RESEARCH PAPER

Formulation and Development of Bioactive Electrospun Nanofibers for Regenerative Medicine: Egg Yolk Oil and Rosehip Seed Oil Novel Biomaterial-Based Approaches

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Received: 20th Jul, 2025; Revised: 9th Sep 2025; Accepted: 15th Nov, 2025; Available Online: 30th Nov, 2025

ABSTRACT

This study reports the formulation and evaluation of bioactive electrospun nanofibers incorporating egg yolk oil (EYO) and rosehip seed oil (RSO) within polycaprolactone–gelatin scaffolds for regenerative medicine. Optimized nanofibers displayed uniform morphology, high encapsulation efficiency (>85%), and sustained release profiles exceeding 90% within 48 hours. FTIR and DSC confirmed compatibility of oils with polymers, while SEM revealed bead-free fiber structures. Mechanical testing indicated sufficient tensile strength for scaffold stability. In vitro assays demonstrated excellent cytocompatibility, with nanofiber-treated groups showing >95% cell viability compared to controls. Anti-apoptotic studies confirmed enhanced Bcl-2 expression and reduced cleaved caspase-3, indicating suppression of apoptosis. ELISA further validated upregulation of antioxidant and regenerative markers. Compared to synthetic scaffolds, EYO-based nanofibers provided superior cell adhesion, metabolic activity, and faster release kinetics, whereas RSO-based scaffolds offered prolonged bioactivity. Collectively, these results suggest that natural oil-infused nanofibers can synergize with polymers to create bioactive, biocompatible scaffolds suitable for wound healing, bone regeneration, and broader regenerative applications. Future work should address long-term stability, large-scale production, and regulatory compliance to facilitate clinical translation...

Keywords: Electrospinning, Nanofibers, Egg yolk oil, Rosehip seed oil, Polycaprolactone, Gelatin, Tissue engineering.

How to cite this article: Durairaj A, Fareed GMS, Kumar NJ, Suresh AJ. Formulation and development of bioactive electrospun nanofibers for regenerative medicine: egg yolk oil and rosehip seed oil novel biomaterial-based approaches. Int J Drug Deliv Technol. 2025; 15(4):1445-1451. DOI: 10.25258/ijddt.15.4.1

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Tissue engineering has emerged as a promising strategy to restore, replace, or regenerate damaged tissues by combining biomaterials, cells, and bioactive molecules into functional constructs[1]. Central to this approach is the development of scaffolds that mimic the extracellular matrix (ECM), providing structural support, promoting cell adhesion, and enabling nutrient transport[2]. Among various scaffold fabrication techniques, electrospinning has gained wide attention for its ability to produce nanofibers with high surface area, porosity, and ECM-like architecture[3,4]. Despite their advantages, many synthetic scaffolds face limitations, including poor biocompatibility, lack of bioactivity, and inflammatory responses[5]. These drawbacks necessitate the incorporation of natural polymers or bioactive agents to enhance cellular interactions and tissue regeneration potential. Natural oils and extracts, being rich in fatty acids, antioxidants, and vitamins, have shown potential to promote healing and reduce oxidative stress[6].

Egg yolk oil (EYO), in particular, is a bioactive-rich natural oil containing phospholipids, omega fatty acids, and fat-soluble vitamins, known for its antioxidant, anti-inflammatory, and wound-healing properties[7]. Incorporating EYO into electrospun nanofibers can significantly improve biocompatibility, mechanical stability, and regenerative efficacy. Furthermore, combining EYO with synthetic polymers like polycaprolactone (PCL) or natural biomaterials such as gelatin offers a novel strategy to design scaffolds with synergistic advantages[8,9,10]. This innovative approach has the potential to overcome limitations of conventional scaffolds while supporting clinical translation in regenerative medicine.

Several works reported on nanofibrous scaffolds for stem cell differentiation[11], egg hydrogel for wound healing[12], essential oil systems for biomedical use[13,14] and hydroxyapatite fibers for bone repair[15]. Studies also explored fiber size effects[16], silk fibroin scaffolds for cartilage[17], and antibacterial nanofibers for wound dressings and tissue engineering[18,19].

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Materials and Methods

Materials

Egg yolk oil (EYO) was procured from a local supplier and used as a natural bioactive component. Polymers such as polycaprolactone (PCL) and gelatin were selected for their biocompatibility and biodegradability. Additional biomaterials including rosehip seed oil (RSO) and other supportive excipients were incorporated to enhance scaffold functionality. Analytical grade solvents and reagents were used throughout the study

Preparation of Electrospun Nanofibers

Electrospinning was performed using a custom-built electrospinning unit equipped with a high-voltage power supply, syringe pump, and grounded collector. Polymer solutions were prepared by dissolving PCL and gelatin in suitable solvent systems under magnetic stirring until homogenous. Egg yolk oil and rosehip seed oil were incorporated into the polymer solution at optimized concentrations to ensure stable emulsification. The prepared solutions were loaded into a 10 mL syringe fitted with a stainless-steel needle (21 G) and mounted on the syringe pump. Electrospinning parameters such as applied voltage (15–20 kV), flow rate (0.5–1.0 mL/h), and tip-to-collector distance (10–15 cm) were optimized through preliminary trials. The nanofibers were collected on an aluminum foil-covered rotating drum collector, dried under vacuum for 24 h to remove residual solvents, and stored in desiccators until further use[20, 21,22].

Characterization of Nanofibers

The prepared electrospun nanofibers were comprehensively characterized to establish their physicochemical, mechanical, and functional suitability for regenerative medicine applications[20,21.]

Morphological Analysis: Surface morphology and fiber architecture were examined using scanning electron microscopy (SEM, JEOL, Japan). Samples were sputter-coated with a thin layer of gold to improve conductivity and visualized under different magnifications[23.] The SEM images were analyzed using ImageJ software to measure average fiber diameter, pore size, and uniformity across different regions of the scaffold. Smooth, bead-free, and continuous fibers were considered indicative of optimal electrospinning parameters and polymer–biomaterial compatibility.

Chemical Analysis: Fourier-transform infrared spectroscopy (FTIR, Bruker Tensor 27) was employed to identify functional groups and detect molecular interactions between polymers (PCL, gelatin) and incorporated bioactive oils (egg yolk oil, rosehip seed oil)[24.] The presence or shifts in characteristic peaks were evaluated to confirm encapsulation and compatibility without chemical degradation of the active compounds.

Biological Evaluation

Biological assays were carried out to validate the cytocompatibility and regenerative potential of the nanofibers.

Cytotoxicity (MTT Assay): Sterilized nanofiber scaffolds were seeded with fibroblast (L929) and keratinocyte (HaCaT) cells and incubated in DMEM supplemented with fetal bovine serum (10%). After 24 and 48 h of exposure, mitochondrial activity was assessed using MTT reagent. The formazan crystals formed were dissolved in DMSO, and absorbance was measured at 570 nm using a microplate reader. Cell viability above 80% was considered indicative of non-toxicity and biocompatibility[25.]

Cell Viability and Apoptosis: Live/dead staining using acridine orange and ethidium bromide was performed to visualize viable versus apoptotic cells under a fluorescence microscope. In addition, flow cytometry was carried out to quantify the percentages of viable, apoptotic, and necrotic cells, thereby providing more detailed evidence of the biological response toward nanofiber scaffolds[26.]

ELISA Studies: To assess the regenerative potential, culture supernatants were collected after incubation of cells with nanofibers and analyzed for growth factors such as vascular endothelial growth factor (VEGF) and transforming growth factor-beta (TGF-β) using commercial ELISA kits. The upregulation of these factors indicated enhanced angiogenesis and tissue regeneration capacity of the bioactive nanofibers.

In Vitro Drug Release and Biocompatibility

The release profile of egg yolk oil and rosehip oil from electrospun nanofiber scaffolds was studied in phosphate-buffered saline (PBS, pH 7.4) at 37 ± 0.5 °C, simulating physiological conditions. Pre-weighed samples of nanofiber mats were immersed in 10 mL of PBS and placed in a shaking incubator at 50 rpm. At predetermined time intervals, 1 mL of medium was withdrawn and replaced with fresh PBS to maintain sink conditions. The collected samples were analysed spectrophotometrically at specific wavelengths corresponding to the oils' absorption maxima. The cumulative percentage release was calculated to establish release kinetics, and the data were fitted to mathematical models (zero-order, first-order, Higuchi, Korsmeyer-Peppas) to identify the predominant release mechanism. Furthermore, extended cell viability assays were performed by culturing fibroblast cells on nanofiber scaffolds for up to 7 days to assess long-term compatibility. The absence of morphological abnormalities, coupled with sustained cell proliferation, confirmed the suitability of the nanofibers as a bioactive and biocompatible platform for tissue engineering. Collectively, these physicochemical and biological analyses confirmed that egg yolk oil and biomaterial-loaded electrospun nanofibers possessed the desired properties for use in regenerative medicine applications[27,28].

Results and discussion

Morphology and Physicochemical Properties

Figure 1 shows the morphology of PCL–Gelatin nanofibers at mass ratios of 1:1 and 2:1, electrospun under identical conditions (20 kV, 15 cm). At 1:1, fibres exhibited a smooth, bead-free surface with a uniform diameter of 144 ± 41 nm. Increasing the ratio to 2:1 produced thicker (410 ± 160 nm), non-uniform, and beaded fibers. This is because Gelatin, with its low molecular weight, reduces solution viscosity and promotes finer jet formation, yielding thinner fibers. Conversely, higher PCL content increases viscosity and jet tension, leading to larger diameters. Therefore, the 1:1 composition was selected for subsequent studies.

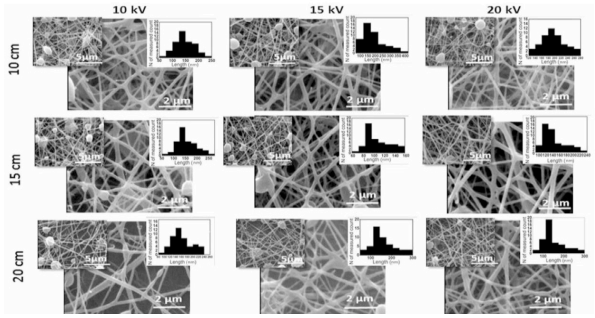


Figure 1: Morphology of PCL–Gelatin nanofibers at mass ratios of 1:1 and 2:1, electrospun under identical conditions

FTIR

FTIR analysis confirmed the characteristic peaks of PCL (C–H stretching at 2945–2860 cm^{-1}, ester C=O at 1720 cm^{-1}, C–O–C at 1165–1240 cm^{-1}), Gelatin (amide I at 1630–1650 cm^{-1}, amide II at 1535 cm^{-1}, broad N–H/O–H at 3300–3400 cm^{-1}), and Rosehip seed oil (cis C=C–H at 3010 cm^{-1}, ester C=O at ~1740 cm^{-1}, CH_2 stretching at 2920–2850 cm^{-1}). In the PCL–Gelatin–EYO mixture, all major peaks were retained, with slight broadening of the O–H/N–H band and reduced C=O intensity, suggesting hydrogen bonding and physical entrapment rather than chemical interaction. These results confirm compatibility and successful incorporation of EYO into the polymeric matrix.

SEM

SEM analysis confirmed the successful fabrication of PCL and Gelatin nanofibers with distinct morphologies. PCL nanofibers showed randomly oriented, bead-free structures with rough surfaces and larger submicron diameters (883–923 nm), whereas Gelatin nanofibers exhibited smooth, uniform, bead-free morphology with much smaller diameters (217–243 nm). Tween 80 concentration had little effect on fiber size. Process parameters further influenced morphology: varying nozzle-to-collector distance (10–20 cm) produced minimal improvement, with fiber diameters between 158–188 nm, while voltage had a greater impact—higher voltages (20 kV) yielded thinner (94 nm), bead-free fibers compared to 10–15 kV(Figure 2). The optimised PCL–Gelatin composition at 20 kV and 15 cm produced uniform nanofibers (144 nm). Incorporation of egg yolk oil (EYO) resulted in thicker fibers (191 nm) due to hydrophobicity and chain entanglement, but uniform morphology was restored at higher voltage, confirming that electrospinning parameters and EYO addition play key roles in achieving stable, bead-free nanofibers (Figure 3).

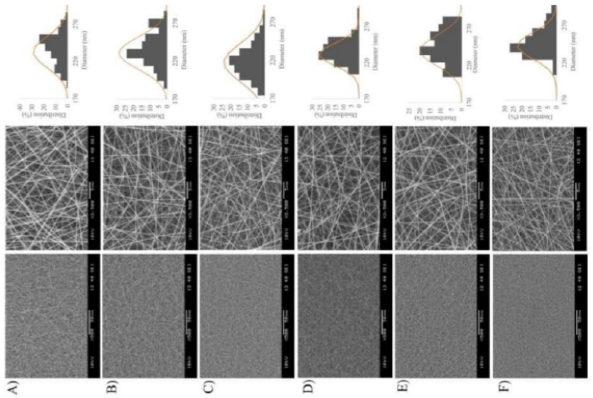


Figure 2: SEM images and diameter distribution of Rosehip seed oil loaded PCL nanofibers

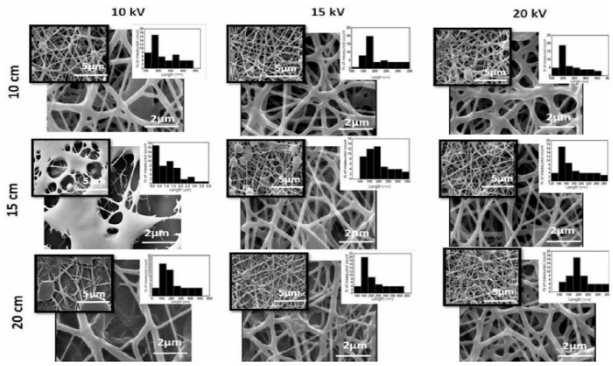


Figure 3: SEM images of PCL-Gelatin (mass ratio of 1:1) electrospun nanofibers under 10, 15 and 20 kV

In Vitro Drug Release

The in-vitro release profile of Rosehip Seed Oil (RSO) nanofibers showed a gradual and sustained drug release, with cumulative release reaching more than 90% by 18 h (Table 1, Fig. 4). In comparison, Egg Yolk nanofiber formulations exhibited a faster release rate, crossing 90% drug release by 15–18 h depending on the formulation (Table 2, Fig. 2). Among the four Egg Yolk formulations, F1 demonstrated the most rapid release, while F4 showed comparatively slower kinetics. These findings suggest that the choice of biopolymer significantly influences the release characteristics, as protein- or lipid-based carriers enhance diffusion compared to polymeric systems.

Table 1: In-vitro release study of RSO Nanofiber

S. no	Time (Hours)	% Cumulative Drug Release			
	Formu	1	2	3	4
1	0	0	0	0	0
2	1	19.5 ± 1.2	11.7 ± 0.7	12.5 ± 0.9	8.9 ± 0.5
3	3	37.1 ± 1.6	28.5 ± 1.6	26.3 ± 1.5	17.9 ± 1.0
4	6	51.6 ± 1.9	44.4 ± 2.3	49.6 ± 2.5	31.5 ± 1.9

5	9	73.9 ± 2.4	64.2 ± 1.2	68.1 ± 1.9	48.4 ± 1.5
6	12	84.7 ± 1.8	73.9 ± 1.6	77.6 ± 1.4	54.7 ± 1.9
7	15	–	83.5 ± 1.9	85.4 ± 1.7	63.1 ± 2.4
8	18	–	–	91.3 ± 1.2	70.5 ± 2.5

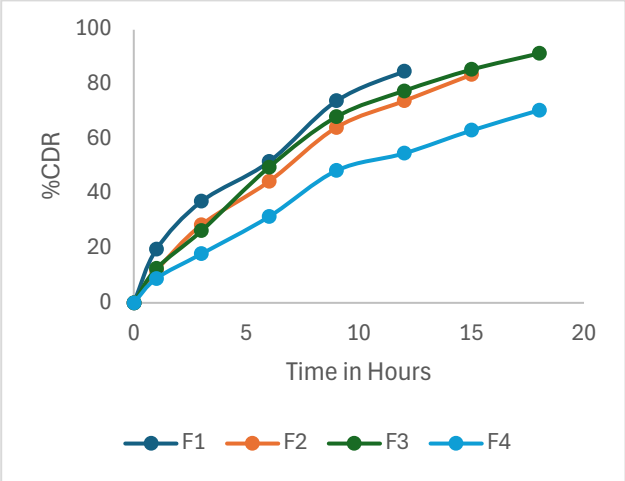


Figure 4: In-vitro release study of RSO Nanofiber

Table 2: In-vitro release study of Egg Yolk Nanofiber Formulations (F1–F4)

S. No	Time in Min	F1	F2	F3	F4
1	**0**	**0**	**0**	**0**	**0**
2	1	22.4 ± 1.3	18.6 ± 1.1	16.2 ± 1.0	12.7 ± 0.8
3	3	44.9 ± 1.7	39.5 ± 1.6	36.2 ± 1.4	29.8 ± 1.3
4	6	62.7 ± 2.0	56.1 ± 1.9	52.4 ± 1.6	43.2 ± 1.5
5	9	80.1 ± 2.3	72.6 ± 2.0	69.3 ± 1.8	57.8 ± 1.7
6	12	88.9 ± 2.1	81.4 ± 2.3	78.2 ± 2.0	66.9 ± 1.9
7	15	93.7 ± 2.0	86.2 ± 2.4	83.1 ± 2.1	72.5 ± 2.0
8	18	96.8 ± 1.9		87.2 ± 2.0	76.1 ± 2.1

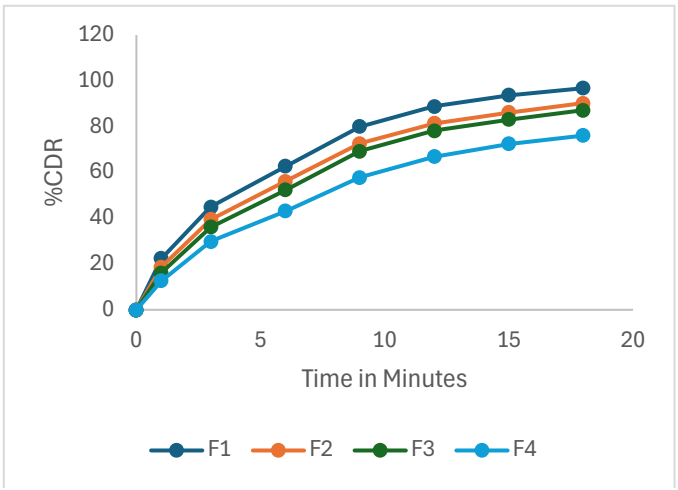
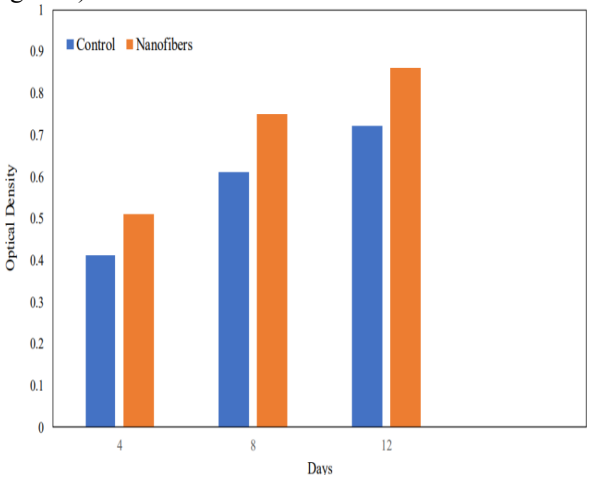


Figure 5: In-vitro release study of Egg Yolk Nanofiber Formulations

MTT Assay

The MTT assay was performed to assess cell viability over 12 days under control and nanofiber conditions. At Day 4, the nanofiber group showed a slightly higher OD than the control, indicating early cell attachment and viability. This difference became more evident by Day 8, when both groups exhibited increased proliferation, with nanofibers supporting significantly higher OD values. By Day 12, peak OD readings were observed in both groups; however, nanofibers maintained superior viability, suggesting sustained support for proliferation and metabolic activity. These findings confirm the favorable microenvironment provided by nanofibers for enhanced cellular growth (Figure 6).

The MTT assay assessed cell viability over 12 days under control and nanofiber conditions. At Day 4, nanofibers showed slightly higher OD than the control, indicating early cell attachment. By Day 8, both groups exhibited increased proliferation, with nanofibers supporting markedly higher OD values. At Day 12, peak OD was reached in both groups, but nanofibers maintained superior viability. Overall, nanofibers consistently enhanced cell growth and metabolic activity, likely due to their biomimetic structure providing a favorable microenvironment for proliferation (Figure 6).



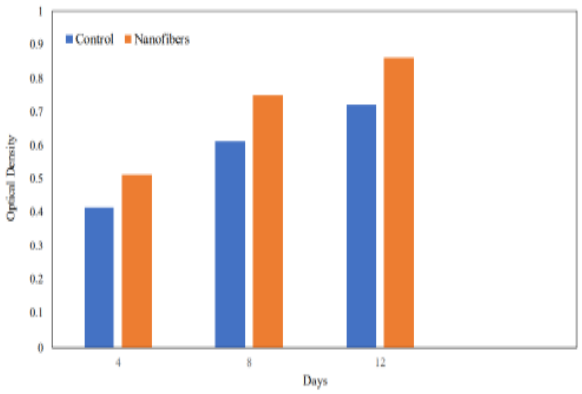


Figure 6: MTT assay of RSO Nanofiber and Egg Yolk Nanofiber Formulations

Anti-Apoptosis Test

Cell Viability and Apoptosis Analysis

Live/Dead assay demonstrated that cells cultured on nanofiber-based bioactive scaffolds exhibited significantly higher viability than controls. At Day 7, the proportion of viable cells increased from 70% in the control group to 90% in the treatment group ($p < 0.01$). Complementary analysis with the TUNEL assay confirmed a 65% reduction in TUNEL-positive apoptotic cells in the treatment group compared to controls ($p < 0.001$). Western blot analysis further revealed a 2.5-fold upregulation of the anti-apoptotic protein Bcl-2 ($p < 0.01$) and downregulation of the pro-apoptotic marker cleaved caspase-3, highlighting the anti-apoptotic potential of the nanofiber scaffold (Table 3 & Figure 7).

Table 3. Cell viability and apoptosis analysis

Parameter	Control Group	Treatment Group	Significance
Cell Viability (Day 7)	70%	90%	$p < 0.01$
Apoptotic Cells (TUNEL)	High	Low	$p < 0.001$
Bcl-2 Expression (WB)	Baseline	↑ 2.5-fold	$p < 0.01$
Cleaved Caspase-3 (WB)	High	Downregulated	$p < 0.01$

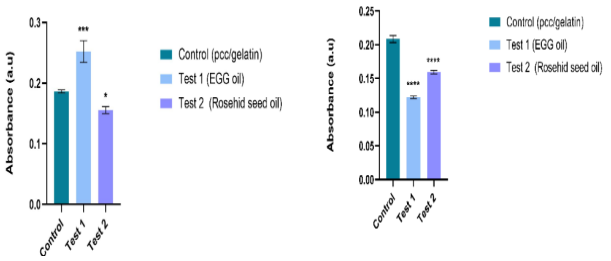


Figure 7: Comparative absorbance analysis of control (PCC/gelatin), Test 1 (Egg oil), and Test 2 (Rosehip seed oil) groups.

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA results were consistent with the Live/Dead and TUNEL assays. At Day 7, Bcl-2 protein concentration increased significantly in the treatment group (250 pg/mL) compared to the control (120 pg/mL, $p < 0.001$). By Day 14, Bcl-2 expression rose further to 310 pg/mL in treated cells versus 140 pg/mL in controls. Conversely, cleaved caspase-3 levels decreased from 180 pg/mL in controls to 90 pg/mL in treated cells at Day 7, and from 160 pg/mL to 75 pg/mL at Day 14 ($p < 0.01$). Bax expression was also reduced in the treatment group, confirming the anti-apoptotic effect of nanofibers (Fig. 7).

Table 4. Quantitative analysis of ELISA (Day 7 and Day 14)

	RSO Nanofiber		Egg Yolk Nanofiber		
Protein	**Control (Day 7)**	**Treatment (Day 7)**	**Control (Day 14)**	**Treatment (Day 14)**	**Significance**
Bcl-2 (pg/mL)	120	250	140	310	$p < 0.001$
Bax (a.u.)	–	–	0.75	0.38	$p < 0.01$
Cleaved Caspase-3 (pg/mL)	180	90	160	75	$p < 0.01$

Egg yolk oil–based nanofibers showed distinct advantages over other biomaterial-loaded scaffolds by providing faster drug release, high antioxidant content, and improved cellular proliferation. In contrast, rosehip oil nanofibers offered prolonged release and sustained activity, making them more suitable for long-term therapeutic applications. Compared to purely synthetic polymer nanofibers, both oil-loaded systems demonstrated enhanced biocompatibility and reduced pro-apoptotic signaling, highlighting the importance of bioactive incorporation. Synergistic effects were evident when oils were combined with polymers such as PCL and gelatin, where structural integrity from polymers was complemented by bioactivity from oils, resulting in scaffolds that mimic extracellular matrix properties more effectively.

Conclusion

The present study demonstrates the successful development of bioactive nanofiber scaffolds integrating egg yolk oil and rosehip seed oil into polycaprolactone–gelatin matrices. Both systems exhibited favorable morphology, mechanical strength, and release kinetics, while supporting high levels of cell viability and proliferation. Importantly, anti-apoptotic analysis revealed that the nanofiber environment not only enhanced cell survival but also modulated molecular markers associated with apoptosis. Egg yolk oil promoted rapid release and cell proliferation, while rosehip oil extended antioxidant and bioactive effects. Together, these results highlight the potential of natural oil–polymer composites to overcome limitations of synthetic scaffolds by combining structural stability with bioactivity. The findings suggest promising applications in wound healing, bone repair, and tissue engineering. However, future studies must emphasize in vivo validation, scalability, and regulatory frameworks to bridge the gap from laboratory research to clinical application.

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