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Allograft contamination during suture preparation for anterior cruciate ligament reconstruction: an ex vivo study

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Abstract

Purpose Effects of suture preparation on graft contamination remain unknown in anterior cruciate ligament reconstruction (ACLR). This study aimed to evaluate the incidence of allograft contamination at different time points of graft preparation and investigate differences in contamination between different sites of the allografts.

Methods Fourteen hamstring tendon (HT), 9 quadriceps tendon (QT), and 9 bone–patellar tendon–bone (BTB) allografts were harvested, sterilised, and stored following routine procedures. Graft suture preparation was performed with baseball stitching for soft tissue and bone drilling for bone plug. The time was recorded simultaneously. The graft was kept moist in a standard operating room environment for 30 min after the initiation of preparation. The specimens were obtained from the middle and both ends of each graft for culture at three different time points: pre-suturing, post-suturing, and 30 min after the initiation of preparation. A total of 192 specimens were transferred to the microbiology laboratory for culture, identification, and semi-quantitative assessment. Culture results were classified as negative, poor, and abundant based on the extent of growth. Contamination level was recorded as low or high corresponding to culture results of poor or abundant.

Results The duration of suture preparation was 348, 301, and 246 s for HT, QT, and BTB ($P=0.090$). The specimens had a positive culture rate of 41/192 (21.4%), of which 21 were from the ends and 20 from the middle. More positive samples with abundant bacterial growth were detected from the ends than from the middles post-suturing (7/8 vs. 1/7, $P=0.010$) and at 30 min (6/11 vs. 0/11, $P=0.012$). The total graft contamination rate was significantly higher at 30 min (19/32, 59.4%) than pre-suturing (4/32, 15.6%) and post-suturing (9/32, 28.1%) ($P<0.001$). The contamination rate with abundant bacterial growth was higher post-suturing (7/32, 21.9%) than pre-suturing (0%). No statistically significant differences were found among the three types of allografts.

Conclusion The contamination rate increases significantly at 30 min compared with pre-suturing and post-suturing. Suture preparation may have introduced the high-level contamination, to which the ends of the graft were more prone than the middle. Therefore, routine prophylactic decontamination after suture preparation should be considered, especially for the ends of the grafts.

Keywords Anterior cruciate ligament reconstruction · Allograft · Suture preparation · Contamination

Chenliang Wu, Xiuyuan Zhang and Yi Qiao contributed equally to this work.

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Abbreviations

ACLR	Anterior cruciate ligament reconstruction
HT	Hamstring tendon
QT	Quadriceps tendon
BTB	Bone–patellar tendon–bone
S_M	Sample from the middle
S_E	Sample from each end
ICC	Intra-class correlation coefficients

Introduction

Postoperative deep knee infection is a rare but devastating complication following anterior cruciate ligament reconstruction (ACLR), with the incidence rate ranging from 0.14 to 2.6% [12, 20, 37]. Several studies have shown an elevated risk of infection using allografts for ACLR [11, 12], to which graft contamination could be an important contributor. Most relevant studies have evaluated accidental contamination, such as dropping on the floor and decontamination methods, such as irrigation, mechanical agitation, or soaking with disinfection solution [3, 6, 25, 28, 33, 36]. However, limited number of studies have focussed on the potential risk of contamination during normal preparation of the graft [22, 23].

Suture preparation, as an essential part of graft preparation, involves suturing for tendon or drilling for bone plug according to graft types [27]. The risk of graft contamination may increase during this process due to the contact of the graft with potential contamination sources, such as gloves, clamps, needles, and sutures [14, 41, 42]. Moreover, the sites of graft with more frequent contact during this manipulation might be theoretically exposed to a higher risk of contamination. These have been mentioned in several studies, but have never been proven [12, 23].

Operative time has been considered as an independent risk factor for postoperative infection in ACLR, but without further investigation of the underlying mechanisms [2, 7, 9, 21, 26]. The increasing contamination rate of irrigation fluid and surgical instrument over time may be one reason [8, 42]. Similarly, the graft would also be exposed to potential airborne microbial contamination in an operating room during the waiting period after suturing due to arthroscopic preparations [1, 23, 39]. However, it needs to be confirmed whether the contamination rate of the graft increases after the waiting period.

Therefore, the primary goal of the current study was to evaluate the incidence of allograft contamination before graft suturing, after graft suturing, and before implantation. The secondary goal was to investigate differences in contamination due to suture preparation between different sites of the allografts. It was hypothesised that the expected contamination rate of allografts could significantly increase after graft suturing with further increase before implantation, with the ends of the graft being more prone to contamination than the middle due to manipulation.

Materials and methods

Pretreatment

The procurement, sterilisation, and storage of allografts was performed following routine procedures [24, 40]. All specimens were obtained from a tissue bank aiming for medical study. As shown in the flowchart (Fig. 1), all allografts were harvested from fresh Chinese Han cadavers under sterile conditions and immediately frozen at − 80 °C for 30 days. For further sterilisation, the allografts were soaked in 75% alcohol for 2 h and γ-irradiated with 25 kGy for 2 h after thawing [40]. The surfaces of grafts were swabbed for culture to confirm their sterility [15, 43]. Finally, 14 hamstring tendons (HT, from 8 men and 6 women, aged 37–58 years), including semitendinosus and gracilis tendons, 9 quadriceps tendons (QT, from 5 men and 4 women, aged 41–58 years), and 9 bone–patellar tendon–bone (BTB, from 5 men and 4 women, aged 38–58 years) were available and stored at − 80 °C before use.

Graft preparation

The simulated surgery was performed under sterile conditions at a temperature of 22 °C in the operating room, which

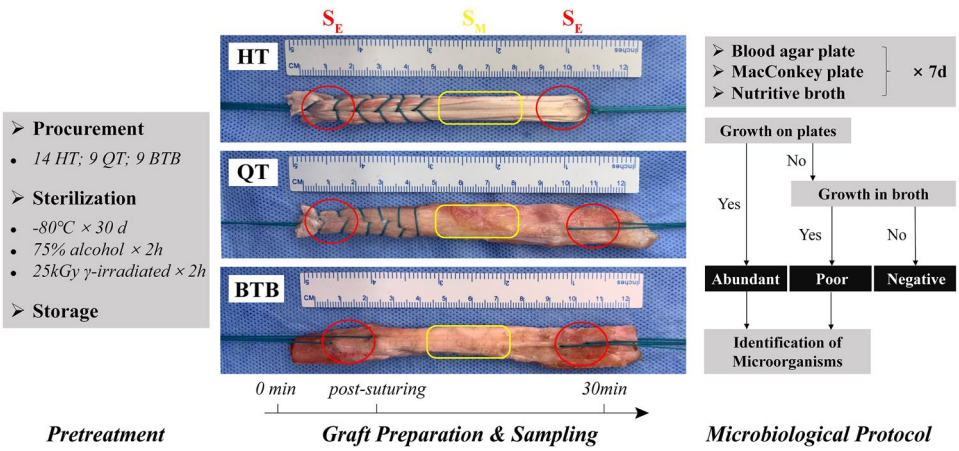

Fig. 1 The flowchart of the study design. HT, hamstring tendon; QT, quadriceps tendon; BTB, bone–patellar tendon–bone; S_E, sampling sites of the ends; S_M, sampling sites of the middle

was equipped with a laminar airflow system. The surgeons wore standard sterile disposable surgical gowns and two pairs of surgical gloves [46]. The grafts were thawed in sterile normal saline at room temperature. Timing was started when the graft was removed from the plastic. Clamps were used for fixation of the two ends of the graft throughout the preparation process. Suture preparation was performed by two experienced surgeons for each end of the graft with the use of non-absorbable sutures (No. 2 Ethibond; Ethicon). The HTs were prepared as single-bundle quadruple-strand grafts. The baseball stitch that crossed the midline of the graft was used for the end with the soft tissue. Two 2-mm drill holes were made to accommodate the passage of the sutures for the end with a bone plug. The end of suture preparation was marked by the completion of tightening of the suture following last throw. The duration of the suture preparation was recorded by two independent examiners using the timer accurate to the seconds, and the results were expressed as the average of the two measurements. The graft was stretched, sized, and kept moist in normal saline-soaked gauze on the preparation table until 30 min after the timing to simulate the waiting period before implantation in clinical practice.

Sampling method

The samples were obtained using a knife or rongeur from the middle and both ends of each graft for culture at each time point. The sampling process was performed as fast as possible and unnecessary contact with the graft was avoided. The sample from the middle (S_M) was 30 mm in length and 5 mm in width, while the sample from each end (S_E) was 15 mm in length and 5 mm in width with sutures on for comparison. The middle and both ends of the graft were obtained as S_{1M} and S_{1E} at the initiation of graft suturing, S_{2M} and S_{2E} at the completion of graft suturing, and S_{3M} and S_{3E} at 30 min. Each sample was collected in an empty sterile container and immediately transferred to a microbiology laboratory.

Microbiological protocol

Each sample was rolled onto a blood agar plate and MacConkey plate for 20 s and then transferred to a nutritive broth bottle for enrichment culture as previous described [3]. The plates and broth bottles were incubated at 37 °C in 5% CO_2 for 7 days. After 7 days with no growth in bottles or plates, the result was considered negative. If there was growth on the plates, it was considered abundant, and the contamination level was recorded as high; if growth was detected only in the nutritive broth but not on the plates, it was considered poor, and the contamination level was recorded as low [16, 22]. The culture result of the whole graft was considered positive if either S_M or S_E was positive. Colonial morphology and Gram stain assessments were performed for all the isolated organisms using standard microbiological methods. The microorganisms were identified using the VITEK 2 Compact automated identification system (BioMérieux, Marcy-I'Etoile, France).

Statistical analysis

The primary outcome variable was specified as total graft contamination rate between different time points. According to previous studies [3, 16, 22] and unpublished data from 50 patients with ACLR in our institution, the contamination rate at initiation and completion of graft preparation was assumed as 0.1 and 0.4. Therefore, a sample size of 32 was determined with an alpha of 0.05 and power of 0.8.

One-way ANOVA for normally distributed variables or Kruskal–Wallis test for non-normally distributed variables was used for quantitative data. The Chi-square test and Fisher's exact test with Bonferroni corrections were used for qualitative data. The inter-observer reliability of the measurements was calculated using intra-class correlation coefficients (ICC). Statistical significance was set at $P < 0.05$. Analyses were performed using SPSS (version 25.0; IBM Corp., Armonk, NY, USA).

Results

The time needed for suture preparation was 348 ± 102 s, 301 ± 112 s, and 246 ± 47 s for HT, QT, and BTB, respectively. No differences were observed between the groups ($P = 0.090$). The ICCs showed excellent inter-observer reliability in all measurements (0.997 for HT, 0.996 for QT, 0.998 for BTB).

The allograft culture results of the 192 samples are summarised in Table 1. Positive culture appeared in 41 (21.4%) samples, of which 21 were from the ends and 20 from the middle. The most common organisms identified in the study were *Staphylococcus warneri* (11/41), *Staphylococcus epidermidis* (7/41), and *Bacillus* species (5/41) (Fig. 2). No differences in the contamination rate were observed between S_E and S_M (Table 2). However, a higher proportion of positive samples with abundant bacterial growth was detected in S_E than in S_M post-suturing (7/8 vs. 1/7, $P = 0.010$) and at 30 min (6/11 vs. 0/11, $P = 0.012$) (Fig. 3).

When considering contamination of the whole graft, the total number of grafts with positive culture was 4/32 (12.5%) at pre-suturing, 9/32 (28.1%) at post-suturing, and 19/32 (59.4%) at 30 min. The graft contamination rate was significantly higher at 30 min than pre-suturing ($P = 0.003$) and post-suturing ($P = 0.035$). The number of grafts with abundant bacterial growth was 0 at pre-suturing, 7/32 (21.9%) at post-suturing, and 6/32 (18.6%) at 30 min, which showed

Table 1 Details of culture results of 192 samples from 32 allografts

	S_{1E}	S_{1M}	S_{2E}	S_{2M}	S_{3E}	S_{3M}
HT 1						*S. sciuri*
HT 2					*M. lentus*	*M. lentus*
HT 3						
HT 4						
HT 5						*S. warneri*
HT 6						
HT 7			*S. epidermidis*[a]	*S. warneri*		*S. warneri*
HT 8						
HT 9		*S. warneri*	*S. haemolyticus*[a]	*S. warneri*	*S. haemolyticus*[a]	
HT 10	*Bacillus* spp.		*Bacillus* spp.	*Bacillus* spp.	*Bacillus* spp.	
HT 11						
HT 12						
HT 13			*S. epidermidis*[a]	*S. epidermidis*[a]	*S. epidermidis*[a]	
HT 14						*S. saprophyticus*
QT 1						
QT 2						
QT 3						
QT 4					*S. aureus*[a]	
QT 5		*S. hominis*			*S. hominis*	*S. hominis*
QT 6			*E. coli*[a]	*S. capitis*	*S. epidermidis*	*S. capitis*
QT 7				*S. warneri*		*S. warneri*
QT 8						*S. epidermidis*
QT 9						
BTB 1						
BTB 2			*S. warneri*[a]	*Bacillus* spp.	*S. warneri*[a]	
BTB 3						
BTB 4			*E. coli*[a]		*E. coli*[a]	
BTB 5			*S. warneri*[a]		*S. warneri*[a]	
BTB 6						*e. durans*
BTB 7	*S. cohnii* ssp.				*S. cohnii* ssp.	
BTB 8						
BTB 9						*S. epidermidis*

S_E samples from the ends; S_M samples from the middle; S_1 samples at pre-suturing; S_2 samples at post-suturing; S_3 samples at 30 min; *HT* hamstring tendon; *QT* quadriceps tendon; *BTB* bone–patellar tendon–bone; S., *Staphylococcus*; M., *Micrococcus*; K., *Kocuria*; E., *Escherichia*. e., *Enterococcus*

[a]The specimens with abundant bacterial growth

significantly higher contamination risk from post-suturing than pre-suturing ($P = 0.032$) (Fig. 4). Furthermore, there was no significant difference in contamination rate among HT, QT, and BTB allografts at any time point (Table 3).

Discussion

The most important finding of this study was that the total graft contamination rate significantly increased at 30 min (59.4%) compared with pre-suturing (12.5%) and post-suturing (28.1%), while suture preparation increased graft contamination risk with abundant bacterial growth from 0% to 21.9%, especially the end parts of the grafts.

Every step of the graft preparation procedure may introduce contamination due to various manipulations and air exposure [23, 33]. Determining which step would significantly increase the contamination risk might help with the development of decontamination strategies. However, few studies have obtained samples from different time points, and none of them involved comparisons between different time points from harvesting to implantation [19, 23]. Our results suggest that graft suture preparation is a crucial process for introducing high-level contamination. The waiting period for implantation was associated with

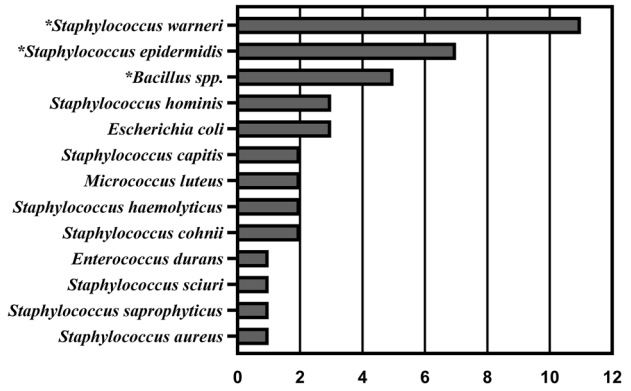

Fig. 2 Types of organisms isolated from 41 culture-positive samples. The three most common organisms identified were marked with *

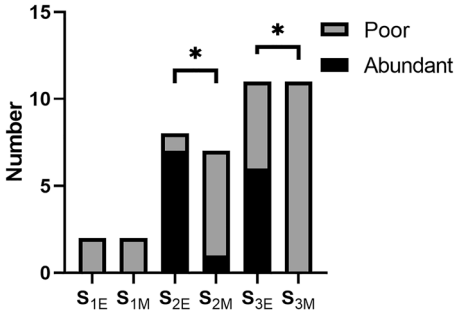

Fig. 3 The number and proportion of positive samples at three time points between different sites from 32 allografts. S_E, samples from the ends; S_M, samples from the middle; S_1, samples at pre-suturing; S_2, samples at post-suturing; S_3, samples at 30 min. *Significant differences between sites in proportion of positive samples with abundant bacterial growth ($P < 0.05$)

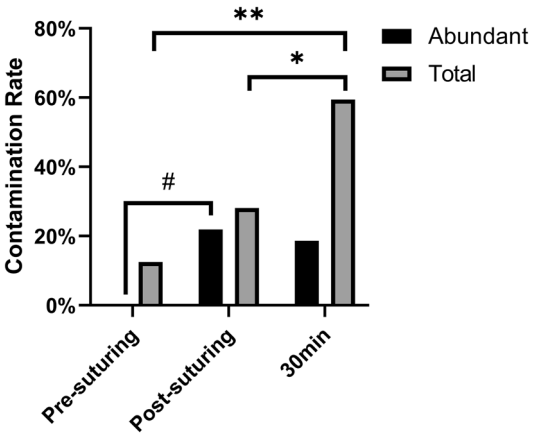

Fig. 4 Contamination rate of allografts with positive culture and abundant bacterial growth. S_E, samples from the ends. #Significant difference for total contamination rate, $P < 0.05$; *significant difference for contamination rate with abundant bacterial growth, $P < 0.05$; **$P < 0.01$

an increased contamination risk of allografts where more than two-thirds were low-level contamination. It implies that the complex manipulation may introduce high-level contamination risk, while a long period of air exposure could pose a low-level contamination risk. The specific cutoff value of the exposure period before implantation needs to be further determined.

Although a significant difference in the risk of infection between different graft choices has been demonstrated, the underlying mechanism has still not been clarified [5, 12, 26, 29]. All the processes of graft harvesting, soft tissue removal, and suture preparation may contribute to graft-based differences of contamination [7, 12, 23, 29]. Compared with autografts, allografts do not require the former two processes, which makes it a perfect choice to study the impact of suture preparation separately. Time consumption using different techniques for graft suturing was found to be different in the literature [13]; however, no study has focussed on differences in suture preparation between soft tissue and bone plug. In the current study, it was found that the two different methods, tendon suturing for soft tissue and bone drilling for bone plug, showed no significant differences in time consumption, contamination rate, and contamination level among the three types of allografts.

It is meaningful to focus on the differences in contamination risk between the sites of the graft. The intra-tunnel portion of the graft, under different physiological environments

Table 2 The number of samples with positive cultures at each time point

	S_{1E}	S_{1M}	S_{2E}	S_{2M}	S_{3E}	S_{3M}	$S_{E\ Total}$	$S_{M\ Total}$
HT	1	1	4	4	4	5	9	10
QT	0	1	1	2	3	4	4	7
BTB	1	0	3	1	4	2	8	3
Total	2	2	8	7	11	11	21	20
Total[a]	0	0	7	1	6	0	13	1

S_E samples from the ends; S_M samples from the middle; S_1 samples at pre-suturing; S_2 samples at post-suturing; S_3 samples at 30 min; *HT* hamstring tendon; *QT* quadriceps tendon; *BTB* bone–patellar tendon–bone

[a]Specimens with abundant bacterial growth

Table 3 The number and percentage of contaminated allografts at each time point

	Pre-suturing	Post-suturing	30 min
HT	2 (14.3%)	4 (28.6%)	8 (57.1%)
QT	1 (11.1%)	2 (22.2%)	5 (55.6%)
BTB	1 (11.1%)	3 (33.3%)	6 (66.7%)
Total	4 (12.5%)	9 (28.1%)	19 (59.4%)
Total[a]	0	7 (21.9%)	6 (18.6%)

HT hamstring tendon; *QT* quadriceps tendon; *BTB* bone–patellar tendon–bone

[a]Grafts with abundant bacterial growth

compared with the intra-articular portion, may contribute to different effects, such as tunnel widening after implantation, if the contamination exists [17, 18]. In this study, we found no difference in the contamination rate between both ends and the middle part of a certain graft. However, the ends of the grafts were susceptible to high-level contamination and relatively highly virulent species, such as *S. aureus* and *E. coli*. Therefore, it is necessary to pay more attention to decontamination measures for the ends of the grafts.

The graft contamination rates of ACLR varied from 2 to 23% in previous studies [3, 4, 30, 33, 36]. However, our study reported a relatively high contamination rate after suture preparation (28.1%) and at 30 min (59.4%), which could be explained by the different experimental conditions. The sampling method, an important parameter, is quite different among studies. By swabbing the surface of graft, Nakayama et al. and Guelich et al. found a relatively low contamination rate for autograft (2%) and allografts (9.7%), respectively [22, 30]. Taking excess tendon tissue from the graft is a more reliable and commonly used method, by which the contamination rate reaches more than 10% [4, 6, 19, 33, 36]. However, the specimens reported in previous studies were mostly limited to 5 × 5 mm in dimension and obtained from leftovers so as not to disturb the integrity of the graft, which made it difficult to represent the actual contamination of the whole graft. In the current laboratory study, each specimen, from either the middle or both ends, was obtained as large as possible to increase sensitivity, which is nearly several fold larger than that in previous studies. Furthermore, bacteria-stained suture material in arthroscopic surgery has been reported as a potential source of contamination in several recent studies [8, 38, 45]. Bartek et al. showed a non-negligible contamination rate of 28.4% during ACLR and meniscus surgery [8]. Therefore, sutures were obtained together with the tissue in the current study. All these factors could increase the detection rate of positive cultures compared to previous studies.

To date, the association between graft contamination and clinical infection has not been demonstrated. This was attributed to the low infection prevalence and relatively small cohorts in published studies [3, 4, 23]. This study provided another possible explanation that the graft contamination rate could be underestimated by previous sampling methods. This indicated that a considerable number of contaminated grafts may have been misclassified as uncontaminated grafts in previous studies, leading to failure to detect differences in clinical infection between groups. Further studies are required to prove this conjecture by comparing the graft contamination rate of different sampling methods.

Graft preparation with intraoperative vancomycin was reported to dramatically reduce the incidence of postoperative infection [7, 31, 34, 35]. Recent studies have confirmed its safety and efficacy [10, 31, 32, 44]. One possible mechanism has been suggested by Pérez-Prieto et al. that it could fully eradicate the contamination of graft [33]. This was consistent with our findings that the vast majority of organisms identified from allografts in the current study, such as coagulase-negative *Staphylococcus* and *Bacillus* species, have been reported as the main pathogens in cases of postoperative infection and are susceptible to vancomycin [10, 31, 33].

There are certain limitations to the current study. First, the sample size was not sufficiently large to analyse each subgroup separately and to detect significant differences between allograft types. Second, the study focussed on the changes in contamination rate over time, but lacked a control group. The increasing high-level contamination rate after suture preparation was actually attributed to the combined effect of suturing and air exposure. Though air exposure had no impact on high-level contamination risk in the current study when comparing post-suturing and at 30 min, it could be more rigorous to assess the separate effect of suturing on graft contamination by setting a control group including grafts placed in saline-soaked gauze without any manipulation. Third, the potential risk of contamination caused by sampling procedure itself was almost inevitable and hard to evaluate, which might overestimate the contamination rate. Besides, it is difficult to simulate a truly individualised surgical time before graft implantation. The time interval from the start of graft preparation to implantation in this study was normalised to 30 min, which was based on a previous study [23] and clinical experience. Finally, this was designed as a laboratory study on allografts without clinical data on infection because it is not achievable and ethical in actual surgery to obtain specimens in sufficiently large dimensions with sutures.

Despite these limitations, the present study provides a novel finding that allograft contamination during preparation could vary not only between the different time points but also between the different graft sites. Therefore, routine prophylactic decontamination after suture preparation should

be considered in day-to-day clinical practice, especially for the ends of the grafts.

Conclusion

The contamination rate of allografts increased significantly at 30 min compared with pre-suturing and post-suturing. Suture preparation may have introduced the high-level contamination, to which the ends of the graft were more prone than the middle.

Author contributions CW, SZ and JZ contributed to conceptualisation. CW, XZ, YQ and SZ contributed to data curation and experiment manipulation. CW, JC and WS contributed to formal analysis and writing. CW, ZY and JJ contributed to measurement. CX and JX contributed to editing and revising. SZ, GX and JZ were responsible for the project administration and supervision.

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Declarations

Conflict of interest None declared.

Ethics approval All specimens were obtained from a tissue bank aiming for medical study. Given that this study was carried out as a laboratory study without patient involvement, no ethical approval was required.

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