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Research article

Mechanical properties of extensive calcified costal cartilage: An experimental study

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ABSTRACT

Background: Autologous costal cartilage is widely used as nasal augmentation or nasal reconstruction material. However, no study has focused on the mechanical difference between no calcified costal cartilage and extensive calcified costal cartilage at present. Our study aims to study the loading behavior of calcified costal cartilage under tensile and compressive stress.
Method: Human costal cartilage specimen was obtained from five extensive calcified costal cartilage patients and classified into four groups (group A: no calcified costal cartilage; group B: calcified costal cartilage; group C: no calcified costal cartilage after transplantation in BALB/c nude mice for half a year; group D: calcified costal cartilage after transplantation in BALB/c nude mice for half a year). Young's modulus, stress relaxation slope, and relaxation amount were analyzed through tensile and compressive tests using a material testing machine.
Results: We included five female patients with extensive calcified costal cartilage. Group B exhibited significantly higher Young's modulus in both the tensile and compressive tests ($p < 0.05$ in tensile test, $p < 0.01$ in compressive test), higher relaxation slope ($P < 0.01$) and higher relaxation amount ($p < 0.05$ in compression test). After transplantation, the Young's modulus of calcified and non-calcified costal cartilage decreased, except that the calcified costal cartilage increased slightly in the tensile test. The final relaxation slope and relaxation amount had increased at different degrees, but the changes did not change significantly before and after transplantation ($P > 0.05$).
Conclusion: Our results showed that the stiffness of calcified cartilage would increase 30.06% under tension and 126.31% under compression. This study may provide new insights to researchers focusing on extensive calcified costal cartilage can be used for autologous graft material.

1. Introduction

Due to the increase of revision rhinoplasty, and East Asian nose features of thick skin and poor tip projection, autologous costal cartilage is widely used as nasal augmentation or nasal reconstruction material [1,2]. The function of the cartilage graft can not only

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provide sufficient support for the soft tissue and maintain a stable structure for a long time, but also provide strong elasticity to resist the internal force of the nose. In nasal reconstruction, cartilage is also needed to bear the pressure of the transferred flap and to prevent flap contracture [2]. Wee et al. conducted a meta-analysis on 491 cases of complications after autologous costal cartilage rhinoplasty. The results showed that the proportion of cases with cartilage absorption after surgery was only 0.22% [3]. Therefore, for the maintenance of long-term morphology, autologous costal cartilage has great advantages because of its relatively low absorption rate. Regretfully, Sunwoo WS et al. found that the incidence of moderate and severe calcification of costal cartilage was 10.8%, and the incidence of women was much higher than that of men [4]. However, we believe that these patients should still choose autologous costal cartilage. After all, the probability of complications of prosthesis exposure and capsule contracture was lower, and the possibility of postoperative warping of calcified costal cartilage was also lower. However, some scholars believed that prosthesis or prosthesis combined with costal cartilage could be used for rhinoplasty, which could reduce the carving time.

At present, there are few experimental and clinical studies on calcified costal cartilage at home and abroad. Our previous retrospective cohort study evaluated patients' satisfaction with extensive calcified costal cartilage compared with no calcified costal cartilage after costal cartilage-based rhinoplasty. There was no significant difference in satisfaction and complications after 1 year of follow-up [5]. To date, no study has focused on the mechanical difference between no calcified costal cartilage and extensive calcified costal cartilage. Our study aims to study the loading behavior of calcified costal cartilage under tensile and compressive stress.

2. Materials and methods

2.1. Specimen collection and preparation

Patients underwent a computed tomographic (CT) scan of the chest with three-dimensional reconstruction to evaluate the rib cartilage (Fig. 1). Because the biomechanics of costal cartilage is different in patients of different ages and genders, we try to keep these conditions consistent when we included patients [6]. Thus, we included five extensive calcified costal cartilage patients aged 24–28 years old. The five patients who underwent open augmentation rhinoplasty with extensive rib cartilage calcification were included in the study and the degree of calcification was level 4 (76%–100%) measured from the entire rib cartilage on CT [7]. We collected the remaining costal cartilage after operation, which these samples contain calcified cartilage. Then, to acquire the segments with calcification, the remaining costal cartilage was placed under X-ray to confirm the calcified part (Fig. 1), and divided into 4 groups of the same size group A: no calcified costal cartilage; group B: calcified costal cartilage; group C: no calcified costal cartilage after transplantation; group D: calcified costal cartilage after transplantation. Groups A and B underwent tensile and compressive tests immediately. Groups C and D were subcutaneously transplanted into nude mice, and the mechanical experiments were carried out after half a year.

For tensile testing, the specimen was made into a semicircular niche-shaped defect at the midpoint of each long side with a corneal trephine. Thus, it can be pulled apart at the midpoint. Then, the processed specimen was loaded on the fixture clamps of the tensile machine (Fig. 2). For compressive testing, the specimen was drilled off into a circular with a diameter of 4 mm and depth of 6 mm corneal trephine. Then, the circular cartilage graft was used for compressive testing (Fig. 2). Groups A and B were carried out above steps.

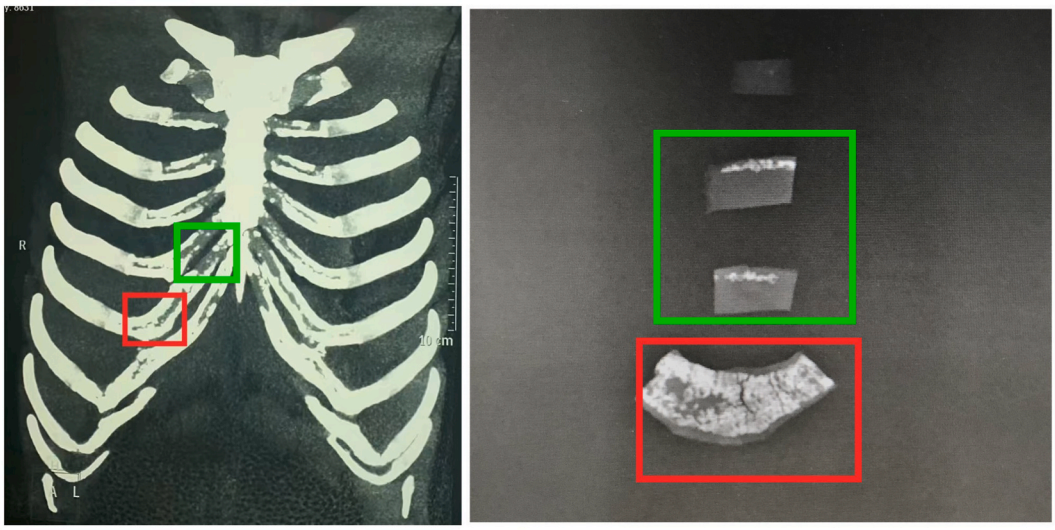

Fig. 1. The left image was 29-year-old female, which showed the three-dimensional reconstruction to evaluate the rib cartilage. The right image showed the red box part of left one under X-ray, to confirm the extensive calcified costal cartilage, and the degree of calcification was level 4 (76%–100%). The green box part showed the non-calcified cartilage. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

BALB/c nude mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. The animal experiment strictly adhered to the ethical guidelines of the National Guide for the Care and Use of Laboratory Animals. Five BALB/c nude mice aged 7 weeks were housed in well-ventilated and temperature-controlled cages. The mice were anesthetized by intraperitoneal injection of 0.5% pentobarbital sodium (50 mg/kg). The mass (gram/g) and volume (milliliter/ml) of the graft were measured. Then, we transplanted groups C and D from the same patient into the subcutaneous area of the back of mice (Fig. 3). The grafts were harvested after half a year from the mice. The grafts underwent tensile and compressive testing after the mass and volume of the graft were measured again. All measurements were taken three times to get the average value. There were five specimens included in each group, and all 20 specimens from the four groups were used for each test.

2.2. Tensile and compressive testing

Our study tested the stress-strain relationship and stress relaxation in tension and compression. We used Young's elastic modulus to compare the stiffness of the four groups. It is a physical quantity that describes the resistance of solid materials to deformation, the ratio of stress to strain when the material is deformed by force. The larger its value is, the less likely the material is to deform. In addition, to compare the viscoelasticity of the four groups of cartilage, the stress relaxation slope and relaxation amount were analyzed by tensile and compression tests [8,9].

Our tests used a 5967 Universal Testing Machine (Instron, Norwood, MA) and 500 N sensor (Instron, Norwood, MA). The experiment was carried out at room temperature and the air humidity was maintained at 60% with an ultrasonic humidifier. For tensile testing, the specimens were stretched at 10 mm/min via clamps. After the stress reached 2 MPa, the tissue was allowed to relax for 10 min. Similarly, for compressive testing, the specimens were compressed at 10 mm/min via the indenters. After the stress reached 2 MPa, the tissue was allowed to relax for 10 min. After the tests, the experimental data were collected and outputted by Bluehill software and the mechanical curves were plotted using Origin 9 (Origin Lab Corporation, US). Young's modulus was calculated from the stress–strain resistance data [Stress (MPa) = load (N)/sample pressure sectional area (mm^2). Strain (%) = compression

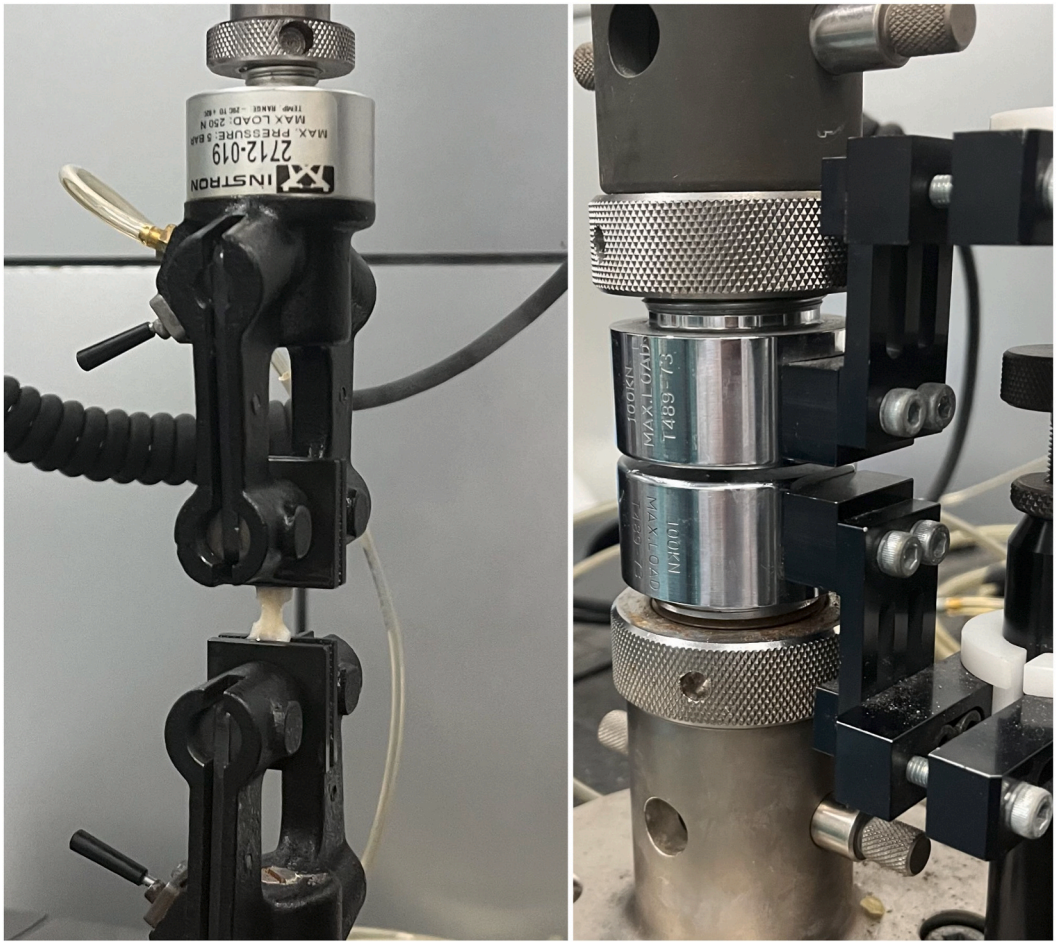

Fig. 2. The left one was the tensile test setup. The cartilage specimen with niche-shaped defect at the midpoint of each long side, which was subjected to clamps. The right one was compressive test setup. The cartilage circular piece specimen was subjected between the indenters.

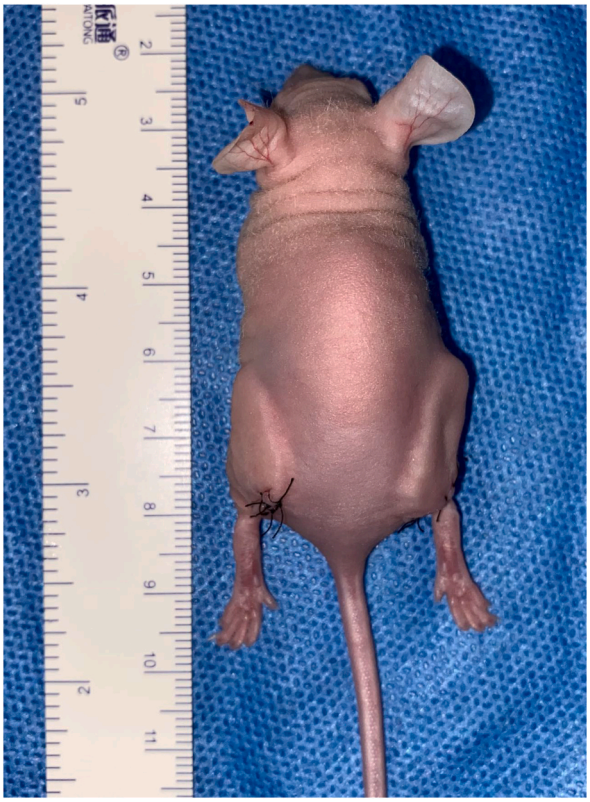

Fig. 3. We transplanted groups C and D from the same patient into the subcutaneous area of the back of BALB/c nude mice.

displacement (mm)/sample compression plate height (mm)]. In the stress relaxation test, the linear slope of the curve over the last 100 s was measured from the relaxation values (final relaxation slope), and the reduced stress amount from the start till the end of the test (time span = 600 s) was measured (relaxation amount).

2.3. Data analysis

GraphPad Prism 8 (GraphPad Software, US) was used for data analysis of the values of Young's modulus, final stress, relaxation slope, and relaxation amount between the four groups of cartilages. Group A vs B, group A vs C, and group B vs D were performed paired t-tests. The difference was considered significance at $p < 0.05$.

3. Results

We included five female patients and the average age was 26.33 ± 1.50. They were extensive calcified costal cartilage patients and harvested the sixth costal cartilage. We also measured the mass (gram/g) and volume (milliliter/ml) of the graft before and after transplant which showed in Table 1. However, they did not show difference after transplant ($P > 0.05$).

Mechanical curves of the four groups were presented in Figs. 4 and 5. Fig. 4 was Stress- Strain curves of the four groups. Fig. 4A was

Table 1
Difference between the four groups in tensile and compressive tests.

		Group A	Group B	Group C	Group D
Mass (g)		0.45 ± 0.02	0.46 ± 0.01	0.44 ± 0.02	0.46 ± 0.01
Volume (ml)		0.39 ± 0.01	0.37 ± 0.02	0.38 ± 0.02	0.36 ± 0.01
Tensile text					
	Young's Modulus (MPa)	26.68	34.70	23.49	40.34
	Final Relaxation Slope (-MPa/s)	0.06	0.28	0.07	0.31
	Relaxation Amount (MPa)	0.58	0.80	0.59	0.86
Compressive test					
	Young's Modulus (MPa)	29.46	66.67	28.76	64.89
	Final Relaxation Slope (-MPa/s)	0.12	0.34	0.14	0.39
	Relaxation Amount (MPa)	0.70	1.05	0.80	1.10

the tensile test curve and B was the compressive curve. Fig. 5 was Relaxation-Time curves of the four groups. Fig. 5A was the tensile test curve and B was compressive curve.The Young's modulus, final relaxation slope, and relaxation number of the four groups were summarized in Table 1 and their difference was drawn in Fig. 6 (Fig. 6A and B) showed the Young's modulus of the four groups. In the tensile test, the average Young's modulus were 26.68 MPa in Group A, 34.70 MPa in Group B, 23.49 MPa in Group C, and 40.34 MPa in Group D (A vs B, $p < 0.05$; C vs D, $p < 0.01$). In the compressive test, the average Young's modulus were 29.46 MPa in Group A, 66.67 MPa in Group B, 28.76 MPa in Group C, and 64.89 MPa in Group D (A vs B, $p < 0.01$; C vs D, $p < 0.01$). Differences were shown in the comparison of the final relaxation slope in the four groups (Fig. 6C, D). In the tensile test, the average relaxation slopes were −0.06 MPa/s in Group A, −0.28 MPa/s in Group B, −0.07 MPa/s in Group C and −0.31 MPa/s in Group D (A vs B, $p < 0.01$; C vs D, $p < 0.01$); in the compressive test, the average relaxation slopes were −0.12 MPa/s in Group A, −0.34 MPa/s in Group B, −0.14 MPa/s in Group C and −0.39 MPa/s in Group D (A vs B, $p < 0.01$; C vs D, $p < 0.01$). The relaxation amounts of the four groups were slightly dissimilar (Fig. 6E, F). In the tensile test, the average relaxation amount was 0.58 MPa in Group A, 0.80 MPa in Group B, 0.59 MPa in Group C, and 0.86 MPa in Group D (C vs D, $p < 0.01$). In the compressive test, the average relaxation amount was 0.70 MPa in Group A, 1.05 MPa in Group B, 0.80 MPa in Group C, and 1.10 MPa in Group D (A vs B, $p < 0.05$).

4. Discussion

At present, costal cartilage is widely used as a stable autologous material. However, costal cartilage calcification is inevitable. Thus, we investigated the presence of any significant difference in the mechanical properties between no calcified costal cartilage and extensive calcified costal cartilage. To clarify the difference, we collected human remaining costal cartilage after operation and performed tensile and compressive mechanical tests using a testing machine before and after transplantation in mice.

From our results, we can see that the quality and volume of cartilage, whether calcified or non-calcified, did not change significantly before and after transplantation in BALB/c nude mice. The reported rate of resorption with autologous costal cartilage in rhinoplasty was less than 1% incidence [10]. Although it has been verified that the calcification of costal cartilage would increase the absorption rate, the specific results should be verified from the clinic [11]. At least, we did not see a significant difference from our experiments. Young's modulus is an index to measure the difficulty of elastic deformation of materials. From the tensile stress-strain curve and compressive stress-strain curve (Fig. 5), the calcified costal cartilage was less likely to strain, indicating that it is more elastic and stronger at the same stress level. Especially in the tensile and compression test, Young's modulus of calcified costal cartilage was 30.06% and 126.31% higher than that of non-calcified costal cartilage. The modulus of calcified and non-calcified costal cartilage decreased, except that the calcified costal cartilage increased slightly in the tensile test, but the changes before and after transplantation did not change significantly ($P > 0.05$), after transplantation in mice half a year. The reported calcification may increase the risk of deformation, which is worthy of further verification [12]. From the tensile stress-relaxation curve and compressive stress-relaxation curve (Fig. 6), the calcified costal cartilage decreased most when the strain length remained unchanged after a stress level was applied, indicating that its elasticity decreased and it was easier to relax (Fig. 7C and D, E, F) showed that the final relaxation slope and relaxation amount of calcified costal cartilage were the largest in both tension and compression tests, and the results were like that after transplantation in mice.

It can be seen from our experiments that the Young's modulus of calcified costal cartilage was higher and the mechanical strength was better, but the stress relaxation speed was faster and the elasticity was relatively poor. More importantly, the mechanical strength of transplanted cartilage did not change after half a year ($P > 0.05$). In addition, compared to diced cartilage grafts which were large-scale clinical application in rhinoplasty, the Young's modulus of costal cartilage is 2.41–3.48 times than that, which shows that the costal cartilage grafts will be more stable [13,14]. These mechanical properties help the cartilage frame to resist the absorption and deformation, thereby ensuring the cosmetic result.

Costal cartilage is mainly composed of collagen fibers, proteoglycans, and water. Collagen fibers are mainly type II collagen, interwoven into a network to form a framework and provide attachment points for proteoglycans. The biomechanical properties of

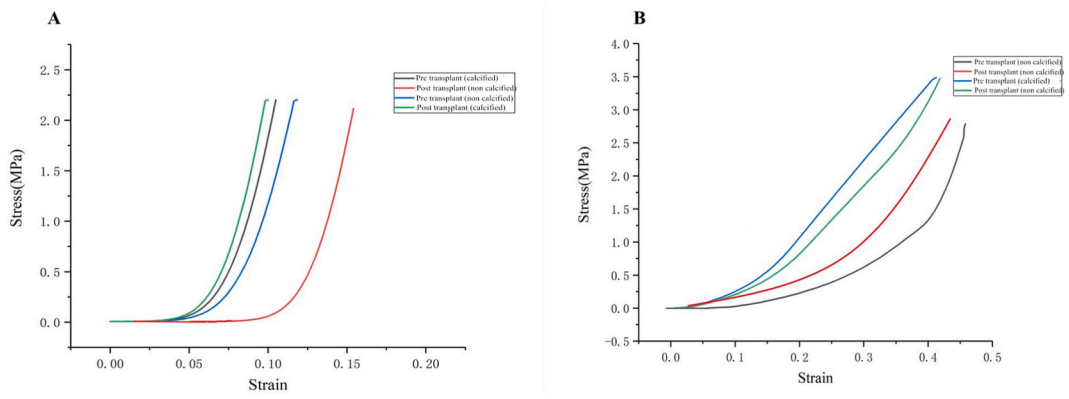

Fig. 4. Stress-Strain curves of the four groups. A was the tensile test curve and B was the compressive curve.

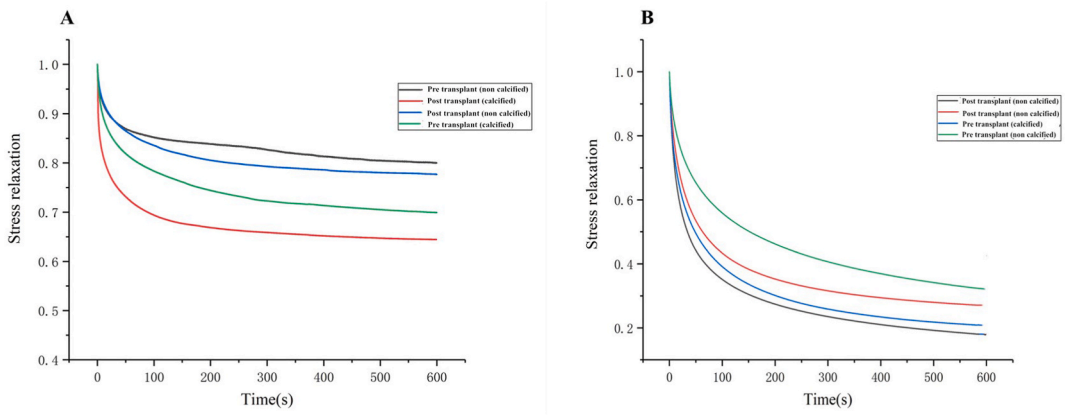

Fig. 5. Relaxation-Time curves of the four groups. A was the tensile test curve and B was compressive curve.

cartilage mainly depend on the cross-linked collagen network. The orientation, organization, and degradation of collagen affect the tensile modulus and strength of cartilage [15]. The rapid relaxation of cartilage indicates that the fibers rearrange or move under the action of stress; slow relaxation indicates that the properties of cartilage gradually tend to an elastic solid, and the collagen fibers degenerate to form a relatively fixed and difficult to move state. When cartilage bears compressive stress, collagen fibers only play an auxiliary role, and proteoglycan is the main undertaker, which is a hydrophilic macromolecule with negative charge. A large number of positively charged ions are retained between the molecules to maintain high osmotic pressure in the cartilage matrix, to regulate the flow of water in the cartilage matrix.

When the cartilage is compressed, water will flow out, and the proteoglycan will compress reversibly, reducing the permeability of the cartilage matrix, making it difficult for water to flow out further, which plays an important role in maintaining the mechanical properties of cartilage [16]. Studies have shown that costal cartilage calcification is different from rib bones [17]. The average mineral density of the calcifications and the calcification volume were less than adjacent ribs. Therefore, the calcified costal cartilage cannot be simply regarded as the same bone as ribs. Forman et al. showed that the increase of calcified volume lead to the increase of cartilage stiffness, about 2.3–3.8 times, but the volumetric percentage of calcification was from 0% to just 24% [11]. The reason for the difference compared with our results may be that their values used a cantilever beam loading model of whole segments, not a piece of prepared costal cartilage, and they used a numeric finite element model. Thus, the calculation results of stiffness factor were different from ours by physical experiments and they cannot be directly compared. Calcified costal cartilage leads to the reduction of collagen fibers and proteoglycans, reduces the reorganization ability of proteoglycans to collagen fibers, and leads to the deformation of the collagen fiber frame under stress, resulting in higher terminal stress relaxation and lower elasticity. It is known that collagen in cartilage works mainly in tension, while proteoglycans provide mainly compressive mechanical properties [18]. Therefore, the biological performance of calcified costal cartilage will decline. The proteoglycan structure has an electrical nature and positive structure provided by positive ions dissolved in tissue water [19]. The electrical repulsion of the negatively charged groups is an origin of the stress bringing cartilage sample to its initial shape [20,21]. Therefore, calcified cartilage had poor deformation under the same stress, compared with non-calcified costal cartilage. And the cartilage fracture was located at the calcification aggregation site. Because this part of cartilage collagen fiber breaks, the cartilage loses its ability to resist stretching.

However, our study has some limitations. Firstly, the specimen size is not large enough to generalize the result. Because there are not many calcified costal cartilages itself and the remaining costal cartilage after operation needs sufficient, the sample size that can be included in the group is less. Then, the environment in BALB/c nude mice is also different from that in humans, so the results may be different. Thus, further research can be conducted on calcified costal cartilage specimens of people transplanted for more than half a year. In the future work, we will continue to collect samples, further improve and enrich this part of the research, and make the results more reliable.

5. Conclusion

Our results showed that the stiffness of the calcified cartilage would increase 30.06% under tension and 126.31% under compression. The modulus of calcified and non-calcified costal cartilage decreased after transplantation in BALB/c nude mice, but the changes did not show significantly ($P > 0.05$). In addition, the quality and volume of cartilage, whether calcified or non-calcified, did not change significantly before and after transplantation. Thus, it is feasible that extensive calcified costal cartilage can be used for autologous graft material, such as augmentation, rhinoplasty, nasal reconstruction, ear reconstruction and so on.

Ethical approval

All procedures performed in the studies were in accordance with the ethical standards of the committee of the Peking Union Medical College Plastic Surgery Hospital. All applicable institutional and/or national guidelines for the care and use of animals were

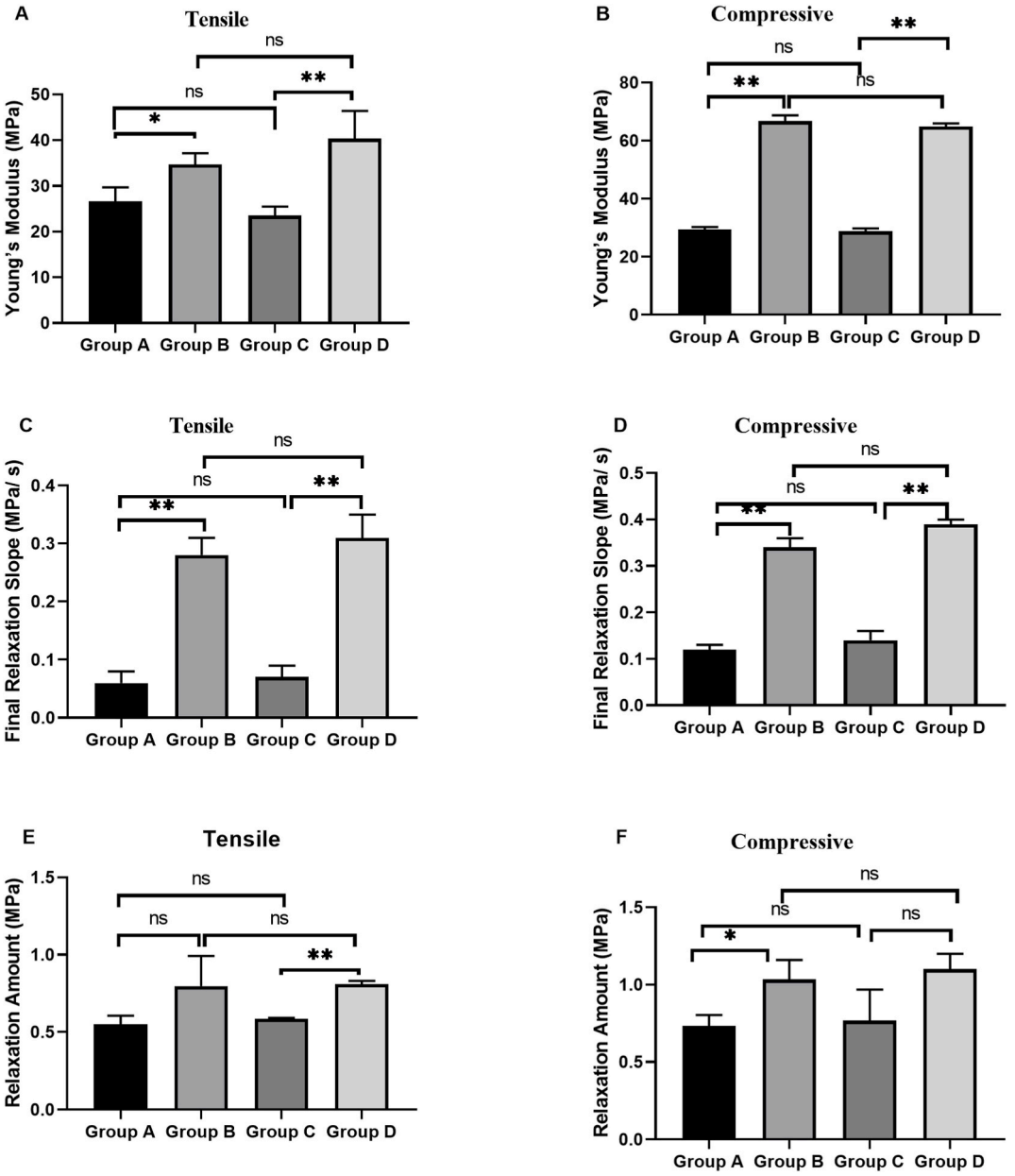

Fig. 6. Comparison of the Young's modulus (A and B), final stress relaxation slope (C and D), and relaxation amount (E and F) of the four groups (*$p < 0.05$, **$p < 0.01$).

followed. The study was approved by the institutional ethics committee.

Informed consent

Informed consents were obtained from the patients in this study.

Declaration

Author contribution statement

Xin Wang: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper. Wenfang Dong: Conceived and designed the experiments; Performed the experiments; Contributed reagents,

materials, analysis tools or data. Huan Wang; Jianjun You; Yihao Xu: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data. Ruobing Zheng: Contributed reagents, materials, analysis tools or data. Fei Fan: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no competing interests.

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