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Combating Microbial Contamination with Robust Polymeric Nanofibers: Elemental Effect on the Mussel-inspired Crosslinking of Electrospun Gelatin

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Abstract

Designing biocompatible nanofibrous mats capable of preventing microbial colonization from resident and nosocomial bacteria for an extended period remains an unmet clinical need. In the present work, we designed antibiotic free durable antimicrobial nanofiber mats by taking advantage of synergistic interactions between polydopamine (pDA) and metal ions with varying degree of antimicrobial properties (Ag\(^{+}\), Mg\(^{2+}\), Ca\(^{2+}\) and Zn\(^{2+}\)). Microscopic analysis showed successful pDA-mediated crosslinking of the gelatin nanofibers which further improves by the inclusion of Ag\(^{+}\), Mg\(^{2+}\) and Ca\(^{2+}\) ions as supported by mechanical and thermal studies. Spectroscopic results reinforce the presence of strong interactions between pDA and metal ions in the composite nanofibers, leading to generation of robust polymeric nanofibers. We further showed that strong pDA-Ag interactions attenuated the cell cytotoxicity and anti-cell proliferative properties of silver ions for immortalized keratinocytes and primary human dermal fibroblasts. pDA-/Ca\(^{2+}\)/Zn\(^{2+}\) interactions rendered the composite structure sterile against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium* strains whereas the silver ion-incorporated composite mats displayed broad spectrum antibacterial activity against both Gram-positive/-negative bacteria and yeast strains. We showed that the strong pDA-Ag interactions help retaining long-term antimicrobial activity of the mats for at least 40 days while attenuating mammalian cell cytotoxicity of silver ions for skin cells. Overall, the results suggest the potential of pDA-metal ion interactions for engineering sterile nanofibrous mats and expanding the antibiotic armamentarium against drug-resistant pathogens.

**Keywords:** Antimicrobial, Polydopamine Crosslinking, Electrospinning, Gelatin, Metal Ions, Tissue engineering
1. Introduction

Decades of overuse and misuse of antibiotics has resulted in the evolution of antibiotic resistant bacterial strains. Every year in the United States, at least 2 million people become infected with antibiotic resistant bacterial strains, and approximately 23,000 people die annually due to failure in combating such resistant strains.\textsuperscript{1} This highlights the need to discover new generation antimicrobial agents that can promote broad spectrum antimicrobial activity against antibiotic resistant strains. In the era of increasing evolution of antibiotic resistant pathogens, alternative strategies are needed to combat microbial colonization in both healthcare and industrial settings.\textsuperscript{2,3} A growing interest in the use of metal ions as antimicrobials has surfaced to augment the ongoing battle against antibiotic resistant bacterial strains. Silver is one of the most common examples of antimicrobial metal ions, and had been used extensively until the beginning of the antibiotics era.\textsuperscript{4} Alkaline earth and transition metal ions also possess antimicrobial activity against Gram-positive and Gram-negative planktonic bacteria, though higher concentrations are required to achieve similar effects when compared to silver or mercury ions.\textsuperscript{5-7}

Metals/metal ions with inherent antimicrobial properties have been used in a number of healthcare and personal care products. For example, silver wound dressings Acticoat\textsuperscript{TM} have shown 99% reduction in bacterial viability including \textit{Methicillin-resistant Staphylococcus aureus} (MRSA), \textit{P. aeruginosa}, \textit{Vancomycin-resistant Enterococci} (VRE), and \textit{Candida albicans}.\textsuperscript{8} Ag alloy and hydrogel coated catheters have benefited the patients by reducing the incidences of nosocomial urinary tract infections.\textsuperscript{9} Biocompatible coating designed using the combination Ag and Ti have demonstrated antimicrobial effectiveness against \textit{S. aureus} and \textit{Klebsiella pneumonia}.\textsuperscript{10} Cu-based coatings have shown antimicrobial activity against \textit{Escherichia coli}, including a verocytotoxigenic \textit{E. coli}, \textit{Listeria monocytogenes}, \textit{Salmonella enterica}, Campylobacter jejuni, \textit{Mycobacterium tuberculosis},
vancomycin-resistant *Enterococci*, methicillin-resistant *S. aureus*.\textsuperscript{11-16} Moreover, the Cu/Ag ionizers are commonly used to control *Legionella* in drinking water systems in hospitals to prevent nosocomial infections.\textsuperscript{17} However, one of the critical factors that limit their enhanced therapeutic utility is the metal ion induced cytotoxicity at higher concentrations which can cause adverse tissue reactions including accumulation in organs, allergy, carcinoma etc. Hence, this drawback, possibly due to limited understanding of metal ion-polymer systems, constitutes a major barrier for exploration of metals/metal ions and their integration in designing anti-infective materials. Therefore, the proposal of novel schemes to overcome metal-ions induced concerns, exploration of novel metal ions and greater insight into the metal- ion-polymer systems are of central interest for designing next generation advanced anti-infective materials.

Electrospinning is a cost-effective method available for producing nanofibrous mats with high surface areas, porosities, good mechanical strength and a morphology that imitates the dimensions of extra-cellular matrix.\textsuperscript{18,19} These morphological and structural features help to enhance haemostasis, fluid absorption, gas permeation and cell proliferation, which makes nanofibrous mats ideal candidates for biomedical applications.\textsuperscript{20} Numerous reports are available pertaining to antibiotics-laden electrospun nanofibers to generate wound dressings with antimicrobial potential. For example, the fabrication of electrospun polycaprolactone (PCL) mats containing rifampicin and polyvinyl Alcohol (PVA) mats containing gentamicin has been reported.\textsuperscript{21,22} While these studies demonstrate successful incorporation of antibiotics inside electrospun nanofibers and confirm their antimicrobial activity, concerns on the development of resistant strains persist as the effective drug concentration depletes in the biological milieu with duration of treatment. Therefore, sustained release of an effective dose of antimicrobials for an extended period of time is desirable in averting microbial colonization and limiting the evolution of antimicrobial resistance. In our previous work, we
have developed durable antimicrobial wound dressings taking advantage of the strong interfacial interactions between polyhydroxy antibiotics and gelatin and their in-situ crosslinking with polydopamine using ammonium carbonate diffusion method (ADM). Polydopamine crosslinking resulted in sustained release of the antibiotics containing $\geq 5$ –OH groups with complete retention of antimicrobial activity for more than 20 days. Furthermore, the antibiotic loaded mats promoted wound healing in a porcine model of partial thickness burns when compared to mats without antibiotics.

Taking advantage of the strong chelating ability of catechol groups in polydopamine (pDA) for metal ions, we design robust polymeric (gelatin) nanofibers via synergistic effect of metal ions (Mg, Zn Ca and Ag) and pDA chemistry which promotes nanofibers cross-linking. We comprehensively characterized the newly developed mineralized composite materials for structural, bonding, morphological, thermal and mechanical properties. The analysis unravels many critical aspects of metal ions-polymer interaction and reveals the robustness of resultant materials. Interestingly, pDA-mineralized composite structures, produced by our facile strategy (Scheme 1), demonstrated excellent long-term anti-microbial activities and good biocompatibility for skin cells.
Scheme 1. Schematic illustration of A) Fabrication of polydopamine crosslinked metal ion (M^{n+}) loaded gelatin mats using electrospinning technique followed by post-spinning ammonium carbonate diffusion. B) Interactions involved in the formation of stable Gel_{pDA}_M^{n+} architecture for controlled release of the metal ions.

2. MATERIALS AND METHODS

2.1. Materials and Reagents. Gelatin (Gel, Type A from porcine skin), dopamine hydrochloride (DA), silver nitrate (AgNO_3), Anhydrous calcium chloride (CaCl_2), Anhydrous magnesium chloride (MgCl_2), zinc chloride (ZnCl_2), 2,2,2-trifluoroethanol (TFE), FITC-conjugated anti-α-tubulin, Fluoromount™, Hoechst and ammonium carbonate were procured from Sigma-Aldrich (Singapore). Mueller-Hinton agar (MHA) and Sabouraud’s Dextrose Agar (SDA) were purchased from Acumedia, Neogen Corporation, Michigan, USA. Dulbecco’s Modified Eagle’s Medium (DMEM) growth medium was from Gibco®. All other cell culture reagents were obtained from Life Technologies Thermo Fisher Scientific, Singapore.
2.2. Bacterial Strains used in the present study: Table 1 shows the various microbial strains have been used for the antimicrobial assessment of different mats.

**Table 1.** Various microbial strains used to evaluate the antimicrobial potential of various nanofibrous mats.

<table>
<thead>
<tr>
<th>Gram positive bacterial strains</th>
<th>Gram negative bacterial strains</th>
<th>Yeast Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA 9808R (from eye)</td>
<td>PA 9027 (ATCC)</td>
<td>CA 10231 (ATCC)</td>
</tr>
<tr>
<td>MRSA 700699 (ATCC)</td>
<td>PA 01 (ATCC)</td>
<td>CA DF001976R (from colon)</td>
</tr>
<tr>
<td><em>B. subtilis</em> 6633 (ATCC)</td>
<td>KP 10031 (ATCC)</td>
<td></td>
</tr>
<tr>
<td><em>B. cereus</em> 11778 (ATCC)</td>
<td>KP DM4299 (from eye)</td>
<td></td>
</tr>
<tr>
<td>VRE 1001</td>
<td>AB 19606 (ATCC)</td>
<td></td>
</tr>
<tr>
<td>VRE 1002</td>
<td>AB 1001</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations Used in the Table:** MRSA, Methicillin-resistant *S. aureus*; VRE, Vancomycin-resistant Enterococci; PA, *Pseudomonas aeruginosa*; KP, *Klebsiella pneumoniae*; AB, *Acinetobacter baumannii*; CA, *Candida albicans*.

2.3. Electrospinning of Metal and Dopamine Incorporated Gelatin Mats. For electrospinning of gelatin nanofibers, 10% (w/v) gelatin solution was prepared in TFE and kept for overnight stirring to homogenize. The composition of the dope solution employed to prepare dopamine and metal ions integrated gelatin mats is compiled as Table 2 and images for all the solutions are shown in Figure S1. In the electrospinning unit, the dope solution was fed into the standard polypropylene syringe connected to a 27G (Braun, internal diameter ~ 0.4 mm) stainless steel nozzle and mounted on a syringe pump (KDS-100, kD Scientific, USA). The nanofibers were collected on the aluminum foil wrapped collector plate, which was placed at the distance (SD) of 12-15 cm from the syringe tip by applying high voltage (Gamma High Voltage Research, Inc., FL, USA) in the range of 7-14 KV at 0.5-1.0 mL/h feed rate. The optimized electrospinning parameters for the preparation of various nanofiber mats with the assigned sample codes are given in Table 2. All electrospinning experiments were performed at 25 °C in air. The nanofibrous mats were dried in a vacuum desiccator for 72 h to remove any residual solvent and later stored in a dry cabinet for future experiments.
### Table 2. Electrospinning details and parameters for various gelatin mats prepared in the present study and their acronyms used.

<table>
<thead>
<tr>
<th>Sample Abbreviation</th>
<th>Details</th>
<th>Dope Solution Composition</th>
<th>Electrospinning Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>ES_Gel</td>
<td>Pristine gelatin (Gel) mats</td>
<td>10% Gelatin (w/v, in TFE)</td>
<td>Voltage =11.5 kV, SD = 12 cm, Flow Rate = 1mL/h</td>
</tr>
<tr>
<td>Gel_DA</td>
<td>Dopamine (DA) incorporated gelatin mats</td>
<td>10% Gelatin + 2% DA (w/w of gelatin) in TFE</td>
<td>Voltage =11.5 kV, SD = 12 cm, Flow Rate = 1mL/h</td>
</tr>
<tr>
<td>Gel_Ag</td>
<td>Silver (Ag⁺) incorporated gelatin mats</td>
<td>10% Gelatin + 1.2% Ag⁺ (w/w of gelatin) in 90% TFE</td>
<td>Voltage =11.5 kV, SD = 12 cm, Flow Rate = 1mL/h</td>
</tr>
<tr>
<td>Gel_Mg</td>
<td>Magnesium (Mg²⁺) incorporated gelatin mats</td>
<td>10% Gelatin + 1.2% Mg²⁺ (w/w of gelatin) in 90% TFE</td>
<td>Voltage =14.0 kV, SD = 15 cm, Flow Rate = 0.8mL/h</td>
</tr>
<tr>
<td>Gel_Ca</td>
<td>Calcium (Ca²⁺) incorporated gelatin mats</td>
<td>10% Gelatin + 1.2% Ca²⁺ (w/w of gelatin) in 90% TFE</td>
<td>Voltage =11.5 kV, SD = 12 cm, Flow Rate = 1mL/h</td>
</tr>
<tr>
<td>Gel_Zn</td>
<td>Zinc (Zn²⁺) incorporated gelatin mats</td>
<td>10% Gelatin + 1.2% Zn²⁺ (w/w of gelatin) in 90% TFE</td>
<td>Voltage =7.0 kV, SD = 14 cm, Flow Rate = 0.5mL/h</td>
</tr>
<tr>
<td>Gel_DA_Ag</td>
<td>Silver (Ag⁺) and Dopamine incorporated gelatin mats</td>
<td>10% Gelatin + 2% DA + 1.2% Ag⁺ in 90% TFE</td>
<td>Voltage =11.5 kV, SD = 12 cm, Flow Rate = 0.8mL/h</td>
</tr>
<tr>
<td>Gel_DA_Mg</td>
<td>Magnesium (Mg²⁺) and Dopamine incorporated gelatin mats</td>
<td>10% Gelatin + 2% DA + 1.2% Mg²⁺ in 90% TFE</td>
<td>Voltage =14.0 kV, SD = 15 cm, Flow Rate = 1mL/h</td>
</tr>
<tr>
<td>Gel_DA_Ca</td>
<td>Calcium (Ca²⁺) and Dopamine incorporated gelatin mats</td>
<td>10% Gelatin + 2% DA + 1.2% Ca²⁺ in 90% TFE</td>
<td>Voltage =11.5 kV, SD = 12 cm, Flow Rate = 0.8mL/h</td>
</tr>
<tr>
<td>Gel_DA_Zn</td>
<td>Zinc (Zn²⁺) and Dopamine incorporated gelatin mats</td>
<td>10% Gelatin + 2% DA + 1.2% Zn²⁺ in 90% TFE</td>
<td>Voltage =10.5 kV, SD = 14.5 cm, Flow Rate = 0.6mL/h</td>
</tr>
</tbody>
</table>

#### 2.4. Ammonium Carbonate Mediated Crosslinking for Dopamine Incorporated Gelatin Mats. To accomplish polydopamine (pDA) crosslinking and metal carbonate structuring in dopamine incorporated mats, the mats were kept in a sealed desiccator containing ammonium carbonate powder in a petri plate for 24 h, which we designate as the ammonium carbonate diffusion method (ADM). Thus, prepared crosslinked mats were then vacuum dried for 24 h.
and used for further studies. The crosslinked mats are named as Gel_pDA, Gel_pDA_Ag, Gel_pDA_Mg, Gel_pDA_Ca and Gel_pDA_Zn for better clarity.

2.5. Mechanical Properties of the Nanofiber Mats. Tensile properties of the mats were determined using table top tensile testing machine (Instron 5345, USA) following the ASTMD882-02 protocol standard. Each fiber mat was cut into 1 cm × 3 cm rectangular shape strips and the thickness of the samples was determined by a digital micrometre. Stress-strain curves were documented employing the cross-section speed of 1 mm min⁻¹. Mechanical properties (tensile strength, Young’s Modulus, failure strain and work of failure) of different mats were then estimated using the recorded stress-strain curves. At least three samples were tested for each sample type and the average values were recorded.

2.6. Morphological Analysis using Field-Emission Scanning Electron Microscopy (FE-SEM) & Transmission Electron Microscopy (TEM). Morphology of different nanofiber mats were examined using field-emission scanning electron microscope (FE-SEM; JEOL - JSM6701F, the Netherlands) and transmission electron microscope (TEM; JEOL JEM-3010). For FE-SEM analysis, the samples were first sputter coated with platinum (JEOL JSC-1200 fine coater, Japan) and viewed at an accelerating voltage of 5kV. To estimate the average fiber diameter of various nanofibrous mats, ~50 distinct individual fibers from different areas were randomly selected and used for measuring their diameter using Image J software. In the TEM study, the fibers were collected on gold-coated copper specimen grids without staining.

2.7. Dynamic Contact Angle Analysis. To investigate the surface wettability of the mats, we monitored the time-dependent deviations in the water contact angle on various electrospun mats using VCA Optima Surface Analysis System (AST products, MA, USA). For this, 1µL of MilliQ water was dropped onto the fiber mats collected on glass cover slips and
photographed continuously at different time intervals including 0, 5, 10, 20, 30, 40, 50, 60, 90 & 120 s. The average values were reported from two independent experiments.

2.8. X-Ray Photoelectron Spectroscopy. XPS studies were executed in a Kratos AXIS UltraVLD (Kratos Analytical Ltd) system using base pressure of ~10^{-9} Torr. Photoemission was induced by Al Kα (1486.71 eV) radiation using physical electronics 04-548 dual Mg/Al anode. XPSPEAK 4.1 is used to curve resolve the XPS data after subtracting the Shirley background. The curve resolved spectra were fit with the minimum number of peaks which were needed to reproduce the spectral features with a 75% Gaussian/ 25% Lorentzian peak shape using a Gaussian–Lorentzian product function.

2.9. Thermogravimetric Analysis. The Q500 thermal analyzer (TA Instruments, DE, USA) was used to perform the thermogravimetric analysis (TGA) of GEL, metal incorporated gelatin mats and all the dopamine incorporated metal loaded gelatin mats at a heating rate of 20 °C/min from 25-900 °C in a dynamic nitrogen atmosphere having a flow rate of 60 mL/min.

2.10. Radial disc diffusion assay. The antimicrobial properties of various metal loaded pDA crosslinked mats were assessed using Kirby–Bauer radial disc diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI). For this, Gram positive and Gram negative bacterial cultures (at a concentration of 0.5 McFarland standards) were spread onto the surface of sterile Muller Hinton agar (MHA) plates using a cotton swab in 9 cm diameter Petri dishes. To evaluate their antifungal efficiency, yeast cultures (at a concentration of 0.5 McFarland standards) were spread onto the surface of sterile SDA plates. Various metal loaded mats (Gel_pDA_Ag, Gel_pDA_Mg, Gel_pDA_Zn and Gel_pDA_Ca) were placed in the dimension of 1 cm × 1 cm on the top of the swabbed cultures and incubated at 35°C ± 2°C for 24 h in both bacterial and yeast cultures. The zone of inhibition
observed for various mats were then estimated to determine their antimicrobial efficiency. The assay was performed in two independent duplicates and the average zone of inhibition value was reported.

The Gel_pDA_Ag mat was also evaluated for their antimicrobial potential using growth inhibition assay following CLSI guidelines. For this assay, 10 ± 0.2 mg of the Gel_pDA_Ag mat was incubated in 2 mL of MH broth containing bacterial cultures (at $10^6$ CFU/mL) for 24 h at 37°C. The bacterial culture without the mat was used as the positive control. After 24 h of incubation, one-log (10-fold) serial dilutions of the bacterial suspension (with and without the mat) were prepared in PBS and 100 μL of each dilution was pour-plated on MHA plates and incubated at 37°C for 24 h for colony-forming units (CFUs) enumeration. The antimicrobial data was reported as the $\log_{10}$ reduction in CFU/ml for Gel_pDA_Ag mat in comparison to untreated control.

2.11. Biocompatibility evaluation and cell proliferation studies. For cytotoxicity assessment and cell proliferation studies of various gelatin mats, human dermal fibroblast cells (hDFs) and HaCaT (immortalized Keratinocytes) were first grown in DMEM medium (Gibco®) supplemented with 10% (v/v) fetal bovine serum, 50 U mL$^{-1}$ penicillin and 50 mg mL$^{-1}$ streptomycin in a humidified incubator at 37 °C and 5% CO$_2$. After 3 days, the cells were passaged once and allowed to proliferate for another 3 days before harvesting. For harvesting, cells were washed with 10mM PBS to remove non-adherent cells before undergoing trypsin-EDTA treatment and centrifugation at 300 rpm for 5 minutes to obtain a cell pallet. Fresh complete medium was then added to dissolve the cell pallet and cell counting was performed using a haemocytometer. For cell seeding, mats were spun on 15mm coverslips and were sterilized under UV light for 2 hours.
For biocompatibility studies, hDFs were then seeded on various electrospun mats placed at the bottom of the 12-well plates (Nunc®) at the density of $8 \times 10^3$ cells per well. CellTiter 96® Aqueous One solution cell proliferation assay kit (Promega) was used to determine cell viability according to the manufacturer’s instruction. Briefly, cells growing on the mat-coated coverslips placed in a 12-well plate containing 500 $\mu$L of cell culture medium for 24 h were incubated with 50 $\mu$L of MTS tetrazolium solution at 37 °C for 2 h. In this assay, metabolically active cells react with the tetrazolium salt present in MTS reagent and thus produce a soluble purple formazan dye with absorption maxima at 490 nm. After 2 h, the absorbance was recorded at 490 nm using a microplate reader (Infinite M200 Pro, Tecan, Mannedorf, Switzerland) and the relative cell viability was calculated. Each treatment was performed in three independent triplicates. For confocal imaging, mats were washed with PBS after 24 h of cell growth to remove the non-adherent cells and the adherent cells were fixed with 3% paraformaldehyde. Subsequently, the cells were fluorescently labeled with Alexa Fluor 569 phalloidin (Molecular Probes®) and FITC conjugated anti-α-tubulin to visualize cellular morphologies and Hoechst 33342 to visualize nuclei. Coverslips were then mounted on glass slides using Fluoromount™ and the confocal imaging was conducted by a Zeiss LSM800 laser scanning microscope (Carl Zeiss Microimaging Inc., NY, USA) using a 40× oil immersion objective lens. At least 20 different microscopic fields were analyzed for each sample.

For performing cell adhesion and proliferation studies for Gel_pDA_Ag and Gel_pDA_Zn mats, the samples were placed in 24-well plates and secured with stainless steel rings to prevent them from lifting off from the coverslips. Subsequently, the mats were washed with PBS twice for 15 minutes to remove debris and residual solvent before being soaked in complete media overnight for conditioning. Both hDFs and HaCaT cells were then seeded at a density of $8 \times 10^3$ cells per well and a plain coverslip served as a positive control.
At Days 1, 4 and 7 post seeding (p.s.), cell proliferation on the mats was assessed by MTS assay as described earlier. To visualize cellular morphologies on various substrates, the media was removed from the wells at day 7 p.s., and washed once with PBS. Subsequently, the cells were then fixed with 4% v/v formaldehyde. After fixation, cells were permeabilised with 0.3% v/v Triton X-100 and subsequently washed thrice with PBS to remove residual detergent. Cells were then stained with Alexa Fluor 647-Phalloidin (actin filaments) and Hoesht (nuclei). Confocal imaging was then performed as described above. To evaluate the cellular morphology and adhesion on the mats, FE-SEM (JEOL – JSM6701F, the Netherlands) analysis was used. Initially, complete media was removed from the wells and cells were washed with PBS. Cells were then fixed using 3% v/v glutaraldehyde. After fixation, subsequent dehydration of the cellular constructs was done using increasing concentrations of diluted ethanol followed by absolute ethanol, and finally with HMDS. The samples were then sputter coated with platinum (JEOL JSC-1200 fine coater, Japan) and viewed at an accelerating voltage of 10kV.

3. RESULTS AND DISCUSSION

3.1. Effect of metal ions on electrospinnability and morphology of as-electrospun gelatin mats. We first studied the effect of metal ions and dopamine alone and then their combined effect on electrospinning process parameters and fiber morphology. Previous studies have reported the effects of metal ions and salts on the morphology of electrospun gelatin nanofibers. A decrease in the diameter of as-electrospun gelatin nanofibers was noted in the presence of di/mono-valent ions (Ca$^{2+}$, Na$^{+}$ or K$^{+}$) in the dope solution owing to the enhanced Coulomb interaction forces during electrospinning. To infer the effect of metal ions on nanofibers, conditions were optimized to generate bead-free fibers and the morphological effects were ascertained by determining the average diameter of the nanofibers. Table 2 shows that the presence of metal ions in the dope solution strongly
influenced the electrospinning parameters. In the presence of Ag\(^+\) and Ca\(^{2+}\) ions in the dope solution, decreasing the flow rate conferred bead-free nanofibers whereas a higher field strength was required in the presence of Mg\(^{2+}\) ions. However, for Zn\(^{2+}\) a lower field strength and flow rate were required to achieve optimum electrospinnability.
Figure 1. Field emission scanning electron micrographs of electrospun gelatin mats. a) ES_Gel, b) Gel_Ag, c) Gel_Mg, d) Gel_Ca, e) Gel_Zn, f) Gel_DA, g) Gel_DA_Ag, h) Gel_DA_Mg, i) Gel_DA_Ca, j) Gel_DA_Zn, k) Gel_pDA, l) Gel_pDA_Ag, m) Gel_pDA_Mg, n) Gel_pDA_Ca, o) Gel_pDA_Zn. Scale bar = 1 μm. Inset in the figures shows the TEM images for the respective sample and the number written on the bottom-left corner of each SEM image corresponds to its enumerated average diameter value.
SEM studies indicated the formation of smooth gelatin nanofibers with an average diameter of $522\pm97$ nm (Fig. 1a). Incorporating metal ions into the gelatin solution was found to have varying effects on the morphologies and diameters of the gelatin nanofibers (Figure 1b-e). In the presence of $\text{Ag}^{+}$ and $\text{Ca}^{2+}$ ions, the average diameter of nanofibers decreased substantially when compared to pristine gelatin mats (Figure 1b and d, Figure S2). The nanofibers formed upon addition of $\text{Zn}^{2+}$ ions displayed “welded junctions” at the fiber cross over points with a marginal decrease in the average diameter in comparison to pristine gelatin fibers (Figure 1e). Reduction in the average diameter of gelatin nanofibers upon integration of metal ions could be due to the enhanced conductivity of the electrospinning dope solution. The presence of metal ions resulted in generation of a charged solution jet which experiences greater coulombic interactions, resulting in thinning of the fibers. Similar decrease in average fiber diameters were previously unveiled for PEO and Gelatin/PCL nanofibers upon adding $\text{Na}^{+}$ ion and $\text{Ca}^{2+}$ ions, respectively.$^{24,25}$ Aggregation and random crosslinking of the gelatin nanofibers was observed upon addition of $\text{Mg}^{2+}$ ions, leading to increased abundance of welded junctions and merged nanofibers (Figure 1c). This anomalous behavior could be attributed to the inherent ability of $\text{Mg}^{2+}$ ions to form stable complexes/chelates with groups like $\text{–COOH}$, $\text{–OH}$ and $\text{–NH}_2$ available in gelatin which promoted inter-molecular interaction among gelatin molecules, leading to their accretion and thus their increased fiber diameter and crosslinked morphology.$^{26}$ TEM images for metal ion loaded fibers, displayed in the insets, revealed the presence of loosely adhered metal salt aggregates on the surface of the nanofibers.

Next, we investigated the electrospinnability of gelatin containing DA alone and DA with metal ions. As was observed before for metal ions, no change in field strength and flow rate was required for DA with silver, magnesium and calcium ions. However, higher field strength was required for the dope solution containing DA and $\text{Zn}^{2+}$ ions than for the
preparation of Gel_Zn nanofibers. SEM studies indicated the presence of few welded junctions at the intersecting points of Gel_DA nanofibers (Figure 1f) which increased dramatically upon adding Ag\(^+\) and Ca\(^{2+}\) ions (Figure 1g and i), suggesting that these metal ions interacted with dopamine as well as gelatin molecules to induce enhanced fiber crosslinking. Interestingly, in the presence of magnesium and DA, the morphology of the mats looked similar to Gel_Mg (without dopamine) mat, though Gel_DA_Mg mats appeared more porous than the former. Analyzing the morphology of Gel_DA_Mg (Figure 1h) and Gel_Mg (Figure 1c) mats revealed systematic crosslinking/networking of gelatin nanofibers in the presence of DA and Mg\(^{2+}\) ions, whereas, random aggregation/agglomeration of gelatin nanofibers was observed with Mg\(^{2+}\) alone. Interestingly, the junction nanofibers formed in the presence of Zn\(^{2+}\) ions disappeared after dopamine incorporation in Gel_DA_Zn mats (Figure 1j), suggesting that DA-metal ions chelation may have prevented metal ion interaction with gelatin nanofibers. In support of this, UV spectra of DA recorded in the presence of Zn\(^{2+}\) ions indicated substantial shift of DA peak from 284 to 298 nm, thus confirming dopamine-Zn\(^{2+}\) complexation. (Figure S3).

Analysis of the average diameter of the nanofibers indicated no apparent change in the diameter of nanofibers for Gel_DA in comparison to pristine ES_Gel mats. However, introducing Ag\(^+\) and Ca\(^{2+}\) ions together with dopamine significantly decreased the average diameter of gelatin nanofibers, though Zn\(^{2+}\) ions had negligible influence on diameter distribution when compared to Gel_DA (Figure S2). On the other hand, the average diameter of Gel_DA_Mg nanofibers was found to be marginally higher than Gel_DA nanofibers. TEM images of the nanofibers showed an absence of metal aggregates/particulates on the fiber surface, which was previously observed for nanofibers prepared from gelatin containing metal ions (Figure 1g-j insets). Thus, the presence of DA in gelatin-metal ion dope solutions allowed smooth dispersion of metal ions, thus promoting the formation of smooth nanofibers.
Recently, we reported that the ammonium carbonate exposure of Ca\(^{2+}\) ions and catecholamine containing collagen mats generated in situ mineralization and polycatecholamine cross-linking of electrospun collagen mats.\(^27\) This methodology (named as ammonium carbonate diffusion method, ADM) provided facile fabrication of nanofibrous mats with outstanding mechanical and osteoconductive properties. Thus, we investigated the influence of simultaneous in situ mineralization and polydopamine crosslinking of gelatin nanofibers containing DA and metal ions. SEM studies indicated a significant increase in welded junctions and extensive branching of the nanofibers after ADM, confirming fiber crosslinking through oxidative polymerization of dopamine \(i.e.\) transformation of dopamine to polydopamine (pDA) (Figure 1k-o). In general, the average diameter of the nanofibers increased significantly for all the mats in comparison to mats containing metal ions only. When compared to Gel_pDA mats, the average diameter of the crosslinked mats remained unaltered for Gel_pDA_Zn mats, whereas a decrease was observed for Gel_pDA_Ag and Gel_pDA_Ca mats (Figure S2c). TEM images also suggested the formation of sparsely distributed mineral particles inside along the length of the nanofibers for Gel_pDA_Ag, Gel_pDA_Ca, and Gel_pDA_Zn mats. Thus, morphological analysis confirmed the formation of smooth pDA coating and mineralization of gelatin nanofibers after ADM. Contrary to our methodology, functionalization of electrospun nanofibers with pDA by conventional Tris-HCl route followed by exposure to silver ions, often resulted in rough surfaces with heterogeneous aggregates of pDA and silver nanoparticles, whereas incorporation of dopamine and metal ions followed by subsequent exposure to ADM generated smooth pDA coating and crosslinking of electrospun gelatin and mineralization in situ.\(^28-30\)

3.2. Effect of metal ions on bonding environment of dopamine loaded gelatin and pDA crosslinked mats. Next, we analyzed the bonding environment and composition of metal and
dopamine incorporated gelatin mats before and after ADM treatment by XPS. High resolution C 1s, N 1s, O 1s, Cl 2p, Ag 3d, Mg 2p, Ca 2p and Zn 2p spectra for different samples are shown in Figure 2a-h. All the mats revealed the presence of C 1s, N 1s and O 1s peaks due to the presence of carbon, nitrogen and oxygen moieties in gelatin, dopamine and polydopamine. On the other hand, Ag 3d, Mg 2p, Ca 2p and Zn 2p peaks also turned up in specific samples where the treatment of these mats was performed using their corresponding metal salts (Figure 2e-h). Although the Ag 3d peak was relatively weaker than Mg 2p, Ca 2p and Zn 2p peak for both AC treated and untreated samples, the observation of different foreign elements (Ag, Mg, Ca and Zn) in various core level spectra confirmed the presence of metal ions in gelatin mats.

**Figure 2.** High resolution (a) C 1s, N 1s (b), O 1s (c), Cl 2p (d), Ag 3d (e), Mg 2p (f), Ca 2p (g) and Zn 2p (h) for different composite structures. Herein S1- Gel_DA_Ag, S2-
Gel_DA_Mg, S3- Gel_DA_Ca, S4-Gel_DA_Zn, S5- Gel_pDA_Ag, S6-Gel_pDA_Mg, S7- Gel_pDA_Ca, S8-Gel_pDA_Zn.

As one of the key aims of our study was to unveil the effect of incorporating metal ions on the dopamine polymerization and pDA crosslinking of gelatin nanofibers, we performed deeper investigations on various bonding patterns in different metal incorporated samples. For comprehensive analysis, the deconvolution of high resolution C 1s and N 1s core level spectra was performed using various Gaussian-Lorentzian components, Figure 3a and b. For reference, the C 1s and N 1s spectra of Gel_DA was also included. The deconvolution of C 1s spectra revealed four peaks, namely C₁, C₂, C₃ and C₄, which are assigned to C-C/C-H, C-N, C-O and C=O bonding, respectively.²⁷,³¹-³³ On the other hand, the deconvolution of N 1s spectra resulted into three peaks, namely N₁, N₂, and N₃, which were allotted to R₂NH, RNH₂ and C=NR bonding, respectively.²⁷,³¹-³³ The examination of these peaks, especially C=NR bonding (N₃ peak) in N 1s spectra, can be used to understand the dopamine polymerization and pDA mediated crosslinking in gelatin. This was owed to the formation of more imine (C=NR) functionalities upon pDA formation and crosslinking of gelatin with pDA (Scheme 1).³¹ Hence, an area ratio method was used to quantify the bonding content and the results were summarized in Table 3. The variation of C=NR bonding in terms of absolute change and relative change for various samples was also estimated and plotted (Figure S4). Interestingly, metal ion incorporated mats (Gel_DA_X, where X = Metal ion) even before ammonium carbonate treatment showed comparatively higher C=NR bonding than metal ion deprived Gel_DA mat (Figure S4). This could be attributed to metal ions triggering dopamine polymerization during electrospinning and activation of gelatin crosslinking even without performing AC treatment. Further increase in the C=NR bonding was reported after ammonium carbonate treatment for Gel_pDA_Ag, Gel_pDA_Mg and Gel_pDA_Ca samples, indicative of increased pDA mediated crosslinking in these samples, concurring with the SEM results. Anomalously, relative change of C=NR
bonding was found to be highest for the Gel_pDA_Zn mat, although the SEM micrograph barely displayed any crosslinked morphology. To explain this incongruity, we hypothesis that the increase in C=NR bonding in Gel_pDA_Zn mat is may be due to the formation of very small pDA oligomers that created a uniform coating on gelatin nanofibers, rather than crosslinking them, leading to increased fiber diameter (767 ± 103 nm) compared to AC untreated Gel_DA_Zn mat (531 ± 117 nm). This reasoning was also supported by the shifting of dopamine peak from 280 nm (monomeric peak) to 298 nm (small oligomer peak) as reported earlier by Wu et al (Figure S3).\textsuperscript{34}
**Figure 3.** Deconvolution of high resolution a) C 1s and b) N 1s spectra for different composite structures.

We have also studied extensively the core level spectra, namely Ag 3d, Mg 2p, Ca 2p and Zn 2p, to reveal the metal bonding state in the nanofibrous composite mats (**Figure 2e-h**). High resolution Ag 3d spectra (**Figure 2e**) of Gel_DA_Ag and Gel_pDA_Ag demonstrated 3d_{5/2} peak at ~ 368.3-368.4 eV. Based on binding energy (BE) of Ag 3d_{5/2} peak,
we suggest Ag to be present in both forms; metallic Ag and Ag bonded to oxygen.\textsuperscript{35} Similar behavior was observed while examining Mg 2p spectra where mats without and with AC treatment showed peaks at similar BE. It is well known that the peak position of Mg shifts toward higher BE side when Mg is bonded to oxygen.\textsuperscript{36,37} The BE position for Mg in metallic state was reported at around 49.4-49.6 eV while the BE for Mg bonded to oxygen can be in the range of \(~50.3-51\) eV and above.\textsuperscript{36,37} Thus, based on the peak position of our Mg 2p spectra, we expect the presence of Mg in both metallic and Mg bonded to oxygen states. To directly probe various bonding states in Mg 2p spectra, we performed deconvolution of Gel\_DA\_Mg and Gel\_pDA\_Mg (\textbf{Figure S5a}). During the deconvolution, we followed the work by Corneille et al. who assigned Mg metallic peak at 49.6 eV and Mg bonded to oxygen peak at 50.8 eV.\textsuperscript{36} In our case, the deconvolution of Mg 2p spectra for both the samples revealed two major distinct peaks namely M\textsubscript{1} (49.7 eV) and M\textsubscript{2} (50.75 eV) corresponding to metallic Mg and Mg bonded to oxygen, respectively. In addition, a weak M\textsubscript{3} (52.0 eV) peak was observed toward slightly higher BE side which was also resulted from bonding of Mg with oxygen. We used an area ratio method to calculate the amount of Mg in metallic and Mg bonded to oxygen states, \textbf{Table 3}. From the results, we found that the bonding of Mg with oxygen increased after AC treatment which was expected to increase the robustness of produced nanofibers.

Furthermore, for the Zn containing samples, we observed from Zn 2p spectra that the Zn 2p\textsubscript{3/2} and Zn 2p\textsubscript{1/2} peaks experienced shifts toward higher BE side after AC treatment (Gel\_pDA\_Zn) with respect to Gel\_DA\_Zn mat. This triggered us to further analyze the bonding states of Zn in these samples. Woll\textsuperscript{38} and Biesinger et al.\textsuperscript{39} suggested that metallic Zn and Zn bonded to oxygen (Zn-O) peaks appear at BE of 1021.4 eV and 1021.7 eV, respectively. The marginal BE difference between the two states of Zn thus created complexity for bonding analysis of Zn. Based on extensive literature survey, we observed
that the metallic Zn peak usually appears at around 1021-1021.4 eV while Zn-O peak originates at around 1021.7-1022 eV and above.\textsuperscript{38-40} Upon closer inspection, we observed the presence of Zn 2p\textsubscript{3/2} peak at $\sim$1021.1 eV in Gel\textsubscript{DA} Zn which shifted to $\sim$ 1021.7-1021.8 eV in Gel\textsubscript{pDA} Zn sample. These spectra suggest that Zn in these samples certainly exist in both metallic and oxide states. Therefore, we performed the deconvolution of Zn 2p\textsubscript{3/2} spectra while fixing the BE of 1021.1 eV for metallic Zn (peak Z\textsubscript{1}) and BE of 1021.8 eV for Zn-O (peak Z\textsubscript{2}) as shown in Figure S5b. In addition, a weak Z\textsubscript{3} peak towards higher BE side was also observed in both the samples which could be assigned to ZnCl\textsubscript{2} in addition to contribution of Zn-O in this peak as well.\textsuperscript{38-40} The results clearly indicate enhanced Zn-O bonding in case of Gel\textsubscript{pDA} Zn mats compared to Gel\textsubscript{DA} Zn mat (Table S1).

Next, we focused on Ca 2p spectra. The peak positions of various important Ca compounds in Ca 2p\textsubscript{3/2} envelopes are observed at 346-6-347.1 eV for CaCO\textsubscript{3} and CaO, at 347.5 eV for CaSO\textsubscript{4}, at 347.6 eV for CaBr, at 348 for CaCl\textsubscript{2}, at 348.2 eV for Ca(NO\textsubscript{3})\textsubscript{2}, etc.\textsuperscript{41,42} In our case, we observed Ca 2p\textsubscript{3/2} peak at 346.9-347 eV for both the Gel\textsubscript{DA} Ca and Gel\textsubscript{pDA} Ca mats. These results therefore indicate that there was a possibility of Ca carbonate and oxide formation, despite carbonate peaks not present in C 1s spectra which may be due to the dominance of carbon (from gelatin) in the C 1s spectra. Overall, XPS analysis provided critical insights into the bonding environment of composites mats such as metal ion-triggered crosslinking of DA containing gelatin fibers and enhancement of crosslinking after AC treatment due to synergistic effects.
Table 3. Bonding content of different constituent peaks in C 1s and N 1s spectra for various samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>C-C/C-H (%)</th>
<th>C-N (%)</th>
<th>C-O (%)</th>
<th>C=O (%)</th>
<th>R NH (%)</th>
<th>RNH₂ (%)</th>
<th>C=NR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel_DA</td>
<td>39.2</td>
<td>22.2</td>
<td>7.9</td>
<td>30.7</td>
<td>93</td>
<td>5.1</td>
<td>1.9</td>
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<tr>
<td>Gel_DA_Ag</td>
<td>37</td>
<td>23</td>
<td>8</td>
<td>32</td>
<td>77.8</td>
<td>20</td>
<td>2.2</td>
</tr>
<tr>
<td>Gel_pDA_Ag</td>
<td>78.3</td>
<td>10.7</td>
<td>2.5</td>
<td>8.5</td>
<td>83.2</td>
<td>14.2</td>
<td>2.6</td>
</tr>
<tr>
<td>Gel_DA_Mg</td>
<td>67</td>
<td>12.5</td>
<td>7</td>
<td>13.5</td>
<td>80</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>Gel_pDA_Mg</td>
<td>49</td>
<td>16.8</td>
<td>8</td>
<td>26.2</td>
<td>82.5</td>
<td>15</td>
<td>2.5</td>
</tr>
<tr>
<td>Gel_DA_Ca</td>
<td>70</td>
<td>19</td>
<td>2</td>
<td>9</td>
<td>66</td>
<td>29</td>
<td>5</td>
</tr>
<tr>
<td>Gel_pDA_Ca</td>
<td>70.4</td>
<td>15</td>
<td>3.2</td>
<td>11.4</td>
<td>71</td>
<td>24</td>
<td>5</td>
</tr>
<tr>
<td>Gel_DA_Zn</td>
<td>66.5</td>
<td>17</td>
<td>3.5</td>
<td>13</td>
<td>68.5</td>
<td>26.5</td>
<td>5</td>
</tr>
<tr>
<td>Gel_pDA_Zn</td>
<td>56</td>
<td>23</td>
<td>4</td>
<td>17</td>
<td>70</td>
<td>23</td>
<td>7</td>
</tr>
</tbody>
</table>

3.3. Effect of DA and metal ions on mechanical properties of electrospun gelatin mats.

To confirm the observations that the presence of metal ions could trigger the oxidative polymerization of dopamine, we investigated the mechanical properties of the gelatin nanofibers prepared under various conditions (Table 4). Representative stress-strain curves used to extract four different tensile parameters are shown in Supporting Information (Figure S6). The results indicated that except for Ca²⁺ ions, the presence of other ions in the dope solution increased the Young’s modulus of the nanofibers in comparison to pristine gelatin mats. Among the metal ions, Mg²⁺ conferred the maximum increase in tensile stress and stiffness, consistent with the network-like morphology of as-electrospun mats. The increase in tensile strength upon incorporation of Ag⁺, Mg²⁺ and Zn²⁺ ions was accompanied by concomitant loss of elastic properties, as indicated by significant decrease in tensile strain.

The presence of dopamine had a similar effect as the metal ions in enhancing the Young’s modulus at the expense of elastic properties. To probe the effect of metal ions, we compared the mechanical properties of Gel_DA and metal ion-containing Gel_DA mats which revealed distinct results for different metal ions. The Young’s modulus and tensile strength were significantly increased and elasticity was slightly increased for Gel_DA_Mg and Gel_DA_Ca mats with respect to Gel_DA mat. The nanofibers prepared from a dope
solution containing Ag\(^+\) ions and DA showed a decrease in tensile stiffness and increase in failure strain, thus transforming the brittle behaviour of Gel_DA mats into a ductile material. Among metal containing Gel_DA mats, Gel_DA_Zn mats displayed poorer tensile properties. This again supports our previous hypothesis that strong interactions among DA and Zn\(^{2+}\) discourage the metal-gelatin nanofiber interactions that thus compromises junction configuration/crosslinking. The presence of calcium ions together with dopamine conferred the maximum increase in tensile modulus and tensile strength than other ions, indicating superior reinforcing ability of calcium ions for dopamine loaded gelatin mats.

**Table 4.** Effects of metal ions on mechanical properties of electrospun nanofibers prepared under various conditions. \(^{†}\)The changes in the mechanical properties were compared with nanofibers prepared without any metal ions. Statistical significant differences were indicated by asterisks: *, p≤0.05; **, p<0.01; ***, p<0.001 and ****, p<0.0001 by t-test or 1-way ANOVA.

<table>
<thead>
<tr>
<th>Sample(^{†})</th>
<th>Tensile strength (σ), MPa</th>
<th>Tensile modulus (E(^{'}), ) MPa</th>
<th>Strain (ε(_b)), %</th>
<th>Toughness (J(_{lc})), MJ.m(^{-3})</th>
</tr>
</thead>
<tbody>
<tr>
<td>ES_Gel</td>
<td>2.30 ± 0.18</td>
<td>62.9 ± 9.0</td>
<td>19.04 ± 2.78</td>
<td>0.23 ± 0.07</td>
</tr>
<tr>
<td>Gel_Ag</td>
<td>3.80 ± 0.34(****)</td>
<td>202.1 ± 35.84(****)</td>
<td>8.74 ± 2.66(****)</td>
<td>0.19± 0.05</td>
</tr>
<tr>
<td>Gel_Mg</td>
<td>4.2 ± 0.67(****)</td>
<td>188.5 ± 25.31(****)</td>
<td>5.44 ± 1.05(****)</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>Gel_Ca</td>
<td>3.39 ± 0.22(**)</td>
<td>69.08 ± 8.67(*)</td>
<td>22.24 ± 2.33(**)</td>
<td>0.54 ± 0.10(****)</td>
</tr>
<tr>
<td>Gel_Zn</td>
<td>2.94 ± 0.14(*)</td>
<td>182.2 ± 24.6(****)</td>
<td>8.45 ± 0.89(****)</td>
<td>0.16 ± 0.02</td>
</tr>
<tr>
<td>Gel_DA</td>
<td>2.56 ± 0.22</td>
<td>132.1 ± 24.01</td>
<td>5.278 ± 1.04</td>
<td>0.06896 ± 0.012</td>
</tr>
<tr>
<td>Gel_DA_Ag</td>
<td>3.78 ± 0.24(*)</td>
<td>90.86 ± 12.41(*)</td>
<td>21.83 ± 2.62(**)</td>
<td>0.53 ± 0.10(****)</td>
</tr>
<tr>
<td>Gel_DA_Mg</td>
<td>5.63 ± 0.86(**)</td>
<td>209.9 ± 61.1(**)</td>
<td>8.70 ± 1.87(**)</td>
<td>0.27 ± 0.086(**)</td>
</tr>
<tr>
<td>Gel_DA_Ca</td>
<td>9.34 ± 2.39(**)</td>
<td>631.8 ± 46.05(**)</td>
<td>12.35 ± 2.54(**)</td>
<td>0.86 ± 0.14(**)</td>
</tr>
<tr>
<td>Gel_DA_Zn</td>
<td>2.22 ± 0.14(*)</td>
<td>51.29 ± 1.7(**)</td>
<td>8.24 ± 1.08(*)</td>
<td>0.095± 0.021</td>
</tr>
<tr>
<td>Gel_pDA</td>
<td>2.93 ± 0.69(*)</td>
<td>157.1 ± 36.1(*)</td>
<td>25.91 ± 4.51(*)</td>
<td>0.52 ± 0.14(*)</td>
</tr>
<tr>
<td>Gel_pDA_Ag</td>
<td>6.97 ± 0.58(*)</td>
<td>414.5 ± 67.02(*)</td>
<td>8.02 ± 4.93(*)</td>
<td>0.26 ± 0.053(*)</td>
</tr>
<tr>
<td>Gel_pDA_Mg</td>
<td>6.45 ± 1.6(*)</td>
<td>461.6 ± 161.2(*)</td>
<td>4.66 ± 2.38(*)</td>
<td>0.16 ± 0.04(*)</td>
</tr>
<tr>
<td>Gel_pDA_Ca</td>
<td>12.4 ± 0.46(**)</td>
<td>778.2 ± 123.3(**)</td>
<td>6.09 ± 0.493(**)</td>
<td>0.46 ± 0.10(*)</td>
</tr>
<tr>
<td>Gel_pDA_Zn</td>
<td>3.253 ± 0.49</td>
<td>77.06 ± 8.761(*)</td>
<td>16.46 ± 2.89(*)</td>
<td>0.33 ± 0.10(*)</td>
</tr>
</tbody>
</table>

More dramatic changes in mechanical properties were observed after ammonium carbonate exposure of metal ions containing Gel_DA mats (Table 4). The results indicated that the ammonium carbonate exposure of gelatin mats containing Ag\(^+\), Mg\(^{2+}\) and Ca\(^{2+}\) ions (Gel_pDA_Ag, Gel_pDA_Mg and Gel_pDA_Ca) displayed more brittle-like behaviour than
Gel_pDA and Gel_pDA_Zn mats which conferred both the brittle and ductile like stress-strain curves. Among the divalent cations, Ca$^{2+}$ ions conferred the maximum improvement in mechanical properties followed by Mg$^{2+}$ ions whereas Zn$^{2+}$ ions decreased the tensile properties with respect to Gel_pDA. The improved mechanical properties of Gel_pDA_Ag, Gel_pDA_Mg and Gel_pDA_Ca, when compared to Gel_pDA, were attributed to pDA-metal ions mediated increase in inter-fiber interactions and cross-linking. In support of our observations, two previous studies reported similar enhancement in tensile properties of electrospun nanofibers by incorporation of mineral nanoparticles or nanotubes that were surface coated with polydopamine at low mineral content.\textsuperscript{43,44} However, poor mechanical properties of Gel_pDA_Zn with respect to Gel_pDA was due to formation of DA-Zn complex which in turn contributed to the loss of inter-fiber interactions and formation of welded junctions (Fig. S3).

3.4. Effect of metal ions on thermal properties of electrospun gelatin mats. Given the ability of metal ions to greatly influence the structure and bonding environment of gelatin nanofibers, the incorporation of metal ions was also expected to have considerable effect on thermal stability. Thermogravimetric analysis (TGA) and differential thermogravimetric analysis (DTA) were performed to assess the thermal stability of different electrospun gelatin-based mats. (Figure 4 & Table 5) Pristine ES_Gel mats underwent weight loss in three stages: first stage weight loss (W$_1$) between 30 and 100 °C, which represents the evaporation of physiosorbed water molecules; second stage weight loss (W$_2$) between 200 and 500 °C, which specifies thermal degradation of gelatin nanofibers; and third stage weight loss (W$_3$) above 500 °C, which corresponds to the carbonization of residual organic components. The peak weight loss temperatures for first, second and third stages are assigned as $^1T_{\text{Dehydration}}$, $^2T_{\text{max}}$, and $^3T_{\text{max}}$, respectively. The $T_i$ (temperature at which the second stage weight loss begin), $T_{1/2}$ (half decomposition temperature) and $W_{\text{Res}}$ (residual weight) were
also estimated to get insight into the dopamine and metal ions interactions on the thermal properties of gelatin mats.

Figure 4. TGA and DTA curves for electrospun gelatin mats incorporated with (a and b) metal ions, (c and d) metal ions and dopamine, and (e and f) metal ions and polydopamine.

Thorough analysis of the TGA result leads us to following conclusions: i) Integrating metal ions (without dopamine) impacts the thermal properties of gelatin nanofibers (Figure 4a and b). Integration of alkaline earth metal ions (Ca$^{2+}$ and Mg$^{2+}$) significantly enhances the $^{1}T_{\text{Dehydration}}$, $T_1$, and $T_{1/2}$ temperatures (Table 5) with increase in $W_1$ and $W_3$ values (Table S2).
These results indicate that Ca$^{2+}$ and Mg$^{2+}$ incorporation promotes and strengthens water interactions with the gelatin matrix and leads to higher $^1T_{\text{Dehydration}}$ and $W_1$ records. Further increases in the $T_1$, $T_{1/2}$ and $W_3$ values designate the stabilization of the gelatin nanofiber skeleton by Ca$^{2+}$/Mg$^{2+}$ ions that postponed its degradation to higher temperatures. On the contrary, transition metals (Ag$^+$/Zn$^{2+}$) showed negligible effects on the thermal properties of gelatin nanofibers. Considering the $^1T_{\text{Dehydration}}$, $T_1$, $T_{1/2}$ and $W_3$ parameters, the thermal stability of the metal incorporated mats follows the following order

$$\text{Gel}_\text{Mg}^{2+} > \text{Gel}_\text{Ca}^{2+} > \text{Gel}_\text{Zn}^{2+} > \text{Gel}_\text{Ag}^+ > \text{ES}_\text{Gel}$$

ii) Adding dopamine alone also helped improve the thermal properties of gelatin nanofibers in term of boosted $T_1$, $^1T_{\text{Dehydration}}$, $T_{1/2}$, and $W_3$ values, which was due to the enhanced inter-fiber interactions in the presence of dopamine molecules. iii) Integration of both metal ions and dopamine within the gelatin matrix further enhanced its thermal properties. Consistent with the trends in mechanical properties, Gel_DA_Mg, Gel_DA_Ag and Gel_DA_Ca showed significant enhancements in almost all the thermal parameters including $^1T_{\text{Dehydration}}$, $T_1$, $T_{1/2}$ and $W_3$ values compared to gelatin and metal ion loaded mats without dopamine. This could be due to the remarkable complexation properties of Mg$^{2+}$, Ag$^+$, Ca$^{2+}$ ions and their ability to promote dopamine polymerization which encourages the crosslinking of gelatin nanofibers, thus leading to improved thermal properties. Like without dopamine mats, Zn$^{2+}$ ions with dopamine do not pose any significant impact on the thermal properties. Interestingly, all dopamine/metal enriched samples showed trifurcation in the $^2T_{\text{max}}$ peak supporting multiple interactions within the gelatin matrix due to the availability of both dopamine and polydopamine components. iv) All the crosslinked samples, particularly Gel_pDA_Ag and Gel_pDA_Ca, showed the best thermal parameters in terms of having high $^2T_{\text{max}}$ and $^3T_{\text{max}}$ values. In the crosslinked samples, trifurcations were transformed into bifurcations which may be due to the conversion of dopamine into polydopamine during crosslinking treatment.
Table 5. Thermal properties of various electrospun gelatin mats.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>$T_1$ ($^\circ$C)</th>
<th>$T_{Dehydration}$ ($^\circ$C)</th>
<th>$T_{max}$ ($^\circ$C)</th>
<th>$T_{1/2}$ ($^\circ$C)</th>
<th>$T_{max}$ ($^\circ$C)</th>
</tr>
</thead>
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<tr>
<td>ES_Gel</td>
<td>180.1</td>
<td>69</td>
<td>335.8</td>
<td>349.3</td>
<td>NA</td>
</tr>
<tr>
<td>Gel_Ca</td>
<td>192.8</td>
<td>85</td>
<td>335, 324.3</td>
<td>363.1</td>
<td>742.7, 612.8</td>
</tr>
<tr>
<td>Gel_Mg</td>
<td>219.2</td>
<td>92.6</td>
<td>333, 342.3</td>
<td>367</td>
<td>663</td>
</tr>
<tr>
<td>Gel_Ag</td>
<td>182</td>
<td>61.3</td>
<td>342.9</td>
<td>350.8</td>
<td>NA</td>
</tr>
<tr>
<td>Gel_Zn</td>
<td>185</td>
<td>66.2</td>
<td>336.1</td>
<td>353.4</td>
<td>NA</td>
</tr>
<tr>
<td>Gel_DA</td>
<td>190.9</td>
<td>77</td>
<td>324.3</td>
<td>352.3</td>
<td>746.3</td>
</tr>
<tr>
<td>Gel_DA_Ca</td>
<td>192.8</td>
<td>81.4</td>
<td>335, 345.4, 353.2</td>
<td>365</td>
<td>738, 614.7</td>
</tr>
<tr>
<td>Gel_DA_Mg</td>
<td>223.6</td>
<td>72.6</td>
<td>319, 343.9, 330.7</td>
<td>367.6</td>
<td>656</td>
</tr>
<tr>
<td>Gel_DA_Ag</td>
<td>204</td>
<td>85.7</td>
<td>327.3, 313.1, 345.86</td>
<td>353</td>
<td>752.6</td>
</tr>
<tr>
<td>Gel_DA_Zn</td>
<td>188.4</td>
<td>81.3</td>
<td>326.3, 343.9, 361.5</td>
<td>358</td>
<td>NA</td>
</tr>
<tr>
<td>Gel_pDA</td>
<td>182</td>
<td>79.4</td>
<td>330.7, 341.5</td>
<td>350</td>
<td>NA</td>
</tr>
<tr>
<td>Gel_pDA_Ca</td>
<td>213</td>
<td>72.6</td>
<td>326.3, 420</td>
<td>352</td>
<td>628, 713.5</td>
</tr>
<tr>
<td>Gel_pDA_Mg</td>
<td>217</td>
<td>81.4</td>
<td>330.7, 416.3</td>
<td>364.5</td>
<td>NA</td>
</tr>
<tr>
<td>Gel_pDA_Ag</td>
<td>190.9</td>
<td>79.4</td>
<td>337.5, 345.9</td>
<td>355.8</td>
<td>747.7</td>
</tr>
<tr>
<td>Gel_pDA_Zn</td>
<td>201.6</td>
<td>79.4</td>
<td>321.9</td>
<td>354.8</td>
<td>NA</td>
</tr>
</tbody>
</table>

3.5. Effect of metal ions on surface wettability of electrospun gelatin mats. Surface wettability is one of the key determinants of cell adhesion and proliferation.\(^{45}\) Thus, we investigated how metal ions altered the surface wettability of ES_Gel and Gel_DA fibers before and after ADM. The surface wettability of all the mats was determined by measuring the water contact angle (WCA) in dynamic mode for 2 minutes. For simplicity, we compared the final WCA (after 2 min) of all these samples (Figure 5). Pristine gelatin showed WCA of $25.5 \pm 3.2^\circ$
Figure 5. Photographs showing the water contact angle (WCA) on various electrospun gelatin mats captured after 2 mins of dropping the water drop.

Upon incorporating metal ions to pristine gelatin mats, an increase in WCA was observed for all the mats except Gel_Mg sample. This increase in the contact angle in metal ion incorporated gelatin mats may be due to the involvement of the hydrophilic groups (–OH/–NH$_2$) of gelatin chains in forming chelates with the available metal ions as reported earlier by Tanaka.$^{46}$ In contrast, the lower WCA of Mg-loaded mats could be due to the accessibility of more magnesium ions, owing to their small size, and their greater affinity to form hydrates. The WCA dropped to 0° in Gel_DA mats due to the higher hydrophilicity provided by the polar groups in the dopamine structure. Incorporation of metal ions together with DA did not alter the final WCA values for Gel_DA_Ag mats, whereas the values increased for Gel_DA_Mg, Gel_DA_Zn and Gel_DA_Mg mats. These results suggest variable interactions between the biopolymer, DA and metal ions that might contribute to the differences in surface hydrophilicity.
WCA values increased significantly for Gel_pDA mats when compared to ES_Gel or Gel_DA mats, indicating polydopamine coatings decreased the aqueous wettability of pristine gelatin mats, consistent with previous results. However, mats containing silver and magnesium (Gel_pDA_Ag & Gel_pDA_Mg) displayed lower values than Gel_pDA. Among the divalent cations, Gel_pDA_Zn mats displayed the highest contact angle, indicating that co-ordination complex formed between the transition metal ion and pDA rendered the surface hydrophobic.\textsuperscript{47} In support of this, we recorded the UV spectra of dopamine (DA) with or without divalent cations at pH 8.5. The absorption peak of dopamine at 284 nm was shifted to 297 nm in the presence of zinc indicating strong interactions between Zn$^{2+}$ and DA whereas no apparent difference was observed in the presence of Ca$^{2+}$ and Mg$^{2+}$ ions (Fig. S3). Thus, the interaction of polydopamine with different metal ions conferred varying degrees of wettability: surface hydrophilicity increased in the presence of silver and magnesium, decreased in the presence of zinc, and remained unaltered in the presence of calcium.

3.6. Cytocompatibility of composite mats for human dermal fibroblasts. The cytocompatibility of the composite mats was ascertained in hDF by immunofluorescence confocal imaging. The hDFs cells were cultured on the mats for 24 h and the cellular integrity was assessed by staining the nuclei, $\alpha$-tubulin and actin of hDFs. In particular, we focused our attention on Gel_Ag and Gel_pDA_Ag as well as Gel_Zn and Gel_pDA_Zn mats to discern the effect of metal ions with and without pDA crosslinking. The hDF cells seeded on ES_Gel and Gel_pDA mats retained spindle shaped morphologies with intact cytoskeleton components and nuclei which were comparable to the cells seeded on tissue culture plates, indicating good biocompatibility of the mats (Figure 6). Cells seeded on Gel_Ag mats appeared rounded and truncated, confirming the cytotoxic effect of silver ions for hDFs. Interestingly, cells exposed to Gel_pDA_Ag mats retained their regular spindle-shaped morphology with intact cytoskeletal components and nuclei (Figure 6d), suggesting that the
strong interactions between silver ion and pDA attenuated the toxic effect of silver. Cells cultured on both Gel_Zn and Gel_pDA_Zn mats appeared healthy. To further confirm these results, we determined the metabolic activity of hDFs, seeded on Gel_Ag and Gel_pDA_Ag mats by MTS assay. The results showed significant decrease in cell viability (14.6±7.2%, p<0.0001) of hDFs seeded on Gel_Ag mats, which was significantly improved in Gel_pDA_Ag mats (74.0±17.4%, p<0.001). Cell growth profile on Gel_Ca/Gel_Mg and Gel_pDA_Ca/Gel_pDA_Mg mats was not affected, confirming their lack of adverse effects for the mammalian cells (Figure S7).
Figure 6. Cytocompatibility of mats in human dermal fibroblasts. (a-f) Confocal images of hDFs grown on different electrospun gelatin mat samples. Cells were cultured on ES_Gel (a), Gel_pDA (b), Gel_Ag (c), Gel_pDA_Ag (d), Gel_Zn (e), and Gel_pDA_Zn (f) for 24 h, stained for α-tubulin (green), actin (red) and nuclei (blue) and imaged using confocal microscopy, 40X objective. At least 5 different fields per samples were imaged and a representative image is presented. Scale bar = 20 um. (g) Cell viability (%) of hDFs cultured
on various mats for 24 h was determined using MTS-based assay and plotted, mean +/- SEM, 
n = 3 in triplicates.

Intrigued by the marked decrease in cytotoxicity of Gel_pDA_Ag and increased 
hydrophobicity of Gel_pDA_Zn mats, we then determined the cell proliferative properties of 
these mats, by monitoring the cell mitochondrial metabolic activity using MTS assay at day 
1, day 4 and day 7 post seeding (p.s.) of HaCaT and hDFs. As shown in Fig. 7a, 
Gel_pDA_Ag mats displayed significant increase in the metabolic activity for HaCaT cell 
lines when compared to cells cultivated on Gel_pDA_Zn or coverslips. Zanette et al., 
reported that limited exposure of HaCaT cell lines to silver nanoparticles resulted in marked 
decrease in cell proliferative properties, suggesting heightened toxicity of silver.48 The lack of 
cytotoxic effects for HaCaT cell lines and the increased cell proliferative properties of the 
epidermal cell lines seeded on Gel_pDA_Ag mats augment our hypothesis that pDA 
chelation of silver decreased the toxicity and adverse effects of the ions on cell proliferative 
properties.

For hDFs, both Gel_pDA_Ag and Gel_pDa_Zn mats displayed similar increase in cell 
proliferation as that of cells cultivated on coverslips at day 1 and day 4 p.s. (Fig. 7b). A 
higher metabolic activity was observed for cells cultivated on coverslips at day 7 p.s., than 
Gel_pDA_Ag and Gel_pDA_Zn mats. It has been shown that sliver wound care products 
displayed significant cytotoxic effects on HaCaT, primary keratinocytes and dermal 
fibroblasts.49,50 However, MTS results attained in the present study demonstrate excellent 
biocompatibility of Gel_pDA_Ag and Gel_pDA_Zn mats for skin cells. Immunofluorescent 
confocal and SEM images further indicate that cells (hDFs and HaCaT) seeded on 
Gel_pDA_Ag and Gel_pDA_Zn mats adhered in greater densities than coverslips (Fig. 7c). 
In particular, hDFs were well spread and formed a confluent monolayer on all the substrates 
even at day 4 p.s. Thus, the pDA crosslinking attenuated the toxicity and anti-proliferative
properties of silver and these results establish the excellent biocompatibility of mineralized mats for skin cells.

**Figure 7.** Cell proliferative properties of mineralized mats. Metabolic activity of HaCaT cell lines (a) and hDFs (b) seeded on Gel_pDA_Ag and Gel_pDA_Zn mats. Cells seeded on coverslips (CS) served as the control. c) Confocal images showing the morphology of HaCaT and hDFs seeded on Gel_pDA_Ag and Gel_pDA_Zn mats. Scale bar = 20 μm. The bottom panel shows the SEM images of hDFs seeded on various substrates. Scale bar = 20 μm.
3.7. Antimicrobial Properties of Composite Mats. Recently, we reported that the polypyrrogallol coating of catheters from a solution containing magnesium ions displayed potent antimicrobial and antibiofilm activities against MRSA strains.\textsuperscript{51} Based on the XPS data that metal ions did interact with polydopamine, we determined the antimicrobial properties of Gel\_pDA mats containing various metal ions by the disc diffusion method (Table 6). The range of pathogens included Gram-negative and Gram-positive bacteria, as well as yeasts. Gel\_pDA mats did not display a clear zone of inhibition (ZOI) against any of the tested pathogens, confirming that pDA alone lacks antimicrobial properties, augmenting our previous results.\textsuperscript{52} Similarly, except for Gel\_Ag mats, disc diffusion assay results indicated that gelatin mats containing other metal ions (Gel\_Mg, Gel\_Zn and Gel\_Ca) lacked any antimicrobial activities (Figure S8). However, Gel\_pDA\_Mg mats displayed a clear ZOI against MRSA and \textit{B. cereus} strains but lacked any antibacterial activity against VRE and \textit{B. subtilis} strains (Table 6). Gel\_pDA\_Zn and Gel\_pDA\_Ca mats displayed clear ZOI against all the Gram-positive strains tested. Against the Gram-negative bacteria, no antimicrobial activity was detected for Gel\_pDA\_Ca mats, whereas Gel\_pDA\_Zn/Gel\_pDA\_Mg mats displayed contact inhibition as no bacterial growth was observed on the tops and bottoms of the mats. Gel\_pDA\_Ag mats displayed potent broad spectrum antimicrobial properties against the panel of Gram-negative/-positive and yeast strains. These results demonstrate that Ag-pDA chelation retained the antimicrobial activity of silver, whereas similar interactions with zinc, calcium and magnesium conferred potent antimicrobial properties.

To ascertain if silver complexation with pDA retained the bactericidal properties, we exposed the bacterial cells (~10\textsuperscript{6} CFU/ml) to Gel\_pDA\_Ag mats for 24 h under proliferative conditions and determined the viable bacteria. The results suggest 2-8 log\textsubscript{10} decrease in bacterial viability, depending on the nature of strains, confirming bactericidal properties of Gel\_pDA\_Ag (Table S3). Next, we investigated the long-term antimicrobial activity by
determining ZOI for Gel_Ag and Gel_pDA_Ag mats against *P. aeruginosa*, following the protocol reported before.\textsuperscript{23,31} For Gel_Ag mats, burst release of silver ions resulted in an increase in ZOI values within 24 h which decreased with increasing soaking time in PBS (Fig. 8b). ZOI value for Gel_pDA_Ag mats, however, remained similar throughout the course of the study, and both the mats retained substantial antimicrobial properties even after 40-day of immersion in PBS.

![Figure 8](image)

**Figure 8.** a) Representative digital photos showing the long-term antimicrobial activity of Gel_Ag and Gel_pDA_Ag mats against *P. aeruginosa* ATCC 9027 strains. b) Graph showing the changes in ZOI, after soaking in PBS for different time intervals. Note a slight increase in ZOI values for Gel_pDA_Ag mats immediately after immersion in PBS which later become consistent with increasing time. Each ZOI value represents an average of two independent experiments, determined by disc diffusion assay.

Together with XPS results, these observations demonstrate that the chelating ability of polydopamine allowed the controlled release of Ag\(^+\) ions, thus attenuating its cytotoxicity for hDFs while retaining long-term antimicrobial activity. These observations corroborate with the results from others that polydopamine or polyDOPA coating following functionalization
of antimicrobial ions such as Cu$^{2+}$ or Ag$^+$ attenuated mammalian cell cytotoxicity while retaining antimicrobial properties.\textsuperscript{28,53-55} It has been demonstrated that commercial wound dressings containing silver or chlorohexidine displayed heightened mammalian cytotoxic effects and anti-proliferative properties.\textsuperscript{50,56} Strategies like the one reported here using polycatecholamine coatings would represent a valuable proposition for better wound management. The synergistic antimicrobial effects of pDA with biologically relevant Mg$^{2+}$, Ca$^{2+}$ and Zn$^{2+}$ ions may provide a simplistic approach towards bacterial decontamination wherein the use of antibiotics or antiseptics are restricted.

Table 6. Antimicrobial activity (in term of zone of inhibition) for various metal-ion loaded gelatin mats crosslinked with polydopamine against different microbial strains.

<table>
<thead>
<tr>
<th>Strains</th>
<th>GEL_pDA</th>
<th>GEL_pDA_Ag</th>
<th>GEL_pDA_Mg</th>
<th>GEL_pDA_Ca</th>
<th>GEL_pDA_Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram Positive Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methicillin-resistant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (MRSA) DM09808R</td>
<td>-</td>
<td>3.5cm</td>
<td>3.0cm</td>
<td>2cm</td>
<td>2.1cm</td>
</tr>
<tr>
<td><em>B.cereus</em> 11778</td>
<td>-</td>
<td>1.9cm</td>
<td>1.3cm</td>
<td>1.5cm</td>
<td>1.3cm</td>
</tr>
<tr>
<td><em>B.subtilis</em> 6633</td>
<td>-</td>
<td>1.4cm</td>
<td>-</td>
<td>1.4cm</td>
<td>2.4cm</td>
</tr>
<tr>
<td>Vancomycin-resistant</td>
<td>-</td>
<td>1.2cm</td>
<td>-</td>
<td>1.7cm</td>
<td>1.5cm</td>
</tr>
<tr>
<td><em>enterococci</em> (VRE) 1001</td>
<td>-</td>
<td>1.6cm</td>
<td>-</td>
<td>1.1cm</td>
<td>1.3cm</td>
</tr>
<tr>
<td><strong>Gram Negative Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P.aeruginosa</em> 9027</td>
<td>-</td>
<td>2.1cm</td>
<td>1.4cm</td>
<td>-</td>
<td>1.6cm</td>
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<tr>
<td><em>P.aeruginosa</em> 01</td>
<td>-</td>
<td>2.4cm</td>
<td>1.4cm</td>
<td>-</td>
<td>0.8cm</td>
</tr>
<tr>
<td><em>A.baumannii</em> 19606</td>
<td>-</td>
<td>4.5cm</td>
<td>1.6cm</td>
<td>-</td>
<td>1.3cm</td>
</tr>
<tr>
<td><em>A.baumannii</em> 1001</td>
<td>-</td>
<td>3.0cm</td>
<td>1.2cm</td>
<td>-</td>
<td>1cm</td>
</tr>
<tr>
<td><em>K.pneumoniae</em> DM4299</td>
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<td>2.8cm</td>
<td>1.5cm</td>
<td>-</td>
<td>1.3cm</td>
</tr>
<tr>
<td><em>K.pneumoniae</em> 10031</td>
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<td>2.2cm</td>
<td>1.5cm</td>
<td>-</td>
<td>0.8cm</td>
</tr>
<tr>
<td><strong>Yeast</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>C.albicans</em> DF0001976R</td>
<td>-</td>
<td>1cm</td>
<td>-</td>
<td>-</td>
<td>0.9cm</td>
</tr>
<tr>
<td><em>C.albicans</em> 10231</td>
<td>-</td>
<td>1cm</td>
<td>-</td>
<td>-</td>
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</tr>
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</table>
4. Conclusions

Crosslinking of gelatin nanofibers can be done via oxidation of dopamine to polydopamine under alkaline conditions. Using this knowledge, we have proposed a method to create a gelatin-based electrospun scaffold that is durable, water resistant, biocompatible and exhibits long lasting anti-microbial activity. We have observed that mats containing silver and zinc ions possess superior anti-microbial activity and also proposed that the interaction between magnesium and calcium ions with polydopamine can also confer good anti-microbial activity. With the rising incidences of antibiotic resistant pathogens, the mineralized antimicrobial mats with cell supportive properties can lower costs without adding other antimicrobial agents. Based on all the mentioned desirable properties, we propose the potential applications of these multifunctional nanofibrous mats as anti-infective wound dressings, benign tissue engineering scaffolds and safe inserts or implants with enhanced tissue regeneration properties.

Supporting Information

Figures showing the photographs of different gelatin solutions for fabricating electrospun mats, diameter distribution curves for different mats, UV-Visible absorbance plot showing dopamine interactions with different metal ions, variation of C=NR bonding in different mats, deconvolution of Mg 2p and Zn 2p$_{3/2}$ spectra for various composite mat samples, stress-strain curves for various mats, confocal images revealing the morphology of human dermal fibroblasts on various calcium and magnesium loaded gelatin mats and disc diffusion assay images showing the antimicrobial effectiveness of various electrospun gelatin mats. Tables showing bonding content of different constituent peaks in Mg 2p and Zn 2p spectra, thermal properties for various electrospun gelatin mats in term of weight loss and bacterial viability results for Gel_pDA_Ag mats.
Acknowledgements

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Nanofibrous Mats with Excellent Antimicrobial Properties
Good Biocompatibility
Optimum Wettability
Enhanced Mechanical Properties
Improved Thermal Stability

Polydopamine Crosslinked Metal Ions Loaded Gelatin Mats

Ammonium Carbonate Vapor Treatment, 24h