AN EXPERIMENTAL STUDY ON THE USE OF FIBRIN GLUE IN CORNEAL WOUND REPAIR IN RABBITS

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(In the name of ALLAH, the Most Beneficent, and the Most Merciful)

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ABSTRAK

Objektif

Untuk menilai keputusan klinikal dan perubahan histopatologi selepas penggunaan gam fibrin bagi penyembuhan hirisan surgikal yang berbeza pada kornea mata arnab.

Tatacara

Sepuluh ekor arnab terlibat dalam kajian ini. Arnab-arnab ini dibahagikan kepada dua kumpulan, A dan B dengan setiap kumpulan mengandungi 5 ekor arnab. Hirisan surgikal sepanjang 6 mm di buat di bahagian tengah kornea arnab dalam kumpulan A, manakala bagi kumpulan B pula hirisan surgikal sepanjang 6 mm dibuat di bahagian pinggir kornea. Semua hirisan surgikal ini kemudiannya dicantum dengan menggunakan gam fibrin. Pemeriksaan klinikal berkala dijalankan pada hari yang ke 1, 3, 7, 14 dan 21 selepas prosedur dengan menggunakan lampu slip mudah alih. Pemeriksaan dilakukan untuk menilai percantuman hirisan surgikal, kejernihan kornea dan kedalaman ruang hadapan mata. Enukliasi dilakukan pada hari ke 21 kajian. Penilaian histopatologi dilakukan untuk melihat inflamasi pada stroma, pertumbuhan pembuluh darah, pembentukan granuloma dan kerenggangan hirisan surgikal secara mikroskopik.

Keputusan

Sebanyak lapan puluh peratus mata arnab dalam kumpulan A mencapai percantuman hirisan surgikal yang baik mulai hari pertama selepas prosedur dijalankan dan sebanyak 100% pada hari ketiga. Sepanjang kajian ini, tiada kebocoran pada hirisan surgikal dilaporkan berlaku dalam kumpulan B. Kesemua subjek mempunyai kornea yang jernih dan ruang hadapan mata yang dalam di akhir kajian. Pemeriksaan histopatologi menunjukkan pertumbuhan pembuluh darah pada darjah yang rendah dalam kumpulan B. Kebanyakan subjek mengalami reaksi inflamasi ringan, tetapi tiada peembentukan granuloma atau kerenggangan hirisan surgikal secara mikroskopik dilaporkan dalam kedua-dua kumpulan yang dikaji.

Kesimpulan

Gam fibrin telah menunjukkan keputusan klinikal yang memuaskan dengan percantuman hirisan surgical yang baik, kornea yang jernih dan ruang hadapan mata yang dalam. Keputusan klinikal ini adalah selaras dengan keputusan histopatologi yang menunjukkan reaksi inflamasi ringan tanpa pembentukan granuloma mahupun kerengganggan secara mikroskopik.

ABSTRACT

Objective

To evaluate the clinical outcomes and histopathological changes after using fibrin glue for repairing different types of corneal wound in rabbits.

Methodology

Ten rabbits were involved in this study. They were divided into two groups A and B, with each group consisting of 5 rabbits. Full thickness central corneal wound of 6 mm in length was performed in group A and peripheral wound of 6 mm was made in group B. The wounds then were closed using the fibrin glue. Serial clinical examination was conducted using portable Slit-lamp at day 1, 3, 7, 14 and day 21 post procedure to evaluate wound opposition, corneal clarity and anterior chamber depth. Enucleation was done on day 21 of study. Histopathology evaluation was performed to look for stromal inflammation, vascularization, granuloma formation and microscopic wound gapping.

Results

Eighty percent of eyes in group A achieved good wound opposition by first day and 100% by third day post surgery. No wound leak was reported in group B throughout duration of study. Clear cornea with deep anterior chamber obtained in all subjects at the end of study.

Histopathology examination revealed stromal vascularization of scanty degree in group B. Majority of subjects had shown mild inflammatory reaction but no granuloma formation or microscopic wound gapping was reported in the two groups of study.

Conclusion

Fibrin glue has shown satisfactory clinical outcomes with good wound opposition, clear cornea and deep anterior chamber. These clinical findings were concomitant with the histopathological results which revealed acceptable inflammatory reaction without any granuloma formation or microscopic gapping.

Chapter 1
INTRODUCTION

1 INTRODUCTION

Cornea is considered the main refracting part of the optical system of the eye. To function as a major optical element it must remain transparent, allowing for the formation of an image on the retina. In addition, light scatter can alter the ability of the retina to obtain a clear image. Cornea possesses the unique characteristics of an orderly arrangement of stromal collagen fibrils and a lack of blood vessels that result in tissue transparency. Any kind of mechanical or biological injury can affect the integrity and transparency of corneal tissue and eventually impairment of vision occurs. Moreover, inadequate healing of corneal wound can result in scar formation with decreased or loss of vision.

Small self sealing perforating corneal wound could be managed with simple patch or bandage contact lens which promoting the healing process. However, repairing of large corneal wounds often make challenging to ophthalmologist and need good surgical skill. Unfortunately, closure of these large wounds by using the conventional suturing method usually bothersome for surgeon and patient as well.

Many complications of corneal suturing technique have been documented including tissue trauma, infection and inflammation. It can result in uneven healing causing regular and irregular astigmatism. Also its time consuming and sutures might become loose or broken post operatively. These complications still occur even surgical repair with suture have done with extremely precision. Hence, the research for alternative method to replace suture has been considered e.g. fibrin glue.

The fibrin glue has been used in surgery since the early 1980s. In the 1990s it became more public and commercially available and general study of potential ophthalmic applications of this material was undertaken. Its usage has increased in numerous surgical fields including ophthalmic surgery.

However there are few reports on fibrin glue applications in repairing clear corneal wound. To the best of our knowledge, there is no previous study conducted in repairing corneal wound larger than 3mm in length with fibrin glue alone.

1.1 CORNEAL WOUND HEALING

Corneal wound healing as in other parts of the body is the end result of a sequence of events which are controlled by many factors. This events or interactions are complex often occurring simultaneously and are influenced by many factors. Elsewhere in the body wound healing culminates in scar formation and vascularisation whereas one of the most crucial aspects of corneal wound healing is how the healing processes aim to minimize these end results in which would otherwise have serious visual consequences.

In fact, corneal wound healing after full-thickness incisions is much slower from wound healing at other sites of the body, which is due to the avascular nature of this tissue (lack of blood and lymphatic vessels). The cornea is composed of three main laminated membranes, the epithelium, the stroma and the endothelium. Because these laminated membranes are structurally quite different, each one has its own characteristic sequence of healing events as will be described below.

1.1.1 Epithelial Wound Healing

The human corneal epithelium is made up of five to seven layers of nonkeratinised squamous epithelial cells which regularly arranged. The upper most layers consist of two to four outer flattened cell layers of squamous cells. In the middle there is a layer of wing cells that are usually one to three cell layers thick which covered the inner most layer of a single mitotically active columnar basal layer.

The basal epithelial cells tightly adhere to their basement membrane through a series of adhesion complexes. The process of corneal epithelium healing can be divided into three distinct that in reality are overlapping continuous phases. These are cell migration by sliding of superficial cells to cover the denuded surface, cell proliferation and cell adhesion (Zeiske et al. 2000).

Within a few hours after epithelial wounding polymorphonuclear leukocytes and neutrophils appear within the wounded area. Cells adjacent to the wound commence migration to re-establish the integrity of the ocular surface epithelium. It has been postulated that the basal and wing cells participate in the formation of the leading edge of the migrating cells (Gipson et al. 1994). Rounding and retraction of epithelial cells at the wound edge, with loss of surface microvilli, can be observed before the basal cells start flattening and separating.

The migrating cells then undergo flattening and elongation and cell proliferation occurs to repopulate the wounded area. Adhesion complex components (Hemidesmosom) takes place within 15 hours of injury (Klatte et al. 1989). The corneal epithelium contains no connective tissue and therefore is not subject to fibrosis or vascularization.

1.1.2 Stromal Wound Healing

The human corneal stroma constitutes about 90% of corneal thickness. It is composed almost entirely of an extracellular matrix and kertocytes. The stroma can be subdivided into thick Bowman's layer and thick Descemet's layer and the lamellar stroma in between. Due to corneal stromal avascularity, wound healing is slower than in other connective tissues.

Following an incisional injury the stromal defect initially fills with a fibrin clot and the adjacent matrix imbibes fluid and becomes edematous in area adjacent to the wound. After that stromal wounding keratocytes are detected within 2-6 hours and undergo proliferation and migration.

Keratocytes start to undergo fibroblastic transformation with resulting expansion of the fibroblast population by mitosis after approximately 48 to 72 hours which reaches its peak between three to six days (Tuft et al. 1993). Within the first 24 to 48 hours, fibroblasts migrate toward the edge of the wound and synthesize collagens. With increasing collagen deposition, the tensile strength of stroma wound increase continuously for at least 6 months. The fibroblasts produce collagens, glycoproteins and proteoglycans which form the new stromal extracellular matrix.

The healing epithelium elaborates cytokines which stimulate transformation of stromal keratocytes into fibroblasts and secrete extracellular matrix. Keratocyte activity is not seen until the corneal wound has been fully covered by new epithelium. So it is clear that the epithelial-stromal interaction is important in corneal wound healing.

At the 3 month point the cellular activity has returned to normal whereas the number of cells continues to be elevated. The formation of corneal stromal scar is a dynamic process and clinical changes can be noted by slit-lamp biomicroscopy for several years post wounding. However the strength of corneal scar never reaches that of uninjured corneal tissue.

1.1.3 Endothelial Wound Healing

The corneal endothelial integrity is crucial in maintaining stromal transparency. It functions as an active barrier in preventing leakage of the aqueous humor into the corneal stroma. The corneal stroma which can absorb considerable amounts of fluid is kept in its normal deturgescent state by the endothelial active transport and barrier functions.

Any leakage at the level of corneal endothelium leads to corneal edema and opacification and thus to severe visual impairment. Corneal endothelial wound healing is critical to the reestablishment of normal corneal deturgescence and transparency after both surgical and traumatic injury.

The corneal endothelium have minimal or no capacity to replicate and is considered to be a non-replicating tissue. Endothelial wound healing is largely replaced by migration and spreading of existing endothelial cells to cover a defect. Cells surrounding the defect enlarge and slide over that area to fill the defect and restore the endothelial integrity (Olsen et al. 1984).

As a result of this process, mean cell size tends to be greater than the rest of the endothelial cells. In addition the endothelium is responsible for the deposition of a new Descemet layer throughout the wound area.

1.2 CORNEAL WOUND CLOSURE

The corneal tissue remodeling after injury is critical since it serves an important role in refracting and focusing light rays which necessary for clear vision. Hence, methods of corneal wound repair after traumatic or surgical injury are of significant clinical and research importance.

1.2.1 Suturing Method

Through the centuries, surgeons have been using sutures made up of different materials to repair corneal tissue. But these may have certain disadvantages like foreign body reaction, infection, inflammation and scarring. In addition, it is time consuming and the final appearance of the healed wound is not always satisfactory.

The use of sutures may also give rise to granular lesions through the foreign body reaction induced by the sutures, which usually requires a second operation for their removal. An intense sensation of a foreign body during the postoperative period is also common. This sometimes even requires the removal of some of the stitches if they have not been reabsorbed within the desired period, meaning more maneuvers and therefore discomfort.

Currently, most of the corneal wounds are repaired by using sutures. Depending on the pattern and extent of injury in a corneal wound, multiple sutures are often needed to realign the edges of damaged tissue in an effort to restore the structural integrity of the cornea. However, sutures are not ideal because it often yields uneven healing resulting in a regular or an irregular astigmatism. In addition, suture material does not actively participate in wound healing and the procedure is inherently invasive (Nirankari et al. 1983).

Furthermore the use of suture materials requires a high degree of surgical skill and is associated with suture-related complications such as buttonholes, suture abscesses, astigmatism, tissue necrosis and giant papillary conjunctivitis. It also requires removal by an ophthalmologist often months after the operation and each time a suture is removed, trauma occurs and there is a new opportunity for infection. In the search of solutions to these problems, surgeons began to think of an alternative to suture which is devoid of its inherent disadvantages. Consequently concept of tissue adhesive was introduced.

1.2.2 Tissue Adhesive

Tissue adhesive has been defined as any substance with characteristics that allow for polymerization, holding tissues together or serving as a barrier to leakage. It is one of the alternative methods to conventional suturing technique which has some added advantages.

The potential advantages of sutureless surgery are well known. These advantages include, shortening the duration of surgical procedures, minimizing post operative inflammation and scar and reducing the possibility of postoperative symptoms and discomfort (Koranyi et al. 2004).

The adhesive material to be an ideal must meet a number of design requirements which can be summarized as the following:

1. Should be biocompatible, degradable and easy to apply.
2. It should adhere to the moist corneal surface and seal the wound to withstand high intra-ocular pressure.
3. Rapidly cure to seal the corneal wound in a controlled manner.
4. Maintain the structural integrity of the eye.
5. Possess solute diffusion properties favorable for normal corneal healing.

A variety of adhesive substances can be applied locally during surgery for wound closure, sealing and hemostasis. The two main categories of tissue adhesives are synthetic in the form of cyanoacrylate derivatives and biologic in the form of fibrin based glue.