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Decellularized human amniotic membrane: more is needed for an efficient dressing for protection of burns against antibiotic-resistant bacteria isolated from burn patients

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ABSTRACT

Human amniotic membranes (HAMs) have attracted the attention of burn surgeons for decades due to favorable properties such as their antibacterial activity and promising support of cell proliferation. On the other hand, as a major implication in the health of burn patients, the prevalence of bacteria resistant to multiple antibiotics is increasing due to overuse of antibiotics. The aim of this study was to investigate whether HAMs (both fresh and acellular) are an effective antibacterial agent against antibiotic-resistant bacteria isolated from burn patients. Therefore, a HAM was decellularized and tested for its antibacterial activity. Decellularization of the tissue was confirmed by hematoxylin and eosin (H&E) and 4,6-diamidino-2-phenylindole (DAPI) staining. In addition, the cyto-biocompatibility of the acellular HAM was proven by the cell viability test (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide, MTT) and scanning electron microscopy (SEM). The resistant bacteria were isolated from burns, identified, and tested for their susceptibility to antibiotics using both the antibiogram and polymerase chain reaction (PCR) techniques. Among the isolated bacteria, three bla_{IMP} gene-positive *Pseudomonas aeruginosa* strains were chosen for their high resistance to the tested antibiotics. The antibacterial activity of the HAM was also tested for *Klebsiella pneumoniae* (American Type Culture Collection (ATCC)

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700603) as a resistant ATCC bacterium; *Staphylococcus aureus* (mecA positive); and three standard strains of ATCC bacteria including *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27833), and *S. aureus* (ATCC 25923). Antibacterial assay revealed that only the latter three bacteria were susceptible to the HAM. All the data obtained from this study suggest that an alternative strategy is required to complement HAM grafting in order to fully protect burns from nosocomial infections.

1. Introduction

Human amniotic membrane (HAM) is a multilayer membrane consisting of epithelial cells, fibroblasts, and basement membrane [1]. Some of its favorable characteristics make HAM a promising scaffold for tissue engineering applications. Functioning as an extracellular matrix (ECM), the basement membrane of HAM is rich in hyaluronic acid [2], collagen types I, III, IV, V and VI, lamini, elastin, fibronectin, proteoglycans, etc. [1,3]. The presence of such components in the ECM endows some unique properties to HAM. For example, HAM promotes cell proliferation and maturation. In addition to its easy availability and cost-effectiveness, some of the potential benefits of HAM in tissue engineering applications include its low immunogenicity and high antibacterial property [4,5]. The application of HAM in skin tissue engineering has been suggested for many years now [5]. This ideal scaffold has been used widely in skin dressing [6,7], neurosurgery [8], and ophthalmic [9] and vaginal surgeries [10] in its fresh or decellularized forms. Decellularization of the HAM has been reported to enhance its cell proliferation-supporting function and reduce its immunogenicity [3,11].

Many studies have reported the antibacterial property of the HAM [1,4,5]. In addition to its antibacterial activity, HAM promotes the healing of infected wounds [4]. On the other hand, the prevalence of antibiotic-resistant bacteria is increasing due to the overuse of antibiotics, especially in developing countries. Development of multidrug-resistant (MDR) strains has become a major concern in the health-care community [12–14]. The aim of this study was to investigate whether HAM (both fresh and decellularized) can protect burns from antibiotic-resistant infections, as an ideal burn wound dressing.

2. Materials and methods

2.1. Preparation of decellularized HAM

2.1.1. Tissue collection

The placentas were collected, and the HAM was separated by a procedure described in our previous work [15]. Briefly, the tissues were obtained from consenting mothers at the time of their cesarean section deliveries. The samples were screened serologically for the possibility of human hepatitis virus types B and C, syphilis, human immunodeficiency virus types I and II, gonorrhea, cytomegalovirus, and toxoplasmosis. The tissues were embedded in an antibiotic-supplemented phosphate-buffered saline (PBS). The human placenta collection was carried out in accordance with the Declaration of Helsinki [16].

The fresh placentas were rinsed with sterile distilled water. After removal of residual blood from the tissue, the amniotic membrane was separated from the chorion and used for the following decellularization process. All steps were carried out under sterile conditions.

2.1.2. Decellularization

For the purpose of decellularization, the fresh HAM was treated with 0.5 M NaOH and 0.2% ethylenediaminetetraacetic acid (EDTA) for 30 s and 30 min, respectively. The tissue was then embedded in 5% ammonium chloride and shaken vigorously, followed by scraping with a cell culture scraper. Both the decellularized and fresh samples were washed with sterile PBS, maintained at −80 °C, and then freeze-dried under vacuum. The tissues were sealed with nylon and stored in room temperature until the following experiments.

2.1.3. Characterizations

The tissue was fixed with 10% natural-buffered formalin (Sigma), followed by dehydration through a graduated series of increasing ethanol and embedment in paraffin. The samples were sectioned with thicknesses of 4 μm. The fresh HAM (Fig. 1A, C, and D) and acellular (Fig. 1B, E and F) tissues were stained with hematoxylin and eosin (H&E) (Fig. 1A and B) and 4′,6-diamidino-2-phenylindole (DAPI) (Sigma–Aldrich, USA; Fig. 1C–F), and then they were viewed under light and ultraviolet microscopes, respectively, to confirm decellularization of the tissue. The results confirmed that the cells were successfully removed from the tissue after the decellularization process (Fig. 1).

2.2. Isolation and identification of resistant bacteria

2.2.1. Isolation and identification of bacteria from burn wounds

The bacteria were collected from the wound exudate of patients with burns admitted to the Burn Unit at the Shahid Motahari Hospital (Tehran, Iran). The bacteria were then identified using standard methods such as ‘‘oxidase,’’ ‘‘triple sugar iron,’’ ‘‘oxidative–fermentative,’’ and ‘‘motility’’ tests [17].

2.2.2. Polymerase chain reaction

The polymerase chain reaction (PCR) technique was used for screening of the β-lactamase imipenemase (bla_{IMP}) gene, encoding a protein conferring widespread resistance in