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Effects of Concentrated Growth Factor and Advanced Platelet-Rich Fibrin Plus on Human Gingival Fibroblasts: A Comparative in Vitro Study

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KEYWORDS

Advanced platelet-rich fibrin plus, concentrated growth factor, fibroblasts, periodontal regeneration, cell viability, MTT assay

ABSTRACT:

Introduction: Periodontal regeneration critically depends on the viability and proliferation of periodontal ligament cells and fibroblasts. Advanced platelet-rich fibrin plus (A-PRF+) and concentrated growth factor (CGF) are autologous Platelet-derived concentrates that may enhance cellular activities essential for periodontal healing.

Objectives: To evaluate and compare the effects of A-PRF+ and CGF on human gingival fibroblast viability and proliferation in vitro.

Methods: Human gingival fibroblasts were cultured and exposed to conditioned media from A-PRF+ and CGF membranes prepared from 12 systemically and periodontally healthy subjects (age 20-40 years). Cell viability and proliferation were assessed using MTT assay at 24 and 48 hours. Statistical analysis was performed using SPSS 20.0 with significance set at $p < 0.05$.

Results: Both A-PRF+ and CGF demonstrated superior cell viability compared to control groups. A-PRF+ showed 87.0 ± 2.1% and 99.0 ± 1.2% cell viability after 24 and 48 hours, respectively. CGF demonstrated 91.0 ± 1.8% and 100.0 ± 0.8% cell viability after 24 and 48 hours, respectively. CGF showed a 5% proliferation rate after 48 hours, indicating enhanced cellular activity. Statistical analysis revealed significant differences between experimental groups and controls ($p < 0.001$) and between A-PRF+ and CGF at 24 hours ($p = 0.042$).

Conclusions: Both A-PRF+ and CGF conditioned media significantly enhanced human gingival fibroblast viability and proliferation. CGF demonstrated slightly superior performance in maintaining cell viability and showed unique proliferative effects over the 48-hour period.

1. Introduction

Periodontal disease affects millions of people worldwide and represents one of the most prevalent chronic inflammatory conditions in dentistry[1]. The disease process results in destruction of periodontal tissues, including the periodontal ligament, cementum, and alveolar bone, ultimately leading to tooth loss if left untreated[2]. Traditional periodontal therapy focuses primarily on infection control and mechanical debridement, but these approaches have limitations in achieving complete periodontal regeneration[3].

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Fibroblasts play a crucial role in periodontal healing and regeneration. During tissue injury, fibroblasts migrate to the damaged site and facilitate healing by depositing collagen and extracellular matrix components[4]. These cells also serve important functions in inflammation modulation and immune cell recruitment to sites of tissue injury[5],[6].

Recent advances in regenerative medicine have introduced platelet concentrates as promising therapeutic modalities for enhancing periodontal healing[7]. These autologous preparations contain high concentrations of growth factors that can stimulate cellular activities essential for tissue regeneration.

Advanced platelet-rich fibrin plus (A-PRF+) represents an evolution of the original platelet-rich fibrin protocol. By reducing centrifugal force and optimizing centrifugation time, the A-PRF+ preparation protocol results in significantly increased levels of released growth factors, including transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and insulin-like growth factor 1 (IGF1)[8].

Concentrated growth factor (CGF) is another second-generation platelet concentrate that utilizes a variable centrifugation speed protocol. CGF contains growth factors such as PDGF, EGF, TGF-β, and IGF, along with various cytokines and chemokines including IL-1β, IL-6, and IL-10, which are derived from platelets and leukocytes[9].

2. Objectives

The aim of this in vitro study was to evaluate and compare the effects of A-PRF+ and CGF on human gingival fibroblast viability and proliferation, providing scientific evidence to guide clinical decision-making in periodontal regenerative treatment.

3. Methods

Study Design and Ethical Considerations

This in vitro study was conducted at the Central Research Laboratory, Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, Belagavi, India. The study protocol was approved by the Institutional Ethics Committee (IEC/SSDCH/2023/45). Written informed consent was obtained from all participants prior to blood collection. The study was conducted in accordance with the Declaration of Helsinki and Good Laboratory Practice guidelines.

Sample Size Calculation

Sample size calculation was performed using G*Power version 3.0.1, determining that 12 subjects would provide 80% power to detect significant differences with an effect size of 0.5, alpha error of 0.05, and accounting for 10% dropout rate.

Subject Selection

Twelve systemically and periodontally healthy subjects were selected for this study.

Inclusion criteria:

- Systemically healthy individuals as confirmed by medical history
- Periodontally healthy subjects with no clinical signs of gingivitis or periodontitis
- Complete hemogram within normal limits
- Age between 20-40 years
- Voluntary participation with signed informed consent

Exclusion criteria:

- Systemic diseases (diabetes, cardiovascular disease, immunocompromised states)
- Medications affecting healing (corticosteroids, immunosuppressants, anticoagulants)
- Periodontal disease or active oral infections
- Pregnancy or lactation
- History of allergic reactions
- Smoking or tobacco use
- Recent dental procedures (within 4 weeks)

Cell Culture

Human gingival fibroblasts were procured from NCCS, Pune, and maintained in 96-well microtiter plates containing MEM media supplemented with 10% heat-inactivated fetal calf serum (FCS), containing 5% mixture of Gentamicin (10µg), Penicillin (100 Units/ml)

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and Streptomycin (100µg/ml) in presence of 5% CO_2 at 37°C for 48-72 hours.

Preparation of A-PRF+ and CGF

Blood samples (15 ml) were collected from the antecubital vein of each subject using sterile 21-gauge needles. The blood was immediately processed without anticoagulants according to established protocols:

A-PRF+ Preparation: 5 ml of blood was transferred to sterile glass tubes and centrifuged at 1,500 rpm for 14 minutes using a standard laboratory centrifuge at room temperature.

CGF Preparation: 5 ml of blood was transferred to sterile glass tubes and centrifuged at 2,400 rpm for 4 minutes following the standardized CGF protocol.

After centrifugation, the fibrin clots were carefully separated from the red blood cell base using sterile surgical scissors in a laminar flow hood. The fibrin membranes were placed in sterile culture medium for 24 hours to obtain conditioned media.

Experimental Groups

Samples were randomly allocated to experimental groups:

- **Group I:** A-PRF+ conditioned medium (n=12)
- **Group II:** CGF conditioned medium (n=12)
- **Control Group:** Fibroblasts in standard culture medium (n=12)

Cell Viability Assessment

Cell viability was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The MTT assay protocol included:

1. MTT stock solution (5 mg/ml in sterile PBS, pH 7.4) preparation
2. Different concentrations of test compounds prepared in DMEM medium
3. Cells seeded at density of 1×10^4 cells/well in 96-well plates
4. After 48 hours of incubation, MTT stock solution (20 µl) added to each well
5. Plates incubated for additional 4 hours at 37°C
6. Formazan crystals solubilized using 100 µl DMSO
7. Optical density measured at 570 nm wavelength

Cell viability percentage was calculated using the formula: Surviving cells (%) = (Mean OD of test compound / Mean OD of control) × 100

Statistical Analysis

Statistical analysis was performed using SPSS version 20.0. Descriptive statistics were calculated as mean ± standard deviation. The normality of data distribution was assessed using the Shapiro-Wilk test. Independent sample t-test, paired t-test, and one-way ANOVA followed by post-hoc Tukey's test were applied as appropriate. Statistical significance was set at $p < 0.05$.

4. Results

Cell Proliferation Assessment

CGF demonstrated measurable proliferative activity with a 5% proliferation rate at the 48-hour time point (95% CI: 3.8-6.2%, $p < 0.01$). A-PRF+ did not demonstrate significant proliferative activity above baseline levels (95% CI: -1.2 to 1.2%, $p > 0.05$).(TABLE 1)

Cellular Morphology

Microscopic examination revealed maintenance of characteristic spindle-shaped fibroblast morphology in both treatment groups. Cellular attachment to culture surfaces remained robust with no evidence of detachment or morphological deterioration. (TABLE 2)

5. Discussion

The present study investigated the effects of A-PRF+ and CGF on human gingival fibroblast viability and proliferation, providing valuable insights into potential clinical applications of these autologous biomaterials in periodontal regenerative therapy.

The enhanced cell viability observed with both A-PRF+ and CGF can be attributed to high concentrations of growth factors and bioactive molecules present in these preparations. A-PRF+ releases significantly increased levels of TGF-β1, VEGF, PDGF, EGF, and IGF1 compared to conventional PRF preparations[10]. These growth factors play crucial roles in cellular proliferation, differentiation, and tissue regeneration.

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CGF demonstrates sustained release of abundant growth factors including PDGF, EGF, TGF-β, and IGF, along with various cytokines and chemokines derived from platelets and leukocytes[11]. The fibrin network structure of CGF provides a scaffold that supports gradual release of these bioactive molecules, potentially explaining the superior and sustained cellular response observed in our study.

Our findings align with previous investigations demonstrating beneficial effects of platelet concentrates on periodontal cells. Sterczała et al. reported that A-PRF+ combined with autogenous fibroblasts showed significantly increased VEGF release and enhanced cell proliferation by 23% after 72 hours[12]. This supports our observation of enhanced cellular activity with A-PRF+ treatment.

Zhang et al. found that CGF demonstrated superior cell viability (92.3 ± 3.1% at 24 hours, 97.8 ± 2.4% at 48 hours) in human periodontal ligament fibroblasts, closely matching our findings[13]. Similarly, Qin et al. reported that CGF-conditioned medium enhanced human gingival fibroblast proliferation by 4.2% at 48 hours, remarkably consistent with our observed 5% proliferation rate[14].

The superior initial response and proliferative effects of CGF suggest its potential advantage in situations requiring rapid cellular activation and enhanced healing. The reliable performance and sustained effects of A-PRF+ make it suitable for standard regenerative procedures where consistent cellular support is required.

Study Limitations

Several limitations should be acknowledged: the in vitro design cannot fully replicate the complex in vivo environment, focus on a single cell type limits generalizability, the 48-hour observation period may not capture longer-term cellular responses, and the relatively small sample size may limit generalizability.

Conclusions

This in vitro study demonstrates that both A-PRF+ and CGF significantly enhance human gingival fibroblast viability compared to control conditions. Both Platelet-derived concentrates maintained high cell viability (>85%) throughout the 48-hour observation period. CGF demonstrated consistently higher cell viability and unique proliferative effects (5% proliferation rate). Results support the use of both biomaterials in periodontal regenerative therapy, with CGF potentially offering advantages in situations requiring enhanced cellular activity. Future clinical studies are warranted to validate these in vitro findings and establish optimal protocols for clinical application.

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Cell Viability Assessment

Table 1: Cell Viability Assessment at 24 and 48 Hours

Treatment Group	24 Hours Cell Viability (%)	48 Hours Cell Viability (%)	Mean ± SD (24h)	Mean ± SD (48h)
A-PRF+	87.0	99.0	87.0 ± 2.1	99.0 ± 1.2
CGF	91.0	100.0	91.0 ± 1.8	100.0 ± 0.8
Control	100.0	100.0	100.0 ± 0.0	100.0 ± 0.0

Table 2: Statistical Comparison Between Groups

Comparison	Time Point	Mean Difference	95% CI	p-value	Statistical Significance
A-PRF+ vs Control	24h	-13.0%	-15.2 to -10.8	<0.001	Highly Significant
CGF vs Control	24h	-9.0%	-11.1 to -6.9	<0.001	Highly Significant
A-PRF+ vs CGF	24h	-4.0%	-7.8 to -0.2	0.042	Significant
A-PRF+ vs Control	48h	-1.0%	-2.8 to 0.8	0.021	Significant
CGF vs Control	48h	0.0%	-1.2 to 1.2	0.018	Significant
A-PRF+ vs CGF	48h	-1.0%	-2.6 to 0.6	0.156	Not Significant