

Commentary

Lessons learned from conventional animals: Encouragement to use specific-pathogen-free animals

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ABSTRACT

Experimental rabbits provide evidence for translational research regarding the pathogenesis or treatment of human diseases. We developed a novel method for regenerating the middle ear mucosa using autologous cultured nasal mucosal epithelial cell sheets, and evaluated the wound healing process in the middle ear mucosa of experimental rabbits. Nonetheless, vigilant microbiological monitoring of experimental animals is essential to effectively prevent a decline in their health conditions, which may affect the research results. We experimented with contamination of *Pasteurella multocida* in non-specific-pathogen-free (SPF) rabbits (without microbiological monitoring). Most non-SPF rabbits had otitis media, whereas SPF rabbits did not, which affected their results during the mucosal regeneration study. The contamination was resolved by changing the experimental design from using non-SPF rabbits to that using SPF rabbits. It is crucial to use the SPF animals for any surgical intervention studies.

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In pre-clinical regenerative medicine and tissue engineering, mice may not be used for transplant surgery due to their small size. Rats are often used in preclinical medicine, and rabbits are used when dealing with smaller organs such as eyes [1], knee joints [2], or the trachea [3]. We have studied regenerative strategies for the middle ear mucosa as a subset of the respiratory epithelia, with an aim to improve the post-operative middle ear mucosa regeneration on the middle ear bone surfaces using autologous cultured nasal mucosal epithelial cell sheets applied after surgery. A successful rabbit preclinical study regarding wound healing of the middle ear [4] led to the publication of a human clinical study [5]. However, when conventional rabbits were used as experimental models, the preclinical study did not work at all.

We pre-screened rabbits for infections, such as sinusitis and otitis media, through nasal examination and endoscopic examination of the eardrum, and those with an evidence of infection were excluded (Fig. 1). However, an acute suppurative otitis media almost always developed in the middle ear mucosa wound-healing rabbit model (Fig. 2a and b). Some rabbits exhibited subcutaneous abscesses near the skin incision (Fig. 2c). Bacterial tests of nasal mucus, ear discharge, and subcutaneous abscesses detected *P. multocida*. By contrast, all SPF rabbits undergoing a routine microbiological monitoring did not exhibit any ear discharge (Fig. 2d and e). As is frequently the case, conventional animals screened before operation were not the same as the SPF animals.

P. multocida is a pathogen commonly infecting diverse animal hosts worldwide. In rabbits, infections due to *P. multocida* are commonly known as “snuffles”, that is, rabbit rhinitis. *P. multocida* can also infect humans bitten or licked by various carrier animals [6–8], and can cause sepsis in immuno-compromised individuals [9,10]. The prevalence of *P. multocida* infection in rabbits housed under laboratory conditions or on breeding farms has been reported to be between 15.8% and 94% [11]. *P. multocida* can infect rabbits asymptotically and develop into an opportunistic infection when the host's immune system is compromised [12]. The infection route can be through droplet infection, contact infection,

Abbreviations: SPF, specific-pathogen-free; *P. multocida*, *Pasteurella multocida*.

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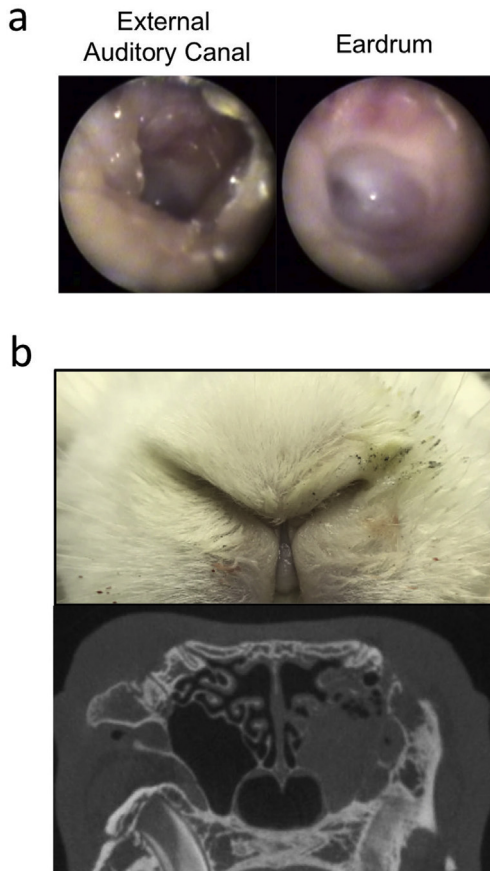


Fig. 1. Examination of conventional rabbits prior to middle ear experimental protocols for mucosal harvest and regeneration. (a) Direct eardrum endoscopic observation in a conventional rabbit. Left panel is external auditory canal; earwax is observed. Right panel is rabbit eardrum; eardrum cannot be observed due to discharge from otitis media. (b) Body surface examination. Upper panel: nasal mucus on the nose tip. Lower panel: computed tomography (CT) image of rabbit nasal sinus showing soft tissue density in the left maxillary.

or birth canal infection from bacteria-positive rabbits. Infection with *P. multocida* causes acute otitis media, pneumonia, conjunctiva inflammation, septicemia, abscess formation, and rhinitis. Contents of the nasal discharge and abscesses are usually characterized by a creamy white pus. Although *P. multocida* is usually asymptomatic in rabbit carriers, opportunistic infections with virulent *P. multocida* can result from stress in animals or a declining health condition. In our case, *Pasteurella* infection seemed to be exacerbated by a direct surgical invasion or operative stresses after removal of the middle ear mucosa, as no ear infection was observed in the sham-operated animals without removal of the mucosa (Fig. 2b and e).

The contamination of *P. multocida* was resolved by changing the rabbit vendor. If we had not proceeded with the experiment with suitable modifications in the experimental protocol, this project would not have provided solutions to the encountered contamination. We hope that our experience will be of use to the reader who promotes or supports the application of a regenerative therapy.

Declaration of Competing Interest

Co-author Masayuki Yamato is an equity holder of CellSeed Inc.; Tokyo Women's Medical University currently receives research funding from CellSeed, Inc. Dr. M. Yamato is also an advisor of commercial efforts, Helios, and NIPPI (Japan).

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Fig. 2. Conventional rabbits (a–c) and SPF rabbits with microbiological monitoring (d, e) after middle ear surgery. (a) Endoscopic observation of eardrum: middle ear mucosal removal producing ear discharge from 1 week post-surgery and observed continually until the rabbit was sacrificed. (b) Micro-CT finding 8 weeks after middle ear mucosa removal in the left ear, and simultaneously in right ear (control sham), the only fenestration of middle ear bone without mucosal removal. Micro-CT findings demonstrate soft tissue density lesions (white asterisk) and significant middle ear bone hyperplasia in only the left ear. Black asterisk is bone-hyperplasia area. (c) Subcutaneous abscess near the skin incision during sacrifice. Creamy white purulence attributed to *P. multocida* infection was observed. (d) Endoscopic observation of eardrum in SPF rabbits showing no detectable ear discharge, including the external auditory canal. (e) Micro-CT 8 weeks after surgical middle ear mucosa removal. The middle ear mucosa removal results from the right ear. Left ear (control sham) simultaneously opens the middle ear bone without mucosa removal. Micro-CT findings demonstrate only middle ear bone hyperplasia, and no soft tissue density lesion in the middle ear cavity. The white arrow is the bone-hyperplasia area.

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