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Morphological, Functional and Biochemical Characterization of Canine Gingival Fibroblasts

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As dogs are good models for *in vivo* studies, it is interesting to evaluate the behavior of canine gingival fibroblasts (CGF) *in vitro*, so that these cells could be seeded on a matrix and later studied *in vivo*. The aim of this study was to perform a morphological, functional and biochemical analysis of CGF, comparing it with human gingival fibroblasts (HGF), as well as to evaluate the change of their characteristics over several passages. Using gingival fibroblasts from 3 dogs and 3 humans in the subculture (Sub), first (P1), third (P3), fifth (P5) and seventh (P7) passages, the following parameters were assessed: cell morphology, spreading, adhesion, viability and total protein content. The results showed no major differences between the passages in terms of morphology and spreading, and a tendency of greater adhesion and viability for HGF when compared with CGF. The total protein content was significantly higher for HGF. HGF exhibited greater functional and biochemical activity *in vitro* compared to CGF. Higher numbers at Sub were observed for both CGF and HGF in all evaluated parameters. The differences do not prevent the use of CGF for tissue engineering, but its use seems to be more appropriate in the subculture or first passage.

Key Words: fibroblasts, cell culture techniques, tissue engineering.

Introduction

Tissue engineering has been widely studied and its main objective is to obtain biological substitutes that improve, maintain or restore the function of damaged tissues and organs. Recent technological advances in tissue engineering suggest its application in several medical fields, including periodontology (1). The cell culture technique allows the study of the biological behavior of cells, considering the kinetics of cell proliferation and the biosynthesis of many components of the extracellular matrix (2).

The culture of fibroblasts is interesting for the development of a material to replace autogenous soft tissue, but it is necessary first a deep understanding of these cells. As part of a heterogeneous population of cells of mesenchymal origin, fibroblasts have a central role in the extracellular matrix remodeling. Under normal conditions, they are responsible for the production and maintenance of the connective tissue matrix (3), and are essential for gingival and periodontal health. When seeded, they can be used in esthetic and reconstructive surgery (4) to improve tissue repair in a variety of conditions.

The use of gingival fibroblast cultures *in vitro* is feasible due to the fact that these cells exhibit morphology and spatial distribution similar to the *in vivo* system (5). Moreover, as dogs are good models for *in vivo* studies, it is important to evaluate the behavior of canine gingival fibroblasts (CGF) *in vitro*, comparing them with human gingival fibroblasts (HGF), so that CGF could be further seeded on a matrix and studied *in vivo* in animal studies. The aims of this study were to perform a morphological, functional and biochemical CGF analysis *in vitro*, by comparing these cells with HGF, and also to analyze the change of these characteristics over several passages.

Material and Methods

Cell Culture

CGF and HGF, established in previous studies from healthy keratinized tissue by the explant technique (6) and stored in liquid nitrogen in the subculture, were used. These previous studies were approved by the Institutional Animal Research Committee (Protocol #06.1.634.53.8) and Ethics Committee (Protocol # 2007.1.1234.58.5). After being defrosted, cells in the subculture were transferred to 75 cm^2 flasks (Nunc, Roskilde, Denmark) containing Dulbecco's Modified Eagle Medium (DMEM; Gibco Invitrogen Corporation, Grand Island, NY, USA) supplemented with 10% of fetal bovine serum (FBS; Gibco BRL, Gaithersburg, MD, USA), 50 ug/mL of vancomycin (Acros Organics, Geel, Belgium), 10 mg/mL of gentamycin (Gibco Invitrogen) and 50 mg/mL of fungizone (Gibco Invitrogen). Cultures were maintained in humid atmosphere at 37°C and 5% CO_2, and medium was changed every 2 days. After reaching confluence, cells were harvested using a solution of trypsin and EDTA at 0.05% (Gibco Invitrogen) and plated on polystyrene or glass coverslips (Fisher Scientific), in 24-well-plates (Nunc) at a density of $2x10^4$ cells *per* well.

Cells were cultivated for up to 21 days. The remaining cells were transferred to 75 cm^2 flasks for the analysis of the next passages. The experiments were performed until the seventh passage.

Morphological Characterization

Cell morphology and stages of adhesion and spreading were assayed by direct fluorescence at 30 min, 4 and 24 h, as described by de Oliveira and Nanci (7). Briefly, cells seeded on glass coverslips were fixed using 4% paraformaldehyde in 0.1 M sodium phosphate buffer (PB), pH 7.2, for 10 min. Then, they were permeabilized with 0.5% Triton X-100 in PB for 10 min. Alexa fluor 488 (green fluorescence)-conjugated phalloidin (1:200) (Molecular Probes, Invitrogen, OR, USA), and 300 nM 4',6-diamidino-2-phenylindole, dihydrochloride (DAPI, Molecular Probes) were used to detect respectively the actin cytoskeleton and cell nucleus. After being mounted with an anti-fade kit (Vectashield; Vector Laboratories, Burlingame, CA, USA), the samples were examined under epifluorescence using a Leica DMLB light microscope (Leica, Bensheim, Germany), with HCX PL Fluotar (x40/0.75) objectives, outfitted with a Leica CD 300F digital camera. The acquired digital images were processed with Adobe Photoshop software version 7.0 (Adobe Systems Inc., San Jose, CA, USA).

According to Rajaraman et al. (8), to assess the stage of adhesion and spreading, the proportion of cells at stage 1 (round cells), 2 (round cells with filopodia), 3 (cells with cytoplasmic webbing), and 4 (well flattened cells) was qualitatively analyzed.

Functional Characterization

Cell Adhesion

Quantitative evaluation of cell adhesion was determined by hemocytometer at 30 min, 2 and 4 h. After each period, the culture medium was removed and the samples washed with phosphate buffered saline (PBS, Gibco) at 37° C to remove non-adherent cells. Then, cells were harvested, and the number of attached cells was determined using a hemocytometer (Hausser Scientific, Horsham, PA, USA) in a phase inverted microscope (Zeiss, Jena, Germany). Cell adhesion was expressed as a percentage of the initial cell number.

Total Cell Number

The total cell number was determined by hemocytometer at days 3, 7 and 10. The cells were enzymatically detached from polystyrene using 1 mL of 0.05% trypsin/EDTA (Gibco). The total number of cells/well was determined after Trypan blue (Sigma) staining using a hemocytometer (Hausser Scientific), as described for cell adhesion.

Cell Viability

Cell viability was evaluated by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) (Sigma-Aldrich) assay at days 3, 7 and 10. Cells were incubated with 10% MTT (5 mg/mL) in culture medium at 37 °C for 4 h. The medium was then aspirated from the well, and 1 ml of acid isopropanol (0.04 N HCl in isopropanol) was added to each well. The plates were then stirred on a plate shaker for 5 min, and 200 µL of this solution was transferred to a 96-well plate (Fisher Scientific, Pittsburgh, PA, USA). The optical density was read at 570 nm on the plate reader (µQuant; Bio-Tek Instruments Inc., Winooski, VT, USA) and data were expressed as absorbance.

Biochemical Characterization

The total protein content was determined by a modification of the Lowry method (9) at days 7, 14 and 21. Briefly, proteins were extracted from each well with 0.1% sodium lauryl sulphate (Sigma) for 30 min and mixed 1:1 with Lowry solution (Sigma) for 20 min at room temperature. The extract was diluted in Folin and Ciocalteau's phenol reagent (Sigma) for 30 min at room temperature. Absorbance was measured at 680 nm using a spectrophotometer (Cecil CE3021, Cambridge, UK). The total protein content was calculated from a standard curve and expressed as micrograms of protein per mililiter (µg/mL).

Statistical Analysis

Data presented in this study are the mean of the results of three sets of cultures for HGF and CGF, established from three different patients and dogs, respectively. All the experiments were carried out in quintuplicate (n=5). Comparisons were performed using the nonparametric Mann-Whitney U test, for independent samples, when comparing two groups, and Kruskal-Wallis, when comparing three or more independent groups, followed by Dunn's Method for multiple comparisons between two groups (significance level: 5%).

Results

HGF and CGF proliferated in parallel orientation, respecting the space between cell membranes. Under conditions of cell confluence, there was no stacking, disruption or cell death. The cells exhibited a fusiform shape with a central nucleus and typical cytoplasmic processes, which are typical morphological features of this cell type. HGF presented a higher speed to achieve cell confluence in the flasks.

Morphological analysis revealed the presence of cells in the stage 1 of adhesion and spreading (round cells) at 30 min (Fig. 1A, D); a greater number of cells attached at 2 h, ranging from stage 1 to 3 of adhesion (cells with cytoplasmic webbing) (Fig. 1B and 1E), and this number was even higher at 24 h, with all cells in advanced stages

of spreading (Fig. 1C and 1F). No differences were observed between passages in cell morphology and stages of cell adhesion and spreading. Comparing HGF and CGF, it was noted that HGF were apparently a little more spread and with a higher diameter at 24 h (Fig. 1C and 1F).

Count with the hemocytometer showed a statistically significant increase in HGF and CGF adhesion over the experimental periods in all the evaluated passages ($p>0.001$) and a decrease of cell adhesion in advanced passages, when compared with the subculture and initial passages (HGF - 30 min: $p<0.001$, 2 and 4 h: $p=0.007$; CGF – 10 min and 4h: $p<0.001$, 2 h: $p=0.004$) (Table 1, Fig. 2). A higher percentage of cell adhesion for HGF, compared to CGF, was observed in advanced passages, with statistically significant difference in P5 ($p<0.001$) and P7 ($p=0.020$) at 30 min (Fig. 2A), and in P5 at 2h ($p=0.011$) (Fig. 2B) and 4h ($p<0.001$) (Fig. 2C).

For the total cell number, a statistically significant increase was observed for both cell types over the

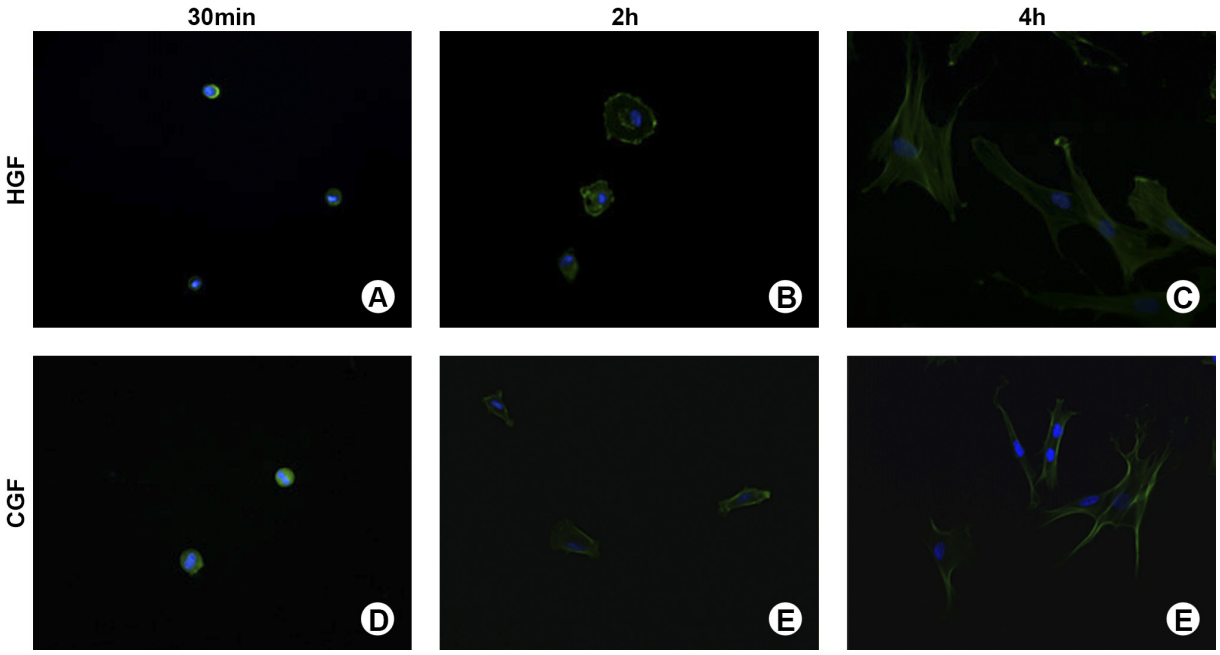


Figure 1. Epifluorescence of human gingival fibroblasts (HGF - A, B, C) and canine gingival fibroblasts (CGF - D, E, F), seeded on glass coverslips at 30 min (A, D), 4 h (B, E) and 24 h (C, F). Green fluorescence shows actin cytoskeleton, and blue fluorescence shows cell nuclei. Note the following: in A and D, the presence of cells in the stage 1 of adhesion; in B and E, cells ranging from stage 1 to 3 of adhesion; in C and F, the larger number of cells attached in advanced stages of spreading (40´ objective).

Table 1. Quantitative analysis (mean ± SD) of cell adhesion (%) of HGF and CGF at 30 min, 2 and 4 h

Passage	HGF				CGF			
	30 min	2 h	4 h	Intragroup Difference	30 min	2 h	4 h	Intragroup Difference
Sub	19 ± 8 abA	39 ± 17 aB	58 ± 21 aB	p<0.001	18 ± 6 aA	27 ± 6 abA	40 ± 4 adB	p<0.001
P1	23 ± 6 aA	31 ± 6 abA	54 ± 11 aB	p<0.001	20 ± 8 aA	30 ± 8 aA	51 ± 16 aB	p<0.001
P3	16 ± 6 abcA	34 ± 13 abB	46 ± 16 abB	p<0.001	13 ± 5 abA	27 ± 7 abA	43 ± 8 acB	p<0.001
P5	14 ± 4 bdA	29 ± 6 abB	45 ± 6 abC	p<0.001	9 ± 3 bA	21 ± 8 abB	33 ± 8 bdB	p<0.001
P7	11 ± 4 cdA	24 ± 5 bB	40 ± 6 bC	p<0.001	8 ± 3 bA	20 ± 4 bB	35 ± 7 cdC	p<0.001
Intergroup difference	p<0.001	p=0.007	p=0.007		p<0.001	p=0.004	p<0.001	

HGF: Human gingival fibroblasts. CGF: Canine gingival fibroblasts. Kruskal-Wallis one-way analysis of variance on ranks. Dunn's Method, significance level of 5%. Between-groups (a, b, c, d) and intragroup (A, B, C) comparisons - different letters indicate statistically significant difference.

experimental periods (p<0.001), and it was significantly higher in the subculture and initial passages for HGF and CGF (HGF - day 3: p=0.001, days 7 and 10: p<0.001; CGF - days 3, 7 and 10: p<0.001) (Table 2, Fig. 3). A higher total cell number was observed for HGF, when compared with CGF, with statistically significant difference in Sub (p=0.002), P3 (p=0.001), P5 (p<0.001) and P7 (p=0.005) at day 3 (Fig. 3A); Sub (p=0.028), P1 (p<0.001), P5 (p<0.001) and P7 (p<0.001) at day 7 (Fig. 3B); and P1 (p<0.001), P3 (p=0.029), P5 (p<0.001) and P7 (p<0.001) at day 10 (Fig. 3C).

MTT assay also showed a significant increase in cell viability over the experimental periods (p<0.001) for both cell types, CGF and HGF, and a significant decrease in cell viability in advanced passages (HGF - days 3, 7 and 10: p<0.001; CGF - day 3: p=0.004, days 7 and 10: p<0.001) (Table 3, Fig. 4). When comparing HGF and CGF, at day 3 it was observed a higher viability for HGF in Sub (p=0.011), P5 (p<0.001) and P7 (p=0.025) (Fig.4A); at day 7 in the Sub (p=0.021), P1 (p=0.001), P5 (p<0.001) and P7 (p<0.001) (Fig. 4B) and in all passages at day 10 (p<0.001) (Fig. 4C).

In all the passages and experimental periods evaluated, the total protein content was significantly higher for HGF than for CGF (day 7 - Sub: p=0.013, P1: p<0.001, P3: p=0.010, P5 and P7: p<0.001; day 14 - Sub: p=0.009, P1, P3, P5 and P7: p<0.001; day 21- Sub: p=0.018, P1, P3 P5 and P7: p<0.001) (Table 4, Fig. 5). For both cell types, HGF and CGF, a significant increase in the total protein content over the experimental periods was noted (HGF - Sub: p=0.003, P1, P3, P5 and P5: p<0.001; CGF - P1: p=0.003, Sub, P3, P5 and P7: p<0.001), while a significant decrease was observed along the passages (p<0.001) (Table 4, Figure 5).

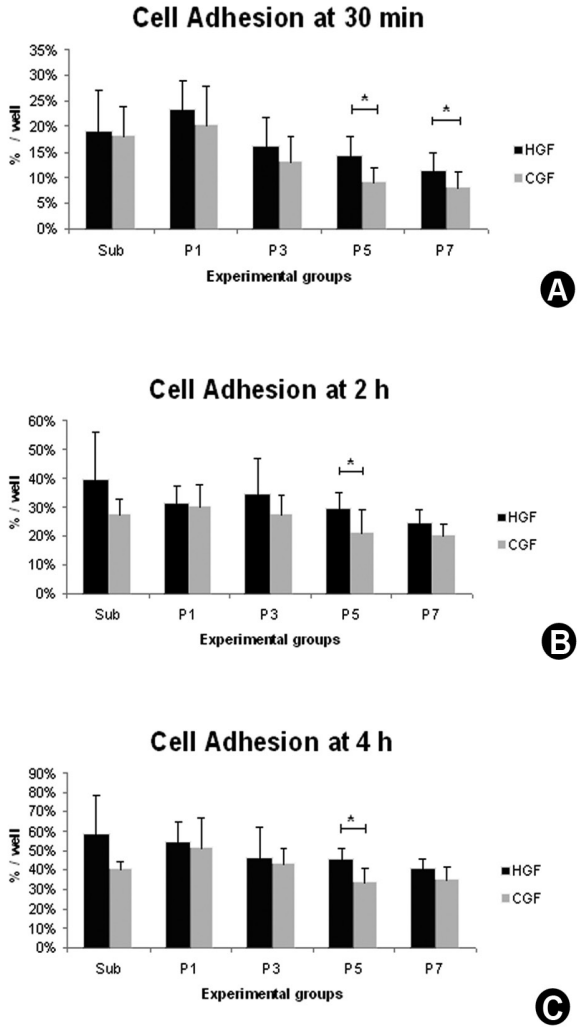
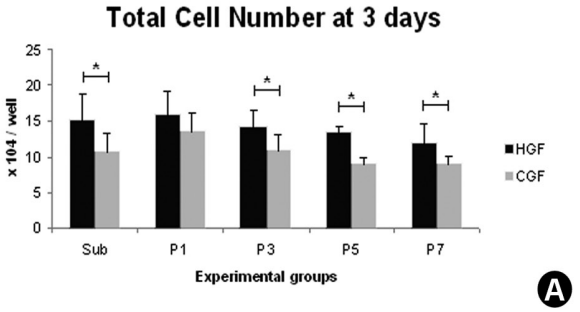


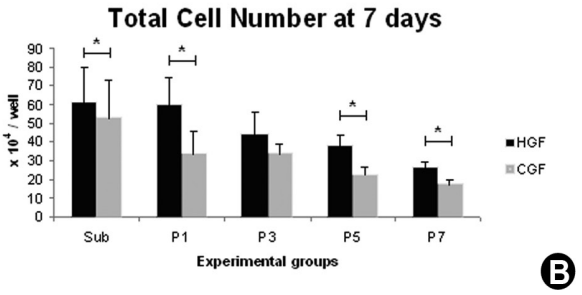
Figure 2. Cell adhesion of human gingival fibroblasts (HGF) and canine gingival fibroblasts (CGF) in the subculture (Sub) and passages 1 (P1), 3 (P3), 5 (P5) and 7 (P7) at 30 min (A), 2 h (B) and 4 h (C), expressed as a percentage of the initial number of cells. Data are reported as mean ± standard deviation. Asterisks (*) indicate p<0.05.

Table 2. Quantitative analysis (mean ± SD) of total cell number (x10⁴) of HGF and CGF at days 3, 7 and 10

Passage	HGF				CGF			
	3 days	7 days	10 days	Intragroup Difference	3 days	7 days	10 days	Intragroup Difference
Sub	15.1 ± 3.7 aA	61.0 ± 19.1 aB	76.0 ± 13.9 abB	p<0.001	10,6 ± 2.7 abA	52.3 ± 21.1 aB	67.3 ± 18.1 aB	p<0.001
P1	15.7 ± 3.6 aA	59.2 ± 15.3 aB	87.3 ± 19.4 aC	p<0.001	13.5 ± 2.8 aA	33.4 ± 12.6 abB	55.2 ± 12.6 aB	p<0.001
P3	14.0 ± 2.5 abA	43.3 ± 12.7 abB	57.1 ± 13.9 bB	p<0.001	10.8 ± 2.3 abA	33.4 ± 5.5 aB	47.3 ± 8.9 aB	p<0.001
P5	13.3 ± 1.1 abA	37.5 ± 6.3 bcB	56.3 ± 12.1 bC	p<0.001	8.9 ± 1.1 bA	21.9 ± 4.9 bB	35.3 ± 8.1 bC	p<0.001
P7	11.7 ± 2.9 bA	25.4 ± 3.5 cB	38.1 ± 4.1 cC	p<0.001	8.8 ± 1.4 bA	16.7 ± 3.0 cB	30.7 ± 2.5 bC	p<0.001
Intergroup difference	p=0.010	p<0.001	p<0.001		p<0.001	p<0.001	p<0.001	

HGF: Human gingival fibroblasts. CGF: Canine gingival fibroblasts. Kruskal-Wallis one-way analysis of variance on ranks. Dunn's Method, significance level of 5%. Intergroup (a, b, c, d) and intragroup (A, B, C) comparisons - different letters indicate statistically significant difference.





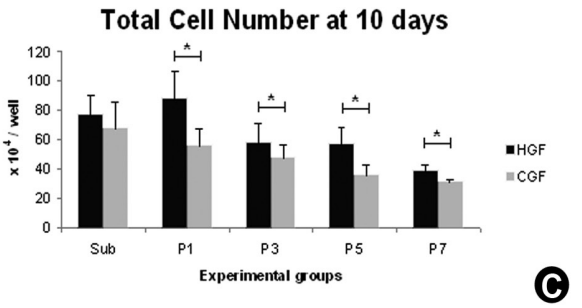
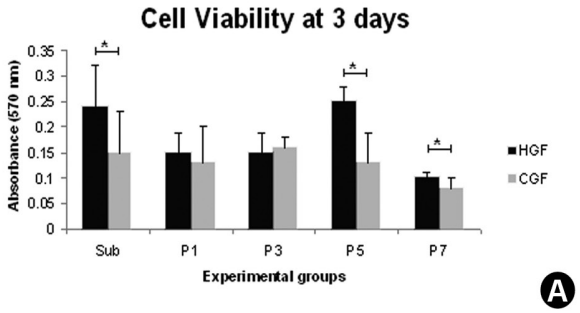
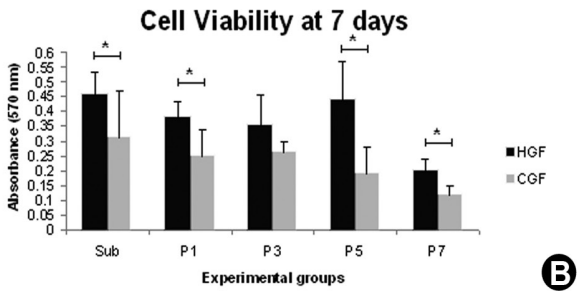


Figure 3. Total cell number of human gingival fibroblasts (HGF) and canine gingival fibroblasts (CGF) in the subculture (Sub) and passages 1 (P1), 3(P3), 5 (P5) and 7 (P7) at days 3 (A), 7 (B) and 10 (C). Data are reported as mean ± standard deviation. Asterisks (*) indicate p<0.05.





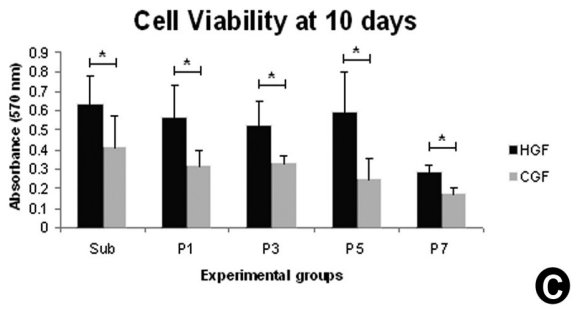


Figure 4. Cell viability of human gingival fibroblasts (HGF) and canine gingival fibroblasts (CGF) in subculture (Sub) and passages 1 (P1), 3 (P3), 5 (P5) and 7 (P7) at days 3 (A), 7 (B) and 10 (C), expressed as absorbance. Data are expressed as mean ± standard deviation. Asterisks (*) indicate p<0.05.

Table 3. Quantitative analysis (mean ± SD) of cell viability (absorbance, 570 nm) of HGF and CGF at days 3, 7 and 10

Passage	HGF				CGF			
	3 days	7 days	10 days	Intragroup difference	3days	7 days	10 days	Intragroup Difference
Sub	0.24 ± 0.08 abA	0.47 ± 0.75 aB	0.64 ± 0.15 aB	p<0.001	0.15 ± 0.09 abA	0.32 ± 0.17 aB	0.41 ± 0.17 aB	p<0.001
P1	0.15 ± 0.05 bcA	0.38 ± 0.06 aB	0.56 ± 0.17 aB	p<0.001	0.13 ± 0.08 abA	0.25 ± 0.09 aAB	0.31 ± 0.08 aB	p<0.001
P3	0.15 ± 0.05 cA	0.36 ± 0.12 aB	0.52 ± 0.14 aB	p<0.001	0.16 ± 0.02 aA	0.26 ± 0.04 aB	0.33 ± 0.04 aB	p<0.001
P5	0.25 ± 0.03 aA	0.45 ± 0.13 aB	0.59 ± 0.22 aB	p<0.001	0.14 ± 0.06 abA	0.19 ± 0.09 abAB	0.25 ± 0.11 abB	p<0.001
P7	0.10 ± 0.01 cA	0.21 ± 0.04 bB	0.29 ± 0.05 bB	p<0.001	0.08 ± 0.02 bA	0.13 ± 0.03 bB	0.17 ± 0.04 bB	p<0.001
Intergroup difference	p<0.001	p<0.001	p<0.001		p=0.004	p<0.001	p<0.001	

HGF: Human gingival fibroblasts. CGF: Canine gingival fibroblasts. Kruskal-Wallis one-way analysis of variance on ranks. Dunn's Method, significance level of 5%. Intergroup (a, b, c, d) and intragroup (A, B, C) comparisons - different letters indicate statistically significant difference.

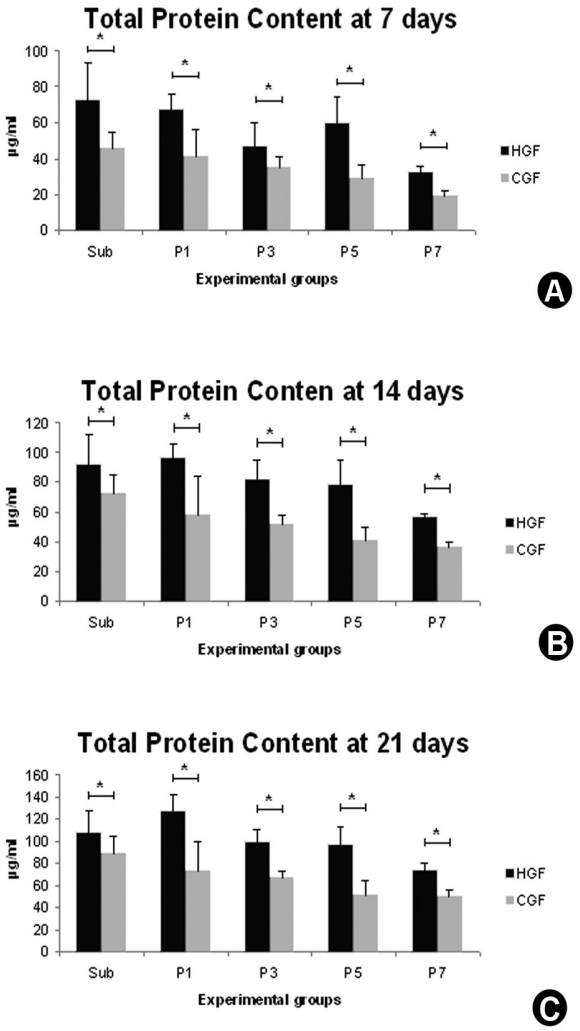


Figure 5. Total protein content of human gingival fibroblasts (HGF) and canine gingival fibroblasts (CGF) in subculture (Sub) and passages 1 (P1), 3(P3), 5 (P5) and 7 (P7) at days 7 (A), 14 (B) and 21 (C), expressed as micrograms of protein per mililiter (µg/mL). Data are expressed as mean ± standard deviation. Asterisks (*) indicate p<0.05.

Discussion

Tissue engineering comes into the research fields as a promising solution to repair and reconstruct lost tissues. In the last decades there have been great advances in this area, which led to its application in different medical fields, including periodontology. Some authors have been evaluating non-enzymatic methods of gingival fibroblasts isolation *in vitro* for the use in gingival augmentation (6,10-14). Gingival fibroblast culture in different matrices has been studied and has shown promising results in soft tissues regeneration (12-14). Fibroblasts are responsible for the normal growth of the organism and are essential in cases where tissue repairs are necessary, being one of the first cells to appear at injured sites. The current concept that both, gingival fibroblasts (15,16) and periodontal ligament fibroblasts (17-21), have the capacity to regenerate the periodontium has stimulated an interest in the development of clinical methods for the regularization of these cell types (6).

Cell transplantation in matrices has been explored as a way to create new human tissues (22). However, studies in animal models involving cell culture in a three-dimensional scaffold are necessary until this methodology is well established. In Dentistry, dogs have been used as a good model for *in vivo* studies. A cell-based therapy for oral tissues regeneration should use autogenous cells, because ingrowth of immunocompetent cells of the host organism might destroy allografts or xenografts (23). Therefore, to evaluate *in vivo* the efficiency of grafts obtained from three-dimensional cell culture techniques in dogs, it is important first to know how their cells behave *in vitro*, since they must be viable, have good spreading activity and maintain their ability to produce proteins in order to be successfully used in tissue engineering.

Table 4. Quantitative analysis (mean ± SD) of total protein content (µg/mL) of HGF and CGF at days 7, 14 and 21

Passage	HGF				CGF			
	7 days	14 days	21 days	Intragroup Difference	7 days	14 days	21 days	Intragroup Difference
Sub	72.4 ± 21.5 aA	91.5 ± 20.8 aAB	106.4 ± 21.0 abB	p=0.003	45.6 ± 08.9 aA	72.2 ± 12.9 aB	88.2 ± 16.0 aB	p<0.001
P1	66.5 ± 9.5 aA	95.9 ± 9.6 aB	125.9 ± 16.3 aC	p<0.001	41.0 ± 15.6 abA	58.1 ± 26.1 abAB	72.9 ± 27.0 abB	p=0.003
P3	46.2 ± 13.6 bA	81.0 ± 13.6 aB	98.6 ± 12.7 abB	p<0.001	34.3 ± 7.0 abA	51.2 ± 6.9 abB	66.5 ± 06.7 abC	p<0.001
P5	59.1 ± 15.5 acA	77.8 ± 17.4 aAB	95.0 ± 17.4 bB	p<0.001	28.6 ± 8.0 bcA	40.3 ± 9.6 bcAB	51.2 ± 13.56 bcB	p<0.001
P7	32.1 ± 3.8 bA	55.8 ± 3.1 bB	73.1 ± 7.3 cC	p<0.001	18.6 ± 3.3 cA	36.2 ± 3.7 cB	49.7 ± 6.0 cC	p<0.001
Intergroup difference	p<0.001	p<0.001	p<0.001		p<0.001	p<0.001	p<0.001	

HGF: Human gingival fibroblasts. CGF: Canine gingival fibroblasts. Kruskal-Wallis one-way analysis of variance on ranks. Dunn's Method, significance level of 5%. Intergroup (a, b, c, d) and intragroup (A, B, C) comparisons - different letters indicate statistically significant difference.

In the present study, CGF were evaluated in culture systems, considering their morphological, functional and biochemical characteristics, in comparison with HGF. Also, the change of these characteristics over several passages was analyzed. This is a relevant study, as it is the first one that evaluates and compares the characteristics of both HGF and CGF. The understanding of dog's cells permits a safer application of these cells in three-dimensional systems, and their *in vivo* study. In confluence, CGF showed a tendency of lower cell adhesion, total cell number, viability and protein content when compared to HGF. Moreover, both cell types exhibited a decrease in these characteristics over the passages, without differences in cell morphology.

Both cell types, HGF and CGF, showed a good growth rate and proliferation in parallel orientation, respecting the space that must exist between the cell membranes, as shown by Martelli Jr. et al. (24). It was observed by direct fluorescence that HGF are larger in size and more elongated when compared with CGF, which explains the higher speed of HGF to achieve cell confluence in flasks. A decreased potential of cell growth over several passages was also noted, which is consistent with Palioto et al. (25), who observed a decrease in the growth rate of HGF and human periodontal ligament fibroblasts after successive passages. The authors (25) also observed that near to the fifteenth passage, the cultures do not progress as well as initially.

Although the results showed a tendency of higher cell adhesion for HGF, this difference was statistically significant only in some passages. On the other hand, significantly higher values of total cell number, cell viability and total protein content were noted for HGF. These results corroborate with those of a recent study (13), in which HGF showed higher cell viability than CGF when seeded on a three-dimensional scaffold.

In summary, the findings of the present study demonstrated that although HGF and CGF present similar morphological characteristics, HGF exhibit greater functional and biochemical activity *in vitro*. The differences between HGF and CGF do not prevent the use of CGF in culture studies; however its use seems to be more appropriate in the subculture or first passage, as there was a reduction in the performance of fibroblasts in both HGF and CGF after several passages.

Resumo

Como os cães são um bom modelo para estudos *in vivo*, é interessante avaliar o comportamento de fibroblastos gengivais de cão (CGF) *in vitro*, para que essas células possam ser cultivadas em uma matriz e estudadas *in vivo* posteriormente. O objetivo do presente estudo foi realizar uma análise morfológica, funcional e bioquímica de CGF, comparando-os a fibroblastos gengivais humanos (HGF), bem como avaliar as alterações dessas características ao longo de várias passagens. Usando fibroblastos gengivais de 3 cães e 3 indivíduos na subcultura (Sub), primeira (P1), terceira (P3), quinta (P5) e sétima (P7) passagens, os seguintes parâmetros foram avaliados: morfologia, espraiamento, adesão, viabilidade e conteúdo de proteína total. Os resultados mostraram não haver diferenças significativas quanto à morfologia e espraiamento, e uma tendência a maior adesão e viabilidade para HGF, quando comparados a CGF. O conteúdo de proteína total foi significativamente maior para HGF. HGF exibiram maior atividade funcional e bioquímica *in vitro* quando comparados a CGF. Maiores valores na Sub foram observados para ambos, CGF e HGF, em todos os parâmetros avaliados. As diferenças não impedem o uso de CGF na engenharia tecidual, contudo, seu uso é mais apropriado na subcultura ou primeira passagem.

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