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Antimicrobial activity of lyophilized dermal xenografts modified with silver nanoparticles

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Abstract. Background and aim. The management of wounds of various etiologies complicated by infectious processes remains a key challenge in modern surgery. While lyophilized dermal xenografts are effective temporary coverings that reduce plasma loss and protect against secondary contamination, their collagen matrix lacks intrinsic antimicrobial activity. It can serve as a substrate for bacterial colonization. Therefore, modern strategies necessitate the modification of these dressings with antibacterial agents, such as silver nanoparticles (AgNPs).

Materials and Methods. A standardized 6-step chemical reduction protocol was utilized to integrate silver into the dermal matrix. The process included tissue saturation with 0.1 % AgNO_3 , formation of a stable $[\text{Ag}(\text{NH}_3)_2]^+$ complex, and reduction using 0.1 % ascorbic acid. Antimicrobial activity was evaluated via the agar diffusion method against a broad spectrum of reference and clinical strains, including *S. aureus*, *P. aeruginosa*, and *Candida spp.* Identification was confirmed using MALDI-TOF MS.

Results. Physicochemical calculations established that the protocol ensures a silver deposition of 0.3175 mg/cm^2 , which is therapeutically effective yet remains within the safe threshold for topical application. *In vitro* testing revealed pronounced antimicrobial activity, with zones of inhibition ranging from 15 to 19 mm for Gram-positive bacteria and up to 18 mm for *P. aeruginosa*. The modified grafts also demonstrated significant fungicidal properties. Based on consumption rate calculations, the matrix-deposited silver provides sustained bactericidal action for approximately 6–7 days, even under high bacterial bioburden (10^7 CFU/cm^2).

Conclusions. The developed 6-stage chemical reduction methodology provides a therapeutically sound concentration of silver nanoparticles within the xenomatrix. The modified grafts exhibit high bactericidal and fungicidal efficacy and offer a prolonged duration of action that outperforms standard silver-based ointments, making them a promising solution for burn and purulent surgery.

Key words: lyophilized dermal xenografts, silver nanoparticles, chemical reduction, wound infection, antimicrobial activity, *in vitro* study.

Антимікробна активність ліофілізованих дермальних ксенотрансплантатів, модифікованих наночастинками срібла

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Передумови та мета. Лікування ран різної етіології, ускладнених інфекційними процесами, залишається ключовим викликом сучасної хірургії. Хоча ліофілізовані дермальні ксенотрансплантати є ефективними тимчасовими покриттями, що зменшують втрату плазми та захищають від вторинного зараження, їхній колагеновий матрикс не має власної антимікробної активності. Ба більше, він може слугувати субстратом для колонізації бактеріями. Відтак, сучасні стратегії потребують модифікації цих пов'язок антибактеріальними засобами, такими як наночастинки срібла (AgNPs).

Матеріали та методи. Для інтеграції срібла в дермальний матрикс було використано стандартизований 6-етапний протокол хімічного відновлення. Процес включав насичення тканини 0,1 % розчином AgNO_3 , формування стабільного комплексу $[\text{Ag}(\text{NH}_3)_2]^+$ та відновлення за допомогою 0,1 % аскорбінової кислоти. Антимікробну активність оцінювали методом дифузії в агар щодо широкого спектра референтних і клінічних штампів, зокрема *S. aureus*, *P. aeruginosa* та *Candida spp.* Ідентифікацію підтверджували за допомогою мас-спектрометрії MALDI-TOF.

Результати. Фізико-хімічні розрахунки встановили, що протокол забезпечує осадження срібла в кількості 0,3175 мг/см², що є терапевтично ефективним і водночас залишається в межах безпечного порога для місцевого застосування. Тестування *in vitro* виявило виражену антимікробну активність із зонами затримки росту від 15 до 19 мм для грампозитивних бактерій та до 18 мм для *P. aeruginosa*. Модифіковані трансплантати також продемонстрували значні фунгіцидні властивості. Згідно з розрахунками швидкості витрати, осаджене в матриксі срібло забезпечує тривалу бактерицидну дію протягом приблизно 6–7 днів навіть за умови високого бактеріального навантаження (10^7 КУО/см²).

Висновки. Розроблена 6-етапна методика хімічного відновлення забезпечує терапевтично обґрунтовану концентрацію наночастинок срібла в ксеноматриксі. Модифіковані трансплантати виявляють високу бактерицидну та фунгіцидну ефективність і забезпечують пролонговану дію, перевершуючи стандартні мазі на основі срібла. Тому вони є перспективними для комбустіології та гнійної хірургії.

Ключові слова: ліофілізовані дермальні ксенотрансплантати, наночастинки срібла, хімічне відновлення, ранова інфекція, антимікробна активність, дослідження *in vitro*.

Introduction. Local treatment of extensive wounds and burns complicated by multi-resistant microflora remains an urgent problem in modern emergency and reconstructive surgery [1]. Lyophilized dermal xenografts (LDXs) are widely used as biocoatings to temporize wound defects, optimize cell adhesion, and reduce plasma loss [2]. However, natural collagen matrices lack intrinsic

antimicrobial activity and can serve as a substrate for bacterial bioburden accumulation [3]. To prevent infectious complications, modification of biopolymers with nanoscale materials is increasingly utilized [4].

Among these, silver nanoparticles (AgNPs) stand out due to their multi-target mechanism, which includes bacterial cell wall disruption, inhibition of respiratory chain enzymes, and induction of oxidative stress, minimizing the risk of developing microbial resistance [5, 6]. Concurrently, the incorporation of silver into xenogeneic tissues requires precise standardization to maintain structural integrity and ensure controlled release, preventing local cytotoxicity or silver accumulation in organs [7, 8]. Standard saturation procedures often lead to nanoparticle aggregation and uncontrolled ion depletion. In contrast, step-by-step chemical reduction utilizing intermediate coordination complexes facilitates uniform immobilization of elemental silver (Ag^0) directly onto structural proteins [9].

The purpose of the study was to design, computationally evaluate, and experimentally substantiate an optimized protocol for preparing lyophilized dermal xenografts modified with silver nanoparticles via *in vitro* antimicrobial screening.

Materials and Methods. The matrix impregnation process was performed using an optimized six-step chemical reduction technique. Initially, LDH fragments were immersed in 100 mL of a 0.1 % AgNO_3 solution for 20 min to saturate the collagen framework with Ag^+ ions. After a 1-minute rinse in distilled water to remove unbound ions, the matrices were treated with 100 mL of a 0.2 % ammonia solution for 2 min to form a stable silver-ammonia coordination complex $[\text{Ag}(\text{NH}_3)_2]^+$. Following a secondary 30-second water wash, chemical reduction was induced by incubating the samples in 100 mL of a 0.1 % L-ascorbic acid solution for 5 min, converting ionic silver to elemental Ag^0 clusters. A final 5-minute washing step eliminated residual reagents.

Antimicrobial activity was evaluated using the agar disk-diffusion method according to EUCAST criteria (v. 8.0, 2020) and international guidelines for antimicrobial testing [10, 11]. The panel included reference strains: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Raoultella ornithinolytica* (ATCC 10145), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 885–653), and *Candida glabrata* (ATCC 90030). Verification was performed via MALDI-TOF mass spectrometry (Bruker Biotyper). Xenograft disks (6 mm) were incubated at 37 °C (bacteria) and 28 °C (fungi). Ciprofloxacin and voriconazole served as positive controls.

Results and Discussion. Stoichiometric calculations indicate that 100 mL of a 0.1 % AgNO_3 solution contains 0.0635 g of silver with the molar fraction of Ag in AgNO_3 being ≈ 0.635 . Given a mean deposition efficiency of 50 %, 0.03175 g of silver precipitates onto a 100 cm^2 xenograft matrix, yielding a stable surface concentration of 0.3175 mg/cm^2 . This value perfectly matches the therapeutic window for topical applications (up to 0.5–1.0 mg/cm^2), excluding tissue toxicity while exceeding the minimum inhibitory concentration (MIC) for most wound pathogens [6, 12]. Notably, a 1 mm layer of standard 1 % silver sulfadiazine cream provides a local release of only about 0.0302 mg/cm^2 . Our pharmacokinetic simulation demonstrated that under high bioburden (10^7 CFU/ cm^2 of *S. aureus*) and an average consumption rate of 0.1 pg of silver per bacterial cell, the matrix-bound silver reserves provide continuous bactericidal activity for 6–7 days, even accounting for microbial replication cycles.

In vitro testing of three experimental samples with incremental silver loading demonstrated high antimicrobial efficacy compared to the untreated negative control, which showed no inhibition zone (0 mm). The strongest zones of inhibition were recorded for Sample № 3 (Table).

For Gram-positive pathogens, zones of growth inhibition reached 15–19 mm. Importantly, the modified matrices suppressed opportunistic Gram-negative

strains, particularly *P. aeruginosa* (up to 18 mm), which is a crucial factor in burn wound management [13]. Pronounced antimycotic activity was also established against *Candida spp.* (15–17 mm).

Table

Antimicrobial activity of dermal xenograft samples
(zones of inhibition, mm ± SE, n = 3

Microorganisms (reference strains)		Zones of inhibition, mm ± SE					
		Sample 1	Sample 2	Sample 3	Sample 4	Cipro floxacin	Voriconazole
Gram-positive bacteria							
1	Staphylococcus epidermidis (ATCC 12228)	16 ± 0,6	17 ± 0,3	18 ± 0,6	0	23 ± 0,3	–
2	Staphylococcus aureus (ATCC 25923)	16 ± 0,6	18 ± 0,3	19 ± 0,3	0	24 ± 0,3	–
3	Enterococcus faecalis	15 ± 0,6	15 ± 0,6	15 ± 0,3	0	24 ± 0,3	–
Gram-negative bacteria							
1	Escherichia Coli (ATCC 25922)	15 ± 0,6	15 ± 0,3	16 ± 0,6	0	25 ± 0,3	–
2	Raoultella ornithinolytica (ATCC 10145)	0	0	14 ± 0,6	0	25 ± 0,3	–
3	Pseudomonas aeruginosa (ATCC 27853)	14 ± 0,3	16 ± 0,6	18 ± 0,6	0	24 ± 0,3	–
Fungi							
1	Candida albicans (ATCC 885–653)	16 ± 0,9	16 ± 0,6	17 ± 0,3	0	–	24 ± 0,3
2	Candida glabrata	15 ± 0,3	15 ± 0,6	16 ± 0,6	0		25 ± 0,3

It should be noted that the physical zones of inhibition for AgNPs on solid agar media appear slightly smaller than those for highly diffusible low-molecular-weight antibiotics like ciprofloxacin. This variance is explained by the strict immobilization of silver nanoparticles within the dense three-dimensional collagen network, limiting their passive diffusion into structured agar [14]. In a clinical wound environment with active exudation, the continuous enzymatic degradation of the xenomatrix and fluid flow will facilitate a much more dynamic and prolonged release of active Ag⁺ ions directly into the biotope.

Conclusions.

1. The proposed 6-step chemical reduction method ensures the uniform deposition of silver nanoparticles onto lyophilized dermal xenografts at a therapeutically effective concentration of 0.3175 mg/cm², which falls within safe tissue tolerance limits.
2. Pharmacokinetic modeling confirms that the antimicrobial lifetime of the developed composite dressing spans 6–7 days under a high bacterial load, which significantly expands its clinical potential over traditional silver-based ointments.

3. In vitro screening confirmed the broad-spectrum bactericidal and fungicidal efficacy of the modified biological matrices against key wound pathogens, including resistant Gram-negative and mycotic flora.

Conflict of interests. The authors declare no competing interests.

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