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Biocompatibility of curcumin-activated platelet-rich fibrin: an in vitro study

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Abstract

Objectives

To assess the short-term cytocompatibility of curcumin-activated platelet-rich fibrin (C-PRF) compared with conventional platelet-rich fibrin (PRF) using HFF-2 human fibroblasts.

Materials and Methods

One venous blood preparation from a healthy volunteer was used to prepare conventional PRF and C-PRF. Commercial curcumin powder containing 95% curcuminoids was solublized in dimethyl sulfoxide (DMSO) to obtain a nominal 100 mM stock. Working solutions of 1–20 mM was prepared, and 0.5 mL of the 20 mM solution was added to 8.5 mL blood before PRF centrifugation. PRF and C-PRF were prepared as 100 mg/mL (w/v) stocks in culture medium and diluted to 6.25–100 µg/mL. HFF-2 cells were exposed for 24 h and assessed by MTT and phase-contrast microscopy. Duplicate wells were technical replicates.

Results

Mean observed viability for PRF was 98.23%, 95.37%, 82.81%, 41.89% and 29.33% at 6.25, 12.5, 25, 50 and 100 µg/mL, respectively; corresponding C-PRF values were 99.15%, 97.38%, 90.49%, 80.92% and 37.56%. C-PRF showed higher observed viability at every concentration, with the largest difference at 50 µg/mL. No inferential p-values were calculated because only one biological preparation was tested.

Conclusions

C-PRF showed higher observed fibroblast metabolic activity than conventional PRF in this single-preparation experiment, particularly at 50 µg/mL. The findings provide preliminary evidence of short-term cytocompatibility but do not demonstrate enhanced periodontal regeneration.

Clinical Relevance:

C-PRF warrants further investigation using multiple independent blood donors, repeated biological preparations and periodontal cell models before regenerative or clinical conclusions are drawn.

Introduction

Periodontitis is a chronic inflammatory disease that can result in loss of the alveolar bone, cementum and periodontal ligament. Regenerative periodontal therapy aims to regenerate these tissues rather than

merely arrest disease progression. Current regenerative approaches include guided tissue regeneration, grafting procedures, enamel matrix derivatives and biologically active materials. Their outcomes can vary according to defect characteristics, host response and the biological properties of the regenerative material [1].

Platelet-rich fibrin (PRF) is an autologous platelet concentrate prepared without anticoagulants. The resulting fibrin network contains platelets and leukocytes and can serve as a biologically active scaffold. PRF has been investigated for tissue healing and periodontal regeneration because its fibrin architecture and cellular components provide a local environment for the release of mediators involved in wound repair [2, 5]. In vitro studies have also reported effects of PRF-derived preparations on fibroblast viability and proliferation [7].

Curcumin is a polyphenolic component of turmeric (Curcuma longa) that has been investigated for anti-inflammatory, antioxidant and antimicrobial activities. Experimental studies have also examined its effects on fibroblast responses and osteogenic differentiation. Curcumin has been reported to modulate wound-healing-associated signalling in human gingival fibroblasts and to influence osteogenic differentiation of human periodontal ligament stem cells [4, 9]. However, these findings concern curcumin itself or other experimental systems and cannot be assumed to demonstrate an effect of curcumin incorporated into PRF.

Combining curcumin with PRF could theoretically provide a fibrin-based delivery matrix together with the biological activity associated with curcuminoids. Before such a combination can be considered for periodontal applications, its short-term cellular compatibility needs to be characterized. The present study therefore compared conventional PRF and curcumin-activated PRF (C-PRF) using HFF-2 human fibroblasts. Cell metabolic activity was assessed using the MTT assay, and cell morphology was examined qualitatively by phase-contrast microscopy. The study was designed as a preliminary single-preparation experiment and was not intended to demonstrate periodontal regeneration.

Materials and Methods

Study design and ethical considerations

This ex vivo experimental study compared conventional PRF with curcumin-activated PRF (C-PRF) in an in vitro fibroblast model. The study was approved by the Institutional Ethical Committee of SRM Kattankulathur Dental College and Hospital (Ethics Approval No. SRMIEC-ST0526-8394). The procedures were conducted in accordance with the ethical principles of the Declaration of Helsinki and its later amendments. Written informed consent was obtained from the volunteer before venous blood collection.

Preparation of curcumin solution

Commercially available curcumin powder (Saptamveda Premium Curcumin; SAPTAMVEDA, India; manufacturer part/model number S. Curcumin. new; ASIN B08HV2CC89) was used. According to the manufacturer's product information, the product contains 95% curcuminoids. The preparation procedure was adapted from Chang et al. [3]. Briefly, a nominal 100 mM curcumin stock solution was prepared by solubilizing curcumin powder in dimethyl sulfoxide (DMSO). The stock solution was stored at − 20°C in amber-colored containers and protected from light. Immediately before use, working solutions at 1, 2, 5, 10, 15 and 20 mM were prepared by serial dilution of the stock solution using DMSO [3].

Preparation of conventional PRF and C-PRF

Peripheral venous blood (17 mL) was collected without anticoagulant from a systemically healthy, non-smoking 25-year-old male volunteer. An aliquot of 8.5 mL was used for conventional PRF. For C-PRF, 0.5 mL of the 20 mM curcumin working solution was added to 8.5 mL of freshly collected blood, giving a nominal total mixture volume of 9.0 mL. The mixture was immediately transferred to a sterile 10 mL vacuum tube and centrifuged under the same conditions as the conventional PRF sample. Both preparations were centrifuged using a REMI R-4C tabletop centrifuge at 2700 rpm (approximately 145 × g) for 12 min at 22°C. The PRF clots were then separated from the red-cell fraction and processed for cell-culture testing [5]. Based on the nominal concentration of the added working solution, the blood-curcumin mixture contained approximately 1.11 mM curcumin before centrifugation. If the 20 mM working solution was prepared entirely in DMSO, the corresponding DMSO concentration in the 9.0 mL mixture was 5.56% (v/v). The post-centrifugation curcumin concentration within the C-PRF was not chemically quantified.

The sequential blood collection, curcumin addition, centrifugation and PRF clot-processing steps are illustrated in Fig. 1.

Cell culture and MTT assay

HFF-2 human dermal fibroblast cells were procured from the National Centre for Cell Science (NCCS), Pune, India. Cells were propagated in 25-cm² vented flasks in Dulbecco's Modified Eagle Medium (DMEM; Invitrogen, USA) containing 10% fetal bovine serum (FBS; Thermo Fisher Scientific, USA) and 1% penicillin–streptomycin (Life Technologies, USA). Cultures were maintained under standard incubation conditions of 37°C and 5% CO_2 in a humidified environment. Once the cultures attained approximately 70–80% confluence, the fibroblasts were transferred to 96-well plates at a seeding density of 1×10^4 cells per well and allowed to remain undisturbed for 48 h to facilitate formation of a consistent cellular monolayer [6].

Conventional PRF (Sample C) and C-PRF (Sample T) were weighed separately and prepared as stock solutions at 100 mg/mL (w/v) using culture medium. Each stock was serially diluted with culture

medium to obtain 6.25, 12.5, 25, 50 and 100 µg/mL. Untreated cells maintained under the same culture conditions served as the negative control. Cells were exposed to each preparation for 24 h.

Following the treatment period, 50 µL of MTT solution (5 mg/mL prepared in phosphate-buffered saline; Sigma-Aldrich, USA) was dispensed into each well. The plates were then maintained at 37°C for 2 h to allow formation of formazan. Subsequently, the culture medium was aspirated, and the resulting formazan crystals were solubilized by adding 150 µL of DMSO. Optical density was measured at 570 nm using a TECAN Spark microplate reader, as specified in the instrument output. Cell viability was expressed as a percentage relative to the untreated control and calculated using the following equation: Cell viability (%) = [absorbance of treated cells / absorbance of untreated control cells] × 100.

Morphological assessment

Following the 24-h exposure period and before the MTT endpoint, fibroblast morphology was examined by phase-contrast microscopy. Cell density, adherence and general fibroblast-like morphology were assessed qualitatively. Representative photomicrographs were acquired at the tested concentrations. Each image included a 50 µm scale bar (Fig. 2).

Experimental replication and statistical analysis

The experiment was performed once using one biological PRF preparation and one biological C-PRF preparation obtained from the same blood collection. Two wells per concentration were used as technical replicates. Accordingly, the wells were not treated as independent biological observations. Cell viability was summarized descriptively as mean ± standard deviation of the technical duplicates. No inferential statistical test or p-value was calculated because independent biological replicates were not available. The untreated control was normalized to 100%. A 70% viability threshold was used descriptively as a commonly applied cytotoxicity reference consistent with ISO 10993-5; this threshold was not used for hypothesis testing.

Results

Cell viability

The raw plate-reader output contained duplicate absorbance measurements for each concentration. After normalization to the untreated control, the observed mean cell viability for conventional PRF was 98.23 ± 0.60%, 95.37 ± 0.52%, 82.81 ± 0.52%, 41.89 ± 0.26% and 29.33 ± 0.26% at 6.25, 12.5, 25, 50 and 100 µg/mL, respectively. The corresponding values for C-PRF were 99.15 ± 0.35%, 97.38 ± 1.12%, 90.49 ± 0.69%, 80.92 ± 0.26% and 37.56 ± 0.52%. Thus, C-PRF showed a higher observed mean viability than conventional PRF at all tested concentrations, with the largest absolute difference at 50 µg/mL (approximately 39 percentage points). At 50 µg/mL, the C-PRF preparation remained above the 70% reference level, whereas conventional PRF was below it. At 100 µg/mL, both preparations were below

70% (Table 1; Fig. 2& 3). Because only one biological preparation was tested, these differences are descriptive and do not establish statistical significance.

Table 1
Descriptive cell viability of HFF-2 fibroblasts after 24 h exposure to conventional PRF and C-PRF.

Concentration (µg/mL)	Conventional PRF Mean ± SD (%)	C-PRF Mean ± SD (%)	Descriptive observation
0 (untreated control)	100.00	100.00	Reference
6.25	98.23 ± 0.60	99.15 ± 0.35	Both ≥ 70%
12.5	95.37 ± 0.52	97.38 ± 1.12	Both ≥ 70%
25	82.81 ± 0.52	90.49 ± 0.69	Both ≥ 70%
50	41.89 ± 0.26	80.92 ± 0.26	C-PRF ≥ 70%; PRF < 70%
100	29.33 ± 0.26	37.56 ± 0.52	Both < 70%

Values are descriptive means ± SD of duplicate technical wells from a single biological preparation. No inferential p-values were calculated. The 0 µg/mL condition represents the untreated control normalized to 100%.

Morphological observations

Untreated cells displayed predominantly adherent, elongated or spindle-shaped fibroblast-like morphology with a dense monolayer. At 6.25 and 12.5 µg/mL, both PRF and C-PRF preparations showed broadly preserved fibroblast-like morphology and cellular density. At 25 µg/mL, the cultures remained comparatively dense, although focal areas of altered morphology were visible. At 50 and 100 µg/mL, greater areas of reduced density, cellular rounding/retraction and altered morphology were observed, particularly in the conventional PRF-treated cultures. The C-PRF cultures appeared to retain greater cellular density than conventional PRF at the corresponding concentrations. These observations were qualitative and were not subjected to quantitative image analysis (Fig. 2).

Discussion

This preliminary in vitro study compared conventional PRF with a curcumin-activated formulation using HFF-2 human fibroblasts. The main observation was that C-PRF produced higher observed cell metabolic activity than conventional PRF at each tested concentration, with the largest difference at 50 µg/mL. The morphological images showed a broadly similar pattern: lower concentrations retained a denser fibroblast-like appearance, whereas higher concentrations were associated with greater morphological alteration. Because the experiment contained only one biological preparation, these observations should be considered exploratory rather than evidence of a reproducible treatment effect.

PRF provides a fibrin-based biological matrix containing platelets and leukocytes and has been investigated for its effects on tissue repair and fibroblast responses [2, 5, 7]. The present experiment extends this concept by incorporating a commercially available curcuminoid-containing preparation during PRF formation. The higher observed viability of C-PRF at the tested concentrations, particularly at 50 µg/mL, suggests that incorporation of the curcuminoid preparation did not produce an obvious additional loss of short-term fibroblast metabolic activity in this experiment. However, the experiment cannot determine whether the observed difference resulted from curcuminoids themselves, interactions between curcuminoids and the fibrin matrix, altered release of PRF-derived factors, or other components of the commercial preparation.

A possible biological basis for the observed difference may involve the reported effects of curcuminoids on oxidative stress, inflammatory signalling, and cellular responses. Curcumin has been shown to modulate wound-healing-associated signalling in human gingival fibroblasts and to influence osteogenic differentiation of periodontal ligament stem cells [4, 9]. In the context of C-PRF, curcuminoids may also interact with the fibrin matrix or influence the release or activity of bioactive components present within PRF. Such interactions could potentially contribute to the higher metabolic activity observed with C-PRF, particularly at 50 µg/mL. However, these explanations remain mechanistic hypotheses because oxidative stress, apoptosis, proliferation, growth-factor release, and intracellular signalling pathways were not assessed in the present study. Therefore, the observed MTT response should not be interpreted as evidence of a specific causal mechanism. Future studies incorporating molecular and functional endpoints will be necessary to determine whether the observed cytocompatibility is associated with modulation of oxidative, inflammatory, or regenerative signalling pathways.

Previous work has demonstrated that curcumin can influence cellular responses in several experimental systems. Chang et al. evaluated curcumin dissolved in DMSO and examined concentration-dependent effects on osteosarcoma and healthy osteoblast cells [3]. Rujirachotiwat and Suttamanatwong reported effects of curcumin on growth-factor-related signalling in a human gingival fibroblast wound-healing model [9], while Shi et al. reported that osteogenic potential were observed after exposure to curcumin on osteogenic differentiation of human periodontal ligament stem cells [4]. These studies provide biological context for investigating curcumin-containing biomaterials, but they should not be interpreted as direct evidence that the present C-PRF formulation has regenerative effects.

The concentration-dependent reduction in MTT-derived metabolic activity at higher concentrations is also relevant. Conventional PRF fell below the 70% cytotoxicity reference at 50 and 100 µg/mL, whereas C-PRF remained above that reference at 50 µg/mL but not at 100 µg/mL. ISO 10993-5 describes a reduction in cell viability of more than 30% compared with the control as a cytotoxicity criterion for the relevant in vitro cytotoxicity framework [10]. In the present study, however, this threshold is used only as a descriptive reference because the experiment had a single biological preparation and therefore cannot provide a reliable estimate of population-level variability.

Several methodological limitations are important. First, the study used one blood collection and one PRF/C-PRF preparation, with duplicate wells serving only as technical replicates. Consequently, the findings cannot be generalized to biological variability among donors, and no valid inferential comparison between treatments can be made from the present experiment. Second, HFF-2 cells are a dermal/foreskin fibroblast line and do not reproduce the phenotype or behaviour of periodontal ligament or gingival fibroblasts. Third, the MTT assay reflects cellular metabolic activity rather than a direct measurement of proliferation, differentiation or tissue regeneration. Fourth, the curcumin product was a commercial preparation reported by the manufacturer to contain 95% curcuminoids; the individual curcuminoid composition and the amount of curcuminoid retained within the C-PRF after centrifugation were not chemically quantified. Finally, the nominal DMSO exposure during C-PRF preparation was not independently assessed for biological effects. These limitations warrant replication using multiple independent donors and controlled curcumin preparations.

Future studies should include multiple independent blood donors and repeated biological preparations, appropriate vehicle controls, and chemically characterized curcumin or curcuminoid preparations. Additional endpoints such as live/dead staining, apoptosis, proliferation, extracellular-matrix production, migration and osteogenic markers could help distinguish cytocompatibility from functional regenerative effects. Studies using periodontal ligament and gingival fibroblasts, followed by appropriate preclinical models, would be required before clinical implications can be considered.

Conclusion

In this single-preparation in vitro experiment, C-PRF showed higher observed HFF-2 fibroblast metabolic activity than conventional PRF across the tested concentrations, with the greatest difference at 50 µg/mL. Lower concentrations were associated with broadly preserved fibroblast morphology, whereas higher concentrations showed greater morphological alteration and reduced metabolic activity. These findings provide preliminary evidence of short-term cellular compatibility but do not demonstrate enhanced periodontal regeneration. Replication using multiple independent biological preparations and clinically relevant periodontal cell models is required.

Declarations

Author Contribution

Ram Avinash Babu: Conceptualization, methodology, experimental investigation, data analysis, interpretation of results, writing—original draft, writing—review and editing. Kalaivani Varatharajan: Conceptualization, methodology, interpretation, validation, supervision, writing—original draft, writing—review and editing. Saravanan AV: Conceptualization, interpretation, supervision, writing—review and editing. Prem B. Rajula M: Conceptualization, interpretation, supervision, writing—review and editing. Chandrika Ravichandran: Sample preparation, experimental investigation, interpretation, writing—review and editing. Harshawarthini Murugan: Sample preparation, experimental investigation, interpretation,

writing—review and editing. Divya V C: writing—review and editing. Sibyl Siluvai: writing—review and editing. Anju s Sreedhar: writing—review and editing. Pranavi J: writing—review and editing. All authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

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Data Availability

The raw plate-reader dataset and derived cell-viability calculations may be shared by the corresponding author upon reasonable request, provided that institutional and ethical requirements are met.

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Figures

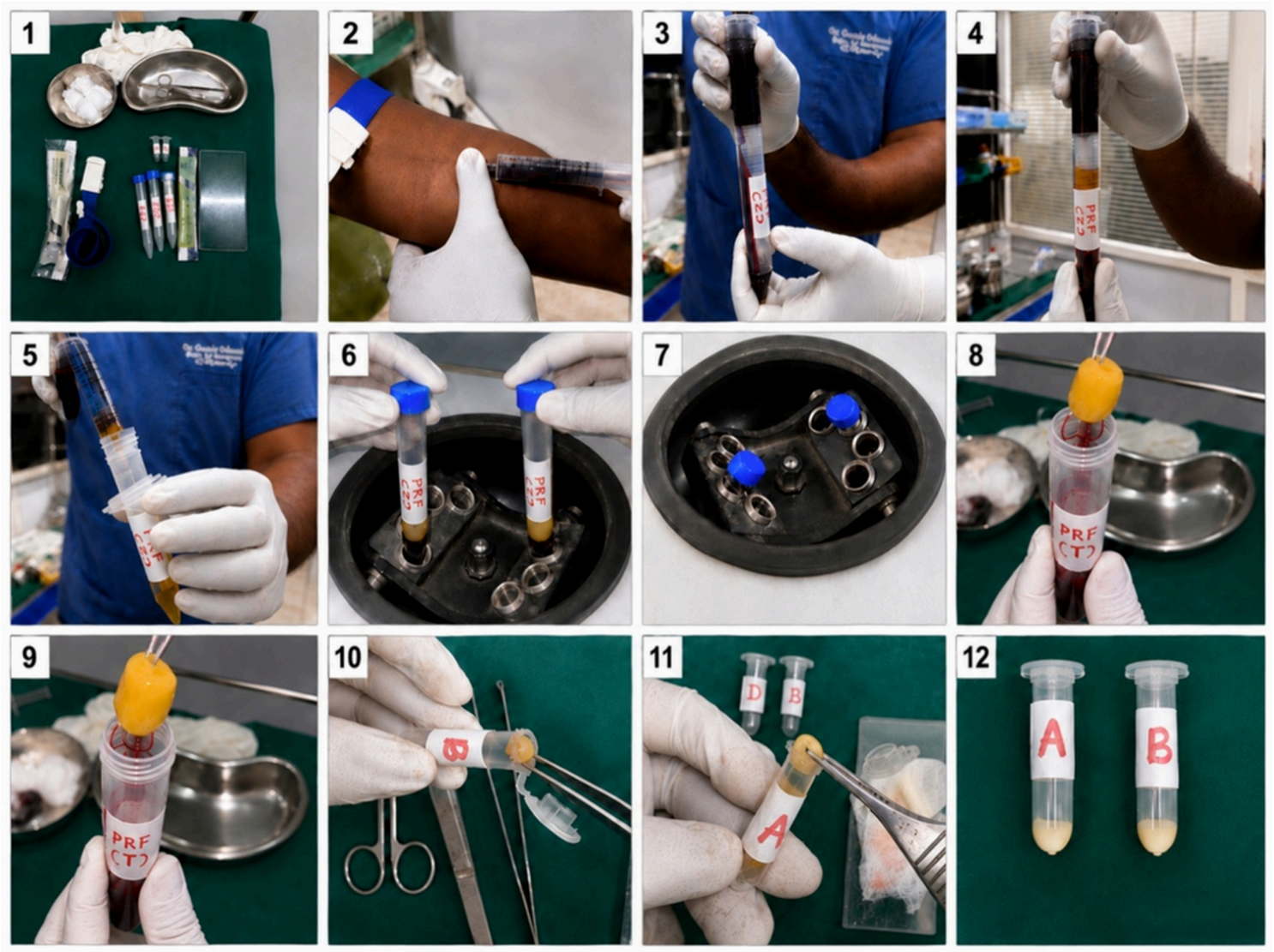


Figure 1

Sequential photographic workflow showing venous blood collection, preparation of conventional PRF and curcumin-activated PRF (C-PRF), centrifugation, retrieval of the fibrin clot and subsequent processing of the PRF preparations. Panels are numbered 1–12 according to the sequence of the experimental procedure.

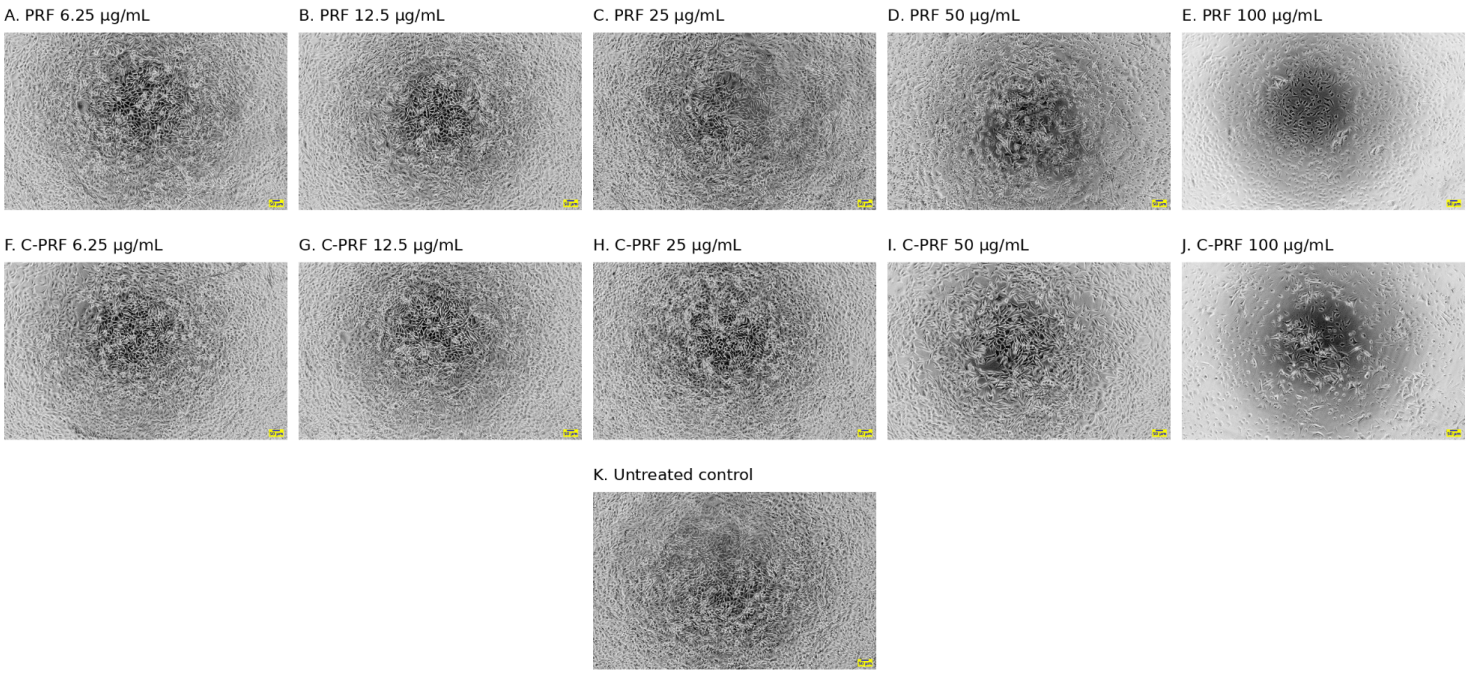


Figure 2

Representative phase-contrast micrographs of HFF-2 fibroblasts after 24 h exposure to conventional PRF (A–E) and C-PRF (F–J) at 6.25, 12.5, 25, 50 and 100 µg/mL, respectively; untreated control (K). Images were acquired with a 50 µm scale bar. Morphological findings are qualitative.

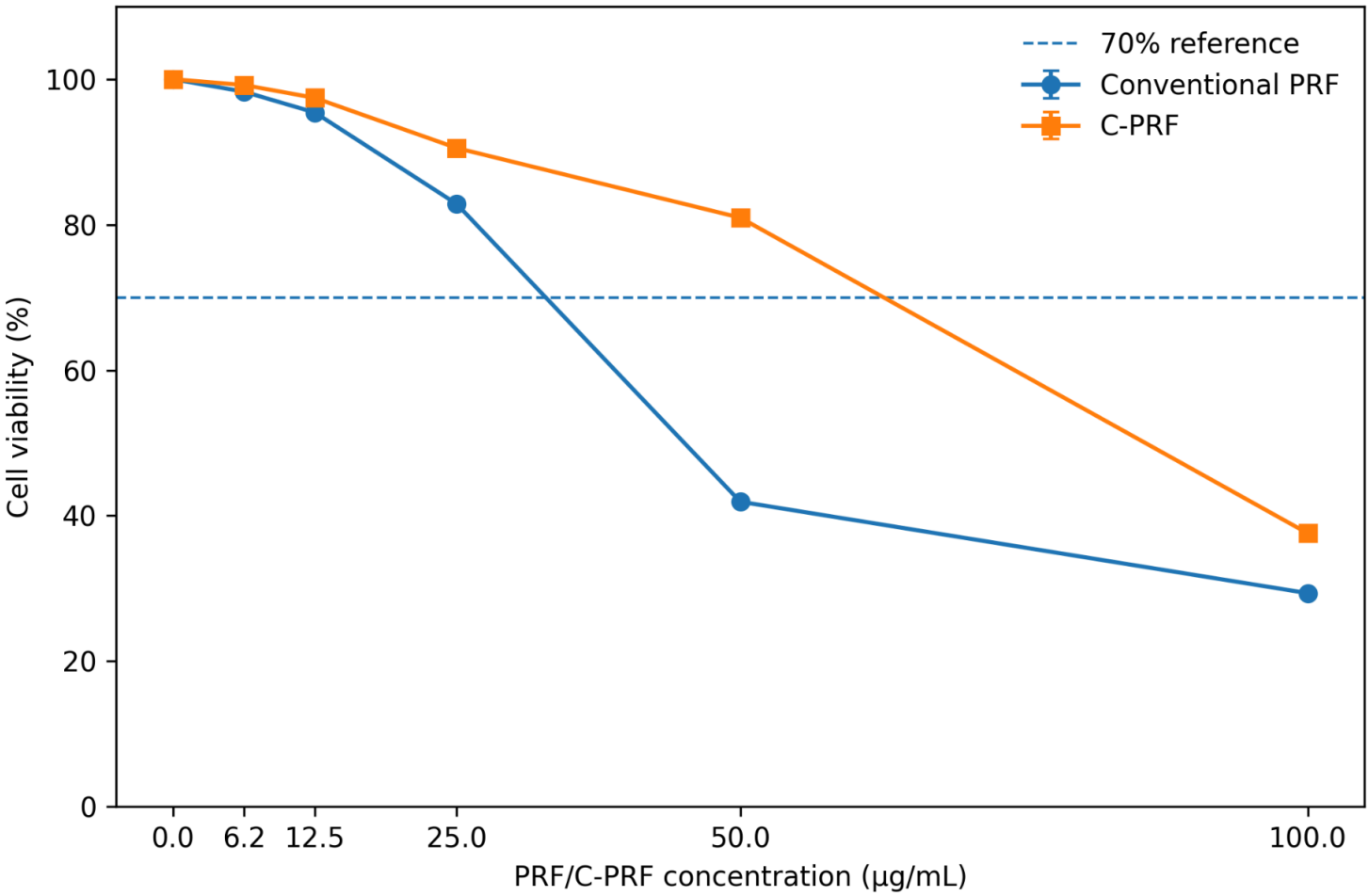


Figure 3

Descriptive cell viability of HFF-2 fibroblasts after 24 h exposure to conventional PRF and C-PRF. Points represent means of duplicate technical wells and error bars represent SD. The dashed line indicates the 70% cytotoxicity reference. No inferential statistical comparison was performed.