AN *IN VITRO* STUDY OF GENOTOXICITY OF LOCALLY PRODUCED BOVINE PERICARDIUM

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AN *IN VITRO* STUDY OF GENOTOXICITY OF LOCALLY PRODUCED BOVINE PERICARDIUM

by

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LIST OF ABBREVIATIONS

+S9	With S9
2AA	2 Aminoanthracene
ABB-C	An Organic Bovine Bone in Collagen
BP	Bovine Pericardium
BP-C	Bovine Pericardium- Collagen
BT	Mucogingival Bilaminar Technique
CAL	Clinical Attachment Level
CPRT	Combined Periodontal Regenerative Technique
DFDBA	Demineralized Freeze- Dried Bone Allograft
EMD	Enamel Matrix Derivative Protein
e-PTFE	Expanded Polytetraflouroethylene
GA	Genetic Analysis
GBR	Guided Bone Regeneration
GM	Glucose Minimal Agar
GR	Gingival Recession
GTR	Guided Tissue Regeneration
ISH	*In Situ Hybridization*
ISO	International Organization for Standardization
mg	Milligram
mm	Millimeter
NB	Northern Bolting
NMP	N-methyle-2-pyrolidune
OECD	Organization for Economic Cooperation and Development

PCR	Polymerase Chain Reaction
PPD	Probing Pocket Depth
S9	Metabolic Activation System
-S9	Without S9
ug	Microgram
VB Salts	Vogel- Bonner Salts
VPD	Vertical Pocket Depth
WHO	World Health Organization

KAJIAN IN VITRO GENOTOKSISITI PERIKARDIUM BOVINE KELUARAN TEMPATAN

ABSTRAK

‘Bovine pericardium’ boleh dianggap sebagai salah satu jenis bahan implan kolagen kerana ia mengandungi satu jaringan fiber kolagen selepas protein yang boleh di degradasi dikeluarkan. Ia merupakan bahan yang mudah diserap secara biologi dan satu membran yang mempunyai kesesuaian bio yang tinggi. Ia juga tergolong dalam kumpulan bahan (GTR) yang mempunyai penyerapan secara bio.

Fungsi utama bahan ini ialah sebagai penstabil dan pelindung bahagian surgeri semasa proses pemulihan peringkat yang berlainan. Ia juga digunakan untuk membenarkan penjanaan semula ligamen periodontal, tulang alveolar dasar dan memulakan proses pembentukan semula tulang dengan menghalang pertumbuhan tisu lembut dalaman.

Tujuan kajian ini dijalankan untuk menentukan genotoksisiti ‘bovine pericardium’ keluaran setempat oleh Bank Tisu, Universiti Sains Malaysia. Bahan ‘bovine pericardium’ di inkubasi dengan sejenis bacterium varian ‘genotype’ khusus iaitu *Salmonella typhimurium* yang membawa mutasi dalam beberapa gen (Ujian Ames). Penilaian ujian adalah berdasarkan bilangan koloni ‘revertant’.

Empat jenis ujian (TA98, TA1537, TA100 and TA1535) dengan dan tanpa keaktifan metabolik (S9) digunakan. Keputusan menunjukkan bahawa nombor purata koloni ‘revertant’ setiap piring dengan ‘bovine pericardium’ berkurangan dua kali ganda dengan kawalan yang negatif. Bilangan nombor koloni ‘revertant’ yang berkurangan dua kali ganda dengan bahan yang diuji menunjukkan bahawa ‘Bovine Pericardium’ tidak mutagenik dalam keadaan yang diuji.

AN *IN VITRO* STUDY OF GENOTOXICITY OF LOCALLY PRODUCED BOVINE PERICARDIUM

ABSTRACT

Bovine pericardium (BP) material can be considered another type of collagen implant material because it consists of a mesh of collagen fiber after the degradable proteins have been removed and it is a truly bioabsorbable and a highly biocompatible membrane which belongs to the family of bioabsorbable guided tissue regeneration (GTR) material.

The main function of this material is to work as a stabilizing and protecting barrier for the surgical site during the different stages of the healing process and it is used to allow the underlying alveolar bone, periodontal ligament to regenerate and begin the bone remodeling process by preventing soft tissue in-growth.

The aim of this study was to determine the genotoxicity of locally produced bovine pericardium (BP) by Tissue Bank, Universti Sains Malaysia. Bovine pericardium material was incubated with special genotype variants of the bacterium, *Salmonella typhimurium* that produce mutations in several genes (Ames test). The evaluation is based on the number of revertant colonies.

Four tester strains (TA 98, TA 1537, TA 100 and TA 1535) were used both with and without metabolic activation (S9). The result showed the average number of revertants colonies per plate treated with bovine pericardium was less than double that of the negative control. The absences of increase in the number of revertant colonies by at

least double with the test material indicated that bovine pericardium (BP) was non-mutagenic under the present test condition.

CHAPTER 1 - INTRODUCTION

1.1 Biomaterials

Biomaterial by definition is a nonviable material used in a medical device, intended to interact with biological system (William's, 1987).

Biomaterials improve the quality of life for an ever increasing number of people each year. The range of application is vast and includes such things as joint and limb replacement, artificial arteries and skin, contact lenses and dentures. While the implementation of some of these materials may be for medical reasons such as the replacement of the diseased tissue required to extend life expectancies, other reasons may include purely aesthetic ones including breast implants. This increasing demand arises from an ageing population with higher quality of life expectation. The biomaterials community is producing new and improved implant materials and techniques to meet this demand but also to aid the treatment of younger patients where the necessary properties are even more demanding. A counter force to this technological push is the increasing level of regulation and the threat of litigation. To meet these conflicting needs it is necessary to have reliable methods of characterization of the material and material/host tissue interactions (Czernuszka, 1996).

1.1.1 Development of Biomaterials

The development of biomaterials used for growing human tissue is a long-term process of multidisciplinary research activities. Materials are selected and tested according to the intended application. Criteria for material selection can be toxicology, biocompatibility, biostability or biodegradability, mass transfer, surface properties, hygienic design, scale-up, cost and other physical or biochemical properties. The resulting and generally the most important criteria for material selection are quality and safety of the final tissue

engineered product. Thus regulations of legal authorities for safety and quality in general and also specific for biomaterials should be followed (Grosskinsky, 2006).

1.1.2 Biomaterials Classifications

Biomedical materials can be divided roughly into three main types governed by the tissue response. In broad terms, inert (more strictly, nearly inert) materials illicit no or minimal tissue response. Active materials encourage bonding to surrounding tissue with, for example, new bone growth being stimulated. Degradable or resorbable materials are incorporated into the surrounding tissue, or may even dissolve completely over a period of time. Metals are typically inert, ceramics may be inert, active or resorbable and polymers may be inert or resorbable (Czernuszka, 1996).

a. Bioinert Biomaterials

The term bioinert refers to any material that once placed in the human body has minimal interaction with its surrounding tissue e.g. of these are stainless steel, titanium, alumina, partially stabilized zirconia and ultra high molecular weight polyethylene. Generally a fibrous capsule might form around bioinert implants hence its biofunctionality relies on tissue integration through the implant (Czernuszka, 1996).

b. Bioactive Biomaterials

Bioactive refers to a material which upon being placed within the human body interacts with the surrounding bone and in some cases, even soft tissue. This occurs through a time–dependent kinetic modification of the surface, triggered by their implantation within the living bone. An ion exchange reaction between the bioactive implant and surrounding body fluids results in the formation of a biologically active carbonate apatite layer on the

implant that is chemically and crystallographically equivalent to the mineral phase in bone e.g. synthetic hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$)] (Czernuszka, 1996).

c. Bioresorbable Biomaterials

Bioresorbable refers to a material that upon placement within the human body starts to dissolve (resorb) and slowly be replaced by advancing tissue (such as bone). Common examples of bioresorbable materials are tricalcium phosphate [Ca_3 (PO_4) $_2$], polylactic, polyglycolic acid, copolymers, collagen, calcium oxide, calcium carbonate and gypsum that have been utilized during the last three decades (Czernuszka, 1996).

1.1.3 General Properties of Biomaterials

The main property required of a biomaterial is that it does not elicit an adverse reaction when placed into service. However the range of applications for biomaterials is large, and the number of different biomaterials is also significant. However in general, metallic biomaterials are used for load bearing applications and must have sufficient fatigue strength to endure the rigors of daily activity e.g. walking and chewing. Ceramic biomaterials are generally used for their hardness and wear resistance for applications such as articulating surfaces in joints and in teeth as well as bone bonding surfaces in implants and polymeric materials are usually used for their flexibility and stability, also for low friction articulating surfaces (Czernuszka, 1996).

1.1.4 General Applications of Biomaterials

a. Orthopedic Applications

Metallic, ceramic and polymeric biomaterials are used in orthopedic applications. Metallic materials are normally used for load bearing members such as pins and plates and femoral stems. Ceramics such as alumina and zirconia are used for wear applications in joint replacements while hydroxyapatite is used for bone bonding applications to assist implant integration. Polymers such as ultra high molecular weight polyethylene are used as articulating surfaces against ceramic components in joint replacements and porous alumina has been used as a bone spacer to replace large sections of bone which have had to be removed due to disease (Czernuszka, 1996).

b. Cardiovascular Applications

Many different biomaterials are used in cardiovascular applications depending on the specific application and the design. For instance, carbon in heart valves and polyurethanes for pacemaker leads (Czernuszka, 1996).

c. Cosmetic Surgery

Materials such as silicones have been used in cosmetic surgery for applications such as breast augmentation (Czernuszka, 1996).

d. Dental Applications

Metallic biomaterials have been used as pins for anchoring tooth implants and as parts of orthodontic devices. Ceramics have been used in tooth implants including alumina

and dental porcelains. Hydroxyapatite has been used for coatings on metallic pins and to fill large bone voids resulting from disease or trauma.

1.2 Barrier Membranes

The use of guided tissue regeneration techniques to treat periodontal defects is now commonplace. The protocol employes barrier membranes to regenerate periodontal ligament, cementum and bone by excluding the faster growing soft tissue cells from the defect space. Clinical studies have widely demonstrated superiority of this treatment modality over traditional open flap debridement techniques (Heinze, 2004).

A variety of membranes are available in the marketplace, starting from non-resorbable polytetraflouroethylene (PTF) membranes to resorbable collagen and polylactide (PLA) and polyglycolic acid (PGA) membranes, all of which exhibit satisfactory clinical outcomes. However a set of design requirements need to be met for a periodontal membrane to be effective, in short these are biocompatibility, cell exclusion, space maintenance, tissue integration, ease of use and biological activity. Most of the membranes currently available have incorporated these criteria into their design to varying degrees (Heinze, 2004).

A membrane that features optimal space maintenance has to be stiff so that it does not collapse over the defect it spans. This is particularly true if no graft materials are used in the defect to support the membrane mechanically from underneath. If a membrane collapses into the defect space, the potentially regenerated volume is reduced and will not allow optimal clinical outcome. The membrane should be stiff enough to withstand the pressures exerted by the overlying flaps and to withstand external forces like

mastication, until the blood clot building underneath the membrane has matured enough to support it (Heinze, 2004).

Several different materials have been used in case reports and clinical studies. Early studies used a millipore filter. While more recent studies have evaluated expanded polytetrafluoroethylene membranes. Results using polytetrafluoroethylene membranes to treat intraosseous defects show substantial bone fill averaging approximately 3 to 5 mm either with or without augmentation with graft materials. The fact that this membrane requires a second surgical procedure to remove it led to studies using biodegradable membranes (Blumenthal *et al.*, 1990).

1.2.1 Types of Barrier Membranes

a. Non-Resorbable Barrier Membranes

Much of current understanding of guided tissue regeneration is based on studies utilizing expanded polytetraflouroethylene (e-PTFE) membranes although they are used less frequently now. The clinical effectiveness of expanded polytetraflouroethylene (e- PTFE) membranes is dependent upon the surgical placement technique and maintenance of tissue coverage over the membrane. Preservation of the keratinized gingiva and a relatively thick overlying surgical flap are critical in order to avoid perforation of the flap by the membrane during healing. After the surgical area has been flapped, the defect is degranulated and the root surface is scaled and root planed (Kao *et al*., 2005).

The expanded polytetraflouroethylene (e-PTF) membrane is trimmed to adapt to tooth configuration, secured by e-PTFE sutures and the flap is repositioned. After membrane

placement, healing is allowed to proceed for 4 to 6 weeks. A second surgery is performed to remove the membrane (Kao *et al*., 2005).

The major problems with non-resorbable membranes are the fact that the membrane is not tissue compatible and often becomes exposed to the oral environment during healing. Upon exposure, the membrane is contaminated and colonized by oral micro flora (Simion *et al*., 1995).

b. Resorbable Membranes

Several studies have shown that contamination of the surgical field can result in decreased formation of new attachment. If the non-resorbable membrane becomes exposed, the infection can be temporarily managed with a topical application of chlorhexidine. This complication has led to the development and more popular use of bioabsorbable membranes. There are basically three types of bioabsorbable membranes; polyglycoside synthetic polymers (e.g. polylactic acid, polyglycolic acid, co-polymers), collagen e.g. bovine pericardium (BP) and calcium sulphate (Kao *et al.*, 2005).

Several features make these bioabsorbable membranes easier to manage clinically; they are more tissue compatible than non-resorbable membranes, the timing for bioabsorption can be regulated and a second surgical procedure is not required to retrieve the non-resorbable membrane (Kao *et al.*, 2005).

CHAPTER 2 - LITERATURE REVIEW

2.1 Guided Tissue Regeneration

Guided Tissue Regeneration (GTR) can be described as procedures attempting to regenerate lost periodontal structures through differential tissue responses. It typically refers to regeneration of periodontal attachment. Barrier techniques, using materials such as expanded polytetraflouroethylene (ePTFE), polyglactin, polylactic acid, calcium sulfate and collagen are employed in the hope of excluding epithelium and the gingival corium from the root in the belief that they interfere with regeneration (American Academy of Periodontology, 2001).

However, in the 1996 World Workshop in periodontics guided tissue regeneration (GTR) was defined as procedures attempting to regenerate lost periodontal structures through differential tissue responses. Barriers are employed in the hope of excluding epithelium and gingival corium from the root surface during periodontal regeneration. They further defined periodontal regeneration as the regeneration of the tooth's supporting tissues, including alveolar bone, periodontal ligament and cementum over a previously diseased root surface.

The ideal treatment is to recover (i.e. regenerate) the periodontal tissues that have been destroyed by disease. Several surgical techniques have been developed in the attempt to regenerate periodontal tissues including guided tissue regeneration (GTR), bone grafting (BG) and the use of enamel matrix derivative (EMD) proteins (Esposito *et al*., 2004).

With guided tissue regeneration (GTR), a biocompatible barrier (either resorbable or non-resorbable) is surgically positioned around the root to seal the bone defect and

protect the blood clot. A Cochrance review has shown that guided tissue regeneration (GTR) is a more effective than open flap debridement. However, it was also observed that there was a marked variability of results with guided tissue regeneration (GTR) among various randomized controlled trials (RCTs). Grafting techniques may include autogenous bone grafting, demineralized freeze-dried bone allograft (DFDBA), animal-derived graft materials (xenografts) and alloplasts such as hydroxyapatite (Esposito *et al*., 2004).

Both guided tissue regeneration (GTR) and grafting procedures are based on the concept of selective exclusion of epithelial cells from colonizing the wound and maintaining the blood clot to regenerate the periodontal tissues. In addition, bone grafts may possess osteoinductive and osteoconductive properties (Esposito *et al*., 2004).

Periodontal regeneration mediated by enamel matrix derivative (EMD) is based on a different concept. It is believed that EMD used in periodontal lesions mimics the development of the tooth-supporting apparatus during tooth formation (Hammarstrom, 1997).

2.1.1 History of Guided Tissue Regeneration

The rationale for the use of guided tissue regeneration (GTR) was first described in 1976 by Melcher who suggested that differences in the behaviour and characteristics of attachment cells lead to repair of the periodontium by epithelium instead of regeneration with periodontal progenitor cells.

Melcher (1976) suggested that cells that repopulate the root surface after periodontal surgery would determine the type of attachment that form on the root surface during healing.

From this hypothesis have come procedures using barrier membranes to allow selective cellular repopulation of the root surface during periodontal regenerative attempts. In theory, these barriers retard apical migration of epithelium and exclude gingival connective tissue from the healing wound, thus favoring healing influenced primarily from the periodontal ligament space and adjacent alveolar bone (American Academy of Periodontology, 1993).

During the past 20 years, several materials and techniques have been developed and tested for enhancing periodontal regeneration which includes flap debridement, allogenic and alloplastic grafting and the use of non-resorbable and resorbable barrier membranes as regenerative techniques. One the most predictable regenerative therapies are treatment of three-walled intrabony defect. This defect can be repaired with 2 to 2.5 mm of bone fill and results in significant gains in clinical probing attachment and decrease in probing depths (Becker, 1999).

2.1.2 Function of Guided Tissue Regeneration

Nyman *et al*. (1982) suggested the placement of a physical barrier between the flap and the root surface to exclude gingival connective tissue and epithelium from the healing process, giving the periodontal ligament cells the opportunity to repopulate the coagulum on the root surface.

There are several guided tissue regeneration (GTR) materials and techniques. The most common approach among the various guided tissue regeneration (GTR) techniques is the placement of a biocompatible barrier between the flap and the periodontal defect. Some of these barriers are absorbable, whereas others are non absorbable (Melcher, 1976).

Barriers offer three advantages during wound healing. First, exclusion of the epithelium and gingival connective tissue cells from the periodontal defect during healing; permits pluripotential cells from the periodontal ligament and the alveolar bone to repopulate the periodontal defect, favoring periodontal regeneration as the defect heals. Second, barriers maintain space between the defect and the barrier, allowing the entry of the regenerative cells from the periodontal ligament and alveolar bone. Finally, the barrier helps to stabilize the clot which may enhance regeneration (Melcher, 1976).

Membrane barrier techniques are based on criteria proposed by Melcher who described the biologic behavior of different tissues (gingival epithelium, connective tissue, periodontal ligament, alveolar bone) during wound healing. The goal of membrane barrier procedures is to guide proliferation of the different tissues during healing after therapy (selective cell repopulation) (Melcher, 1976).

Cells that have the capability to form bone, cementum and periodontal ligament must occupy the defect to stimulate regeneration of the tissues. The progenitor cells reside in the periodontal ligament or alveolar bone or both which remain around the tooth or bony defect. Placement of a physical barrier between the gingival flap and the defect before flap repositioning and suturing prevents gingival epithelium and connective tissue

(undesirable cells) from contacting the space created by the barrier. It also facilitates repopulation of the defect by regenerative cells (Rowe *et al*., 1996).

Different types of membrane materials have been developed; the biomaterial and physical characteristics of the rnembranes used can significantly influence barrier function, biocompatibility, cell conclusiveness, space making, tissue integration and clinical manageability which are criteria that must be considered in the design of materials used for regenerative procedures (Scantlebury, 1993).

These materials should also be safe, efficient, biocompatible, cost effective and easy to use. In addition they must remain in place until regeneration is complete and not interfere with newly formed tissue; the clinical and histological results of various barriers have generally been favorable. However, none of the materials has been found to be ideal for every clinical situation because each material has specific benefits and certain associated drawbacks (Meffert, 1986).

2.2 Types of Guided Tissue Regeneration

2.2.1 Non-resorbable Membranes

The first studies used non-resorbable materials such as cellulose filters (millipore filter) and expanded polytetrafluoroethylene (e-PTFE) (Gore-Tex Regenerative Material). These materials were not originally manufactured for use in medical or dental procedures. Cellulose filters and expanded polytetrafluoroethylene (ePTFE) were chosen as barrier materials because they allowed the passage of liquid and nutritional products through the barrier but their micro porosity excluded cell passage (Gottlow, 1993).

a. Cellulose Filters

Nyman *et al.* (1982) conducted the initial studies with the use of cellulose filters in primates to exclude connective tissue and gingival epithelium, allowing cells from the periodontal ligament to repopulate the wound. The periodontal ligament, cementum and alveolar bone on the facial aspect of the cuspid teeth were removed and cellulose filters were placed over the defects. Histological examination showed regeneration of the alveolar bone and new attachment of new cementum with inserting periodontal ligament fibers. However, disadvantages of the use of cellulose filters include exfoliation, premature removal and the need for a second surgical procedure for their removal.

b. Expanded Polytetrafluoroethylene Membranes

They are widely used in many animal and human studies and these membranes have been considered the gold standard with which other types of membranes are compared. Membranes made of expanded polytetrafluoroethylene (e-PTFE) are composed of a matrix of polytetrafluoroethylene (PTFE) nodes and fibrils in a microstructure that vary in porosity. Expanded polytetrafluoroethylene (e-PTFE) is recognized for its inertness and tissue compatibility (Gore, 1986).

The porous microstructures allow the ingrowth and attachment of connective tissue for stabilization of the healing wound complex and inhibition of epithelial migration (Gray and Hancock, 1998).

These membrane barriers consist of two parts. The first part is a coronal border (open microstructure collar) that facilitates early clot formation and collagen fiber penetration to stabilize the membrane into place. The collar may also stop apical proliferation. The second part is an occlusive portion that prevents gingival tissues outside the barrier from interfering with the healing process at the defect site (Caffesse and Quinones, 1992).

There are two configurations of expanded polytetrafluoroethylene (e-PTFE) membranes, transgingival and submerged that can be used in different situations. The transgingival design is used to treat defects that are associated with structures that extend through the gingiva such as teeth. The submerged design is used in situations where there is no communication with the oral environment such as bony defects (Becker, 1996).

Titanium-reinforced ePTFE membranes were designed to increase the tent like effect which is an advantage when the defect does not have inadequate space. Therefore titanium-reinforced ePTFE membranes were created to be used in situations where the anatomy of the defect may cause non-reinforced material to collapse into the defect space or where more space is needed for the desired regeneration (Becker, 1996).

The results of the present study showed that the membrane appeared to the well tolerated by the soft tissue with no inflammation or drainage. The membrane also provided an effective barrier allowing bone deposition in the osseous defects. The advantage of the use of this membrane is that it can be left exposed in the oral cavity without the risk of compromising the bone regeneration process (Becker, 1996).

The main disadvantage of the use of expanded polytetrafluoroethylene (ePTFE) membranes is that a second surgical procedure is required for their removal which increases the cost and surgical trauma to the patient. However, with the use of these membranes, clinicians have control over the length of time that the membrane remains in place. The principle advantage is that the membrane retains its functional characteristics long enough for adequate healing to occur and then it can be eliminated immediately. After removal there is no possibility of breakdown products interfering with the maturation of the regenerated tissue; they are more predictable with less risk for long-term complications and easier in clinical management. The use of expanded polytetrafluoroethylene (ePTFE) membranes may be advantageous in situations where there is the anticipation of soft tissue management problems and when complete flap closure cannot be achieved. If premature removal of the membrane is required it can be accomplished without interfering with the regenerated tissues (Becker, 1996).

2.2.2 Resorbable Membranes

The avoidance of a second surgical procedure is the main advantage of using resorbable membranes. A disadvantage of using bioresorbable rnembranes is that material exposure or flap dehiscence can cause postoperative tissue management problems. Material exposure after surgery can lead to bacterial growth, alteration of fibroblast morphology and migration, all of which may jeopardize the success of the regeneration process. Another common problem is the difficulty in preventing membrane collapse into the defect which can result in inadequate space making (Anson, 1996).

Resorbability may be associated with degradation through enzymatic activity (biodegradation) or hydrolization (bioabsorption) as a cellular response from the

surrounding tissue. The inflammatory response should be minimal and reversible and must not interfere with regeneration (Anson, 1996).

a. Collagen Membranes

Collagen is a natural component of the periodontal tissues, weakly immunogenic, favorable tissue response, malleable, hence can be formed, shaped and manipulated, semi permeable, allowing nutrient passage and gas exchange, possesses haemostatic properties through its ability to aggregate platelets, supports cell proliferation via lattice structure and cell-binding domains, facilitates early wound stabilization and maturation, is chemotactic for fibroblasts and promotes cell migration and is absorbed naturally being replaced by host tissues (Wang and MacNeil, 1998).

Bovine pericardium (BP) material can be considered another type of collagen implant material because it consists of a mesh of collagen fibers after the degradable proteins have been removed and it has been used in surgical procedures when guided tissue regeneration is needed. Its main function is to work as a stabilizing and protecting barrier for the surgical site during the different stages of the healing process also allowing the underlying alveolar bone and periodontal ligament to regenerate and begin the bone remodeling process by preventing soft tissue ingrowth. It has many features such as being a natural biological membrane, easy to handle (flexible and adaptable), remodels and is resilient, making it suturable and also has different configurations (Anson and Marchand, 1996).

b. Polylactic Acid

It is a bioresorbable matrix barrier composed of a blend of polylactic acid that is softened with citric acid for malleability and to facilitate clinical handling. It (Guidor) was the first resorbable barrier to be approved by the Food and Drug Administration (FDA) for membrane barrier techniques. This device is a multilayered matrix. The layer that is in contact with the bone or tooth (the inner layer) features small circular perforations and several space holders to ensure enough room for the formation of new attachment. Whereas the layer in contact with the gingival tissue (the outer layer) has larger rectangular perforations to allow rapid in growth of gingival tissue into the interspace between the two layers, preventing or minimizing epithelial down growth. The resorption process of the material is programmed to ensure barrier function for a minimum of 6 weeks after which it slowly resorbs. Complete resorption occurs at approximately 12 months (Lundgren, 1995).

Several studies have demonstrated the efficacy of polylactic acid membranes to allow the formation of new attachment and bone in the treatment of interproximal defects and gingival recession in primates as well as infrabony defects and class II furcation defects in humans (Lundgren, 1995).

The results obtained in these studies showed that the use of this matrix barrier around teeth resulted in reduced probing depths, a gain in clinical attachment and a very low incidence of gingival pathologic disease, gingival recession and device exposure (Laurell, 1994).

c. Polyglycolic Acid and Polylactic Acid

There are bioresorbable membranes made of polyglycolic acid and polylactic acid (Resolut) that consist of an occlusive film with a bonded, randomly oriented, fiber matrix located on each surface. The film bonds the fibers and separates the soft tissue from the defect. The random arrangement of the fibers and the openness of the fibrous matrix encourage the ingrowth of connective tissue and inhibit apical migration of the epithelium. The fiber matrix is the primary structural component that provides adequate strength for space making during the initial phases of healing (2 to 4 weeks) (Hardwick *et al.,* 1995).

Simion *et al.* (1996) compared the use of resorbable membranes made of polyglycolic acid and polylactic acid with expanded polytetrafluoroethylene (ePTFE) membranes for membrane barrier procedures. This study showed that there was a significantly greater amount of bone regeneration obtained with the use of expanded polytetrafluoroethylene (ePTFE) membranes compared with the resorbable membranes. This difference may be due to several factors, e.g. the fixation screws prevented ePTFE membrane collapse, the stiffness of the resorbable material was not sufficient and as the membrane resorbed, the space making capability of the barrier decreased.

d. Synthetic Liquid Polymer

It is a polymer of lactic acid which is dissolved in N-methyl-2-pyrroliduone (NMP). This material begins as a solution that sets to a firm consistency on contact with water or other aqueous solution (Atrisorb). When outside the oral cavity, the membrane is a partially set solution which allows it to be trimmed to the dimensions of the defect before

intraoral placement. The barrier is then adapted to the defect and sets in a firm consistency. This barrier has the advantage of being rigid enough for placement but flexible enough to be adapted to the defect. The barrier adheres directly to dental structures; therefore sutures are not required (Polson, 1995).

Chemically the material is a polymer that is resorbed through the process of hydrolysis. The rate of resorption is controlled and the membrane is present during the critical period of healing, preventing epithelial migration and isolating the periodontal defect. Alternatively, it can be used by placing graft material in the defect to ensure a tentlike position of the membrane, applying the liquid polymer directly to the surgical site and then allowing contact with surrounding fluids which initiates the set-up of the polymer in the firm consistency (Polson, 1995).

e. Polyglactin

It is a woven mesh barrier made of polyglactin 910 (Vicryl) and a resorption rate of 30 to 90 days. The results of several studies have questioned the use of polyglactin for guided tissue regeneration procedures, reporting that the mesh provides an insufficient barrier because of fragmentation of the material. The integrity of the mesh is lost after 14 days and the cervical sealing between the mesh and the adjacent tooth may not be perfect, allowing for the growth of connective tissue and epithelium between the root surface and the barrier (Lundgren, 1995).

f. Calcium Sulphate

It is medical-grade calcium sulphate commonly known as plaster of paris which has been used after immediate implant placement as part of a bone graft placed around the implants. Barrier composed of medical-grade calcium sulphate can be placed over bone grafts for clot stabilization and to exclude undesirable tissues (gingival connective tissue and epithelium). The advantages of this material include providing a source of calcium in the early mineralization process and particle retention (Sottosanti, 1997).

This material is available in sterile kits that contain exact amounts of medical-grade calcium sulphate powder and a syringe that is prefilled with cap set. When mixed together, these substances create a moldable plaster that can conform to the desired shape even in the presence of blood. Sutures are not required because this mixture is adhesive. Calcium sulphate dissolves in approximately 30 days without an inflammatory reaction and it does not attract bacteria or support infection (Anson, 1996).

The rationale for using medical-grade calcium sulphate for guided tissue regeneration (GTR) procedures includes complete resorption within 3 to 4 weeks, biocompatibility (causes no increase in inflammation), adaptability (does not need to be cut before placement), porosity (allows fluid exchange but excludes the passage of epithelium and connective tissue), minimal postoperative discomfort, clot protection during the early stages of healing, soft tissue growth over exposed calcium sulphate, lack of infection with material exposure and less effect on cellular morphology (Anson, 1996).

g. Acellular Dermal Allograft

It is a relatively new type of a bioresorbable grafting material which is acellular human cadaver skin that has been obtained from tissue skin (Alloderm). The material has undergone a process of deepithelialization and decellularization to eliminate the targets of rejection response, leaving an immunologically inert avascular connective tissue (Shulman, 1996).

Shulman (1996) evaluated the alloderm material which appeared to become completely and permanently incorporated into the surrounding tissue after 6 weeks when used as a membrane barrier. The use of a cellular dermal allograft has several advantages because it does not contain cellular material which eliminates the possibility of rejection because of the presence of major histocompatibility complex class 1 and 2 antigens. The materials used for membrane barriers must have certain properties such as being memory free, easy to place and adapt, biocompatible and able to be covered by soft tissue and remain revered. If the material is bioresorbable it must be predictable and remain intact as a barrier for 6 weeks with complete resorption in less than 6 month (Shulman, 1996).

2.3 Clinical Studies

Joly *et al.* (2002) examined 10 systemically healthy patients with ages ranging from 35-56 years and these patients had intrabony osseous defects around mandibular canines and premolars. Clinically and radiographically more bone fill was demonstrated in sites treated with guided tissue regeneration (GTR).

Kerdvongbundit *et al.* (1999) examined 20 patients with range age of 30-65 years to evaluate the regenerative potential of the periodontal tissue in class 11 furcation defects in mandibular molars using reconstructive surgery based on the guided tissue regeneration (GTR) technique versus the coronally positioned flap (CPF) technique. After 12 months following surgical treatment, both GTR and CPF procedures showed gains in new clinical attachment levels. When comparing parameters between the two surgical procedures, GTR molars showed significantly more improvement in probing depth as well as vertical and horizontal attachment level of the interradicular osseous defect than CPF molars.

Paolantonio (2002) examined 45 systemically healthy, non-smoking patients aged between 27-51 years, a Miller's Class I or II gingival recession was treated for coverage: 15 patients underwent mucogingival bilaminar technique (BT), 15 GTR by a bioabsorbable membrane and 15 combined periodontal regenerative technique (CPRT) by collagen membrane and collagen-incorporated hydroxyapatite. BT, GTR and CPRT successfully treated gingival recession defects, obtaining comparable percentages of root coverage but BT and CPRT created a thick gingival tissue significantly greater than that achieved with GTR.