BMC Urology

RESEARCH Open Access

The ratio of COL2A1:COL1A1 in dartos tissue patients with hypospadias

Prahara Yuri[1*], Hotman Christinus[1], Zico Yusuf Alfarizi[1] and Medina Ndoye[2]

Abstract

Background The inelasticity of dartos tissue and the regulation of collagen expression are significant factors in the pathophysiology of chordee associated with hypospadias. While the COL2A1:COL1A1 ratio is recognised as a measure of cell differentiation, there is yet to be a study specifically examining this ratio in hypospadias. The aim of this study was to determine the COL2A1:COL1A1 ratio.

Methods We collected 55 samples of dartos tissue, comprising 35 from patients with hypospadias procured from urethroplasty procedures and 20 from patients with phimosis collected during circumcision without any lichen cases at our institution. The gene expression levels of COL1A1 and COL2A1 in the dartos tissue were analyzed using reverse-transcriptase polymerase chain reaction (qPCR).

Results Based on the type of penile abnormality, the expression levels of COL1A1 and COL2A1 measured by qPCR were downregulated in hypospadias, with value of 0.83 (0.38–2.53) and 0.43 (0.10–5.66), respectively, compared to phimosis, which had levels of 1.85 (1.24–4.61) and 0.94 (0.26–2.47) ($p < 0.001$). The expression levels of COL1A1 and COL2A1 were also significantly downregulated among mild, moderate, severe penile curvature, and control groups ($p < 0.001$ and $p = 0.02$). However, the COL2A1:COL1A1 ratio did not show statistically significant differences based on penile abnormalities and curvature ($p > 0.05$).

Conclusion The expression levels of COL1A1 and COL2A1 are significantly downregulated in patients with hypospadias and ventral curvature when compared to those in the phimosis group. However, the COL2A1:COL1A1 ratio was not significant.

Keywords Hypospadias, Chordee, Ventral penile curvature, COL1A1, COL2A1, Gene expression

Background

Hypospadias is a significant health concern, not only because of its high occurrence but also due to its psychological effects on patients and the complications associated with its surgical repair [1–3]. The severity of hypospadias can be classified as mild or severe based on factors such as penile length, glans size and shape, urethral plate quality, and degree of ventral curvature [4]. The severity of the curvature is linked to the disproportion between the dorsal and ventral sides of the penis, periurethral fibrosis, and disorganized fibrotic dartos tissue in the ventral skin [5–7].

*Correspondence:
Prahara Yuri
prahara.yuri@ugm.ac.id
[1]Urology Division, Department of Surgery, Faculty of Medicine, Nursing, and Public Health, Sardjito Hospital, Gadjah Mada University, Yogyakarta 55281, Indonesia
[2]Urology Department HOGIP UCAD, Cheikh Anta Diop University, Dakar, Senegal

In hypospadias, the dartos fascia appears thick and inelastic [8], and on the ventral side, it is consistently absent distal to the ectopic opening [9]. Patients with hypospadias show reduced amounts of total collagen and an altered ECM composition in the dartos fascia [10].

Type I collagen is the most abundant form of collagen, and it plays a crucial role in maintaining skin elasticity. Type II collagen, a fibrillar type, is primarily found in cartilage but also occurs in various embryonic epithelia [11]. Collagen interactions contribute to tissue stability, strength, and help maintain its integrity under stress [12]. To date, no studies have examined type II collagen in the dartos fascia of patients with hypospadias. Therefore, we investigated the collagen type I alpha 1 chain gene (COL1A1) and collagen type II alpha 1 chain gene (COL2A1) in patients with hypospadias.

Methods

In this cross-sectional study, we enrolled 55 participants, comprising 20 controls with phimosis, 20 with proximal hypospadias, and 15 with distal hypospadias by excluding the presence of lichen cases from 2017 to 2020 period at our institution. A pediatric urologist (PY) used sharp dissecting scissors to collect dartos tissue during surgery. Dartos tissue from the midline was collected from patients undergoing urethroplasty for hypospadias, whereas periurethral dartos tissue was obtained using sleeve methods from patients in the circumcision group for phimosis. All the specimens were obtained after degloving. In the control group, phimosis patients were sampled as in a previous study [10]. The collected tissue samples were preserved in RNAlater solution and stored at -80 °C.

Penile curvature measurement

Measurements were performed following the induction of an artificial erection by injecting normal saline into the corpus cavernosum with a 25G winged needle prior to degloving. A goniometer was used for the assessment. Penile curvature was categorized as mild (less than 30°), moderate (30–60°), or severe (greater than 60°).

Table 1 Primer gene sequences

Gene	Forward	Reverse
COL1A1	TACAGCGTCACTGTCGATGGC	TCAATCACTGTCTTGCC CCAG
COL2A1	CTGAGACAGCATGACGCCG	GGCTGCGGATGCTCTCAAT
GADPH	AGGTGAAGGTCGGAGTCAACG	GATGACAAGCTTCCCGT TCTCAG

RNA extraction and cDNA synthesis

Dartos tissues obtained from urethroplasty and circumcision were preserved in RNAlater solution (Ambion, AM7021). RNA extraction was performed using Genezol RNA solution (GENEzolTM, Cat. No. GZR100) in Eppendorf tubes. The concentration and purity of the isolated RNA were measured using the NanoDrop method. cDNA synthesis was performed using the Reverse Transcriptase Excel kit (RP1300, SMOBIO, Hsinchu City, Taiwan) under the following conditions: denaturation at 25 °C for 10 min, annealing at 42 °C for 50 min, and extension at 85 °C for 5 min.

The cDNA was then amplified and quantified using a Taq Master Mix kit (GoTaq® Green Master Mix, Cat. M7122) using forward and reverse primers for COL1A1, COL2A1, and GAPDH (Table 1). For qPCR, denaturation was performed at 94 °C for 2 min followed by 40 cycles of amplification under the same denaturation conditions. Annealing was performed at 56 °C for 30 s, followed by extension at 72 °C for 1 min and a final extension at 72 °C for 10 min. The PCR products were separated on a 2% agarose gel with a 100-bp DNA ladder (Bioron, Germany, Cat. No. 306009) (Fig. 1). Finally, we used ImageJ software for the quantification of gene expression with densitometric analysis of electrophoresis bands on agarose gel.

Statistical analysis

The normality of the data distribution was assessed using the Kolmogorov–Smirnov test. For data that did not follow a normal distribution, Kruskal–Wallis and Mann–Whitney U tests were applied, while one-way ANOVA was used for normally distributed data. Statistical significance was set at $P<0.05$.

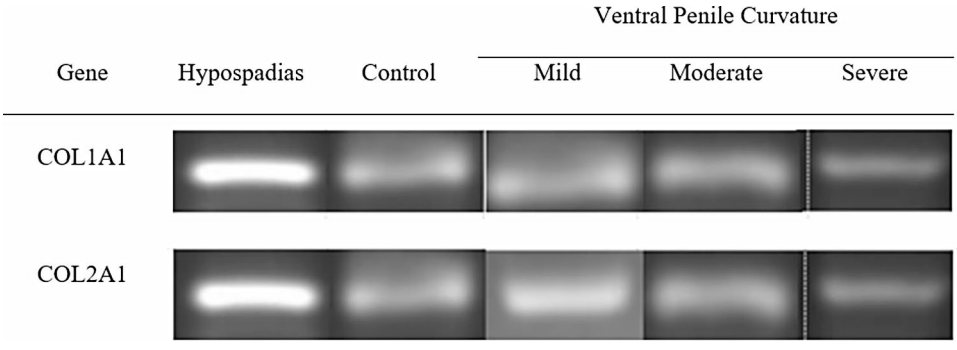

Fig. 1 COL1A1 and COL2A1 representative of q-PCR

Table 2 Study characteristics

Characteristics	Med. (min - max); *N* (%)	*P* value
Age (years)		0.001
Hypospadias	7 (1–25)	
Normal Penis	6 (1–13)	
Hypospadias	35 (63)	N/A
Distal		
Glandular	3 (5.5)	
Subcoronal	3 (5.5)	
Midshaft	9 (16.4)	
Proximal		
Penoscrotal	13 (23.6)	
Scrotal	6 (10.9)	
Perineal	1 (1.8)	
Normal Penis	20 (36.4)	
Penile Curvature		N/A
Mild (< 30 degree)	11 (20)	
Moderate (30–60 degree)	10 (18.2)	
Severe (> 60 degree)	14 (25.5)	
No Curvature	20 (36.4)	

Table 3 COL1A1 and COL2A1 expression based on penile abnormality and curvature

	Col1A1	Col2A1	Col2A1:Col1A1 Ratio
Based on penile abnormality			
Hypospadias	0.97(0.38–2.53)	0.71(0.10–5.66)	0.70(0.14–2.24)
Normal penis	2.07(1.24–4.61)	1.16(0.26–2.47)	0.58(0.15–1.47)
p-value	< 0.001	< 0.001	0.54
Based on Curvature			
Mild	1.50(0.57–2.53)	1.09(0.27–5.66)	0.71(0.14–2.24)
Moderate	0.80(0.59–1.06)	0.54(0.22–1.58)	0.65(0.29–1.66)
Severe	0.68(0.38–0.89)	0.54(0.10–1.26)	0.74(0.21–1.50)
No Curvature	2.07(1.24–4.61)	1.16(0.26–2.47)	0.58(0.15–1.47)
p-value	< 0.001	0.02	0.50

Results

The median age of the patients in our study was 7 years in the hypospadias group and 6 years in the control group. Among the patients with hypospadias, 14 had severe penile curvature, ten had moderate curvature, and 11 had mild curvature. Further details regarding the patient characteristics are provided in Table 2.

Compared to the hypospadias group, which had values of 0.97 (0.38–2.53) and 0.71 (0.10–5.66), respectively. qPCR results indicated that COL1A1 and COL2A1 expression was significantly downregulated in the dartos tissue of hypospadias patients compared to phimosis patients ($p<0.001$). Regarding penile curvature, we found that COL1A1 and COL2A1 expression was significantly higher in mild curvature cases [1.50 (0.57–2.53) and 1.09 (0.27–5.66)] than in moderate [0.80 (0.59–1.06) and 0.54 (0.22–1.58)] and severe curvature cases [0.68 (0.38–0.89) and 0.54 (0.10–1.26)] ($p<0.001$ and $p=0.02$, respectively).

COL1A1, COL2A1, and COL2A1:COL1A1 ratios are shown in Table 3. We observed that the severe hypospadias group had a higher COL2A1:COL1A1 ratio [0.74 (0.21–1.50)] compared to the phimosis samples [0.58 (0.15–1.47)], mild hypospadias [0.71 (0.14–2.24)], and moderate hypospadias [0.65 (0.29–1.66)], though this difference was not statistically significant ($p>0.05$) (Fig. 2).

Discussion

The pathophysiology of hypospadias is not fully understood [10]. While the tissue proximal to the meatus displays normal morphology, hypospadias specimens distal from the meatus exhibit abnormal characteristics, including granular areas and less organized smooth muscles around large blood vessel channels, in contrast to the structure seen in healthy tissue [13].

Under the urethral plate, Snodgrass et al. [14] discovered well-vascularized connective tissue composed of collagen, smooth muscle, blood vessels, and nerves with no signs of fibrous bands or dysplastic tissue. Similarly, Erol et al. [13] identified large blood vessels, glands, and muscles, suggesting a connection to the corpus spongiosum. Additionally, Hayashi et al. [15] utilized transmission electron microscopy (TEM) to observe nerves, smooth muscle cells, and capillaries, further supporting its similarity to the corpus spongiosum. It was discovered that the glans tissue beneath the urethral plate displayed the same cytokeratin staining as the normal urethra, suggesting that in hypospadias, the glans under the urethral plate represent an incomplete attempt at urethral formation [13]. Some studies support the findings of a well-vascularized tissue area beneath the urethral plate [13–15].

Camoglio et al. [16] observed that the penis in hypospadias was stiffer than that of normal penis; although not explicitly stated in the publication, elastography of the tissue surrounding the tunica spongiosum indicated significantly increased stiffness in hypospadias compared to the normal penis. Similarly, Spinoit et al. [17] reported that 70% of the patients with hypospadias exhibited structural abnormalities in the dartos fascia. Resection of dartos tissue often helps straighten the penis in patients with chordee, indicating that the pathophysiology of this condition is associated with dartos tissue [17]. Structural abnormalities in the dartos tissue are closely related to the clinical severity of congenital penile malformations. The composition of the fibromuscular dartos tissue along the penile shaft determines the elasticity of the subcutaneous tissue and skin mobility [17, 18]. Based on the nature of each subtype of collagen and its distribution, the level of expression and type of collagen will affect the consistency of the tissue [19].

Atmoko et al. [20] reported that the dartos tissue in hypospadias had a thicker, less elastic consistency but with lower levels of total collagen and elastin, and a

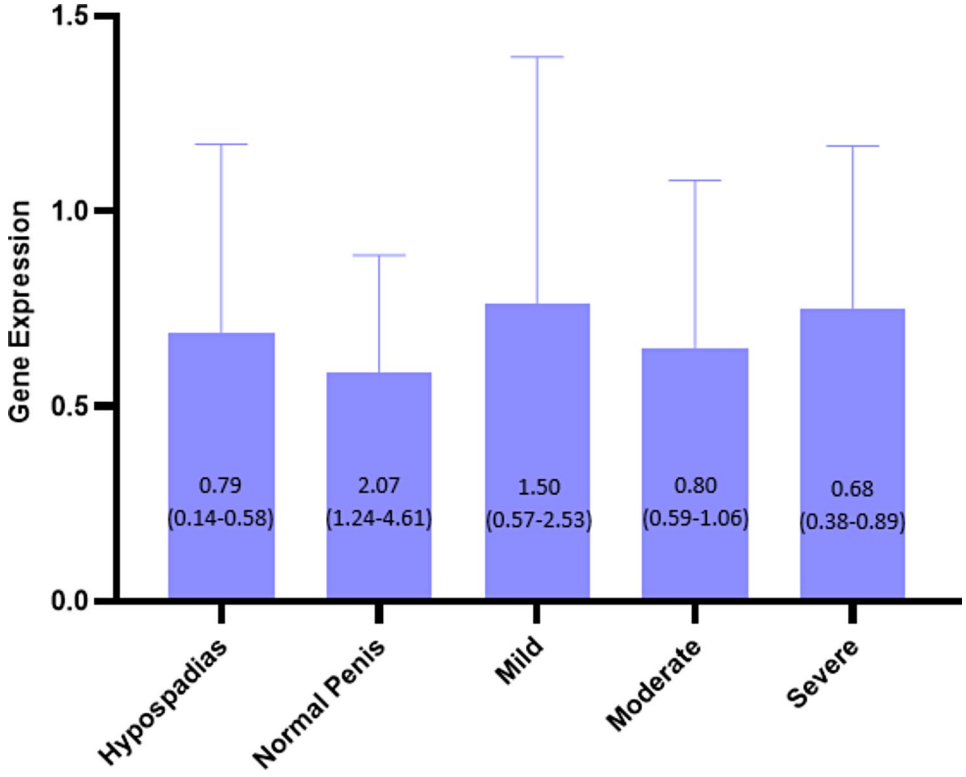

Fig. 2 COL2A1 and COL1A1 Ratio in Hypospadias and Control Group

higher reticulin-to-collagen ratio. Our previous study in hypospadias compared to normal penis showed that expression of COL1A1 and COL6A1 might affect dartos tissue elasticity [10], but no difference in vascularity [21]. Our results support the idea that total collagen expression was reduced in patients with hypospadias compared to those with a normal penis, as we observed lower levels of COL1A1 and COL2A1 mRNA in the hypospadias group.

Type I collagen is notably soft and fragile when unbound, but can form a denser matrix network by cross-linking with other collagen types [22]. Our previous study indicated that mRNA levels of COL1A1 and COL6A1 were downregulated in the dartos tissue of patients with moderate to severe hypospadias, and there was also a moderate correlation observed between COL1A1 and COL6A1 [11]. Hypospadias arise from abnormal growth of the urethral fold and ventral foreskin of the penis, reflecting failure in the canalization of the urethral fold [23]. Furthermore, cell differentiation can be assessed by measuring the ratio of COL2A1 to COL1A1 [24].

Type II collagen is primarily expressed in cartilage tissue, but proteins derived from the translation of COL2A1 have also been detected in malignant melanoma and breast cancer [26]. This indicates that type II collagen can be found in non-cartilage tissues, which aligns with our findings. Additionally, proteins from COL2A1 mRNA have been linked to chemokines such as transforming growth factor-β (TGF-β), which plays a role in tissue fibrosis and possesses anti-inflammatory properties [25]. This indirectly supports the notion that collagen expression may be correlated with the expression of other collagen subtypes.

Collagen type II has a structure similar to collagen I. Collagen type II has a homotrimeric molecular structure with a chain composition $[\alpha1(II)]_3$ [26]. Several studies have suggested COL2A1:COL1A1 ratio usage in the cell differentiation index [24], anabolic chondrocyte marker [27] and osteoarthritis severity [28]. In addition to our study, no study has discussed the COL2A1:COL1A1 ratio in dartos tissue of hypospadias patients. We found no differences between hypospadias and phimosis in COL2A1:COL1A1 ratio.

Lastly, the thick and inelastic dartos tissue seen in patients with hypospadias does not necessarily indicate higher collagen levels. In our study, we discovered that this tissue showed lower expression levels of COL1A1 and COL2A1, although this was not reflected in the COL2A1: COL1A1 ratio. Furthermore, mRNA levels of COL2A1 do not always correlate with protein expression, as fluctuations in COL2A1 and COL1A1 protein levels can arise from transcriptional and post-transcriptional modifications [29]. This research represents the first assessment of COL2A1 and COL1A1 gene expression

and its ratio in the dartos tissue of hypospadias patients. However, our study has limitations, including the small sample size and the absence of immunohistochemical examinations to validate this finding.

Conclusion

COL1A1 and COL2A1 expression was downregulated in hypospadias patients. As penile curvature became more severe, the expression of COL1A1 and COL2A1 was more downregulated, but there was no difference in the COL2A1:COL1A1 ratio.

Abbreviations

COL1A1 Collagen type I alpha I chain gene
COL2A1 Collagen type II alpha I chain gene

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12894-024-01688-1.

Supplementary Material 1

Acknowledgements

None.

Author contributions

PY concepted and designed the study, analyzed and interpreted the patient data. PY, HC, ZY and MN analysed and wrote the manuscript. All authors read and approved the final manuscript.

Funding

This research had been received funding from *Hibah Dana Masyarakat Penelitian FK-KMK UGM*.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The Ethical Committee of the Faculty of Medicine, Universitas Gadjah Mada/Dr. Sardjito General Hospital approved this study (KE/FK/0699/2019). All parents provided written informed consent for their participation in this study.

Competing interests

The authors declare no competing interests.

Received: 25 February 2024 / Accepted: 26 December 2024
Published online: 04 January 2025

References

1. Bouty A, Ayers KL, Pask A, Heloury Y, Sinclair AH. The genetic and environmental factors underlying Hypospadias. Sex Dev. 2015;9(5):239–59.
2. Schönbucher VB, Landolt MA, Gobet R, Weber DM. Psychosexual Development of children and adolescents with Hypospadias. J Sex Med. 2008;5(6):1365–73.
3. Appeadu-Mensah W, Hesse AJ, Glover-Addy H, Osei-Nketiah S, Etwire V, Sarpong P. Complications of hypospadias surgery: experience in a tertiary hospital of a developing country. Afr J Paediatr Surg. 2015;12(4):211.
4. Gnech M, van Uitert A, Kennedy U, Skott M, Zachou A, Burgu B, Castagnetti M, O'Kelly F, Quaedackers J, Rawashdeh YF, Silay MS. European Association of Urology/European Society for Paediatric Urology Guidelines on Paediatric Urology: Summary of the 2024 updates. Eur Urol. 2024 Apr 15.
5. Dessanti A, Iannuccelli M, Falchetti D, Sotgiu S, Scanu AM, Feo C. Correction of congenital chordee penis by 'ventral separation and outward rotation of corpora'. J Pediatr Surg. 2002;37(9):1347–50.
6. Djakovic N, Nyarangi-Dix J, Özturk A, Hohenfellner M. Hypospadias Adv Urol. 2008;2008:1–7.
7. Rodríguez Fernández V, López Ramón y Cajal C, Marín Ortiz E, Sarmiento Carrera N. Accurate diagnosis of severe hypospadias using 2D and 3D Ultrasounds. Case Rep Obstet Gynecol. 2016;2016:1–4.
8. Yuri P, Gunadi, Lestari RP, Fardilla FP, Dachlan I. Expression of mRNA vascular endothelial growth factor in hypospadias patients. BMC Urol. 2021;21(1):163.
9. Chaudhary G, Yhoshu E, Chauhan U, Ahmed I, Gupta MK, Piplani R et al. Anatomical study of hypospadias penis using magnetic resonance imaging in children. J Pediatr Urol. 2021;S1477513121005222.
10. Yuri P, Gunadi, Lestari RP, Fardilla FP, Setyaningsih WAW, Arfian N, et al. The impact of COL1A1 and COL6A1 expression on hypospadias and penile curvature severity. BMC Urol. 2020;20(1):189.
11. Kolpakova-Hart E, Nicolae C, Zhou J, Olsen BR. Col2-Cre recombinase is co-expressed with endogenous type II collagen in embryonic renal epithelium and drives development of polycystic kidney disease following inactivation of ciliary genes. Matrix Biol. 2008;27(6):505–12. https://doi.org/10.1016/j.matbio.2008.05.002.
12. Wu Z, Korntner SH, Mullen AM, Zeugolis DI. Collagen type II: from biosynthesis to advanced biomaterials for cartilage engineering. Biomater Biosyst. 2021;4:100030. https://doi.org/10.1016/j.bbiosy.2021.100030. Published 2021 Nov 22.
13. Erol A, Baskin LS, Li YW, Liu WH. Anatomical studies of the urethral plate: why preservation of the urethral plate is important in hypospadias repair. BJU Int. 2001;85(6):728–34. https://doi.org/10.1046/j.1464-410x.2000.00486.x.
14. Snodgrass W, Patterson K, Plaire JC, Grady R, Mitchell ME. Histology of the urethral plate: implications for hypospadias repair. J Urol. 2000;164(3 Pt 2):988-9; discussion 989 – 90. https://doi.org/10.1097/00005392-200009020-00017. PMID: 10958724.
15. Hayashi Y, Mizuno K, Kojima Y, Moritoki Y, Nishio H, Kato T, et al. Characterization of the urethral plate and the underlying tissue defined by expression of collagen subtypes and microarchitecture in hypospadias: histology of urethral plate in hypospadias. Int J Urol. 2011;18(4):317–22.
16. Camoglio FS, Bruno C, Zambaldo S, Zampieri N. Hypospadias anatomy: elastosonographic evaluation of the normal and hypospadic penis. J Pediatr Urol. 2016;12(4):199. e1-199.e5.
17. Spinoit AF, Van Praet C, Groen LA, Van Laecke E, Praet M, Hoebeke P. Congenital Penile Pathology is Associated with abnormal development of the Dartos muscle: a prospective study of primary penile surgery at a Tertiary Referral Center. J Urol. 2015;193(5):1620–4. https://doi.org/10.1016/j.juro.2014.10.090.
18. Spinoit AF, Radford A, Ashraf J, Gopal M, Subramaniam R. Modified tubularized incised plate urethroplasty in distal hypospadias repair: stepwise technique with validated functional and cosmetic outcome. J Pediatr Urol. 2017;13(1):86–7. https://doi.org/10.1016/j.jpurol.2016.09.014.
19. Bielajew BJ, Hu JC, Athanasiou KA. Collagen: quantification, biomechanics and role of minor subtypes in cartilage. Nat Rev Mater. 2020;5(10):730–47.
20. Atmoko W, Shalmont G, Situmorang GR, Wahyudi I, Tanurahardja B, Rodjani A. Abnormal dartos fascia in buried penis and hypospadias: evidence from histopathology. J Pediatr Urol. 2018;14(6):536. https://doi.org/10.1016/j.jpurol.2018.04.034. e1-536.e7. Epub 2018 Jun 21. PMID: 30554609.
21. Yuri P, Gunadi, Lestari RP, Fardilla FP, Dachlan I. Expression of mRNA vascular endothelial growth factor in hypospadias patients. BMC Urol. 2021;21(1):163. https://doi.org/10.1186/s12894-021-00930-4. PMID: 34837995; PMCID: PMC8627631.
22. Amirrah IN, Lokanathan Y, Zulkiflee I, Wee MFMR, Motta A, Fauzi MB. A Comprehensive Review on Collagen Type I Development of Biomaterials for tissue Engineering: from biosynthesis to Bioscaffold. Biomedicines. 2022;10(9):2307. https://doi.org/10.3390/biomedicines10092307. PMID: 36140407; PMCID: PMC9496548.
23. Baskin L, Shen J, Sinclair A, Cao M, Liu X, Liu G, Isaacson D, Overland M, Li Y, Cunha GR. Development of the human penis and clitoris. Differ 2018 Sep-Oct;103:74–85. https://doi.org/10.1016/j.diff.2018.08.001. Epub 2018 Aug 23. PMID: 30249413; PMCID: PMC6234061.
24. Marlovits S, Hombauer M, Truppe M, Vècsei V, Schlegel W. Changes in the ratio of type-I and type-II collagen expression during monolayer culture of human chondrocytes. J Bone Joint Surg Br. 2004;86(2):286–95. https://doi.org/10.1302/0301-620x.86b2.14918.

25. Talluri B, Amar K, Saul M, Shireen T, Konjufca V, Ma J, et al. COL2A1 is a Novel Biomarker of Melanoma Tumor repopulating cells. Biomedicines. 2020;8(9):360.
26. Bächinger HP, Mizuno K, Vranka, Janice, Boudko. Sergei Collagen Formation Struct. 2010. https://doi.org/10.1016/b978-008045382-8.00698-5.
27. Kuettner KE, Memoli VA, Pauli BU, Wrobel NC, Thonar EJ, Daniel JC. Synthesis of cartilage matrix by mammalian chondrocytes in vitro. II. Maintenance of collagen and proteoglycan phenotype. J Cell Biol. 1982;93(3):751–7. https://doi.org/10.1083/jcb.93.3.751.
28. Zhong L, Huang X, Karperien M, Post J. Correlation between Gene expression and osteoarthritis progression in human. IJMS. 2016;17(7):1126.
29. Asopa V, Vincent T, Saklatvala J. The effects of Age and Cell isolation on collagen II synthesis by articular chondrocytes: evidence for transcriptional and posttranscriptional regulation. Biomed Res Int. 2020;2020:1–9.

Publisher's note