



Fabrication and Characterization of Electrospun PCL/GelMA Composite Scaffolds for Muscle Tissue Engineering

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Abstract

BACKGROUND: Muscle tissue engineering seeks to develop biomimetic scaffolds capable of restoring or replacing damaged muscle by promoting cell alignment, proliferation, and differentiation within a controlled microenvironment. This study presents a novel hybrid scaffold combining electrospun polycaprolactone (PCL) fibers with gelatin methacryloyl (GelMA) hydrogel. The scaffold integrates the topographical guidance of aligned PCL fibers with the supportive 3D matrix of GelMA to promote muscle tissue formation.

METHODS: Electrospun PCL fibers (random/aligned) were incorporated into GelMA hydrogel to form a composite scaffold. Fiber morphology and orientation were analyzed using scanning electron microscopy (SEM), and surface modification of PCL fibers following plasma treatment was confirmed via Fourier transform infrared (FTIR) spectroscopy. Mechanical properties were assessed through tensile testing, and viscoelastic behavior was evaluated via rheometry. Cell viability and proliferation were assessed using live/dead and metabolic assays. Myogenic differentiation was evaluated by immunofluorescence staining of myosin heavy chain 2 (MYH2), and myotube formation was quantified by fusion index.

RESULTS: Aligned PCL fibers significantly enhanced scaffold mechanics, with tensile strength and Young's modulus increasing five- and six-fold, respectively, compared to randomly oriented fibers. The fiber-reinforced GelMA scaffold showed a 45-fold increase in storage modulus (G') relative to GelMA alone. Enhanced cell viability and proliferation were observed. F-actin staining confirmed cell alignment along the fiber axis. MYH2 expression indicated improved myogenic differentiation, with the highest fusion index reaching 34.3%.

CONCLUSION: The PCL/GelMA hybrid scaffold exhibits excellent mechanical and biological performance, highlighting its potential for skeletal muscle tissue regeneration.

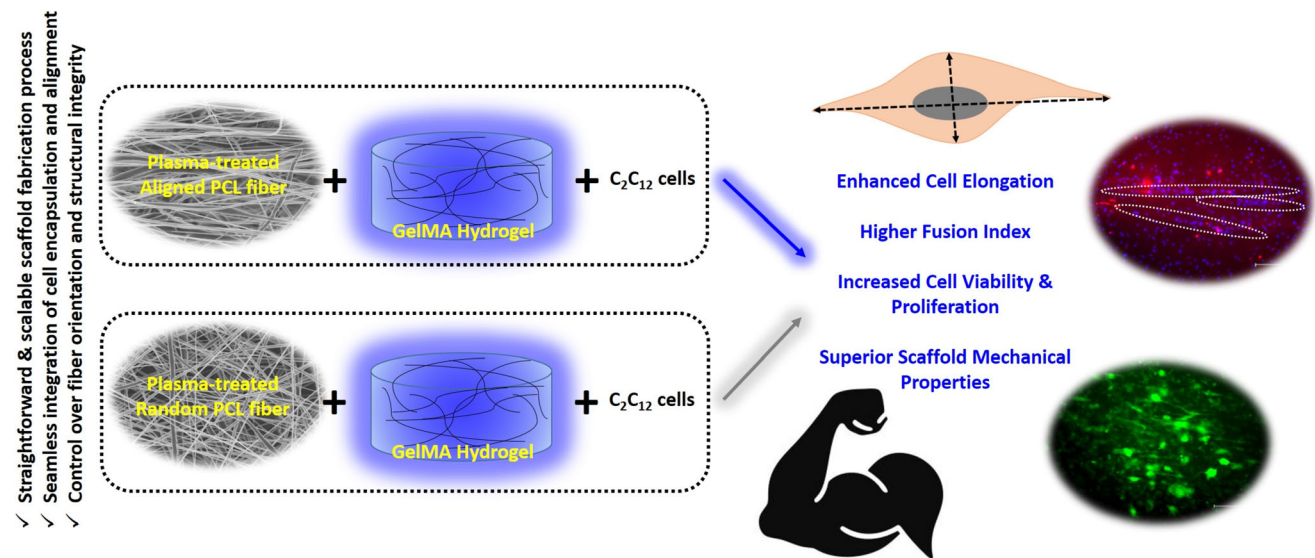
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GRAPHICAL ABSTRACT



Keywords GelMA hydrogel · Electrospun PCL fibers · Muscle tissue engineering · Hybrid scaffold · Fiber orientation

1 Introduction

Skeletal muscle injuries, whether due to trauma, degenerative diseases, or congenital disorders, pose significant challenges for clinical treatment due to the limited regenerative capacity of adult muscle tissue [1]. Skeletal muscle has a limited ability to regenerate after injury, particularly when large portions of muscle are lost or damaged. Current treatment strategies, such as surgical repairs or the use of autologous muscle grafts, have limitations in terms of their success, scalability, and ability to restore full functionality [2]. As a result, there is an urgent need for innovative approaches in tissue engineering that can provide effective solutions for skeletal muscle regeneration. One promising approach for engineering skeletal muscle tissue is the development of biomaterials that can mimic the natural extracellular matrix (ECM), providing both mechanical support and biological cues to guide cellular behavior, particularly myogenic differentiation. Skeletal muscle tissue has a unique architecture in which muscle fibers are highly organized and aligned along a specific orientation to enable coordinated contraction and movement. For tissue engineering to effectively replicate muscle tissue, scaffolds must guide the alignment of muscle cells in a similar manner [3]. Therefore, scaffolds that encourage cellular alignment along these axes are critical for forming functional tissue. Additionally, the scaffold should provide a supportive environment for cell encapsulation, which mimics the hydrated nature of the native muscle ECM [4]. However, the challenge remains in creating a biomaterial

that can effectively combine both the ability to encapsulate cells and direct their alignment in a 3D matrix.

Most tissue engineering approaches have addressed either cellular encapsulation or cellular alignment. Chen et al. [5] developed a novel 3D porous collagen scaffold with concave microgrooves to engineer highly aligned, multi-layered skeletal muscle tissues, promoting myoblast alignment, myosin heavy chain expression, and muscle ECM synthesis for potential use in tissue repair studies. While microgrooved hydrogels are effective for directing cell alignment and generating tissue constructs with specific directional properties, they are constrained by limited cell behavior and insufficient 3D ECM interaction. Similarly, Cha et al. [6] developed a multiscale scaffold combining electrospun nanofibers and PEG hydrogel micropatterns to investigate the effects of topographical cues on skeletal muscle cell alignment, elongation, and myogenesis. Yang et al. [7] engineered a photopolymerizable methacrylate glycol chitosan/type 1 collagen hydrogel for the efficient encapsulation of cells, positioning it as a promising scaffold for skeletal muscle tissue engineering applications. Ansari et al. [8] developed an injectable 3D Arginine-Glycine-Aspartic (RGD)-coupled alginate hydrogel scaffold for encapsulating gingival mesenchymal stem cells and assessed the ability of the encapsulated cells for myogenic differentiation. Jun et al. [9] created macroporous and injectable fibrin-based construct to encapsulate human umbilical cord stem cells for muscle tissue engineering application.

Amidst numerous studies focusing on either cell alignment or encapsulation, few have successfully integrated these two strategies in a single, cohesive scaffold that mimics the complexities of native muscle tissue and enhancing functional tissue regeneration. Lee et al. [10] introduced aligned gelatin-based microribbons (μ RBs) as 3D macroporous hydrogels for guiding smooth-muscle cell alignment and regeneration, wherein it supports direct cell encapsulation, enhances cell proliferation, matrix deposition, and retention of the contractile phenotype. Hwangbo et al. [11] developed a gelatin methacryloyl (GelMA) structure with uniaxially aligned C2C12 cells using an in situ crosslinking process during extrusion, optimizing biophysical parameters to enhance cell alignment and myogenic activity, and demonstrated improved muscle regeneration in a mouse model of volumetric muscle loss compared to conventional crosslinking methods. Bian et al. [12] presented a versatile cell/hydrogel (fibrinogen/collagen type 1) micromolding approach using polydimethylsiloxane molds to fabricate large, thick, and functional skeletal muscle tissue networks with aligned, densely packed myofibers, demonstrating controlled tissue architecture, high cell viability, and spontaneous contractions. Yeo et al. [13] developed a cell-laden scaffold that integrates polycaprolactone (PCL) macrosized struts, aligned nanofibers from optimized electrospinning, and cell-printed myoblasts thereby emphasizing cell encapsulation and alignment.

Our study presents an innovative strategy that seamlessly integrates cell alignment with hydrogel embedding through a simple yet effective fabrication process. Unlike previous approaches that addressed these aspects separately, our method combines plasma-treated electrospun PCL fibers with GelMA hydrogel to create a biomimetic scaffold that simultaneously provides mechanical support, topographical guidance, and a physiologically relevant 3D microenvironment. The novelty of this approach lies in: (1) the straightforward fabrication process that allows precise control over fiber orientation while maintaining structural integrity, (2) the synergistic combination of plasma-treated PCL fibers and GelMA hydrogel that enhances both cell–matrix interactions and mechanical properties, and (3) the scalable nature of the fabrication process that enables the creation of complex, multi-layered constructs. By optimizing these key features, this study aims to demonstrate enhanced myogenic differentiation and advance the field of muscle tissue engineering, particularly for treating large-scale muscle injuries where current therapeutic options are limited.

2 Materials and methods

2.1 Chemicals and reagents

All chemicals and solvents were used as received from the manufacturer unless otherwise specified. Fetal bovine serum (FBS) was purchased from GIBCO. The 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent and 1% Penicillin–Streptomycin 100 $\times$ were sourced from WelGene. Donor equine serum was acquired from Cytiva. Other chemicals, including 2,2,2-Trifluoroethanol (TFE), Polycaprolactone (PCL), Gelatin methacryloyl (GelMA), 2-Hydroxy-4-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure2959), Triton X-100, bovine serum albumin (BSA), 4',6-diamidino-2-phenylindole (DAPI), Albumin-fluorescein isothiocyanate conjugate (FTIC-Albumin), and Dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St Louis, MO, USA). Invitrogen™ LIVE/DEAD™ Viability/Cytotoxicity Kit, paraformaldehyde, and Goat anti-Mouse IgG (H + L) Secondary Antibody conjugated with Rhodamine were obtained from Thermo Fisher Scientific (Waltham, MA, USA). Myosin-2 (MYH2) antibody was purchased from Santa Cruz Biotechnology (Dallas, TX, USA).

2.2 Electrospun PCL fiber preparation

PCL pellets were dissolved in TFE to prepare a 10% (w/v) PCL solution. The solution was then incubated in an oven at 60 °C for 24 h. Electrospinning was performed using an ESR-200RD electrospinning system (NanoNC, Seoul, Korea). To produce aligned fibers, the 10% PCL solution was loaded into a 23G syringe. A voltage of 19 kV was applied, with a collector-to-needle distance of 5 cm and a collector rotation speed of 2000 rpm. For random fibers, the same PCL solution was used, with a collector-to-needle distance of 15 cm and a rotation speed of 400 rpm. Fiber samples were collected on a grounded collector wrapped in aluminum foil. To enhance cell attachment, the electrospun PCL fibers were subjected to plasma treatment at 60 W for 1 min using a plasma system (Covance, Femto Science Inc., Seoul, Korea). The treated fibers were cut into 1.5 $\times$ 1.5 cm pieces and sterilized with ethanol. Prior to cell seeding and hydrogel casting, fibers were rinsed twice with Dulbecco's phosphate-buffered saline (DPBS) and disinfected under UV light for 10 min.

2.3 Chemical and physical properties of PCL fiber

Plasma treatment induced changes in functional groups on the PCL fibers were analyzed using iS50 Fourier transform infrared (FT-IR) spectroscopy (Thermo Fisher Scientific,

Waltham, MA, USA) in the wavenumber range of 500–4000 cm^{-1} in attenuated total reflectance (ATR) mode. The tensile strength and Young's modulus of PCL fibers were evaluated using a Universal Testing Machine (UTM) (34SC-1, Instron, MA, USA). The fibers, with a thickness of 0.1 mm and a length of 1.5 cm, were subjected to testing at a rate of 10 mm/min. In case of aligned electrospun fibers, the tensile direction was aligned along the orientation of the electrospun fibers.

2.4 Surface characterization of PCL fiber

The surface morphology of both random and aligned PCL fibers was examined using a scanning electron microscope (SEM) (SU8600, Hitachi, Tokyo, Japan; Smart Materials Research Center for IoT supported by the Korean Basic Science Institute, NFEC-2023-02-285654). Samples were coated with platinum under vacuum for 120 s, and images were acquired at a magnification of 3000 $\times$ with a scale bar of 10 μm . The surface hydrophilicity of the fibers before and after plasma treatment was assessed by contact angle goniometry (DSA100, KRUSS, Hamburg, Germany). The contact angle values were averaged from three measurements per sample. Protein adhesion onto PCL fibers was evaluated by incubating the PCL fibers with FITC-BSA (10 mg/mL) dissolved in PBS for 24 h at 37 °C in a 5% CO_2 atmosphere. Fluorescence images were captured using an EVOS M5000 microscope (Invitrogen, Waltham, MA, USA), and fluorescence intensity was quantified using ImageJ software. Data were averaged from 10 independent measurements.

2.5 Preparation of PCL/GelMA hydrogel scaffold via photo-crosslinking

A 5% (w/v) GelMA solution was prepared by dissolving GelMA in serum-free DMEM, with stirring at 50 °C until complete dissolution. To initiate the photo-crosslinking process, 0.1% (v/v) Irgacure2959 photoinitiator was incorporated into the GelMA solution. The hydrogel precursor was then drop-cast onto a scaffold of electrospun PCL fibers. UV irradiation was applied using the AS00-4H86F UV LED spot curing system (Altis UVcare) equipped with a CL06 lens (365 nm wavelength, 6 mm spot size). The sample was exposed to UV light at an intensity of 5 mW/cm^2 for 30 s, sufficient to induce photo-crosslinking and form the PCL/GelMA hydrogel scaffold.

2.6 Rheological characterization of PCL/GelMA hydrogel scaffold

The rheological properties of the PCL/GelMA hydrogel scaffold were evaluated using a rheometer (MCR92, Anton

Paar, Graz, Austria) with a 25 mm parallel plate geometry. Frequency sweep tests were performed at 37 °C with a constant shear strain of 1%, while the frequency varied between 0.1 and 10 Hz. The storage modulus (G') and loss modulus (G'') were measured and recorded to assess the mechanical behavior of the hydrogel scaffold.

2.7 Preparation of cell-laden PCL/GelMA Hydrogel Scaffold

C2C12 mouse myoblasts (sourced from ATCC) were cultured in growth medium composed of Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin–Streptomycin (100 $\times$). Once the cells reached approximately 80% confluence, they were detached using trypsin, and a cell suspension was prepared for seeding. Suspended C2C12 myoblasts were seeded at a density of 8×10^3 cells/ cm^2 onto electrospun PCL fiber mats (1.5×1.5 cm), which had been pre-incubated in growth medium at 37 °C in a 5% CO_2 atmosphere. Pre-incubation was carried out to support cell attachment and interaction with the fiber surface. The cells were allowed to attach to the PCL fiber mats overnight before adding a GelMA hydrogel solution. The scaffolds were then subjected to UV irradiation (as described in Sect. 2.5) for 30 s to crosslink the hydrogel, stabilizing the construct and promoting cell-hydrogel interactions at the fiber–gel interface. The resulting cell-laden PCL/GelMA scaffolds were subsequently used for the cell proliferation and viability assays described in Sects. 2.8 and 2.9. To initiate myogenic differentiation, the culture medium was switched to differentiation medium (DMEM containing 2% donor equine serum and 1% Penicillin–Streptomycin (100 $\times$)), which was refreshed every 2 days.

2.8 Cell proliferation evaluation using MTT assay

The proliferation of C2C12 cells on the PCL/GelMA hydrogel scaffolds was assessed quantitatively using the MTT assay on days 1, 4, and 7 post-seeding. The cell-laden scaffolds, prepared as described in Sect. 2.7, were placed in 24-well non-treated tissue culture plates, with 1 mL of growth medium added to each well. MTT reagent (0.006 g/mL in PBS) was introduced at a volume of 50 μL per well containing 450 μL of medium, and the plates were incubated for 2 h at 37 °C. After incubation, the supernatant was carefully removed, and the formazan crystals formed were dissolved in DMSO. Absorbance was measured at 540 nm using a microplate reader (Epoch, BioTek, VT, USA). The absorbance values obtained were used as an indirect measure of cell proliferation, based on the metabolic activity of viable cells.

2.9 Cell viability using live/dead assay

Cell viability on the cell-laden PCL/GelMA scaffolds was qualitatively and quantitatively assessed using the LIVE/DEAD™ Viability/Cytotoxicity Kit (Invitrogen, USA). Scaffolds prepared as described in Sect. 2.7 were placed in 24-well non-treated tissue culture plates, with 1 mL of growth medium added to each well. For viability staining, the scaffolds were incubated with a staining solution containing 2 μ M calcein AM and 4 μ M ethidium homodimer-1 for 1 h at 37 °C under dark conditions. Following staining, scaffolds were incubated in PBS for 2 h to remove any residual dye. Fluorescence microscopy was used to visualize live (green) and dead (red) cells on days 1 and 4 of differentiation. Quantitative analysis of cell viability was performed by counting live (green) and dead (red) cells in multiple randomly selected fields using ImageJ (NIH, USA). The percentage of viable cells was calculated using the following equation:

$$\text{Cell viability(\%)} = \left[\frac{\text{Number of Live cells}}{\text{Total number of cells}} \right] * 100$$

2.10 Immunofluorescence staining

Immunofluorescence staining was performed to visualize cytoskeletal organization and assess myogenic differentiation with the cell-laden PCL/GelMA hydrogel scaffolds. On day 7 of differentiation, scaffolds were fixed in 4% paraformaldehyde, followed by permeabilization with 0.1% Triton X-100. The scaffolds were then incubated with a red fluorescent phalloidin conjugate to label F-actin. After thorough washing with PBS, DAPI was applied for 30 min to stain cell nuclei.

To assess myogenic differentiation, myosin heavy chain (MyHC) expression was evaluated on day 14 of differentiation. Fixed and permeabilized scaffolds were incubated with an anti-MyHC primary antibody, followed by incubation with a Rhodamine-conjugated secondary antibody. Fluorescence images were captured using an EVOS M5000 microscope (Invitrogen, Waltham, MA, USA). The fusion index, defined as the percentage of nuclei within multinucleated cells relative to the total number of nuclei, was calculated as follows:

$$\text{Fusion index(\%)} = \left[\frac{\text{Number of nuclei in multinucleated myotubes}}{\text{Total number of nuclei}} \right] * 100$$

2.11 Statistical analysis

Data are presented as the mean $\pm$ standard deviation (SD). All experiments, unless otherwise specified, were

conducted in triplicate. Statistical analysis was performed using one-way analysis of variance (ANOVA), using GraphPad Prism 9 software (GraphPad Software, San Diego, CA, USA). Differences between groups were considered statistically significant at p -value < 0.01 , with higher levels of significance reported as p -value < 0.0001 where applicable.

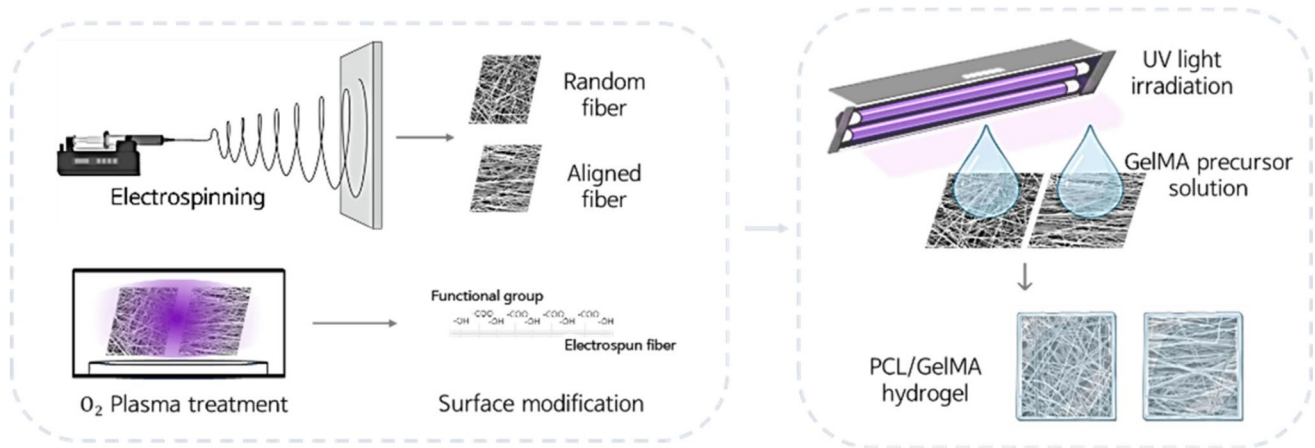
3 Results

3.1 Fabrication of PCL/GelMA hydrogel scaffold

The fabrication process, as illustrated in Scheme 1, involves four critical steps. *Electrospinning of PCL fibers*: First, PCL fibers are electrospun with controlled orientation by adjusting the collector rotation speed and needle-to-collector distance. Higher rotation speeds (2000 rpm) and shorter distance (5 cm) produce aligned fibers, while lower (400 rpm) and greater distance (15 cm) result in random fiber arrangements. *Plasma Treatment*: Second, the PCL fibers undergo plasma treatment, which introduces oxygen-containing functional groups on the fiber surface, improving hydrophilicity and protein adsorption capacity. *GelMA incorporation*: Third, GelMA solution is applied to the surface-modified fibers, ensuring thorough infiltration of the fiber network. *UV-crosslinking*: Finally, UV-mediated crosslinking is performed, during which the methacrylate groups in GelMA undergo free radical polymerization, forming a stable network that physically interlocks with the surface-modified PCL fibers.

3.2 Physical, chemical, and mechanical characterization of electrospun PCL fibers

Structural characterization of electrospun PCL fibers was systematically performed through SEM analysis (Fig. 1A and B), which demonstrated effective control over fiber orientation through key electrospinning parameters. The orientation of fibers was primarily controlled by adjusting the rotating collector's speed and the needle-to-collector distance. For aligned fibers, the reduced needle-to-collector distance (5 cm) enhanced electrostatic guidance of the fibers, while the higher rotation speed (2000 rpm) induced greater stretching, resulting in uniform fiber orientation along the collector's direction. Conversely, lower rotation speeds (400 rpm) and increased needle-to-collector distances (15 cm) produced random fiber arrangements due to reduced directional forces. Quantitative analysis of fiber orientation using ImageJ OrientationJ plugin confirmed these observations. Random fibers showed a broad angular distribution across -90° to 90° , while aligned fibers exhibited a sharp peak distribution at 0° , indicating high



Scheme 1 Schematic representation of the fabrication process for the PCL/GelMA hydrogel hybrid scaffold

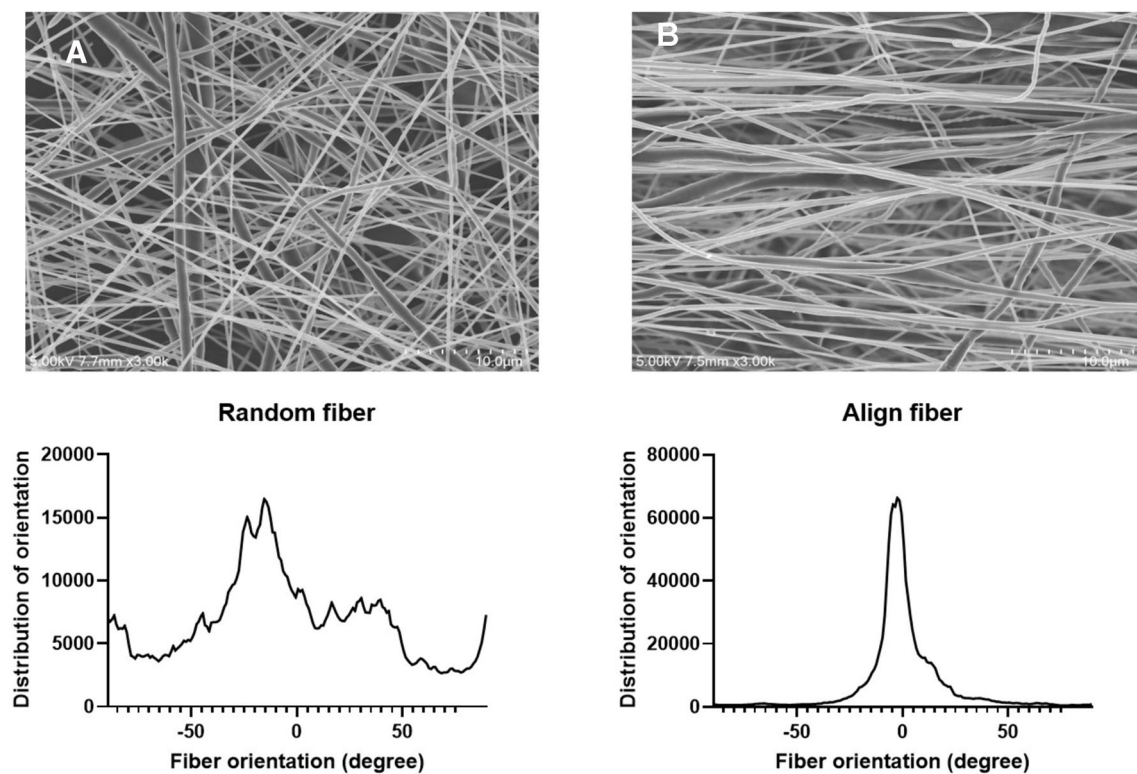


Fig. 1 Representative scanning electron microscope (SEM) images of electrospun PCL fibers: **A** random orientation and **B** aligned orientation. Fiber orientation distribution is shown in the corresponding graph

directional uniformity. This controlled fiber orientation is crucial for tissue engineering applications, as it can provide specific directional cues for cellular behavior. To further assess the structural integrity of the fibers after incorporation into the GelMA hydrogel, we conducted SEM imaging of the electrospun PCL/GelMA composite scaffold. The SEM results (Fig. S1) clearly demonstrate that the aligned fiber morphology is preserved following GelMA integration. In particular, Fig. S1b shows well-oriented, continuous fibers with no observable disruption

that would indicate a loss of alignment, in contrast to the randomly arranged network shown in image Fig. S1a. This confirms that the structural integrity of the aligned nanofibers is retained within the composite scaffold, supporting their utility in guiding cell alignment and tissue organization.

Surface modification of PCL fibers through oxygen plasma treatment was verified using complementary analytical techniques. FT-IR analysis confirmed the successful introduction of functional groups on the fiber surface

(Fig. S2). The appearance of characteristic peaks at 3300 cm^{-1} (hydroxyl groups, $-\text{OH}$) and 1640 cm^{-1} (carbonyl/carboxyl groups, C=O/-COOH) demonstrated effective surface functionalization. These findings confirm that the surface modifications were successfully incorporated into the fibers, validating the effectiveness of the oxygen plasma treatment. The enhancement of the hydrophilic properties of the plasma-treated fibers was further assessed using contact angle measurements. The data revealed a significant reduction in the contact angle for both random and aligned fibers compared to their untreated counterparts (Fig. S3a and Fig. S3b). Specifically, the contact angle for untreated random fibers was $108.6 \pm 2.7^\circ$, while that of untreated aligned fibers was $111.7 \pm 1.9^\circ$, with no significant difference observed between the two groups. After plasma treatment, the contact angles of the random and aligned fibers were reduced to $44.9 \pm 5.4^\circ$ and $45.5 \pm 1.3^\circ$, respectively, with no significant difference between the two groups. These values represent a 58.6 and 59.3% reduction in contact angle compared to the untreated fibers, respectively. These results collectively demonstrate that oxygen plasma treatment effectively modified the surface of PCL fibers, introducing functional groups that significantly enhanced the hydrophilicity of the fibers. Consequently, the treatment improved the surface characteristics, making it more conducive to cell adhesion and attachment.

To assess cell adhesion between the plasma-treated and untreated groups, fibers were immersed in FITC-conjugated BSA solution, and the fluorescence intensity of the protein adhered to the fibers was measured. The plasma-treated group exhibited significantly higher fluorescence intensity, indicating increased attachment of the fluorescently labeled BSA (Fig. S4a), which correlates with enhanced protein adsorption on the fiber surface. Since protein adsorption is a critical step for cell attachment [14], the higher fluorescence intensity observed for plasma-treated fibers suggests improved conditions for cell adhesion. Quantification of the fluorescence intensity (as given in Fig. S4b), using the ImageJ software, revealed lower values for the untreated fibers, with fluorescence intensities of 15.7 and 14.5 a.u. for random and aligned fibers, respectively. In contrast, the plasma-treated random and aligned fibers showed substantially higher fluorescence intensities of 67.8 and 52.2 a.u., respectively. These results confirm that the plasma-treated fibers support enhanced cell adhesion compared to their untreated counterparts.

A tensile test was conducted to evaluate the mechanical properties of the fibers based on their structural arrangement. Tensile strength, which refers to the maximum stress a material can withstand, and Young's modulus, a measure of material rigidity calculated as the ratio of stress to strain, were determined for both random and aligned fibers. The

average tensile strength of the random fibers was $3.0 \pm 0.2\text{ MPa}$ (Fig. S5a), and the average Young's modulus was $14.3 \pm 0.5\text{ MPa}$ (Fig. S5b). In contrast, the aligned fibers exhibited significantly higher values, with a tensile strength of $16.4 \pm 1.8\text{ MPa}$ and Young's modulus of $95.7 \pm 16.0\text{ MPa}$. The observed difference in mechanical strength between the two groups can be attributed to the alignment of the fibers. Aligned fibers, with their structure oriented parallel to the tensile direction, are better able to distribute stress, which leads to delayed cracking and increased tensile strength and stiffness [15]. In contrast, randomly arranged fibers, where the tensile direction does not match the fiber orientation, cannot dissipate stress effectively, resulting in faster cracking. These results confirm that the mechanical properties of the fibers can be modulated by changing their structural arrangement. Furthermore, previous studies have demonstrated that providing mechanical stimulation, such as stretching the matrix during cell culture, can enhance cell elongation and alignment [16–18]. Fibers with higher tensile strength and Young's modulus, therefore, have increased utility for applications requiring greater resistance to external stimuli.

The comprehensive characterization of the plasma treated electrospun PCL fibers demonstrates the successful development of electrospun PCL fibers with controlled orientation and enhanced surface properties. The combination of structural organization, improved surface chemistry, and robust mechanical properties creates an optimal substrate for cell attachment and guidance in muscle tissue engineering applications [19, 20].

3.3 Rheological analysis of PCL/GelMA composite scaffolds

The viscoelastic properties of the GelMA hydrogel and PCL/GelMA composite scaffolds were characterized through rheological analysis across a frequency range of 0.1–10 Hz. In all samples, the G' consistently exceeded the G'' , indicating the formation of stable hydrogel network with predominantly elastic behavior rather than viscous flow characteristics. This elastic dominance is crucial for maintaining scaffold integrity during cell culture and tissue development.

The integration of PCL fibers into the GelMA hydrogel significantly enhanced the mechanical properties of the composite scaffold (Fig. 2A–D). The average G' of the GelMA hydrogel was $38.1 \pm 2.1\text{ Pa}$, while the PCL-integrated hydrogel showed a higher value of $85.5 \pm 12.5\text{ Pa}$, demonstrating that the incorporation of PCL fibers enhanced the mechanical strength of the hydrogel. Additionally, when an extra hydrogel layer incorporating PCL fibers was added to the matrix, the G' increased substantially to $1713.0 \pm 387.7\text{ Pa}$, approximately 45 times higher

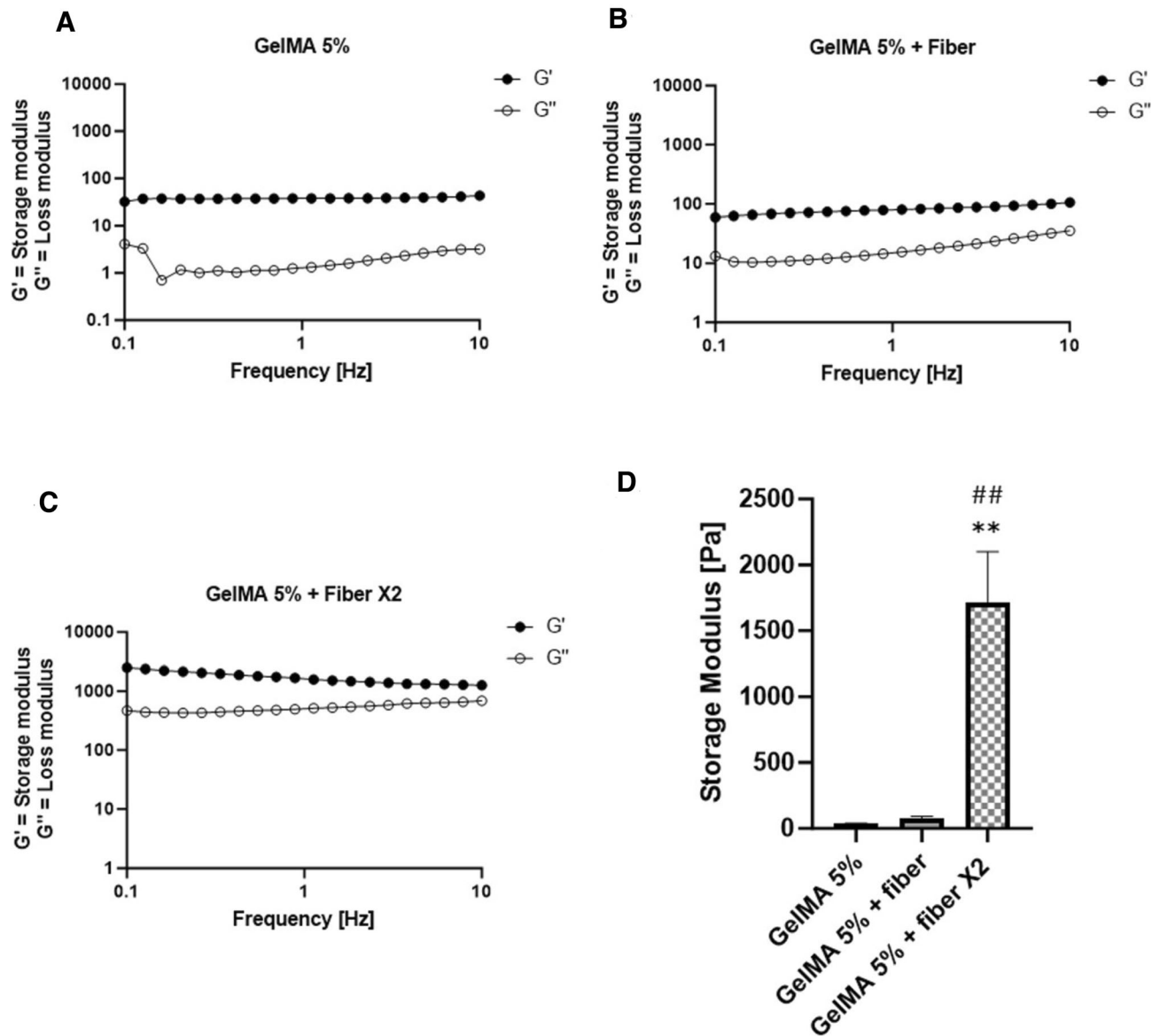


Fig. 2 Rheological characterization of the hydrogels: **A** GelMA hydrogel, **B** PCL fiber-incorporated GelMA hydrogel, and **C** two-layered PCL fiber-incorporated GelMA hydrogel. **D** Quantification of the storage modulus (G') for all three groups. Bars represent

mean $\pm$ standard deviation. Significant differences are noted as p-value (**, ##) < 0.0001 . The symbol ** indicates difference from GelMA 5% group, ## indicates difference from GelMA 5% + fiber group

than that of the GelMA hydrogel. This significant increase in mechanical strength highlights the effectiveness of adding layers to the 3D matrix, which not only improves the structural integrity but also allows for the control of fiber orientation, promoting the growth and alignment of cells within each layer.

The observed mechanical properties of the scaffold are highly relevant for tissue engineering applications, with the G' values falling within the range reported for various soft tissues [21, 22], indicating compatibility with native tissue

environments. The ability to enhance mechanical properties through fiber incorporation and the layered structure provides design flexibility for various tissue engineering applications [23–25]. Additionally, the increased mechanical strength of the composite scaffold enhances support for cell growth and muscle tissue development, while maintaining the beneficial properties of both the hydrogel and fiber components, allowing it to withstand contractile forces during muscle formation.

3.4 Analysis of cell proliferation and viability in PCL/GelMA composite scaffolds

MTT assay was used to indirectly evaluate cell proliferation based on the metabolic activity of viable cells. The results from the MTT assay (Fig. 3A) demonstrate a clear temporal and experimental group-dependent trend in cell viability across the different culture conditions. On day 1, cells cultured on 2D plates exhibited lower absorbance at 540 nm compared to the other experimental groups, which could be attributed to the initial stage of adaptation to the 2D culture environment, where cells may not yet have fully adhered or adjusted to the flat surface. Furthermore, the high absorbance observed in the experimental groups containing random fibers, aligned fibers, and fibers (random/aligned) embedded GelMA hydrogel may suggest that these 3D culture environments provide enhanced initial cell attachment or a more favorable microenvironment that supports cell viability early in the culture period. These conditions are likely to promote better adhesion and spreading, which might be responsible for the higher optical density (O.D.) values observed in these groups on day 1.

By day 4, cells cultured on the 2D plates demonstrated significantly higher absorbance compared to those on random fibers, aligned fibers, and random fiber + GelMA hydrogel groups. This result aligns with the general understanding that 2D cultures often provide superior conditions for cell proliferation in the early stages due to their simplicity and more accessible nutrient and oxygen diffusion. However, the groups incorporating GelMA hydrogel (random fiber + GelMA hydrogel and aligned fiber + GelMA hydrogel) exhibited higher absorbance than those cultured on only electrospun fibers, suggesting that the presence of the hydrogel enhanced cell viability and proliferation. The hydrogel likely provided additional structural support and biochemical cues that facilitated cell survival, even though the values remained lower than those of the 2D culture group. This reinforces the importance of the 3D environment in supporting cell growth, despite the initial slower proliferation observed.

By day 7, the trend continued with 2D cultures showing the highest O.D. values, indicating continued superior cell proliferation. However, the groups with GelMA hydrogel exhibited higher absorbance than the electrospun fibers alone, with the random fiber + GelMA hydrogel group approaching the O.D. values of the 2D group. Most notably, the aligned fiber + GelMA hydrogel group showed O.D. values that exceeded those of the 2D culture, suggesting that the combination of aligned fibers and GelMA hydrogel creates a highly favorable environment for cell viability and proliferation. This could be due to the combined benefits of the hydrogel's biocompatibility and the

aligned fiber scaffold's ability to guide cell orientation and movement, which is closer to the native 3D tissue architecture. The fibers alone groups might not have offered optimal cell–matrix interactions or the 3D microenvironment necessary for sustained cell proliferation, leading to slower growth compared to the more supportive 2D or hydrogel-based conditions.

Checking cell viability in cell differentiation experiments is crucial to ensure that the observed differentiation effects are not confounded by cell death or stress. High cell viability indicates that the cells are alive and healthy, providing confidence that any changes in cell behavior, such as differentiation, are due to the experimental conditions rather than poor cell health. Live/Dead assay was used to assess cell viability qualitatively. On day 1 of the cell differentiation experiment, all experimental groups—2D cell culture, random fiber, aligned fiber, random fiber + GelMA hydrogel, and aligned fiber + GelMA hydrogel—showed similar percentages of cell viability (Fig. 3B–D), indicating that initial cell health and survival were comparable across all conditions. This observation suggests that, initially, the cells were able to survive and adhere equally well in all the environments. By day 4, however, a marked difference was observed in cell viability. Cells encapsulated within the random fiber + GelMA hydrogel and aligned fiber + GelMA hydrogel groups exhibited significantly higher cell viability compared to cells cultured on the 2D tissue culture plates. The GelMA hydrogel groups, both with random and aligned fibers, showed enhanced cell survival, suggesting that the 3D environment provided by the hydrogels may be better suited for supporting cell viability over time compared to the 2D culture. This enhanced survival in GelMA-containing scaffolds can be attributed to several factors: (1) the presence of cell-adhesive motifs in GelMA supporting cell–matrix interactions, (2) the 3D architecture providing better cell–cell contact, and (3) the hydrogel network offering a more physiologically relevant mechanical environment.

3.5 Analysis of cell morphology, alignment and myogenic differentiation in PCL/GelMA composite scaffolds

On day 7 of the differentiation experiment, F-actin was immunostained to evaluate the cytoskeletal structure of the cells (Fig. 4A). The aspect ratio (Fig. 4B) and orientation (Fig. 4C–G) of the cells were subsequently analyzed using ImageJ software. Cell elongation can be quantified by comparing the length and width of a cell, which is often represented using its aspect ratio. The aspect ratio is defined as the ratio of the longest dimension (typically the length) to the shortest dimension (typically the width). For

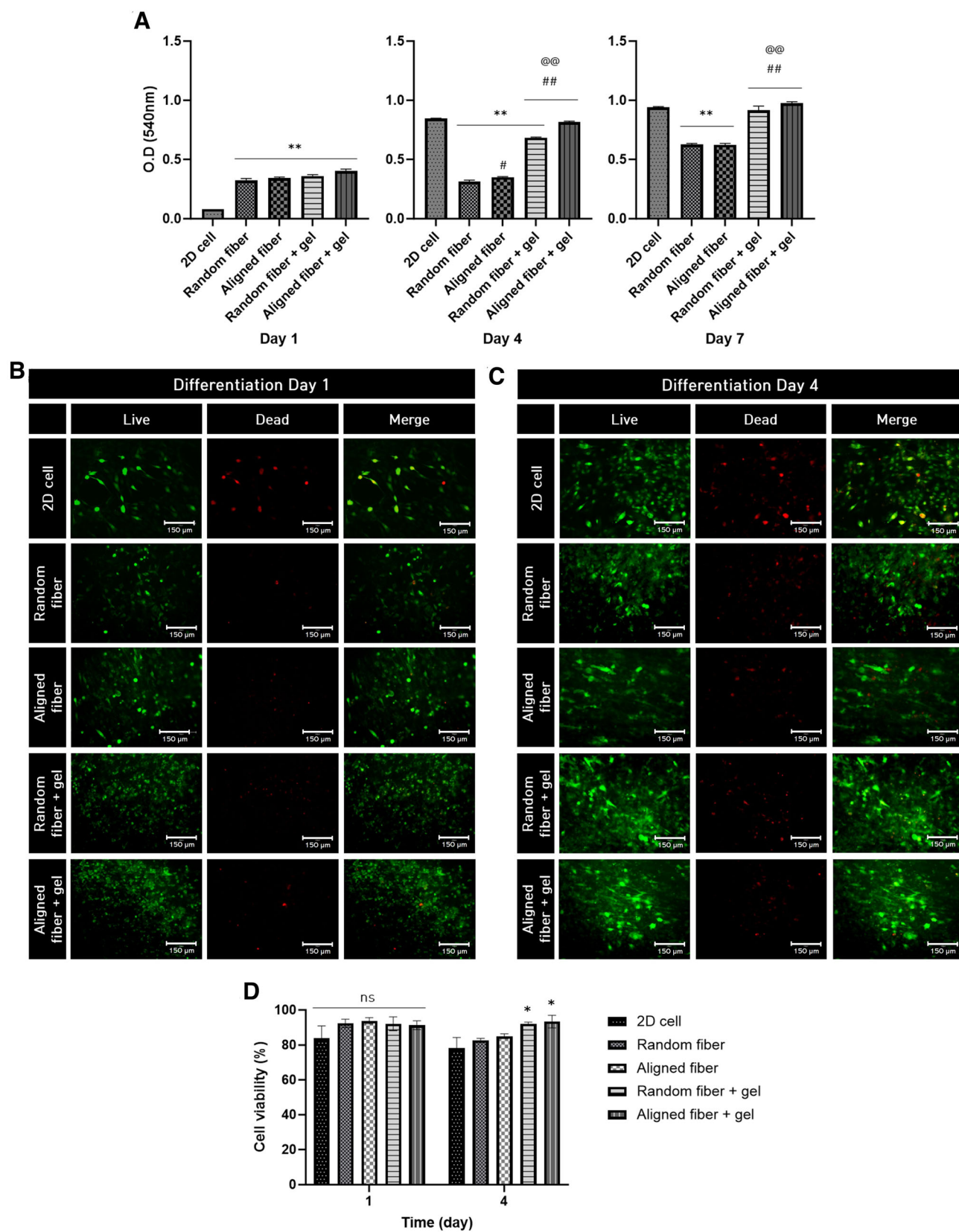


Fig. 3 **A** Cell proliferation analysis using the MTT assay on days 1, 4, and 7. Bars represent mean $\pm$ standard deviation. Significant differences are noted as p-value (**, ##, @@) < 0.0001 and p-value (#) < 0.01 . The symbol ** indicates difference from 2D cell group, # and ## indicates difference from random fiber group, @@ indicates difference from aligned fiber group. Fluorescent images of the Live/Dead assay for cell viability (Scale bar: 150 μ m) on **B** day 1, **C** day 4, and **D** quantification of fluorescent intensity using ImageJ analysis. Bars represent mean $\pm$ standard deviation. Significant differences are noted as p-value (*) < 0.01 . The symbol * indicates difference from 2D cell group

elongated cells, the aspect ratio will be greater than 1, while for spherical or round cells, the aspect ratio will be close to 1. An increase in the aspect ratio indicates greater cell elongation, as the length increases relative to the width. The aspect ratio analysis revealed that the aligned fiber + GelMA hydrogel group exhibited the highest aspect ratio, which was significantly greater compared to the 2D cell culture group and the random fiber + GelMA hydrogel group. The aspect ratio results indicate that the cells cultured on the aligned fiber + GelMA hydrogel scaffold displayed a more elongated morphology compared to those cultured on 2D plates or random fiber + GelMA hydrogel scaffolds. The significantly higher aspect ratio in the aligned fiber + GelMA hydrogel group suggests that the aligned fibers and the hydrogel matrix together provide a favorable environment for cells to elongate and adopt a more elongated shape, which is often associated with differentiated, more functionally mature cells.

To further assess cellular alignment, orientation analysis was performed based on F-actin staining images (Fig. 4C–G). The aligned fiber and aligned fiber + GelMA groups exhibited a distinct orientation peak around 0° , indicating strong alignment of cells along the fiber axis. In contrast, both the random fiber and random fiber + GelMA groups displayed broad, non-directional orientation distributions, suggesting that without fiber alignment, the cells do not adopt a specific orientation. Similarly, the 2D culture group showed a random orientation pattern, reflecting the lack of structural guidance cues on flat surfaces. The presence of GelMA hydrogel in the random fiber + GelMA group did not significantly improve alignment compared to the random fiber-only group, indicating that hydrogel alone is insufficient to guide cell orientation in the absence of structural cues.

Immunofluorescence staining for myosin heavy chain 2 (MYH2) was performed to assess muscle cell differentiation in various experimental groups (Fig. 5A). The fusion index [26], a measure of myotube formation, was calculated for cells cultured on different scaffolds (Fig. 5B). Cells cultured on random fibers and aligned fibers exhibited fusion index values of 13.8 and 20.6%, respectively.

Incorporating GelMA hydrogel further enhanced the fusion index, with random fiber + GelMA and aligned fiber + GelMA hydrogel groups showing values of 22.0 and 34.3%, respectively. The aligned fiber + GelMA group had the highest fusion index, significantly outperforming all other groups, including the 2D cell culture group, indicating a more favorable environment for myotube formation in these conditions.

4 Discussion

For effective muscle tissue engineering, providing a 3D microenvironment that supports both cell alignment and intimate cell–matrix interactions is crucial for recapitulating native tissue architecture and function [27]. Three-dimensional scaffolds offer superior conditions for cell survival, proliferation, and differentiation by enabling enhanced cell–cell and cell–matrix interactions compared to conventional 2D cultures [28, 29].

In this study, we present a novel strategy combining aligned electrospun PCL fibers and GelMA hydrogel to fabricate structurally anisotropic, bioactive 3D scaffolds for skeletal muscle tissue engineering. This composite system addresses critical challenges in recapitulating the mechanical, biochemical, and topographical cues necessary for effective myogenesis. The electrospinning of PCL fibers with controlled orientation, followed by plasma treatment and GelMA incorporation, offers a tunable platform that addresses key limitations in traditional 2D cultures systems and unmodified polymer scaffolds. The scaffold components were carefully selected based on their specific properties and functions. GelMA, derived from gelatin through methacryloyl modification, preserves the inherent bioactivity of natural ECM proteins while gaining photocrosslinking capability [30, 31]. This dual functionality enables both cell–matrix interactions through integrin-binding motifs and precise control over mechanical properties through UV-mediated crosslinking. PCL was selected for its favorable mechanical properties, processability, and FDA approval for biomedical applications [32, 33]. However, its hydrophobic nature necessitates surface modification for optimal cell response [34], which was addressed through plasma treatment in this study.

One of the central findings of this study is the critical role of fiber alignment in guiding cell behavior. Orientation of electrospun fibers plays a crucial role in guiding cellular behavior and determining scaffold properties [35]. By manipulating electrospinning parameters such as collector rotation speed and needle-to-collector distance, we achieved precise control over fiber orientation [36, 37]. Aligned PCL fibers significantly enhance directional uniformity, which is known to provide topographical cues that

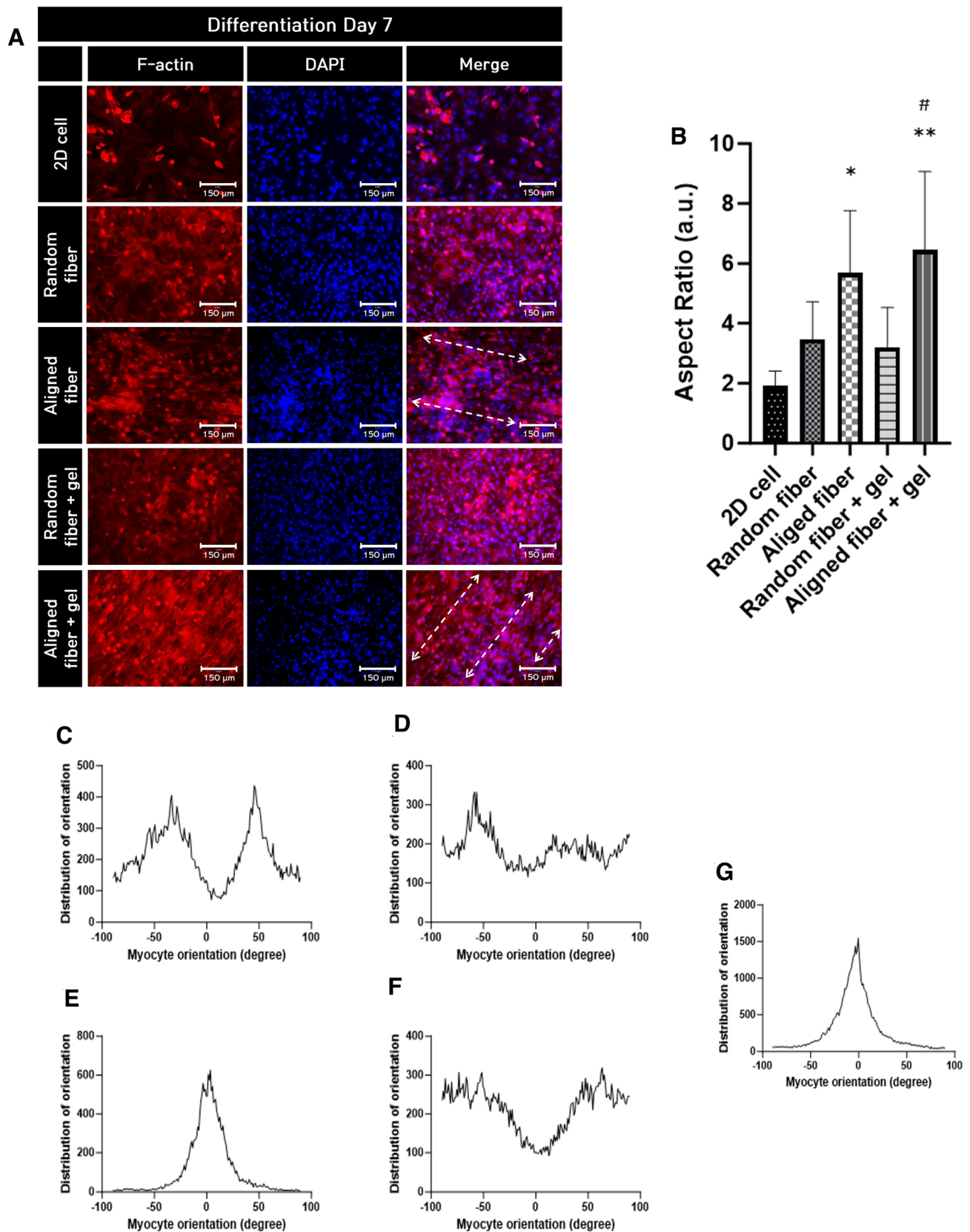


Fig. 4 **A** Fluorescent images of F-actin staining (Scale bar: 150 μ m) on day 7 of differentiation. **B** Graph showing the aspect ratio. Bars represent mean $\pm$ standard deviation. Significant differences are noted as p-value (**) < 0.0001 and p-value (*, #) < 0.01. The symbol * and ** indicates difference from 2D cell group, # indicates difference from random fiber + gel group, and **C–G** Cell orientation analysis using ImageJ for all experimental groups, presented in the same order as shown in panel (A)

influence cell orientation and cytoskeletal organization [38–41]. SEM and orientation analysis confirmed these alignment effects, which are especially crucial in myogenic differentiation, where parallel alignment mimics native muscle tissue architecture.

Surface modification through oxygen plasma treatment effectively improved the hydrophilicity and bioactivity of the PCL fibers. FT-IR and contact angle measurements confirmed the successful introduction of hydroxyl and carboxyl groups, resulting in significantly lower water contact angles and improved protein adsorption. These surface modifications translated into enhanced BSA

adhesion, supporting the notion that oxygen plasma treatment creates a more cell-conductive environment. Enhanced protein adsorption is a prerequisite for integrin-mediated adhesion [14], which subsequently facilitates better cell spreading, survival, and downstream differentiation.

The mechanical characterization revealed another important dimension in scaffold optimization. Tensile strength and Young's modulus were measured specifically on the aligned and random PCL fiber mats prior to hydrogel incorporation, providing insights into the structural integrity and anisotropic mechanical behavior of the fibrous scaffold. Aligned fibers demonstrated superior tensile strength and stiffness compared to their randomly oriented counterparts, consistent with previous literature showing that uniaxial fiber orientation enables better stress distribution along the fiber axis. This mechanical robustness is beneficial not only for maintaining structural integrity [15] but also for supporting cellular alignment and mechanotransduction during myogenic differentiation

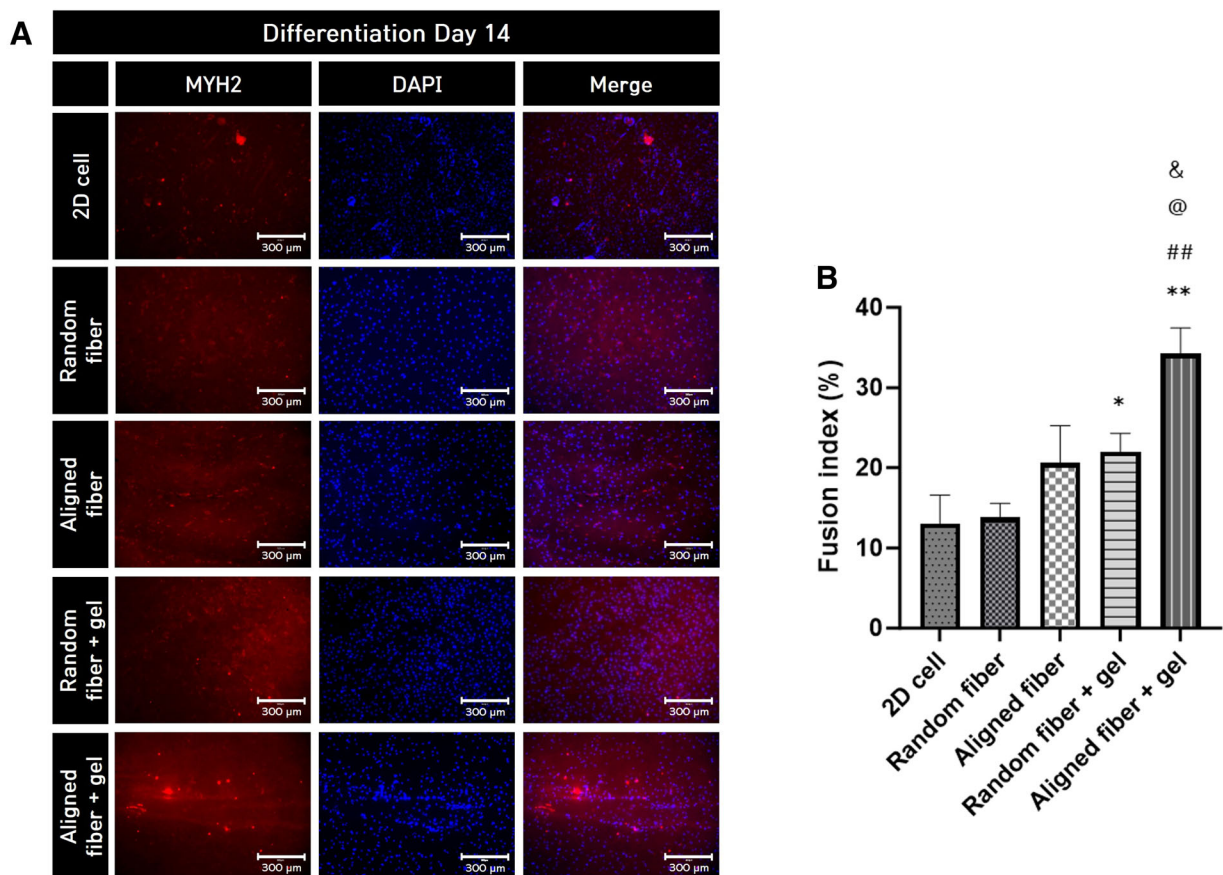


Fig. 5 **A** Fluorescent images of MYH2 staining (Scale bar: 300 μ m) on day 14 of differentiation. **B** Graph depicting the fusion index (%) of the cells. Bars represent mean $\pm$ standard deviation. Significant differences are noted as p-value (**) < 0.0001 and p-value (*, @,

&) < 0.01. The symbol * and ** indicates difference from 2D cell group, ## indicates difference from random fiber group, @ indicates difference from aligned fiber group, & indicates difference from random fiber + gel group

[16–18]. While the modulus values of electrospun PCL fibers exceed those of native muscle tissue, their aligned architecture mimics the directional mechanical environment critical for myoblast elongation and fusion.

Moreover, the incorporation of these fibers into GelMA hydrogel matrices substantially augmented the viscoelastic properties of the resulting composite. Rheological data showed that layered constructs—particularly those incorporating PCL fibers—exhibited significantly higher storage moduli (G'), underscoring their potential to support cell-laden 3D matrices under mechanical loading conditions encountered in dynamic tissue environments [23–25]. These enhanced storage and loss moduli reflect improved stiffness under dynamic loading conditions of the fiber-reinforced GelMA hydrogels and suggest that they provide a more physiologically relevant 3D microenvironment for skeletal muscle function and regeneration.

The composite scaffolds also demonstrated promising biological performance. Cell proliferation assays revealed that although 2D cultures initially supported faster proliferation, the combination of PCL fibers and GelMA hydrogel—especially with aligned fibers—led to sustained and eventually superior cell growth by day 7. This is in line with previous reports suggesting that the 3D microenvironment provided by hydrogels offers more physiologically relevant conditions that support long-term cell viability. The enhanced performance of aligned fiber + GelMA constructs may be attributed to synergistic effects between topographical cues from aligned fibers and the biochemical signals offered by GelMA, including RGD motifs and the ability to retain growth factors.

Cell morphology and alignment further underscore the importance of scaffold design. The aspect ratio and orientation analyses of F-actin-stained cells revealed that only aligned fiber-containing groups induced highly elongated and directionally aligned cytoskeletal structures, resembling the native morphology of developing myotubes. This level of guidance was not replicated in random fiber or 2D control groups, highlighting the unique advantage of anisotropic scaffolds in replicating the aligned architecture of skeletal muscle.

Most notably, myogenic differentiation, as indicated by MYH2 expression and fusion index measurements, was markedly enhanced in the aligned fiber + GelMA group. The fusion index in this group not only surpassed that of all other 3D conditions but also exceeded the values observed in standard 2D cultures, suggesting that these scaffolds provide an optimal microenvironment for myoblast fusion and myotube formation. The data supports the hypothesis that alignment and biomimetic 3D structure are both crucial in recapitulating the developmental cues necessary for functional muscle tissue regeneration.

Taken together, these findings demonstrate that the combination of plasma-treated aligned PCL fibers and GelMA hydrogel produces a composite scaffold that excels in mechanical performance, supports robust cell viability and proliferation, promotes cytoskeletal alignment, and significantly enhances myogenic differentiation. The integrated fabrication process—including fiber alignment, surface treatment, and hydrogel incorporation—collectively contributes to a microenvironment that recapitulates key structural and functional aspects of native muscle tissue. These outcomes position the PCL/GelMA composite as a highly promising platform for skeletal muscle tissue engineering applications. Future studies should explore long-term *in vivo* integration, vascularization potential, and responsiveness to dynamic mechanical stimuli to further validate its clinical applicability.

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Authors contributions Min Ji Hong: Writing—original draft, Methodology, Formal analysis, Data curation. Goeun Bae: Formal analysis, Data curation. Won-Gun Koh: Writing—review & editing, Methodology, Conceptualization. Karthika Muthuramalingam: Writing—original draft, Supervision, Investigation. Hyun Jong Lee: Writing—review & editing, Writing—original draft, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Data availability The data that supports the findings in this study are available from the corresponding authors upon reasonable request.

Declarations

Conflict of interest The authors declare that they have no known competing interests.

Ethical approval Not applicable as there is no use of animal models for the experiments. All authors approve the submission.

References

1. Han WM, Jang YC, García AJ. Engineered matrices for skeletal muscle satellite cell engraftment and function. *Matrix Biol.* 2017;60–61:96–109.
2. Shayan M, Huang NF. Pre-clinical cell therapeutic approaches for repair of volumetric muscle loss. *Bioengineering (Basel).* 2020;7:97.
3. Cheng Y-W, Shiowski DJ, Ball RL, Whitehead KA, Feinberg AW. Engineering aligned skeletal muscle tissue using decellularized plant-derived scaffolds. *ACS Biomater Sci Eng.* 2020;6:3046–54.
4. Del Bakhshayesh AR, Asadi N, Alihemmati A, Tayefi Nasrabadi H, Montaseri A, Davaran S, et al. An overview of advanced biocompatible and biomimetic materials for creation of

- replacement structures in the musculoskeletal systems: focusing on cartilage tissue engineering. *J Biol Eng*. 2019;13:85.
5. Chen S, Nakamoto T, Kawazoe N, Chen G. Engineering multi-layered skeletal muscle tissue by using 3D microgrooved collagen scaffolds. *Biomaterials*. 2015;73:23–31.
 6. Cha SH, Lee HJ, Koh W-G. Study of myoblast differentiation using multi-dimensional scaffolds consisting of nano and micropatterns. *Biomater Res*. 2017;21:1.
 7. Yang P, Li C, Lee M, Marzanyan A, Zhao Z, Ting K, et al. Photopolymerizable hydrogel-encapsulated fibromodulin-reprogrammed cells for muscle regeneration. *Tissue Eng Part A*. 2020;26:1112–22.
 8. Ansari S, Chen C, Xu X, Annabi N, Zadeh HH, Wu BM, et al. Muscle tissue engineering using gingival mesenchymal stem cells encapsulated in alginate hydrogels containing multiple growth factors. *Ann Biomed Eng*. 2016;44:1908–20.
 9. Liu J, Xu HH, Zhou H, Weir MD, Chen Q, Trotman CA. Human umbilical cord stem cell encapsulation in novel macroporous and injectable fibrin for muscle tissue engineering. *Acta Biomater*. 2013;9:4688–97.
 10. Lee S, Tong X, Han LH, Behn A, Yang F. Winner of the Young Investigator Award of the Society for Biomaterials (USA) for 2016, 10th World Biomaterials Congress, May 17–22, 2016, Montreal QC, Canada: aligned microribbon-like hydrogels for guiding three-dimensional smooth muscle tissue regeneration. *J Biomed Mater Res A*. 2016;104:1064–71.
 11. Hwangbo H, Lee H, Jin EJ, Lee J, Jo Y, Ryu D, et al. Bio-printing of aligned GelMa-based cell-laden structure for muscle tissue regeneration. *Bioact Mater*. 2022;8:57–70.
 12. Bian W, Bursac N. Engineered skeletal muscle tissue networks with controllable architecture. *Biomaterials*. 2009;30:1401–12.
 13. Yeo M, Lee H, Kim GH. Combining a micro/nano-hierarchical scaffold with cell-printing of myoblasts induces cell alignment and differentiation favorable to skeletal muscle tissue regeneration. *Biofabrication*. 2016;8:035021.
 14. Nishida K, Baba K, Murakami D, Tanaka M. Nanoscopic analyses of cell-adhesive protein adsorption on poly(2-methoxyethyl acrylate) surfaces. *Biomater Sci*. 2022;10:2953–63.
 15. Jaganathan S, Kandasamy R, Venkatachalam R, Gunalan M, Dhairiyasamy R. Advances in optimizing mechanical performance of 3d-printed polymer composites: a microstructural and processing enhancements review. *Adv Polym Technol*. 2024;2024:3168252.
 16. Lu K, Seidel T, Cao-Ehlker X, Dorn T, Batcha AMN, Schneider CM, et al. Progressive stretch enhances growth and maturation of 3D stem-cell-derived myocardium. *Theranostics*. 2021;11:6138–53.
 17. Takemoto F, Uchida-Fukuhara Y, Kamioka H, Okamura H, Ikegame M. Mechanical stretching determines the orientation of osteoblast migration and cell division. *Anat Sci Int*. 2023;98:521–8.
 18. Fang B, Liu Y, Zheng D, Shan S, Wang C, Gao Y, et al. The effects of mechanical stretch on the biological characteristics of human adipose-derived stem cells. *J Cell Mol Med*. 2019;23:4244–55.
 19. Guex AG, Kocher FM, Fortunato G, Körner E, Hegemann D, Carrel TP, et al. Fine-tuning of substrate architecture and surface chemistry promotes muscle tissue development. *Acta Biomater*. 2012;8:1481–9.
 20. Alshangiti DM, El-damhougy TK, Zaher A, Madani M, Mohamady Ghobashy M. Revolutionizing biomedicine: advancements, applications, and prospects of nanocomposite macromolecular carbohydrate-based hydrogel biomaterials: a review. *RSC Adv*. 2023;13:35251–91.
 21. Cox TR, Erler JT. Remodeling and homeostasis of the extracellular matrix: implications for fibrotic diseases and cancer. *Dis Model Mech*. 2011;4:165–78.
 22. Goder Orbach D, Roitman I, Coster Kimhi G, Zilberman M. Formulation-property effects in novel injectable and resilient natural polymer-based hydrogels for soft tissue regeneration. *Polymers (Basel)*. 2024;16:2879.
 23. Pei B, Wang W, Fan Y, Wang X, Watari F, Li X. Fiber-reinforced scaffolds in soft tissue engineering. *Regen Biomater*. 2017;4:257–68.
 24. Tamayol A, Akbari M, Annabi N, Paul A, Khademhosseini A, Juncker D. Fiber-based tissue engineering: progress, challenges, and opportunities. *Biotechnol Adv*. 2013;31:669–87.
 25. Robles KN, Zahra Ft, Mu R, Giorgio T. Advances in electrospun poly(ϵ -caprolactone)-based nanofibrous scaffolds for tissue engineering. *Polymers (Basel)*. 2024;16:2853.
 26. Jones JM, Player DJ, Martin NRW, Capel AJ, Lewis MP, Mudera V. An assessment of myotube morphology, matrix deformation, and myogenic mrna expression in custom-built and commercially available engineered muscle chamber configurations. *Front Physiol*. 2018;9:483.
 27. Gahlawat S, Oruc D, Paul N, Ragheb M, Patel S, Fasasi O, et al. Tissue engineered 3d constructs for volumetric muscle loss. *Ann Biomed Eng*. 2024;52:2325–47.
 28. Li Y, Yang ST. Effects of three-dimensional scaffolds on cell organization and tissue development. *Biotechnol Bioprocess Eng*. 2001;6:311–25.
 29. Ng R, Zang R, Yang KK, Liu N, Yang S-T. Three-dimensional fibrous scaffolds with microstructures and nanotextures for tissue engineering. *RSC Adv*. 2012;2:10110–24.
 30. Xiang L, Cui W. Biomedical application of photo-crosslinked gelatin hydrogels. *J Leather Sci Eng*. 2021;3:3.
 31. Sun M, Sun X, Wang Z, Guo S, Yu G, Yang H. Synthesis and properties of gelatin methacryloyl (gelma) hydrogels and their recent applications in load-bearing tissue. *Polymers (Basel)*. 2018;10:1290.
 32. Rahimkhoei V, Padervand M, Hedayat M, Seidi F, Dawi EA, Akbari A. Biomedical applications of electrospun polycaprolactone-based carbohydrate polymers: a review. *Int J Biol Macromol*. 2023;253:126642.
 33. Kayan GÖ, Kayan A. Polycaprolactone composites/blends and their applications especially in water treatment. *ChemEngineering*. 2023;7:104.
 34. Dwivedi R, Kumar S, Pandey R, Mahajan A, Nandana D, Katti DS, et al. Polycaprolactone as biomaterial for bone scaffolds: review of literature. *J Oral Biol Craniofac Res*. 2020;10:381–8.
 35. Aviss KJ, Gough JE, Downes S. Aligned electrospun polymer fibres for skeletal muscle regeneration. *Eur Cell Mater*. 2010;19:193–204.
 36. Robinson AJ, Pérez-Nava A, Ali SC, González-Campos JB, Holloway JL, Cosgriff-Hernandez EM. Comparative analysis of fiber alignment methods in electrospinning. *Matter*. 2021;4:821–44.
 37. Medeiros GB, Lima FdA, de Almeida DS, Guerra VG, Aguiar ML. Modification and functionalization of fibers formed by electrospinning: a review. *Membranes (Basel)*. 2022;12:861.
 38. Li X, Wang X, Yao D, Jiang J, Guo X, Gao Y, et al. Effects of aligned and random fibers with different diameter on cell behaviors. *Colloids Surf B Biointerfaces*. 2018;171:461–7.
 39. Ferraris S, Spriano S, Scalia AC, Cochis A, Rimondini L, Cruz-Maya I, et al. Topographical and biomechanical guidance of electrospun fibers for biomedical applications. *Polymers (Basel)*. 2020;12:2896.
 40. Santos AEA, Guadalupe JL, Albergaria JDS, Almeida IA, Moreira AMS, Copola AGL, et al. Random cellulose acetate nanofibers: a breakthrough for cultivated meat production. *Front Nutr*. 2024;10:1297926.

41. Flores-Rojas GG, Gómez-Lazaro B, López-Saucedo F, Vera-Graziano R, Bucio E, Mendizábal E. Electrospun scaffolds for tissue engineering: a review. *Macromol.* 2023;3:524–53.

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