



Porous micro-/nano-fibrous silver-coated polymeric scaffolds with tunable mechanical properties for wound healing applications

Parvathalu Kalakonda¹ · Shalini Thudumu¹ · Soujanya Laxmi Mynepalli¹ · Rajitha Kathi¹ · Vasudeva reddy Yatham¹ · Pritam Mandal⁴ · Sreenivas Banne³ · Pranay Bhaskar Kalakonda⁶ · Murali Banavoth² · Moses Kigozi⁵ · Bala Bhaskar Podili¹

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Abstract

Electro-spun scaffolds of poly(glycerol sebacate)/poly(ϵ -caprolactone)(PGS/PCL) poly(methyl methacrylate)–poly(ϵ -caprolactone)(PMMA/PCL) have been using for biomedical applications of tissues engineering due to their attractive thermal, optical, and mechanical properties. In this work, we fabricate both nano-/micro-fibrous scaffolds from a hybrid material composite of PMMA/PCL and PGS/PCL fiber using a standard electro-spinning method and coat one side of the scaffolds with silver nanoparticles (AgNPs). This method of AgNPs coating prevents the scaffold (substrate) from melting behavior, and AgNPs coating reduces the pore diameter and increases fiber diameter, which improves their thermo-mechanical properties. Thereafter, we compare the mechanical properties of pristine PGS/PCL scaffold polymers; PMMA/PCL polymers with the AgNPs-coated PGS/PCL, PMMA/PCL scaffolds. The PMMA/PCL composite fibrous scaffolds show a higher modulus (E), tensile strength (TS), and lower tensile strain without any post-processing. These AgNPs-coated composite fibrous scaffolds provide potential applications in biomedical research fields, such as in fabricating of surgical meshes, bandages, and Band-Aids. Further, the enhanced antibacterial activities of AgNPs, which are used as a coating to our hybrid composite scaffolds, contribute to wound healing.

Keywords Fibrous scaffold · Electro-spinning · Silver coating · Tensile modulus · Composite

Introduction

Chronic wounds and burns are becoming more difficult to resolve issues, and it has been increasingly one of the major public health issues. Recent studies show that more than 5 million patients are contrived by chronic wounds in the USA itself [1, 2]. Skin is the largest organ in humans, many important biological functions, and acts as the primary protecting layer against environmental pathogens. When the skin's barrier is breached, the chances of microbial infections are increased [3–5]. The infections hinder wounds against bacterial infections, which can be favorably taken care of through the use of wound dressings by fabricated AgNPs-coated composite fibrous scaffolds due to their antimicrobial activity of AgNPs [6, 7].

Previously, natural polymers (gelatin, collagen) and their nano-composites have been used for biomedical applications [8–10]. A nano-composite of gelatin containing silver nanoparticles was proposed as a potential material for antibacterial applications. It was observed that the nano-composite gel has antimicrobial effects on a diversified of bacteria strains, such as *E. coli*, *S. aureus*, and *B. cereus* [11, 12]. The Gram-positive pathogens show more significant antibacterial activity than Gram-negative pathogens. The metal nanoparticles were used as additive materials for wound/burn dressings to inhibit microbial activity [13, 14], but they showed less efficient than silver nanoparticles, because these materials do not have foldable behavior, which is required for the effective application of wound dressings. In this regard, synthetic polymers scaffold plays a vital role to stand wound dressing due to their high degree of foldable behavior and biocompatibility. In addition, synthetic polymers offer desirable higher mechanical properties than hydrogel materials. Electro-spun scaffolds possess good morphological features, making them excellent material for tissue engineering, and wound healing applications [15, 16].

Recently, PGS was reported a prospective biopolymer in the development of scaffold hybrid materials for biomedical industrial applications due to its unique thermo-mechanical and biological properties [17–20]. The functional groups of PGS polymer have also been approved by the FDA [20–23] and PGS elastomeric polymer has been attracted as a significant biopolymer because of its easy synthesis process. This polymer has higher biodegradability and biocompatibility, and it has identified as a promising polymer material candidate for tissue engineering industrial applications [24–33]. Recently, some studies have found that PGS/PCL composite-based scaffolds showed great cellular response in several biocompatibility testings. However, recent studies also claimed that pristine PGS polymer, with low molecular weights, is not suitable candidate for the electro-spinning fabrication process. The methodology procedure and formation of PGS nanofibers could however be achieved by addition of other polymer materials such as gelatin or poly(3-caprolactone) (PCL) and polymethyl methacrylate (PMMA). PCL and PMMA polymers and their functional groups are FDA approved. These biodegradable hydrophobic polymers have been exhibited poor cell attachment due to their hydrophobicity property. PGS, PCL, and PMMA show unique physical

properties, due to their novel material properties. In addition, we speculate that scaffold coatings by AgNPs, which have significant antibacterial activity, should enhance the antimicrobial activity of fabricated composite scaffold to inhibit bacterial colonization in wound healing applications. Thus, the synthesis or fabrication process of a micron-sized fibrous scaffold having with silver nanoparticles and possessing desirable thermo-mechanical characteristics is of great importance in biomaterial industry [34–44]. Previously, nano-coatings of antibacterial or antimicrobial composite materials, such as metal nanoparticles, and antibiotics on the nano-composite of micro-/nano-fibrous scaffolds have been shown a great potential activity to minimize microbial infections. But the release of nano-coating on the scaffold shows safety concerns, due to their possible toxicity [45–47]. However, microbial infections are common problems associated with medical surgeries and wound healing treatment. It is important to design a scaffold fibrous material with antibacterial agent coating for many tissue engineering and wound healing applications. In this context, we put our focus on investigating the promise of the composite scaffolds with an antibacterial activity, which can better function as tissue engineering skin grafts for wound and burn treatment.

In this work, we fabricated PGS/PCL, PMMA/PCL composite scaffold samples by the electro-spinning process. These nano-composite scaffolds are found to be stable without any further post-processing treatment [33]. The 1:1 ratio of PGS/PCL showed better mechanical properties compared to other reported combinations [48–51]. We coat the scaffolds with silver nanoparticles AgNPs on the surface of fiber scaffolds and measure their thermal, mechanical, and compositional activity. We find the AgNPs coating on the surface of the scaffold enhances their mechanical properties, viscoelastic properties, and thermal stability and acts as desirable antibacterial agents for biomedical applications [52–56].

Methods and characterization

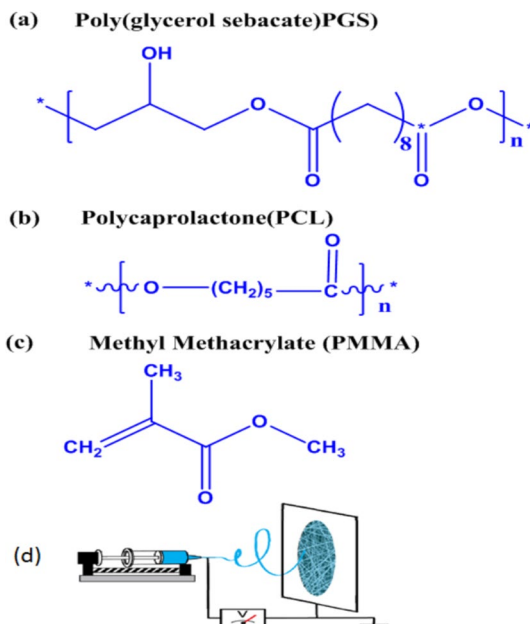
Materials

The chemicals (e.g., PGS, PCL, and PMMA) and solvents (chloroform, ethanol) were purchased from Sigma-Aldrich. Chemical structures of PGS, PCL, and PMMA are shown in Fig. 1. Then the PGS and PCL, PMMA, and PCL blends were dissolved with 1:1 in chloroform: ethanol (9: 1) solution. The concentration of polymer 20% (w/v) is used, and enough time (about 10 h) was allowed for the solutions to mix properly at normal room temperature to get a homogeneous mixture before the electro-spinning process.

Mechanical characterization

The mechanical characteristics were measured using DMA 800 system (TA instrument) at a rate of 1 mm/s, keeping system at room temperature with a 5-N load cell. For the measurements of tensile strength, we used standard ASTM-D 882 technique,

Fig. 1 Molecular structure of **a** PGS, **b** PCL, and **c** PMMA representing the ester bonds and alkaline groups. **d** Schematic of typical electro-spinning design



which has been calibrated with testing of plastic sheets and thickness of less than 0.25 mm.

Thermal analysis

Differential scanning calorimetry (DSC-204 Netzsch) is used to measure heat capacity and the heating/cooling behavior of scaffolds at a heating rate of 3 °C/min.

Thermogravimetric analysis (TGA-209F-Netzsch) is used to measure thermal stability of AgNPs-coated scaffolds under atmospheric N₂ over a temperature range of 27–800 °C at a heating rate of 3 °C/min.

Microstructure characterization

The details of morphology of hybrid composite fibrous scaffold were carried out using the scanning electron microscopy (HR-SEM-Nova Nano 630).

Fourier transform infrared spectroscopy (FTIR)

The chemical compositions and their functional groups in nano-composite fibrous scaffold were recorded using a Fourier transform infrared spectroscopy (FTIR-6700 ranging from 4000 to 400 cm⁻¹).

Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

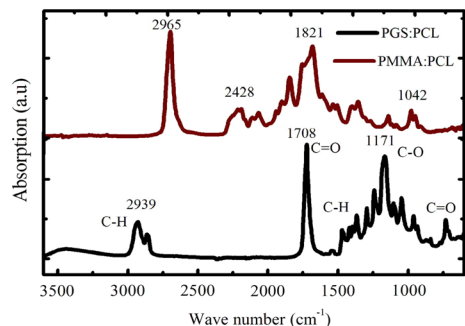
The activity of Ag^+ ions in deionized water (DI) was measured by a Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) to know details of Ag^+ ions release as a part of their antibacterial activity.

Results and discussion

In this work, we used the electro-spinning method (Fig. 1) to make micro-/nano-fibrous scaffold of PGS/PCL (1:1), PMMA/PCL and then coated with 150-nm-thick silver nanoparticles layer (AgNPs) in a controlled method using RF sputtering procedure. The electro-spinning technique is used to make microcomposite scaffolds, and it does not require any further post-processing process. The main challenge during the sputtering of AgNPs coating on the surface of scaffolds is not to increase the substrate temperature (which may likely happen because of the incidence of AgNPs on the scaffold surface, converting the kinetic energy of NPs to heat energy). PCL-based composite scaffolds have a glass transition below 60 °C, and the polymer can melt during the fabrication process if the substrate temperature was not properly controlled. In our procedure, we controlled substrate temperature, which was maintained below 40 °C to avoid any PCL polymer melting. The AgNPs showed better adhesion to the polymer which aids to improve the thermo-mechanical properties of scaffold. The mechanical properties of scaffold materials rely on the micro-structure of the scaffold and AgNPs coating. The benefit of RF sputtering over other techniques is to control the exact thickness of AgNPs coating on the scaffolds. In this study, along with the fabrication of AgNPs coating nano-composite scaffolds of PGS/PCL and PMMA/PCL, we focused on the comparison of their thermo-mechanical properties with other reported nano-composite scaffolds. It is expected that AgNPs coating acts as an antibacterial agent for Band-Aid, elastic heaters, strain gauges, temperature sensors, and flexible electronic applications. However, it is important to study different functional groups on the scaffolds to understand the structure of AgNPs-coated fibrous scaffolds.

The FTIR analysis was probed to study the chemical compositions of fibrous composite scaffolds (Fig. 2). All of the functional groups of PGS and PMMA

Fig. 2 FTIR spectra of the fibrous scaffold of PGS/PCL and PMMA/PCL



polymers overlapped with PCL polymer bands. Two primary bands were assigned at 1723 cm^{-1} corresponding to stretching vibration of carboxyl (C=O) and 1167 cm^{-1} , attributed to stretching vibration of the ether group (C-O) in the spectrum [51]. Other stretching bands were also observed, such as symmetric C-H stretching at 2926 cm^{-1} , C-O , and C-C stretching vibrations at 1295 cm^{-1} . Methyl groups were also stretching [51] C-H at 1360 , 2930 , 2860 cm^{-1} , and the ester band C-O vibration was observed at 1166 , 1723 , 930 , and 1287 cm^{-1} . FTIR revealed that PGS, PCL, and PMMA polymer characteristics were still present, even though polymers were greatly reduced in their microstructure. The scaffold films are thin enough, and their functional characteristics of polymers can still hold surface-active materials.

The surface morphology of both fibrous scaffolds is shown in Fig. 3. The diameter and porosity of the scaffold play a vital role in improving mechanical characteristics of the scaffold. The Ag coatings (150 nm thick) on the surface of scaffold fiber improve mechanical characteristics due to reduction of pore size, increasing diameter, and stronger interfacial interactions between the scaffold fiber and silver nanoparticles, Fig. 4. The diameter of pristine scaffolds was measured as 400 nm using statistical analysis of the normal distribution of SEM images with the help of ImageJ software, Fig. 5. The diameter of pristine fibrous scaffolds increases and pore size decreases after AgNPs coating on scaffolds.

The mechanical characteristics were measured using a fibrous scaffold with and without silver nanoparticles (AgNPs) coating. Both fibrous scaffolds were used for mechanical measurements having rectangular shape and an average thickness of $100\text{--}200\text{ }\mu\text{m}$. In Fig. 6A, the imbedded figure shows the linear region of the stress–strain curve for both scaffolds in $10\text{--}15\%$ strain range. The mechanical characteristics of scaffolds depend on their microstructure parameters such as fiber

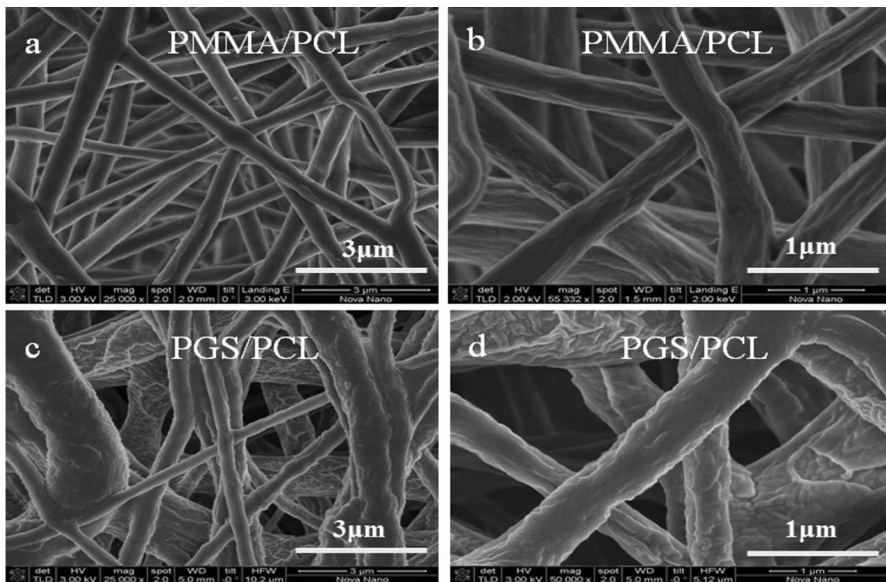


Fig. 3 SEM images of the pristine fibrous of **a, b** PGS/PCL and **c, d** PMMA/PCL scaffolds

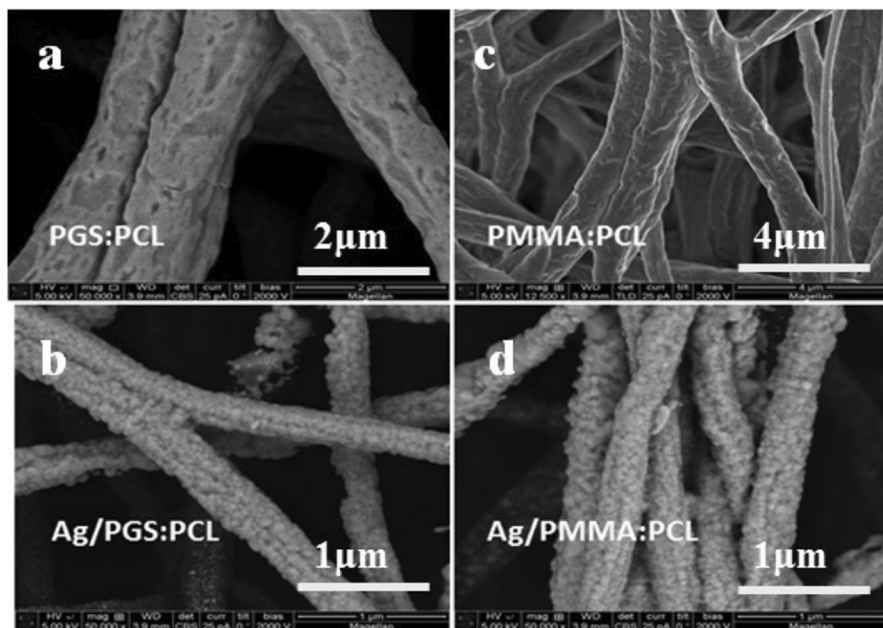


Fig. 4 SEM images of the pristine fibrous **a** PGS/PCL, **c** PMMA/PCL scaffold and AgNPs-coated fibrous scaffolds, **b** 150-nm AgNP-PGS/PCL, **d** 150-nm AgNP-PMMA/PCL

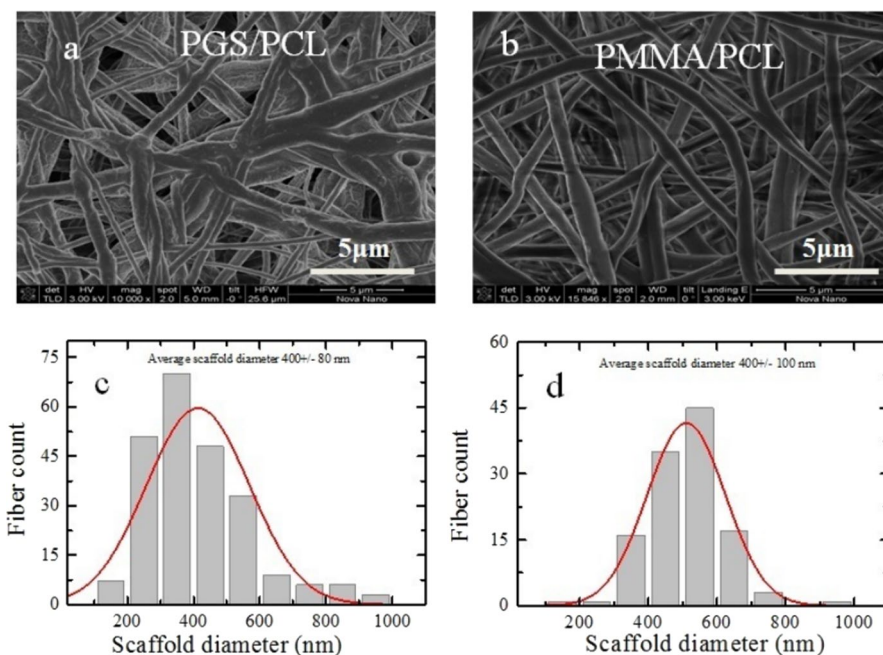


Fig. 5 SEM images of the pristine fibrous **a** PGS/PCL, **b** PMMA/PCL scaffolds. Pristine fibrous scaffold distribution of diameter **c** PGS/PCL and **d** PMMA/PCL

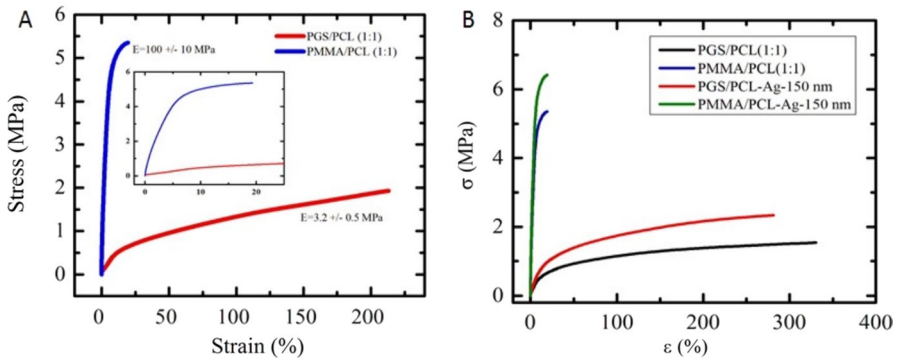


Fig. 6 **A** The tensile stress (σ) versus tensile strain (ϵ) of fibrous scaffold of PGS/PCL and PMMA/PCL, **B** the tensile stress (σ) versus tensile strain (ϵ) of the fibrous scaffold of Ag-coated PGS/PCL and PMMA/PCL

diameter, pore diameter, and outer coating of metal. The tensile modulus of pristine PGS/PCL (1:1) scaffolds showed about 3.3 MPa, whereas PMMA/PCL showed about 100 MPa. The mechanical properties of AgNPs-coated scaffolds increase 3–4 times higher than the pristine scaffolds, Fig. 6B.

The pristine ultimate tensile strength (UTS) of PMMA/PCL scaffold showed approximately 30 times higher compared to the pristine PGS/PCL polymer scaffold. Interestingly, the tensile strain increased about 250% for PGS/PCL scaffold, whereas PMMA/PCL scaffold showed only 25% strain (Fig. 6B). The AgNPs coating on scaffold may help to make brittle scaffold due to stronger interfacial interaction between AgNPs and scaffold fiber. The PMMA/PCL fibrous scaffold showed higher degree of modulus (E), higher UTS, and higher stiffness compared to PGS/PCL fibrous scaffold due to tightly packed morphological structure with higher diameter (Fig. 2C). The morphological stretching of the scaffold fiber after mechanical testing is shown in Fig. 7. The PGS/PCL scaffold fiber showed

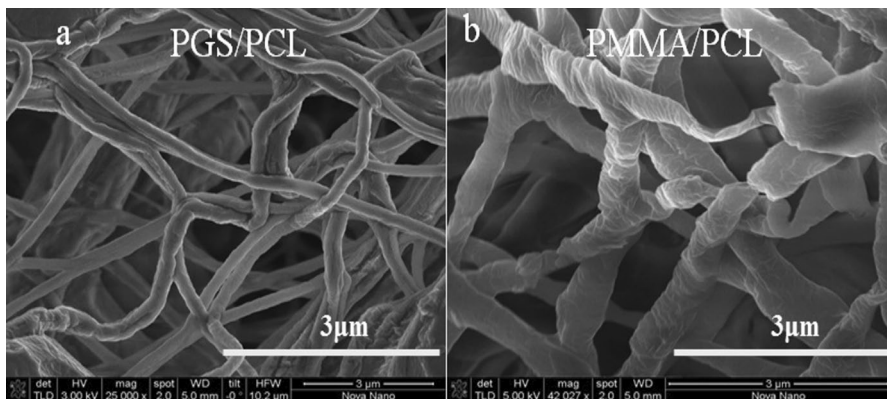


Fig. 7 SEM images of the pristine fibrous PGS/PCL and PMMA/PCL scaffolds after mechanical testing

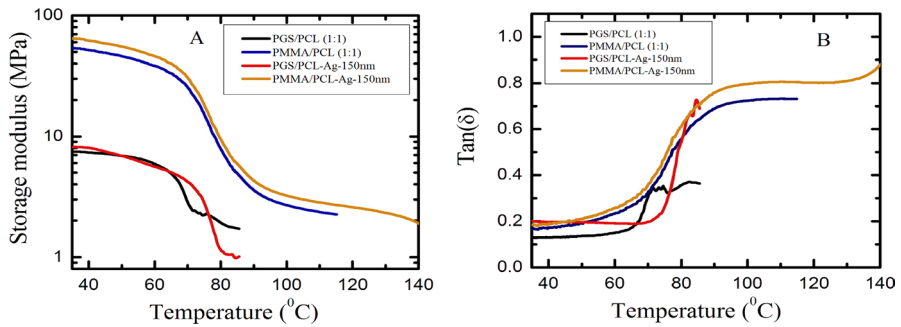
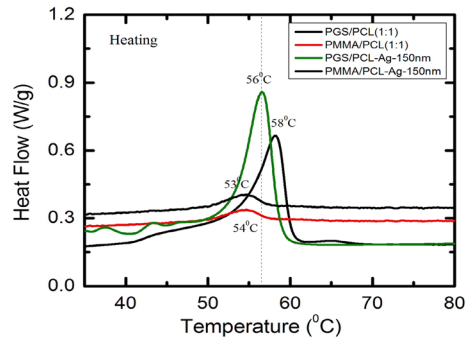


Fig. 8 **A** The storage modulus is a function of temperature measurements for both PGS/PCL and PMMA/PCL scaffolds, **B** damping ratio ($\tan \delta$) versus temperature measurements for both PGS/PCL and PMMA/PCL scaffolds

Fig. 9 Differential scanning calorimetry measurements of pristine and Ag-coated fibrous scaffold of PGS/PCL, PMMA/PCL



a twisting and stretching behavior after mechanical stretching, whereas PMMA/PCL scaffold showed lower twisting and tape-type stretching behavior. As a result, one should note that PMMA/PCL scaffold is a good material for expanded wound healing applications. We speculate that the primary cause behind scaffolds greatly improved mechanical properties after AgNPs coating is the increased friction between the fibers. It might also be due to the interlocking of fibers, which avoids any slippage, and increases the friction between adjacent fibers which is observed more on the surface of fiber.

The Ag coating induced restricted thermal motion of micro-scaffold fiber through the altering melting and glass transition temperature of fiber. We assessed the melting transition temperature and their glass transition of both fibrous scaffolds using DMA technique (Fig. 8). The glass transition temperature of both pristine fibrous scaffolds showed a broader glass transition temperature 60–90 °C. The storage modulus (E') of PMMA/PCL & Ag-PMMA/PCL showed much higher values than PGS/PCL & Ag-PGS/PCL fibrous scaffold (Fig. 8A) due to the heat transfer between the fibers and the AgNPs coating. Both pristine scaffold samples started fracturing at a lower temperature due to the melting of fibers, whereas the mechanical fracture of Ag-coated scaffold was shifted

Fig. 10 Thermogravimetric analysis (TGA) measurement was taken for both pristine PGS/PCL, PMMA/PCL scaffolds and Ag-coated PGS/PCL, PMMA/PCL scaffolds under nitrogen atmosphere. The mass loss in fibers associated with burning fibrous scaffolds was reduced in Ag-coated scaffolds

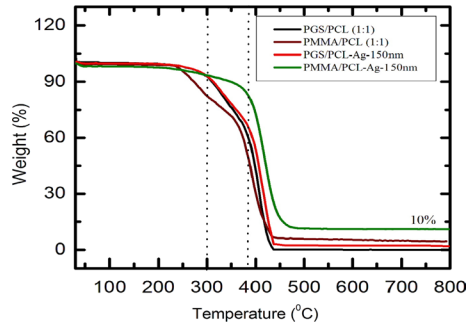
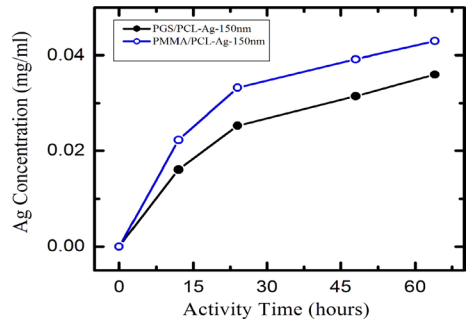


Fig. 11 Cumulative release of Ag⁺ ion concentration of Ag-coated fibrous PGS/PCL and PMMA/PCL scaffolds in DI water as a function of time using ICP-OES



to a higher temperature, suggesting that Ag acted as a strong antibacterial agent (mechanical integrity) to the pristine scaffold. The Ag coating restricts the fiber motion, leading to increased stiffness of the scaffolds, resulting in their mechanical reinforcement.

The origin of the mechanical improvement of fibrous scaffolds and Ag-coated scaffolds was assessed using differential scanning calorimetry (DSC). We examined scaffold crystalline behavior and stiffness, both of which would lead to enhancing modulus (E) and lower melting temperature (T_m). Our analysis showed a sharp melting peak at 58 °C (Fig. 9), and the melting temperature for both scaffolds showed a reduction in the temperature of about 2 °C. The endothermic peak slightly increased for Ag-coated fibrous scaffold which suggests an increased crystalline behavior due to the restricted thermal motion of microfibers and micro-/nano-confinement of Ag coating. The glass transition temperature of the micro-fibrous scaffold was broad and close to the melting temperature from DSC and was analyzed from DMA measurements.

We also examined both fibrous scaffold's thermal stability using TGA technique shown in Fig. 10. The mass degradation of fiber in Ag-coated fibrous scaffold associated with burning off was reduced. The mass degradation of Ag-coated scaffolds was probably decreased because of heat transfer from silver nanoparticles to fibers which led to stronger interfacial interaction. The mass loss of scaffolds showed two regimes which might be because of the degradation of the temperature of two polymers that phase separate before they degrade completely. The thermal stability of both Ag-coated scaffolds was seen to be improved about 10 °C. The Ag-coated

sample left 10% weight even at 800 °C, and it might be due to the nano-confinement of Ag coating on micro-scaffold composites.

We emphasize that silver nanoparticles are a potential candidate for wound healing due to their antibacterial activity. The concentration of Ag^+ ions releases from the scaffolds needed to be identified. The release of Ag^+ ions from Ag-coated fiber scaffolds was investigated by the total immersion method in DI water media to investigate the antibacterial activity. The Ag-coated scaffolds were in DI water (2 ml) for several days before that concentration of Ag^+ ions was measured by ICP-OES (Fig. 11). The release profile of Ag^+ ion for both Ag-coated scaffolds was found to show similar trends (Fig. 10). We estimated that approximately 5–10% of AgNPs were released even after 3 days. The amount of AgNPs coating on the fiber surface was observed to be significantly high even after 3 days of activity, and we compared the coating levels before studying their activity. We suggest that the controlled release of Ag^+ coating from both scaffolds prevents possible toxicity while processing antibacterial activity on these scaffolds. Therefore, we highlighted Ag-coated scaffolds hold great potential promise in many biomedical applications such as a wound healing dressings, surgical meshes, and antibacterial sutures.

Conclusion

In this work, we fabricated silver nanoparticles (AgNPs) and coated composite fibrous scaffolds of PGS/PCL and PMMA/PCL using the electro-spinning technique and RF sputtering. By utilizing RF coating method, we successfully fabricated uniform coating on the surface of the electro-spun blend of PGS/PCL and PMMA/PCL polymers. The FTIR technique presented that the PGS, PCL, and PMMA polymer features in the composite scaffold were not entirely lost although the structure was greatly changed. This indicates the polymer scaffold does not change chemical characteristics and surface activity. The mechanical characteristics of scaffolds were improved probably because of the increased friction between fibers after Ag coating. We believe that the reason for the enhancement in mechanical characteristics by adding such nano-coating is due to the intermeshing of fibers. The coating prevents slippage and increases frictional forces between adjoining fibers on top of each other. We speculate that these fibers lost less mass due to the heat transfer of Ag^+ coating, which improved the thermal stability of scaffolds. We used AgNPs coating on the scaffolds to enhance the antibacterial activity and control the release of Ag^+ ions on the surface of the scaffolds, observing that the release of Ag^+ ions was steady with the time and did not vary for any of the AgNPs-coated scaffolds. We highlighted that the AgNPs coating on the surface of scaffolds provides potential applications such as surgical meshes, bandages, and possible antibacterial activity in wound healing and, finally, emphasize that many metal nanoparticles were used as additive materials for wound dressing to inhibit microbial activity, but they were less efficient than silver nanoparticles, because these materials do not have foldable behavior which is required for effective applications of wound dressings. In this regard, AgNPs coating on the surface of PGS/PCL, PMMA/PCL composites scaffolds provides higher mechanical properties with desirable biomedical applications.

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Declarations

Conflict of interest There are no conflicts to declare.

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Authors and Affiliations

Parvathalu Kalakonda¹ · Shalini Thudumu¹ · Soujanya Laxmi Mynepalli¹ · Rajitha Kathi¹ · Vasudeva reddy Yatham¹ · Pritam Mandal⁴ · Sreenivas Banne³ · Pranay Bhaskar Kalakonda⁶ · Murali Banavoth² · Moses Kigozi⁵ · Bala Bhaskar Podili¹

✉ Parvathalu Kalakonda
parvathalu.k@gmail.com; parvathalu@wpi.edu; pkalakon@andrew.cmu.edu

¹ Department of Physics, Smart Nanomaterial Research Laboratory, Government City PG College (A), Osmania University, Hyderabad 500002, India

² School of Chemistry, University of Hyderabad, Hyderabad, India 500046

³ Department of Chemistry and Biosciences, Rice University-BRC, Houston, TX 77005, USA

⁴ Department of Physics, Michigan Technological University, Houghton, MI 4993, USA

⁵ Department of Chemistry, Faculty of Science and Education, Busitema University, Kampala, Uganda

⁶ Department of Materials Science and Engineering, North Carolina State University, Raleigh, NC 27695, USA