In The Name Of GOD
Ahvaz Jundishapur University of Medical Sciences
Faculty of medicine

Thesis for Orthopaedic Surgery doctorate degree

Title:
Evaluation of the Osteoinductive and Osteoconductive Effect of the Amniotic Membrane in Bone Defects due to Open Fractures in Rabbits

Author:
Ahmad Abbaszadeh, MD

Supervisors:
Seyed Shahnam Moosavi, MD

Advisor:
Mohammad Ali Ghasemi, MD

Registration No:
U-93188

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Abstract

Introduction: The repair of long bone segmental defects is one of the most challenging problems in orthopaedic surgery. In this study, the researchers decided to carry out animal experiments on the use of HAM in the bone defect to evaluate the osteoinductive effects of it, and to also use it as a guide for the regular production of bone, without waiting for membrane production (MASQUELET method).

Materials and Methods: Twenty New Zealand white male adult rabbits were used in the study, and divided into 4 groups. The surgical site was prepared with the purpose of working on the left forearm diaphysis. In each radius, a bone defect of 15mm in length was created. The fixation of the radius was not done because the support of the ulna was sufficient. The defect was not filled with anything in group 1; however, a tube shaped HAM was inserted in group 2, a Tendon shaped HAM was utilized for group 3 and a Tube shaped HAM + bone graft (DBM) was used in group 4.

Results: Bone formation was radiographically observed in the defects which had been implanted using tube shaped HAM (group 2), that was complete in 60% and partially complete in 40% of the cases. No bone formation was seen at up to 8 weeks after surgery in group 1 and 3. A small amount of bone formation was observed at both ends and the ulnar site of the defect in group 4.

Conclusion: These results of the study indicate that tube shaped HAM could have an osteoconductive and osteoinductive effects in large segmental bone defects.

Key Words: Human Amniotic membrane (HAM), rabbits, osteoconductive, osteoinductive.
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Chapter 1:

Introduction &
literature review
Introduction

Repair of long bone segmental defects is one of the challenging problems in orthopaedic surgery. Current treatment options include bone grafting (auto or allogenic), vascularized bone graft and distraction osteogenesis. However, these techniques often involve multistage surgical procedures, inhibit early limb function, and require several revision procedures to maintain acceptable alignment and achieve osseous healing.(1)

Segmental bone defects resulting from traumatic injuries are complicated problems with significant long-term morbidity. Historically, due to the difficulty in managing segmental long bone defects, amputation was the preferred treatment.(2) Traditional bone graft techniques are limited by uncontrollable graft resorption, even when the recipient site is well vascularized. More recently, the use of an antibiotic cement spacer followed by grafting within this space confirmed by an induced biomembrane has been described as a potential treatment strategy.(2)

Among bone reconstruction techniques, the induced membrane technique, proposed in 1986 by Masquelet, has rarely been studied or evaluated in the surgical literature until recently.(3) Masquelet technique, which is the use of a temporary cement spacer followed by staged bone grafting, is a recent treatment strategy to manage a posttraumatic bone defect.(2)

Critical size defect (CSD) is defined as the defect with the minimum length that cannot be spontaneously bridged leading to non-union. Such defects are generally accepted to be 1.5 to 2 times the diameter of the long bone diaphysis, but they vary according to the host and the bone.(4)

Although many methods for bone reconstruction exist, they all have specific indications and limitations. Established methods are distraction osteogenesis and bone transport, or bone grafting, including autologous bone
grafts, bone marrow aspirate, allografts, bone substitutes or growth factors. Furthermore, the concept of an induced-membrane represents another strategy for bone regeneration and particularly in cases of large bone defects secondary to trauma, infection or tumor excision.

This method involves a two-stage procedure, where a ‘biological’ membrane is induced as a foreign body response after application of a cement spacer at the first stage, acting as a ‘chamber’ for the insertion of autologous bone-graft at the second stage. It has been shown that this induced membrane possesses osteoinductive, osteogenic and angiogenic properties and several clinical studies have demonstrated satisfactory results. Finally, the concept of Guided Bone Regeneration (GBR) using a bioabsorbable or non-resorbable membrane that acts as a barrier to prevent soft-tissue invasion into the defect and forms a ‘chamber’ to ‘guide’ the bone regeneration process is also used for bone reconstruction.(4)

The guided bone regeneration (GBR) technique promotes the increase of osteoblastic proliferation and synthesis of bone matrix. The whole process is controlled by complex molecular interactions that act upon mesenchymal cells, producing their proliferation and differentiation.(5)

The clinical application of amniotic membrane (AM) for GTR, while fulfilling the current goals of GTR (cell exclusion, space maintenance, tissue integration, and ease of use) is also in line with the modern concept of biomechanical GTR.(6)

The human amniotic membrane (HAM) is a biomaterial with lacking any vascular tissue which is consists of three layers including a single epithelial cell layer, intermediate basement membrane layer, and a mesenchymal cell layer. Certain characteristics of HAM make it an extremely useful biomaterial for therapeutic purposes including: anti-inflammatory, anti-scarring, promotion of epithelialization (7,8), anti-pain properties(10,11), mechanical
properties(12), enhanced healing of wounds (13,14), adhesive effect of the stromal layer (15,16), anti-adhesive effect of the epithelial layer (17,18) low immunogenicity property (19,20), antibacterial and antiviral activity (21,22), also anticancer effects (23) and induction of apoptosis (24).

HAM has been used in clinic for treatment of various diseases such as: coverage of skin defects, anti-inflammation in burned skin or ulcers (25,26), corneal surface reconstruction (9,27), ocular surface reconstruction (17,28), control of wound infection (29), treatment of oral facial defects (30), bladder augmentation (31), and reconstruction of long ureteral defects (32).

In this study, due to the properties of amniotic membrane and due to the fact that most bone defects caused by open fractures which increases the chance of infection, we decided in animal studies, use of amniotic membrane in the bone defect, to evaluate the osteoinductive effect of AM, also use it as a guide for the regular production of bone, and not wait for membrane production (MASQUELET method), and faster to do the second stage of surgery (Bone graft). Also we help patient to come back to work earlier.

**Bone Tissue**

The mechanical properties of bone are readily apparent. Its tensile strength nearly equals that of cast iron, but it is three times lighter and ten times more flexible. Yet bone is not a homogenous inert material like iron, or the plastics and metals that form most orthopaedic implants. Its matrix consists of organic and inorganic components, and it is covered on its internal and external surfaces by cells and cell processes.

An elaborate system of lacunae, canals or tunnels containing cells and cell processes, blood vessels, lymphatics, and nerves permeates the matrix, and various specialized cell populations responsible for maintaining the tissue lie
within the matrix lacunae and on the bone surfaces. In most people bone appears to remain unchanged for decades, but this appearance is deceptive; it is constantly changing in response to mechanical and hormonal signals. Over a lifetime, the skeleton is fully turned over multiple times, adjusting its alignment to altered loads.

Mature bones consist of a central fatty or hematopoietic marrow supported and surrounded by bone tissue and periosteum.

Although the three component tissues of bone differ in composition, structure, and function, they are not independent. Marrow can serve as a source of bone cells, marrow blood vessels form a critical part of the bone circulatory system, and disorders or mechanical disruption of the marrow can affect the activities of bone and periosteal cells.

Bone consists of mesenchymal cells embedded within an abundant extracellular matrix. The matrix contains mineral that gives the tissue great strength and stiffness in compression and bending. The organic component of the bone matrix, primarily type I collagen, and contributes to bone strength, but also gives bone the plasticity that allows substantial deformation without fracture. Bone matrix also contains various cytokines, including growth factors that stimulate bone formation.

These growth factors appear to have important roles in normal bone metabolism and in fracture healing. The periosteum, consisting of two layers—an outer fibrous layer and an inner, more cellular and vascular cambium layer—covers the external bone surfaces and participates in the healing of many types of fractures.

The thicker, more cellular periosteum of infants and children has a more extensive vascular supply than that of adults.
Perhaps because of these differences, the periosteum of children is more active in fracture healing. Two types of bone can be distinguished by their mechanical and biological properties:

Woven or immature bone, and lamellar or mature bone. Woven bone forms the embryonic skeleton and is replaced by lamellar bone during development and growth. Woven bone also forms the initial fracture repair tissue and is replaced by lamellar bone as the fracture remodels under mechanical load.

Compared with lamellar bone, woven bone has a more rapid rate of deposition and resorption, an irregular woven pattern of matrix collagen fibrils consistent with its name, approximately four times the amount of osteocytes per unit volume, and an irregular pattern of matrix mineralization. The frequent patchwork formation of woven bone and the spotty pattern of mineralization create an irregular radiographic appearance that distinguishes the woven bone found in fracture callus from lamellar bone. Because of its lack of collagen fibril orientation, irregular mineralization, and relatively high cell content and water concentration, woven bone is less stiff and more easily deformed than lamellar bone.

**Amniotic membrane**

**Embryology**

Following implantation of the blastocyst in the endometrium, its wall becomes the chorion, the outer layer of membranes surrounding the fetus. The chorion has many villi on its outer surface which are more pronounced at the site of the placenta and attenuated outside this area resulting in formation of chorion frondosum (villous chorion) and chorion laeve (nonvillous chorion), respectively. In the same early stages, cleavage occurs within the cellular mass of the embryo and the trophoblasts. The innermost trophoblastic cells, the so
called amniogenic cells, form the amniotic membrane (amnion) which is visible by day 10 post-conception (Fig. 1).

By the end of the third month of gestation, the exocoelomic or chorionic cavity separates the chorion leave from the amnion. The amniotic sac fills with fluid, expands gradually and adheres to the inner surface of the chorion; the chorionic cavity disappears but the two layers do not fuse histologically and remain separable (Fig. 2). The amnion is a thick and hard but flexible sheet of tissue at full term.

**Histology and physiology**

In 1962 Bourne described several layers in the amniotic membrane. The innermost layer, adjacent to the amniotic fluid, is a single homogenous layer of cuboidal epithelial cells which is presumably derived from embryonic ectoderm. This epithelium is firmly fixed to a basement membrane which is in turn attached to a condensed acellular layer composed of collagen types I, II, and V.

Amniotic epithelial cells have many microvilli at the apical surface and some processes at the basal aspect which extend toward the basement membrane. These processes have a hemidesmosome type of attachment with multiple filaments. These cells have a large irregular nucleus with a large homogenous nucleolus as well as many intracytoplasmic organelles and pinocytic vesicles. The ultrastructure of amniotic epithelial cells suggests that it has an active secretory function as well as intra- and transcellular transport functions. The distribution of collagen type IV subchains in the basement membrane of the amnion resembles that of the conjunctiva but not the cornea.

External to the amniotic membrane epithelium is a layer of mesenchymal fibroblast- like cells which are probably derived from the mesodermal embryonic plate. These cells are scattered in a full term membrane. The outer-most layer of the amnion is the zona spongiosa which is an almost acellular
layer adjacent to the laeve chorion. Human amniotic membrane is totally free of smooth muscle cells, nerve fibers and lymphatics or blood vessels (Fig.3).

The tractional resistance of the amniotic membrane is related mainly to the condensed layer of interstitial collagens type I and II. Collagens type V and VI are less important in this respect. Collagen type I is the main interstitial collagen in tissues with high tractional resistance such as bone and tendon, but in other tissues, collagen III is known as the main factor of integrity and firmness. Elastin exists in small amounts in the amniotic membrane and its elasticity is mainly due to collagen type III. Owing to the presence of interstitial collagens, one of the important properties of amniotic membrane is its resistance to proteolytic factors.

Figure 1- Embryonic membranes and placenta are derived from the outer cell mass and the fetus is derived from the inner cell mass of the blastocyst.
Interstitial collagens of the amniotic membrane are produced by various cells: collagens type I and III are mainly produced by mesenchymal cells, basal membrane proteins of the amnion such as collagen type IV, fibronectin and
laminin are mainly produced by amniotic epithelial cells. The amniotic membrane is not just a simple avascular membrane, it has multiple metabolic functions such as transport of water and soluble materials and production of bioactive factors including vasoactive peptides, growth factors, and cytokines.

**Properties**

Certain characteristics of amniotic membrane make it a suitable substrate for ocular surgery:

1) **Promotion of Epithelialization**

Amniotic membrane serves as a basement membrane which facilitates epithelial cell migration, reinforces adhesion of basal epithelial cells, promotes epithelial differentiation and prevents epithelial apoptosis. It also improves corneal sensation and tear stability by an unknown mechanism. It produces various growth factors which can stimulate epithelialization. It is believed that amniotic membrane can promote expansion and maintenance of progenitor epithelial cells in vivo. It can also produce endothelin-1 and parathyroid hormone related protein. The epithelial cells of the membrane produce brain natriuretic peptide and corticotrophin releasing hormone which have roles in increasing cellular proliferation and calcium metabolism.9 In the year 2000, Koizumi et al demonstrated that cryopreserved amniotic membrane can express mRNA for epidermal growth factor (EGF) as well as two growth factor receptors including hepatocyte growth factor (HGF) and keratocyte growth factor (KGF) in addition to producing the related growth factors.

Amniotic membrane can accelerate epithelial healing through several mechanisms of action mentioned above. Its basement membrane serves as a safe and suitable bed for the growth of epithelial cells. Laminin isoforms, present in the basement membrane, facilitate adhesion and expansion of corneal epithelial
cells. The ability of the basement membrane of the amnion to support expansion of progenitor cells can explain application of AMT for treatment of partial limbal stem cell deficiency.

It has also been reported that amniotic membrane can act as a bandage contact lens allowing epithelialization to occur under its cover. Moistened by tear, amniotic membrane can provide a wet media for ocular surface re-epithelialization. It also has good permeability providing sufficient oxygenation for epithelial cells which is in contrast to many synthetic materials.

2) Inhibition of Fibrosis

Fibroblasts are naturally responsible for scar formation during wound healing and are activated by transforming growth factor β (TGF-β). Amniotic membrane inhibits expression of TGF-β receptors in fibroblasts resulting in less fibrosis. It has been shown that amniotic membrane suppresses TGF-β signaling of fibroblasts in the cornea and limbus and in the conjunctiva and pterygia. Chui and Tseng applied dispase-treated amniotic membrane with and without corneal epithelial cells onto the corneal stroma of rabbit eyes and found that amniotic membrane inhibits keratocyte differentiation to myofibroblast and helps maintain corneal transparency.

3) Inhibition of Inflammation and Angiogenesis

AMT has been reported to be effective for treating severe neurotrophic corneal ulcers. The exact mechanism of the anti-inflammatory properties of amniotic membrane is not clear. It is postulated to serve as a barrier, decreasing influx of inflammatory cells to the affected area and consequently reducing inflammatory mediators. When applied as a patch in vivo, amniotic membrane entraps T lymphocytes. In an experimental study on rabbit eyes, the stromal matrix of the amniotic membrane attracted inflammatory cells. Additionally, other anti-inflammatory factors such as tissue inhibitors of metalloproteinase,
interleukin-10 (IL-10), and IL-1 receptor antagonists as well as endostatin which inhibits endothelial cell proliferation, angiogenesis, and tumor growth have been isolated in human amniotic membrane. The presence of proteinase inhibitors may facilitate wound healing. Thrombospondin-1, an antiangiogenic factor, is secreted by the epithelium of amniotic membrane. IL-1α and IL-1β, two very potent pro-inflammatory mediators, are suppressed by the stromal matrix of the amniotic membrane.

In 2001 Shimmura et al reported that amniotic membrane reduces inflammation through entrapment of inflammatory cells. They applied cryopreserved amniotic membrane onto injured ocular surface for one week and performed immunohistochemical studies afterwards. Cells underwent staining for CD4, CD8, CD14, and CD20 and TUNEL staining for apoptosis (TUNEL: Terminal deoxynucleotidyl transferase-mediated dUTP Nick End Labeling). The results revealed that CD4, CD8, and CD14 positive cells, the main inflammatory cells, existed in large numbers in the amniotic membrane and that most of the entrapped cells were involved in apoptosis. It has also been shown that cultivating corneal epithelial cells on amniotic membrane matrix results in suppression of IL-1α and IL-1β.27 Secretion of certain antiangiogenic factors, such as thrombospondin-1 and TIMP 1-4 may also explain anti-inflammatory properties of the amniotic membrane. It has also been reported that corneal neovascularization is significantly suppressed by amniotic membrane.

4) Lack of Immunogenicity

It was initially believed that amniotic epithelial cells do not express HLA-A, B, or DR anti-gens, however subsequent studies showed that both amniotic epithelial and mesenchymal cells and fibroblasts express all HLA class I molecules including class Ia (HLA-A, B, C, DR) and class Ib (HLA-G, E)
antigens. However, HLA class II antigens are not expressed by amnion epithelial cells.

INFγ and other immunologic factors have been recognized in the amniotic membrane. It seems that in the presence of viable epithelial cells, amniotic membrane may induce immunologic reactions. One study revealed that transplantation of fresh amniotic membrane is associated with a mild inflammatory reaction probably due to expression of HLA-I antigens by viable epithelial cells. However cryopreserved amniotic membrane does not lead to immunologic rejection. The main reason is loss of epithelial cells due to cryopreservation. FDA- approved methods of amniotic membrane processing include Delbeco modified eagle medium (DMEM) or cryopreservation in glycerol 50%, both of which result in the death of all amniotic epithelial cells leading to non-immunogenicity. Human amniotic membrane has the ability to suppress T lymphocytes in allografted limbus cells, this implies immuno-suppressive properties which can increase the success rate of grafting.

5) Antimicrobial and Antiviral Properties

Amniotic membrane may decrease the risk of infection due to antimicrobial properties. Amniotic membrane has cystatin E, the analogue of cysteine proteinase inhibitor, which exhibits antiviral properties. Kjaergaard et al have also shown in vitro antimicrobial effects of the amnion and chorion against certain micro-organisms. Further studies are needed to verify the antimicrobial and antiviral characteristics of the amniotic membrane.

Amniotic membrane may function as a barrier against bacterial infiltration by adhesion to the wound surface, reducing bacterial load. In clean surgical wounds, the hemostatic property of collagen fibers in amniotic basement membrane prevents hematoma formation reducing microbial accumulation and thereby the risk of infection. Adhesion to the wound surface prevents dead
space formation and serous discharge accumulation, which is another mechanism of action against infection. Furthermore, formation of fibrin filaments during wound healing results in adhesion of the wound bed to amniotic membrane collagens leading to bacterial entrapment and stimulation of phagocyte migration. There is a report that amniotic membrane may decrease bacterial proliferation even in contaminated wounds.

6) High Hydraulic Conductivity

This property of the amniotic membrane makes it attractive for revision of leaking blebs after glaucoma filtering surgeries.

7) Other Properties

Amniotic membrane acts as a biologic dressing resulting in significant pain relief in burns owing to adhesion to the wound surface and coverage of dermal nerve endings. It also prevents wound surface drying, which accelerates wound healing.

Amniotic membrane preparation

Amniotic membrane may be contaminated by normal vaginal flora during normal vaginal delivery, therefore amniotic membrane is obtained under sterile conditions through elective cesarean section after a full-term pregnancy.(39) The donor is initially evaluated for the possibility of blood borne infectious by taking a careful history of high risk sexual behavior, intravenous drug abuse, blood transfusion or malignant disease and by performing physical examination specifically for tattoos and needle marks. Following identification of a potential donor, she is informed about the method of obtaining the tissue and its usage. After obtaining written consent, serum samples of the potential donor are tested for anti-HIV-1 and 2 antibodies, hepatitis B surface antigen, hepatitis B core
antigen, anti-hepatitis C virus antibody, and rapid plasma reagin test for syphilis. The serologic tests are repeated 6 months later in seronegative subjects to recognize infections in the window period of the previous tests. Until confirmation of the seronegative state of the donor, the amniotic membrane is preserved at -80°C. This screening, does not, however, exclude the possibility of infections with pathogens for which no test is available such as Creutzfeldt-Jakob disease. Contamination of the tissue may also occur during preparation and processing procedures, therefore absolute aseptic techniques should be applied at all stages.

Donor material including placenta and fetal membranes are placed in a sterile organ bag composed of bilayered polyethylene containing RPMI-1640 solution within it in a sterile container and transferred to the preparation unit at 4°C temperature. Based on FDA-approved standards, good tissue practice principles, and standards of the American Association of Tissue Banks, tissue processing should be performed in clean room condition with neatness class of 100 according to the 209E classification. Tissue processing may also be performed under a lamellar flow hood in a clean room with neatness class of less than 100. There are several methods of tissue preparation and preservation which are described hereunder.

1) Heat-dried Amniotic Membrane

   In this method, after preparing the tissue under a lamellar flow hood in a clean room with a neatness class of 100, the tissue in dried overnight in an oven at 40±2°C. It is then sterilized using 25 KGY gamma irradiation. In this method, the membrane loses many of its biologic properties due to the high temperature employed and serves as a biologic dressing which is often used for management of burns.

2) Air-dried Amniotic Membrane
چکیده
مقدمه:
ترمیم دیفکت‌های استخوانی بلند یکی از مشکلات مورد بحث در ارتوپدی می‌باشد. در این مطالعه محققان تصمیم گرفتند تا با مطالعه روی حیوانات و استفاده از غشا آمونیوتیک در دیفکت استخوانی به بررسی اثرات استئواینداکتیو آن پیردازند، همچنین از آن به عنوان یک گاید برای تولید منظم استخوان استفاده کنند و منتظر جهت تولید غشاء (که در روش ماسکوله استفاده می‌شود) نباشیم.

روش کار:
بیست عدد خرگوش بالغ سفید نر در این مطالعه استفاده شد و به چهار گروه تقسیم شدند. محل جراحی بر روی دیافیز ساعد چپ آماده شد. در هر رادیوس یک دیفکت استخوانی به طول 15mm ایجاد شد. فیکسیشن رادیوس به عمل سه بعدی آماده شد. در گروه یک (کنترل) هیچ‌چیزی به کار نرفت. در گروه دوم غشا آمونیوتیک لوله‌ای به کار رفت. در گروه سوم غشا آمونیوتیک به شکل تاندون (پرکردن کل دیفکت) استفاده شد. و در گروه چهار غشا آمونیوتیک لوله‌ای شکل و یون ساخته شد.

نتایج:
تولید استخوان در گروه دوم مشاهده شد. که در 06 درصد کامل و در 15 درصد نسبی بود. هیچ‌چیزی مشابه در گروه یک و سه مشاهده نشد. مقدار کمی تولید استخوان در دو طرف دیفکت و سمت اولنار دیفکت در گروه چهار مشاهده شد.

بحث و نتیجه‌گیری:
نتایج این مطالعه نشان دادند که غشا آمونیوتیک لوله‌ای کمک می‌کند تا اثرات استئواینداکتیو و استئوکانداکتیو در دیفکت‌های استخوانی بزرگ داشته باشند.

واژگان کلیدی:
غشا آمونیوتیک، خرگوش، استئواینداکتیو، استئوکانداکتیو
تقدیم:

پس از طالب علمیان، هنرمندان و فرهنگمندان، شوراهای علمی و فرهنگی، جوی اورکانزیون توانسته‌ام و از این نسبت، که سخن‌وران، در ستودن او بمانند و شمارندگان، شمارندن نعمت‌های او ندانند و کوشندگان، حق او را گزاردن نتوانند، نسبت آنها را در این نامه دلم قرار دهم.

ما حاصل آموخته‌ها را تقدیم می‌کنم به آنان که مهر آسمانی شان آرام بخش آلام زمینی ام است، تقدیم به مقدس‌ترین واژه‌ها در لغت نامه دلم.

به استوارترین تکیه‌گاهم، دستان پرمهر پدرم به سبزترین نگاه زندگیم، چشمان سبز مادرم که هرچه آموختم در مکتب عشق شما آموختم و هرچه بکوشم قطره‌ای از دریای بی‌کران مهربانی‌تان را سپاس نتوانم بگویم.

امروز هس تیم به امید شماست و فردا کلید باغ به‌شتم رضای شما تقدیم به خواهر و برادر هایم

که با هم آغاز کردیم، در کنار هم آموختیم و به امید هم به آینده چشم می‌دوژیم. قلبم لبریز از عشق به شماست و خوشبختی تان منتهای آرایند.

راه آوردی گران سنگتر از این ارزان نشد تا به خاک پایتان نثار کنم، باشد که حاصل تلاشم نسیم گونه غبار خستگی‌تان را بزداید.

بوسه بر دستان پرمهرتان و در نهایت تقدیم به استاد گرامیم جناب آقای دکتر سید شهنام موسوی که بدون راهنمایی‌های ایشان تامین این پایان نامه بسیار مشکل می‌نمود.

از جناب آقای دکتر محمد علی قاسمی و سرکار خانم دکتر نسترن رنجبری به دلیل یاریها و راهنمایی‌های بی‌چشم‌داشت ایشان که بسیاری از سخت‌های را برایم آسان‌تر نمودند.

دو نهایت نگه‌بان:

امکان‌هاим به‌خوبی که به‌شکن‌های مروزی که به‌خوبی بازی‌ها بی‌تنا، که‌ای این پیام که‌یس باشیم‌ها، صد می‌شود.

از جناب آقای دکتر مالک، رئیس دانشگاه علوم پزشکی که به‌دارم گام‌ها، جای‌ها می‌کردیم، صد می‌شود.
فرم شماره 11: صورتجلسه دفاع از پایان نامه

با تاییدات خداوند متعال جلسه دفاع از پایان نامه آقای/آمام... در

رشته.../п/کم. به شماره دانشجویی .../11111111 تحت عنوان ((

که ماهنامه (اصفهان) می‌باشد بعد از انجام کارها در مجامع به

حضور سید حسن، مشاور و هیأت داوران در محل

در تاریخ .../11111111 نشکل و با موافقیت از پایان نامه خود دفاع نموده و

موفق به کسب نمره .../11111111 (به حروف .../11111111) با رتبه .../11111111 گرده است.

استاد (ان) راهنما:

امضاء

استاد (ان) مشاور:

امضاء

هیأت داوران:

امضاء

مدیر گروه:

امضاء

دکترعلی علی فراست

Marvelous...
دانشگاه علوم پزشکی و خدمات بهداشتی درمانی جنوب شیراز
دانشکده پزشکی
پایان نامه
جهت اخذ مدرک دکترای تخصصی در رشته ارتودودی

عنوان:
بررسی اثرات استئواینداکتیو و استئوکانداکتیو غشای آمنوتویک در بون دیفک های ناشی از شکستگی باز در خرگوش

نگارنده:
دکتر احمد عباس زاده

اساتید راهنما:
جناب آقای دکتر سید شهنام موسوی

اساتید مشاور:
جناب آقای دکتر محمد علی قاسمی

شماره پایان نامه:
U-93188

تاريخ تصویب پایان نامه:
۹۳/۱۱/۱۵

تاريخ دفاع پایان نامه:
۹۵/۳/۴