DOI: 10.15825/1995-1191-2026-1-206-211

TECHNIQUE FOR DECELLULARIZATION OF CORNEAL LENTICULES FOR SUBSEQUENT KERATOPLASTY

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This article addresses strategies for reducing corneal graft rejection and explores alternatives to full-thickness corneal transplantation. The objective of the study is to develop a technique for removing cellular material from human corneal lenticules obtained during refractive surgery for use as a biomaterial in keratoplasty. A decellularization procedure was developed in which lenticules were immersed in a 1% aqueous solution of sodium lauryl sulfate for 24 hours, followed by detergent removal using a buffered 0.9% NaCl solution for 5 days. Decellularization effectiveness was confirmed by the absence of genetic material using Hoechst staining and hematoxylin & eosin staining. Histological analysis of the biomaterial after preservation for various periods demonstrated no signs of biodegradation of the acellular lenticules, which remained sterile for at least 12 weeks. A comparative experimental study in rabbits demonstrated the efficacy and safety of the proposed technique.

Keywords: keratoplasty, corneal lenticule, rabbit, experiment, histology.

INTRODUCTION

The demand for donor corneal tissue remains high, driving ongoing research aimed at reducing graft rejection rates and identifying alternatives to full-thickness corneal transplants [1, 2]. The aim of this study is to develop a technique for decellularizing human corneal lenticules obtained during refractive surgery, with a view to their subsequent use as biomaterials in keratoplasty.

MATERIALS AND METHODS

A procedure was proposed for removing cellular components from surgical specimens. Corneal lenticules obtained during refractive laser surgery were immersed in a 1% aqueous solution of sodium lauryl sulfate (SLS) under aseptic and antiseptic conditions. The stromal fragments were maintained in the solution for 24 hours, after which the residual SLS was removed by sequential rinsing of the lenticules in sterile buffered 0.9% NaCl solution, with five washing cycles of 24 hours each. All procedures were performed in a Class II laminar flow hood. As there are currently no universally accepted standards for assessing decellularization efficiency, evaluation was based on the absence of detectable genetic material, as determined by Hoechst staining and hematoxylin and eosin (H&E) staining [3].

The required concentration of the SLS solution and incubation time for lenticule processing were determined experimentally. Corneal stromal fragments were treated under the following conditions: 0.1% SLS for 1 day (n = 10), 0.1% SLS for 2 days (n = 10), 1% SLS for 1 day (n = 10), while untreated lenticules served as controls (n = 10). Next, the samples were stained with Hoechst dye (Invitrogen, Cat. No. HN-3570, Lot 822389) at a dilution of 1:300 and incubated for 10 minutes. The lenticules were then washed twice in Tris–Tween buffer (pH 7.6) for 5 minutes each and mounted in Fluoromount (Diagnostic Biosystems, REF K024, Lot P 939-B). Fluorescence visualization was performed using a Nikon Eclipse 80i research microscope equipped with a DIH-M epifluorescence attachment.

To rule out possible biodegradation of lenticules following decellularization, histological analysis was performed after storage in sterile tubes containing buffered saline solution for varying durations. The biomaterial was kept in a dark environment at 2–5 °C for 7, 16, 21, 42, 70, and 86 days. Fresh, untreated lenticules served as the control group (No. 7).

Prior to histological examination, the storage media were tested for sterility; no microbial growth was detected. All lenticules were then fixed in 10% neutral formalin, rinsed in running water, dehydrated through graded alcohols, and embedded in paraffin. Histological sections were prepared and stained with H&E.

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The specimens were examined under a Leica DM LB2 microscope at magnifications of 50×, 100×, 200×, and 400×. Digital imaging and documentation were performed using a Leica Aperio CS2 slide scanner.

To evaluate the efficacy and safety of the proposed decellularization technique, a comparative experimental study was conducted using an animal model. Acellular lenticules (Group 1, n = 10) and untreated lenticules (Group 2, n = 10) were transplanted into sexually mature Soviet Chinchilla male rabbits. Under aseptic conditions and intravenous anesthesia, the animals underwent standardized corneal injury in the form of superficial keratectomy (diameter 4.5 mm, depth approximately 150 μm). Lenticule transplantation was performed according to a previously described technique [4].

Postoperatively, the animals received standard ophthalmic therapy, including antimicrobial agents (adjusted for antibiotic sensitivity), anti-inflammatory treatment, and keratoprotective support: moxifloxacin 0.5% or tobramycin 0.3% (three times daily for 14 days), dexamethasone 0.1% (for 21 days), and ofloxacin ointment 0.3% (twice daily for 10 days).

The rabbits were examined daily and were euthanized at predefined time points (1, 4, and 8 weeks) via intravenous overdose of an anesthetic combination (zolazepam hydrochloride + tiletamine hydrochloride), following photographic documentation. Enucleated eyeballs were then subjected to histological processing and analysis using the methodology described above.

RESULTS

The following results were obtained from experiments on the decellularization of corneal stromal lenticules. In untreated lenticules (control), numerous keratocyte nuclei were clearly visualized. In contrast, treated samples exhibited only background fluorescence and isolated nuclear elements, indicating the presence of minimal residual nucleic acid fragments (Fig. 1). The median (Me) number of cell nuclei per field of view was 6.5 [6.0; 7.75] in the group incubated in 0.1% SLS for 1 day, 2.5 [2.0; 3.0] in the group incubated in 0.1% SLS

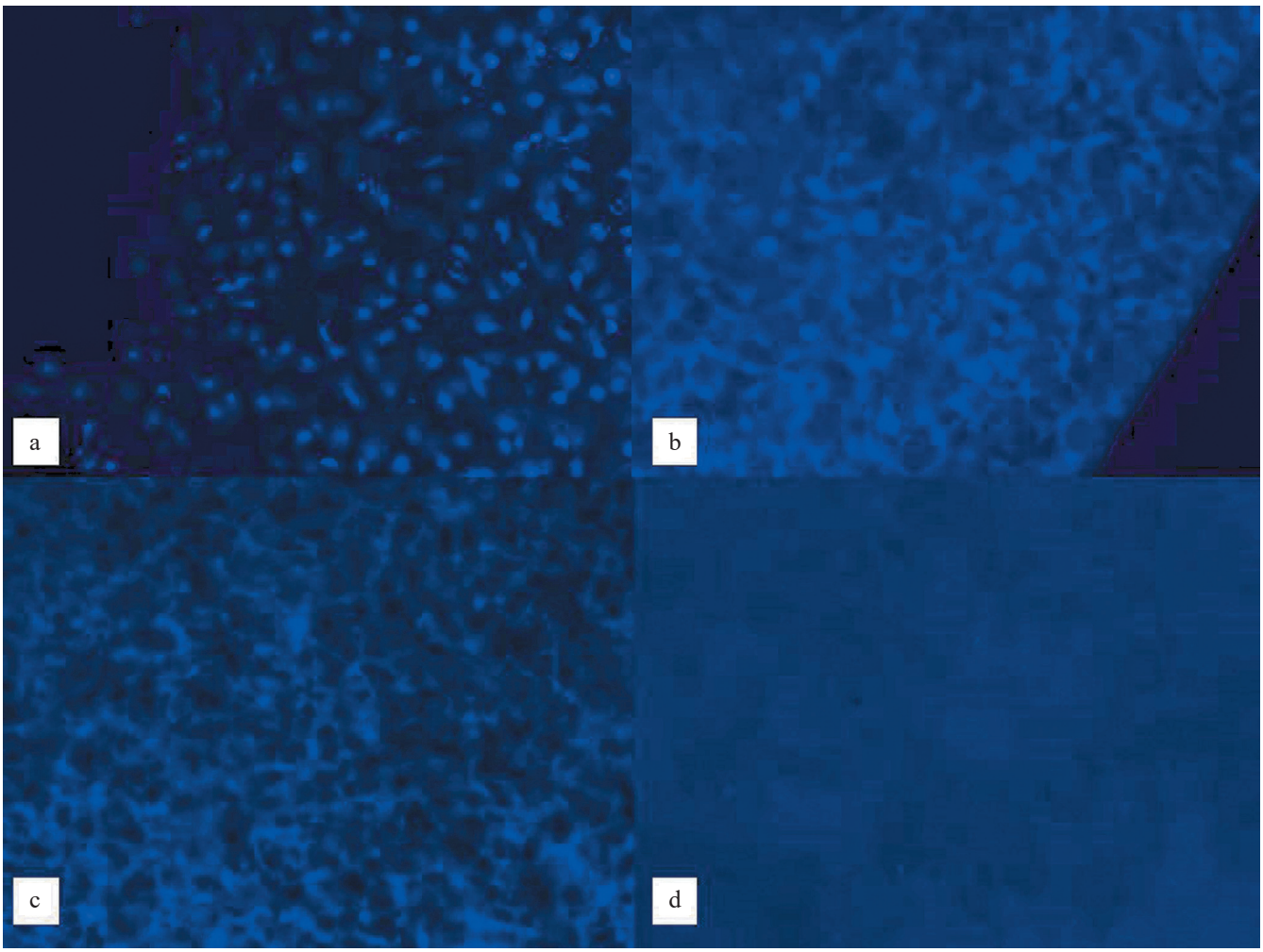

Fig. 1. Fragments of the corneal stroma stained with Hoechst dye (×100): (a) control; (b) lenticule exposed to a 0.1% SLS solution for 1 day; (c) lenticule exposed to a 0.1% SLS solution for 2 days; (d) lenticule exposed to a 1% SLS solution for 1 day

for 2 days, and 1.0 [0.25; 1.0] in the group treated with 1% SLS for 1 day. These differences were statistically significant (Mann–Whitney U test). Based on these findings, treatment with a 1% SLS solution for 24 hours was identified as the optimal protocol for effective decellularization of corneal lenticules.

Histological examination of lenticules preserved for varying durations and stained with H&E demonstrated that, irrespective of exposure time to the 1% SLS solution, the stromal architecture differed from that of untreated tissue. Specifically, treated samples were characterized by the absence of cellular elements, more uniform hydration, and slight fraying at the lenticule edges.

Specifically, sample No. 7 (control) represented a corneal stromal lenticule obtained by femtosecond (Fs) laser extraction without additional processing. In this sample, the stroma contained a normal population of keratocytes – basophilic, elongated spindle-shaped cells located between stromal fibers. The stromal fibers were densely packed, and the edges appeared sharply delineated. In contrast to experimental samples (Nos. 1–6), the presence of isolated slit-like spaces suggested uneven stromal edema (Fig. 2).

Additionally, rounded microcavities, occasionally connected by thin bridges, were observed, likely reflecting microcavitation effects induced by femtosecond laser exposure. It can be assumed that immersion in the decellularization solution, followed by preservation in buffered 0.9% NaCl, leads not only to the removal of cellular components but also to uniform stromal hydration (swelling) accompanied by moderate disruption of collagen fiber organization.

In the *in vivo* experiment, the early postoperative course (up to 10–14 days) was similar in both groups and was characterized by a moderate inflammatory response, including episcleral vessel injection, serous conjunctival discharge, and perifocal corneal edema. Corneal sutures remained intact, and the grafts were well adapted to the recipient corneal surface.

However, from days 10–14 onward, animals in Group II (untreated lenticules) exhibited a marked increase in inflammation, including expansion of corneal

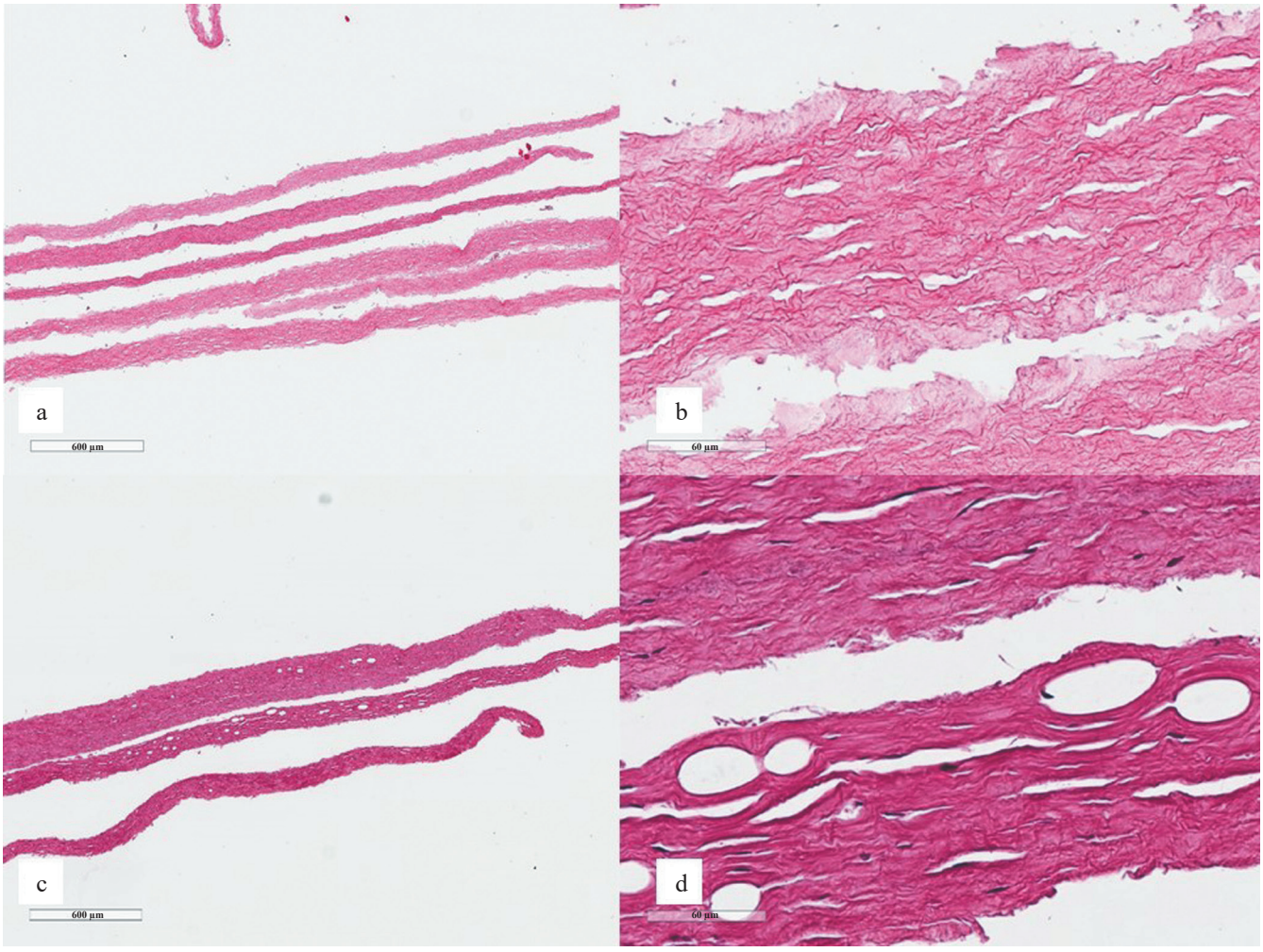

Fig. 2. Microslide of decellularized corneal material and intact lenticules (H&E stain): (a, b) lenticules subjected to decellularization, showing moderate stromal hydration due to exposure to saline solution, with visible slit-like spaces and microcavities; (c, d) intact lenticules containing normal keratocyte density, with compact stroma and microcavities connected by bridges

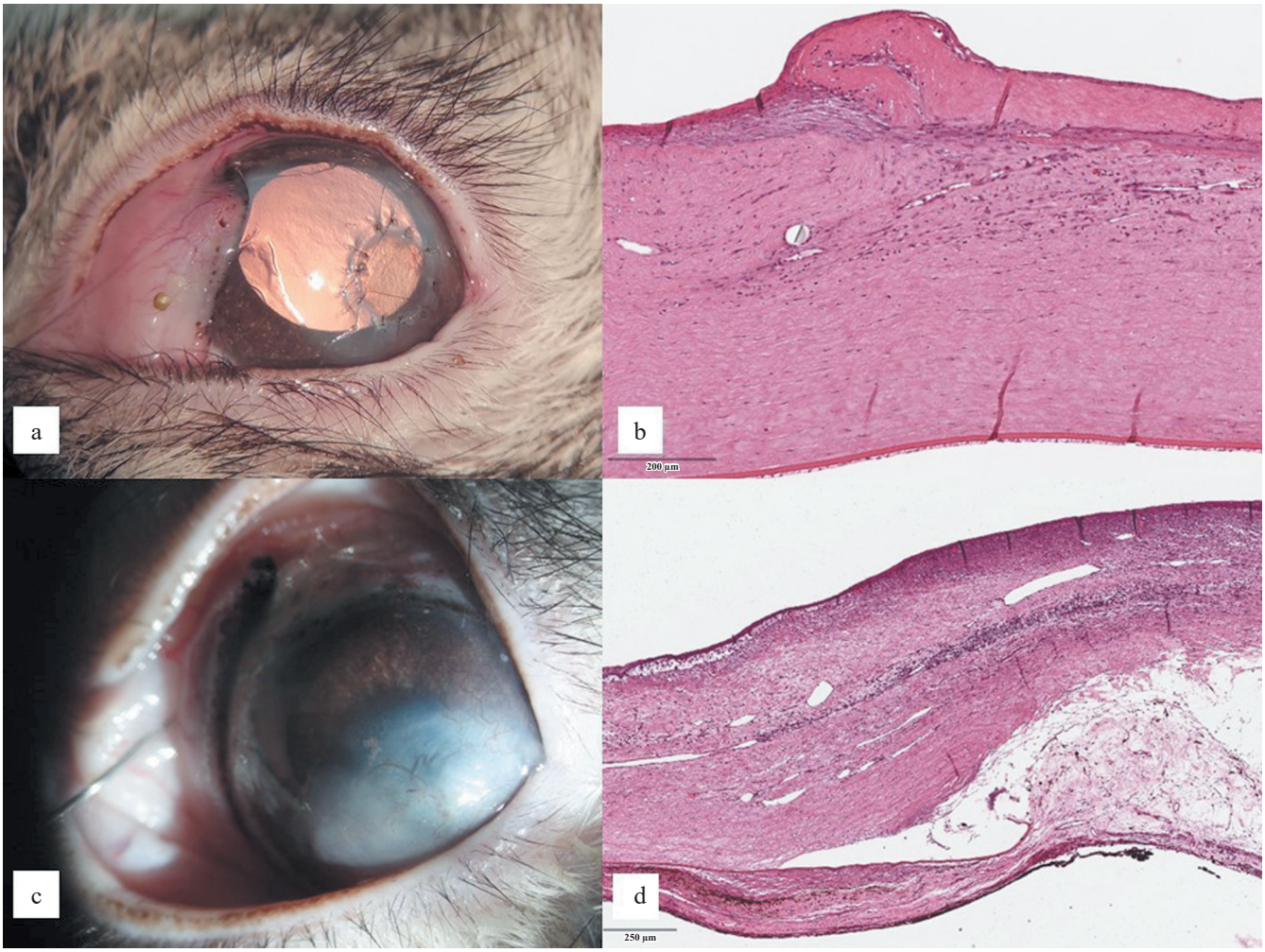

Fig. 3. Clinical and pathological correlation during the postoperative period: (a) photo of the anterior segment of a Group I rabbit, 8-week observation; (b) corneal micropreparation from a Group I rabbit, H&E stain; (c) photo of the anterior segment of a Group II rabbit, 8-week observation; (d) corneal micropreparation from a Group II rabbit, H&E stain

edema, neovascularization, profuse mixed conjunctival discharge, iris vessel injection, and the presence of cellular suspension in the anterior chamber. Anti-inflammatory therapy was intensified with the addition of betadine instillations, while corneal cultures remained sterile. Between 30 and 60 days, this group developed vascularized corneal opacification and extensive anterior synechiae. Light microscopy revealed xenograft degradation and pronounced inflammatory cell infiltration (lymphocytes, eosinophils) at the implantation site, consistent with immune-mediated graft rejection [5–6].

In contrast, the postoperative course in Group I (acellular lenticules) remained favorable throughout the study. The grafts retained transparency, corneal edema resolved by week 8, and the corneal surface became fully re-epithelialized. Histological analysis demonstrated recellularization of the initially acellular stromal matrix by recipient cells (Fig. 3).

CONCLUSION

The proposed technique for decellularizing human corneal lenticules obtained during refractive surgery ensures an effective and sufficient level of cellular removal, as demonstrated by comparative transplantation experiments using decellularized and intact lenticules in a rabbit model.

The procedure is technically simple and can be implemented directly in a clinical setting, enabling rapid preparation of graft material. Furthermore, the decellularized lenticules can be preserved for at least 3 months without evidence of structural degradation, allowing for the establishment of a readily available tissue reserve for emergency tectonic keratoplasty.

The authors declare no conflict of interest.

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The article was submitted to the journal on 14.07.2025