CHAPTER II
LITERATURE REVIEW

Over the last decade, the magnets have been improved in terms of their materials: magnetic, physical, chemical, biological properties and corrosion resistance until they are suitable for clinical application.

This chapter was divided into five parts as follows:

I. The magnetic materials

II. Clinical applications of magnets in orthodontics

III. Corrosion

IV. The biological effects of corrosion products released from the magnets

V. The biocompatibility testing and fibroblast cell culture

I. The magnetic materials

The magnets are categorized as soft and hard magnets according to the coercivity and obvious magnetism. The soft magnet has low coercivity and obvious magnetism only while it is in a magnetic field. While the hard magnet still has high coercivity and high magnetization after removed from the applied magnetic field.

A number of different magnetic materials are now available and have been improved in their properties, such as coercivity and maximum energy product, since the discovery of samarium-cobalt and neodymium-iron-boron permanent magnets.

The magnets provide the magnetic field which is called the flux density of the magnets that induces changes in the surrounding medium. This flux can attract or repel other magnets and attract other iron-containing materials. The force produced by any two magnets is inversely proportional to the square of the distance between them.

There are many factors that influence the magnetic forces generated by the magnets, such as different types of magnets, size and shape, distance between the

magnets, and temperature. Moreover, the properties of permanent magnets can be altered by mechanical treatments, corrosion, and radiation (David, 1998).

Types and composition of permanent magnet

    1) Magnetite or lodestone

The Fe_3O_4 material which is a naturally-occuring oxide of iron. It was the first permanent magnet material discovered but today it is not even considered to be a hard magnetic material.

    2) Permanent magnet steels

Carbon, tungsten, or chromium is added to iron to increase coercivity, hysteresis, loss, and maximum energy product.

    3) Alnico alloys

These alloys consist mainly of iron, cobalt, nickel and aluminum with small amounts of other metals such as copper. These constituents formed a finely intermixed two phase alloys consisting of a strongly magnetic phase (Fe-Co) and a very weekly magnetic phase (Ni-Al). Their magnetic properties were superior to other materials available at that time but they were very hard and brittle.

    4) Hard ferrites

The hard ferrites, also known as ceramic magnets, are good permanent magnets. The hard ferrites are usually either barium or strontium ferrite ($BaO \cdot 6Fe_2O_3$ or $SrO \cdot 6Fe_2O_3$). They are relatively cheap to produce and commercially remain the most important permanent magnet materials.

    5) Platinum-cobalt

These alloys consist of Pt and Co. Although its magnetic properties are improved, its cost makes it impractical. So, this magnet material is no longer in use.

    6) Samarium-cobalt

These materials were developed in the late 1960S, based on alloys of the rare earths with the 3d transition series ferro-magnets, iron, cobalt and nickel. They had high anisotropies, high Curie temperatures, high coercivity, and high saturation magnetization.

7) Neodymium-iron-boron

The main ingredient of this alloy contains $Nd_2Fe_{14}B$, a very hard magnetic phase. The addition of a small amount of boron is found to improve its properties. In general $Nd_2Fe_{14}B$ has rather poor temperature stability of it magnetic properties and poor corrosion resistance. The addition of small amount of copper and cobalt to the main $Nd_2Fe_{14}B$ composition is found to improve both coercivity and corrosion resistance without significantly reducing the remanence. It has greater coercivity and energy product than samarium-cobalt.

8) Nanostructured neodymium-iron-boron

This permanent magnet material is formed by using a two-phase material with ultrafine (i.e. nanostructured) grain structure consisting of 90% soft phase (such as α -Fe) and only 10% hard phase (such as Nd2Fe14B). It is a high-performance and low-cost permanent magnet.

9) Samarium-iron-nitride

The rare earth transition metal compounds of general composition R_2T_{17}, where R is a rare earth and T is a 3d transition metal. There are chemically more stable than other rare earth transition metal compounds. This property is useful for producing materials with good corrosion resistance, which has been one of the main drawbacks of the NdFeB materials.

II. Clinical applications of magnets in orthodontics

The permanent magnets are being increasingly used in orthodontic and orthopedic treatment for a variety of purposes.

Magnetic brackets

Originally, Kawata et al.(1977) designed the magnetic bracket made from iron-cobalt and chrome alloy, but it was unsuccessful and subsequently replaced by rare earth magnets which produced sufficient force for tooth movement (Kawata and Matsuga,1979; Kawata et al., 1987).

Tooth movement

The magnets exerting either repelling or attracting force can be used in the same way similar to coil springs and elastic elements to control the tooth position. They could deliver light continuous forces with or without archwires (Muller, 1984; Blechman, 1985).

The distalization of maxillary molars by using repelling magnets could move molar rapidly within a relatively short period. These magnets were easy to be inserted, well tolerated, and did not require patient cooperation except for maintaining good oral hygiene (Blechman and Smiley, 1978; Gianelly et al., 1989; Bondemark and Kurol, 1992).

Deimpaction

The magnets are clearly the "thinking man's" appliances for deimpaction of teeth. An ideal treatment approach should attempt to mimic the normal eruption pattern.

The magnetic attraction system was introduced in different approaches for the use of fixed and removable magnets in treatment of various impacted teeth (Sandler et al., 1989; Sandler and Springate, 1991; Darendeliler and Friedli, 1994). This treatment procedure was less trauma and poses less risk of infection than conventional orthodontic treatment.

Tooth intrusion

The removable and fixed appliances with acrylic bite blocks incorporating magnets have been used as the Active Vertical Corrector (AVC) (Dellinger, 1986; Woods and Nanda, 1988), and the Magnetic Activator Device IV (MAD IV) (Darendeliler et al., 1995) for correcting the anterior openbite. These provided a simple reciprocal force system to rapidly intrude posterior teeth.

Tooth extrusion

A subgingival crown-root fracture presents the dentist with a difficult restorative problem. Up to now, the treatment options have usually been limited to extrusion of the remaining root. The alternative method of orthodontic extrusion (force eruption) was the application with attractive magnets (Bondemark et al., 1997). Good force control at short distances, no friction, and no material fatique of permanent magnets resulted in successful rapid extrusion without any soft tissue dehiscence, aberrant root mobility, or root resorption.

Expansion

Intramaxillary expansion and orthopaedic movement of the palatal shelves have been used in orthodontics for many years. The repulsive magnetic forces were used for palatal expansion in monkeys (Vardimon *et al.*, 1987). Furthermore, the maxillary expansion by using a Magnetic Expansion Device (MED) was introduced to correct the crossbite (Darendeliler *et al.*, 1993). These expansions were quite feasible, slower, and significantly gentler to the involved tissues than conventional jackscrew expansion. These could avoid such side effects as root resorption and alveolar dehiscence.

Functional appliances

The Functional Orthopaedic Magnetic Appliances, i.e. FOMA II and III were introduced for correction of Class II and III malocclusions, respectively (Vardimon *et al.*,1989; 1990). Moreover, the Magnetic Activator Device (MAD) was developed as a two-piece functional orthopedic appliance. Several types have been designed to treat the different clinical problems, i.e. mandibular lateral deviation (MAD I), Class II malocclusion (MADII), Class III (MAD III), and openbite (MAD IV) (Darendeliler and Joho, 1992; 1993; Darendeliler *et al.*, 1993).

The functional magnetic system (FMS) can decrease the incidence of treatment failure associated with conventional mechanotherapy. Because of less bulkiness, the functional magnetic devices contribute to improve patient cooperation, nearly full-time wear, and possibility of functioning during the wear.

Fixed retention

The neodymium-iron boron micro-magnetic used as a fixed retainer can retain central incisors that have been brought together to close a median diastema (Springate and Sandler, 1991).

The permanent magnetic devices offer a novel approach in generating intramaxillary and intermaxillary force. They can effectively provide three directional control forces; however, the use of magnets for orthodontic treatment is currently limited because it is hard to manufacture. In addition, the high price of magnets is still a factor of consideration.

III. Corrosion

Definition of terms:

Tarnish - A process by which a metal surface is dulled in brightness or discolored through the formation of a chemical film, such as sulfide and oxide.

Corrosion - A chemical or electrochemical process through which a metal is attacked by natural agents, such as air and water, resulting in partial or complete dissolution, deterioration, or weakening of any solid substance. The corrosion rate may actually increase with time.

Corrosion products - The chemical compounds that are produced by the metal undergo chemical or electrochemical reaction with nonmetallic elements in the environment. They may accelerate, retard, or have no influence on the subsequent deterioration of the metal surface.

The specific ions may play a major role in the corrosion of certain alloys. For example, oxygen and chloride are implicated in the corrosion, whereas sulfur is probably most significant in surface tarnish developed on alloys that contain silver, although chloride has also been identified as a contributor.

The most common example of corrosion is rusting of iron. The iron combines with oxygen in air and water to form hydrated oxide of iron. This oxide compound shows porous and this metal is bulkier, weaker, and more brittle than the non-oxide form of iron.

Classification of corrosion reaction (Marek, 1996)

    1) Chemical corrosion

It is a direct combination of metallic and nonmetallic elements. This type is exemplified by oxidation, halogenation or sulfurization reactions. Such corrosion is also referred to as *dry corrosion* because it occurs in the absence of water or other fluid electrolytes.

    2) Electrochemical or electrolytic corrosion

This type is referred to as *wet corrosion* because it requires the presence of water or other fluid electrolytes. It also requires a pathway for the transport of electrons,

an electrical current, if the process is to continue. The chemical corrosion is seldom isolated and almost invariably is accompanied by a second type of corrosion.

Electrochemical corrosion

The electrochemical cell is composed of four components: an anode, a cathode, an electrolyte, and an ammeter. The anode is the surface where positive ions are formed, i.e., the metal surface is corroding while free electrons are produced; so that sometimes it is referred to as oxidation reaction.

The reaction may be described as

$$M^o \rightarrow M^+ + e^- \qquad (1)$$

At the cathode or cathodic site, a reaction must occur that consumes the free electrons produced at the anode. Numerous possibilities exist and are dependent on the environment. For example, metal ions may be removed from the solution to form metal atoms, hydrogen ions may be converted to hydrogen gas or hydroxyl ions may be formed:

$$M^+ + e^- \rightarrow M^o \qquad (2)$$
$$2H^- + 2e^- \rightarrow H_2 \uparrow \qquad (3)$$
$$2H_2O + O_2 + 4e^- \rightarrow 4(OH)^- \qquad (4)$$

These processes are referred to as reduction reactions. The electrolyte serves to supply the ions needed at the cathode and to carry away the corrosion products at the anode.

In order for electrochemical corrosion to be an ongoing process, the production of electrons by the oxidation reactions at the anode must be exactly balanced by the consumption of electrons in the reduction reactions at the cathode. This is an important consideration in determining the rate of a corrosion process and can be used to reduce or eliminate corrosion.

Types and mechanism of corrosion

1) Uniform corrosion

The metal is attacked evenly and throughout, and its mechanical properties diminish proportionately with weight loss. This chemical or electrochemical corrosion is usually found in the exposed surface or wide area. It is rarely seen in orthodontic attachments, since they are not evenly exposed to corrosion agents.

2) Localized or pitting corrosion

This type of corrosion is the most common form of corrosion in orthodontic attachments. It affects the mechanical properties or aspects much more than could be inferred from the weight loss. It happens when the material is improperly treated or contains impurities. Halide ions, especially chloride, cause pitting corrosion.

3) Crevice corrosion (Deposit corrosion or Gasket corrosion)

Crevice corrosion occurs in narrow spaces or shielded areas on a metallic surface, caused by the localized electrochemical process and chemistry changes, such as acidification and depletion in the oxygen content. This commonly takes place at the leakage of a restoration, under a pellicle, or under other surface deposits. It has been implicated as the mechanism involved in the corrosion of orthodontic brackets.

4) Intergranular corrosion

The aspect and metal weight remain approximately constant, but the loss of mechanical properties can result in poor performance or even collapse. This insidious type of attack can go so far as to reduce the metal to grains. For example, heating can make material more susceptible to intergranular corrosion.

5) Galvanic corrosion (Dissimilar metal corrosion)

An accelerated attack is occuring on a less noble metal when electrochemically dissimilar metals are in physical or electrical contact in the presence of a liquid corrosive environment. Such a situation, the potential difference is suddenly short-circuited through the two alloys. The result may be a sharp pain.

6) Stress corrosion

When the mechanical stress is associated with a corrosive environment, the degradation is most apt to occur because of fatique of the metal. The mechanical

distortion and excessive cold working promote corrosion by making the distorted portion of the metal more anodic. The alloy then behaves electrochemically as if two alloys were present.

7) Microbiologically induced corrosion

Undetected until recently in dentistry, but widespread in industry, microbial attack is directed mainly against the metal and occurs especially in non-aerated, sensitized areas such as the junction between mesh and foil.

Any one of these types of electrochemical corrosion is seldom found alone. Generally, two or more mechanisms act simultaneously and so compound the problem.

The general mechanism of corrosion in oral environment

The general mechanism of corrosion and subsequent ions released from metal involve the loss of the passivated layer that covers and protects the metal surface.

This disintegration of a metal may occur through the action of moisture, atmosphere, acid or alkaline solutions, and certain chemicals. Tarnish is often an early indication, the forerunner of corrosion. It may in time form or accumulate elements or compounds that chemically attack the metallic surface. In addition, water, oxygen, and chloride ions are present in saliva and contribute to corrosion attack. Various acids such as phosphoric, acetic, and lactic are presented at times. At the proper concentration and pH, these acids can lead to corrosion (Marek, 1996).

In the oral cavity, numerous variables facilitating the corrosion of the metal are temperature, quantity and quality of saliva, plaque, pH, protein, physical and chemical properties of food and liquids, and general and oral health conditions (Park and Shearer, 1983). Chemical composition of alloys (Geis-Gerstorfer and Weber, 1987), and masticatory habits (Obatake et al., 1991) also influence corrosion.

Corrosive protection

Certain metal develops a thin and highly protective film by oxidation reaction with the environment. This protective film makes this metal passive. Chromium is the best example of passivity that is not readily corroded because it has already been corroded

so rapidly and uniformly. This metal forms a film of a corrosion product, which probably consists of closely packed chromic oxide.

However, tensile stresses and certain ions, such as chloride, can disrupt this protective film of passivating metals and result in rapid stress and pitting corrosion.

A coating with a noble metal may be applied to the surface of a second metal, for instance, a base metal, to prevent corrosion. The coating material must be less active than the base metal; that is, the coating material must be cathodic to the base metal. If its surface becomes scratched or pitted to such a depth that the base metal is exposed to the environment, the base metal will be corroded at a rapid rate. In general, a large anode surface coupled to a small cathode surface result in low corrosion rates. And in the reverse, the anode surface is small and the cathode surface is large. Thus, rapid corrosion is expected where the coating has been scratched.

The paints or other types of organic or inorganic coating are used for protection. The corrosion rate of the more active metal is reduced because the surface area available for the reduction reaction has been decreased.

Aluminum and titanium are other passivating metals that have found application in dentistry.

IV. The biological effects of corrosion products released from the magnets

A number of studies have investigated the corrosion resistance of the magnets. They have demonstrated that there is a release of corrosion products. The metal ions are released and come into contact with cells and tissues in the environment. So, the specific biological potential of the respective dissolved metal ions should be taken into consideration in terms of corrosion products.

The $SmCo_5$ magnet was an inert alloy and corrosion resistant to artificial saliva while the acid resistance of $SmCo_5$ magnet was relatively low (Tsutsui et al., 1979). The neodymium-iron-boron magnets are more likely to be corroded, because of the high iron content, i.e., 72.3% by weight (Blechman and Steger, 1993). The corrosion of rare earth magnets leads to substance loss and to disturbed physical properties (Vardimon and Mueller, 1985). Furthermore, the intraoral magnets are likely to be tarnished and their

attachment site appears somewhat corroded, thus significantly affecting their useful life span (Drago, 1991). The oral bacteria form a biofilm on intra-oral appliances was the possible contribution to corrosion. *Streptococcus sangius*, one of the predominant organisms in the oral cavity, causes the corrosion of $Nd_2Fe_{14}B$ magnets greater than in the absence of organism (Wilson *et al.*, 1995). In addition, the presence of sucrose in a mucin-containing artificial saliva affects the microbial composition of multi-species biofilms growing on $Nd_2Fe_{14}B$ magnets and results in a marked increase in corrosion of magnets (Wilson *et al.*, 1997).

Concerning the corrosion process, it may display functional and esthetic effect, but the greatest significance is biological effect. The magnet must not produce corrosion products which are harmful to the body.

The several studies have evaluated the possible biological effects of the corrosion products released from magnets. The leachable $Nd_2Fe_{14}B$ magnet corrosion products after 5 days had no cytotoxic effect on osteoblastic-like cells, the rat osteosarcoma cell line UMR-106 (Sandler *et al.*, 1989). Moreover, the effect of the corrosion products of uncoated $Nd_2Fe_{14}B$ magnets on the proliferation of human oral mucosal fibroblasts was assessed by the methylene blue uptake /elution technique. The compounds in the corrosion products were examined using quantitative X-ray analysis appeared to consist of mainly iron compounds (94.8%), although a significant amount (3.2%) of neodymium chloride ($NdCl_3$) was found. Fibroblast proliferation in the presence of corrosion products (1, 50, 100%) for 48, 96, and 144 hours was significant lower than with culture medium, but there was no obvious dose response. However, the fibroblasts could proliferate and their attachment was not disrupted (Evans and McDonald, 1995).

The samarium-cobalt and neodymium-iron-boron magnets are extremely susceptible to corrosion, especially in chloride-containing environments. Therefore, the magnetic materials must be securely separated from the oral fluid before being used in clinical applications. Various methods have been used to eliminate the corrosion with varying degrees of success (Riley *et al.*, 1999).

There was a release of a small amount of water soluble, cytotoxic components from both new and clinically used uncoated magnets. The recycling magnet per se might be removed the cytotoxic components and it maintained good biocompatibility. It is recommended that the magnets should be stored in water for 24 hours before clinical use, thereby conceivably decreasing the oral exposure of their cytotoxic agents (Bondemark *et al.*, 1994a).

The magnets should preferably be coated with parylene, acrylic, stainless steel, biocompatibility epoxy resin, paladium-cobalt, or acid proof material (e.g. nickel, chromium, or titanium) in order to reduce their corrosiveness. There were studies that characterized the electrochemical properties, corrosion tendencies and reactivity to the oral environment of $SmCo_5$, Sm_2Co_{17} and $Nd_2Fe_{14}B$. They found that the uncoated magnets were excessively corroded. On the contrary, the coated samples showed only a small amount of corrosion (Bondemark *et al.*, 1994b; Obatake *et al.*, 1991; Vardimon *et al.*, 1991; Vardimon and Mueller, 1985).

The magnets may be used in the oral cavity for a long period of time and are likely corroded. The intraoral magnets are known to release corrosion products, which lead to substance loss and disturbed physical properties. However, only few studies have so far focused on the possible biological effects of these corrosion products on oral tissues and cells. These aspects are unclear and contradictory. Therefore, further studies on the biological effects of corrosion products should be warranted.

V. The biocompatibility testing and fibroblast cell culture

The term of biocompatibility is defined as being harmonious with life and not having toxic or injurious effects on biological function. It is measured on the basis of localized cytotoxicity, systemic response, allergenicity and carcinogenicity (Dorland's illustrated Medical Dictionary, 1994).

It is reasonably possible, for clinical use of any new dental materials, that it should not produce any side effects at a local or systemic level. According to Autian (1974), the biological safety testing of the materials must include three levels of testing:

Level 1: *In vitro* testing in order to establish the toxic, allergic, and carcinogenic

nature of the material

Level 2: In use testing on animal (*in vivo*)

Level 3: In clinical trial

In vitro testings

The pre-clinical tests (Level 1) may be regarded as a screening phase for evaluating materials to be used in the clinical setting.

The *in vitro* methods save lives of animals while simulate specific *in vivo* condition. These methods are more economical than performing experiments with animals. Furthermore, *in vitro* methods allow a more easily controlled test situation and higher statistical accuracy than *in vivo*.

For *in vivo* experiment, it is impossible to investigate continuously cellular and tissue reactions to the material. The results of *in vivo* show wide deviation due to the existence of a number of unknown factors.

The cell culture is one of *in vitro* methods that have frequently been used to study the biocompatibility of dental materials. It can provide a continuous supply of homogenous cellular material for biochemical experiments. The cell culture can be stored in a deep-frozen state and revived without changing their growth rate and genetic composition. The designation of proper cell culture system is an integral component of any *in vitro* experiments. The cultures should be obtained from sources that have performed adequate quality-control tests. There are various types of cells that have been selected for culture procedure.

Fibroblasts have been extensively studied for this method. They can be better observed when isolated from the interlacing fabric of fibers in which they reside *in vivo*. In this environment, the cells migrate out from the explant into the surrounding medium, with their processes adhering to form a cellular network (Fawcett, 1986). Fibroblast can be easily split every week for early passage cell lines including large quantities of well-characterized cell culture.

Fibroblast

1) Development of the fibroblast

Fibroblasts originate from mesenchymal cells. Once differentiated, fibroblasts can replicate by mitotic cell division. When fibroblasts from embryonic tissue are cultured, they are able to undergo about 50 divisions before senescence and death. However, this number is reduced to approximately 20 divisions when fibroblasts from adult tissue are cultured (Ten Cate, 1998).

The fibroblast is capable of serial replication *in vitro* and *in vivo*; when the cell enters the mitotic phase, it loosens its attachment to a surface and becomes spherical. After telophase, the daughter cells flatten onto available surfaces and once again resume an extended form (Goldberg and Rabinovitch, 1988).

2) Structure of the fibroblast

The fibroblast is a spindle-shaped cell with tapering eosinophilic cytoplasmic extensions (Figure 2.1). The resting fibroblast (e.g., in tendons) has a flattened, dark-staining, closed nucleus and little cytoplasm. For the active fibroblast (e.g., in the periodontal ligament) has a pale-staining, open-faced nucleus and much more cytoplasm (Goldberg and Rabinovitch, 1988; Ten Cate, 1998).
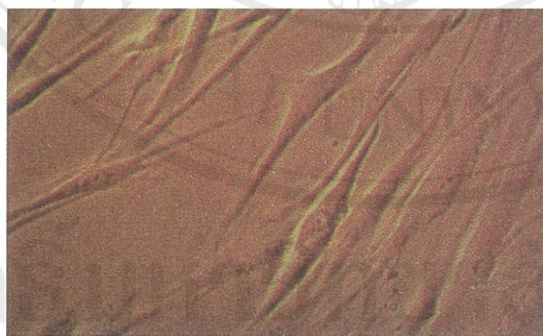

Figure 2.1 Fibroblast cells

The fibroblast contains cytoplasmic organelles and inclusions. There are a number of Golgi complexes and many profiles of rough endoplasmic reticulum, mitochondria, and secretory vesicles. All of these organelles indicate the ability of fibroblasts to multiply and secrete molecules (Ten Cate, 1998).

3) Role of the fibroblast

Fibroblasts are the predominant cell type of connective tissue that produces the precursors of the extracellular matrix components. They have many equally important functions, such as producing and maintaining the ground substance in which they and their fibrous products are enmeshed. Therefore, fibroblasts may be described as the architect, builder, and caretaker of connective tissue. They also play an important role in the development, structure, and function of the tooth (Ten Cate, 1998).

Measurement of viability and growth of cells in culture

A variety of techniques have been proposed for determining the viability and growth of cells in tissue culture (Jakoby and Pastan, 1979).

Measurement of cell viability

Various manipulations of cells, including passaging, freezing, and dissociation from primary tissue, can result in cell death. There are numerous viability assay methods. The most commonly used technique is the staining with dye and salt. Cellular uptake of dye and salt depends on cellular membrane integrity and cellular metabolism. The human diploid cells are difficult to be counted directly; hence, the staining procedure and dye exclusion assay are incorporated to enhance visibility of cells. Dye exclusion tests are routine methods.

To determine the number of surviving cells in a population, Trypan blue dye exclusion assay is usually used for this purpose. Normal healthy cells are able to exclude the dye, but trypan blue will diffuse into cells in which the membrane integrity has been lost (Darlington, 1988).

Measurement of cell growth

1) Visual method

The most commonly used measurement of growth is direct enumeration of cells using a haemocytometer. It is a good technique if only a few samples are counted, while numerous cultures should be counted by electronic enumeration.

2) Electronic systems

They use flow-through cells or apertures for measurement of incorporated dyes or cell numbers.

3) Chemical method

The biochemical synthesis of the components is measured as an expression of cell growth, for example, protein determination, and DNA determination.
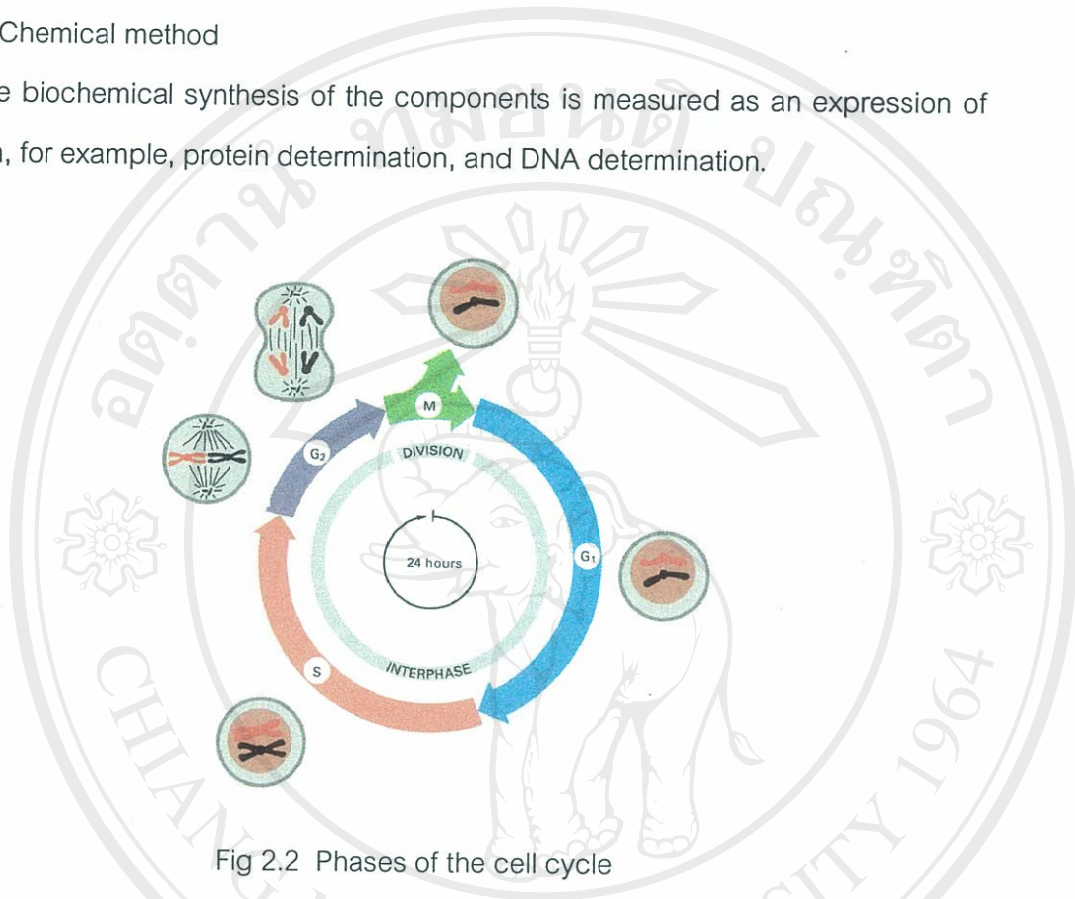

Fig 2.2 Phases of the cell cycle

The division of cells must be regulated and coordinated with both cell growth and DNA replication. In most growing cells, the cell cycle consists of four major phases: mitosis (M) corresponding to the separation of daughter chromosomes and followed by cytokinesis; gap1 (G1) interval preceding DNA synthesis, cell is metabolically active and continuously grows but does not replicate its DNA; synthesis of DNA (S), and gap2 (G2) following DNA synthesis, cell growth continues and proteins are synthesized in preparation for mitosis (Figure 2.2). Eukaryotic cells in G1 are diploid with DNA content of 2n. During S phase, replication increases the DNA content of the cell ranging from 2n to 4n. DNA content then remains at 4n for cells in G2 and M, and decreasing to 2n after cytokinesis. Non-dividing cells exit the cell cycle, entering the quiescent G0 state (Cooper, 2000).
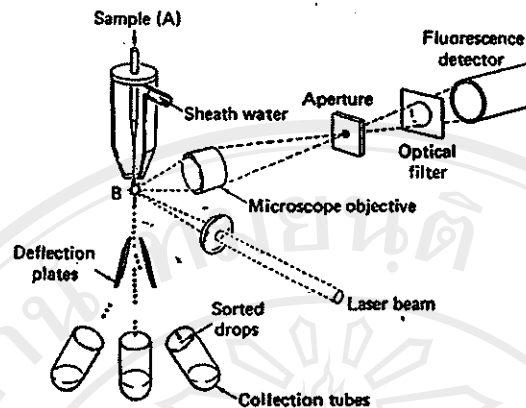
Figure 2.3 A flow system of flow cytometer

For DNA analysis, cells are stained with fluorescent dyes that can bind to DNA. The DNA content of each cell and its location in the cell cycle can be measured by flow cytometer. The flow system was illustrated in Figure 2.3. The fluorescently stained cells in aqueous suspension are transported into the sorter chamber (A) and illuminated by a laser beam (B), one by one, through an intense light that excites the dye. The fluorescence is collected by a microscope objective and projected through an optical filter onto photomultiplier. The resulting fluorescence that is measured and recorded is proportional to the amount of the cellular component which the dye is bound. In addition, drops containing cells can be sorted on the basis of their fluorescence by charge deflection plates (Jakoby and Pastan, 1979; Rodger, 1988).

The flow cytometric analysis permits the enumeration and characterization of cells that are new actively synthesizing DNA by measuring the fluorescence intensity of individual cells. The method may be performed with the incorporation of $[^3H]$thymidine or bromodeoxyuridine (BrdU) or other labeling reagents that are analogs of the DNA precursor. Therefore, this application can lead to distinguish cells in the G_0/G_1, S, and G_2/M phases of the cell cycle in a histogram or a dot plot diagram (Figure 2.4).
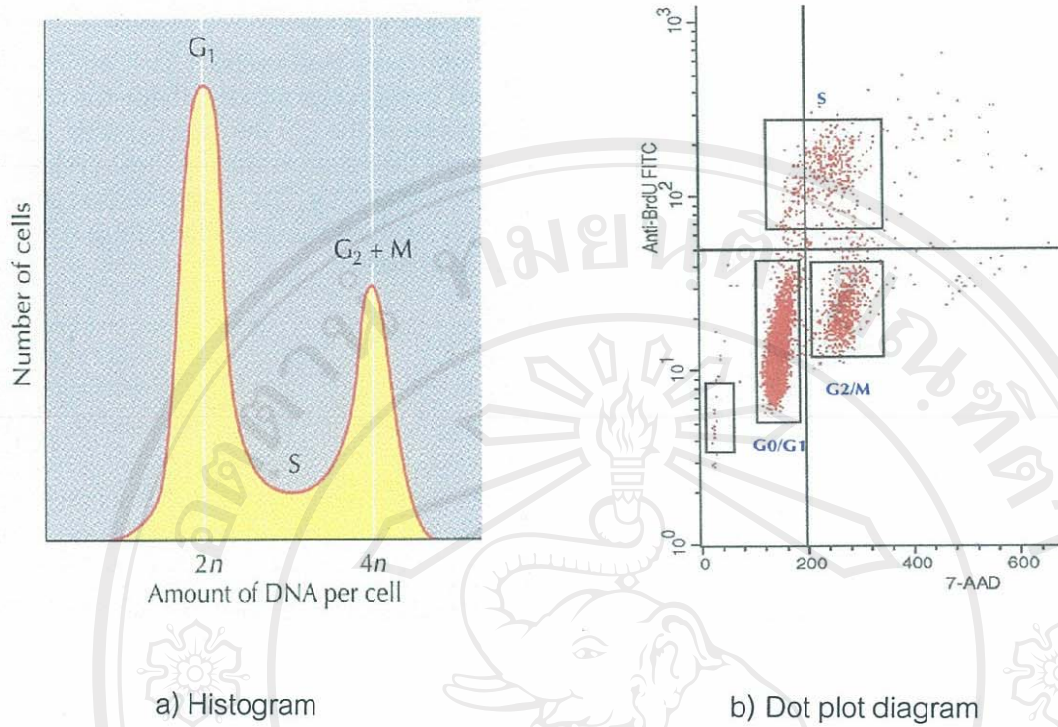
a) Histogram

b) Dot plot diagram

Fig 2.4 Cell cycle analysis by flow cytometry