PLASTIC AND RECONSTRUCTIVE SURGERY

Royal Australasian College of Surgeons

Characterization of costal cartilage allografts

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Key words
allografts, cartilage, orthopaedic surgery, plastic surgery.

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Accepted for publication 26 July 2022.

doi: 10.1111/ans.17967

Abstract

Background: Human costal cartilage remains widely used in the reconstruction of soft tissues, particularly within the field of plastic and orthopaedic surgery. The biologic expense of using autologous human costal cartilage has become superseded by the increasingly common use of irradiated costal cartilage allografts. To date, there has been no histologic investigation of such costal cartilage allografts. This study aims to characterize the histologic variations that exist between different costal cartilage specimens, and to quantify this between specimens in spite of their common anatomical derivation.

Methods: Twenty-five specimens of cadaveric human costal cartilage were obtained from Australian Biotechnologies. Each specimen was irradiated, sectioned and stained with Haematoxylin and Eosin, Masson's trichrome and tetrachrome stains. After being analysed under light microscopy, specimen dimensions, chondrocyte counts and mineral content was quantified and measured.

Results: The median specimen diameter was 8.20 mm, with an interquartile range (IQR) of 1.59 mm. The median measurement from the superficial to basal chondrocyte layer was 1409.91 μm (IQR = 885.59 μm), and the median measurement from superficial to calcified zone was 4146.26 μm (IQR 1441.83 μm). The median chondrocyte area was 442.74 μm^2 (IQR = 2622.72 μm^2) with their total chondrocyte count ranging from 289 to 591 chondrocytes per square millimetre. The median percentages of collagen and mineral content were 45.17% and 71.82%, respectively (IQR = 20.48%, 14.75%).

Conclusion: These findings emphasize the histologic and biochemical degree of variation that exists between specimens of human cadaveric costal cartilage on a microscopic level. This has the potential to influence the selection of costal cartilage allografts for reconstructive purposes.

Introduction

Human costal cartilage has become one of the integral components of human soft tissue reconstruction, particularly within the fields of plastic and orthopaedic surgery. It remains a subject of intense histologic and biological curiosity, given it is a tissue with limited regenerative capacity and great biologic expense. The use of autologous costal cartilage, whilst operatively feasible, carries significant donor site risk and morbidity. This has resulted in increasing demand for irradiated cartilaginous allografts, largely derived from cadaveric donors. Although these allografts come with their own economic expense, their use negates the risk of pneumothorax and cosmetic deformity that could result from its harvest *in vivo*.

The histologic characteristics of hyaline and articular cartilage have been extensively researched. This can lead to the presumption that human costal cartilage is histologically and biologically identical to these alternate cartilage subtypes. However, the histological analysis specifically pertaining to human costal cartilage and its composition remains poorly documented in the literature. Furthermore, there is an inherent assumption that when costal cartilage allografts are requested for use in surgical reconstruction, they are consistent in their histologic composition. The nature of the tissue within a costal cartilage specimen may vary according to age of the donor[1] and anatomical site of harvest. This study is the first of its kind that aims to identify the histologic characteristics of irradiated human costal cartilage, by assessing the degree of variability pertaining to its histologic architecture, collagen content and degree of mineralization. The ability to predict the histologic nature of this tissue could lend itself to a heightened understanding of its biologic behaviour

once implanted into a human recipient. This in turn has the potential to improve postoperative outcomes by establishing and selecting favourable donor cartilage characteristics. The targeted and specific selection of costal cartilage allografts could rationalize its surgical and reconstructive applications, which may prevent postoperative complications that are attributed to the histologic nature of these allografts.

Methods

Twenty-five samples of gamma-irradiated human costal cartilage were ethically obtained from Australian Biotechnologies. Each irradiated sample of cartilage was then sectioned three times, in order to be stained separately with haematoxylin and eosin, Masson's trichrome, and tetrachrome staining. Haematoxylin and eosin staining was used to identify the basic histologic architecture of each specimen. Masson's trichrome stains enabled the quantification of each specimen's collagen content by staining it light green. The tetrachrome stain demonstrated each specimen's osteoid content by staining it red, to correlate with the degree of mineralisation. The three separate stains for each slide were then microscopically examined by the author under 1 mm, 500 and 200 µm magnifications under the light microscope. Electronic screen captures of the most representative histologic sections for each slide were saved. The screen captures relevant to each specimen and their stains were then examined using ImageJ software.[2] The haematoxylin and eosin stains were used to identify the cross-sectional diameter of the histologic zones of cartilage, as well as the total chondrocyte cell counts and chondrocyte surface area. Three measurements were recorded, pertaining to the diameter of the histologic zones of interest. The overall diameter of the specimen in millimetres, the superficial to germinal basal layer measurement at 200 µm magnification, and the superficial to ossified zone at 500 µm magnification (Fig. 1). The total number of chondrocytes detected in each haematoxylin and eosin-stained slide were also calculated using the cell counting function on ImageJ at 1 mm magnification. The average chondrocyte surface area was also derived from these sections, using the ImageJ surface area calculation tool under magnification at 200 µm (Fig. 2).

To calculate the total collagen content within each allograft specimen, the slides stained with Masson's trichrome were examined at 1 mm, 200 and 500 µm magnifications. The quantity of light green staining (representative of collagen content) was extracted from each slide using the chromatic detection software within ImageJ. The total surface area of the light green staining was digitally subtracted from the surrounding tissue, and expressed as a percentage of the total tissue identified within the slide (Fig. 3). This process was repeated for every slide at 1 mm, 200 and 500 µm, and an average percentage of collagen content within each specimen was calculated.

The same technique was used to identify mineral content within the tetrachrome stained slides, whereby the parameters for mineral detection were adjusted to detect the signature red hue within the cartilaginous tissue stained with tetrachrome. The surface area of red- stained tissue was then quantified and expressed as a percentage, denoting the amount of mineralized tissue contained within each specimen (Fig. 4) at all magnifications of 1 mm, 200 and 500 µm. The average mineral content for each specimen was derived from the average percentage mineral content found in each of the magnified slides pertaining to that specimen.

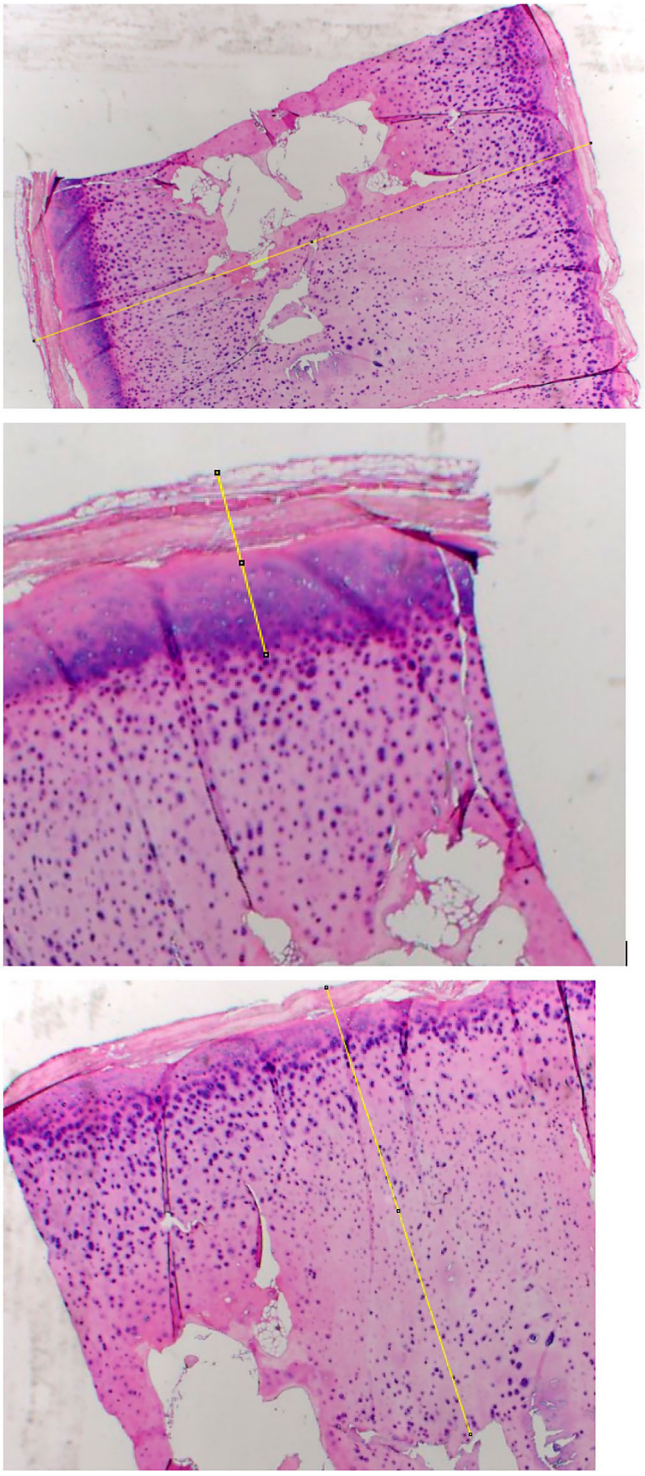


Fig. 1. Specimen diameters, showing superficial to germinal basal layer reference measurement (500 µm magnification) and superficial to ossification layer reference measurement (500 µm magnification).

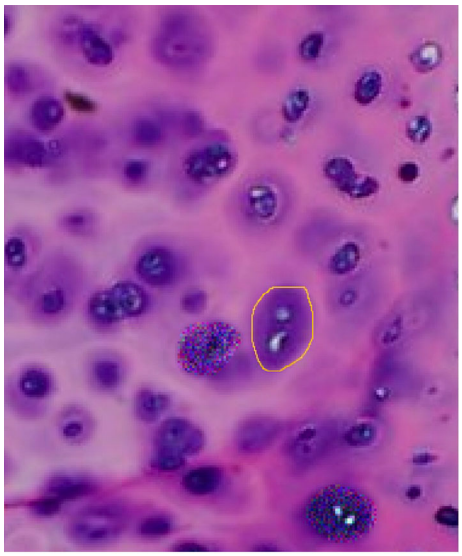


Fig. 2. Cell surface area calculation using haematoxylin and eosin staining at 200 μm magnification.

Results

The measurement of overall specimen diameter ranged from 6.45 to 10.37 mm. The median specimen diameter was 8.20 mm, with the interquartile range (IQR) = 1.59. The specific histological thickness of the superficial to basal layer zone and the superficial to calcified zones were measured in the scale of micrometres (μm) with the range of thicknesses respective to each zone depicted in Table 1. As expected, the width of each specimen from its most superficial layer to the basal or germinal layer of chondrocytes was thinner in comparison to the measurement from the superficial to the calcified layer. The measurements depicting these two standardized zones reflected a median thickness of 1409.91 μm for the superficial to basal layer zone, with an IQR of 885.59 μm. The median thickness of the superficial to calcified layer was 4146.26 μm, IQR = 1441.83 μm. The median chondrocyte area was found to be 4422.74 μm^2, with the 75th percentile (Q_3) at 6000 μm^2, IQR = 2622.72 μm^2. The total chondrocyte counts (per square millimetre) ranged from a maximum of 591, and a minimum of 289, with a median number of 418 chondrocytes. The total percentages of mineral and collagen content from all specimens is represented in Table 2. The median percentage of collagen content within each specimen was found to be 45.17%, with the respective median percentage of 71.82% for mineraloid material. The interquartile range of mineral content was 14.75%, and 20.48% for collagen content.

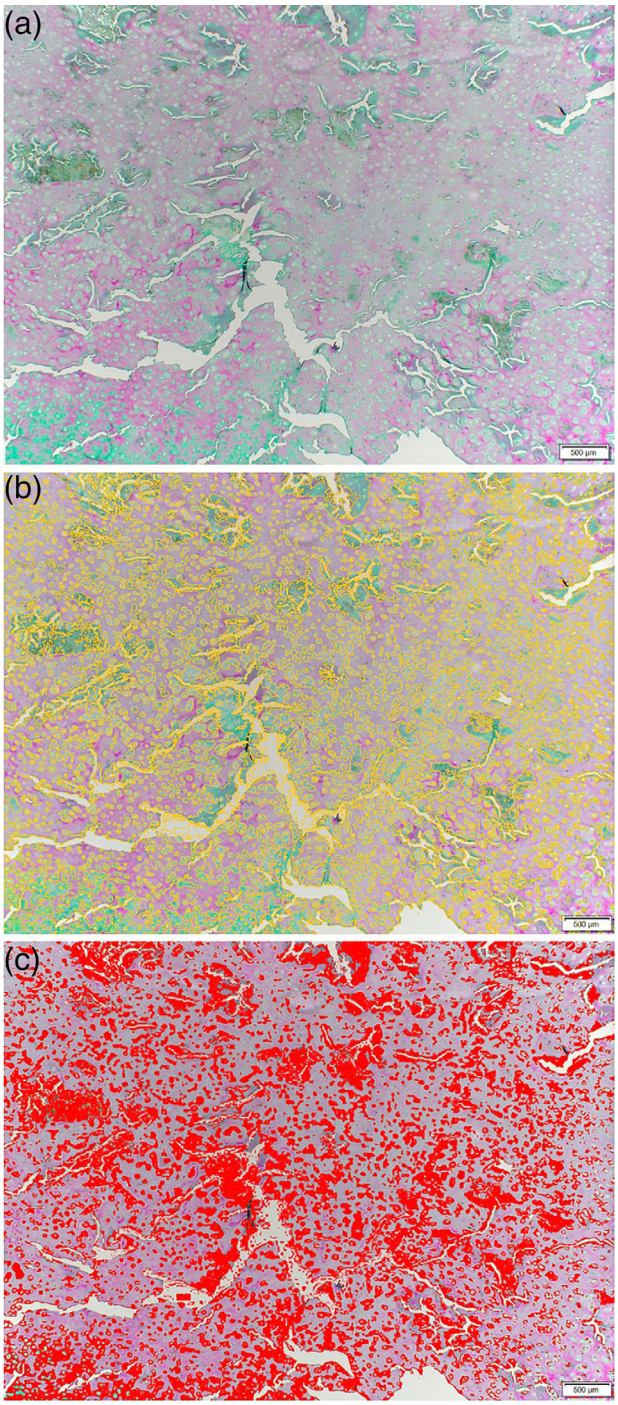


Fig. 3. Light green staining collagen from Masson's trichrome stained slide (a), outlined by the imageJ chromatic detection tool (b) and highlighted to calculate surface area (c).

Discussion

It is evident from the results that considerable variation amongst costal cartilage specimens exists. This variation pertains to differences in specimen size, histologic architecture, and the nature of the tissue composition. The relatively narrow interquartile range of values representing the specimen diameter is consistent with the fact that all specimens have been derived from the same anatomical site, being the costal cartilage of cadaveric human donors. Interestingly, there remained notable variation between the distance measured from the superficial layer to the calcified zone of tissue (IQR = 1441.83 μm, median of 4146.26 μm). The interquartile range is also skewed below the median value, as it is not evenly distributed either side of the mean. This may be accounted for by

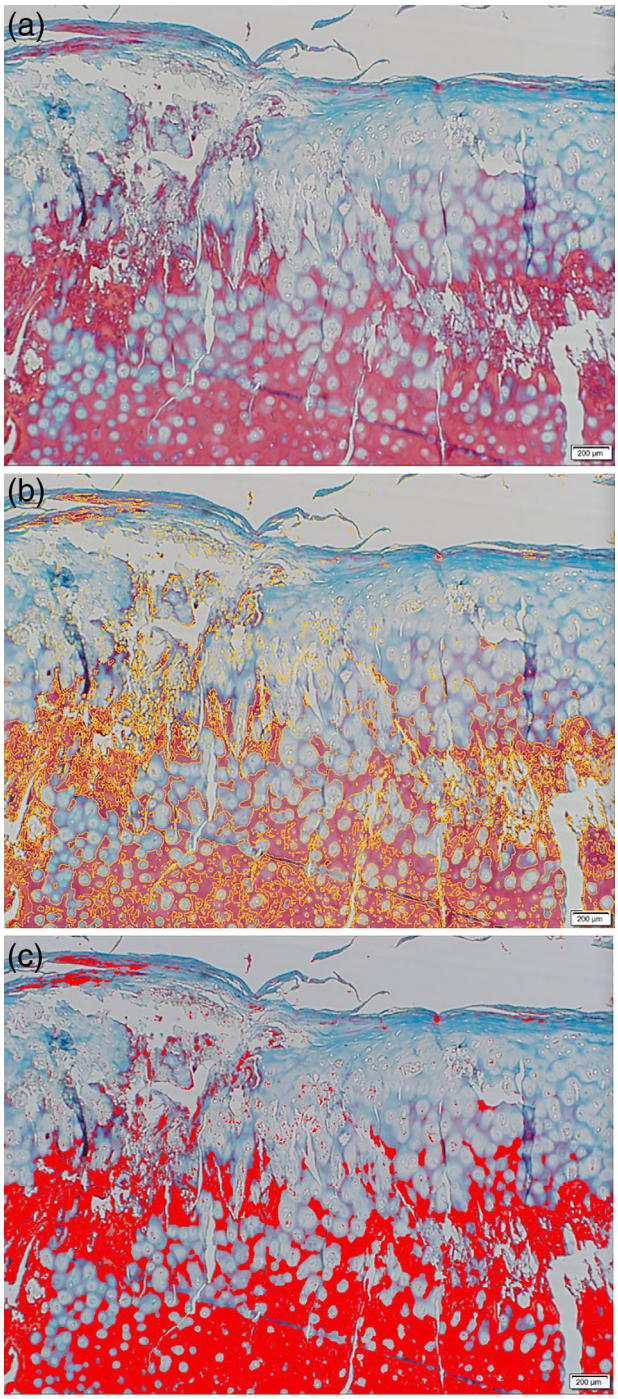


Fig. 4. Red staining mineraloid tissue from tetrachrome stained slide (a), outlined by the imageJ chromatic detection tool (b) and highlighted to calculate surface area (c).

Table 1 Measurement of histological thickness (μm)

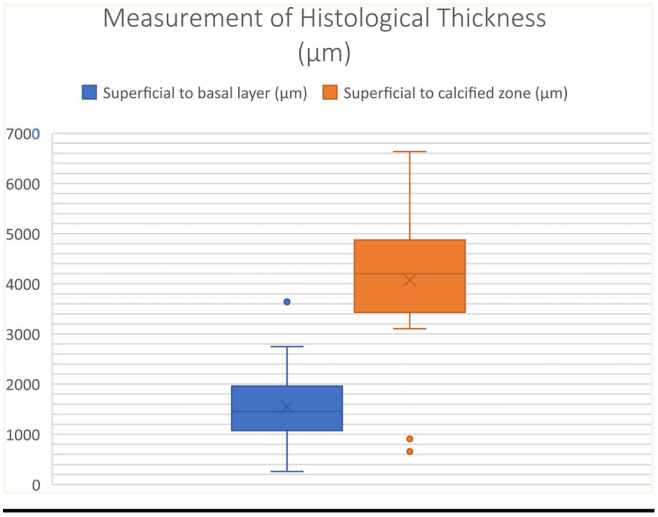
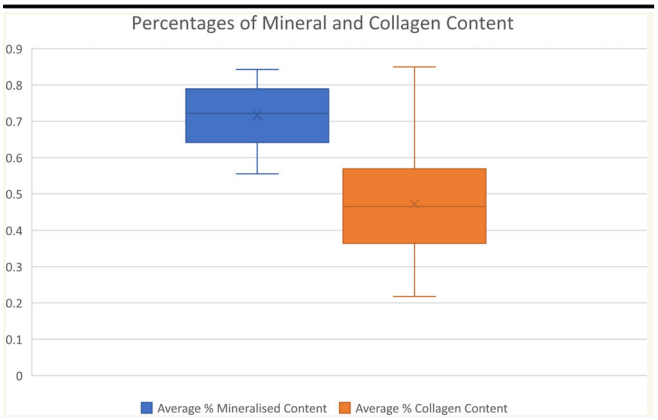


Table 2 Percentages of mineral and collagen content



the variation in age of the cartilaginous specimens and their respective donors, with higher levels of calcification potentially attributable to older specimens. There is a narrower interquartile range for the dataset corresponding to the distance from the superficial layer to the basal layer of germinal chondrocytes (IQR 885.59 μm). This could be explained by the fact that it is a more histologically reproducible measurement which is anatomically defined by the stratum basale at its inferior extremity. Despite this, the interquartile range is skewed superiorly to the median line. This suggests that considerable variation exists amongst these measurements, even though they have all been derived from a standardized anatomical set of landmarks. The standardization of our landmarks was achieved by keeping the reference points of measurement identical to those outlined by He *et al.*,[3] whereby their schematic representation of the zonal architecture of cartilage and its schematic histologic boundaries were used as reference points for the measurements obtained within this study.

All cartilage specimens were inherently composed of chondrocytes. Our results demonstrate that there is significant variation in their histologic area (IQR = 2622.72 μm^2, median 4422.74 μm^2), which implies the 25 cartilage specimens were not morphologically identical. In addition, the differences between the mineraloid composition and the collagenous composition for each specimen demonstrated marked variation, which emphasizes the diversity of chemical composition that exists between allografts derived from the same species. There was a higher

degree of variability in collagen content between specimens (IQR = 20.48%), in comparison to the calcified mineral content (IQR = 14.75%), which may be reflective of age-related degenerative changes, or it may represent baseline morphologic variations dependent on the anatomical location from which the specimen was harvested (e.g. medial sternocostal joint vs. lateral costochondral junction). It can be deduced that a high magnitude of variation may exist between irradiated samples of cadaveric human costal cartilage, irrespective of their derivation from a consistent human anatomical site. These variations in collagen and mineral content can render certain cartilage specimens more favourable than others when selecting allografts for reconstructive rhinoplasty or otoplasty, for instance. A cartilage allograft with a high percentage of mineralization may be less malleable intraoperatively and not have the biologic longevity as a specimen with a low mineral content and a higher percentage of collagen.

Mallinger and Stockinger[4] were amongst the earliest to document how human costal cartilage displays altered histologic characteristics as a consequence of physiologic ageing. They identified how the arrangement of 'amianthoid fibres' within the type II collagen of costal cartilage was a key conformational change that could be appreciated under the electron microscope, and hence be correlated with costal cartilage specimens of older donors. Our study used light rather than electron microscopy and hence would not have been able to identify these characteristic patterns of ageing as they relate to the orientation and diameter of collagenous fibres. Other studies have since explored how patterns of ossification within costal cartilage may be used to estimate human age via light microscopic examination,[5] using the Von Kossa, Haematoxylin–eosin, Congo Red and Safranin staining methods. Similar to our findings, Rejtarová *et al*. noted that characteristic patterns of ossification in costal cartilage were found within their sample of 17 human cadaveric specimens. In contrast, our study used a larger sample ($n = 25$) but did not correlate the degree of ossification with the age of the cadaveric specimen, which was not disclosed. Our study also used tetrachrome staining to determine the degree of ossification within a specimen, as originally documented by Villanueva and Hattner.[6] Ralis and Watkins[7] reiterated the usefulness of their modified tetrachrome stain as it allowed accurate differentiation between ossified components and non-mineralized tissue, similar to our analysis. Our study remains the first of its kind to differentiate and quantify calcified tissue within human costal cartilage in this manner. It is also the first to establish its connective tissue content by staining with Masson's trichrome, which highlights its collagen composition by characteristic light green staining.[8]

In contrast to previously published work, this study is the first to use specific biologic stains to identify and characterize the variability that exists between specimens of irradiated human costal cartilage. The quantification of collagen and mineral content, in conjunction with the identification of its morphologic and ultrastructural inconsistencies, has formed the groundwork to improve our understanding of this tissue which is readily used in reconstructive surgery. Further assessment of these specimens under electron microscopy, micro-CT and *in vitro* mechanical stress-relaxation testing may form the basis of subsequent research to augment our preliminary histologic findings.

Our study highlights the significant variation that exists between different specimens of irradiated costal cartilage, which is defined by certain histologic characteristics. This has the potential to influence how cadaveric costal cartilage allografts are selected for the purpose of soft tissue reconstruction. The demonstration of certain histologic features can assist in the selection of certain allografts which specifically possess higher collagen and lower mineralized content, which can contribute to the predictability of their *in vivo* behaviour. Further studies including more detailed analyses of their biomechanical properties can enhance our understanding of allograft variability *in vivo*. Preliminary analyses such as those undertaken in this study have the potential to assist in the prediction of postoperative longevity of reconstructed soft tissues, and the optimization of post- transplantation outcomes. Ultimately, a more accurate prediction of allograft behaviour can reduce the incidence of revision surgery and optimize patient outcomes following cartilaginous reconstruction.

Acknowledgements

The authors wish to acknowledge Senior Lecturers Dr. Vedran Lovric, Dr. Rema Oliver and their team at SORL in the preparation and staining of the cartilage specimens used for the purpose of this study. We also wish to acknowledge that all patients and medical staff described in this original manuscript have given their voluntary verbal and written consent to be published for the purposes of surgical teaching and education. Open access publishing facilitated by University of New South Wales, as part of the Wiley - University of New South Wales agreement via the Council of Australian University Librarians.

Author contributions

Sonia Sinclair: Formal analysis; investigation; methodology; writing – original draft; writing – review and editing.

Conflict of interest

None declared.

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