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Original research article

Experimental study on clinical and morphological determination of the optimal cannula diameter for lipoaspirate harvest from rabbit inguinal fat pad

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Abstract
Determining the most appropriate cannula diameter for lipoaspirate harvesting is important, both in terms of the quality and composition of the material obtained, and the ease of practical use of the cannula. The size of the cannula is one of the main factors affecting the qualitative characteristics of the obtained lipoaspirate sample for further use of adipose tissue. The purpose of the investigation was to clinically and histomorphometrically determine the optimal cannula diameter for lipoaspirate samples collection from rabbit inguinal fat pad in an experimental study. The methods of Animal models, Surgical procedures, Macroscopic examination, Histological examination, and Morphometric study were applied. There is a direct correlation between the percentage of connective tissue fibres in the lipoaspirate and the diameter of the cannula. The lack of clear criteria for selecting a lipoaspiration cannula is one of the limitations to obtaining generally accepted lipoaspiration protocols with subsequent use of adipose tissue. In this study, the animal experiment determined the most ideal cannula diameter suitable for collecting the largest amount of lipoaspirate for subsequent use.

Keywords: Adipose tissue; Analysis; Experiment; Fragment; Histology

Highlights:
- Optimal cannula diameter for lipoaspirate harvest determined using rabbit inguinal fat pad.
- Connective tissue fibres in lipoaspirate correlate with cannula diameter.
- Lack of clear criteria for cannula selection is a limitation in lipoaspiration protocols.
- Use of a 3mm cannula yields evenly distributed lipoaspirate with practical convenience.

Introduction

Fat autotransplantation, or lipofilling, is one of the most common operations in cosmetology. Lipofilling is the process of transferring fat from one area of the body to a different area, where correction is desired. Normally, this surgery has no associated trauma or potential danger. Since its introduction, lipofilling (also known as lipomodelling) has become increasingly popular. As a soft tissue filler, adipose tissue has many desirable properties: it is easily harvested, autologous, and can be reintegrated into recipient sites (Beck et al., 2014; Erdim et al., 2009; Kirkham et al., 2012; Kotsiubenko et al., 2021). However, fat grafting has an unpredictable resorption rate, so there is great interest in optimising this procedure to ensure consistent graft volume. Fat harvesting, part of fat transfer surgery, is the removal of adipose tissue from the donor site. Lipofilling is the process of removing autologous fat and transplanting it into the desired part of the body using syringe injections (Grishin et al., 2021). The syringe is connected to a special tube, a cannula, which allows the material to be injected under the skin. Different harvest procedures, such as whole fat excision or liposuction cannulas, result in a range of fat particle volumes, which may play a role in the cellular stability of grafts (Markov et al., 2021; Mustafin and Volkov, 1984). Selecting the correct cannula diameter is one of the determining considerations in these operations, but the subject of "correct" cannula diameters for lipofilling is currently unsettled and still leaves room for debate among specialists on the matter.

The first "fat grafting" procedure dates back to 1893, when Gustav Neuber transferred fat the size of a bean from the upper arm to the face. However, at that time, lipofilling did not

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gain due popularity in plastic surgery, and remained an overlooked subject until the late 20th century (Mansurov et al., 2013).

There is an optimal cannula diameter that ensures maximum collection of lipoaspirate from the rabbit's inguinal adipose tissue with minimal side effects or complications. Autologous fat grafting is widely used in cosmetic and reconstructive surgery, but long-term effects remain controversial. Every step in the transfer process can result in mechanical damage to the graft tissue. In particular, during liposuction, the removed adipose tissue splits into individual globules and is exposed to shear forces that can affect the subsequent viability of the fat graft. The optimal size of a liposuction cannula for fat transplantation is unknown (Canizares et al., 2017; Gause et al., 2014; Trivisonno et al., 2014). There are side effects and complications associated with lipoaspirate collection, especially when using different cannula sizes. Using cannulas that are too small can make it difficult to collect enough fat, while large cannulas can cause tissue injury and bleeding. Other possible complications include infection, scarring, uneven fat extraction, and aesthetic concerns. Choosing the right cannula size depends on the individual patient, the area to be treated, and the experience of the technician. Consideration of safety and accuracy are key to achieving the best results and minimising the risk of complications (Łapiński et al., 2021; McGuire et al., 2022; Yuanjing et al., 2021).

The interaction of the components of the liposuction system (suction pump, suction tube, collection container, and cannula) determines the effectiveness of liposuction. In clinical practice, however, no single component is more important than the cannula. The design of the cannula, including the port design, port placement and rod characteristics, is the most important factor affecting the flow resistance, aspiration rate, and the final contour (Sagalevich et al., 2016). There are many design and port placement options available, but functional improvements to the cannula stem have been largely ignored.

The most important and variable component of the lipoplasty system is the liposuction cannula. Cannula choice plays a dual role. It has been shown that the addition of a cannula makes the greatest contribution to flow resistance in the lipoaspiration system. Due to direct contact with the layers of adipose tissue, the selected cannula has a noticeable effect on the rate of resection, the final contour, and the quality of the aspirate for possible additional transplantation (Okassova et al., 2021; Sahalevych et al., 2022). Regardless, there is no universal standard for the design and manufacture of liposuction cannulas. Many variants with different port and cannula stem characteristics are available, and there is little clinical advantage to them. Often, the use of a specific cannula depends on the knowledge of the surgeon and the availability of equipment (Avelar, 2021; Frojo et al., 2023; Lukyanenko et al., 2021; Ozsoy et al., 2006). Thus, the proposed study solves several problems associated with the procedure of lipoaspirate collection from inguinal adipose tissue. It helps to determine the optimal cannula diameter to achieve the best results of fat harvesting with minimal damage to the surrounding tissue. This improves the quality of the procedure and reduces the risk of complications. The results of this study could have a significant impact on the practice of surgery and aesthetic medicine, allowing medical professionals to improve fat harvesting techniques and achieve better surgical outcomes, which brings satisfaction to patients.

The design of the port and the intrinsic friction coefficient of the cannula are important variables affecting lipoaspiration efficiency. Determining the most favourable liposuction cannula and hypodermic needle size in relation to adipocyte viability can help to increase fat graft survival. When re-injections are required, storing fat tissue collected during the first procedure can be a practical solution, as long as it is stored at the correct temperature to maximise the number of viable fat cells.

Materials and methods

Animal models

The present study was performed on 32 Californian rabbits, all young (under 7 months of age) weighing 2940 ± 159 g. The sex of the animals was not considered in the experiment. As the sex of the animal has no direct influence on the morphological characteristics of the fibre, it can be considered a minor factor in this particular experiment.

Surgical procedures

The animals were divided into 4 equal groups of 8 animals each. All rabbits underwent the same surgical procedures. General anaesthesia was induced through a combination of ketamine 35 mg/kg and xylazine 5 mg/kg, administered intramuscularly. Adipose tissue was obtained by the Columan method from the fatty tissue of the inguinal region of animals. Lipoaspirate was harvested by manual aspiration using cannulas (Newmed, Pakistan) and 10 ml Luer-Lock syringes. Cannulas with one central and two side holes were used, the inner diameter of the cannula and the diameter of each of the holes were 1.0 mm, 2.0 mm, 3.0 mm, and 4.0 mm. All surgical interventions were performed by the same surgeon. During the adipose tissue harvesting procedure, the frequency of reciprocating movements per minute and the incidence of cannula occlusion were counted for each case.

Macroscopic examination

Fragments of adipose tissue obtained from each sample were examined under magnification in the operating room after extraction and photographed. The lipoaspirates were then left to decant for 10 minutes, the supernatant was extracted, and then the adipose tissue was placed into 5 cm^3 syringes for volume measurement. The obtained quantitative value was translated into a semi-quantitative scale in accordance with the following gradation (0 points – less than 1 cm^3, 1 point – 1–2 cm^3, 2 points – 2–3 cm^3, 3 points – more than 3 cm^3) (Fig. 1). After the measurement, each sample was sent for histological examination.

Histological examination

The subject for histological examination was the fat and stromal-vascular fractions of the lipoaspirate obtained after decantation. The material was preserved in 10% neutral buffered formalin. Next, they were placed in histological cassettes with a thickness under 5 mm, held in a carousel-type tissue processor to obtain paraffin blocks. Representative histological sections with a thickness of 5 microns were obtained from each paraffin block and stained with hematoxylin and eosin according to standard protocol.

Morphometric study

The criteria for semi-quantitative evaluation of histological sections are presented in Table 1.

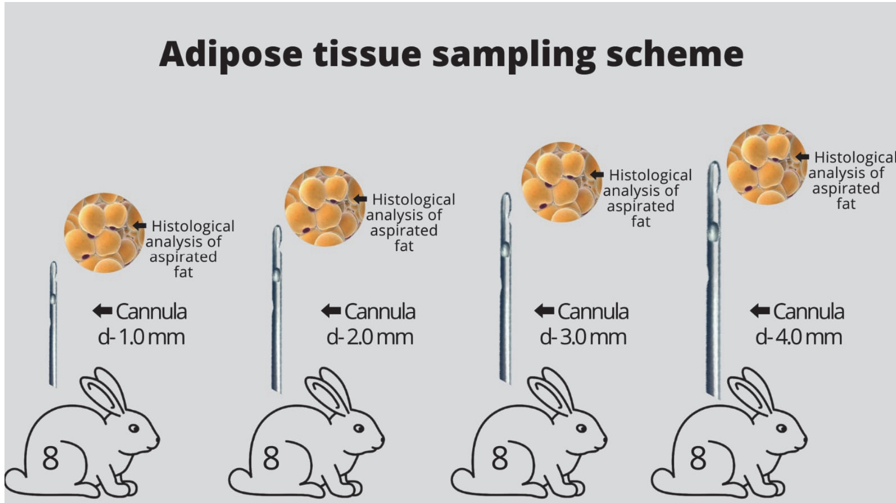


Fig. 1. Lipoaspirate sampling and analysis scheme

Table 1. The system of semi-quantitative evaluation of histological sections

Evaluation criteria	Rating scale			
Description	0	1	2	3
Qualitative characteristics of the lipoaspirate harvested				
Relative amount of adipose tissue in the lipoaspirate, %	<50	50–75	75–90	>90
Relative amount of fibrous tissue in the lipoaspirate, %	<10	50–75	75–90	>90
Size of stromal fragments (fibrous tissue, collagen fibres), mm	–	<1	1–1.5	>1.5
Relative number of artefacts (destruction of adipose tissue), %	<2	2–10	10–30	<30
*Practicality of use per unit of time (1 min)**				
Labour input per operation	Light exertion No effort	Requires moderate exertion and effort	Requires intense exertion and effort	Difficult to apply in routine practice
Frequency of reciprocating movements	<300	300–400	400–500	>500
Frequency of cannula occlusion	–	1–2	3–4	>5

Note: * mean score including arithmetic mean of labour input per unit time, frequency of reciprocating movements, frequency of cannula reinsertion after lavage.

The subjective criteria used to assess the practical usability of a large-diameter cannula included the following factors: ease of holding the cannula during the lipoaspirate collection procedure; stability of the cannula during movement and manipulation, feeling of comfort and control during cannula handling; the ability to accurately position the cannula to achieve the desired area of lipoaspirate collection; ease of use of the cannula in different positions of the patient's body; no inconvenience or discomfort for the patient when using a large diameter cannula; ensuring efficient and rapid lipoaspirate collection with a large diameter cannula. These criteria were used to evaluate the ease and effectiveness of using a large-diameter cannula during the lipoaspirate collection procedure.

The relative amount of fat and stromal-vascular fractions in the composition of lipoaspirate was calculated from the total area occupied by tissues on histological sections in relation to the area of the histological section. The artefacts included tissue with a disturbed morphological pattern characteristic of mechanical damage (destruction of adipose tissue).

Basic morphometric measurements and the taking of images were carried out using a Carle Zeiss digital colour microscope and Image software. Histomorphometric assessment of histological preparations was performed blindly by two independent pathologists at the magnification of the microscope objective (×100), the result being an arithmetic mean value.

Statistical analysis

Statistical analysis was carried out using the χ^2 with correction for continuity (Yates), Fisher's exact test was used for a small number of observations (less than 5). Statistical data processing was performed using IBM SPSS 22.0 statistical analysis software (IBM Corp., Armonk, N.Y.). The differences were considered statistically significant at $p \leq 0.05$.

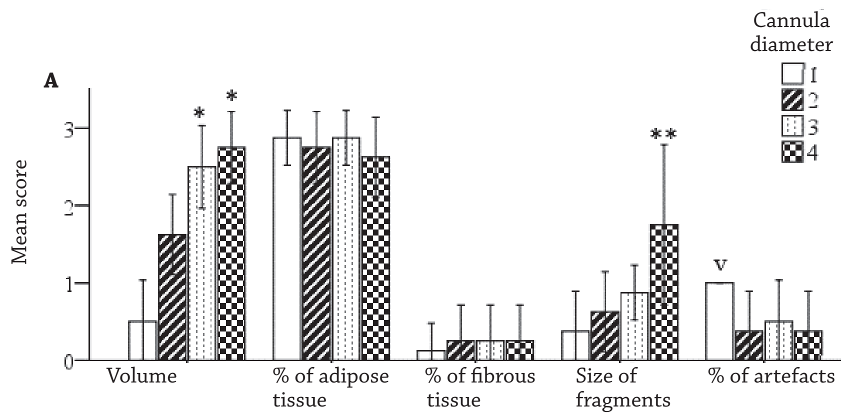
The use of animals in this study was approved by the Bioethics Commission of the Medical University of Karaganda (Approval No. 53, dated 03/15/2021), and the study complied with the requirements of national legislation (Ahn et al., 2021; Beck et al., 2014; Campbell et al., 1987; Canizares et al., 2017; Erdim et al., 2009; Gause et al., 2014; Good Laboratory Practice Standard, 2015; Kirkham et al., 2012; Order of the Minister..., 2020; Ozsoy et al., 2006) and recommendations of the European Convention for the protection of vertebrate animals used for experiments or other scientific purposes (European Convention..., 1986).

Results and discussion

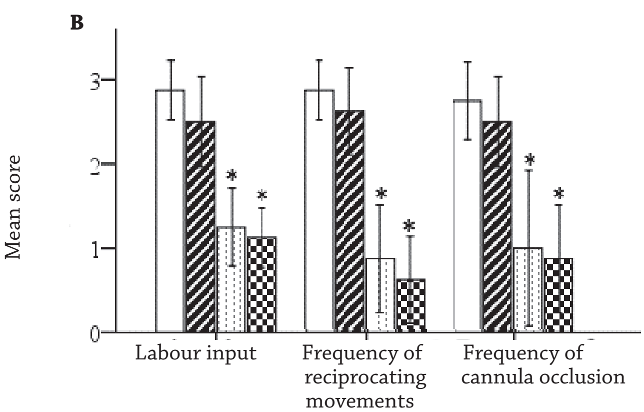
The effect of cannula diameter on the percentage of connective tissue fibres in lipoaspirate was studied to determine the optimal diameter for adipose tissue sampling. Using different cannula diameters, lipoaspirate was collected from the inguinal adipose tissue of rabbits. After collection, the obtained lipoaspirate samples were analysed, including the calculation of the percentage of connective tissue fibres. It was found that the diameter of the cannula influences the percentage of connective tissue fibres in the lipoaspirate. In particular, when using a smaller diameter cannula, a decrease in the percentage of connective tissue fibres was observed, indicating greater purity of adipose tissue samples. Thus, the choice of the optimal cannula diameter is an important factor in obtaining a high-quality lipoaspirate with a minimum content of connective tissue fibres.

Comparative characterisation of the quality of the material obtained in the study groups

Fig. 2 shows the mean semi-quantitative assessment scores for the quality characteristics of the lipoaspirate obtained using cannulas of different diameters. The mean volume of the obtained material in the groups with medium and large cannula diameters was 2.5 cm^3 and 2.8 cm^3, respectively. In the groups with minimum and small cannula diameters it was 0.5 cm^3 and 1.6 cm^3.



A. Qualitative characteristics of the obtained material



B. Subjective assessment of the adipose tissue harvesting procedure

Fig. 2. Comparative assessment of the qualitative characteristics of the lipoaspirate obtained and the subjective assessment of the adipose tissue harvesting procedure using cannulas of different diameters. * statistically significant differences from a cannula with a diameter of 1 mm and 2 mm ($p < 0.05$); ** statistically significant differences from a cannula with a diameter of 1 mm, 2 mm, and 3 mm ($p < 0.05$); v – statistically significant differences from a cannula with a diameter of 2 mm, 3 mm, and 4 mm ($p < 0.05$).

The mean relative amount of fatty tissue in the lipoaspirate in all groups was 90–95% and the mean relative ratio of fibrous tissue was less than 10%. In groups with a minimal, small cannula diameter, the average size of stromal fragments (fibrous tissue, collagen fibres) was less than 1 mm, and in the group with a large cannula diameter in 37.5% of cases, the average size of fragments was 1–1.5 mm, and 25% of cases exceeded 1.5 mm. Artefacts (destruction of adipose tissue) in all groups occupied less than 10% of the area, with less than 2% of artefacts in the medium- and large-diameter cannula groups in 50% and 80% of cases, respectively.

Comparative subjective assessment of the practicality of using cannulas with different diameters

Table 2 presents the mean semi-quantitative score of the subjective assessment of the practicality of using cannulas with different diameters. The mean practicality scores were 2.9 and 2.7 for the minimal and small cannula diameter groups, respectively, and – 1.1 and 0.9 for the medium and large cannula diameter groups. Labour input per unit of time in groups with minimal and small cannula diameter averaged 2.9 and 2.6 points, respectively, and in groups with medium and large diameter – 1.3 points and 1.1 points.

The frequency of reciprocating movements during aspiration of adipose tissue in groups with minimal and small cannula diameter was 2.9 points, and in groups with medium and large cannula diameter – 0.9 points and 0.6 points. In the group with minimal and small cannula diameters, the average frequency of reciprocating movements of more than 500 aspirations was 87.5% and 62.5%, respectively. The frequency of reciprocating movements during aspiration in groups with an average and large cannula diameter of less than 300 was detected in 25% and 37.5% of cases, respectively.

The frequency of cannula occlusion in groups with minimal and small cannula diameters was 2.8 points and 2.5 points, and in groups with medium and large cannula diameter – 1.0 points and 0.9 points. At the same time, in the group with a minimum and small cannula diameter, more than 5 cases of cannula occlusion were observed in 75% and 50% respectively, while in the medium and large diameter groups, only 25% and 37.5% of cases occurred in the cannula occlusion group.

Table 2. Comparative assessment of the qualitative characteristics of the lipoaspirate obtained, and the clinical assessment of the practicality of the adipose tissue harvesting procedure using cannulas of different diameters

Evaluation criteria	Type of cannula			
Description	1	2	3	4
Qualitative characteristics of the lipoaspirate harvested				
Volume of material obtained, cm3	0.5 ± 0.5	1.6 ± 0.5	2.5 ± 0.5	2.8 ± 0.5
Relative amount of adipose tissue in the lipoaspirate, %	2.9 ± 0.4	2.8 ± 0.5	2.9 ± 0.4	2.6 ± 0.5
Relative amount of fibrous tissue in the lipoaspirate, %	0.1 ± 0.4	0.3 ± 0.5	0.3 ± 0.5	0.5 ± 0.5
Size of stromal fragments (fibrous tissue, collagen fibres), mm	0.4 ± 0.5	0.6 ± 0.5	0.9 ± 0.4	1.8 ± 1.0
Artefacts (destruction of adipose tissue), %	1.0 ± 0.0	0.4 ± 0.5	0.5 ± 0.5	0.4 ± 0.5
Practicality of use per unit of time (5 min)*	2.9 ± 0.4	2.7 ± 0.4	1.1 ± 0.6	0.9 ± 0.5
Labour input per unit of time	2.9 ± 0.3	2.6 ± 0.5	1.3 ± 0.5	1.1 ± 0.4
Frequency of reciprocating movements	2.9 ± 0.4	2.9 ± 0.2	0.9 ± 0.6	0.6 ± 0.5
Frequency of cannula insertion (occlusion)	2.8 ± 0.5	2.5 ± 0.5	1.0 ± 0.9	0.9 ± 0.6

Note: * mean score including arithmetic mean of labour input per unit time, frequency of reciprocating movements, frequency of cannula reinsertion after lavage.

A comparative morphological study of the qualitative characteristics of rabbit inguinal lipoaspirate and the subjective assessment of the adipose tissue harvesting procedure using cannulas of different diameters in an animal experiment was carried out. The most significant finding of the study is the establishment of a 3 mm cannula in the experiment as the most suitable and convenient procedure for obtaining lipoaspirate from rabbit inguinal pat (Fig. 3).

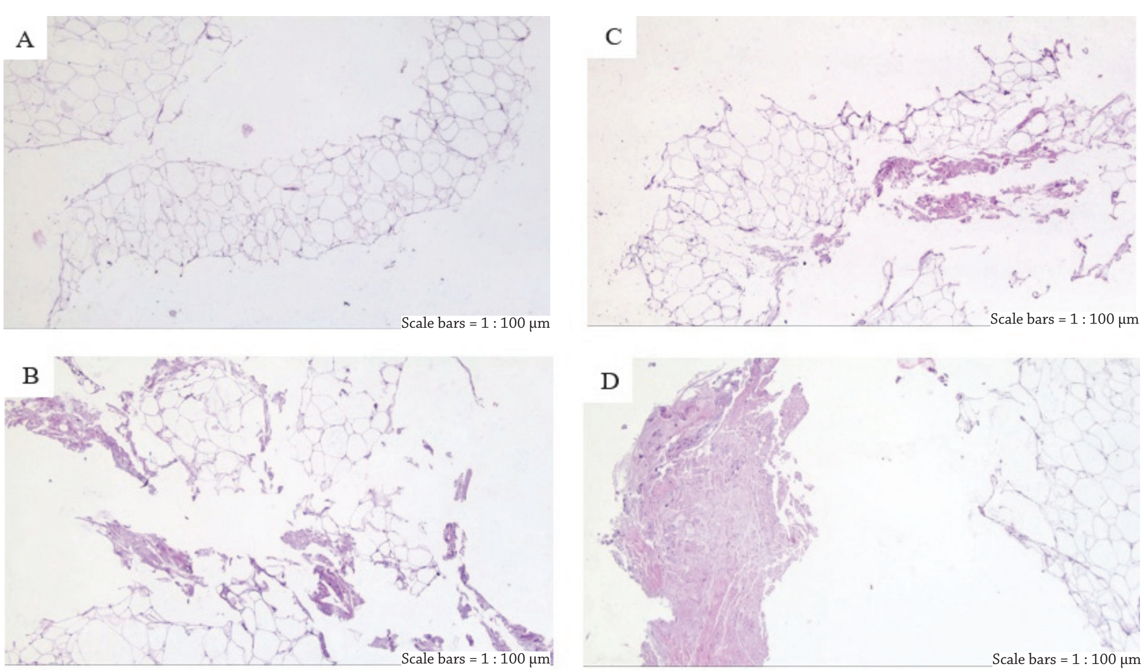


Fig. 3. Histological pattern of the obtained lipoaspirate. Haematoxylin and eosin stain. ×100. (**A**) Small fragments of adipose tissue. A group with a cannula diameter of 1 mm. (**B**) Small fragments of destructed adipose tissue and fibrous tissue. A group with a cannula diameter of 2 mm. (**C**) Small fragments of adipose tissue and fibrous fraction up to 1 mm. A group with a cannula diameter of 3 mm. (**D**) Small fragments of adipose tissue and a large fragment of a fibrous fraction of more than 1.5 mm. A group with a cannula diameter of 4 mm.

An important finding of this experiment was that a cannula with a diameter of 3 mm allows a sufficient volume of lipoaspirate with an optimal ratio of fat to fibrous tissue to be harvested per unit time (1 min). With the 3 mm cannula, the average volume in 1 minute was found to be statistically insignificant compared to the 4 mm cannula, but significantly greater than the minimum and small diameter cannula ($p < 0.05$).

The amount of fibrous tissue is of great clinical importance for the subsequent use of lipoaspirate. In addition to adipose tissue, fibrous tissue, which provides mechanical support and is rich in a stromal-vascular component, has been shown to improve lipoaspirate properties (Bluguermann et al., 2013; Del Vecchio and Rohrich, 2012; Gryshchenko et al., 2019; Kato et al., 2014). The size of the fibrous tissue fragments also affects the quality of the obtained lipoaspirate and the possibilities of its use. The ideal fibrous tissue microfragment should be large enough to contain sufficient mesenchymal tissue, but also should not interfere with nutrient diffusion (Auersvald et al., 2022; Fan et al., 2021; Fontes et al., 2018; Hui and Chui, 2018; James et al., 2018; Tong et al., 2018; Wu and Goldman, 2017). This study shows that using 3 mm and 4 mm cannulas, lipoaspirates containing approximately 10% fibrous tissue (8.9% and 9.3% respectively) can be harvested, but the size of the fibrous tissue fragments when using cannula #3 is limited to 1.5 mm (approximately 1 mm on average), whereas individual large fibrous tissue fragments (up to 2.5 mm), unevenly distributed ($p < 0.05$) were identified when using the 4 mm cannula.

Conclusion

This was the first time an experimental study was conducted to determine the clinical and morphological determination of the optimal cannula diameter for the collection of lipoaspirate from rabbit inguinal adipose tissue. The results of the study confirm that there is an optimal cannula diameter that provides the highest efficiency of lipoaspirate collection with minimal damage to surrounding tissues and the risk of complications. This allows medical specialists to more accurately determine the parameters of the procedure for collecting lipoaspirate from rabbit inguinal adipose tissue, which helps to improve the quality of the procedure and patient outcomes. Further application of these findings can have a significant impact on the practice of surgical interventions and aesthetic medicine.

Mechanical fractionation of adipose tissue causes deterioration of its inherent properties, as each repeated mechanical injury results in a large number of destroyed adipocytes and cellular debris, which can affect graft engraftment and the final outcome of the surgical procedure. We believe that by selecting a cannula with a diameter of 3 mm, a "ready-to-use" optimally sized lipoaspirate with evenly distributed fibrous tissue can be obtained without the need for additional treatment.

It has also been observed that the use of a cannula with a large diameter is subjectively characterised by a higher practical convenience in use. The use of these cannulas when harvesting adipose tissue did not require additional efforts and changes in the pace of the procedure. It was found that using a 3 mm cannula, the labour input, rate of reciprocating movements during aspiration of adipose tissue and incidence of cannula plugging were not statistically significantly different from using a 4 mm cannula, but were significantly lower than using the minimum and small diameter cannula ($p < 0.05$). Thus, a cannula with a diameter of 3.0 mm is optimal for practical use, reducing the volume of effort on the part of the surgeon and mechanical trauma to the surgical site.

The strengths of this study include the identification of the most suitable cannula diameter for harvesting the largest amount of lipoaspirate suitable for subsequent use, based on animal experiments. The study also has several limitations, in particular, a limited number of cannulas with different diameters were studied, and there was no comparative characterisation of the adipose tissue harvesting procedure performed by several surgeons from different anatomical areas. Further studies in this area will expand knowledge to later improve the adipose tissue harvesting procedure and its use. Histomorphometric analysis and assessment using a system of developed subjective criteria showed that a cannula with a diameter of 3 mm for lipoaspirate harvesting from rabbit inguinal fat pads provides a lipoaspirate with uniformly distributed fibrous tissue of an optimal "ready-to-use" size.

Ethical aspects and conflict of interest

The authors have no conflict of interests to declare.

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