



# Comparison of *MMP1* Gene Expression and Collagen Percentage in Response to Polydioxanone, Poly-L-Lactic Acid and Polycaprolactone Thread Implantation in Ageing Rat Skin

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## Abstract

**Background/Aim:** Photoageing causes collagen degradation, leading to sagging skin, increased wrinkles and rough surfaces as signs of skin ageing. UV-B radiation increases reactive oxygen species (ROS) production, affecting the mitogen-activated protein kinase (MAPK) pathways, resulting in the expression of the *MMP1* gene, which can induce collagen fibre degradation. This study aimed to compare the *MMP1* gene expression and collagen percentage in response to polydioxanone (PDO), poly-L-lactic acid (PLLA) and polycaprolactone (PCL) thread implantation in ageing rat skin.

**Methods:** The study involved female Wistar rats, divided into six groups: young rats without UV-B exposure, old rats without UV-B exposure, ageing model rats exposed to UV-B and ageing model rats implanted with PDO, PLLA and PCL threads. UV-B exposure was given 3 times per week for 4 weeks with a total dose of 840 mJ/cm<sup>2</sup>. Parameters assessed included *MMP1* gene expression and skin collagen percentage.

**Results:** Data analysis showed that the ageing model skin organ had higher *MMP1* gene expression ( $p < 0.05$ ) and lower collagen percentage ( $p < 0.05$ ) compared to young rats without UV-B exposure. *MMP1* gene expression in the three thread implantation groups was equivalent to other groups ( $p > 0.05$ ). Collagen percentage was equivalent in the three thread treatment groups ( $p > 0.05$ ), higher ( $p < 0.05$ ) than an ageing model, but did not differ ( $p > 0.05$ ) from young rats without UV-B exposure.

**Conclusions:** The study concludes that the implantation of the three types of threads, PDO, PLLA and PCL, does not reduce *MMP1* gene expression but has an equivalent ability to increase collagen percentage in ageing rat models.

**Key words:** Matrix metalloproteinases; Skin laxity; Collagen; Polydioxanone; Poly-L-lactic acid; Polycaprolactone.

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## Introduction

The skin can reflect a person's health condition and the ageing process.<sup>1</sup> Factors contributing to the ageing process include intrinsic and extrinsic

factors. The most significant extrinsic factor that accelerates skin ageing is exposure to sunlight, which contains ultraviolet (UV) rays; hence, this

type of skin ageing is referred to as photoageing.<sup>2,3</sup> Around 80 % of skin ageing is caused by sun exposure. The clinical features of photoageing include rough skin, dryness, hypo- or hyperpigmentation, deep wrinkles, telangiectasia and precancerous lesions.<sup>4</sup>

The accumulation of UV radiation in collagen fibres within the dermal tissue of the skin increases the production of reactive oxygen species (ROS). The rise in ROS production leads to an increase in free radicals, which activate the signalling pathways of nuclear factor-kappa B (NF- $\kappa$ B) and mitogen-activated protein kinase (MAPK). Both pathways result in the elevation of inflammatory cytokine tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and matrix metalloproteinases (MMPs). The increased expression of MMPs, consisting of *MMP1* and *MMP3*, as well as TNF- $\alpha$ , is associated with skin photoageing.<sup>5</sup> *MMP1* in the extracellular matrix of the dermis can induce the degradation of collagen fibres, leading to deeper wrinkles, reduced skin elasticity, sagging skin and a rough surface, which are signs of skin ageing.<sup>6-8</sup>

Collagen is the main component in the structure of dermal tissue and serves as the primary connective tissue in the dermis. Type I collagen is the most abundant in the dermis, with thick fibres provide firmness to the dermal structure, while type III collagen consists of thin fibres that support the skin.<sup>9,10</sup> The degradation of collagen involves MMP enzymes, categorised as collagenases. Collagenase-type MMPs bind to the interstitial regions of type I, II and III collagen and can damage extracellular membrane molecules and other proteins.<sup>11,12</sup> Specifically, *MMP1* initiates the degradation of type I and III collagen fibres in the skin.<sup>6,7</sup>

As times have progressed, skin health and beauty have become important indicators of overall well-being, leading to an increasing demand for beauty products, including anti-ageing products.<sup>13</sup> One of the clinical treatments involves the use of threads. There are various types of threads, including polydioxanone (PDO), poly-L-lactic acid (PLLA) and polycaprolactone (PCL). These threads can tighten the skin quickly through a mechanical method and stimulate the tissue to produce new collagen (neocollagenesis).<sup>14</sup>

Septiadery's 2016 study showed that PDO thread implantation therapy in rat for four weeks was quite effective in increasing neocollagenesis in

tissue exposed to UV-B radiation.<sup>15</sup> Kapicioğlu et al research in 2019 found a significant increase in collagen fibres in skin implanted with PLLA threads compared to the control, as observed through Masson's trichrome staining.<sup>16</sup> Cho et al study comparing the efficacy of PCL, PLLA and PDO threads revealed that PCL thread implantation was the most effective in enhancing neocollagenesis in the skin, beginning in the fourth week of the study.<sup>14</sup>

Based on these findings, this research aimed to compare the neocollagenesis effects by examining the differences in *MMP1* expression and collagen percentage in response to PDO, PLLA and PCL thread implantation in an ageing mouse model, specifically older rat exposed to UV-B radiation.

## Methods

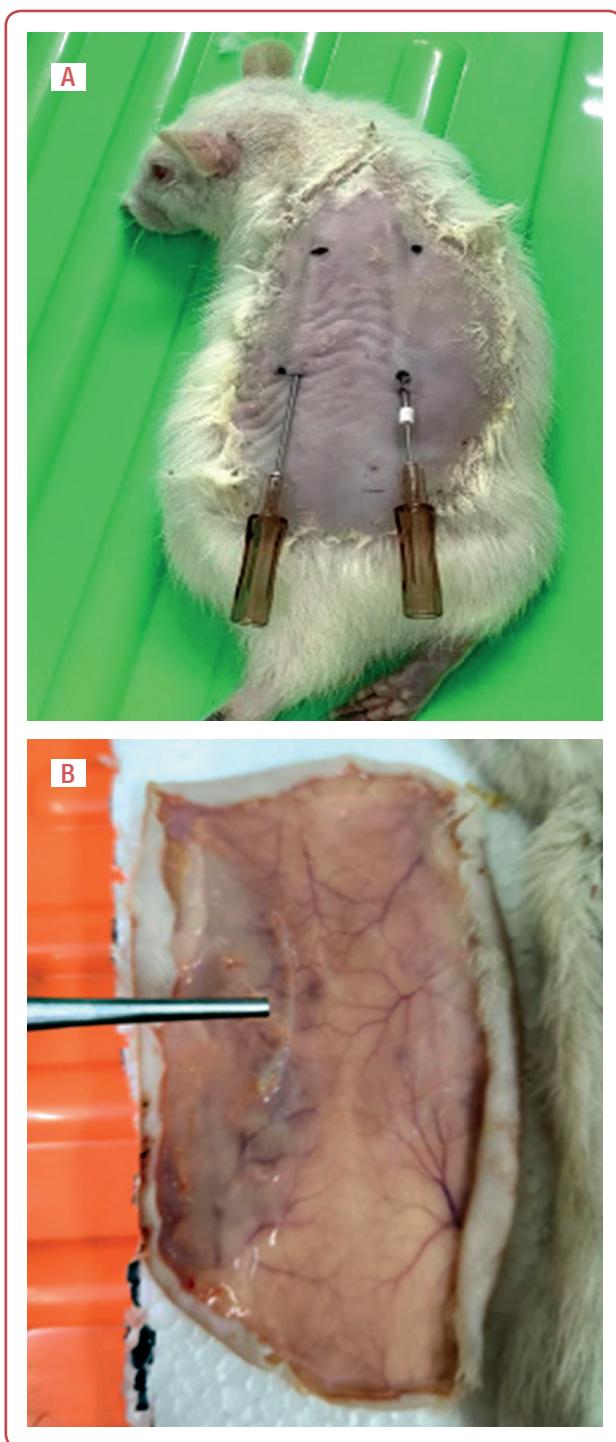
The research was conducted at the Animal Testing Laboratory and the Maranatha Biomedical Research Laboratory – Faculty of Medicine, Maranatha Christian University (FK-UKM), Bandung, with Masson's Trichrome staining performed at the Anatomical Pathology Department of Hasan Sadikin Hospital, Bandung.

## Treatment

In this study, the elderly rats were female rat aged 15-20 months, equivalent to 45-50 years in humans, when women typically enter menopause.<sup>17</sup> The ageing mouse model consisted of 15-20-month-old rat exposed to UV-B radiation. A total of 25 rat aged 15-20 months and 5 rat aged 6-9 months were adapted for one week in cages. On the eighth day, the older rats were randomly divided into five groups, each consisting of five rats. All rats had the hair on their backs shaved in an area of 6x5 cm. The groups included YC (control group of young rat not exposed to UV-B), OC (control group of old rat not exposed to UV-B), AC (control group of old rat exposed to UV-B, ie, ageing model rat), T1 (ageing model rat implanted with PDO thread), T2 (ageing model rat implanted with PLLA thread) and T3 (ageing model rat implanted with PCL thread). All groups received treatments for four weeks.<sup>15</sup>

After the group division, the rats were anaesthetised with ketamine at two mg/kg body weight. An entry point was made into the skin using an

18 G needle and the thread was then inserted into the skin with a cannula (Figure 1). Once the rat regained consciousness, they were exposed to UV-B radiation three times per week, with a dose of  $50 \text{ mJ/cm}^2$  (50 seconds) in the first week,  $70 \text{ mJ/cm}^2$  (60 seconds) in the second week and  $80 \text{ mJ/cm}^2$  (80 seconds) in the third and fourth weeks.<sup>15</sup>



**Figure 1:** (A) Thread implantation in the skin of the mouse's back before the cannula is removed. (B) The pointer shows the thread inside the skin of the mouse's back.

After all treatments were completed, the rats were euthanised by cervical dislocation following an 8-hour fasting period and were injected intraperitoneally with ketamine at a dose of 100 mg/kg body weight for anaesthesia. A skin biopsy measuring 5x5 cm was performed on the rat's backs.

### Examination of *MMP1* gene expression

Skin tissue weighing 50-100 mg was cut into small pieces and placed into 1.5 cc Eppendorf tubes. The skin tissue is flash-frozen with liquid nitrogen to prevent degradation. A total of 0.5 cc of GENEzol™ was added to the tube, the skin tissue was ground until completely broken down, then another 0.5 cc of GENEzol™ was added. The Eppendorf tube was kept at room temperature for 5 minutes, then 0.2 cc of chloroform was added and the tube was sealed with aluminium foil. The tube was vortexed for about 10-15 seconds until the solution became homogeneous, then left to stand for 3 minutes at room temperature. The sample was then centrifuged at 11,200 RPM, 4 °C, for 15 minutes, resulting in the formation of three layers. A total of 0.6 cc of the supernatant was transferred into a new Eppendorf tube, followed by the addition of 0.5 cc of isopropanol, maintaining an isopropanol: supernatant ratio of 1:1. The Eppendorf tube was inverted repeatedly until white strands appear. The tube was centrifuged again at 11,200 RPM, 4 °C, for 10 minutes. After centrifugation, the supernatant was discarded, leaving a white pellet at the bottom of the tube, which was then dried using a tissue base by inverting the microtube for 5-10 minutes. After centrifugation, the supernatant was discarded, leaving a white pellet at the bottom of the tube, which was then dried using a tissue base by inverting the microtube for 5-10 minutes. After drying, 30-50 µL of NFW solution was added, followed by incubation at 55 °C for 10 minutes and the RNA is stored at -80 °C. The total RNA concentration was quantified using absorbance spectrophotometry at 260/280 nm wavelength.

The composition of the PCR mixture was as follows: 10 µL SYBR® Green qPCR Mix + 0.8 µL *MMP1* forward primer + 0.8 µL *MMP1* reverse primer + 1.4 µL PCR water. The PCR mixture was then added to Eppendorf tubes containing 2 µL cDNA, then running the samples in a real-time PCR machine.<sup>18, 19</sup> The sequences of *MMP1* and GAPDH primers are listed in Table 1. *GADPH* is commonly used as a housekeeping gene because it is generally expressed at a constant level across various cell types and experimental conditions, making it a reliable internal control.

**Table 1:** *MMP1 and GAPDH gene*<sup>20, 21</sup>

Gene	Forward primer	Reverse primer
<i>MMP1</i>	5'-CTCCCTTGGACTCACTC ATTCTA -3'	5'-AGAACATCACCTCTCCCC TAAAC -3'
<i>GAPDH</i>	5'-GAGAACCTGCCAAGT ATG -3'	5'-GGAGTTGCTGTTGAAGTC -3'

### Histopathological examination

Rat skin samples were immersed in 10 % phosphate-buffered formalin for 24 hours, after which they underwent embedding for histopathological examination. The materials required included 10 % phosphate-buffered formalin, clearing agent, distilled water, ethyl alcohol, xylene and paraffin. Masson's trichrome stain<sup>22</sup> was used to colour the skin tissue, which was sectioned to a thickness of 5 µm using a microtome. The fixed and stained skin tissue was examined under a microscope

(Olympus CX21LED trinocular microscope with H1600 industrial camera) at magnifications of 4x and 40x. The results were then photographed and the images were imported into Image J 1.54g software. This allowed for the calculation of collagen area pixel counts and the total tissue area pixel counts, enabling the determination of the collagen percentage.<sup>15</sup>

### Data analysis

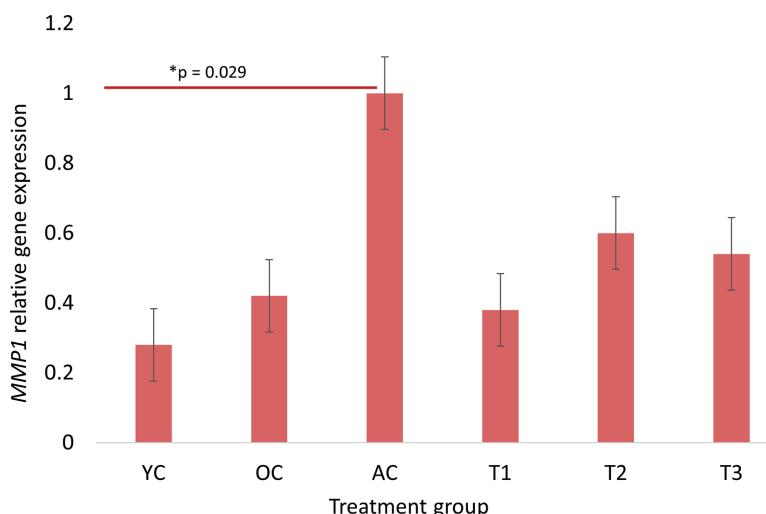
The data for *MMP1* gene expression and collagen percentage were analysed using SPSS software. The *MMP1* gene expression data were found to be normally distributed but heterogeneous, so the statistical analysis was performed using the Kruskal-Wallis test, followed by the Mann-Whitney test. The collagen percentage data were normally distributed and homogeneous, thus a One Way ANOVA was conducted, followed by the Tukey LSD post hoc test for mean differences. Statistical analysis was performed at a 95 % confidence level, with significance determined by a p-value ≤ 0.05, using SPSS software.

## Results

### MMP1 gene expression

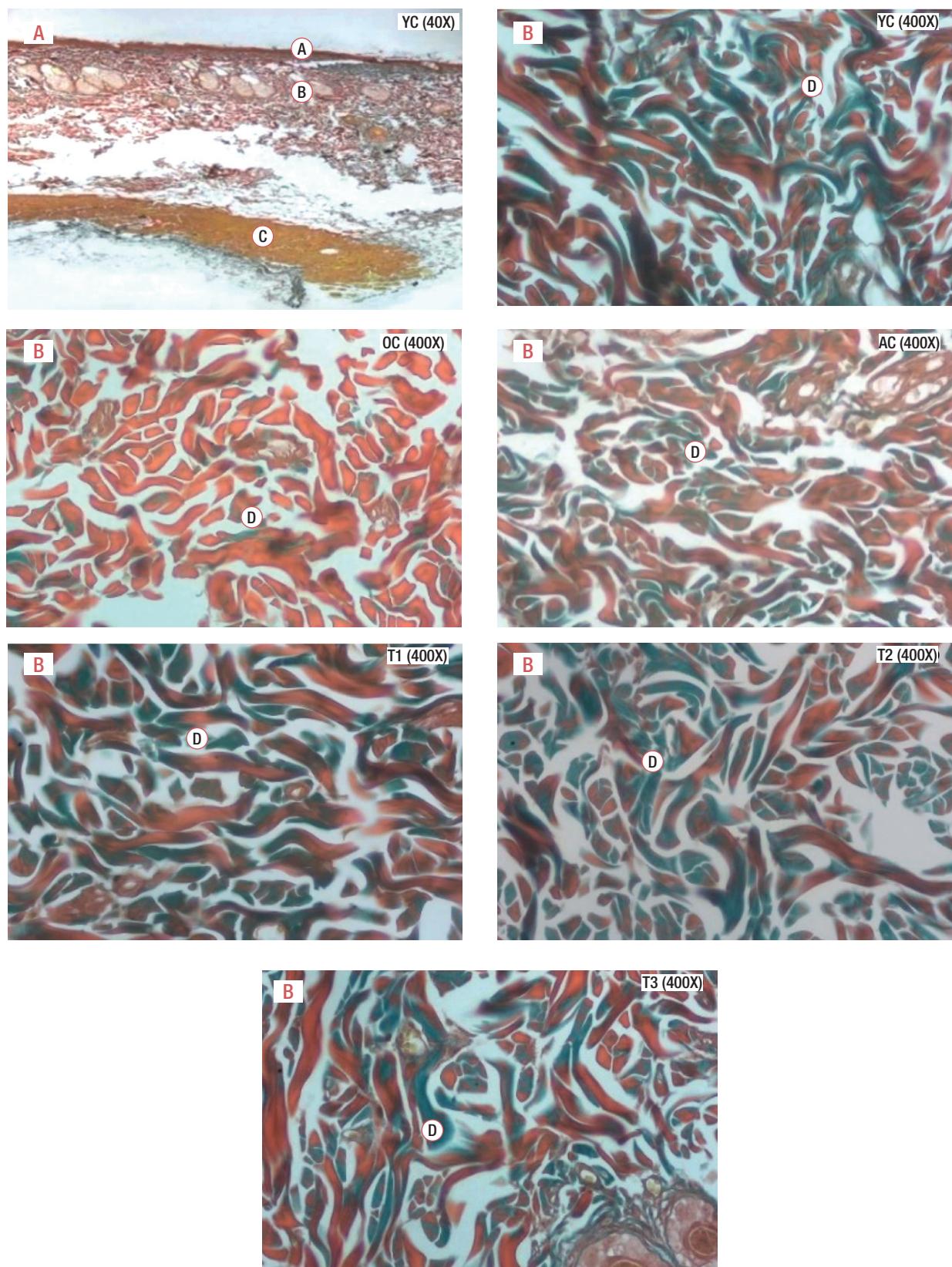
Figure 2 shows that the young rat group without exposure had the lowest *MMP1* gene expression (0.27), while the ageing rat group exhibited the highest *MMP1* gene expression (1.00). The dif-

ference in gene expression between these two groups was significant. The *MMP1* gene expression in the groups of rats treated with the three types of threads showed no significant differences compared to all the other groups.

**Figure 2:** Statistical test results for *MMP1* gene expression

YC: young rat group without UV-B exposure;  
OC: old rat group without UV-B exposure;  
AC: ageing rat group, old rats with UV-B exposure;  
T1: treatment group 1, ageing rats implanted with polydioxanone (PDO) threads;

T2: treatment group 2, ageing rats implanted with poly-L-lactic acid (PLLA) threads;  
T3: treatment group 3, ageing rats implanted with polycaprolactone (PCL) threads; P = p-value;  
\*: significant;



**Figure 3:** Histological features of rat skin stained with Masson's trichrome: A) 40x and B) 400x magnification

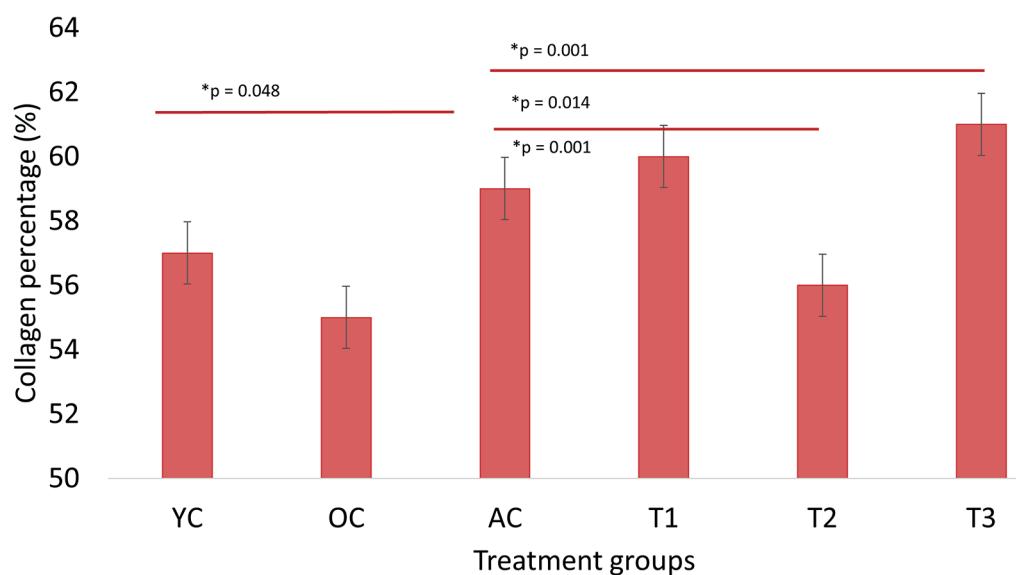
A: epidermis layer of rat skin;  
 B: dermis layer of rat skin;  
 C: hypodermis layer of rat skin;  
 D: collagen fibres appear in bluish-green colour in dermis layer;  
 YC: dermis layer of young rat group without UV-B exposure;  
 OC: dermis layer of old rat group without UV-B exposure;  
 AC: dermis layer of ageing rat group, old rats with UV-B exposure;  
 T1: dermis layer of treatment group 1, ageing rats implanted with polydioxanone (PDO) threads;  
 T2: dermis layer of treatment group 2, ageing rats implanted with poly-L-lactic acid (PLLA);  
 T3: dermis layer of treatment group 3, ageing rats implanted with polycaprolactone (PCL) threads;

## Histological examination

The histological features of rat skin stained with Masson's trichrome at 40x and 400x magnification. At 40x magnification, the layers of rat skin, including the epidermis, dermis and hypodermis can be observed (Figure 3A). Collagen fibres appear bluish-green in the dermis layer of histological image at 400x magnification (Figure 3B).<sup>16,23</sup>

The group of ageing rats model implanted with PDO threads had the highest average collagen percentage (66.01 %), while the untreated age-

ing model group had the lowest (54.40 %). The collagen percentage in the ageing model group was lower than in the young group but showed no difference compared to the old rat group without UV exposure. The collagen percentage in the treatment groups with the three types of threads was higher than in the untreated ageing model group but showed no difference from the young group without UV-B exposure. There was no difference in collagen percentage among the treatment groups with the three types of threads (Figure 4).



**Figure 4:** Tukey LSD test results for collagen percentage comparison in rat groups with Masson's trichrome staining

A: epidermis layer of rat skin;  
YC: young rat group without UV-B exposure;  
OC: old rat group without UV-B exposure;  
AC: ageing rat group, old rats with UV-B exposure;

T1: treatment group 1, ageing rats implanted with polydioxanone (PDO) threads;  
T2: treatment group 2, ageing rats implanted with poly-L-lactic acid (PLLA) threads;  
T3: treatment group 3, ageing rats implanted with polycaprolactone (PCL) threads;  
 $p = p$ -value; \*: significant;

## Discussion

The data analysis results indicate that the skin of the ageing rat model group exhibits higher *MMP1* gene expression and a lower collagen percentage compared to the young rat group without UV-B exposure. The result aligns with the theory that *MMP1* gene expression increases in ageing and photoaged skin. Photoageing from UV-B rays induces ROS, which activates *NF-κB*, *TGF-β* and the transcription factor activator protein-1 (AP-1). The AP-1 transcription factors c-Jun and c-Fos induce *MMP1*, which plays a role in collagen fibre degradation.<sup>8,24</sup> Activation of *NF-κB* also induces *MMP* gene expression.

The *MMP1* gene expression in the rat groups treated with the three types of threads did not differ from that of the ageing model group. This indicates that the implantation of PDO, PLLA and PCL threads does not significantly reduce *MMP1* gene expression.

The collagen percentage was similar across the three treatment groups, higher than in the untreated ageing model group but not different from the young rats without UV-B exposure. This suggests that the implantation of PDO, PLLA and PCL threads increases collagen percentage in the

ageing rat model group, matching that of young rats without UV-B exposure.

Although PDO, PLLA and PCL thread implantation does not decrease *MMP1* expression, it does increase the collagen percentage in the skin of ageing rat model. This finding indicates that thread implantation stimulates an inflammatory response, effectively inducing new collagen formation (neocollagenesis).<sup>14, 25</sup> Numerous molecules in living organisms are involved in this process. *MMP1* is only one molecule that can influence neocollagenesis by specifically initiating the degradation of type I and III collagen fibres.<sup>6,7</sup> Given the complexity of neocollagenesis mechanisms, it is assumed that the increased collagen percentage in the skin of ageing rat model group implanted with threads in this study involves various intracellular molecules such as macrophages, multinucleated giant cells and fibroblasts.<sup>16, 25</sup> However, the *MMP1* gene expression reduction mechanism to inhibit neocollagenesis post-thread implantation is not significant.

Despite the differences in characteristics among PDO, PLLA and PCL threads in terms of materials, durability and mechanical effects, this study found no difference in *MMP1* gene expression or collagen percentage in the skin of ageing rat models implanted with PDO, PLLA and PCL threads. The result may be due to the implantation mechanisms of PDO and PLLA threads, as in the study by Kim et al, which suggests collagen synthesis is stimulated through a subclinical inflammatory reaction,<sup>25</sup> while *MMP1* only acts as an enzyme that can increase collagen degradation via *AP-1* activation, thereby activating *c-Jun* and *c-Fos* in the nucleus.<sup>8, 24</sup> Therefore, future studies can explore the effects of thread implantation on target molecules involved in the neocollagenesis process.

The findings of this study, showing that thread implantation increases collagen percentage or tissue neocollagenesis in ageing rat models, align with previous research in Septiadeary, Kapicioğlu et al, Cho et al.<sup>14-16</sup>

Collagen is a vital component for maintaining skin structure and elasticity. It is the main component of the dermal tissue structure, consisting of natural proteins produced by fibroblasts and the extracellular matrix. Collagen serves as the primary connective tissue in the dermis. It

includes type I, II, III and IV collagen, with type I collagen being the most abundant, comprising 80-85 % of the dermis and providing dermal structure firmness. In contrast, type III collagen comprises 10-15 % of the dermis as a supporting factor.<sup>9, 10, 25</sup>

Threads can stimulate collagen through a biostimulatory reaction, an inflammatory response triggered by macrophage, histiocyte, or multinucleated giant cell encapsulation of microparticles, leading to a granulomatous reaction surrounding the implanted thread in the dermis.<sup>14, 25</sup> Mercik noted that PDO thread implantation affects two mechanisms: the first is mechanical stress in the skin tissue during PDO implantation in the dermis or subcutaneous layer and the second is the stimulation of fibroblasts by PDO material to synthesise collagen.<sup>26</sup> PDO threads are absorbable by the body within six to eight months.<sup>14</sup> Consiglio et al stated that PLLA threads can produce an inflammatory reaction that stimulates collagen formation, with PLLA threads being absorbable within 12 months.<sup>13, 14</sup> Kim et al found that the effect of PLLA threads is a biostimulatory reaction through macrophage, myofibroblast and collagen encapsulation. The initial neocollagenesis reaction is inflammation, followed by microparticle encapsulation. Histologically, granulomatous reactions surrounding PLLA threads, including macrophages and multinucleated giant cells, were observed.<sup>25</sup>

## Conclusion

The study concludes that the implantation of the three types of threads, PDO, PLLA and PCL, does not reduce *MMP1* gene expression but has an equivalent ability to increase collagen percentage in ageing rats models.

## Ethics

This study was approved by the Research Ethics Committee of the Faculty of Medicine, Maranatha Christian University, with approval number 203/KEP/XI/2023, dated 27 November 2023.

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## Conflicts of interest

The authors declare that there is no conflict of interest.

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## Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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Validation: HR

Formal analysis: TLW, HR

Investigation: FKP

Resources: FKP

Data curation: FKP

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Writing - review and editing: TLW, HR

Visualisation: FKP  
Supervision: TLW  
Project administration: FKP  
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