Spectroscopic Characterization of a Novel Tilapia Skin Collagen–Zinc(II) Topical Formulation and Antibacterial Activity Against Clinical Isolates

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Abstract

Tilapia skin collagen (TSC) and zinc(II) ions (Zn(II)) are known for their regenerative and antimicrobial properties. This study reports the development and physicochemical characterization of a novel TSC–Zn(II) biomaterial in hydrogel and membrane (MB) forms for burn wound healing and prevention of opportunistic infections. UV–Vis spectroscopy showed a bathochromic shift in the n→π* transition, indicating complex formation. Raman and FTIR analyses revealed shifts in amide I (νC=O), amide II (νC–N), and amide A (νN–H) bands, confirming Zn(II) coordination. These spectral features support the formation of a stable TSC–Zn(II) complex. Membranes showed excellent biocompatibility in fibroblast assays and strong antimicrobial activity against *S. aureus*, *P. aeruginosa*, and *K. pneumoniae*. With structural stability, sustained Zn(II) release, and broad-spectrum antimicrobial action, the TSC–Zn(II) system emerges as a promising, spectroscopically validated biomaterial for advanced wound care.

Keywords: Fish collagen, Zinc(II), Wound healing, Antimicrobial activity

Introduction

Burn wound infections are a major global health challenge, especially in low- and middle-income countries, with Africa and Latin America accounting for approximately 77% of global cases [1]. These infections are a leading cause of mortality during treatment, often caused by opportunistic or commensal bacteria. Nile tilapia (*Oreochromis niloticus*) skin has emerged as a promising therapeutic dressing due to its collagen-rich matrix, which promotes regeneration, high biocompatibility, low inflammatory response, and stimulation of fibroblast and epithelial growth [2]. Zn(II) is an essential micronutrient with potent antimicrobial activity, including efficacy against multidrug-resistant strains. This study presents the physicochemical and microbiological characterization of a novel tilapia skin collagen (TSC) membrane with Zn(II) [3]. The TSC-Zn(II) material demonstrated structural stability, biocompatibility, and broad-spectrum antimicrobial action, highlighting its potential as a sustainable and translational therapeutic tool for burn wound treatment and prevention of secondary infections.

Experimental

Zn(II) membranes (MB-Zn(II)) preparation:

TSC-Zn(II) gel was prepared by adding Zn(II), thickener, and plasticizer under controlled stirring and temperature conditions. After cooling to room temperature, the gel was formed. MB-Zn(II) membranes were obtained by pipetting the gel onto glass plates and heating for 3 hours.

Electronic and vibrational spectroscopy characterization of TSC and TSC-Zn(II) complexes in aqueous solution and in MB-Zn(II):

TSC-Zn(II) complexes were prepared in different $ZnCl_2$ ratios and characterized by electronic spectroscopy UV–Vis (ES) (200–400 nm), Raman spectroscopy (RS) (0–3600 cm^{-1}, 532 nm laser), and FTIR (0–4500 cm^{-1}). Samples were analysed in solution and membrane forms.

In vitro microbiological activity assays:

TSC-Zn(II) gel and membrane (MB-Zn(II)) were tested against clinical isolates using Minimum Inhibitory Concentration and disk diffusion assays. Gram-positive (*Staphylococcus epidermidis, Staphylococcus aureus*) and Gram-negative (*Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae*) strains were evaluated. Both formulations demonstrated antimicrobial efficacy.

MB-Zn(II) cytotoxic with neutral red in fibroblast culture:

MB-Zn(II) cytotoxicity was evaluated in BALB/c 3T3 fibroblasts using the neutral red uptake assay. Cells were exposed to eight concentrations (0.6–2000 µg/mL) for 24 h, and absorbance was measured at 540 nm. The results showed concentration-dependent biocompatibility.

Analysis of the diffusion capacity of Zn(II) from MB-Zn(II) to aqueous with phenanthroline (Phen):

The release of Zn(II) from MB-Zn(II) was evaluated using phenanthroline complexation and UV-Vis spectroscopy. After diffusion on gelatin disks in citrate buffer (pH 5.5), Zn(II) was detected by its interaction with Phen.

Results and Discussion

ES analysis showed a bathochromic shift from 215 to 225 nm and a hyperchromic effect in the C=O amide bond of TSC upon Zn(II) addition, indicating protein-metal complex formation [4]. RS confirmed collagen integrity with characteristic peaks, and the amide I band shifted from 1664 to 1678 cm^{-1} as Zn(II) concentration increased, suggesting C=O interaction with Zn(II). A $ZnCl_2$ vibration signal at 284 cm^{-1} indicated preservation of the Zn-Cl bond [5]. FTIR analysis of the MB-Zn(II) base showed no significant interaction between Zn(II) and excipients, while incorporation of TSC revealed amide band shifts (amide A, I, II) upon Zn(II) addition, confirming coordination between Zn(II) and collagen functional groups [7]. These findings demonstrate preferential interaction of Zn(II) with TSC, consistent across aqueous and composite systems.

The MB-Zn(II) formulation showed high biocompatibility with fibroblasts, maintaining near 100% cell viability, indicating safety for skin applications. Antimicrobial tests revealed that low Zn(II) concentrations (0.03% w/w) effectively inhibited common skin bacteria in both gel and MB forms. Disk diffusion confirmed clear inhibition zones only in MB-Zn(II) samples, demonstrating potent and specific antimicrobial activity. These results support MB-Zn(II) as a promising agent for preventing wound infections.

The MB-Zn(II) releases Zn(II) ions, confirmed by Phen complexation, which showed a spectral shift from 266 to 271 nm, indicating Zn(II) release through gelatin disks [6]. In citrate buffer (pH 5.5), MB-Zn(II) showed greater solubilization than MB, likely due to the formation of Zn(II)-aquo complexes on the gelatin surface. These hydrated complexes give the material a gelatinous, moist appearance, suggesting Zn(II) acts as a moisture scavenger. This enhanced hydration may improve dressing adherence and handling in wound treatment.

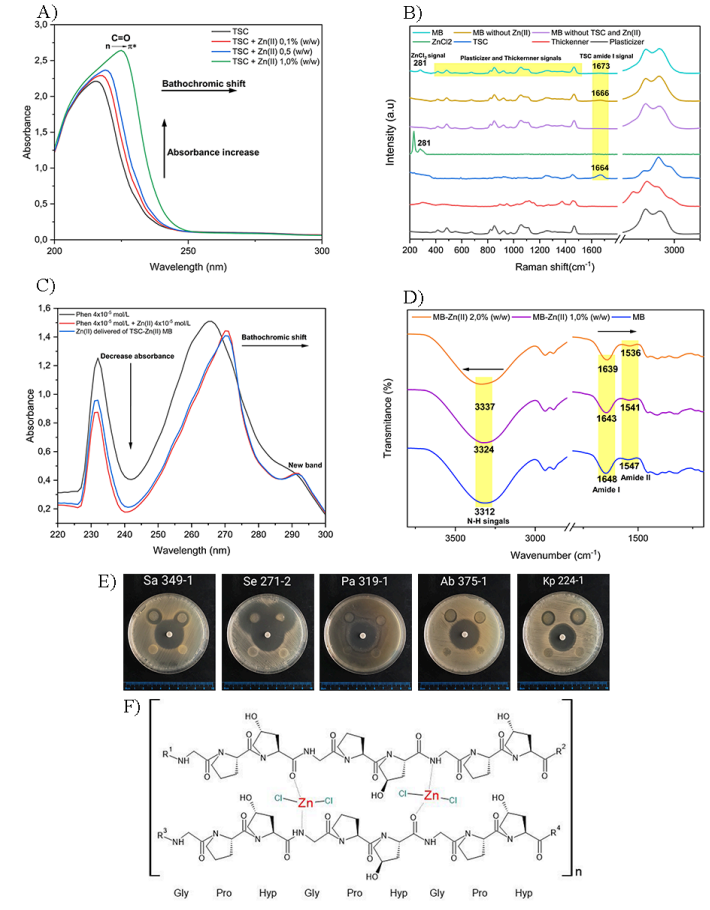

Figure 1. A) ES spectra of TSC-Zn(II); B) RS spectra of MB-Zn(II); C) Phen-Zn(II) complex; D) FTIR of MB-Zn(II); E) Disk diffusion assay, F) Proposed TSC-Zn(II) complex structure

Table 1. TSC-Zn(II) MB viability in fibroblast culture assay

MB concentration (µg/mL)	Cell viability (%)
Positive control	100
0,6	> 95
2,0	> 95
6,3	> 95
20,0	> 95
63,3	> 95
200,0	> 90
633,0	> 90
2000,0	> 95
Negative control	12

Table 2. Minimum inhibitory concentration of TSC-Zn(II) gel

Zn(II) in gel concentration (%)	*S. aureus*	*S. epidermidis*	*A. baumanii*	*P. aeruginosa*	*K. pneumoniae*
0,25	–	–	–	–	–
0,125	–	–	–	+	–
0,0625	–	–	+	+	–
0,03125	+	–	+	+	+
0,01562	+	+	+	+	+
0,00781	+	+	+	+	+

Conclusion

This study developed TSC membranes with Zn(II), demonstrating effective antimicrobial activity, enhanced physicochemical properties, and high biocompatibility with fibroblasts. The membranes show promise for burn wound infection control and healing, using sustainable, low-cost materials. Further *in vivo* studies are needed to confirm clinical applicability.

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