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Polydopamine-modified Collagen Sponge for Improved Anti-inflammatory and Tissue Repair

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Electronic Supplementary Information

Abstract Vascular closure devices (VCDs) play a critical role in preventing bleeding and hematoma formation after percutaneous interventions. Despite effective mechanical hemostasis, the current collagen-based VCDs lack anti-inflammatory and regenerative capabilities. Herein, we report the development of a polydopamine (PDA)-modified collagen sponge (Col@PDA) to enhance the anti-inflammatory and pro-regenerative potential of collagen-based materials. PDA modification not only preserved the sponges' inherent porosity and swelling behavior but also endowed them with immunomodulatory functions. *In vitro*, the modified sponges exhibited superior anti-inflammatory and antioxidant activities in RAW 264.7 macrophages and L929 fibroblasts. In a rat subcutaneous implantation model, Col@PDA attenuated local inflammation, promoted dermal regeneration, and enhanced collagen deposition and angiogenic factor expression. Collectively, PDA modification enables collagen sponges to actively modulate the healing microenvironment, resulting in faster tissue repair than unmodified collagen sponges.

Keywords Vascular closure device; Collagen sponge; Polydopamine; Anti-inflammatory; Tissue repair

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INTRODUCTION

Vascular puncture is an indispensable component in percutaneous interventional procedures.[1–3] Vascular closure devices (VCDs) have become standard tools to mitigate postoperative complications, such as bleeding, pseudoaneurysm, and hematoma[2,3] and their clinical efficacy has been widely validated.[4–6] Current VCDs predominantly utilize biodegradable materials, including collagen,[7] poly(ethylene glycol),[8] and poly(glycolic acid).[9] Among these, collagen sponges are widely employed because of their favorable biocompatibility and platelet-activating properties,[10–15] which enable rapid local thrombus formation and effective mechanical hemostasis. Nonetheless, their capacity to regulate local inflammation and promote tissue repair remains limited and needs further improvement.[16,17]

Polyphenolic compounds, such as epigallocatechin-3-gallate (EGCG), tannic acid, and dopamine, have been reported to possess potent anti-inflammatory activity.[18–22] Polydopamine (PDA), which is formed *via* oxidative self-polymerization of dopamine, has been widely employed for the surface functionalization of biomaterials. This widespread application is attributed to several key features: its catechol-quinone redox cycle scavenges reactive oxygen species (ROS), mitigating oxidative stress and suppressing inflammation;[23–27] it downregulates pro-inflammatory cytokines while upregulating angiogenic factors, including vascular endothelial growth factor (VEGF), thereby accelerating tissue repair;[24,28–30] it promotes platelet adhesion and activation, enhancing coagulation;[28,31–33] and its abundant reactive groups and strong surface adhesiveness enable further multifunctional modifications.[34–36] In this study, we functionalized collagen sponges with polydopamine (PDA) to confer anti-inflammatory and proregenerative properties.

The resulting PDA-modified collagen sponges (Col@PDA) were prepared *via* immersion and characterized for morphology, physicochemical, coagulation, antioxidant, and anti-inflammatory properties. Finally, the material was evaluated in a rat subcutaneous implantation model to assess its *in vivo* anti-inflammatory activity and tissue-regenerative efficacy

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(Scheme 1).

EXPERIMENTAL

Polydopamine-modified collagen sponges were fabricated using the dip-coating method. Briefly, native collagen sponges were immersed in PDA solutions of varying concentrations (0.1, 0.3, and 0.5 mg/mL), prepared by dissolving dopamine hydrochloride in 10 mmol/L Tris-HCl buffer (pH=8.5).[37] The mixture was magnetically stirred for 10 h at room temperature to allow for oxidative self-polymerization of dopamine on the collagen surface. Upon completion of the reaction, the samples were retrieved and rinsed three times with deionized water to remove any unreacted PDA monomers. Subsequently, the samples were flash-frozen in liquid nitrogen and placed in a freeze-dryer for 24 h. The final products, collagen sponges modified with different concentrations of PDA, were labeled as "Col," "Col@PDA1", "Col@PDA3", and "Col@PDA5", corresponding to the initial PDA concentrations.

RESULTS AND DISCUSSION

Preparation and Physicochemical Characterization of PDA-modified Sponges

Polydopamine-modified collagen sponges (Col@PDA) were successfully fabricated using a dip coating strategy. Native collagen sponges were immersed in PDA solutions of varying concentrations for 10 h, followed by thorough rinsing with deionized water to remove unreacted monomers and subsequent lyophilization. Macroscopic observation revealed a distinct color change upon modification. The sponges exhibited a characteristic brown hue that intensified with increasing PDA concentration (Fig. 1a), preliminarily confirming the successful deposition of PDA onto the collagen matrix.

To evaluate the functional recovery of the materials, their swelling behavior was assessed. Upon compression and immersion in liquid, both the native and PDA-modified sponges rapidly expanded and swiftly recovered their original morphology (Fig. 1b, Video S1 in the electronic supplementary information, ESI). This rapid fluid uptake is critical for vascular closure, ensuring immediate contact with blood at the puncture site. Scanning electron microscopy (SEM) analysis further confirmed that the PDA coating did not compromise the intrinsic porous network structure of the collagen sponge (Fig. 1c).

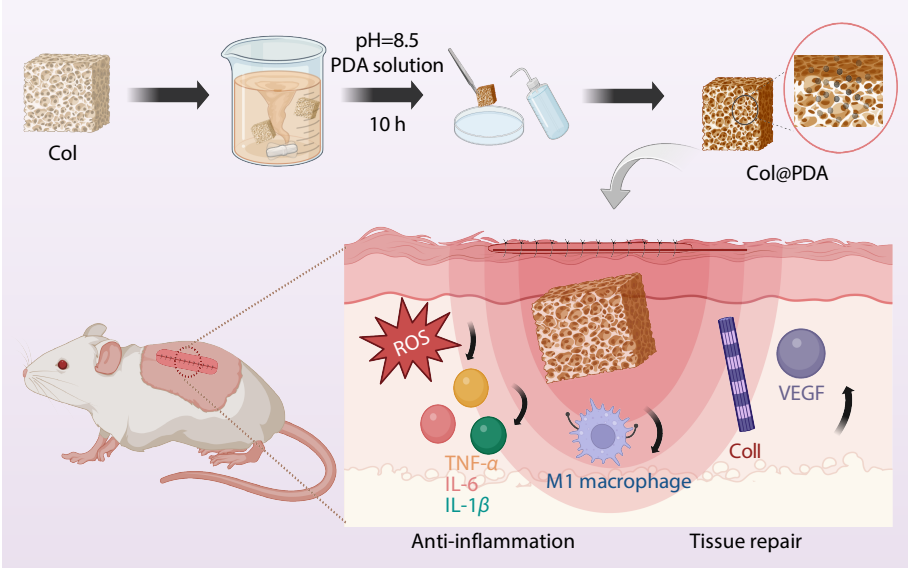
A comprehensive physicochemical characterization was conducted to assess the impact of the modification on the material properties. The volume expansion ratio and liquid absorption tests (Figs. 1d and 1e) indicate that the PDA modification did not significantly alter the liquid absorption capacity. The materials retained their rapid liquid absorption and expansion capabilities, which are prerequisites for effective hemostasis upon blood contact. Porosity measurements *via* ethanol displacement (Fig. 1f) show no statistically significant difference between the Col@PDA groups and the native collagen control, suggesting that the PDA layer was sufficiently thin to prevent pore blockage. Furthermore, mechanical testing (Fig. 1g and Fig. S1 in ESI) demonstrates that both the tensile and compressive strengths remained stable following the modification.

Collectively, these results confirm that the PDA functionalization strategy successfully imparts new bioactive properties while preserving the essential porous architecture, swelling kinetics, and mechanical stability required for vascular sealing applications, thereby laying a solid physicochemical foundation for subsequent biological evaluation.

Evaluation of Hemostatic Performance

Effective hemostasis is a prerequisite for successful tissue repair, particularly in arterial puncture scenarios characterized by high pressure and rapid blood flow. We systematically evaluated the coagulation efficacy of the materials using whole blood clotting time, blood cell adhesion assays, Blood Clotting Index (BCI), and coagulation profile tests (Activated Partial Thromboplastin Time (APTT), Prothrombin Time (PT), and Thrombin Time (TT)).

Whole blood clotting time assays (Fig. 2a) revealed a significant concentration-dependent reduction in clotting time for



Scheme 1 The preparation process and mechanism of action of Col@PDA.

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Col@PDA compared to Col. Notably, the Col@PDA5 group achieved effective hemostasis within 2 min, whereas the native collagen group required approximately 7 min. This improvement was further quantified by measuring the clotting time across groups (Fig. 2b), confirming that PDA modification substantially accelerates the coagulation process. Correspondingly, the Blood Clotting Index (BCI) was significantly lower in the Col@PDA groups (Figs. 2c and 2d), corroborating their enhanced coagulation efficiencies. SEM imaging (Figs. S3a and S3b in ESI) showed an increase in the adhesion of platelets and red blood cells on the surface of the Col@PDA sponges. Coagulation pathway analysis (Fig. 2e) revealed the specific mechanisms involved. While APTT showed no significant difference between groups, indicating minimal impact on the intrinsic pathway, PT and TT were significantly shortened in the Col@PDA5 group compared to the native collagen group. This reduction in PT and TT suggests that the procoagulant effect of PDA modification is mainly attributed to the enhancement of the extrinsic pathway and the accelerated formation of fibrin. The accelerated PT and TT may be at-

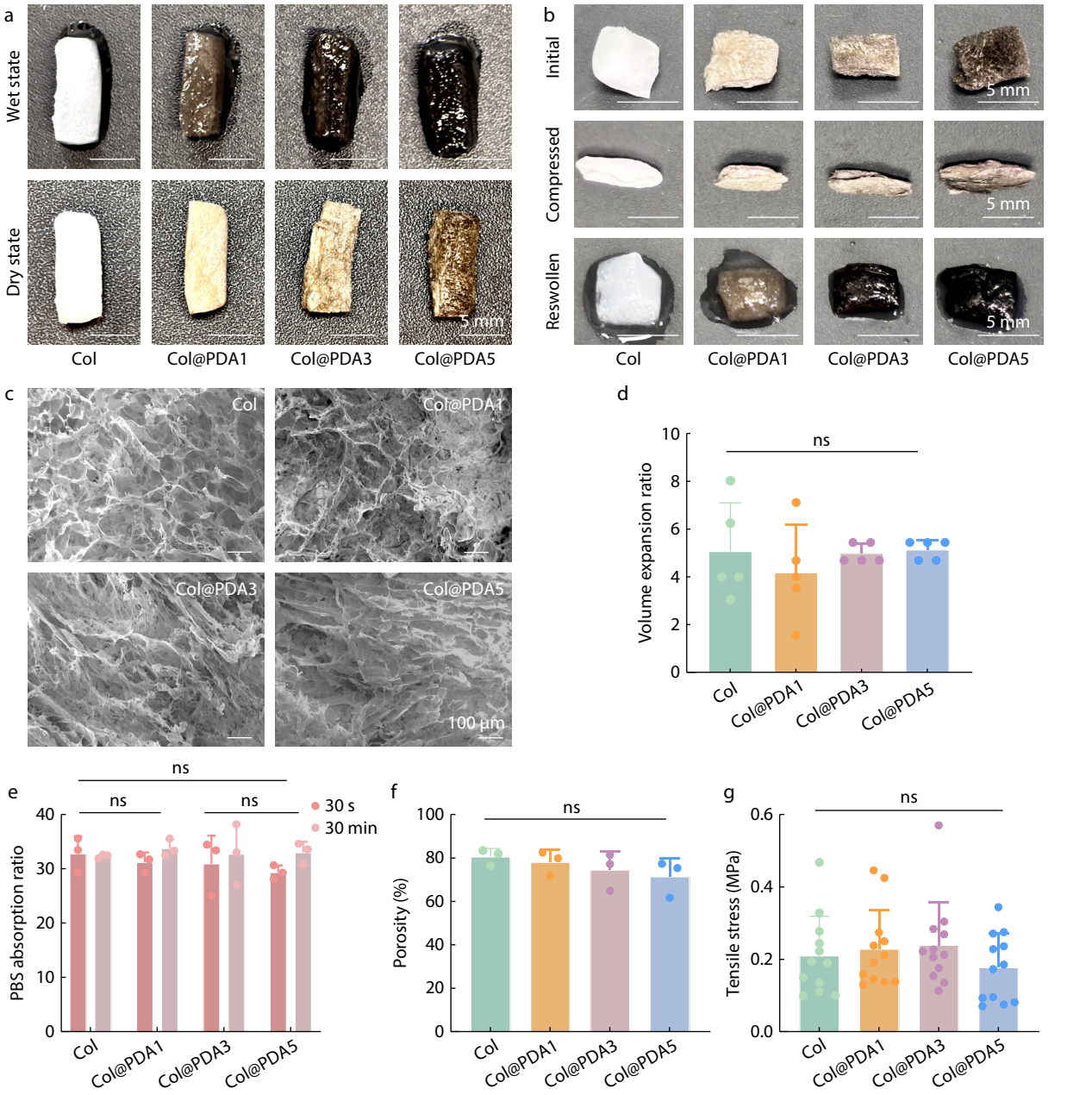

Fig. 1 Structural and physicochemical characterization of Col and Col@PDA Sponges. (a) Appearance of sponges modified with different concentrations of polydopamine after reaction and freeze-drying (All the scales are 5 mm.); (b) Morphology of Col and Col@PDA sponges in their initial, compressed and reswollen size (All the scales are 5 mm.); (c) SEM images of the internal structure of Col and Col@PDA sponges (All the scales are 100 μm.); (d) Volume expansion ratio test, n=5; (e) Instantaneous and saturated absorption ratio test; (f) Porosity of sponges; (g) Tensile strength test, n=12. ns, not significant.

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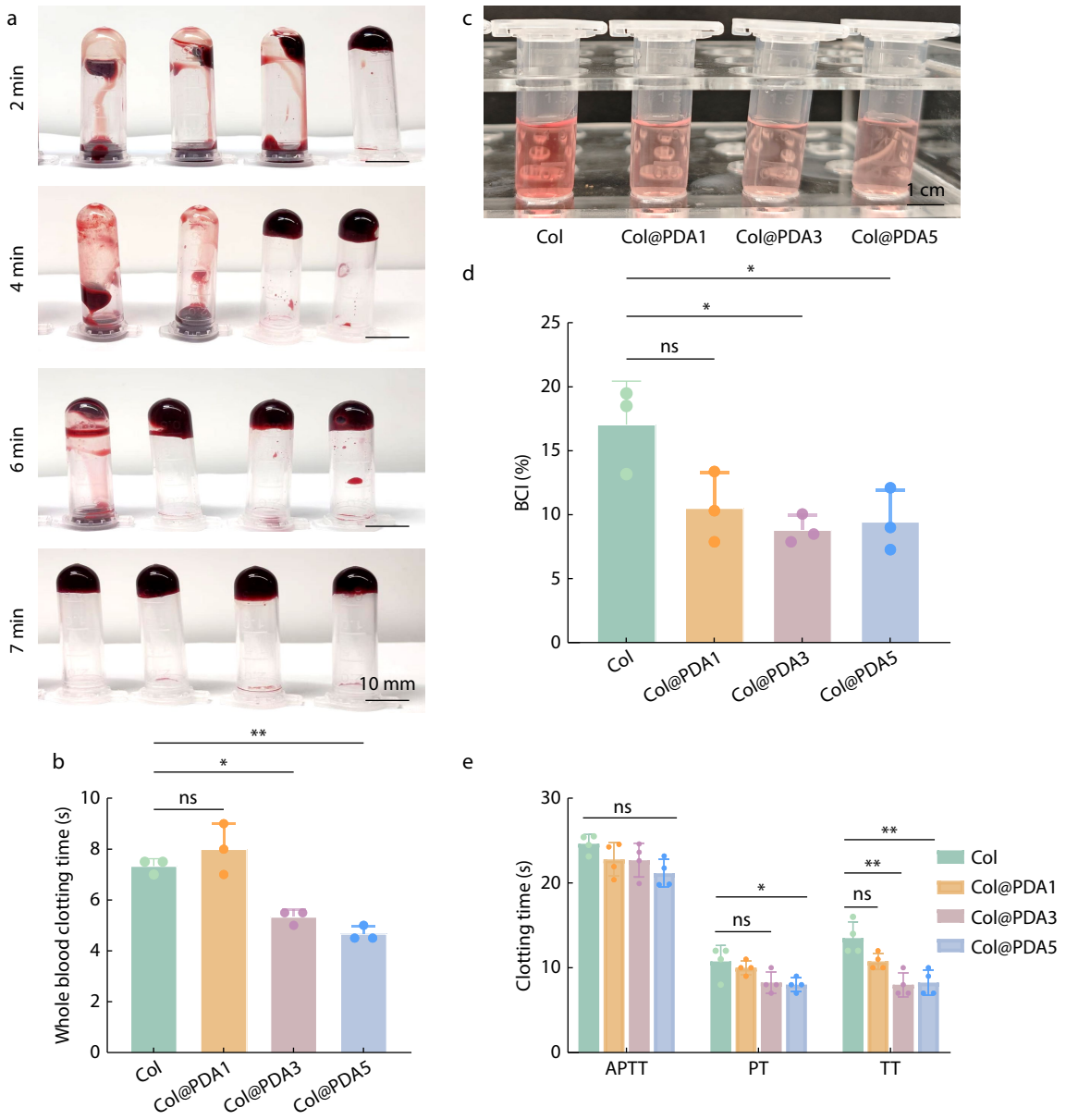

Fig. 2 Hemostatic properties of Col and Col@PDA sponges. (a) Schematic diagram of the whole blood clotting time test (All the scales are 10 mm.); (b) Whole blood clotting time of Col and Col@PDA sponges; (c) Schematic diagram of the whole blood clotting index test; (d) Whole blood clotting index of Col and Col@PDA sponges; (e) APTT, PT, TT tests, n=4. *P<0.033, **P<0.002. ns, not significant.

tributed to the strong protein adsorption capacity of the PDA surface and the increased surface roughness formed by the PDA oligomers, which promote the adsorption and conformational conversion of fibrinogen and other thrombus-related proteins.[38] In summary, PDA modification significantly enhances the pro-coagulant performance of collagen sponges by promoting cell adhesion, activating platelets, and accelerating the extrinsic coagulation cascade.

Assessment of Antioxidant Performance

Excessive accumulation of reactive oxygen species (ROS) in the wound microenvironment can inhibit cell proliferation and angiogenesis, induce persistent inflammation, and impede healing. The catechol structure of polydopamine endows the material with inherent free radical scavenging potential, thereby reducing local oxidative stress and laying the foundation for subsequent resolution of inflammation and tissue repair. We validated the antioxidant capacity of Col@PDA at both molecular and cellular levels. At the molecular level, ABTS radical scavenging assays (Figs. 3a and 3b) demonstrated that the ROS scavenging capacity of the Col@PDA sponges improved with increasing PDA concentration. The Col@PDA5 group achieved an ROS scavenging rate of up to 90.35%. UV-Vis spectroscopy of the supernatant (Fig. 3c) revealed a concentration-dependent decrease in the absorbance at 734 nm, confirming the excellent small-molecule antioxidant activity of the modified sponges. At the cellular level, we evaluated intracellular antioxidant performance using RAW 264.7 macrophages and L929 fibroblasts. Oxidative stress was induced using lipopolysaccharide (LPS) and

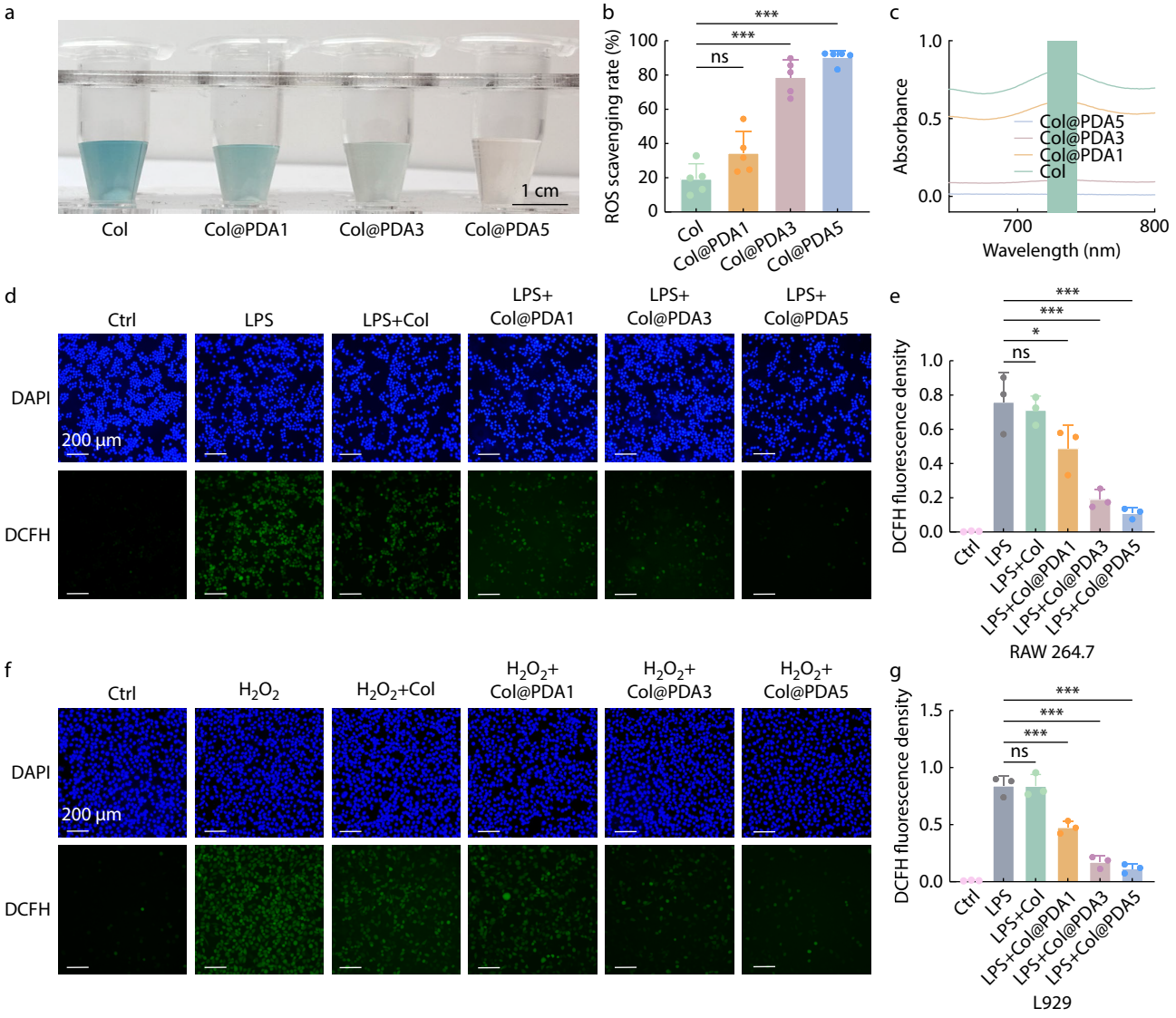

Fig. 3 Antioxidant properties of Col and Col@PDA sponges. (a) Schematic diagram of the ABTS assay for Col and Col@PDA sponges; (b) ABTS assay, n=5; (c) UV-Vis spectra of the supernatant from the ABTS assay; (d) Fluorescence images of DCFH and Hoechst staining captured by an inverted fluorescence microscope showing ROS scavenging in RAW 264.7 cells (All scales are 200 μm.); (e) Relative DCFH fluorescence intensity in RAW 264.7 cells treated with Col and Col@PDA sponges; (f) Fluorescence images of DCFH and Hoechst staining captured by an inverted fluorescence microscope showing ROS scavenging in L929 cells (All scales are 200 μm.); (g) Relative DCFH fluorescence intensity in L929 cells. *P<0.033, ***P<0.001. ns, not significant.

hydrogen peroxide, followed by staining with Hoechst (nuclei) and DCFH-DA (ROS). Fluorescence microscopy images (Figs. 3d and 3f) showed a visible reduction in intracellular green fluorescence with increasing PDA concentrations. Quantitative analysis of fluorescence intensity (Figs. 3e and 3g) confirmed that Col@PDA sponges significantly reduced intracellular ROS levels in both cell lines compared with the native collagen control. These results indicate that PDA modification effectively mitigated oxidative stress within the cellular microenvironment, creating favorable conditions for tissue regeneration.

Evaluation of Anti-inflammatory Performance

Excessive accumulation of inflammatory cells and cytokines in the wound microenvironment is a critical factor that delays healing. Therefore, the modulation of the inflammatory response is essential for tissue repair. We assessed the anti-inflammatory efficacy of Col@PDA by co-culturing RAW 264.7 macrophages with the materials under LPS stimulation to induce M1 phenotype polarization. Supernatants were analyzed *via* ELISA for pro-inflammatory cytokines, and cells were analyzed *via* flow cytometry for polarization markers (Fig. 4a). ELISA results (Figs. 4b–4d) showed that the levels of pro-inflammatory cytokines, including TNF-α, IL-6, and IL-1β, were significantly reduced in the supernatants of cells cultured with Col@PDA compared to Col., indicating that Col@PDA effectively suppressed the release of inflammatory mediators. Given that M1 macrophage polarization is associated with the secretion of high levels of proinflammatory cytokines and exacerbated tissue damage, we further examined the expression of CD86, a canonical marker of the M1 phenotype. Flow cytometry analy-

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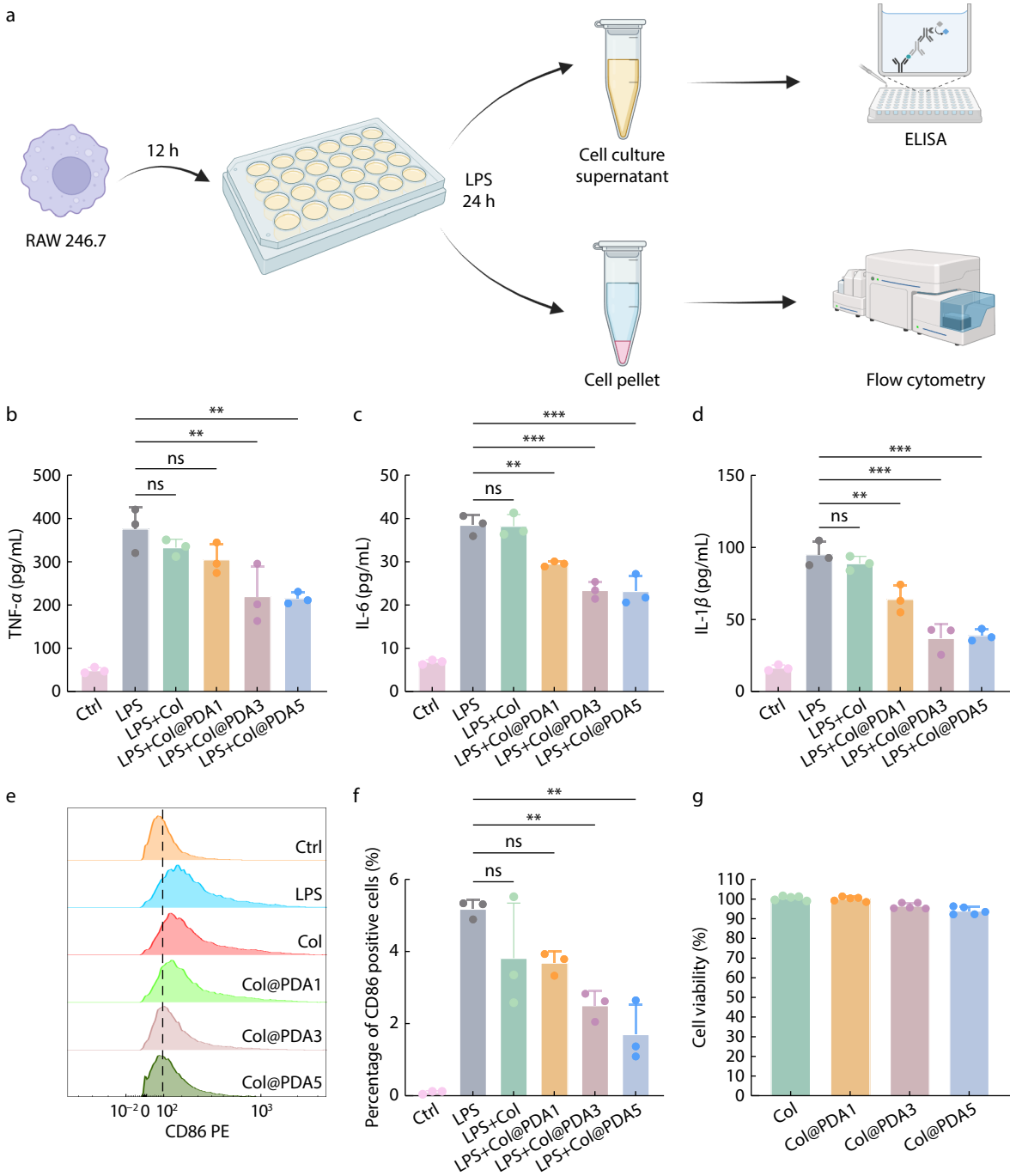


Fig. 4 Anti-inflammatory properties of Col and Col@PDA sponges. (a) Schematic diagram of the experimental procedure for evaluating the anti-inflammatory property; (b–d) Levels of inflammatory cytokines by ELISA tests; (e) Flow cytometry plots showing the expression of the M1 macrophage polarization marker CD86; (f) Percentage of CD86-positive cells measured by flow cytometry for the M1 macrophage polarization assay; (g) Cytocompatibility assay, n=5. $^{**}P$<0.002, $^{***}P$<0.001. ns, not significant.

sis (Figs. 4e and 4f) revealed that the proportion of CD86-positive cells decreased with increasing PDA concentrations. We assessed the cytocompatibility of the collagen sponges and PDA-modified collagen sponges at different PDA concentrations to determine whether they induced cytotoxicity or compromised biosafety. As shown in Fig. 4(g), the cell viability of all groups exceeded 90%, indicating that the PDA-modified collagen sponges had good cytocompatibility. These findings suggest that PDA modification not only directly scavenges ROS but also actively modulates the immune response by downregulating pro-inflammatory cytokines and inhibiting M1 macrophage polarization. This immunomodulatory capability reduces local inflammatory reactions and demonstrates a significant potential for promoting tissue repair.

In vivo Tissue Repair

To validate the therapeutic efficacy of Col@PDA in a physiologi-

cal context, we employed a rat subcutaneous implantation model to assess the resolution of inflammation and tissue repair capabilities. Macroscopic observation of the wounds at 1, 3, and 7 days postoperatively (Fig. S4 in ESI), and histological analysis *via* hematoxylin-eosin staining (H&E) (Fig. 5a) revealed that the Col@PDA groups exhibited significantly reduced inflammatory infiltration and improved healing morphology compared to the Col group.

Quantitative analysis of dermal thickness *via* H&E staining (Fig. 5b) showed that the dermal layer in the Col@PDA group regenerated more rapidly. By day 7, the rate of dermal thickening in the Col@PDA5 group was 22.6% higher than that in the control group. Masson's trichrome staining (Fig. 5c) further demonstrated a significant increase in collagen deposition in the Col@PDA group. Statistical analysis of collagen deposition ratios (Fig. 5d) indicated that by day 7, the Col@PDA5 group exhibited an 18.9% higher increase in collagen deposition compared to the control. These histological findings preliminarily confirmed that PDA-modified collagen sponges alleviated local inflammation and accelerated wound closure.

Immunofluorescence staining was performed to quantify local inflammatory factor levels on day 3. Visual observation (Figs. 6a and 6c) and quantitative analysis (Figs. 6b and 6d) confirmed that the inflammation levels in the Col@PDA groups were lower than those in the unmodified group. Specifically, the fluorescence intensity of TNF-*α* and IL-6 decreased with increasing PDA concentrations, with the Col@PDA5 group exhibiting the lowest expression levels. This

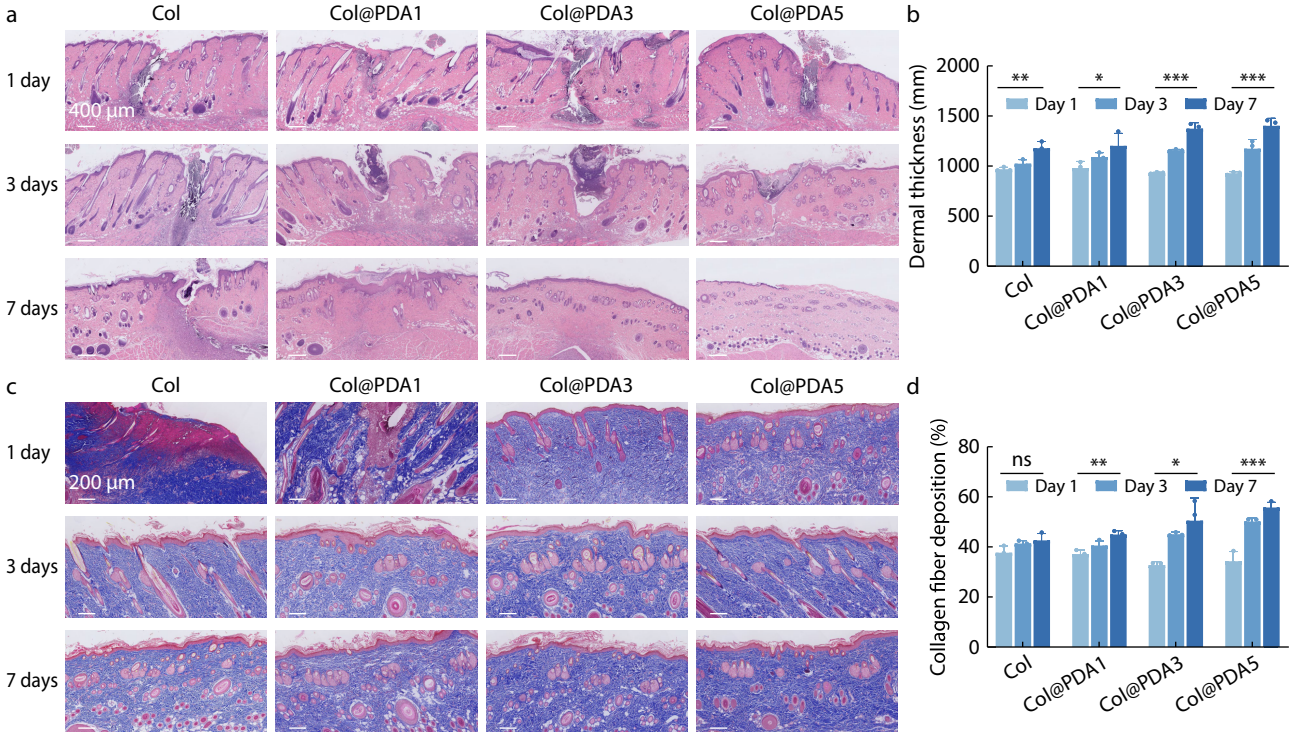
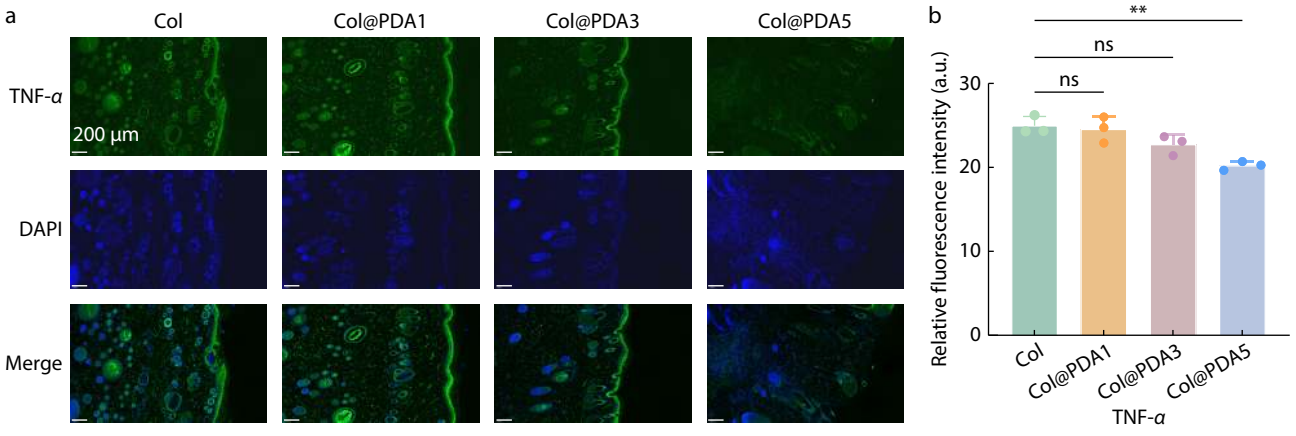


Fig. 5 *In vivo* anti-inflammatory and tissue repair properties of Col and Col@PDA sponges. (a) Hematoxylin and eosin (H&E) stained sections of the wound site on days 1, 3, and 7 post-implantations (All the scales are 400 μm.); (b) Quantification of dermal thickness on day 1, 3, 7; (c) Masson's trichrome-stained sections of the wound site on days 1, 3, and 7 post-implantations (All the scales are 200 μm.); (d) Quantification of collagen deposition ratio on day 1, 3, 7. **P*<0.033, ***P*<0.002, ****P*<0.001. ns, not significant.



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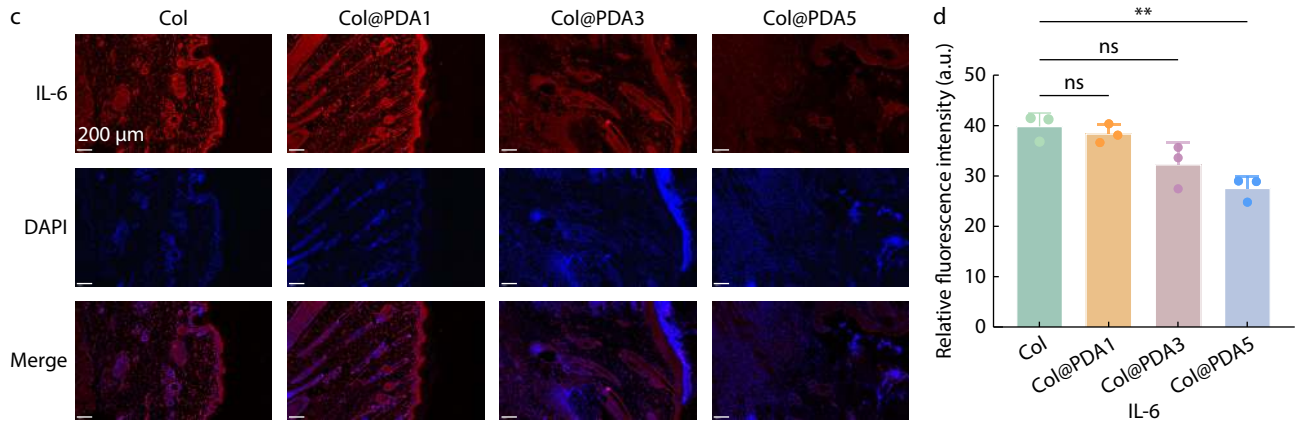

Fig. 6 Immunofluorescence staining of inflammatory factors on day 3 post-implantation in rats. (a) Immunofluorescence staining of TNF-*α* and DAPI in skin wound tissue (All the scales are 200 μm.); (b) Quantification of TNF-*α* fluorescence intensity; (c) Immunofluorescence staining of IL-6 and DAPI in skin wound tissue (All the scales are 200 μm.); (d) Quantification of IL-6 fluorescence intensity. ***P*<0.002. ns, not significant.

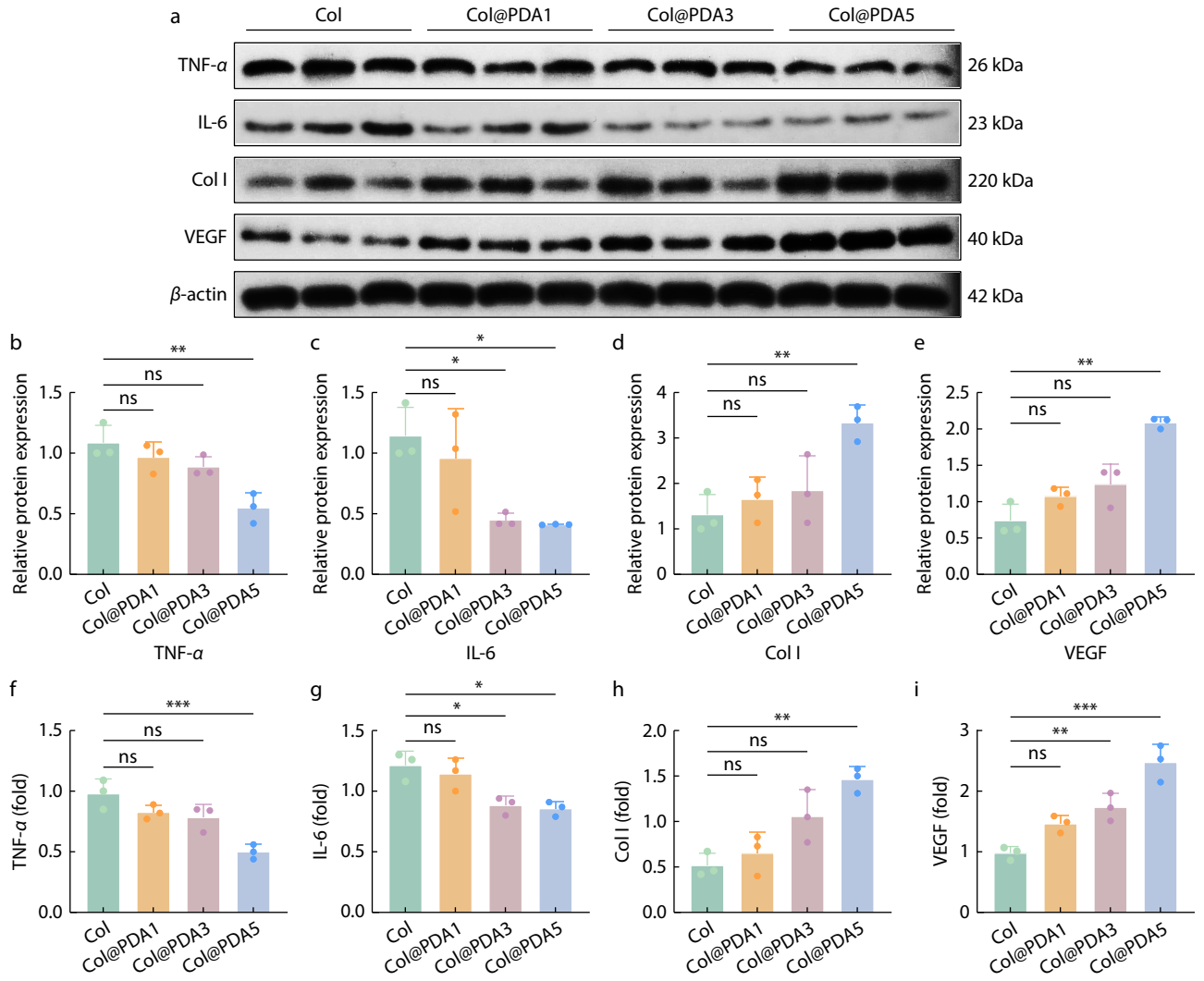

Fig. 7 WB and qPCR analysis of rat subcutaneous implants. (a) Representative Western blotting images for each experimental group; (b–e) Western blotting analysis of inflammatory cytokine and tissue repair marker levels in each experimental group; (f–i) qPCR analysis of inflammatory cytokine and tissue repair marker levels in each experimental group. **P*<0.033, ***P*<0.002, ****P*<0.001. ns, not significant.

confirmed the excellent *in vivo* anti-inflammatory efficacy of the modified materials. Collectively, the speed of tissue repair was approximately doubled, and the tissue healing time was reduced by approximately 50% compared with unmodified collagen sponges.

Molecular biological analyses (Western blot (WB) and Quantitative real-time polymerase chain reaction (qPCR)) provided further mechanistic insights. The protein and mRNA expression levels of pro-inflammatory factors (TNF-*α* and IL-6) were downregulated in the PDA-modified groups compared to the controls (Figs. 7a, 7b, 7c, 7f and 7g), indicating sustained anti-inflammatory performance *in vivo*. The upregulation of VEGF stimulates endothelial cell proliferation and migration, driving angiogenesis, whereas increased Col I expression directly facilitates extracellular matrix reconstruction. The expression of pro-healing indicators including Vascular Endothelial Growth Factor (VEGF) and Type I Collagen (Col I) was significantly upregulated (Figs. 7d, 7e, 7h and 7i). Collectively, these *in vivo* results demonstrated that PDA-modified collagen sponges accelerate tissue repair by mitigating inflammatory responses, promoting angiogenesis, and enhancing collagen deposition. These findings highlight the broad application prospects of Col@PDA in vascular puncture site repair.

CONCLUSIONS

In summary, we have designed a polydopamine-modified collagen sponge (Col@PDA) with enhanced anti-inflammatory and regenerative properties. Comprehensive characterization confirmed that the PDA coating substantially increased the bioactivity of the collagen matrix, while preserving its inherent porosity and liquid absorption capacity. *In vitro* assays showed that PDA coating, *via* its potent reactive oxygen species (ROS) scavenging activity, mitigates cellular oxidative stress, suppresses M1 macrophage polarization, and downregulates pro-inflammatory cytokines such as TNF-*α*, IL-6, and IL-1*β*. An *in vivo* evaluation using a rat subcutaneous implantation model revealed that Col@PDA significantly reduced inflammatory infiltration. It also promotes dermal thickening and collagen deposition while increasing VEGF and Type I collagen expression. These combined effects accelerated tissue regeneration. The speed of tissue repair was approximately doubled, and the tissue healing time was reduced by approximately 50% compared with unmodified collagen sponges. Overall, PDA modification enabled collagen-based closure materials to actively modulate the healing microenvironment. These findings suggest that Col@PDA is a promising candidate for next-generation vascular closure devices, with the potential to improve vascular access site repair.

Conflict of Interests

The authors declare no interest conflict.

Electronic Supplementary Information

Electronic supplementary information (ESI) is available free of charge in the online version of this article at http://doi.org/10.1007/s10118-026-3747-x.

Data Availability Statement

Data are available from the corresponding author on reasonable request.

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Graphical Abstract

Polydopamine-modified Collagen Sponge for Improved Anti-inflammatory and Tissue Repair

Xiao-Yun Han, Wei-Qi Lu, Cheng-Qiang Ye, Ding-Qi Tang, Jia-Rong Wang, Meng Hu, Pei-Hong Ji, Ke-Feng Ren, You-Xiang Wang, and Jian Ji

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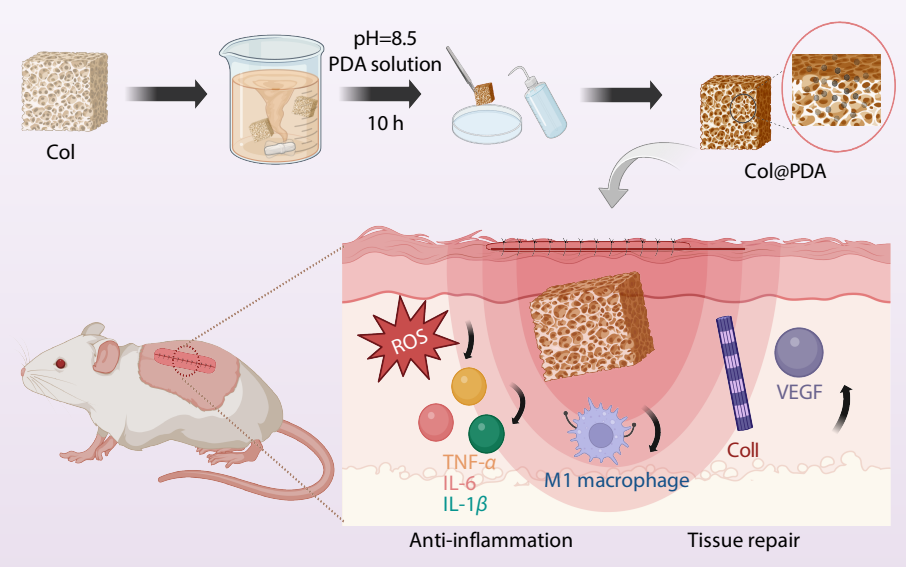
The polydopamine-modified collagen sponge was prepared by immersing the collagen sponge in a polydopamine solution. It exhibits excellent antioxidant and anti-inflammatory effects and can promote tissue repair at wound sites.



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