

Article

Surface Engineering of PEEK Using Ultrashort Laser Pulses: A Pathway to Enhanced Cellular Response

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Abstract

Polyetheretherketone (PEEK) has emerged as a promising biomaterial for orthopedic and craniofacial implants due to its favorable mechanical properties and fatigue resistance; however, its inherent chemical inertness limits effective osseointegration. In this study, femtosecond laser surface modification is explored as a strategy to enhance the bioactivity of PEEK. Based on a previously performed parametric study, controlled micro- and nanoscale surface textures were fabricated using femtosecond laser processing, enabling precise tuning of surface roughness and wettability without the need for additional chemical treatment. The modified surfaces were systematically characterized in terms of morphology, composition, and topography using scanning electron microscopy (SEM), 3D profilometry, and water contact angle measurements. Four optimized femtosecond laser-generated surface architectures were selected for the present investigation and comprehensively characterized, followed by in vitro evaluation of dental pulp stem cell adhesion, morphology, and proliferation. The results indicate that laser-induced micro/nanostructuring enhances the surface properties of PEEK, while supporting cellular attachment and favorable cell–surface interaction. Differences in the biological response were observed among the optimized laser-textured surfaces. These findings highlight the feasibility of femtosecond laser texturing as a clean, reproducible, and scalable approach for the development of next-generation, personalized orthopedic implants.

Keywords: polyetheretherketone (PEEK); femtosecond (fs) laser hierarchical structuring; surface biofunctionalization; bioactivity; osseointegration

1. Introduction

The incidence of orthopedic trauma and degenerative skeletal conditions continues to rise, driven largely by aging populations and increased life expectancy, placing sustained demand on advanced implant technologies [1,2]. In parallel, the development in biomaterials understanding has enabled the design of implants with improved mechanical reliability, resistance and biological performance [3]. Although titanium alloys remain widely used for orthopedic applications, their high stiffness relative to bone may induce stress shielding, while the growing recognition of titanium hypersensitivity underscores the need for alternative biomaterials exhibiting more favorable mechanical behavior and improved compatibility in patients at risk of titanium allergy [4].

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Among these, polyetheretherketone (PEEK) has gained considerable attention as a high-performance polymer for orthopedic, spinal, and craniofacial applications [5,6]. Its elastic modulus is closer to that of cortical bone than conventional metals, which could help minimize load mismatch at the implant–bone interface. In addition, PEEK exhibits excellent fatigue resistance, chemical stability, and radiolucency, the latter being particularly advantageous for postoperative imaging and long-term monitoring [7–9]. Nevertheless, the clinical performance of PEEK is often limited by its biologically inert surface, which practically leads to insufficient osseointegration and weak interfacial bonding, frequently associated with fibrous tissue formation rather than direct bone apposition [10–12].

To overcome this limitation, a range of surface modification strategies has been applied, including plasma treatments, chemical functionalization, and the application of bioactive coatings such as hydroxyapatite and natural biopolymers [13–15]. Although these approaches can enhance surface bioactivity, they often involve additional processing steps, limited long-term stability related to coating adhesion, or contamination with toxic chemicals [16]. In contrast, surface topography modification—particularly at micro- and nanoscale levels—has emerged as a robust means of influencing cell–material interactions without altering the bulk properties of the substrate. It is now well established that hierarchical surface features can modulate protein adsorption, cell adhesion, and subsequent osteogenic responses by mimicking aspects of the natural bone microenvironment [17,18].

Within this context, femtosecond laser processing offers a promising, precise, and versatile surface-engineering alternative to conventional modification techniques. Owing to its ultrashort pulse duration, material removal occurs with minimal thermal diffusion, allowing the fabrication of well-defined micro- and nanostructures even on thermally sensitive polymers such as PEEK [19,20]. By adjusting laser parameters, it becomes possible to tailor surface roughness, wettability and design in a controlled and reproducible manner. Importantly, the process does not require chemical reagents, reducing the risk of contamination with toxic chemical residues while improving process consistency [21–23]. Recent investigations have shown that laser-induced surface structuring can enhance the biological response of both metallic and polymeric biomaterials, particularly with respect to protein adsorption and osteoblastic activity [24,25]. In addition to metallic substrates, laser surface engineering has been successfully applied to a variety of polymeric biomaterials, where controlled modification of surface morphology, chemistry, and wettability has been shown to improve cytocompatibility while preserving the advantageous bulk properties of the polymers. Such laser-induced surface functionalization has emerged as an attractive, solvent-free approach for tailoring cell–material interactions and promoting tissue integration, making it particularly suitable for biomedical applications involving high-performance polymers [26–28]. Nevertheless, there remains a need for systematic studies that correlate specific femtosecond laser-generated surface architectures on PEEK with their corresponding effects on osteointegration-related properties.

Based on a previously performed extensive parametric study, femtosecond laser texturing is employed to generate controlled micro- and nanoscale hierarchical patterns on PEEK substrates, with the aim of improving their surface biofunctionality. The resulting surfaces in the present research are characterized using scanning electron microscopy (SEM), 3D profilometry and water contact angle (WCA) measurements to assess morphological, compositional, and wettability changes. In addition, cellular *in vitro* studies were conducted to investigate the behavior of dental pulp stem cells (DPSCs) using SEM and confocal microscopy, while DPSCs proliferation was evaluated through metabolic activity assays on the modified surfaces. These stem cells were selected as the biological model in the present study because they represent an accessible population of mesenchymal stem cells with high proliferative capacity, multilineage differentiation potential, and well-documented osteogenic capability. Owing to these characteristics, DPSCs are widely used for

evaluating the cytocompatibility and osteoinductive potential of biomaterial surfaces intended for bone tissue engineering and orthopedic applications. Their response to engineered implant surfaces provides valuable information regarding the early events of cell adhesion, proliferation, and subsequent bone regeneration [29–31]. Through this combined approach, the study seeks to clarify the role of femtosecond laser-induced surface features in promoting improved bone–implant interactions and to support the development of next-generation PEEK-based implants with enhanced cell–biomaterial adhesion.

2. Materials and Methods

2.1. Preparation of PEEK Samples

Medical-grade polyetheretherketone (PEEK) rods (diameter 12 mm; Ensinger GmbH, Nufringen, Germany) were used as base material. The rods were sectioned into disc-shaped specimens with a thickness of 5 mm prior laser treatment. To ensure consistent surface conditions, all samples were mechanically polished and subsequently cleaned in an ultrasonic bath using 70% ethanol for 10 min, followed by deionized water for an additional 10 min. The cleaned specimens were then air-dried and stored under ambient conditions until further processing.

2.2. Femtosecond Laser Surface Structuring of PEEK Substrates

Surface modification of the PEEK substrates was carried out using a Ti:sapphire femtosecond laser system (Solstice ACE, MKS Spectra-Physics, Milpitas, CA, USA), operating at a central wavelength of 800 nm, with a pulse duration of 70 fs and a repetition rate of 1 kHz. The maximum output power of the system was 6 W. The laser beam was directed through a galvanometric scanning system (SCANLAB, Puchheim, Germany) equipped with a Z-axis module to enable complex pattern generation. An irradiation wavelength of 800 nm was employed because femtosecond Ti:sapphire laser systems operating at this wavelength enable highly localized material processing through nonlinear absorption mechanisms. The ultrashort pulse duration confines energy deposition both spatially and temporally, minimizing heat diffusion and thermal degradation while producing well-defined micro- and nanostructures on the PEEK surface [19,20,32]. The beam was focused perpendicularly onto the sample surface using a 160 mm f-theta lens, resulting in an approximate spot size of 50 μm . This setup allowed precise and reproducible structuring, including the fabrication of multiscale patterns and geometries with non-uniform features (Figure 1).

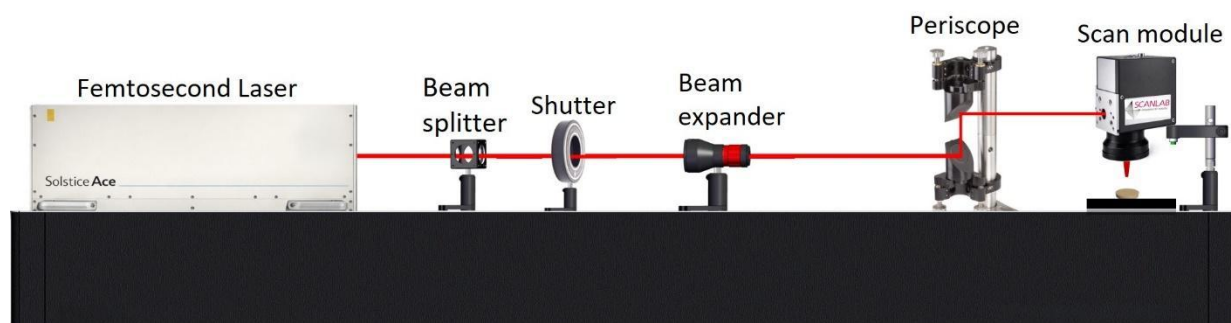


Figure 1. Schematic representation of the Solstice ACE femtosecond laser system integrated with a Scanlab Intelliscan scanning module (SCANLAB, Puchheim, Germany).

The laser beam was oriented perpendicular to the sample surface, and raster scanning was performed while varying key parameters, including laser power P /pulse energy E , scanning speed v , hatch spacing d_x , and focusing conditions. These variations aimed to

determine conditions that minimize thermal effects while avoiding surface damage or deformation, and at the same time promote surface characteristics favorable for cell adhesion [17,32]. The wide parametric range (20–80 mW, scanning speeds of 3.44–32 mm/s, and hatch distances of 45–160 μm) was investigated in our previous research, due to the need of identification of most suitable laser processing conditions for the creation of a reproducible surface texture on a PEEK substrate with minimized thermal distortion. The obtained results showed that by an increase of $P > 20$ mW, considerable material removal was monitored, resolidification of ejected material, and partial disturbance of the microstructures' integrity, which limits their applications for biological evaluations. Thus, processing at 20 mW has shown preservation and accuracy of the created patterns, without the onset of thermal disruption.

Based on that previously performed extensive parametric study [33], conducted under ambient conditions to identify suitable processing parameters ($P = 20$ mW, $v = 3.44$ –32 mm/s, $d_x = 45$ –160 μm), a series of four fs laser hierarchical and multilevel structured PEEK group substrate designs were generated for further biological evaluation in the present research, using an average laser power of 20 mW, corresponding to a pulse energy of 20 μJ at a repetition rate of 1 kHz and a peak laser fluence of approximately 2.04 J/cm² (at 50 μm laser spot diameter), as follows:

- Group 1 (G1-PEEK)—femtosecond laser surface structured PEEK samples structured at 3 levels of lines at $\alpha 60^\circ$ on LASER scanning module at $P = 20$ mW/ $E = 20$ μJ / $F = 2.04$ J/cm², and scanning velocity of $v = 7.6$ mm/s in continuous operation working mode; raster scanning of the samples was performed, as distance b/n the lines at each level of $d_x = 160$ μm ;
- Group 2 (G2-PEEK)—femtosecond laser surface structured PEEK samples structured at 2 levels of lines at $\alpha 45^\circ$ on LASER scanning module at $P = 20$ mW/ $E = 20$ μJ / $F = 2.04$ J/cm², and scanning velocity of $v = 7.6$ mm/s in continuous operation working mode; raster scanning of the samples was performed, as distance b/n the lines at each level of $d_x = 150$ μm ;
- Group 3 (G3-PEEK)—femtosecond laser surface structured PEEK samples structured at 3 levels of lines at $\alpha 60^\circ$ on LASER scanning module at $P = 20$ mW/ $E = 20$ μJ / $F = 2.04$ J/cm², and scanning velocity of $v = 3.44$ mm/s in continuous operation working mode; raster scanning of the samples was performed, as distance b/n the lines at each level of $d_x = 160$ μm ;
- Group 4 (G4-PEEK)—femtosecond laser surface structured PEEK samples structured at 2 levels of lines at $\alpha 45^\circ$ on LASER scanning module at $P = 20$ mW/ $E = 20$ μJ / $F = 2.04$ J/cm², and scanning velocity of $v = 3.44$ mm/s in continuous operation working mode; raster scanning of the samples was performed, as distance b/n the lines at each level of $d_x = 150$ μm .

The processing parameters were selected to ensure reproducible surface texturing while minimizing thermal damage to the polymer. Following laser processing, all samples were ultrasonically cleaned in 70% ethanol to remove residual ablation debris and then air-dried at room temperature. All further analyses were performed with respect to control laser-nonpatterned PEEK tablets (cPEEK). For better comprehension, the differences between the four groups were outlined in Table 1.

Table 1. Differences in the parameters of the femtosecond laser radiation used for the design of groups 1, 2, 3, 4 PEEK samples.

PEEK	Peak Laser Flu- ence [J/cm ²]	Laser Power/Pulse Energy [mW/μJ]	Scanning Velocity [mm/s]	Hatch Distance [μm]	Design
Group 1	2.04	20	7.6	160	3 levels t ± 60°
Group 2	2.04	20	7.6	150	2 levels t ± 45°
Group 3	2.04	20	3.44	160	3 levels t ± 60°
Group 4	2.04	20	3.44	150	2 levels t ± 45°

2.3. Surface Morphology and Topography Analysis

The morphology of the laser-treated PEEK surfaces was examined using scanning electron microscopy (SEM) (Hitachi SU8020, Hitachi High-Technologies, Tokyo, Japan), equipped with a field-emission electron source and a secondary electron detector. Prior to imaging, a thin carbon layer (~20 nm) was deposited onto the samples by sputtering to prevent charging effects. SEM images were acquired at magnifications ranging from 500× to 5000×, depending on the scale of the observed features.

Surface topography and roughness were evaluated using a Zeta-20 3D optical profiler (Zeta Instruments, KLA Corporation, Milpitas, CA, USA). This system operates as an integrated optical microscope with magnification capabilities between 5× and 100× and generates three-dimensional surface reconstructions through vertical scanning. The instrument provides sub-nanometer vertical resolution, enabling detailed characterization of micro- and nanoscale features.

From the obtained 3D surface maps, roughness parameters were calculated, including the arithmetic mean roughness (Ra) and the areal roughness (Sa). The Ra parameter represents the average deviation of the surface profile from the mean line, whereas Sa extends this concept over a defined surface area. For each sample, Sa values were determined as the average of five independent measurements conducted on both modified and untreated regions. Additional visualization and data processing were performed using ProfilOnline 2.0.0 software (Filmetrics, Inc., KLA Corporation, San Diego, CA, USA).

2.4. Wettability Measurements

Surface wettability was assessed using a DSA100 drop shape analyzer (KRÜSS GmbH, Hamburg, Germany) with video-assisted optical contact angle measurements. Dynamic water contact angle (WCA) measurements were carried out at room temperature by depositing a 2 μL droplet of deionized water onto the sample surface and recording its behavior over a period of 3 min. The contact angle values were calculated using ADVANCE software (version 1.14, KRÜSS, Hamburg, Germany), which applies fitting based on the Young–Laplace equation. For each type of laser-treated surface, measurements were performed at three different locations on five independent samples, and the mean WCA[°] was calculated. The obtained values were averaged and compared with those of untreated cPEEK.

2.5. Chemical Structure Characterization

Detailed chemical characterization by means of X-ray diffraction (XRD) and micro-Raman analysis of the four fs-processed PEEK group samples, investigated in the current study with respect to the control ones, was previously performed and published elsewhere [33]. The previously performed XRD and micro-Raman analyses have shown that femtosecond laser texturing did not trigger substantial changes in the chemical composition and the crystalline phase of PEEK samples. The representative XRD peaks and the degree of crystallinity remained unaffected, which showed an insignificant degree of sur-

face amorphization. The micro-Raman results also demonstrated no detectable peak displacements in the typical vibrational bands, which in turn suggests the stability of the polymer's chemical structure.

2.6. Biological Evaluation

The biological response of dental pulp stem cells (DPSCs), isolated from the human dental pulp of 3rd molars, to the four femtosecond laser-textured PEEK surface architectures was evaluated using SEM, confocal microscopy and metabolic activity assays. In addition, the untreated PEEK surface (cPEEK) was included for qualitative SEM observation of the initial cell morphology and attachment. As already mentioned, these stem cells were selected as an *in vitro* model because of their mesenchymal origin, high proliferative activity, and well-established osteogenic differentiation potential, making them a suitable model for evaluating biomaterial surfaces intended for bone regeneration and orthopedic applications. For cells morphology evaluation, after 20 min of UV-decontamination, DPSCs were seeded on samples at 3×10^3 cells/cm² and grown in α -MEM culture medium (LONZA, Basel, Switzerland) supplemented with 1% Glutamax® (HealthWellnessLifestyle Inc., Pasig City, Metro Manila, Philippines), 1% penicillin/streptomycin antimycotic agent (PSA) and 10% decompemented fetal bovine serum (FBS) (*v/v*) until 10 days. After 10 days of culture, cells were fixed in paraformaldehyde 4% (*w/v*, Sigma-Aldrich, Saint-Quentin-Fallavier, France) at 37 °C for 10 min, and permeabilized with Triton X-100 0.5% (*v/v*, Sigma-Aldrich, Saint-Quentin-Fallavier, France) for 5 min and incubated with Alexa® Fluor-488 conjugated-Phalloidin® (1/100 dilution in 0.5% Bovine serum albumin (Thermo-Fischer, Waltham, MA, USA) for 45 min at room temperature in the dark. Nuclei were counter-stained with 4,6-diamidino-2-phenylindole (DAPI, 100 ng/mL, 1/3000 dilution, Sigma-Aldrich, Saint-Quentin-Fallavier, France) for 5 min. Cells were imaged by confocal laser-scanning microscopy (CLSM) 710 NLO, 20× objective, Numerical Aperture 1.4 (Zeiss, Oberkochen, Germany). For SEM imaging, samples were dehydrated, desiccated then coated with a gold–palladium film using ion sputtering (JFC 1100, JEOL, Croissy-sur-Seine, France). SEM observations were performed using SEM (evo 10, Zeiss, Oberkochen, Germany) with an accelerating voltage of 5 kV. Kinetic of DPSCs proliferation was carried out by a water-soluble tetrazolium salt (WST-1®) assay (Roche Diagnostics, Meylan, France) at days 7, 10 and 14. By the manufacturer's protocol, absorbance was measured at 440 nm with a correction at 750 nm, using a FLUOstar Omega microplate reader (version V3.00 R3, BMG Labtech, Aylesbury, UK).

2.7. Statistical Analysis

Statistical analyses were conducted using GraphPad Prism 9.0. The cytocompatibility tests were performed on three independent donors and 2 samples per condition. Results were presented with histograms (mean $\pm$ SEM), and statistical analysis was conducted using the Kruskal–Wallis nonparametric test. When significant differences between groups were identified, Dunn's multiple comparisons test was conducted. Results with *p*-value < 0.05 were considered statistically significant.

3. Results

3.1. PEEK Surface Evaluation After Femtosecond Processing

Representative SEM micrographs of fs laser-structured PEEK samples are presented in Figure 2. As can be clearly seen, the multilevel design of all four groups of fs PEEK samples is characterized by homogeneously repeated microroughness with visible directionality and well-defined edges (especially groups 2 and 4 fsPEEK). Such surface characteristics have been reported to promote enhanced cellular attachment and spreading, as

cells tend to preferentially anchor to anisotropic features and sharp topographical cues [21,22,24]. The group of Fadeeva et al., for example, reported that mammalian cells, including osteoblast-like cells, exhibit selective attachment and guided growth on laser-structured surfaces with defined topographical cues [21]. Similarly, the group of Cunha A. et al. demonstrated that anisotropic microgrooves can direct osteoblastic cell alignment and enhance focal adhesion formation, particularly when the structures are inclined, enabling multidirectional anchoring [22]. Further supporting these observations, the group of Dumas V. et al. showed that femtosecond laser-patterned titanium surfaces significantly influence mesenchymal stem cell (MSC) behavior, leading to enhanced adhesion, elongation, and early osteogenic commitment, especially on oriented micro/nanostructures [25]. In addition, the group of Anselme K. et al. reported that nanoscale surface features modulate protein adsorption and integrin-mediated adhesion, which in turn govern osteoblast spreading and cytoskeletal development [17]. These findings collectively indicate that directional and hierarchical surface architectures, including those oriented at intermediate angles such as 45° , provide favorable conditions for cell attachment, organization, and function.

Furthermore, as can be clearly seen from Figure 2a,c, for groups 1 and 3, respectively, the application of a 60° multilevel scanning strategy results in the formation of characteristic “trulli-shaped” conical microstructures protruding above the baseline surface. Such features are indicative of localized material reorganization and controlled ablation dynamics typical for femtosecond laser processing under overlapping irradiation conditions. Similar conical and spike-like morphologies have been reported on various materials by the group of Vorobyev A.Y. et al., who demonstrated that ultrafast laser irradiation can induce self-organized microcones due to feedback mechanisms between laser energy deposition and surface roughness evolution [19]. In addition, the group of Bonse J. et al. described the formation of hierarchical micro- and nanostructures, including conical protrusions, resulting in cumulative pulse effects and redeposition processes during femtosecond laser treatment of metals, semiconductors, and dielectric materials [20]. From a biological perspective, such high-aspect-ratio features have been shown to influence cell behavior. Gnilitzkyi I. et al. reported that conical and spike-like surface morphologies enhance osteoblastic cell adhesion and spreading by increasing the number of available anchoring sites and promoting focal adhesion contacts [24]. These findings suggest that the observed “trulli-shaped” structures formed at 60° scanning angles may positively contribute to the overall biofunctional performance of the fsPEEK surfaces.

These results are also supported by the 3D Zeta profilometer analysis performed on the four groups laser-structured polymer samples in respect to cPEEK (Figure 3). It is clearly seen that after laser structuring, characteristic, homogeneously distributed micro-“valleys” and “peaks” are generated not only above, but also below the baseline of the PEEK surface. The total peak-to-valley height was measured to be approximately $18\text{ }\mu\text{m}$ for groups 1 and 2, and increased to $\sim 25\text{ }\mu\text{m}$ for groups 3 and 4, indicating a clear dependence on processing parameters. Such bidirectional material redistribution is typical for femtosecond laser ablation, where localized energy deposition leads to simultaneous material removal and redeposition. Similar surface morphologies have been reported by the group of Bonse J. et al., who demonstrated that ultrafast laser irradiation results in hierarchical surface features composed of microgrooves and superimposed nanoscale structures due to cumulative pulse effects and surface instabilities [20]. Likewise, Vorobyev A.Y. et al. described the formation of pronounced peak–valley morphologies with increasing laser fluence, attributed to enhanced ablation efficiency and material ejection dynamics [19]. The group of Wu Y. et al. on the other hand reported that variations in surface roughness significantly influence osteoblast behavior, including adhesion, proliferation, and matrix

formation, with rougher and more complex surfaces providing improved conditions for cell–surface interactions [34].

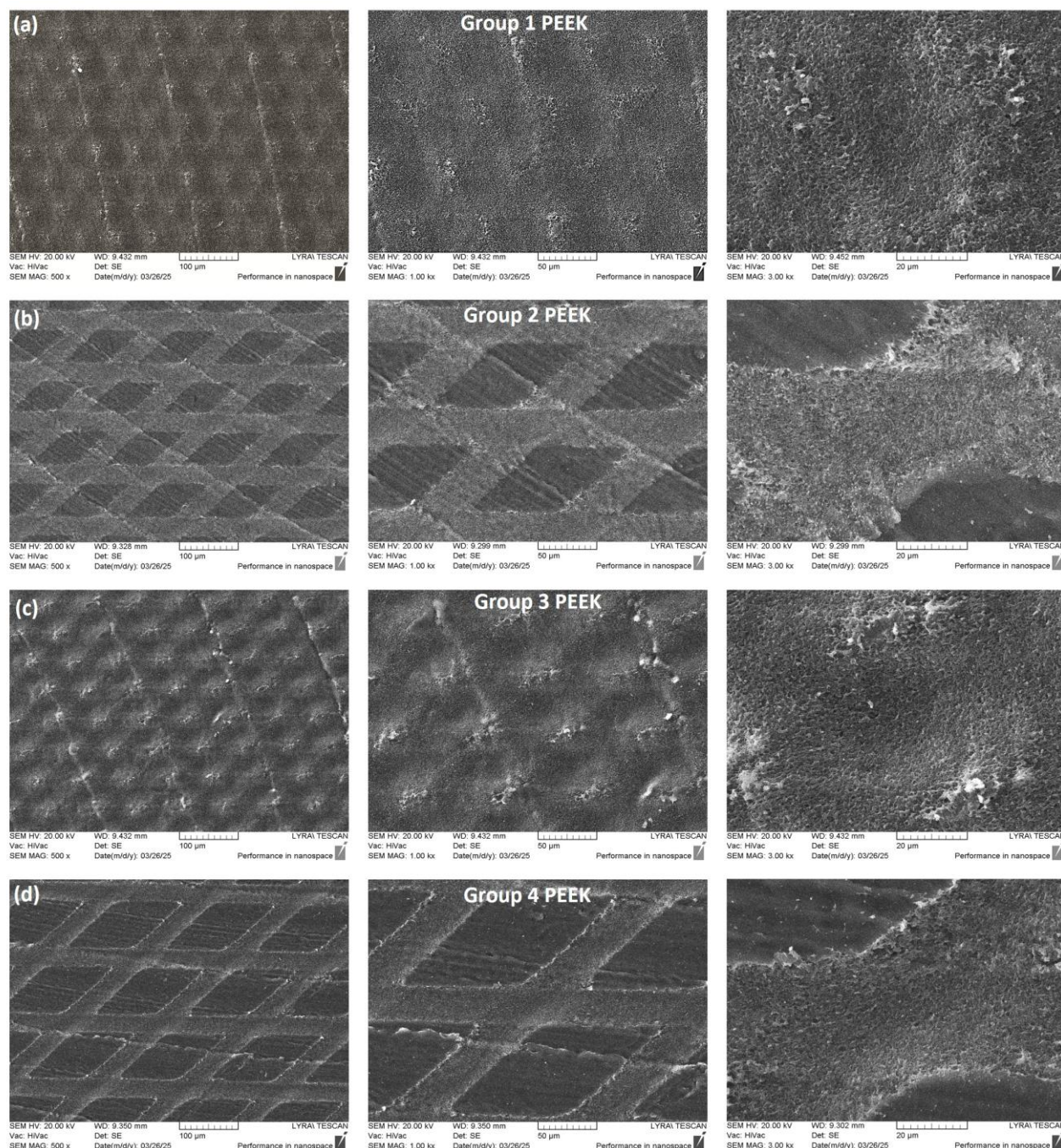


Figure 2. Representative SEM images taken at 500 $\times$, 1.0 and 5.0 k $\times$ of the fs laser multilevel micro-structured designs of PEEK samples: (a) Group 1 PEEK; (b) Group 2 PEEK; (c) Group 3 PEEK; (d) Group 4 PEEK.

The quantitative evaluation of surface roughness demonstrates further that, not only multilevel hierarchical structure was observed, but the total surface area roughness parameter (S_a) of the laser-structured samples was enhanced from 1.25 μm for the control surface to 1.54–1.71 μm for groups 1 and 2, processed at higher scanning velocities and up to 3.21 μm (group 3, three-level structuring) at lower scanning speeds, where more energy was deposited to the surface, leading to intensified material ejection/removal and surface restructuring at the site of interaction. A similar trend has been reported by Stratakis E. et

al., who showed that decreasing scanning speed and increasing laser fluence result in higher surface roughness and more complex hierarchical morphologies due to stronger laser–matter interaction [23].

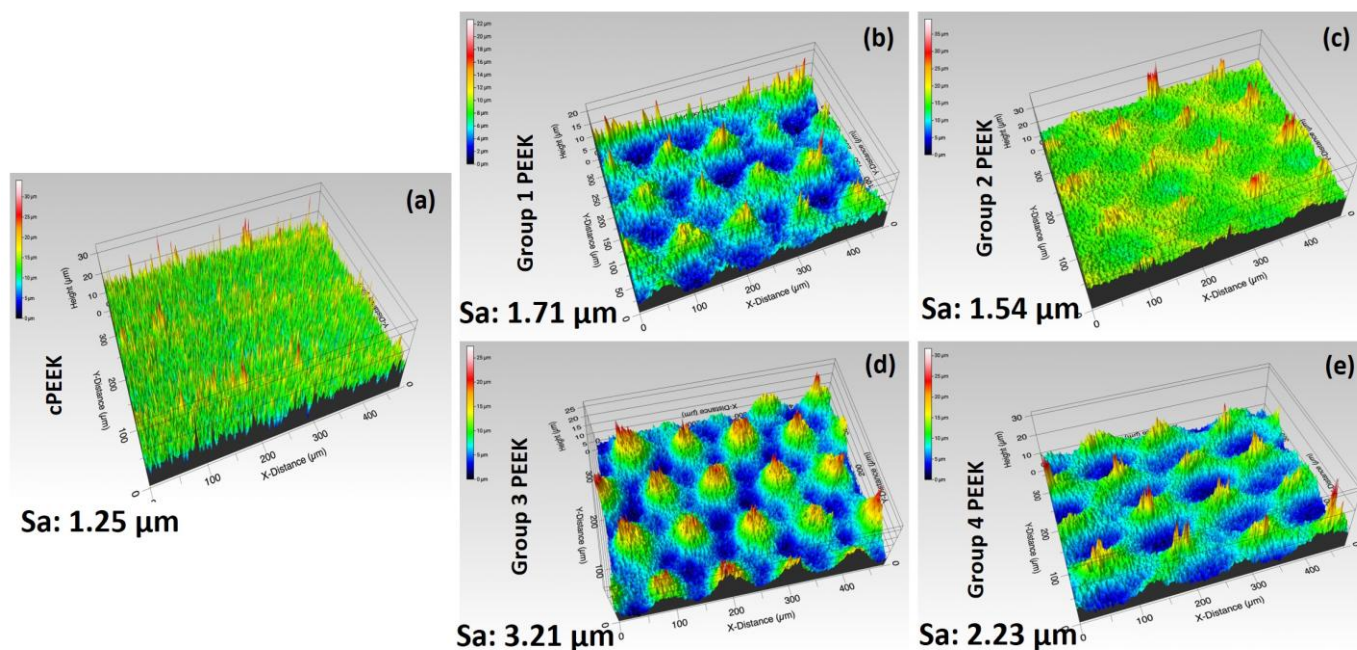


Figure 3. Representative three-dimensional surface reconstruction images (20×) of the fs laser multilevel micro-structured designs of PEEK with respect to control samples: (a) cPEEK; (b) Group 1 PEEK; (c) Group 2 PEEK; (d) Group 3 PEEK; (e) Group 4 PEEK.

From a biological point of view, such increases in surface roughness and the presence of hierarchical peak–valley structures are known to enhance protein adsorption and promote cell adhesion. The group of Zhang Y. et al., for example, demonstrated that surfaces with increased roughness and multiscale features enhance osteogenic responses by modulating cell–material interactions and promoting osteoblast differentiation, as well as improving interfacial contact through increased surface area and topographical complexity [35]. Therefore, the observed topographical modifications in the fsPEEK samples are expected to contribute positively to their biofunctional performance.

3.2. WCA Measurements

Water contact angle measurements (Figure 4a) revealed a clear correlation between surface wettability and the laser-induced surface roughness (Figure 4b). The untreated, polished PEEK surface exhibited a moderately hydrophilic character, with a mean WCA of approximately 74°. Following femtosecond laser structuring, an overall increase in contact angle of about 15–28° was observed, indicating a shift toward more hydrophobic behavior. In particular, surfaces characterized by hierarchical micro- and nanoscale features displayed WCA values exceeding 105°, which is associated with pronounced hydrophobicity. This behavior is closely related to the multiscale surface morphology generated during laser processing. SEM and 3D profilometry analyses revealed the presence of well-defined microstructures superimposed with finer nanoscale features, forming a hierarchical architecture capable of entrapping air within surface cavities. Such air entrapment reduces the effective solid–liquid contact area and promotes a composite solid–air interface beneath the droplet, in agreement with the Cassie–Baxter model [36,37]. As a result, the deposited water droplet exhibits non-uniform spreading behavior, particularly during

the initial stage of contact (first ~60 s), leading to fluctuations in the measured WCA values and the irregular profiles observed in the dynamic measurements—Figure 4a.

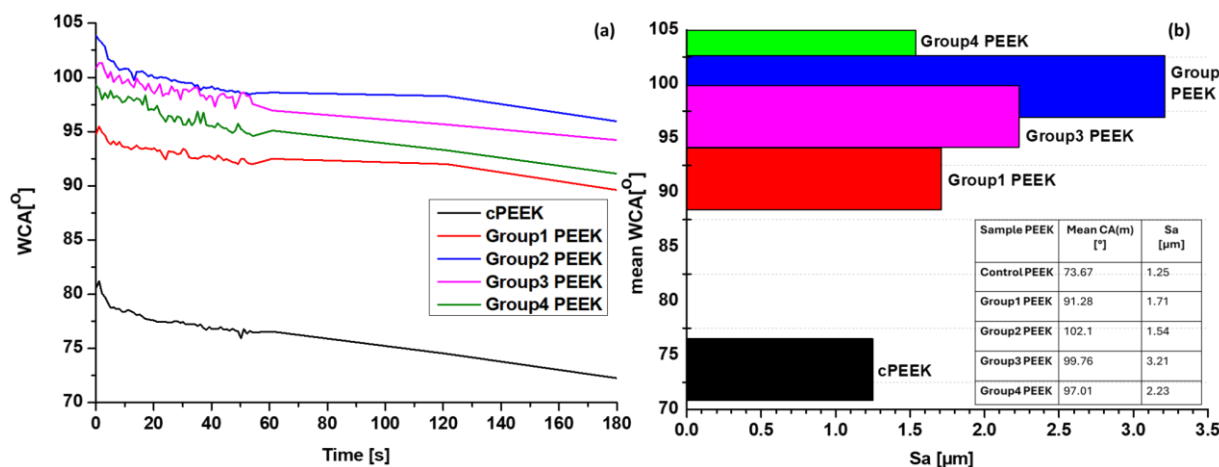


Figure 4. (a) Dynamic WCA evaluation of the fs laser-created designs of PEEK samples for a period of 180 s; (b) Dependence of the mean WCA on the surface roughness S_a .

The wettability results, when considered alongside the surface roughness data, reveal a consistent relationship between femtosecond laser-induced topography and the expected biological response of the modified PEEK surfaces—Figure 4b. As shown also in Figure 3, the increase in areal roughness (S_a) from 1.25 μm for the untreated surface to values as high as 3.21 μm for the multilevel laser-structured samples (Group 3) reflects the formation of a hierarchical micro/nano architecture. This structural complexity directly influences the wetting behavior, as evidenced by the corresponding increase in water contact angle (WCA) values (Figure 4), with highly structured surfaces exhibiting hydrophobic characteristics ($\text{WCA} > 100^\circ$). The transition toward hydrophobicity can be explained by the combined effect of increased roughness and the formation of air-filled cavities within the hierarchical structures, consistent with the Cassie–Baxter model. The initially detected high values of the WCA could be addressed by the Cassie–Baxter wetting regime. This regime explains the water droplet behavior in the first seconds, whereas air trapped below the created nanostructures keeps the liquid from penetration into the solid, resulting in higher contact angle values [36]. The transition to Wenzel state develops gradually in the first 60 s, as the droplet stays on the surface, progressive liquid infiltration into the created surface micro/nano-structures develops, which induces a decrease in the trapped air and enhances the solid–liquid contact zone. These are the first signs of Cassie–Baxter state destabilization and a transformation to Wenzel-like wetting model [37]. As roughness increases, the effective solid–liquid contact area decreases, leading to higher apparent contact angles. In the present case, the progression from moderately rough ($S_a \approx 1.5\text{--}1.7 \mu\text{m}$) to highly structured surfaces ($S_a \approx 3.2 \mu\text{m}$) corresponds to a shift from near-hydrophilic to distinctly hydrophobic behavior, indicating that topography is the dominant factor governing wettability. From a biological perspective, this interplay between surface roughness and wettability is particularly relevant for cell–material interactions. While moderately rough and slightly hydrophilic surfaces are often considered optimal for initial protein adsorption, increased roughness at the micro- and nanoscale provides additional anchoring points for cells, facilitating focal adhesion formation and cytoskeletal organization [21,25]. The group of Zhao G. et al. demonstrated that microstructured surfaces significantly enhance osteoblast adhesion, proliferation, and differentiation by promoting integrin-mediated signaling and improving cell–surface contact [38]. Similarly, Dalby M.J. et al. showed that nanoscale topographical features strongly influence stem

cell adhesion and cytoskeletal arrangement, with hierarchical structures mimicking the extracellular matrix and directing cell behavior at early stages [39]. In the context of laser-structured materials, Cunha A. and coworkers reported that femtosecond laser-generated hierarchical micro/nano architectures enhance osteoblastic cell spreading and focal adhesion formation due to the combined effect of increased roughness and directional topographical cues [22]. These findings collectively support the notion that multiscale surface structuring, as achieved in the present study, provides a favorable microenvironment for cell attachment and function.

Importantly, although higher WCA values are typically associated with reduced wettability, the presence of hierarchical roughness can compensate for this effect by promoting protein adsorption through increased surface area and localized energy variations. In such cases, cell behavior is governed not solely by wettability, but by the combined influence of surface chemistry, roughness, and topographical cues.

As explained above, the Cassie–Baxter model reduces the apparent contact between the water droplet and the surface as a result of air encapsulation; this tendency concerns macroscopic wetting measurements instead of action in the biological environment. The implant surface undergoes prompt protein layer formation because of the physiological conditions after implant integration. The process of protein adsorption is governed not only by the wettability state, but also by surface chemistry, morphology and roughness. The interplay between these processes facilitates a considerable number of adsorption sites and beneficial topographical stimulus for protein binding, including fibronectin and vitronectin, which thereafter promote focal adhesion organization, integrin binding, and cell growth [21]. Thus, while the laser-processed surfaces possess increased apparent WCA, under static air conditions, the increased roughness and multiscale architecture foster protein adsorption and cell adhesion under biological conditions [36,37].

This suggests that the fs-laser-structured PEEK surfaces, particularly those with higher Sa values, provide a favorable microenvironment for cell attachment despite their apparent hydrophobic character. Overall, the correlation between increased Sa roughness, elevated WCA values, and enhanced surface complexity indicates that femtosecond laser structuring enables precise tuning of surface properties. The resulting hierarchical architectures are expected to support improved biological performance by balancing wettability effects with topography-driven cell adhesion mechanisms, making them highly relevant for advanced orthopedic implant applications.

3.3. Biological Evaluation

The ability of DPSCs to adhere and colonize the fs laser-structured PEEK surfaces was also investigated. As presented in Figure 5a, SEM observations revealed the presence of flattened cytoplasmic membrane extensions, suggesting strongly adhered cells on all the studied laser-treated PEEK groups, which is not the case for the cPEEK. To confirm these observations, F-actin (i.e., cytoskeleton) fibers were labelled and visualized using laser-scanning confocal microscopy. The images showed that DPSC adopted an anisotropic organization, suggesting that the cytoskeleton is aligned along the cell axis—Figure 5b. The fs laser-structured PEEK surfaces seem not to have an effect on the cell morphology. Finally, the effect of fs laser-structured PEEK surfaces on the DPSCs proliferation kinetics was assessed by cell metabolic activity from day 7 to day 14. The results showed an increase in the absorbance over time, suggesting that cells proliferate on the fs laser-structured PEEK surfaces. However, no effect of group 3 on DPSCs proliferation was observed as no statistical difference in the absorbance of the reagent was obtained between day 14 and day 7—Figure 5c. To summarize, DPSCs successfully adhered and proliferated on all four femtosecond laser-textured PEEK surface architectures. Differences in proliferation kinetics were observed among the investigated laser-textured groups, indicating that the hierarchical surface archi-

tecture influences the early cellular response. However, the anisotropic organisation of DPSCs suggests the absence of an intrinsic osteogenic effect [40]. Deeper molecular biology characterization of cells is required to confirm this statement.

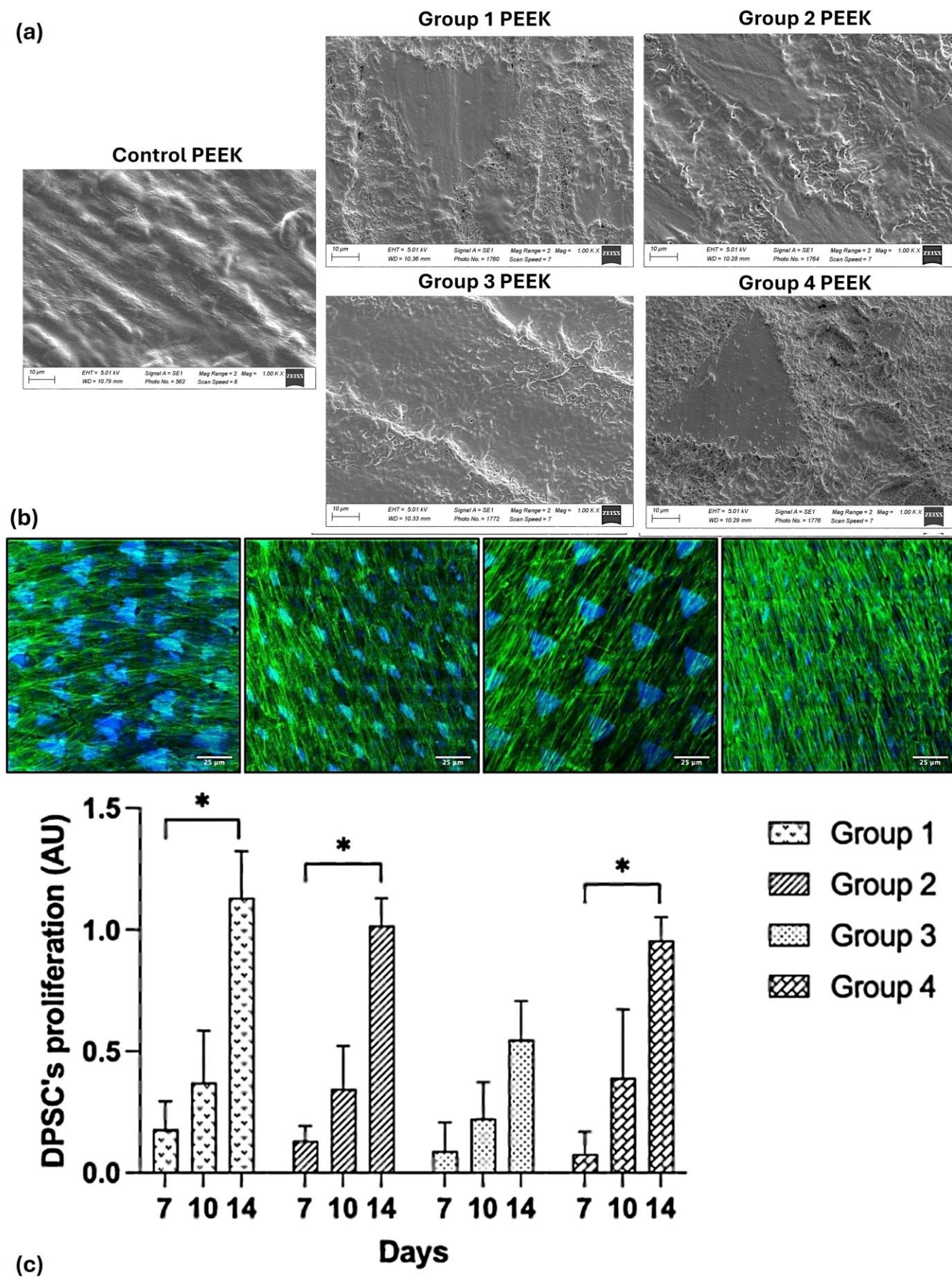


Figure 5. Cell behavior on femtosecond laser-textured PEEK. (a): Representative SEM images taken at 1.0 k $\times$ of DPSCs on the control and the femtosecond laser micro-structured PEEK samples. Scale

bars = 10 μm (b): Confocal microscopy views of DPSCs cultured on the femtosecond laser-textured PEEK 10 days, Green labelling for phalloidin and blue labelling for DAPI. Scale bars = 25 μm . (c): Proliferation assay of DPSCs, cultured on the femtosecond laser micro-textured PEEK for 7, 10, and 14 days. ($n = 3$, * $p < 0.05$ Kruskal–Wallis test).

4. Discussion

One of the major limitations of PEEK in orthopedic and dental applications is its limited bioactivity, which often results in poor bone integration and the formation of fibrous tissue at the implant interface. In the present study, femtosecond laser irradiation was employed to generate hierarchical surface architectures capable of modifying the physical properties of PEEK without altering its bulk characteristics. The results demonstrate that the laser parameters strongly influence the resulting morphology, roughness and wettability, which ultimately affect cellular behavior.

SEM observations and three-dimensional profilometry revealed the formation of regular microstructured patterns composed of peaks and valleys uniformly distributed across the surface. In particular, the three-level scanning strategy produced characteristic conical features and a more pronounced topography. Samples processed at lower scanning velocity exhibited higher roughness values, indicating that a larger amount of laser energy was delivered to the material, promoting enhanced ablation and material redistribution. Similar phenomena have been reported for ultrafast laser processing of various biomaterials, where cumulative pulse effects give rise to hierarchical structures that resemble natural extracellular environments [19,20,23].

Surface roughness increased substantially after laser treatment, reaching its maximum value for Group 3. Such modifications are known to influence protein adsorption and subsequent cell attachment. Previous studies have shown that rough surfaces provide additional anchoring sites for cells and facilitate focal adhesion formation, thereby improving interactions at the cell–material interface [31,32]. Therefore, the observed increase in roughness is expected to contribute positively to the biological performance of the modified PEEK substrates. Laser texturing also affected surface wettability. Although untreated PEEK exhibited moderate hydrophilic behavior, all laser-processed groups showed increased water contact angles, with values above 100° for the most developed structures. This tendency can be attributed mainly to the hierarchical topography generated during processing. According to the Cassie–Baxter model, air pockets trapped inside the micro- and nanostructures reduce the effective contact area between the liquid and the surface, resulting in higher apparent contact angles [36,37]. While surface wettability is often considered an important factor in determining cell response, it is now generally accepted that cell behavior depends on the combined influence of topography, surface energy, chemistry and protein adsorption rather than on a single parameter. In biological studies, the interplay between surface roughness and wettability is particularly critical for PEEK, which is inherently bioinert and requires surface modification to promote cell attachment. While moderately rough and slightly hydrophilic surfaces are generally favorable for initial protein adsorption, increasing micro- and nanoscale roughness introduces additional anchoring sites that enhance focal adhesion formation and cytoskeletal organization. The work of several research groups correlates with the results presented in the current study. For example, the group of Toth J.M. et al. demonstrated that osteoblast adhesion and proliferation on PEEK are strongly influenced by surface morphology and roughness, with smoother injection-molded PEEK showing different cellular responses compared to rougher machined variants [7]. Similarly, Han X. et al. reported that variations in surface roughness of 3D-printed PEEK significantly affect osteoblast metabolic activity and proliferation, highlighting the importance of topography in regulating cell behavior [41]. Further evidence is provided by Mengdi and coworkers, who showed that

nanostructured and composite-modified PEEK surfaces (e.g., hydroxyapatite-containing PEEK) promote osteoblast adhesion and differentiation through enhanced surface bioactivity and local biochemical signaling [42]. In addition, the group of Awaja F. et al. demonstrated that the introduction of oxygen-containing functional groups on PEEK surfaces increases focal adhesion formation, as evidenced by elevated vinculin expression and improved osteoblast attachment [43]. More recently, the group of Yang et al. reported that nanostructured PEEK surfaces not only enhance osteoblast adhesion and proliferation but also modulate immune responses, leading to improved osteogenic activity and bone regeneration potential [44].

Despite the increase in hydrophobicity, the laser-modified surfaces supported DPSC attachment and proliferation. SEM observations showed well-spread cells with numerous cytoplasmic extensions, indicating strong adhesion to the substrate. Confocal microscopy further demonstrated organized actin filaments and elongated cell morphology along the laser-generated structures. The anisotropic organization observed on all samples suggests that the microstructures provided topographical guidance to the cells, in agreement with previous reports describing contact guidance effects on laser-patterned substrates [21,22,25], which is not the case of the control PEEK surface—Figure 5a. The proliferation assays confirmed that all laser-treated surfaces were cytocompatible. This finding agrees well with previous reports demonstrating that appropriately designed laser-generated micro- and nanotopographies on polymer substrates promote cell adhesion and proliferation while maintaining excellent cytocompatibility [27,45]. A progressive increase in metabolic activity was observed between days 7 and 14 for most groups. Interestingly, Group 3, which exhibited the highest roughness and the most developed hierarchical morphology, showed no significant increase in metabolic activity during this period. This observation suggests that increasing roughness does not necessarily lead to a proportional improvement in cell proliferation. Highly developed topographies may alter the balance between proliferation and differentiation processes, as has been reported for stem cells cultured on nanostructured surfaces [39]. Consequently, there appears to be an optimal range of surface complexity that favors cell growth. No major differences in cell morphology were observed among the investigated groups, indicating that all laser-generated structures provided suitable environments for DPSC attachment. Nevertheless, Groups 1, 2 and 4 displayed a more sustained proliferative response, suggesting that moderate roughness combined with hierarchical organization may represent a more favorable surface configuration than excessively rough structures. Although the elongated morphology and alignment of DPSCs observed on the laser-textured PEEK surfaces do not by themselves indicate an intrinsic osteogenic effect, they represent an important early stage of the cellular response to the implant surface. Contact guidance promotes cytoskeletal organization, focal adhesion maturation, and integrin-mediated mechanotransduction, thereby strengthening cell–material interactions and supporting stable cell attachment and proliferation. These early events are considered prerequisites for subsequent extracellular matrix deposition and osteogenic differentiation under appropriate biological and biochemical stimuli. Consequently, while the present study does not demonstrate direct osteoinduction, the improved early cellular response induced by the hierarchical laser-generated topographies is expected to create a more favorable microenvironment for long-term bone formation and implant osseointegration. Moreover, previous studies by Dubus et al. have demonstrated that enhanced *in vivo* bone formation can be achieved even in the absence of direct osteoblast differentiation, highlighting the importance of the early cell–material interactions in determining the long-term biological outcome [46,47]. Building upon these findings, the next stage of our research will involve *in vivo* evaluation of the optimized laser-textured PEEK surfaces to determine whether the enhanced early cellular response

observed *in vitro* translates into improved bone formation and osseointegration under physiological conditions.

Although the present study primarily attributes the improved biological performance to the laser-generated hierarchical surface topography, it should be noted that femtosecond laser irradiation performed in the ambient atmosphere may also induce limited surface oxidation and the formation of oxygen-containing functional groups within the outermost nanometers of the polymer surface. Such subtle chemical modifications cannot be fully resolved by XRD or Raman spectroscopy, which mainly probe the bulk material, and therefore cannot be completely excluded. Previous studies have shown that laser-induced oxidation of polymer surfaces may contribute to protein adsorption and subsequent cell attachment by altering surface energy and interfacial interactions. Nevertheless, the previously performed XRD and micro-Raman characterization of the same laser-treated PEEK surfaces revealed no detectable changes in the bulk crystalline structure or chemical composition, suggesting that any laser-induced chemical modification is confined to a very thin surface layer. Consequently, the enhanced cellular response observed in the present study is considered to arise predominantly from the hierarchical micro/nanostructure and increased surface roughness, while a possible contribution of subtle surface chemical modifications cannot be excluded. Future investigations employing X-ray photoelectron spectroscopy (XPS) will be valuable for elucidating the relative contributions of surface chemistry and topography.

Compared with coating-based or chemical modification approaches, femtosecond laser processing offers several advantages. The technique enables precise and reproducible surface functionalization without the use of chemical reagents and avoids potential problems associated with coating delamination or degradation. In addition, only the surface properties are modified, while the excellent mechanical characteristics of PEEK are preserved. These advantages make ultrafast laser texturing particularly attractive for the fabrication of personalized implants.

Although the present results demonstrate improved cytocompatibility, further investigations are necessary to clarify whether the observed cellular response translates into enhanced osteogenic differentiation. Analysis of osteogenic markers, mineralization studies and gene expression assays would provide additional insight into the mechanisms involved. Moreover, *in vivo* experiments are required to confirm whether the favorable *in vitro* response observed here leads to improved osseointegration under physiological conditions. A limitation of the present study is that untreated PEEK was included only for qualitative SEM evaluation of the initial cell morphology. In contrast, quantitative biological analyses (confocal microscopy and proliferation assay) were performed exclusively for the four optimized laser-textured surfaces. Consequently, although the relative biological performance of the different laser-generated surface architectures could be compared, the magnitude of the biological response with respect to control PEEK could not be quantified. Future studies will include untreated PEEK as a complete experimental control for all biological assays to enable direct quantitative assessment of the effects of femtosecond laser surface structuring on cell attachment, proliferation, and osteogenic behavior.

The results indicate that femtosecond laser-induced hierarchical structuring provides an efficient means of overcoming the biological inertness of PEEK. By controlling the surface architecture, it is possible to create microenvironments that support cell adhesion, orientation and proliferation while maintaining the intrinsic advantages of the polymer. These findings highlight the potential of ultrafast laser processing for the development of advanced PEEK implants intended for orthopedic applications.

5. Conclusions

Hierarchical microstructured surfaces were successfully produced on PEEK using femtosecond laser irradiation under different scanning conditions. The resulting surface architectures exhibited well-defined topographical features and increased roughness compared with untreated PEEK. The degree of surface development depended strongly on the processing parameters, with lower scanning speeds producing more pronounced peak–valley morphologies. Laser texturing induced changes in wettability, leading to increased water contact angles as a consequence of the hierarchical surface organization. Nevertheless, all laser-treated surfaces supported DPSC adhesion and proliferation. Cells displayed normal morphology and cytoskeletal organization, confirming the cytocompatibility of the modified substrates. Although Group 3 presented the highest roughness, its proliferative response was less pronounced, suggesting that excessive surface complexity may not be advantageous and that an optimal topographical range exists.

Taken together, the results demonstrate that femtosecond laser processing represents a precise and chemical-free approach for improving the surface characteristics of PEEK while preserving its bulk properties. The generated hierarchical surface architectures supported DPSC attachment and proliferation *in vitro* and may provide favorable conditions for the early stages of cell–material interactions that are essential for subsequent bone regeneration and long-term osseointegration. Further studies focused on osteogenic differentiation and *in vivo* performance are required to fully assess the clinical potential of these laser-engineered surfaces.

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Abbreviations

The following abbreviations are used in this manuscript:

PEEK	polyetheretherketone
fs	femtosecond
SEM	scanning electron microscopy
WCA	water contact angle
DPSCs	dental pulp stem cells
MSC	mesenchymal stem cell
XRD	X-ray diffraction

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