# THE USE OF MONOLAYER CULTURED CHONDROCYTES AS A CELLULAR MODEL IN DETERMINING THE EFFECT OF *QUR'ĀNIC* RECITATION ON PRIMARY CELLS GROWTH *IN-VITRO*

BY

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# ABSTRACT

In Islamic tradition, the use of *Qurʾānic* recitation as sound healing for therapeutic purposes can be traced back to the times of *Prophet Muhammad* (ﷺ). Known to have an impact on emotional reactions, the number of researches done concerning sound healing has grown. It has been used in dealing with various pyscho-spiritual health problems. It is believed that not only the brain or the auditory cell react to the sound but other cells will respond to it as well. Hence, sound healing may be used to address degenerative diseases such as osteoarthritis. Cartilage is an avascular tissue made of only one cell type, chondrocytes which has minimal self-repair capacity due to the low mitotic property. Once injured and left untreated, degeneration changes will precede ageing and can be progressive. Limitations in currently available treatments for osteoarthritis is noted. These therapies serve only as a temporary measure to alleviate pain but not treating the disease. Seeking alternative ways to facilitate cartilage repair and regeneration thus become crucial. The development of a cellular model can simulate the actual microenvironment of the human body. This study evaluated the effects of the *Qurʾānic* recitation on the growth of chondrocytes. The cellular model was established using serially cultured chondrocytes *in-vitro*. The model consists of five groups exposed individually to (1) *Qurʾānic* recitation of *Surah al-Fatihah* (QR), (2) Arabic poem recitation (APR), (3) Western poem recitation (WPR), (4) speaker with no sound (SP) and (5) control group with no intervention. The exposure duration to all groups was set to 12 minutes. All groups were evaluated for cellular structure and function using microscopic evaluation via standard histology and immunocytochemistry staining. Growth kinetics, scratch assay, chondrogenic genes expression, and sulphated glycosaminoglycan (sGAG) assay are the other relevant parameters included in the study. The microscopic observation of the chondrocytes showed that the QR group maintained its chondrocytic morphology better than the other cell groups. The chondrocytes in other groups exhibited dendritic-like appearance and adopted a more fibroblastic appearance at later passages. Haematoxylin & Eosin (H&E), Alcian blue and Safranin O staining indicated the presence of cartilaginous matrix in all groups. The QR cell showed better collagen type II expression than the other groups. Collagen type I was co-expressed in all groups throughout passages. Overall, growth kinetics profile showed that the number of chondrocytes in all group increased steadily. The cell viability and growth rate are higher for the cells exposed to QR. The QR group also showed a faster and better healing effect on the scratch assay. The proliferation was rapid, and the time to heal was reduced. The overall results showed that the QR exposed cells showed better chondrogenic markers expression, especially collagen type II, than the other groups. The sGAG content increased gradually in all groups. This study suggests that the *Qur'ānic* recitation may facilitate in cartilage repair and regeneration.

# خلاصة البحث

وفقا للنصوص الإسلامية يرجع استخدام تلاوة القرآن كعلاج صوتي للاستشفاء إلى عصر النبي محمد (ﷺ)، ونظرا لتأثيره على ردود الفعل العاطفية فقد ازداد عدد الأبحاث التي أجريت عن العلاج بالصوت، وقد تم استخدامه في التعامل مع مختلف الأمراض الروحية النفسية. من المعتقد أن التفاعل مع الأصوات لا يحدث فقط في الدماغ أو الخلايا السمعية ولكن الخلايا الأخرى تستجيب للأصوات أيضًا، وبالتالي فإنه بالإمكان استخدام العلاج الصوتي لمعالجة الأمراض التنكسية مثل هشاشة العظام. الغضروف هو نسيج لاوعائي مكون من نوع واحد فقط من الخلايا وهي الخلايا الغضروفية والتي لديها قدرة إصلاح ذاتية محدودة بسبب خاصية الانقسام المنخفض فيها، وعندما تصاب وتترك بدون علاج ستستبق التغيرات التنكسية فيها التقدم في العمر وبإمكانها أن تسوء تدريجيا. العلاجات المتاحة حاليا لالتهاب المفاصل العظمي محدودة، وتعمل هذه العلاجات فقط كإجراء مؤقت لتخفيف الألم وليس لشفاء المرض. ولذلك أصبح البحث عن طرق بديلة لتسهيل إصلاح الغضاريف وتجديدها أمرًا بالغ الأهمية. تطوير نموذج خلوي بإمكانه أن يحاكي البيئة الدقيقة الفعلية لجسم الإنسان، وبهذا قيمت هذه الدراسة آثار تلاوة القرآن على نمو الخلايا الغضروفية. تم إنشاء النموذج الخلوي باستخدام خلايا غضروفية مستنبتة في المختبر. تألف النموذج من خمس مجموعات تم تعريضها للأصوات بشكل فردي على كل من: (1) تلاوة لسورة الفاتحة، و (2) تلاوة لقصيدة عربية، و (3) تلاوة لقصيدة غربية، و (4) مكبر صوت بدون صوت، و (5) مجموعة ضابطة بدون أي تدخل. تم تحديد 12 دقيقة كمدة التعريض لجميع المجموعات. تم تقييم جميع المجموعات بناء على البنية والوظيفة الخلوية بالتقييم المجهري النسيجي المبدئي والتصبيغ الكيميائي المناعي. تضمنت المعايير الأخرى المتعلقة في هذه الدراسة كلا من: حركية النمو، فحص الخدش، وتعبير الجينات الغضروفية، وفحص الجليكوزامينوجليكان. أظهرت المعاينة المجهرية للخلايا الغضروفية أن مجموعة تلاوة الفاتحة قد حافظت على مظهرها الغضروفي بشكل أفضل من مجموعات الخلايا الأخرى. أظهرت الخلايا الغضروفية في المجموعات الأخرى مظهرًا شبه تغصني وتبنت مظهرًا ليفيًا أكثر في الأطوار اللاحقة. أظهر تصبيغ الهيماتوكسيلين واليوزين (H&E)، والتصبيغ بزرقة الألسيان والصفرانين وجود مصفوفات غضروفية في جميع المجموعات. أظهرت خلايا تلاوة الفاتحة أفضل تعبير للكولاجين من النوع II بشكل أفضل من المجموعات الأخرى، وتم التعبير ثانويا عن الكولاجين من نوع I في جميع المجموعات عبر الأطوار. بشكل عام أظهر أنماط حركية النمو أن عدد الخلايا الغضروفية في كل المجموعة قد زاد بشكل متدرج، وكان معدل بقاء ونمو الخلايا أعلى في الخلايا المعرضة لتلاوة الفاتحة. أظهرت مجموعة تلاوى الفاتحة أيضا تأثيرا أسرع وأفضل للشفاء في فحص الخدش، وكان الاستكثار سريعا وتم تسريع وقت الشفاء. أظهرت النتائج إجمالا بأن الخلايا المعرضة لتلاوة الفاتحة قد أظهرت تعبيرًا أفضل للمؤشرات الغضروفية، وخاصة الكولاجين من نوع II مقارنة بالمجموعات الأخرى، وقد ازداد محتوى الجليكوزامينوجليكان تدريجيًا في جميع المجموعات. وبهذا أشارت هذه الدراسة إلى أن تلاوة القرآن قد تسهل إصلاح الغضاريف وتجديدها.

# APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Health Sciences.

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# DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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There is a famous saying…..

*"Behind a successful man, stands a woman…and behind a successful WIFE, stands a PROUD HUSBAND…"*

*I dedicate this thesis to my beloved husband, Mohd Syahmi bin Samsudin. Thanks to your support and never-ending love for me during this journey. Your confidence never failed to motivate me. I love you. May Allah bless our marriage till Jannah.*

*To my late son (Muhammad) may Allah bless you and reunite us in Jannah. I love u so much dear (Al-Fatihah).*

*To Muhammad Ali bin Mohd Syahmi, Mama loves you so much. Thank you Allah for Your greatest gift. May you always be successful in whatever you do in your life, here and hereafter. InsyaAllah.*

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# LIST OF ABBREVIATION

| | |
|---|---|
| AA | Antibiotics-Antimycotic |
| APR | Arabic Poem Recitation |
| BSC | Biohazard Safety Cabinet |
| $CaCl_2$ | Calcium Chloride |
| cDNA | Complementary Deoxyribonucleic Acid |
| CELPAD | Centre of Languages and Pre-University Academic Development |
| dB | Decibels |
| DMSO | Dimethylsulfoxide |
| DNA | Deoxyribonucleic Acid |
| ECM | Extracellular Matrix |
| EDTA | Ethylenediaminetetraacetic Acid |
| EtBr | Ethidium Bromide |
| FBS | Foetal Bovine Serum |
| GAGs | Glycosaminoglycan |
| GuHCI | Guanidine Hydrochloride |
| H&E | Haematoxylin & Eosin Staining |
| HCl | Hydrochloric Acid |
| HEPES | 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid |
| KAHS | Kulliyyah of Allied Health Sciences |
| KCl | Potassium Chloride |
| ICC | Immunocytochemistry |
| IIUM | International Islamic University Malaysia |
| $MgCl_2$ | Magnesium Chloride |
| NaOH | Sodium Hydroxide |
| $Na_2HPO_4$ | Sodium Phosphate Dibasic |
| PBS | Phosphate Buffered Saline |
| PCR | Polymerase Chain Reaction |
| PDT | Population Doubling Time |
| RT | Reverse Transcription |
| QR | *Qurʾānic* Recitation |
| RNA | Ribonucleic Acid |
| SD | Standard Deviation |
| SEM | Standard Error of Mean |
| sGAG | Sulphated Glycosaminoglycan |
| SLM | Sound Level Meter |
| SP | Speaker on with no sound |
| SPL | Sound Pressure Level |
| TERM | Tissue Engineering and Regenerative Medicine |
| TAE | Tris-Acetate-EDTA |
| TNCD | Total Number of Cell Doubling |
| UV | Ultraviolet |
| WPR | Western Poem Recitation |

# LIST OF SYMBOLS

| | |
|---|---|
| $^{o}C$ | Degree Celsius |
| MPa | Mega pascal |
| L | Litre |
| ml | Millilitre |
| mg | Milligram |
| rpm | Revolutions per minute |
| µm | Micrometre |
| µM | Micromolar |
| µl | Microlitre |
| µg | Microgram |
| ng | Nanogram |
| ± | Plus-minus |
| = | Equal |
| > | More than |
| < | Less than |

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background of The Study

Sound has been a part of human life as it is widely used or aired while travelling, working, thinking, managing mood, healing, expressing self, and support reminiscence (O'Callaghan, McDermott, Michael, Daveson, Hudson, & Zalcberg, 2014). The authors said that sound based on one's preference will be able to enhance meaning, mood, improve energy levels and work life. The process of healing with sound is as old as the existence of the sound itself (Gelfo, 2012). Apart from psychological or spiritual enhancement, Sulong, Khalil, Dahari and Zakaria (2016) in their study reported that the different types of sound result in different growth rates on certain orchid species. They also deduced that sound was able to provide a positive effect on the *in-vitro* seed germination and plant growth rate. A study done by Lestard and Capella (2016) suggests that human breast cancer cells experienced cell growth arrest and/or apoptosis after exposure to sound. The above examples suggest that sound may have direct or indirect effects on human, physically and spiritually. The interaction between sound and cell has become an exciting topic to be explored (Sarvaiya & Kothari, 2015).

In *Islam, Qurʾānic* recitation has already been used as a method for treatment since the times of *Prophet Muhammad* (ﷺ). According to El-Hady and Kandeel (2017), based on the concept of faith in *Islam, Muslims* accepted that the Holy *Qurʾān* is *Kalamullah* (the words of *Allah*). The Holy *Qurʾān* is one of the revealed scriptures and included in the six articles or pillars of faith (*Arkanul Imān*). Along with this Holy *Qurʾān, Muslims* also believe in *hadith*, on the healing properties that contained in the *Qurʾān* verses. Both the Holy *Qurʾān* and *hadith* are major sources of *Shariʿah* (the

Islamic law). A study conducted by Shafiei, Salari, and Sharifi (2011) proposed that *Qurʾānic* recitation is also one type of music or sound that contained magnificent miracles with specific characteristics and its unique rhythm. This research investigated the healing effect through the incantation of one specific chapter in the Holy *Qurʾān*, which is the *Surah al-Fatiḥah* on the monolayer chondrocytes culture. This study uses the chondrocytes as the subject of interest as the loss of chondrogenic property, and replicative senescence has been well documented in monolayer cultured chondrocytes (Hubka, Dahlin, Meretoja, Kasper, & Mikos, 2014).

According to *Tafsir Ibn Kathir, Surah al-Fatiḥah* contained various virtues that could qualify it as a *ruqyah* or a healing incantation. It was proven during the time of the *Prophet Muhammad* (ﷺ) when a clan leader was inflicted with a disease, and one of the *Sahabah* recited *Surah al-Fatiḥah* upon the sick clan leader, and by God's will, the clan leader was cured of the disease (*Sunan Ibn Majah*, Chapter 14, *Hadith* Number 2156). From this *hadith,* it can be assumed that this particular *Surah* might contain healing properties that can be used as treatment. However, there is no scientific evidence has been documented which support the use of *ruqyah* as treatment for physical illness. Thus, a scientific approaches are required in order to verify that the *Qur'ānic* recitation can be used as a healing modality in treating physical illness.

The advancement in tissue engineering (TE) may provide a solution to this problem. As part of the biological therapy, research in TE has magnified and gains much popularity over the years. TE opens the possibility of circumvention of the use of pharmaceutical drugs or therapies (Mason & Dunnill, 2008). It is an alternative field suggested by scientists to overcome the shortage of organ donor, tissue transplants for diseased and failed or malfunction organ. They are three main components that play significant roles in TE. They are also known as tissue engineering triad, which includes

(1) cell sources, (2) signaling factors and (3) biomaterial scaffolds. The use of *Qurʾānic* recitation in this study, from the scientific context, was intended to represent a signaling factor or a biophysical stimulus in directing the growth and differentiation of chondrocytes. The present author feels that the *Qurʾānic* recitation might have potential effects on the chondrogenic differentiation on the monolayer chondrocytes culture.

### 1.2 Statement of The Problem

There has been an increased of interest in applying *ruqyah* among *Muslims* worldwide. *Ruqyah* can be defined as ways of healing or treating diseases using the incantation of the *Qur'ānic* recitation (Adynata & Idris, 2016). Previous researches associated with the *Qurʾānic* recitation were more focussed on the psychological or spiritual healing aspect compare to the physical context. It has been documented that the sound will affect not only the brain but as well as other cell including both the auditory and non-auditory cells (Norris, 2011). However, there is still a lack of study on the effects of the *ruqyah* towards the cell.

Articular cartilage has limited regenerative capabilities due to the lack of blood supply. Damages to the cartilage may lead to progressive degeneration if left untreated. Treatment such as joint replacement surgery may have a painful effect or post-surgical complications. Furthermore, the healthcare cost to treat cartilage damage is expensive. The duration for post-rehabilitation is time-consuming and regular check-up is necessary for the post-treatment procedures.

This research used monolayer cultured chondrocytes as a cellular model in determining the effect of the *Qurʾānic* recitation on primary cells growth *in-vitro*. The cell used in this study was chondrocytes derived from the rabbit's cartilage. This study looked at the feasibility of using a non-invasive means of treatment by using

the *Qur'ānic* recitation in the combination of tissue engineering technique. The use of the *Qur'ānic* recitation as a means of treatment would be affordable and no extra pain introduced. To date, there is no systematic study and information on the relationship between the *Qurʾānic* recitation and the healing properties at the cellular level. Hence, this study was designed to study the potential of the sound healing using *Qurʾānic* recitation in sustaining the chondrogenic property on serially passaged chondrocytes through the observation of cellular morphology and cartilaginous matrices production in monolayer cell culture setting.

## 1.3 Research Objectives

### 1.3.1 General Objective

To evaluate cellular growth and chondrogenic properties of the *in-vitro* serially passaged monolayer cultured chondrocytes undergoing exposure to various sounds namely a *Qurʾānic* recitation (*Surah al-Fatihah*), an Arabic poem recitation, a Western poem recitation and a switched on-speaker with no sound.

### 1.3.2 Specific Objectives

The study aimed to achieve the following objectives:

a. To identify the optimum time exposure of various sounds on the monolayer cultured chondrocytes based on the 17 times of complete recitation of *Surah al-Fatihah* verses.

b. To evaluate and compare the effects of various sounds exposure on the morphology and phenotypic trait of the monolayer cultured chondrocytes in serial passages using standard histological and immunocytochemistry evaluation.

c. To evaluate and compare the effects of various sounds exposure on the growth and proliferation of the monolayer cultured chondrocytes in serial passages using growth kinetic profile analysis and wound healing assay.

d. To evaluate and compare the effects of various sounds exposure on the chondrogenic properties of monolayer cultured chondrocytes using cartilaginous gene expression analysis and sulphated glycosaminoglycan (sGAG) production assay.

## 1.4 Research Hypothesis

The *Qurʾānic* recitation (*Surah al-Fatihah*) will promote positive effects on the cellular growth and the chondrogenic properties of the *in-vitro* serially passaged monolayer cultured chondrocytes better than the Arabic poem recitation, the Western poem recitation and the switched on-speaker with no sound.

## 1.5 Significance of The Study

This present study managed to relate the use of *Qur'ānic* recitation as sound healing at the cellular level. From the results, it can be seen that different sounds will have different effects on the cell. The *Qur'ānic* recitation provides a positive effect on the cell and might have a significant role in the development and treatment of the cartilage. There was no invasive method used during the study. It showed that this sound healing method will not cause harm or unpleasant effect on the cell or tissue involved. This study might contribute to the decision making especially before any intervention related to articular cartilage degeneration or development is required. The findings from this present study give additional information for future works involving sound healing, particularly, the use of *Qur'ānic* recitation on healing.

## 1.6 Limitation of The Study

This present study used a monolayer cultured chondrocytes, harvested from a commercially available healthy rabbit's knee. A normal trend of healthy cultured chondrocytes can be seen in all groups especially the control. However, to observe the ability of the different sound exposures in influencing the growth and proliferation of the chondrocytes, it is best to use diseased or unhealthy cell. It is hoped that with the used of the unhealthy cell, the healing properties of the sound can be appreciated more.

Animal cell were used in this study. This is not a representative of human sample. The outcome of this study could not be applied to the human until further research on the human cell is conducted. Human cell could be used to observe the effect of sound exposure directly to the human. This study also limited only to the two-dimensional (2D) cell culture setting. It could not represent the actual anatomy of the cartilage. The chondrocytes also lost its phenotypic characteristic during the monolayer culture. Thus, the actual effect of the sound might not be fully understood. Further study on the chondrocytes cultured in three-dimensional (3D) or scaffolds are suggested.

In this study, the method applied during the growth kinetics profile analysis was Trypan blue assay. This method only permits the visualization of dead cells based on the loss of membrane integrity (Lestard & Capella, 2016). The authors mentioned that it could not distinguish between necrotic or late apoptotic cell. It would be interesting to know whether cell death was due to apoptosis, which is a more physiological type of cell death, or necrosis, which would indicate an abrupt, sudden, death. A cell cycle analysis should be done in future.

## 1.7 Definition of Terms

a) Sound

The definition of sound as outlined by the Cambridge dictionary (http://dictionary.cambridge.org/dictionary/english) is "*something that you can hear or that can be heard*". Sound can also be explained as a mechanical wave that will cause agitation in the medium when it passes through it (Lestard, Valente, Lopes, & Capela, 2013).

b) Healing

The definition of the healing by the Cambridge dictionary (http://dictionary.cambridge.org/dictionary/english) is "*the process of becoming well again, especially after a cut or other injury, or of making someone well again or the process in which a bad situation or painful emotion ends or improves*".

c) Therapy

The term therapy is defined by the Cambridge dictionary (http://dictionary.cambridge.org/dictionary/english) as "*a treatment that helps someone feel better, grow stronger, etc., especially after an illness*".

d) Sound healing

Sound healing can be defined as *"the intentional use of sound to promote greater wellness of body, mind, spirit, community, or environment, or to facilitate greater interconnection and balance of these elements through the use of sound*" (Gelfo, 2012).

e) Frequency

The Cambridge dictionary (http://dictionary.cambridge.org/dictionary/english) defined frequency as "*the number of times that a wave, especially a light, sound, or radio wave, is produced within a particular period, especially one second*". Frequency is the number of vibrations per second, also known as cycles per second, Hertz-Hz (Raghu, 2018).

f) Wavelength

From the Cambridge dictionary (http://dictionary.cambridge.org/dictionary/english), wavelength can be decribed as "*the distance between two waves of energy, or the length of the radio wave used by a particular radio station for broadcasting programmes"*. According to Raghu (2018), Wavelength is the length of a single wave or the spacing of a wave.

g) Resonance

Cambridge dictionary (http://dictionary.cambridge.org/dictionary/english) defined resonance as "*the production of a sound as a result of vibration (shaking) of another object*". Raghu (2018) explained when one object vibrating at the same natural frequency with the second object, it will forced the second object into vibrational motion kown as resonance.

h) *Ruqyah*

According to Adynata and Idris (2016), *ruqyah* is a healing incantation using the verses from the Holy *Qurʾān* and it is one of the ways of treating diseases and disorders.