CHAPTER II

LITERATURE REVIEW

There are five parts of literature review as follows:

I. The permanent magnetic materials and magnetic fields

II. The applications of magnets in orthodontics

III. Biological implications of magnetic fields

IV. Fibroblasts in cell culture

V. Investigation methods in the *in vitro* study

I. The permanent magnetic materials and magnetic fields

Magnetism has been known since early human history. It has been realized from the observation that certain naturally occurring stones would attract one another and would also attract small bits of iron, but not other metals. The word "magnetism" came from the name of the district (Magnesia) in Asia Minor, one of the locations where the stones were found (Halliday *et al.*, 1992).

Magnetic materials are traditionally divided into two categories: soft and hard magnetic materials. While the former is easily magnetized and demagnetized, the latter is not. Therefore, these two types are suitable for different applications. For example, the softer the material is, the better it is suited to certain electrical devices, such as a transformer, in which the magnetization must be reversed several times per second (David, 1998; Becker, 1970).

The alternative term of the hard magnet is a permanent magnet, which can be permanently magnetized by the application of magnetic field. The permanent magnet is a passive device used to generate a magnetic field. It can generate stable magnetic field without continuous expenditure of electrical energy flowing in a coil or solenoid to maintain the field. The energy needed to maintain the magnetic field has been stored

previously when the permanent magnet is charged (i.e. magnetized initially to high field strength and then to remanence when the applied field is removed). In addition, the permanent magnet is only used if it has a relatively high magnetization when removed from the applied magnetic field. Therefore, a high remanence of the permanent magnet is a desired property, and this inevitably means a high saturation magnetization.

There are various permanent magnetic materials, such as magnetite or lodestone, permanent magnet steels, alnico alloys, ferrites, platinum-cobalt, samarium-cobalt, and neodymium-iron-boron. There are differences in magnetic properties and applications among these types. The permanent magnets are mainly used in electric motors, generators, loudspeakers, television tubes, moving-coil meters, and magnetic suspension devices and clamps (David, 1998).

The breakthrough in the use of permanent magnets in dentistry came with the introduction of new magnetic alloys, based on cobalt and some rare-earth elements, so-called rare earth magnets. The first well-known rare earth magnet was a samarium-cobalt magnet, which generates magnetic energy about 2 to 6 times greater than the strongest conventional permanent magnet ($AlNiCo_5$). According to the resistance to demagnetization, the rare earth magnet was 20 to 50 times superior to $AlNiCo_5$. In addition, it was reported that there were about as twice the intrinsic coercive force and maximum energy, generated by the samarium-cobalt magnet, as the force and energy derived from the conventional magnets (Cerny, 1978; Becker, 1970).

Neodymium-iron-boron ($Nd_2Fe_{14}B$) is another kind of the rare earth magnet, which was discovered over 20 years ago (Chin 1980). It was shown that the force generated by the neodymium-iron-boron magnet is three times stronger than that generated by the samarium-cobalt magnet (Robinson, 1984). The neodymium-iron-boron permanent magnets have been used in magnetic resonance imaging systems, or MRI, and in various dental applications (David, 1998). However, they are easily corroded. The released corrosion products could shorten the magnet's life. Therefore, various coating materials have been developed to prevent leach out of the corrosion

products and the galvanic action occurring when the magnets were contacted with a different metal (Blechman, 1985).

The permanent magnets generate stable magnetic fields without continuous expenditure of electrical energy. The orbital motions and spins of electrons, called "Amperian currents" within the permanent magnet material, lead to a magnetization within the material and a magnetic field outside. The small bits of iron have been used to reveal the presence of magnetic field, as demonstrated on Figure 2.1. The lines of magnetic field are a geometrical abstraction, which help us to visualize the direction and strength of a magnetic field. The magnetic field induces changes in the medium surrounding the magnet. When a magnetic field has been generated in the medium, the response of the medium is the magnetic induction, also sometimes called the flux density. The relation between the magnetic induction and the magnetic field is a property called the permeability of the medium flux (David, 1998; Halliday and Resnick, 1966).

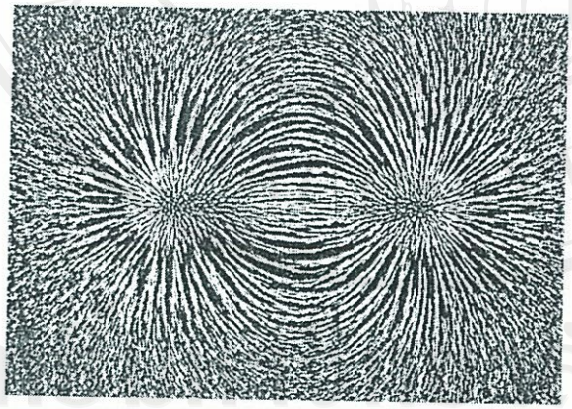

Figure 2.1 Iron fillings sprinkled on a sheet of paper covering a bar magnet. The distribution of the fillings suggests the pattern of lines of the magnetic field (Halliday and Resnick, 1966)

The magnetic field in free space has been schematically represented and characterized by "lines", or called "flux lines". The magnetic flux causes a magnet to attract or repel other magnets, and attract other materials composed of iron. It emerges

from one pole of the magnet, conventionally known as the north pole, and returns to the other, or the south pole, of the magnet as shown in Figure 2.2. Opposite magnetic poles attract one another (thus the north pole of one bar magnet attracts the south pole of another), while similar magnetic poles repel one another (David, 1998; Halliday *et al.*; 1992; Bondemark *et al.*, 1995b).

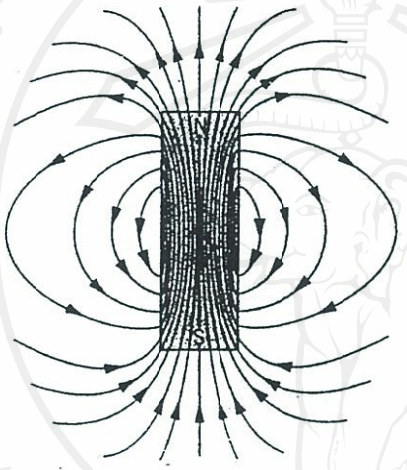

Figure 2.2 The magnetic field lines of a bar magnet. The lines form closed loops, leaving the magnet at its north pole and entering at its south pole.

From the clustering of field lines outside the magnet near its ends or poles, it is assumed that the magnetic field has its greatest magnitude there. A difference between the flux measured at the border as opposed to the center of the magnet was previously reported. The flux density decreases rapidly (exponentially) with increasing distance from the magnets. When the flux density at the pole face contacts of various types of magnetic fields was compared, it was found that the magnets in attractive positions produced the highest flux density, followed by the single magnet and the least from repelling magnets (Noar *et al.* 1996a and 1996b; David, 1998; Bondemark *et al.*, 1995b).

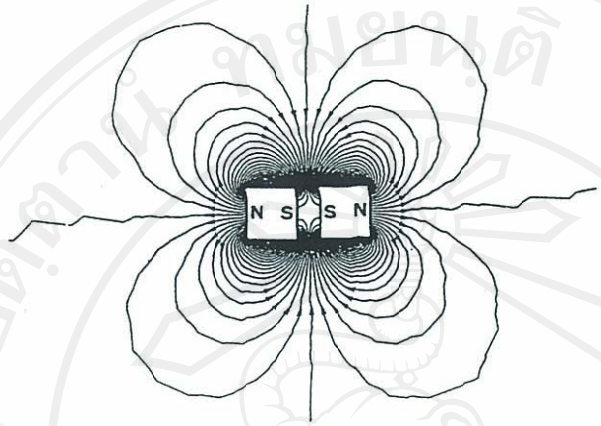

Figure 2.3 A two-dimensional computerized figure shows the magnetic field of repelling magnets, (N) is the north pole and (S) is the south pole.

The flux density or magnetic induction of the magnet can be measured and given a numerical value by using Gaussmeter or Teslameter with a magnetic field probe, called a Hall probe. The magnetic flux is measured in the units of webers. The flux density in webers per square meter (w/m^2) is also known as the magnetic induction, and the flux density of 1 w $/m^2$ is equal to the magnetic induction of 1 tesla. (David, 1998; Bondemark *et al.*, 1995b).

When a magnetic field is generated, a change in energy of the volume of space as well as a force is produced. The magnetic force can be detected by the acceleration of an electrical charge moving in the field (David, 1998). It was found that the force between a pair of magnets was directly related to the flux above each magnet; therefore, the numerical value of force can be calculated from the flux density. The principle of generated forces between two magnets follows the Coulomb's law of force. The magnets exhibit very large force at a small separation, and the force decreases markedly at a larger separation (Fraunhofer, 1992; Mancini *et al.*, 1999).

A number of studies indicate that the maximum magnetic force between two magnets (Figure 2.4) is controlled by several factors, including shapes and axis lengths of magnets, the distance between two magnets, and the verticotransverse displacement from centric spatial orientation of the two magnets. In the contact position of magnets, a long slender cylinder-shaped magnet generates greater maximum force than a short wide disk-shaped magnet although it has less volume. However, the wide disk-shaped magnet can exert greater attractive force (Vardimon *et al.*, 1991; Mancini *et al.*, 1999), exhibit higher range of the verticotransverse displacement from the centric spatial orientation, and exhibit less decline of force than other shapes of magnets (Vardimon *et al.*, 1991). Consequently, it can be concluded that the magnets with larger pole face areas and longer magnetic axes provide higher attractive force (Vardimon *et al.*, 1991; Fraunhofer, 1992; Mancini *et al.*, 1999).

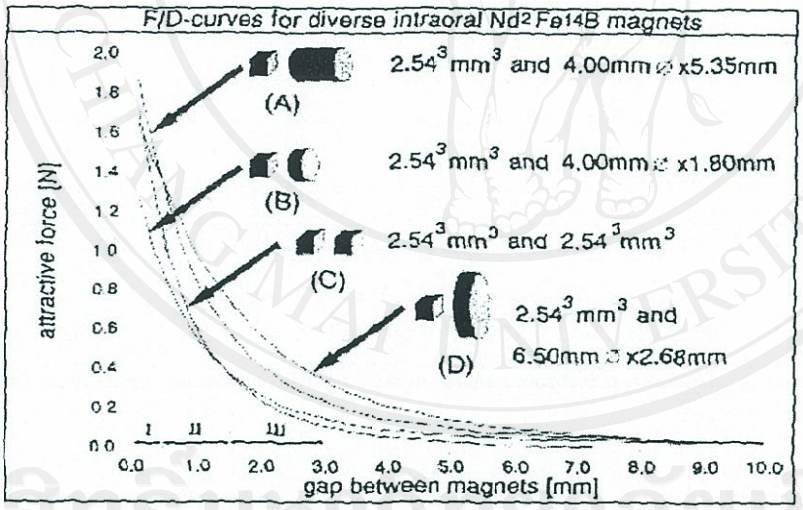

Figure 2.4 A change in force magnitude with respect to the distance between magnets (Vardimon *et al.*, 1991)

II The applications of magnets in orthodontics

The use of magnets in orthodontics was first described in 1978. At that time Blechman and Smiley tried to move the teeth of adolescent cats by using magnetic forces released from the Aluminium-nickel-cobalt magnets (Alnico), which is a kind of

the conventional permanent magnet. This study showed that as the air gap between two magnets decreased the greater the force became and this, in combination with continuous force application, might lead to a shorter period of treatment. It was shown that this tooth movement mechanic is an atraumatic, precise, and efficient method (Blechman and Smiley, 1978).

Up until now, the magnetic forces have been applied in several clinical orthodontic practices including:

1. Distalization of molars (Gianelly *et al.*, 1988 and 1989; Bondemark and Kurol, 1992a; Bondemark *et al.*, 1994a)
2. Artificial eruptive treatment of an unerupted tooth (Vardimon *et al.*, 1991; Darendeliler and Friedli, 1994; Mancini *et al.*, 1999)
3. Correction of anterior open bite (Dellinger, 1986; Darendeliler *et al.*, 1995; Noar *et al.*, 1996a and 1996b)
4. Orthodontic extrusion of crown-root fractured teeth (Bondemark *et al.*, 1997)
5. Tooth movement with and without arch wires (Muller, 1984)
6. Arch expansion (Vardimon, 1987; Darendeliler *et al.*, 1993)
7. Fixed retainer (Springate and Sandler, 1991)
8. Functional appliances (Vardimon *et al.*, 1989 and 1990).

Numerous advantages of magnets in orthodontic applications have been demonstrated, including 1) the absence of friction, 2) no risk on fatigue of force which leads to a decrease in chairside time from unnecessary reactivation (Muller, 1984; Bondemark and Kurol, 1992b), 3) vector-controllable force of sufficient magnitude and duration, 4) no need of patient cooperation, 5) almost no tooth mobility or no discomfort during treatment like using elastics, 6) bodily movement primarily, with minimal rotation, and 7) no reported adverse effects such as root resorption or alveolar bone loss (Blechman, 1985; Blechman and Steger, 1993).

With respect to these benefits, it is therefore assumed that magnetic force is suitable and reliable for orthodontic practices with a predictable and controllable result. Nevertheless, some disadvantages have been shown, such as the force-separation

behavior, the high cost of magnets, and the most important matter regarding the questionable biological effects of the magnets (Muller, 1984; Blechman, 1985; Bondemark and Kurol, 1992b; Fraunhofer, 1992; Blechman and Steger, 1993).

III. Biological implications of magnetic fields

Several *in vivo* studies on various kinds of tissues in both human and animals, as well as the *in vitro* studies, have been performed to indicate whether there are any adverse effects from an exposure to the magnetic fields.

Some studies indicated that the magnetic fields were not harmful and safe for clinical uses. An *in vitro* study was conducted to investigate the proliferation of osteoblast-like cells, the rat osteosarcoma cell line (UMR-106 cells), under the static magnetic field. The amount of uptaken radioactive thymidine was used to assess the proliferation of these cells grown under the magnetic field for 24, 48 and 72 h. The result showed that the cell proliferation was not significantly changed by the magnetic field (Sandler *et al.*, 1989). This finding was in agreement with other *in vitro* studies which indicated that the mouse fibroblast cells still showed normal morphology after exposure to high static magnetic field (190 mT and 220 to 250 mT) for 2 h (Bondemark *et al.*, 1994b and 1994c).

An *in vivo* study was carried out on the biopsies taken from the oral cavity of the cat. The tissues were taken from the areas exposed to magnetic field for nine months. It was found that there were no abnormal or pathological alterations of the oral tissues from these areas (Blechman and Smiley, 1978). Another study, done on the rat sagittal sutures, revealed similar findings, i.e. there were no significant changes in both the growth pattern and the rate of bone deposition of the rat sagittal sutures exposed to 1,000G (100mT) of magnetic field for 1, 3, 5, and 10 days (Camilleri and McDonald, 1993). Moreover, the magnetic fields expressed no cytotoxic effects on the human dental pulp and buccal gingival tissue after the 8 week-exposure to 100 -150 Gauss (10 -15 mT) and 200 - 900 Gauss (20 -90 mT) of static magnetic fields, respectively (Bondemark *et al.*, 1995a and 1998).

Nevertheless, some adverse biological effects of magnetic field were reported. There was an *in vitro* experiment established to study the vital cell functions of the human periodontal fibroblasts grown under 160 to 280 mT of magnetic fields. It was found that their cellular growth and cellular attachment were significantly decreased after exposure to magnetic field for 5 weeks (Linder-Aronson and Lindskog, 1995).

Additionally, a number of *in vivo* studies demonstrated that the static magnetic fields might have some effects on the bone-lining osteoblasts and epithelial recycling system in rats, which led to an increase in bone resorption and the reduced thickness of epithelium after a 4- and 8-week exposure (Linder-Aronson and Lindskog, 1991; and 1995). Similar effects were also found in monkeys at the fourth week (Linder-Aronson *et al.*, 1992; and 1996). However, the affected tissue tended to recover and returned to normal within 4 weeks after removing of the magnets.

Several *in vitro* and *in vivo* studies, as well as orthopedic clinical applications, indicated the possible biological mechanisms of cells, which had been exposed to the magnetic field. It was hypothesized that certain static magnetic fields might stimulate some bioeffects of osteogenesis. These effects might then result in an accelerated rate of osteogenesis in the adjacent tissues (Blechman and Steger, 1995).

Recently, there was a study investigating the effects of a moderate magnitude of static magnetic field on orthodontic tooth movement in rats. The results revealed that 10 to 17 mT of magnetic field did not enhance tooth movement, nor did the histological appearance of periodontal ligament (PDL) during tooth movement alter. However, significantly greater root resorption, increased width of PDL, and greater bone remodeling were found at day 7 of tooth movement. The interesting thing was that these effects were not found at day 14 (Tengku *et al.*, 2000).

According to the overall studies, the controversial issue about the biological effects of magnetic field was still debated. Although, it was indicated that there were no harmful effects from static magnetic field; and furthermore, the affected cells or tissues could recover shortly. However, a confirmation by the method with higher detection should be required.

IV. Fibroblasts in cell culture

Fibroblasts are the predominant cells of connective tissue, which originate from mesenchymal cells. Schroeder (1986) reported that the most numerous cell type in the human being is the fibroblast and that there are about 200 x 10^6 fibroblasts per unit volume of 1 cm^3 connective tissue. The fibroblasts are responsible for the elaboration and turnover of both fiber and ground substance including collagen, proteoglycans, and elastin. Because all tissues of the tooth (except enamel) and its supporting apparatus are connective tissue, fibroblasts play an important role in the development, structure, and function of the tooth and tissue integrity. The fibroblast may therefore be described as an architect, a builder, and a caretaker of connective tissue (Ten Cate, 1998).

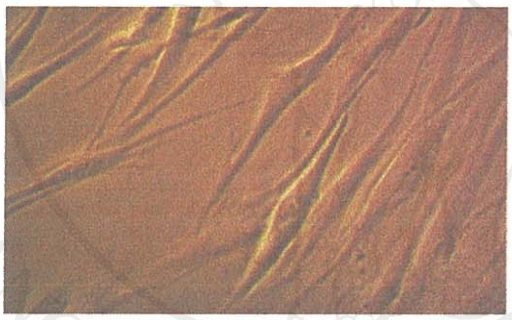

Figure 2.5 The fibroblasts (under a microscope using a 10x objective lens)

The fibroblast is a spindle-shaped cell with tapering eosinophilic cytoplasmic extensions. Its nucleus contains two to four nucleoli, and it has a well-developed Golgi area, rough endoplasmic reticulum, scattered mitochondria, and microfilaments. Its shape can vary from its original simple elongated spindle shape to other shapes, influenced by the nature of the substrate to which it attaches, by the space available for its movement over the substrate, and by stimulation for division (Goldberg and Rabinovitch, 1988). The resting fibroblast has a flattened, dark staining, closed nucleus and little cytoplasm. The active fibroblast (e.g. in the PDL) has a pale-staining, open-faced nucleus and much more cytoplasm. The outline of the fibroblast is difficult to be

discerned. However, it is more easily visualized after stained with iron-hematoxylin (Ten Cate, 1998).

Fibroblasts have been extensively studied in tissue culture. They are capable of serial replication *in vivo* and *in vitro*. When they enters the mitotic phase, they loosen their attachment to a surface and become spherical. After telophase, the daughter cells flatten onto available surfaces and once again resume an extended form. In culture, they can rapidly develop ability to attach to the wall of their container and to move across the surface. Such adherence and mobility are attributed to the development of a system of microfilaments (Schurch *et al.*, 1992). The ability of cells to attach to the surface of the culture vessel is one of the criteria for determining the cell viability.

V. Investigation methods in the *in vitro* study

Jakoby and Pastan (1979) divided the cell culture measurement into four major categories as follows:

a) A visual method, which is the most commonly used measurement of cell growth by employing a hemocytometer in direct counting of cells, is best applied when only a few samples are to be counted. If numerous cultures are to be counted, electronic counting should be considered.

b) Chemical methods, such as protein and DNA determination, are used to analyze the cell viability and growth by biochemical procedures.

c) Electronic systems use flow-through cells or apertures to measure the number of cells that incorporate dyes.

d) A combination of miscellaneous methods

To investigate the viability and growth of the cultured human gingival fibroblasts, the visual method of "The trypan blue dye exclusion assay" and the electronic method of "The flow cytometry" were the methods of choice for this study.

I. The trypan blue dye exclusion assay

A dye exclusion assay is a method used to estimate the number of surviving cells in a population by staining with dyes. It is the most commonly used method, which is based on the assumption that viable cells do not uptake certain dyes such as trypan

blue, whereas nonviable cells do. The number of cells can be counted manually by using a haemocytometer. Automated methods using cell counting devices are more suitable when a large number of samples are counted. A way of estimating cell growth is to determine the number of cells in several microscopic fields and compare this number to a standard in which a known number of cells have been seeded into a tissue culture container. Occasionally, the ability of cells to uptake or incorporate a radioactive or fluorescent compound may also be useful in the determination of cell growth. The integrity of the cell membrane and the general appearance of the suspended cells should be monitored by a phase-contrast microscope. If possible, the ultrastructure of the cells should be examined by a scanning and transmission electron microscope (Jakoby and Pastan, 1979; Darlington, 1988).

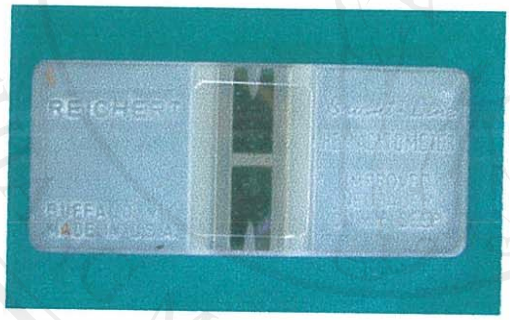

Figure 2.6 A haemocytometer

II. The Flow cytometry

The flow cytometry is the detection, measurement, and analysis of signals as single cell flow in a liquid stream through a beam of light (Figure 2.7). This method is used to determine multiple fluorescent and physical characteristics of a population on a cell-by-cell basis in a quantitative manner. The flow cytometer measures the way a cell scatters light and emits fluorescence, then records and stores a description of each particle that passes through the sample stream. Using this information, the system can determine the size, density, and the DNA content and the protein level for each individual cell (Rodger, 1988).

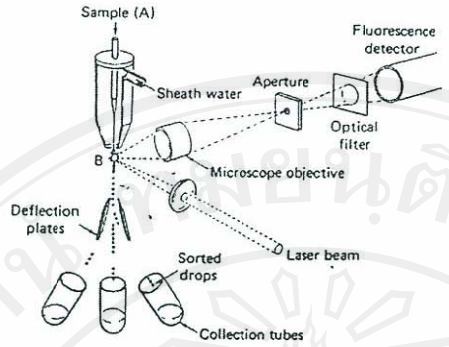

Figure 2.7 The flow cytometric system (Jakoby and Pastan, 1979)

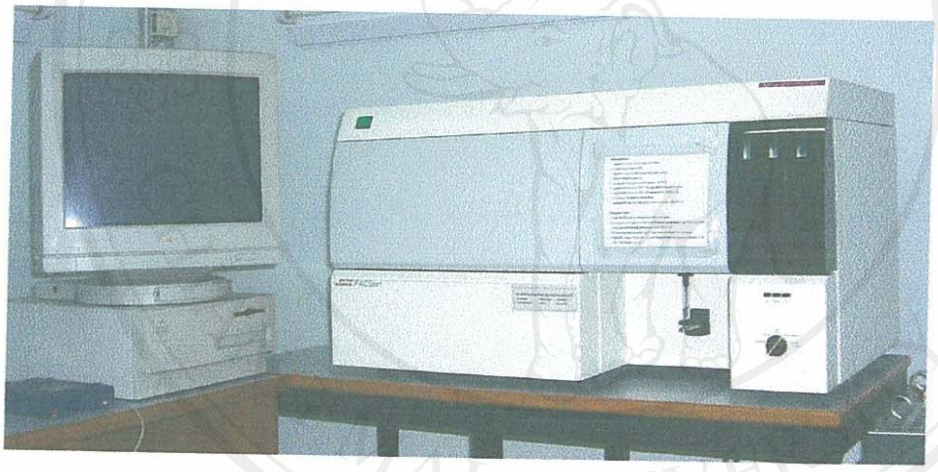

Figure 2.8 A flow cytometer

The most common application of flow cytometric techniques related to the cell cycle is the determination of the fraction of cells in the G_0 + G_1, S, and G_2 + M phases (Figure 2.9). An average duration and variability of each phase, as well as the rates of DNA synthesis can be obtained from the flow cytometric data. In each distribution, the peak at x1 DNA content is produced by diploid (n), G_0 + G_1 phase cells. The peak at x2 DNA (2n) content is produced by G_2 + M phase cells, and the intermediate is produce by S phase cells in which varying amounts of DNA have replicated. The areas under each of these regions of DNA distribution are proportional to the fractions of cells in the corresponding cell cycle phase. It was indicated that the effect of the perturbing

influence on cell cycle progressions could be determined by the time-dependent changes in the DNA distributions resulting from a disturbance study the effect (Figure 2.10)(Jakoby and Pastan, 1979; Rodger, 1988).

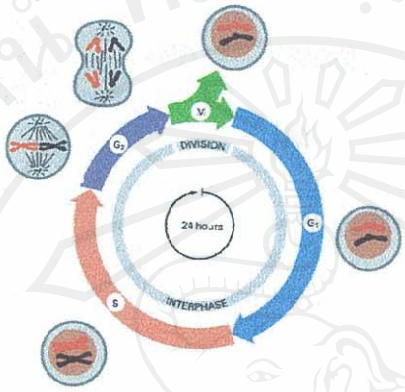

Figure 2.9 A cell cycle diagram

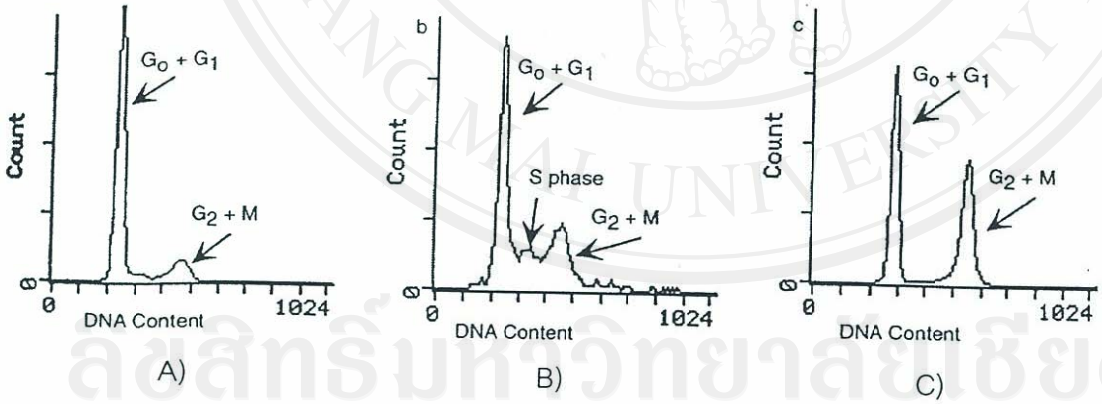

Figure 2.10 Sample graphs of cell cycle analyzed by flow cytometric system

A) A normal cell cycle distribution of stained cultured cells

B) An increase in S phase and G_2

C) Cells arresting in G_2