OPG | Osteoprotegerin |
| o/w | Oil-in-water |
| PBS | Phosphate buffer saline |
| PCL | Poly(ε-caprolactone) |

| | |
|---|---|
| PDA | Polydopamine |
| PEI | Polyethyleneimine |
| PHBVDB | Poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-2,3-dihydroxybutyrate) |
| PI3K | Phosphoinositide 3-kinases |
| PDLLA | Poly(D,L-lactic) acid |
| PLA | Poly(lactic) acid |
| PLGA | Poly(lactic-co-glycolic) acid |
| PLLA | Poly(L-lactic) acid |
| PMMA | Poly(methyl methacrylate) |
| PPE | Personal protective equipment |
| PSA | Particle size analysis |
| PVA | Poly(vinyl) alcohol |
| PVP | Poly(vinylpyrrolidone) |
| p-Akt | Phosphorylated-protein kinase B |
| RES | Reticuloendothelial system |
| RGD | Arginine-glycine-aspartic acid |
| RNA | Ribonucleic acid |
| ROP | Ring opening polymerization |
| ROS | Reactive oxygen species |
| Runx2 | Runt-related transcription factor 2 |
| SC | Subcutaneous |
| SEM | Scanning electron microscopy |
| SK3 | Male rabbit fibroblast |
| *S. aureus* | Staphylococcus aureus |
| TCA | Tricarboxylic acid cycle |
| TGF | Transforming growth factor |
| THF | Tetrahydrofuran |
| TZP | Tetragonal zirconia polycrystals |
| UV-vis | UV-visible spectroscopy |
| w/o | Water-in-oil |
| w/o/w | Water-in-oil-in-water |

## LIST OF SYMBOLS

| | |
|---|---|
| % | Percentage |
| ° | Degree |
| °C | Degree Celsius |
| µL | Microliter |
| µm | Micrometer |
| ~ | Approximately |
| ρ | Density |
| $\theta$ | Angle |
| > | Greater than |
| CFU/mL | Colony-forming unit per milliliter |
| g | Gram |
| $g/cm^3$ | Gram per cubic centimeter |
| g/mol | Gram per mol |
| k | Kilo |
| L | Liter |
| mg | Milligram |
| mg/mL | Milligram per milliliter |
| $mJ/m^2$ | Mega joule per square meter |
| mL | Milliliter |
| mm | Millimeter |
| mM | Millimole |
| nm | Nanometer |
| ppm | Parts per million |
| rpm | Rotation per minute |
| $R^2$ | Correlation coefficient |
| t | Time |
| s | Seconds |
| wt% | Weight percentage |
| w/v% | Weight per volume percentage |

**KONJUGASI KUMPULAN BOAKTIF KEPADA MIKROSFERA POLI(ASID LAKTIK) UNTUK SISTEM PENGHANTARAN UBAT**

## ABSTRAK

Penghasilan mikrosfera poli(asid laktik) (PLA) melalui kaedah emulsi dan pemeruapan pelarut (ESE) mempunyai potensi dalam aplikasi-aplikasi sistem penghantaran ubat (DDS) disebabkan oleh biokeserasian, biodegradasi, tidak toksik dan sifat-sifat mekanikal PLA yang sangat baik. Walau bagaimanapun, sifat PLA yang hidrofobik menyebabkan afiniti sel rendah yang boleh menimbulkan tindak balas keradangan apabila diberikan secara *in vivo*. Oleh itu, dalam kajian ini, fungsian permukaan melalui hidrolisis natrium hidroksida (NaOH) telah dijalankan untuk memperkenalkan kumpulan berfungsi hidrofilik pada mikrosfera PLA. Seterusnya, kejuruteraan permukaan melalui konjugasi dengan faktor pertumbuhan (GFs) iaitu fibroblast asas (bFGF) dan epidermal (EGF) telah dilakukan bagi meningkatkan interaksi isyarat dengan sel khususnya osteoblast. Selain itu, keupayaan PLA sebagai pembawa ubatan telah dibandingkan antara gentamicin dan dexamethasone dari segi kecekapan enkapsulasi, memuat dan profil pembebasan ubatan diikuti dengan penilaian antimikrobial melalui penyebaran agar dalam lubang Kirby-Bauer dan kepekatan minimum perencatan (MIC) pada strain *Staphylococcus aureus* (*S. aureus*) dan *Escherichia coli* (*E. coli*). Gentamicin pada 0.375% mempunyai kecekapan enkapsulasi (42.3%), memuat ($26.5\times10^{-3}$%) dan profil pembebasan ubatan ($R^2$ = 0.9944) yang baik melalui model Higuchi telah diperhatikan. Perencatan zon tertinggi bagi strain bakteria juga diperhatikan pada 0.375% gentamicin mikrosfera PLA sejajar dengan penilaian nilai MIC (0.002 mg/mL). Pengubahsuaian pada mikrosfera PLA juga meningkatkan

perumahan, migrasi dan pembezaan sel yang menyumbang kepada proses penghantaran ubatan yang berjaya. Semua modifikasi yang dilakukan menghasilkan peningkatan percambahan osteoblast dengan peratusan tertinggi sel hidup didapati pada gentamicin EGF konjugasi mikrosfera PLA pada hari ke 5. Sementara itu, semua modifikasi pada mikrosfera PLA meningkatkan migrasi osteoblast berdasarkan penutupan luka adalah 100% selepas tiga hari daripada pembenihan sel menunjukkan peningkatan dalam proses penyembuhan luka. Peningkatan permukaan mikrosfera PLA yang hidrofilik juga menawarkan permukaan yang menggalakkan pelekatan osteoblast seperti yang ditunjukkan oleh pewarnaan positif 4',6-diamidino-2-phenylindole (DAPI) daripada nukleus sel. Selain itu, pengubahsuaian pada mikrosfera PLA dapat meningkatkan keupayaan PLA dalam meningkatkan proses pembezaan sel stem mesenchymal (MSCs) kepada jajaran sel osteogenik kerana warna positif hanya diperhatikan pada mikrosfera PLA yang terubahsuai di mana warna positif tertinggi berlaku dengan kehadiran GFs. Keputusan ini menunjukkan bahawa fungsian dan konjugasi mikrosfera PLA dengan GF meningkatkan hidrofilisiti PLA dan juga sifat tidak toksiknya. Mikrosfera PLA yang dikonjugasi berpotensi sebagai pembawa penghantaran ubat seperti yang dibuktikan dapat meningkatkan pelekatan, lampiran, percambahan dan pembezaan sel dengan profil pembebasan ubatan yang berpanjangan dan berterusan.

**CONJUGATION OF BIOACTIVE GROUPS TO POLY(LACTIC ACID) MICROSPHERES FOR DRUG DELIVERY SYSTEMS**

## ABSTRACT

The development of poly(lactic) acid (PLA) microspheres through the emulsion and solvent evaporation (ESE) method is promising for drug delivery systems (DDS) applications owing to the biocompatibility, biodegradability, non-toxic and excellent mechanical PLA properties. However, hydrophobicity of PLA may result in low cells affinity which can elicit an inflammatory response when administered *in vivo*. Therefore, in this research, surface functionalization via sodium hydroxide (NaOH) hydrolysis has been conducted to introduce hydrophilic functional groups on the PLA microspheres. Further surface engineered by conjugation with the basic fibroblast (bFGF) and epidermal (EGF) growth factors (GFs) were performed in order to improve its signaling interaction with cells specifically osteoblasts. Whereas, the capability of PLA as a drug carrier was compared between gentamicin and dexamethasone in terms of drug encapsulation efficiency, loading and release profile prior to antimicrobial assessments via Kirby-Bauer agar well diffusion and minimum inhibitory concentration (MIC) on *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) strains. It was observed that gentamicin encapsulated at 0.375% were having good encapsulation efficiency (42.3%), loading ($26.5\times10^{-3}$%) and release profile ($R^2$ = 0.9944) of Higuchi model. The highest zone of inhibition for bacterial strains was also observed at 0.375% gentamicin encapsulated PLA microspheres in parallel with the MIC values assessment (0.002 mg/mL). The PLA microspheres modifications done enhanced the cellular homing, migration and differentiation which contribute to a successful drug delivery process. All

the modifications performed resulted in increasing osteoblasts proliferation with the highest percentage of cells viability was observed at gentamicin EGF conjugated PLA microspheres on day 5. While, all modifications on PLA microspheres enhanced the migration of the osteoblasts as wound closure was 100% after three days of the cells seeding indicated enhanced in wound healing process. Increase in hydrophilicity of the surface of PLA microspheres offers favorable surface for osteoblasts attachment which was reflected by positive 4',6-diamidino-2-phenylindole (DAPI) staining of the cells' nuclei. Other than that, the modifications on the PLA microspheres were able to enhance the capability of the PLA in facilitating the differentiation process of mesenchymal stem cells (MSCs) into osteogenic lineage since only positive stain was observed on the modified PLA microspheres in which the highest intensity occurred by the presence of GFs. These results indicated that the functionalization and conjugation of PLA microspheres with GFs improved the hydrophilicity of the PLA with non-toxic properties. The conjugated PLA microspheres has potential to be used as drug delivery vehicle as evidenced it can enhance the cells adherence, attachment, proliferation and differentiation with prolonged and sustained drug release profile.

# CHAPTER 1

# INTRODUCTION

## 1.1 Overview

Drug delivery systems (DDS) are engineered technologies for the controlled release or targeted delivery of therapeutic agents such as drugs to improve health and extend lives (Kalaydina et al., 2018). The controlled release DDS implies therapeutic release kinetic over a prolonged duration advanced rapidly as a better delivery devices to overcome the limitations associated with conventional administration (Liu et al., 2016). Conventional drug intake basically leads to a rapid increase of drug content in plasma which then will decrease below therapeutic window in a short period of time. This will require re-administration of the drugs in order to achieve intended pharmaceutical function (Tamargo et al., 2015). Therefore, DDS has been introduced which enables the drug plasma concentration to be maintained within desired therapeutic range and allows specific pharmaceutical drug delivery to the target site of action more efficiently and at safer condition (Tiwari et al., 2012). This is particularly crucial for the delivery of the therapeutic agents that are rapidly metabolized and removed from the body after administration (Kang and Lee, 2009).

While various devices such as micelles, liposomes, dendrimers, nanoparticles and microspheres (Vasir et al., 2003) have been developed for depot-based delivery system, biodegradable polymeric microspheres is one of the approaches to fulfill the objectives of DDS. From this biodegradable property, they are implanted into the body without the essential for following elimination via the surgical process (Sheikh et al.,

2015). Single administration of drug encapsulated biodegradable polymer microspheres allows the release in a continuous and controlled manner over a sustained period of time, therefore retaining the drug concentration within a target ranges (Ma et al., 2008). This approach can significantly minimize systemic side effect encountered from the repeated administration using conventional methods, thus increase the patient's compliance during drug intake (Freiberg and Zhu, 2004, Versypt et al., 2013, Andhariya et al., 2017). Other than that, this carrier also has the potential to act as a transient mask in order to protect labile and unstable active pharmaceutical ingredients (API) such as peptides, proteins and enzymes from physiological degradation in the local tissue surrounding which resulting in prolong their half-live *in vivo* (Hines and Kaplan, 2013, Szlek et al., 2016).

In the advancement of DDS, biodegradable polymeric biomaterials have been used as healing devices because their suitability and effectiveness in biomedical applications such as biocompatibility, biodegradability and easily tailors into desired properties (Shah et al., 2011). Furthermore, polymeric materials particularly microparticle system are widely studied for its various healing applications such as constant and targeted drug release, inoculation/vaccination and growth factor delivery (Patra et al., 2018). The deficiency in regular dosages can be minimized after the first administration due to the sustainability of drug release and the degradation of residual polymer is cleared through the body (Kamaly et al., 2016).

Among the numerous types of synthetic biodegradable polymers, polyesters are considered as the most feasible biodegradable polymers for drug delivery applications. One of the commercialize polyesters used is poly(lactic) acid (PLA) because it can be

synthesized from the renewable resources which can reduce the energy crisis (Singhvi et al., 2019). Furthermore, PLA can be commercially produced with an extensive range of characteristics. In the past two decades, PLA has been intensively studied and developed in various pharmaceutical and biomedical applications because of its bioresorbable, biocompatible, biodegradable and excellent mechanical properties (Song et al., 2018).

PLA is one of the aliphatic and thermoplastic biodegradable polyesters manufactured synthetically by polymerization of lactic acid monomers or cyclical lactic dimmers (Lopes et al., 2014). Numerous studies in biomedical sectors such as the production, modification and application of PLA microspheres have been prepared and used (Alsaheb et al., 2015). The capability of PLA to be used as a drug carrier has been reported significantly increase in the encapsulation efficiency of restenosis (Fishbein et al., 2000), oridonin (Xing et al., 2007), progesterone (Matsumoto et al., 1999) and bovine serum albumin (BSA) (Gao et al., 2005). According to Roney et al. (2005), small size of PLA microspheres allows its penetration into organic barrier such as blood-brain barrier resulting in effective targeted and constant release of drugs and peptide/protein delivery.

In fact, PLA benefits from biocompatibility, renewability, energy-saving and processability relative to other polymers (Rasal et al., 2010). First, PLA is an excellent material for medicinal needs that involves clips, sutures and DDS as PLA's degradable materials such as carbon dioxide ($CO_2$) and water ($H_2O$) are neither toxic nor cancer-causing to the body (Shah et al., 2011). Second, PLA is made from both degradable and renewable resources such as corn and rice which can help minimize fossil fuel reliance and mitigate society's energy crisis (He et al., 2014). Third, the energy-saving property

gives the new concept of a "low-carbon economy" ultimately invests in PLA (Vink et al., 2003). Lastly, PLA has good thermal processability, so that blow molding, extrusion, film casting and fiber spinning can be used (Rhim et al., 2006, Mokhena et al., 2018). Moreover, due to the numerous required features of PLA such as biodegradability, renewability, thermoplasticity and distinct catalysts with increase biocompatibility, high catalytic activity, low toxicity and exceptional stereoselectivity, PLA is potential applicant used in the biomedical field (Jamshidian et al., 2010).

In drug delivery, undesired effects such as high burst release of drugs and low encapsulation efficiency are encountered due to poor connections between polymers and surface of hydrophilic molecules desorption (Thote et al., 2005). In order to overcome these issues, certain modifications must be made to create active functional groups either by using additives during their manufacture or by direct immobilization (Datta et al., 2013). The blending of PLA with functional nanoparticles can yield a new class of hybrid materials (Ray, 2012). There are various uses of PLA-based hybrid materials that have been well documented and clinically tested on other tissues including cartilage (John et al., 2007), adipose (Mauney et al., 2007), liver (Lv et al., 2006) and bone tissue (Mathieu et al., 2006). PLA also has been applied for implant and therapeutic devices such as screw, fixation rods, plates, sutures and membrane applications such as wound covers as well as dermatological therapy such as facial lipoatrophy and scar rejuvenation (Xiao et al., 2012).

Apart from that, PLA has some limitations in biomedical applications include the degree of degradation via ester group hydrolysis is too low (Bergsma et al., 1995), PLA is low cell affinity since PLA is hydrophobic which can induce an inflammatory reaction

from body host tissues and PLA is not suitable for hard mechanical applications due to its brittle property unless it is modified appropriately (Rasal and Hirt, 2009, Xiao et al., 2012). Hence, surface and bulk PLA modification approaches have been done to be suited in biomedical applications (Xiao et al., 2012). The detailed surface properties including topography, roughness, energy and hydrophilicity are important for interactions with biomacromolecules and biomedical applications of PLA. According to Xiao et al. (2012), pure PLA results in an inflammatory reaction when inserted in body tissues. Thus, surface modification is needed to design the biomaterials with specific characteristics. Commonly, there are two types of methods of modification which are chemical and physical (coating and entrapment) methods. Chemical methods are widely used to produce hydrophilic functional groups on the surface of biomaterials such as amine, carboxyl and hydroxyl. In addition, this approach is the easiest and most effective way to increase the hydrophilicity property of PLA by using alkali hydrolysis treatment (Yang et al., 2003).

Since PLA does not contain reactive functional groups such as amine, carboxyl and hydroxyl, several researchers have concentrated on incorporating bioactive molecules into the material, for instance peptides, proteins and growth factors which resulting in biomimetic microspheres with bioactive functions (Wang et al., 2017). The ester bonds are hydrolyzed in the surface hydrolysis of aliphatic polyester which produced the hydrophilic functional groups. Such active functional groups can be used to conjugate bioactive molecules to regulate protein adsorption or cell adhesion (Stupack et al., 2001). This technique is ideal for numerous applications in implant technology

and tissue engineering as it offers recognition sites and potential to induce faster biodegradation and cell adhesion.

On the other hand, in order to encapsulate drug within PLA microspheres, microencapsulation technique is used. Microencapsulation is the technique of coating or surrounding chemical substances either solid, liquid or gas form within material which capable of releasing its content under certain conditions such as physical force, moisture or pH (Chanana et al., 2013). According to Hwisa et al. (2013), emulsion and solvent evaporation (ESE) technique has been reported to be the most effective in encapsulating either insoluble or poorly soluble drugs in biodegradable microspheres. The effectiveness of DDS preparation technique for microspheres relies on the drug being successfully entrapped in the particles. The understanding of ESE technique from the intrinsic (interaction between materials) and extrinsic (modifiable parameters such as polymer and stabilizer concentration, water-in-oil phase ratio, stirring speed and drug loading) perspectives is important for the development of microspheres with desired drug encapsulation (Iqbal et al., 2015).

Therefore, this study is aimed to assess the ability of surface engineered PLA microspheres without interrupting its bulk properties to improve the cell affinity through sodium hydroxide (NaOH) hydrolysis to produce hydrophilic functional groups. Such active functional groups will be used to conjugate growth factors namely basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) to enhance bioactivity with the surrounding cells in DDS. Other than that, double ESE is performed to encapsulate drugs (gentamicin and dexamethasone) within PLA microspheres to compare their effectiveness in DDS in terms of encapsulation efficiency, drug loading and release

profiles prior to antimicrobial activity assessments. All the modifications done towards PLA microspheres will be assessed via *in vitro* studies in terms of cells cytotoxicity, proliferation, migration, attachment and differentiation assays.

### 1.2 Problem Statements

Instead of the promising features of PLA for biomedical applications, various challenges and barriers in the development of an efficient DDS have still emerged. PLA microspheres' hydrophobicity characteristic compared to the surrounding extracellular matrix (ECM) has unfavorably elicits poor cell affinity and can induce immune response during *in vitro* and *in vivo* administration (Sadeghi et al., 2016). Other than that, accumulation of acidic lactic acid by-products resulting from the disruption of the chain in wet condition appears to cause an inflammatory reaction that could hinder further use of the microspheres for DDS. This condition is much worse for the delivery of highly susceptible macromolecular therapeutic agents such as proteins that could adversely weaken their activities under harsh acidic environments (Makadia and Siegel, 2011).

It is also noteworthy that the PLA surface does not has active functional groups for bioactive molecules attachment to enhance integrin binding with cell membranes and control protein adsorption (Ma et al., 2002, Croll et al., 2004, Duan et al., 2008). Moreover, several undesired effects such as high initial burst during the initial phase of drug release appear to transpire for conventional PLA before a stable release profile can be achieved (Wang et al., 2007). This bursting effect potentially reduces the drug delivery carrier's effective lifetime, thereby impacting both therapeutically and economically on its efficacy.

Besides that, the encapsulation of drugs can also be challenging due to the partitioning of weakly associated drugs from organic phases to external water phases during the process of conventional emulsion for the synthesis of PLA microspheres (Ramazani et al., 2016). It is therefore very important to design an efficient delivery mechanism which has to overcome all the aforementioned shortcomings in order to turn the PLA microspheres' biological potential into medical reality.

In this study, NaOH hydrolysis is chosen for surface functionalization of the PLA microspheres because it introduces reactive functional groups which enhancing PLA hydrophilicity. It is predicted that the improvement of hydrophilicity property will also increase the biocompatibility of the PLA microspheres. Furthermore, polymer surface engineering is potentially used to develop materials that are able to control the cellular adhesion and maintain differentiated phenotypic expression as such modifications involve enriching surfaces with functional domains such as growth factors (GFs).

Figure 1.1 illustrates the action of GFs in signal transduction pathway to increase cellular responses. The GFs such as basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) can promote cellular adhesion through binding to integrin receptors and this interaction also plays a significant role in cell growth, differentiation and overall regulation of cell function (Belair et al., 2014). GFs basically involve in regulating tissue homeostasis and wound healing process (Mina, 2015), while GFs signaling contributes to regulate signals by ECM to determine tissue formation and regeneration process (Lu et al., 2012).

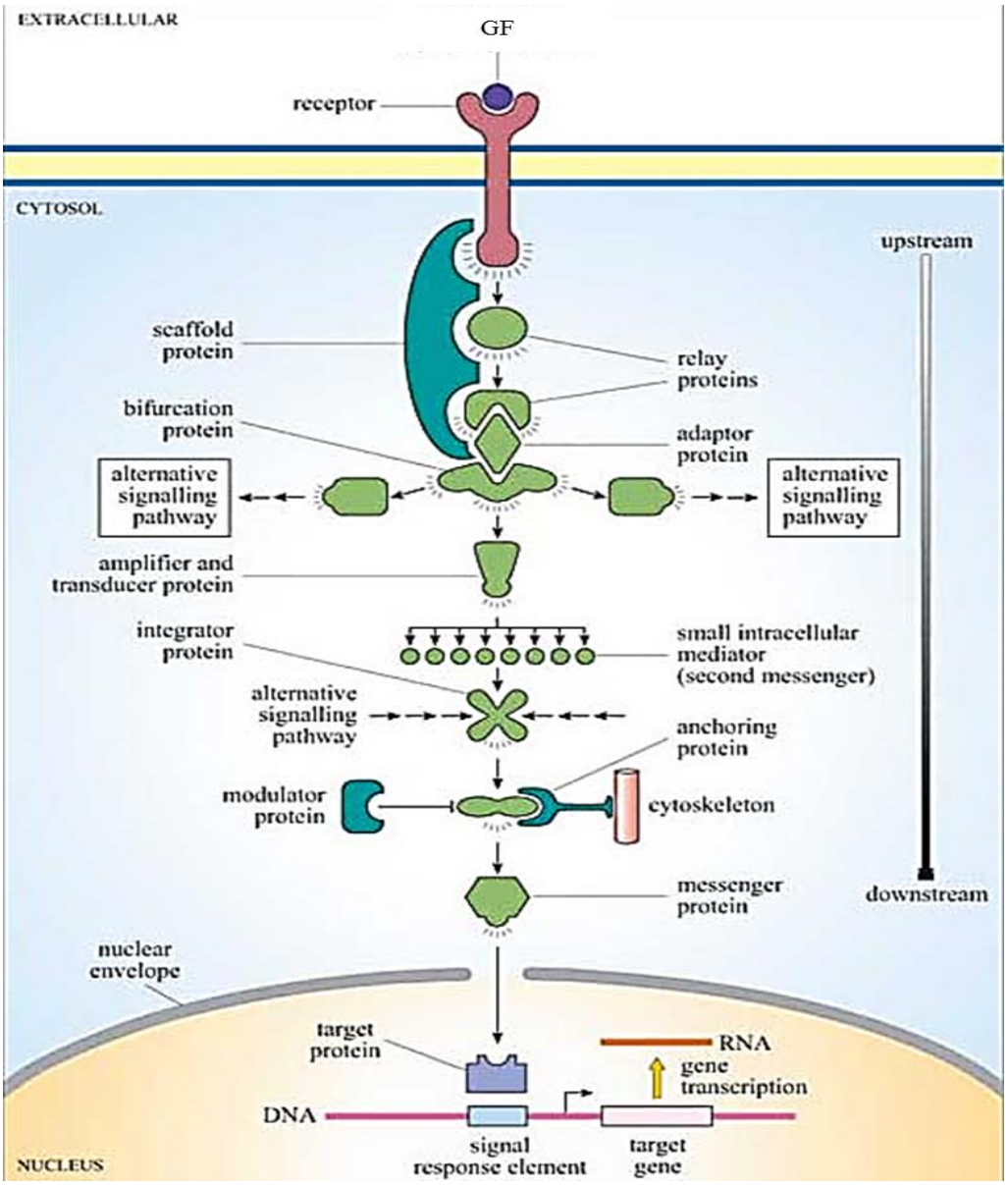


Figure 1.1: Signal transduction pathway involving signaling molecules (GF) leading to cellular responses (expression of a target gene) (Alberts et al., 2002).

Whereas, emulsion and solvent evaporation (ESE) technique has the ability to overcome undesired effects encountered by conventional methods such as high initial burst before a stable release profile can be achieved (Ramazani et al., 2016). In fact, this bursting effect can reduce the drug delivery carrier's effective lifetime which decreasing its efficiency. Therefore, microencapsulation using ESE is chosen to produce PLA

microspheres as it offers to yield a drug carrier that can release in a controlled manner within the therapeutic range for prolong period. This method also eliminates the need for multiple drug administration as exhibited from conventional procedures, therefore enhance the patient's compliance. The drugs to be encapsulated within PLA microspheres such as gentamicin (Abdelghany et al. 2012) and dexamethasone (Rodrigues et al. 2017) basically have the ability to inhibit bacterial infections which crucial for serious infection treatments. All the modifications performed will be evaluated via *in vitro* assays to observe the improvement in biocompatibility property of the PLA microspheres.

### 1.3 Objectives

Previously, several bulk modifications of PLA were reported by other researchers (Castillejos et al., 2018, Standau et al., 2019). However, very limited research on surface modification of PLA especially in the form of microspheres was conducted. Therefore, this research work is aimed to develop an ideal surface modified PLA microspheres for drug delivery applications in terms of improving PLA surface with hydrophilic functional groups via alkaline hydrolysis, further enriching it with GFs via conjugation to increase cellular bioactivity and encapsulating potential drugs prior to *in vitro* studies. The following are the specific objectives for the surface modified PLA microspheres development:

1. To study the effect of surface modification via NaOH hydrolysis towards optimized PLA microspheres properties.
2. To assess the bFGF and EGF growth factors conjugated PLA microspheres properties including conjugation efficiencies and biocompatibility improvements.

3. To evaluate the encapsulation efficiency, drug loading and release profile of gentamicin and dexamethasone encapsulated PLA microspheres.
4. To assess the effectiveness of gentamicin and dexamethasone encapsulated PLA microspheres to inhibit the bacterial infections.
5. To study the biocompatibility property of the PLA microspheres via *in vitro* (toxicity, proliferation, migration, attachment and differentiation) assays.

**1.4 Scope of Study**

In the first stage of study, the optimized fabrication of PLA microspheres via single emulsion and evaporation technique was conducted followed by surface modification of PLA microspheres through catalytic induced hydrolysis method, where the alkaline used was NaOH. The effect of different concentration of NaOH hydrolysis was characterized in terms of chemical bonding, surface and cross sectional morphologies, particle size and size distributions, hydrophobicity/hydrophilicity, surface energy and charges, porosity and molecular weight distribution.

The second stage, bFGF and EGF growth factors were immobilized by linking with carboxyl groups after surface modification under N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDAC) catalyzation. The surface and bulk properties of surface treated and the ability of immobilization with growth factors were studied in the aspect of chemical bonding especially the presence of hydroxyl, carboxyl and amine groups, surface morphology, particle size distributions, hydrophilicity property, surface energy, surface charges and porosity.

The third stage, the fabrication of PLA encapsulated with gentamicin and dexamethasone by using double emulsion solvent evaporation technique was explored. Microspheres properties were studied in the aspect of chemical bonding, surface morphology, particle size distributions, hydrophobicity/hydrophilicity, surface energy, porosity, encapsulation and loading efficiency as well as release profiles. The release of drugs was conducted in the catalyzed accelerated medium at the condition of 37°C.

The fourth stage, the effectiveness of PLA microspheres to deliver gentamicin and dexamethasone in inhibiting *Staphylococcus aureus* and *Escherichia coli* infections was studied via Kirby-Bauer agar well diffusion and micro-broth dilution methods.

Lastly, *in vitro* bioactivity evaluations in terms of PLA biodegradation, cells cytotoxicity, proliferation, migration, attachment and differentiation assays for neat PLA, surface engineered, drugs encapsulated and GFs conjugated PLA microspheres were discussed.

**1.5 Thesis Outline**

This thesis consists of five chapters which are introduction, literature review, methodology, results and discussion and conclusions.

In Chapter 1, a general introduction to the subject is presented, in addition to the problem statements, research objectives, scope of the study and the outline of the thesis.

Chapter 2 presents relevant literature review in support of the remainder of the thesis. This chapter provides a general understanding of drug delivery system, biodegradable polymer, poly(lactic acid) (PLA), its structure, properties and an overview

of its advantages and disadvantages. The later part of this chapter is focused on fabrication of PLA microspheres, surface modification, drugs encapsulation and growth factors conjugation of PLA and the applications. While the last part of this chapter discusses on antimicrobial and *in vitro* studies.

Chapter 3 covers the details of the materials, methodology for preparation of PLA microspheres, surface modification, growth factors conjugation and drugs encapsulation techniques for characterizations prior to drug release profiles, antimicrobial activity evaluations and *in vitro* studies.

Chapter 4 covers the experimental results and discussion of the testing that was done towards the PLA as mentioned in the previous chapter.

Chapter 5 summarizes the conclusions of this research and also includes a few suggestions for future works.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Introduction

Drug delivery system (DDS) could assist and deliver the correct amount of drug to the site of action to maximize the desired therapeutic response in an optimum period of time (Hillery et al., 2001). When a drug is taken, the resulting biological responses such as lowering of blood pressure would occur which determined by the pharmacological properties of the drug (Kang and Lee, 2009). An interaction of the drug with specific receptors at the site of action usually produces these biological effects (Garland, 2017). The improvement of drug-releasing materials has been studied for the last three decades (Coelho et al., 2010). Their suitability for use in tissue engineering especially for cartilage and bone, pharmaceutical and cancer therapy have been well assessed (Nikkola et al., 2009). Drug delivery carriers have numerous advantages compared to the conventional drug administration approaches (Tiwari et al., 2012). Drug delivery has been established in order to conserve effective drug concentration in the bloodstream over a longer period of time, increase efficiency and minimize side effects of the drugs to enhance patients' compliance (Jain, 2008).

## 2.2 Drug Delivery System (DDS)

### 2.2.1 Definition

Drug delivery system (DDS) is a device or formulation that enables the introduction of a therapeutic material into the body and improves its efficiency and

safety by governing the time, place and rate of release of drugs in the body (Bruschi, 2015). This system associates drug delivery procedures with engineered technology and generates the ability to specifically target the rate at which drug gets released and where it is released which can benefits patients in various ways by eliminating multiple administrations (Katie, 2016). Davis (2013) reported that DDS based on biodegradable polymers is beneficial for use as implants that provide a slow and sustain release of a pharmacological agent.

According to the National Institute of Biomedical Imaging and Bioengineering (2018), drug has been used to improve health and the practice of the delivery system has changed extensively in the past few decades and even excessive modifications are anticipated in the near future. Biomedical engineers have contributed to improve understanding on the physiological barriers to deliver drug efficiently, for instance drug movement through cells and tissues and the development of innovative modes of drug delivery that have entered clinical practice (NIH, 2018).

Shaik et al. (2012) stated that the approaches in DDS used in monitoring the pharmacodynamics, pharmacokinetics, efficiency, immunogenicity, biorecognition and non-specific poisonousness which related to polymer science, molecular biology and bioconjugate chemistry. These approaches are aimed to decrease drug loss, prevent unwanted side effects and increase drug bioavailability. Thus, extensive and intensive researches on the development of drug delivery and drug targeting systems have been conducted (Reddy and Swarnalatha, 2010, Bhagwat and Vaidhya, 2013).

DDS is considered ideal when the system achieves inert, biocompatible, mechanically strong, give comfort for the patient, capable of achieving high drug loading, readily processable, safe from accidental release, simple to administration and removal, easy to fabricate and sterilize and free of toxic leachable impurities (Li et al., 2015). The advantages of DDS include maintain drug levels in a desired range, need for less dosing, eliminate over or under dosing, increase patient compliance and prevention of side effects (Wen et al., 2015).

### 2.2.2 Concept/Mechanism of Drug Delivery System

Conventional drug release system increases the drug concentration in the bloodstream and rapid reduction of the drug availability within short period of time (Singh and Lillard Jr, 2009). Huynh and Lee (2014) reported on the conventional system that has been improved with the controlled release system to achieve the objective of sustaining the drug level in the bloodstream for prolonged effect. In the practical of DDS, the concept of controlled release of drug from dosage form is to maintain its concentrations within the therapeutic range which can improve the permeability of drug across the skin and mucosal membranes (Takayama, 2015). The development of controlled release system using polymeric materials for the pharmaceutical application has been studied using poly(lactic-co-glycolic acid) (PLGA) by Gandhi et al. (2012), poly(vinyl alcohol)-graft-poly(ethylene glycol) (PVA-g-PEG) copolymer by Huynh and Lee (2014) and polylactides (PLA) by Sowjanya et al. (2017). There are four major mechanisms of DDS include diffusion, swelling, chemically controlled and degradable.

Figure 2.1 shows the mechanisms of diffusion-controlled drug delivery system. The diffusion-controlled release system is divided into reservoir (membrane-controlled) and monolithic (matrix-controlled) devices (Blagoeva and Nedev, 2006). In reservoir system, the drug is encapsulated as a core within a polymer coat or film. The diffusion process occurs through a membrane that controls the drug or solvent movement between two sides and the diffusion rate is determined by the membrane permeability and the device's geometry (Fu and Kao, 2010). While the transport of the drug is by solution-diffusion mechanism which related to the release characteristic modelling (Varshosaz and Hajian, 2004). In addition, when the device contains dissolved drug, the rate of release decreases exponentially with time because the drug concentration within the device decreases (Dukhin and Labib, 2017). However, if the active agent is in a saturated suspension, then the driving force for release is kept constant (zero order release) until the device is no longer saturated (Andersson et al., 2009).

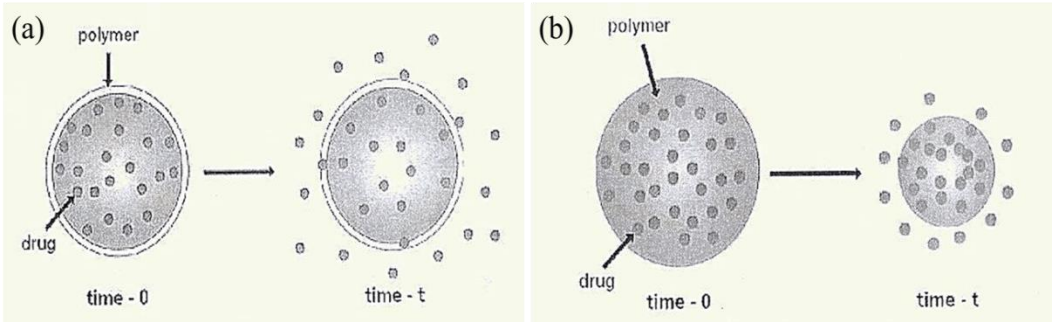


Figure 2.1: Schematic representation of (a) reservoir and (b) monolithic diffusion-controlled drug delivery system mechanisms (Blagoeva and Nedev, 2006).

While in a monolithic system, the drug is dispersed in a matrix and the release is controlled by diffusion from the system which can occur on a macroscopic scale (through pores in the polymer matrix) or on a molecular level (passing between polymer chains) (Andersson et al., 2009). According to Dizaj et al. (2015), the drug solubility and

its dissolution rate of monolithic system significantly influence the drug release kinetics. If the drug is present in the matrix below its solubility limit, it can be dissolved in a polymer matrix whereas if the drug is present above its solubility limit, it is dispersed (Medarevic et al., 2019). For a dispersed drug in a matrix, the dissolution rate is slower than the diffusion rate of the drug, thus the release rate is determined by the dissolution rate (dissolution-controlled), whereas diffusion-controlled accounted for a dissolved drug in a matrix in which the rate is vice versa (Skolakova et al., 2019).

The other mechanism of DDS is water penetration where the process of osmotic pressure from the incoming water pushes the drug out via the semi-permeable membrane (Keraliya et al., 2012). This controlled release system is divided into osmotically controlled and swelling (Patel and Patel, 2010). Study by Harrison (2007) explained that osmotically controlled system contains an osmotically active agent within a rigid housing which separated from the therapeutic agent by a movable wall. Based on the Figure 2.2, water is osmotically driven across the semipermeable wall of the housing under gradient osmotic pressure, thus will increase the pressure in the compartment of the osmotic agent (Siew, 2013, Nidhi et al., 2016). According to Keraliya et al. (2012), osmotic pump is the most promising controlled system used for oral and implantation. The osmotic pump consists of an inner core (containing drug and osmogens) coated with semipermeable membrane and as the core absorbs water the volume is expanded resulting in drug solution release through the delivery ports. While in swelling controlled system, the agent is dispersed in a hydrophilic polymer which is glassy in the dehydrated state (Ahmed, 2015). The therapeutic agent absorbs water or body fluids and swells which leads to the polymer releasing the agent into the outer environment because of

increasing in the formulation of aqueous solvent content (Calo and Khutoryanskiy, 2015).

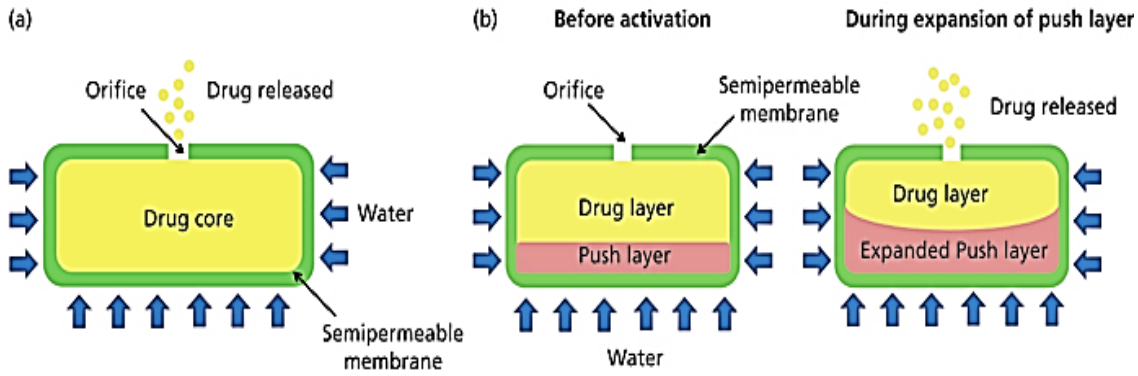


Figure 2.2: Schematic of (a) osmotically controlled system and (b) formation of push layer by osmotic pressure to release out the drug (Siew, 2013).

According to Sawadogo and Nacro (2016), chemically controlled system is where the therapeutic agent can be attached to a polymer backbone with changes its chemical construction in the biological fluid and the disintegration of the backbone by hydrolysis breaks the bond resulting in the releasing of the agent. Huynh and Lee (2014) and Nidhi et al. (2016) reported that the drug also can be dispersed in a biodegradable polymer without any transformation during the release period but later will be slowly degraded.

On the other hand, degradable drug release mechanism is versatile and popular which mostly used in injection of implantation applications (Stewart et al., 2018). Figure 2.3 shows the mechanism of degradable drug release system. The empty polymeric microspheres do not need surgical or removal procedures because of degradability into a non-toxic byproduct property and the depleted microspheres will degrade itself with surrounding environment (Pek et al., 2014, Chen and Liu, 2016). The most critical

criteria in degradable drug release systems is the degradable products are non-toxic and can be excreted out from the body easily in a safe manner (Fu and Kao, 2010).

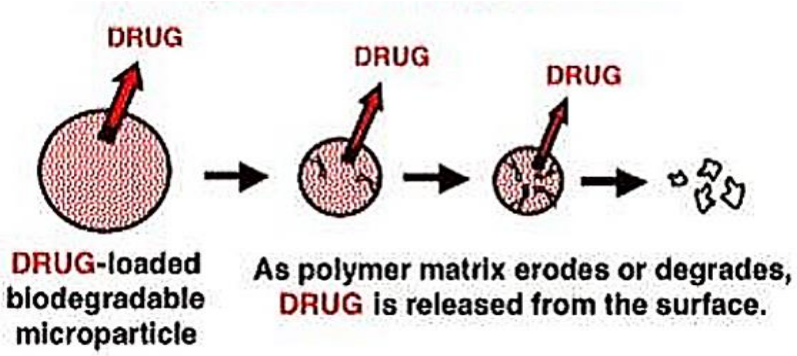


Figure 2.3: Schematic diagram of degradable drug release mechanism (Mustapha, 2014).

### 2.2.3 Type of Drug Delivery System

The objective of targeted drug delivery system is mainly to transfer drug into the targeted cells without any harmful and undesired effects on healthy tissues and organs (Sheikhpour et al., 2017). Targeted drug delivery also focuses on directing the therapeutic concentration of the drug and sustaining availability at the targeted location (Kumari et al., 2015). In order to achieve such objectives, it is crucial to recognize the classification of DDS. DDS can be classified into two types based on solving the desired positions which are active and passive targeting (Yu et al., 2016). Figure 2.4 shows the classification of types in DDS.

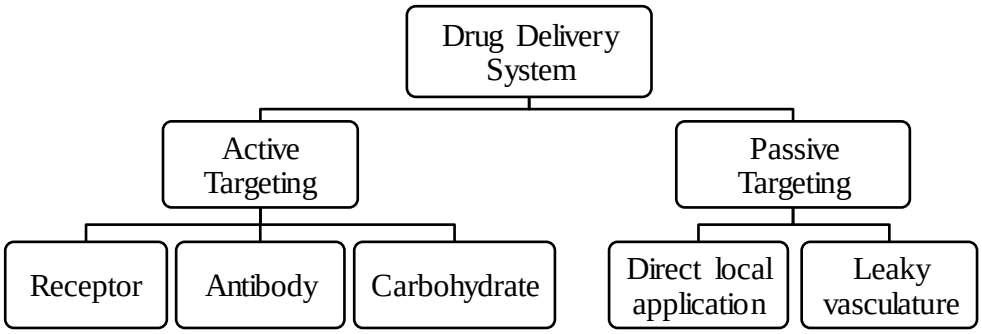


Figure 2.4: Types of drug delivery system (Bhagwat and Vadhya, 2013).

Active targeting is the interaction between ligands on the drug carrier's surface and receptors on the cell's surface which leads to accurate targeting at the desired site (Aguilar, 2013). This system is basically based on a method that delivers a certain amount of therapeutic or diagnostic agents to a targeted disease area within the special organ in the body (Anarjan, 2019). For instance, ∂vß3, ∂vß5 integrins and extra domain B (EDB) fibronectin were discovered in angiogenic vessels using this approach (Nilsson et al., 2001).

On the other hand, passive targeting involves the improvement of chemotherapeutic agents of vascular permeability of tumor cells or tissues (Chen et al., 2016a). This approach normally occurs under certain conditions such as inflammation and hypoxia which are typical for tumours, thus the endothelium of blood vessels becomes more permeable than in the healthy state (Attia et al., 2019). Study by Alexis et al. (2008) reported poly(ethylene) glycol (PEG)ylated nanoparticles have decreased the adsorption of plasma protein on their surface and hepatic filtration via passive targeting. In brief, active targeting changes the natural distribution of a carrier guiding it to a specific cell or organ while passive targeting depends on the natural distribution of the drug and enhanced permeation and retention (EPR) effect (Yu et al., 2016).

### 2.2.4 Route of Administration in Drug Delivery System

The success of the drug administration is determined by the efficacy and safety to deliver the drug at the targeted area with minimal host's defense system interaction (Din et al., 2017). The factors to be considered to achieve therapeutic effect include type of disease, desired effect and can be administered through enteral, parenteral, topical or

inhalation routes (Turner et al., 2011). Figure 2.5 shows the different type of drug administration routes.

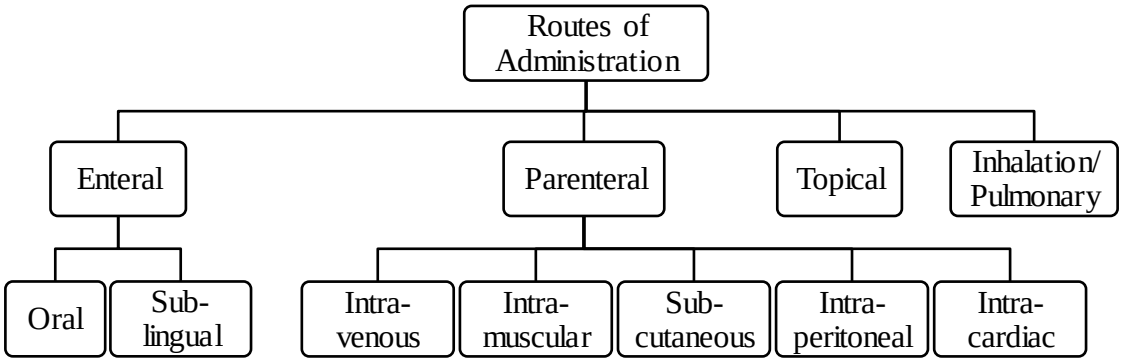


Figure 2.5: Major type of routes of drug administration (Pharma, 2015).

Enteral route is the most common and oldest route to deliver drug because it is simple and comfortable for patients (Ruiz and Montoto, 2018). The drug is absorbed into the systemic circulation through the oral, gastric mucosa, small intestine or rectum and can be divided into oral and sub-lingual routes (Hirota and Shimizu, 2012). According to Gautami (2016), oral route is mostly used for neutral drugs in a form of capsule, tablet, powder, emulsion or syrup. The advantages of orally administered drug include economical, no sterilization needed, suitable to produce local action, larger amount of drug can be given, easily self-administered and toxicity or overdose can be overcome with antidotes (Muheem et al., 2016, Saxen, 2016). Whereas sub-lingual route is the placement of small and lipid soluble drug under the tongue to allow diffusion into the capillary and enters the systemic circulation (Colombo et al., 2007). This type of route basically convenient to patients, rapid success of maximum therapeutic concentration, low infection and the drug not entering hepatic circulation (Maddison et al., 2008).

Based on Figure 2.6, the other route for drug administration is parenteral where poorly absorbed and unstable drug in gastrointestinal tract (GIT) is introduced directly

across body barrier's defense into systemic circulation (Washington et al., 2001, Ahmed and Aljaeid, 2016). Parenteral route is having the highest drug bioavailability, providing the most control over the actual dose of drug delivered to the body and used for treatment of patients that require rapid onset of action (Jin et al., 2015). This route can be divided into five categories; intravenous (IV), intramuscular (IM), subcutaneous (SC), intraperitoneal (IP) and intracardiac (IC). IV route is the most used in parenteral type where the drug is administered into the vein which requires rapid effect and maximum control over drug level circulation, while IM is used for rapid onset and slowly absorbed drug to provide a sustain dose over an extended period of time (Hirota and Shimizu, 2012, Saxen, 2016). On the other hand, SC route requires absorption via simple diffusion which is slower effect compared to IV whereas IP route is used when large amount of blood replacement fluids are needed (Parasuraman et al., 2017). The other parenteral type of drug administration is IC where the drug is injected directly into the heart because very rapid absorption and onset of action are required (Turner et al., 2011).

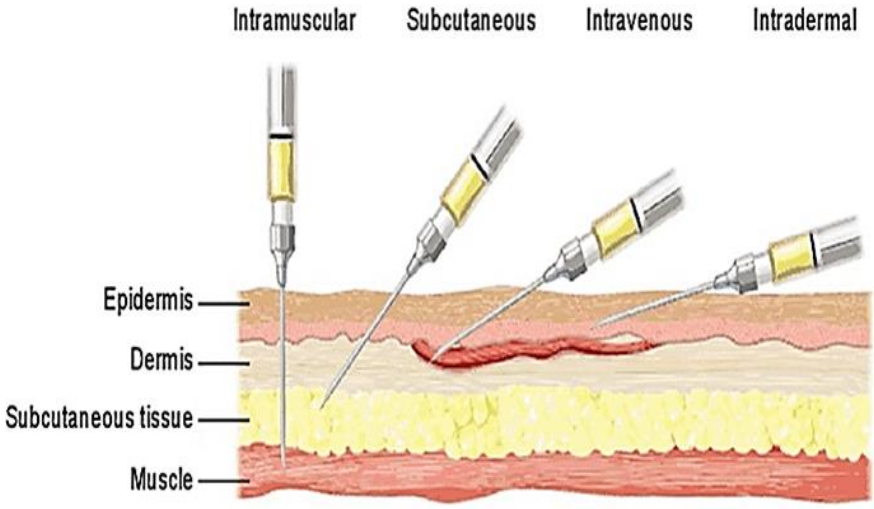


Figure 2.6: Parenteral route of drug administration (Ahmed and Aljaeid, 2016).

For topical route, the application is used directly when a local effect of the drug is desired which categorized into dermal (local) and transdermal (systemic) (Alkilani et al., 2015). Transdermal delivery consists of membrane permeation-controlled, adhesive dispersion-type, matrix diffusion-controlled and micro-reservoir dissolution-controlled systems (Bathe and Kapoor, 2015). The advantages of topical route include lower risk of side effects, steady level of drug in the system and local therapeutic effects (Hirota and Shimizu, 2012). While inhalation or pulmonary route involves volatile drug administration into the nose which directly passes to the lungs and eliminates systemic effects (Ibrahim et al., 2015). The merits of this route are easy, fewer doses required, minimum side effects as well as rapid onset of action and absorption (Hirota and Shimizu, 2012).

### 2.2.5 Drug Delivery System Carrier/Device

The design of novel DDS which ables to transport an effective amount of cargo specifically to the target cell or tissue is one of the main challenges for the biomedical scientific community (Corilla and Vallet-Regi, 2013). Most clinically used drugs in oral or systemic administration are low molecular weight that exhibit short half-life in the bloodstream and a high overall clearance rate (Flynn, 2007). Thus, high initial drug doses are essential to sustain therapeutic concentrations over a prolonged time (Musteata, 2012). According to Devi (2010), some drugs are having an optimum concentration range within maximum benefit is derived, so concentration above or below this ideal range is considered toxic or no therapeutic effect, respectively. On the other hand, slow progress of severe disease treatment has suggested a growing need for multidisciplinary approaches to the therapeutic agents' delivery to the target tissues