



浙江大学爱丁堡大学联合学院
ZJU-UoE INSTITUTE

Periodic mild heat stimuli diminish extracellular matrix synthesis in pellet cultured human chondrocytes

Ito et al.

Presenter: Wu Shaoran

Introduction

- **Extracellular matrix (ECM)** synthesized by chondrocytes is an important component of **articular cartilage (AC)**.
- Matured ECM has firm structure to resist load bearing, and needs a long time to obtain full weight bearing capacity.



chondrocytes

extracellular
matrix

Background

- AC defects lead to physiological diseases, and AC cannot generate automatically. Autologus chondrocyte implantation is a promising therapy for AC regeneration challenged by ECM synthesis.
- To enhance ECM synthesis and maturation, microenvironmental factors have been investigated for decades.



Fig 2: The sagittal (2a) and coronal (2b) images reveal a focal, fluid signal-intensity abnormality (arrows) extending through the articular cartilage of the lateral femoral condyle. Available from: <https://radsources.us/cartilage-defect-of-lateral-femoral-condyle/>

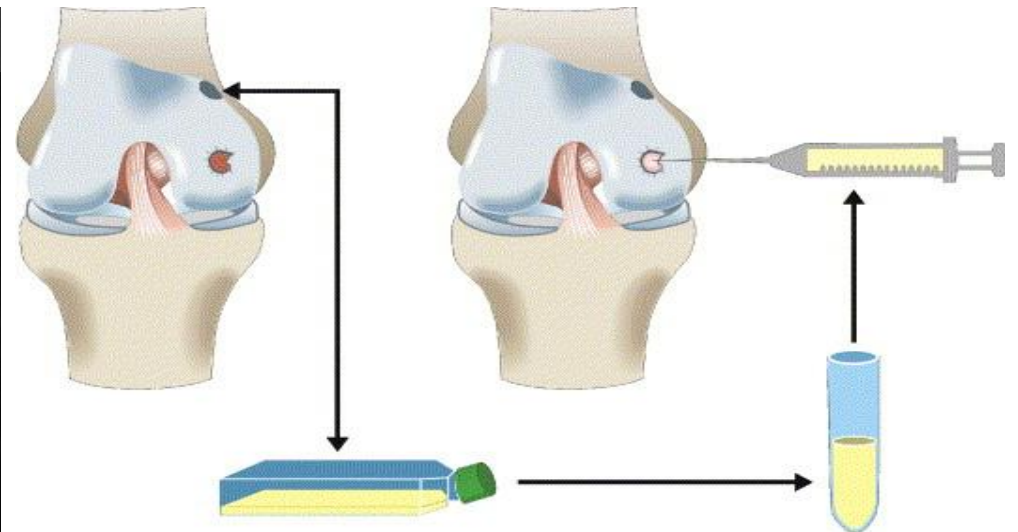


Fig. 1 Basic concept of autologous chondrocyte transplantation (ACT) with cell harvesting, expansion in monolayer cell-culture and implantation. DOI: <https://doi.org/10.1016/j.ejrad.2005.08.009>

Research question

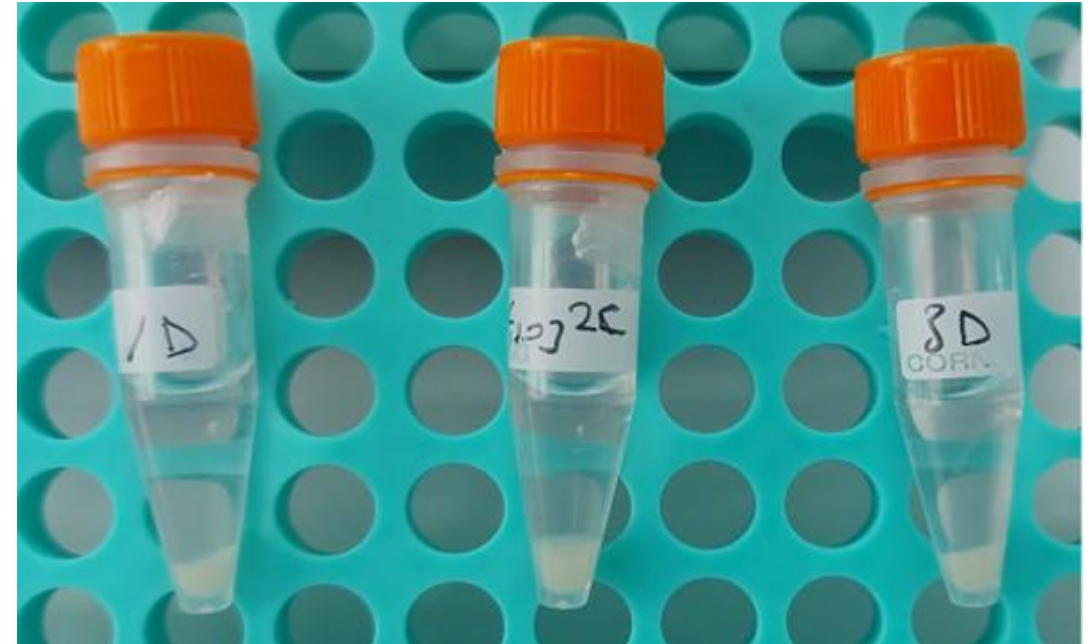
This study explored the effects of periodic heat stimuli to ECM synthesis in pellet cultured chondrocytes.

Hypothesis

Periodic mild heat stimuli enhance ECM synthesis.

Materials

- The chondrocytes were isolated from the AC of a 89-year-old woman, and cultured until passage two.
- Aliquots of 2.5×10^5 cells in tubes were **centrifuged to form pellets**, which provided a **3D environment** to **mimic internal ECM synthesis**.



Available from: <https://services.brieflands.com/cdn/serve/316ab/5ce82ae5ebde6a7399c3b054882fa12b44b88d38/jjm-17-9-148070-g002-preview.png>

Methods

- The controlled experimental method was used in the study.
- The pre-cultured pellets were divided into three groups:
 - experimental group: 32°C + heat stimuli (HS),
 - controlled group: 32°C, 37°C

They were separately cultured under 32°C or 37°C, and the pellets in the experimental HS group were additionally transferred to process HS.

Heat stimuli

- Heat stimuli were given by transferring the pellets into a separate CO₂ incubator **set at 41°C** for 20min/day, 6times/week.

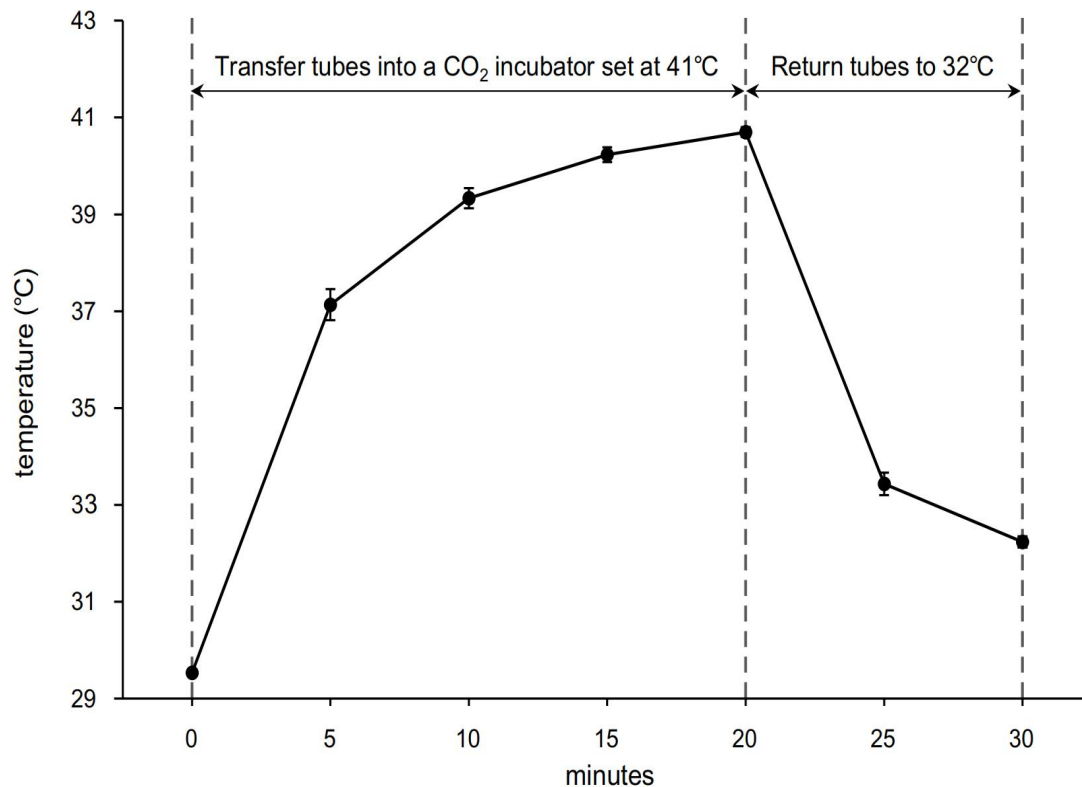


Fig. 1 Temperature changes

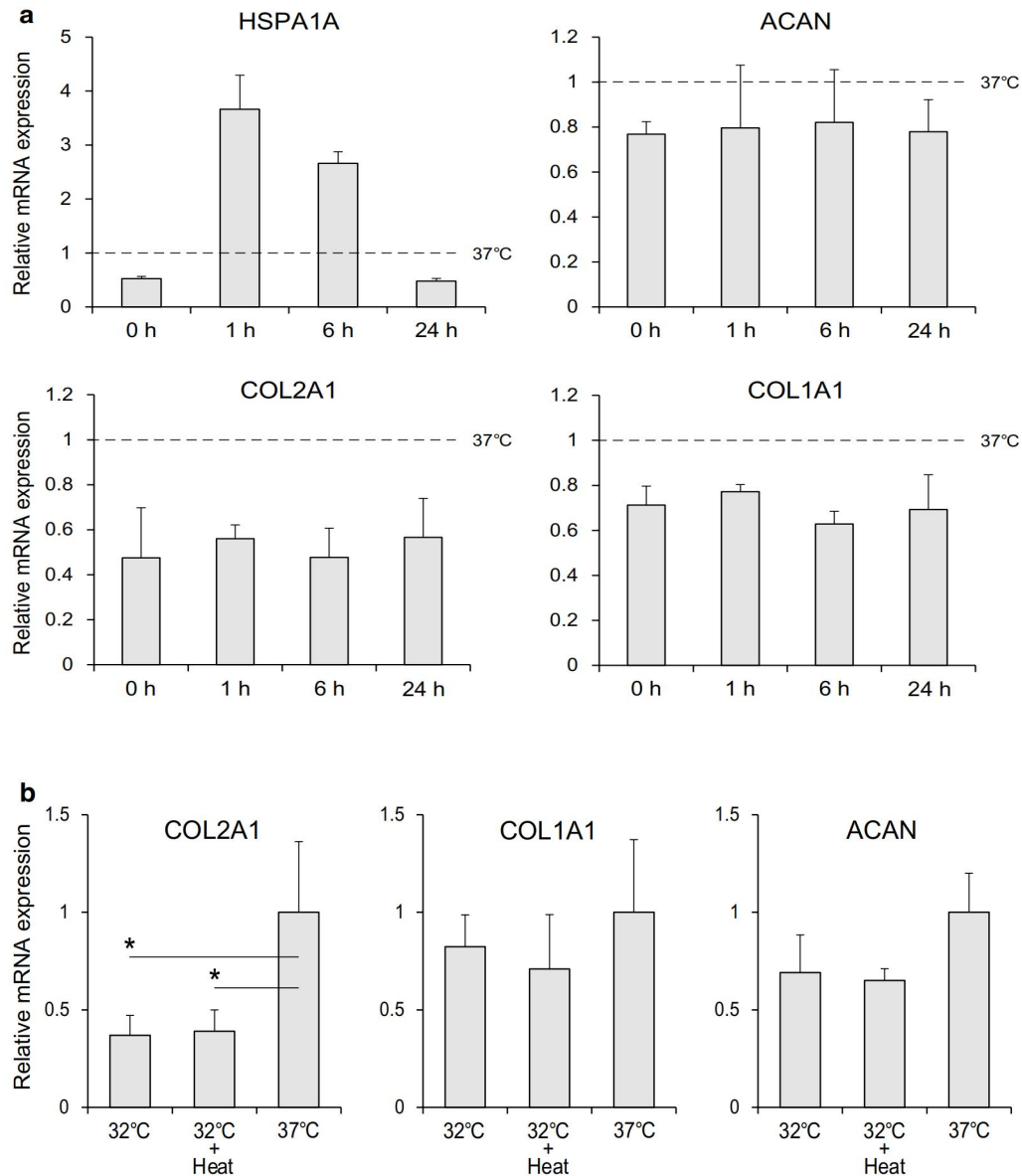
The temperature was **over 40°C at 15 min**, and **immediately dropped** in 10 min after putting back at 20 min.

Related mRNA detect

- Total mRNA was extracted from heat stimulated and controlled pellets before and at 1, 6, 24 h after heat stimulation for **immediate effects**, and on day 7 for **accumulated effects**.
- The mRNA expression of HSPA1A, COL2A1, COL1A1 and ACAN was assed by **qRT-PCR** (quantitative real-time reverse-transcription polymerase chain reaction).

Collagen and proteoglycan detect (on day 21)

- 1) **Macro photography**
- 2) **Wet weight measurement**
- 3) **Quantitative measurement (analyzed with ANOVA):**
 - collagen content–hydroxyproline assay
 - proteoglycan content–DMMB colorimetric assay
 - The values of collagen and proteoglycan were **normalized to DNA content** in order to describe **the amount of these molecules per cell**.



mRNA expression levels

Immediate effects:

- The expression of HSPA1A was upregulated in 1h after HS, but the expression of COL2A1, COL1A1 and ACAN was not changed by HS, and their levels were lower than 37°C group.

Accumulated effects:

- No significant differences in mRNA expression levels between the 32 °C group and the 32 °C+HS on day 7.

Fig. 2 Immediate and accumulative effects of heat stimulus on mRNA expression

(No significant difference between the wet weight of the 32°C and HS group)

- Lower hydroxyproline ($P < 0.05$) & lighter staining intensity
--> **lower collagen content**
- Higher DMMB colorimetric ($P < 0.01$) & deeper staining intensity
--> **higher proteoglycan content**
- The DNA content in HS group was significantly lower.

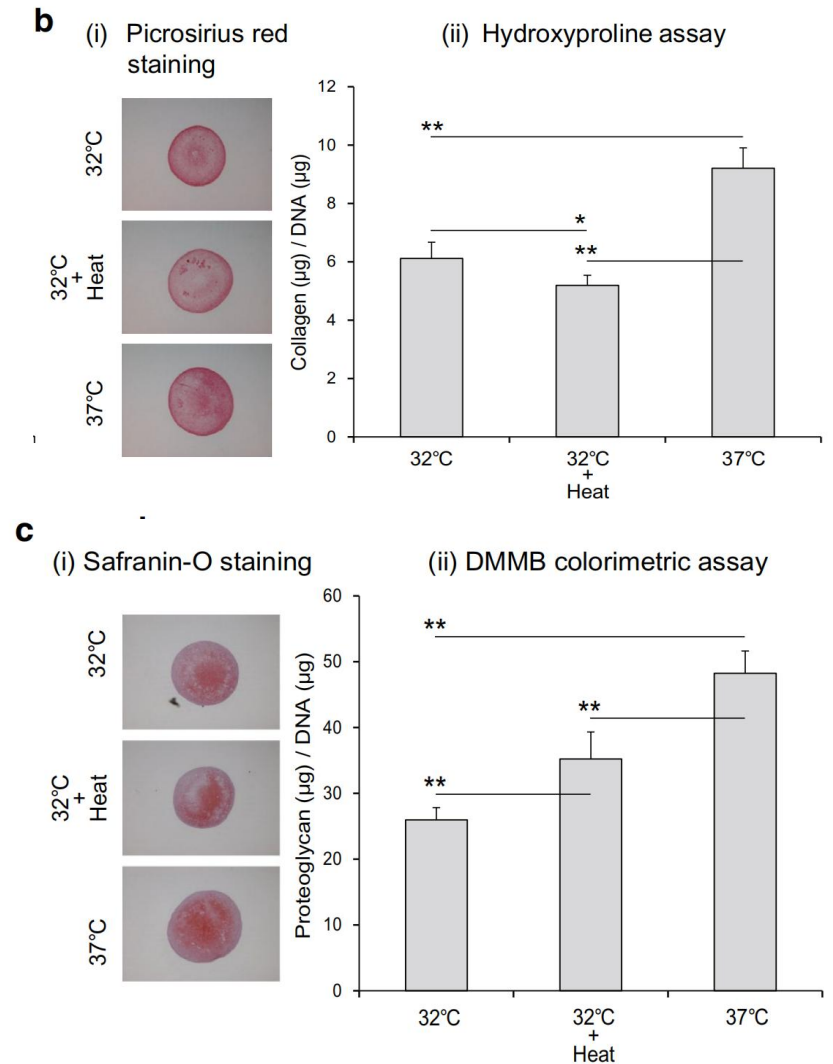


Fig. 3 Collagen and proteoglycan accumulation

Discussion

- The results showed that **the periodic mild HS diminished the accumulation of collagen**, which is the major ECM of AC. (Contrary to hypothesis)
- Additional experiments of 41–45°C performed no positive effects on ECM synthesis.
- Their previous study suggested the negative effect of prolonged exposure to heat stimulation.

Discussion

- No significant difference in the mRNA expression level
 - The inhibition of collagen happened at the translation or post-translation level but not the transcription level.**
- The DNA content was lower in the HS group
 - The cell loss was reported in the HS group.**

CONCLUSION

Periodic mild heat stimuli diminish ECM synthesis due to inhibition of the collagen production and loss of cells.

Limitations

- The results were **derived from one patient only**, and could not be simply generalized.
- ECM synthesis was influenced by **multiple confounding factors**, for example, the **stimulating duration** and **other disease situation** of the patient.
- The results of group 37°C did not obviously contribute to the conclusion. The variable was heat stimuli, so groups focused on with or without HS were enough.

Further study or Improvement

- More confirmation based on patients from various backgrounds are needed.
- Expanded graded experiments of different stimulating duration with comprehensive understanding of the overall patient situation.
- Effects of similar HS in other cartilage tissues, which may help generate a summative conclusion.

Any question?

Thank You For Listening !



<https://forms.office.com/Pages/ResponsePage.aspx?id=xSAmKRCuU0Wsz2jsgDJQCG8MIBA7QOxCvt7819ZOI-hUOTZRSk5DMIQyMjRNNzhOWUE5TUo0RkNOSS4u>