INTRODUCTION:
Low-level laser therapy (LLLT) has been a widely used and well-accepted physical therapy modality for the management of tendon disorders. The physiological effects attributed to LLLT include pain reduction, accelerated tissue healing and reduction of inflammation. Although several authors have demonstrated the potential of LLLT in the facilitation of the tendon healing process, the molecular and biochemical mechanisms remain to be determined. The therapeutic effect of LLLT could be explained by photostimulation of target tissue and cells. However, how tenocytes sense photonic energy and convert it into cascades of cellular and molecular events that ultimately lead to adaptive physiological changes of tendons is not well understood. This study aims to elucidate the effects of LLLT on cultured tenocytes, and to further investigate the biochemical mechanisms of LLLT promoting tenocyte proliferation and regulating extracellular matrix metabolisms.

METHODS:
Tenocytes harvested from Achilles tendons of Sprague-Dawley rats were used in this study. Ga-As laser at 904nm was used to stimulate tenocytes of SD rat. The MTT assay was used to evaluate mitochondria activity of tenocytes after low-level laser. Adenosine triphosphate (ATP), nitric oxide (NO) and intracellular calcium concentration were determined following LLLT. Synthesis of collagen and transforming growth factor-β1 (TGF-β1) were determined and their gene expression was also studied. All the study procedures received approval of the animal care and use committee of National Yang-Ming University.

RESULTS:
Fig. 1 Effects of LLLT on tenocyte proliferation. MTT assay at 24 and 48 hours revealed significant cytostimulatory effects at both 0.5 and 1 J/cm².

Fig. 2 The level of total collagen in medium at 96 hours was measured by Sircol collagen assay kit.

Fig. 3 Laser-treated tenocytes showed a significant increase in ATP but not NO level.

Fig. 4 Photomicrography of tenocytes submitted to 1 J/cm² LLLT showed increased intracellular calcium level at 15 and 30 min.

Fig. 5 Effects of LLLT on PCNA, collagen types I, collagen type III and TGF-β1 gene expression.

Fig. 6 LLLT-enhanced TGF-β1 gene production through ERK pathway

Results of our study showed low-level laser stimulated tenocyte proliferation and collagen synthesis with an optimal dose of 1 J/cm². The associated tenocyte proliferation was mediated by early up-regulation of PCNA. The mechanisms by which collagen synthesis both in protein and mRNA level were likely mediated by intracellular calcium release and upregulation of TGF-β1 through MAPK/ERK pathway.

DISCUSSION:
Our studies on the molecular and cellular mechanisms of LLLT suggested that photons absorbed by the mitochondria; might induce more ATP production and high levels of intracellular calcium, which then activates ERK pathway to induce TGF-β1 production and many gene transcript products responsible for the beneficial effects of LLLT.

SIGNIFICANCE:
The results of this in vitro experiment provided a clearer understanding of the molecular and biochemical mechanisms of tendon healing and matrix metabolism induced by LLLT. The study also provided scientific basis for LLLT in clinical practice.