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The In Vitro Impact of Two Scaffold-Type Structure Dental Ceramics on the Viability, Morphology, and Cellular Migration of Pharyngeal Cancer Cells

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Abstract: There is a growing trend with respect to the use of ceramic materials in dental practice. With an increase in the number of cases of head and neck cancer, the use of dental implants in these patients is subject to controversy. Consequently, the purpose of the present study was to evaluate the impact of two ceramic materials on the viability, proliferation, migration, and structure of the cytoskeleton and nuclei of pharyngeal cancer cells. Therefore, samples of the two ceramic were immersed in artificial saliva with three different pH values in order to better simulate the natural biological environment. A 21-day immersion period was followed by testing of the saliva on pharyngeal cancer cell line Detroit-562 for its viability, morphology, and migration, as well as its effects on the nucleus and cytoskeleton. The results of the study after stimulation of Detroit-562 cells for 72 h with the three types of artificial saliva in which the ceramic materials were immersed indicated the following: (i) viability of cells did not change significantly, with the percentage of viable cells not falling below 90%; (ii) no morphological changes were recorded, with the shape and number of cells being similar to that of the control cells; (iii) the scratch assay method indicated that the two types of ceramics do not stimulate cell migration; and (iv) fluorescence immunocytochemistry revealed that both the nucleus and the cytoskeleton distributions were unaltered, as they were observed in unstimulated cells. The preliminary results of the study indicate that the investigated ceramic materials did not interact unfavorably with tumor cells when immersed in artificial saliva, thereby supporting the possibility of their safe use in cancer patients.

Keywords: ceramic scaffolds; cell viability; MTT; fluorescence immunocytochemistry; pharyngeal carcinoma

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1. Introduction

Technology in the dental industry has been improving continuously over the years, which has led to the possibility of replacing certain materials used in dental practice with others that have better physical properties and are more biocompatible [1]. Such materials include ceramics, which have demonstrated improved quality properties relative to molten metal porcelain systems. Ceramic materials are used in dentistry for their aesthetic qualities due to their transparency and translucency, as well as the fact that they give teeth a natural color and are biocompatible. The material also exhibits optimal mechanical properties, such as increased resistance to bending and tearing, with low abrasive properties and wear resistance [2,3]. These physical and biological characteristics allow ceramics to be used in a wide range of dental applications, such as single restorations, three-unit bridges, crowns over implants, or restorations [4,5].

Dental implants are considered one of the most suitable options for the treatment of edentulous patients [6]. Some patients have reported complications after receiving dental implants. Inflammation of the soft tissue and bone are among the most common complications. Edema, erythema, and hypertrophy are among the clinical manifestations of these conditions [7]. Therefore, the material used in the manufacture of implants must be biocompatible and possess physical properties that ensure that the implant has the desired strength and hardness. Chemically speaking, dental implants can be manufactured from metals, ceramics, or polymers [8]. Among the most used ceramics are those based on calcium phosphate due to their osteoconduction and osteoinduction properties. On the other hand, vitroceramic masses are considered suitable for scaffold generation due to their high surface coverage capacity. However, composite materials made from at least two types of material are currently considered the most appropriate therapeutic approach [9].

Nasopharyngeal carcinoma is one of the most common cancers of the head and neck. Amongst these cancers, oral pharyngeal cancer ranks sixth in global incidence, causing approximately 4% of neoplastic deaths. Several factors contribute to the appearance and development of this disease, including tobacco smoking, alcohol consumption, and the presence of inflammatory conditions in the oral cavity. It is still unknown whether trauma and chronic oral inflammation contribute to the development of pharyngeal cancer [10].

Nonetheless, there has recently been an increase in the number of newly diagnosed cases of head and neck cancer in patients under 40 and even in children and adolescents without pre-existing risk factors [11]. Several other risk factors have also been identified, such as exposure to immunosuppressive and irritating factors related to dental implants and other dental work [7]. Currently, there are several treatment options for nasopharyngeal carcinoma, including radiotherapy in the early stages, chemotherapy, and surgery for more advanced stages. Radiation therapy is often the preferred treatment option due to its effectiveness. However, radiation therapy is not without side effects, which can manifest in the form of complications in the oral mucosa, salivary glands, or dentition [12].

Moreover, the conventional treatment currently used to treat pharyngeal cancer is associated significant morbidity, in addition to the fact that oral rehabilitation may not be adequate in some circumstances. Most pharyngeal cancer patients have difficulty speaking, swallowing, chewing, and expressing themselves, so the impact of treatment on their quality of life and psychological well-being is significant [13]. Several approaches can be adopted in response to these problems, including the use of dental implants, although some clinicians advise against implant placement in patients with head and neck cancer [14]. Ceramic materials have been shown to be effective in bone reconstruction due to their biocompatibility, availability, and their close similarity to inorganic bone components [15]. To date, there is little evidence of a link between dental implants and oral carcinomas [16,17].

Thus, the present study was designed to evaluate two types of ceramics, obtained from Ceramco iC Natural DentineDentsply Sirona (P1) and Ceramco iC Natural Enamel, Dentsply Sirona (P2), respectively at the level of the Detroit 562 pharyngeal cancer cell line. The purpose of the experiment was to recreate an environment as close as possible to actual biological and pathological conditions. Therefore, artificial saliva was prepared with three

pH values (acidic, neutral, and basic), in which the investigated ceramics were immersed for 21 days. Furthermore, to provide a more comprehensive view of the influence of these samples on pharyngeal cancer cells, the effect on cell migration was studied, as well as the impact on cell morphology, cell nuclei, and cytoskeleton structure.

2. Materials and Methods

2.1. Reagents

The subject of this study was two types of ceramic masses that are often used in dentistry for dental prostheses and facets, namely P1, obtained from Ceramco iC Natural Dentine, Dentsply Sirona, and P2, synthesized from Ceramco iC Natural Enamel, Dentsply Sirona. The following reagents were used to obtain artificial saliva: $CaCl_2 \cdot 2H_2O$ (purity > 99.5%) from Honeywell Fluka™ (Charlotte, NC, USA); NaCl (purity > 99.5%) from Chimopar S.A. (Bucharest, Romania); CO (NH2) 2 (purity > 99.5%) from Sigma-Aldrich (St. Louis, MO, USA); KCl (purity > 99.8%) and NaOH pellets (purity > 99.3%) from Chimreactiv (Bucharest, Romania); and HCl 37% from Honeywell Fluka™ (Charlotte, NC, USA).

The following reagents were used for in vitro experiments. Trypsin-EDTA solution, dimethyl sulfoxide (DMSO), fetal bovine serum (FBS), penicillin/streptomycin, and an MTT-based cell growth determination kit were purchased from Sigma Aldrich, Merck KgaA (Darmstadt, Germany). Eagle's Minimum Essential Medium (EMEM–ATCC 30-2003™) was purchased from ATCC (American Type Cell Collection, Lomianki, Poland). All reagents were of analytical grade and purity for cell culture use.

2.2. Preparation of the Artificial Saliva

In order to create an environment as similar as possible to actual biological environment, artificial saliva was prepared with three pH levels: (i) acidic (a) (pH = 3); (ii) neutral (n) (pH = 7), and (iii) basic (b) (pH = 10). For the preparation of artificial saliva, the method described above in the literature was adapted to our laboratory requirements. Therefore, a solution containing 0.40 mg/L NaCl, 0.40 mg/L KCl, 0.80 mg/L $CaCl_2 \cdot 2H_2O$, and 1 mg/L $CO(NH_2)_2$ was prepared in water. In order to adjust the pH, 37% HCl was used for acidic pH, and 10N NaOH was used for basic pH. The pH was measured using a Thermo Scientific Eutech pH 150 portable pH meter with electrodes (Waltham, MA, USA).

2.3. Storage Period of Samples in Artificial Saliva

The two ceramics were prepared according to the method outlined above by Thrivikraman et al. for in vitro evaluation [18]. Using a mortar and pestle, the ceramic materials were ground into an ultrafine powder. Then, the powder was suspended in artificial saliva at a concentration of 2 mg/mL for 21 days. Subsequently, the saliva in which the ceramic powders were suspended was utilized to obtain the test concentrations (5, 10, 25, 50, and 75 µg/mL) for stimulation of the Detroit-562 cell line.

2.4. Cell Culture

The pharyngeal carcinoma cell line Detroit-562, purchased from the American Type Culture Collection (ATCC, code no CCL-138 ™, LGC StandardsGmbH, Wesel, Germany), was used in the present study. Cells were cultured in a culture-specific medium, represented by Eagle's Minimum Essential Medium (EMEM-ATCC® 30-2003™) supplemented with 10% Fetal Bovine Serum and 1% antibiotic mixture (100 U/mL penicillin/100 g/mL streptomycin). During the experiment, the cells were maintained at a standard temperature (37 °C) and under 5% CO_2.

2.5. Cellular Viability Assessment

Cell viability was determined using the MTT method. Thus, the cells were cultured in 96-well plates of 1×10^4 cells/well. Once confluence reached 90%, the cells were stimulated with five concentrations of ceramics (5, 10, 25, 50, and 75 µg/mL) suspended in artificial saliva at the three pH values for 72 h. Cell culture medium was then removed and replaced

with fresh medium in a volume of 100 µL per well. A reagent MTT solution of 10 µL was added to each well, and the plates were incubated at 37 °C for 3 h. Finally, 100 µL/well of solubilization solution was added, and the plates were kept at room temperature, protected from light, for 30 min. Using Cytation 5 (BioTek Instruments Inc., Winooski, VT, USA), absorbents were measured at two wavelengths of 570 nM and 630 nM. The obtained results are expressed as a percentage (viable cells%).

2.6. Cellular Morphology

We then evaluated the effects of the two ceramic samples on pharyngeal cancer cells in terms of cell morphology and confluence. After 72 h, we microscopically evaluated and photographed Detroit-562 cells under bright-field illumination. The pictures were analyzed using Gen5™ microplate data collection and analysis software (BioTek Instruments Inc., Winooski, VT, USA).

2.7. Wound-Healing Assay

A wound-healing (scratch) assay was used to evaluate the effect of the two ceramic types on cell migration. To this end, cells were cultured in 24-well Corning plates (1×10^5 cells per well). When the confluence reached a suitable level, an automatic scratch was made using the AutoScratch TM wound-making tool provided by BioTek® Instruments Inc. (Winooski, VT, USA) as recommended by the manufacturer. The cells were then stimulated with two concentrations (5 and 75 µg/mL) of the two ceramic samples suspended in three different types of saliva (acidic, neutral, and basic). As part of the assessment of the effect on cell migration, photographs of cells were taken at the beginning of the experiment (0 h) and the end of the exposure interval (24 h) with Cytation 1 and processed using Gen5™ microplate data collection and analysis software (BioTek® Instruments Inc., Winooski, VT, USA). The effect on cell migration was quantified by applying the previously described calculation formula to determine the migration rates as a percentage [19].

2.8. Fluorescence Immunocytochemistry

Cells were cultured in 12-well plates of 1×10^5 cells/well. After reaching a confluence of approximately 90%, they were stimulated with the two ceramic materials immersed in artificial saliva at a concentration of 75 µg/mL for 72 h. Then, the cells were washed with ice-cold PBS and fixed with 4% paraformaldehyde for 30 min at 4 °C. Subsequently, the cells were again washed with ice-cold PBS and permeabilized with 2% Triton X-100/1× PBS. Thereafter, a blocking solution of 30% FCS in 0.01% Triton X-100 was added. Cells were incubated overnight with the primary antibody (α-tubulin antibody, Invitrogen by Thermo Fisher Scientific) in a dark humidity chamber at 4 °C. The next day, the secondary antibody (Alexa FlourTM 488, Invitrogen by Thermo Fisher Scientific) was added, and the cells were incubated at room temperature for 30 min. Finally, 40,6-diamidino-2-phenylindole (DAPI) was added for 15 min to visualize the nuclei.

2.9. Statistical Analysis

All data are expressed as means ± SD; the differences were compared by applying one-way ANOVA followed by Dunnett's multiple comparisons post-test with GraphPad Prism version 9.0.0 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). The statistically significant differences between data were labeled with * (* $p < 0.1$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

3. Results

3.1. Cellular Viability Assessment

After 72 h of stimulation with five concentrations (5, 10, 25, 50, and 75 µg/mL), the effect of the ceramic samples (P1 and P2) suspended in artificial saliva at three pH values (acidic, neutral, and basic) on pharyngeal cancer cells was determined.

P1, suspended in acidic saliva, did not have a major impact on the viability of the cells after 72 h. Across all concentrations tested, cell viability was similar to that of unstimulated control cells. There was a slight reduction in cell viability in saliva with a neutral pH, depending on the concentrations tested. However, cell viability was approximately 90% when a concentration of 75 µg/mL was used in comparison to control cells. Additionally, a dose-dependent decrease in cell viability was observed in the case of artificial saliva with basic pH, with a minimum viability value of approximately 94% recorded at a concentration of 75 µg/mL. Therefore, no evidence of a significant change in cell viability was found in any of the samples tested (Figure 1).

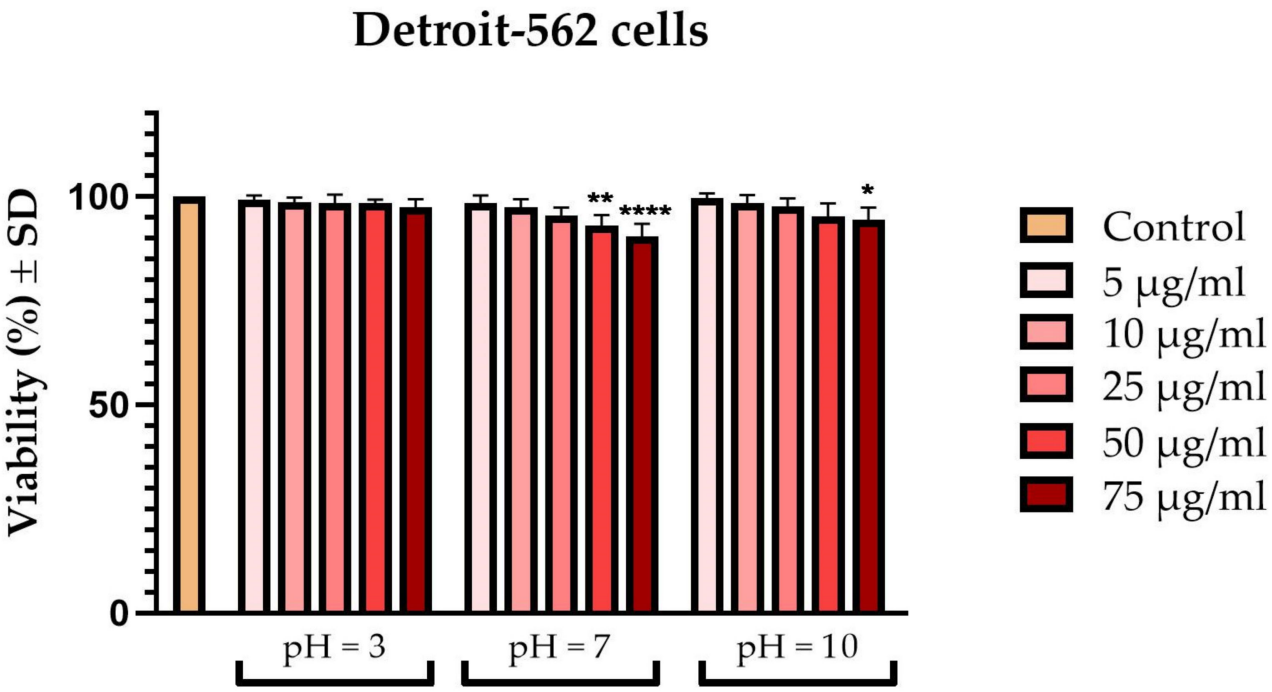

Figure 1. In vitro evaluation of the effect of P1 (5, 10, 25, 50, and 75 µg/mL) suspended in artificial saliva with acidic pH (pH = 3), neutral pH (pH = 7), and basic pH (pH = 10) at the Detroit-562 cell line level after 72 h of treatment. The results are expressed as viability percentages (%) normalized to control cells (unstimulated) and expressed as mean values ± SD of three independent experiments performed in triplicate. The statistical difference between the control group and the P1-treated group was determined by applying one-way ANOVA, followed by Dunnett's multiple post-test comparisons (* $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$).

Despite P2 being suspended in saliva with an acidic pH, no obvious decrease in cell viability was found, which was relatively constant at each of the five concentrations tested. The viability of the cells at a concentration of 5 µg/mL was 98%, whereas at a concentration of 75 µg/mL, the cells were 96% viable. On the other hand, in neutral saliva, a reduction in cell viability was observed, depending on the concentration. The lowest value of cell viability was observed at a concentration of 75 µg/mL, resulting in a cell viability of 91%. With respect to the effect of saliva with basic pH on cell viability, it did not result in significant changes, as the viability of the cells was found to be comparable to that of unstimulated control cells (Figure 2).

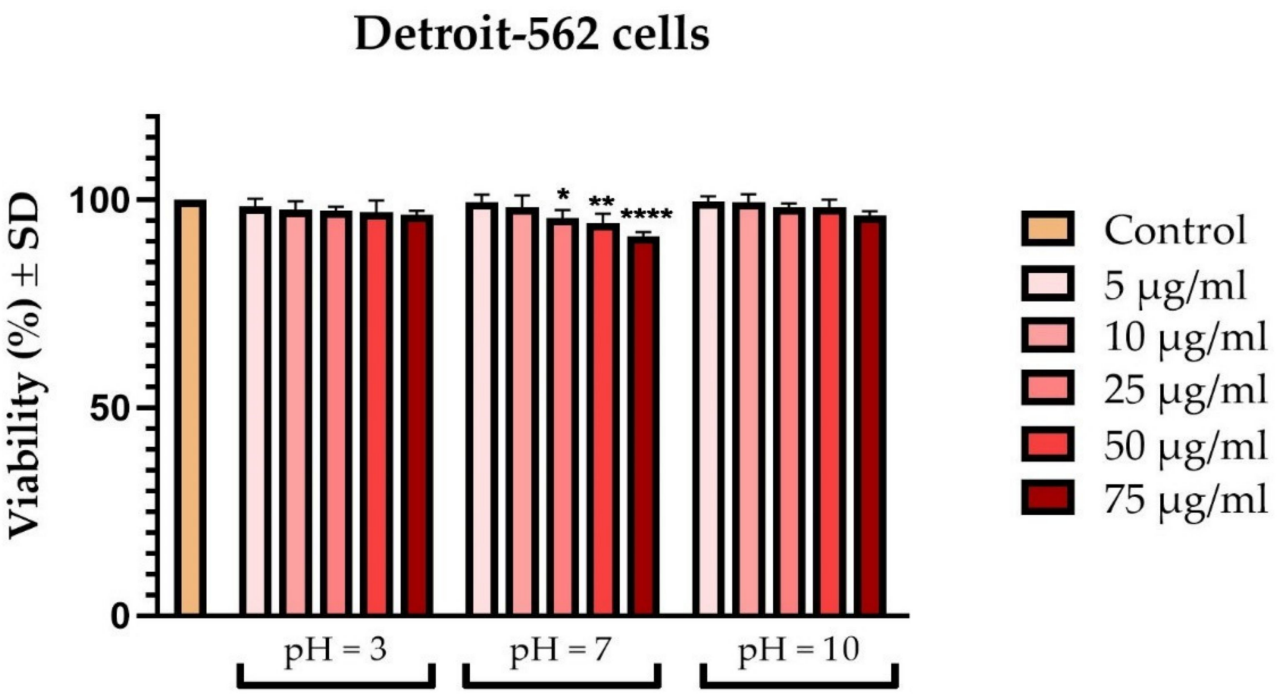

Figure 2. In vitro evaluation of the effect of P2 (5, 10, 25, 50, and 75 µg/mL) suspended in artificial saliva with acidic pH (pH = 3), neutral pH (pH = 7), and basic pH (pH = 10) at the Detroit-562 cell line level after 72 h of treatment. The results are expressed as viability percentages (%) normalized to control cells (unstimulated) and expressed as mean values ± SD of three independent experiments performed in triplicate. The statistical difference between the control group and the P1-treated group was determined by applying one-way ANOVA analysis, followed by Dunnett's multiple post-test comparisons (* $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$).

3.2. Cellular Morphology

We assessed the effect of two types of suspended ceramics in artificial saliva with different pH levels by observing the morphology and confluence of Detroit-562 cells.

P1 tested at three different concentrations (5, 25, and 75 µg/mL) suspended in artificial saliva with acidic, neutral, and basic pH was observed for 72 h in terms of its effects on cell morphology and number. As shown in Figure 3, no significant changes were observed between the three concentrations tested at the different pH levels. A similar morphology and comparable cell confluence were observed in this case in comparison with control cells that had not been stimulated. These results are in line with those obtained previously in the cell viability test (Figure 3).

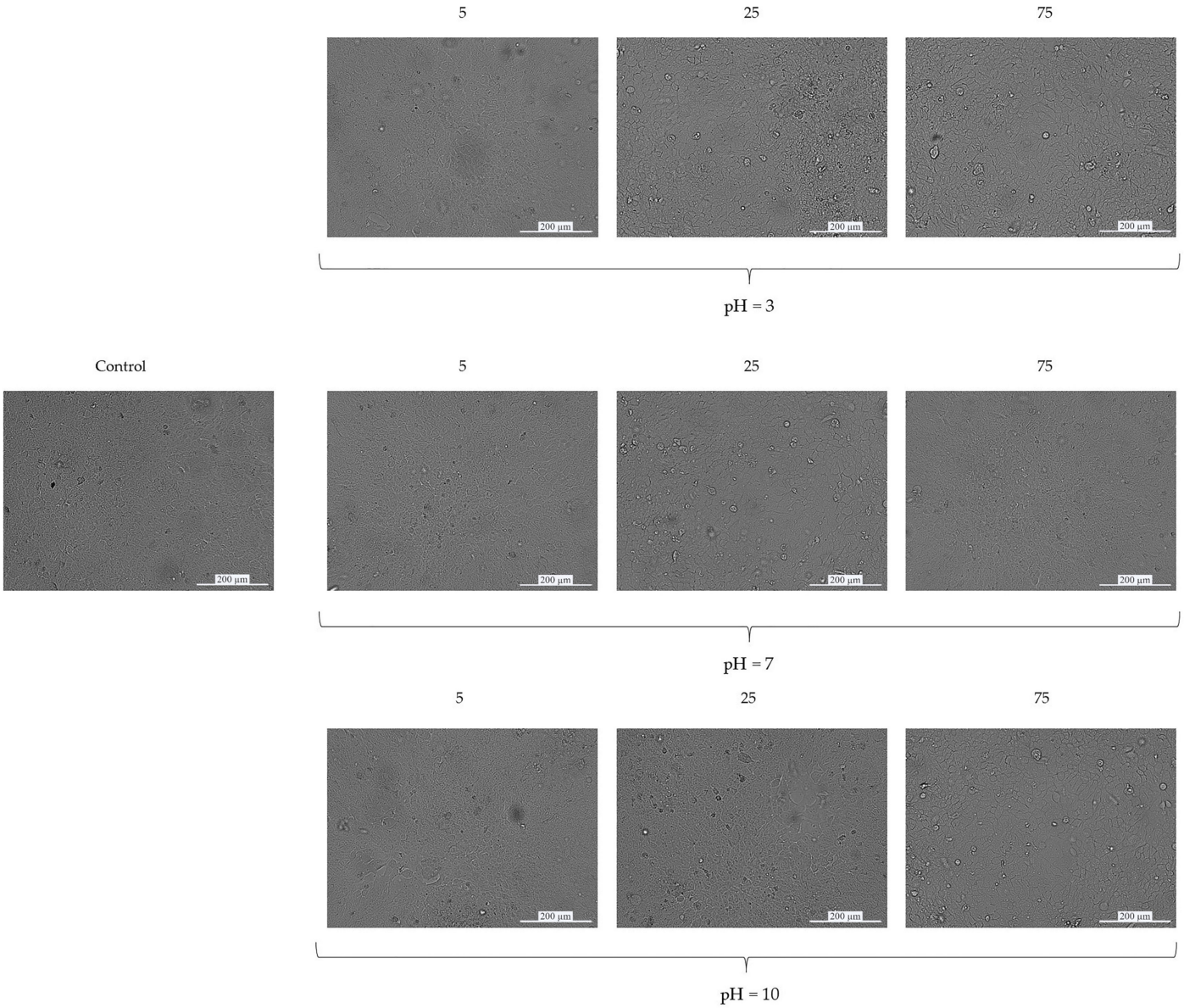

Figure 3. Morphological and shape changes produced by P1 (5, 25, and 75 µg/mL) in Detroit-562 after 72 h of treatment. The morphology of the cells was not altered by P1, with the number, confluence, and shape of cells treated with P1 remaining the same as those observed in control cells. The scale bars indicate 200 µm.

P2 had similar effects on cell morphology and confluence at the Detroit-562 cell line level to those observed in response to P1. Thus, none of the three concentrations tested at different pH values (acidic, neutral, and basic) produced any significant change. The shape and numbers of cells were the same as in unstimulated control cells (Figure 4).

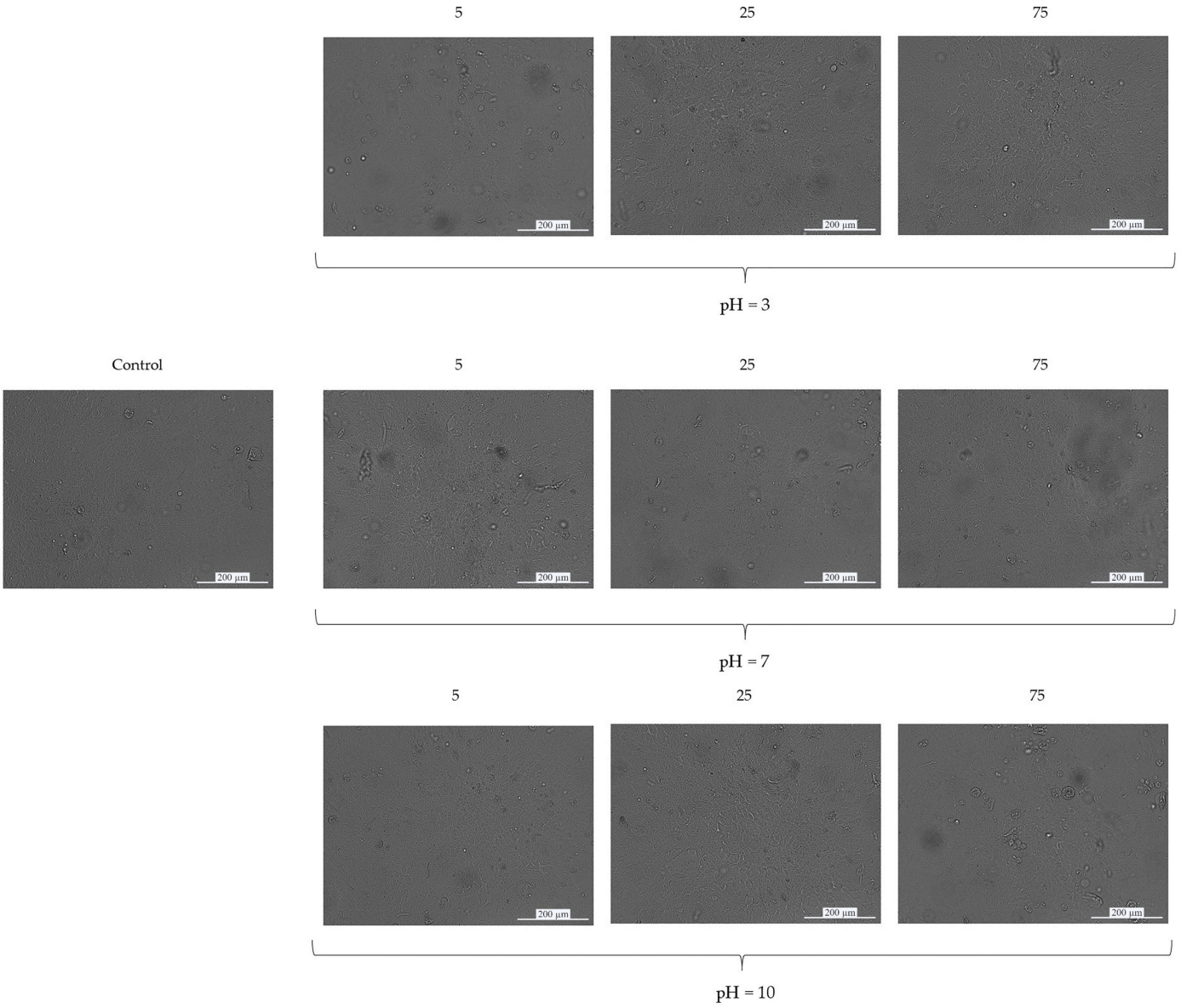

Figure 4. Morphological and shape changes produced by P2 (5, 25, and 75 µg/mL) in Detroit-562 cells after 72 h of treatment. No changes were observed in the morphology of cells treated with P2, as their number, confluence, and shape were similar to those of control cells (unstimulated). The scale bars indicate 200 µm.

3.3. Wound-Healing Assay

To evaluate the influence of the two ceramic samples on the migration of pharyngeal cancer cells, the wound-healing method was applied, in which two concentrations (5 and 75 µg/mL) were suspended in three types of saliva with varying pH values for analysis.

The migration of cells was slightly inhibited at all three analyzed pH levels for P1. Among the concentrations tested, 75 µg/mL at pH 7 showed the most potent inhibition of cell migration. However, the percentage inhibition of cell migration did not differ substantially from that of unstimulated control cells; when the rate of approach of the cells in the control case was approximately 46%, the rate of approach of the cells in the 75 µg/mL sample at acidic pH was approximately 39% (Figure 5).

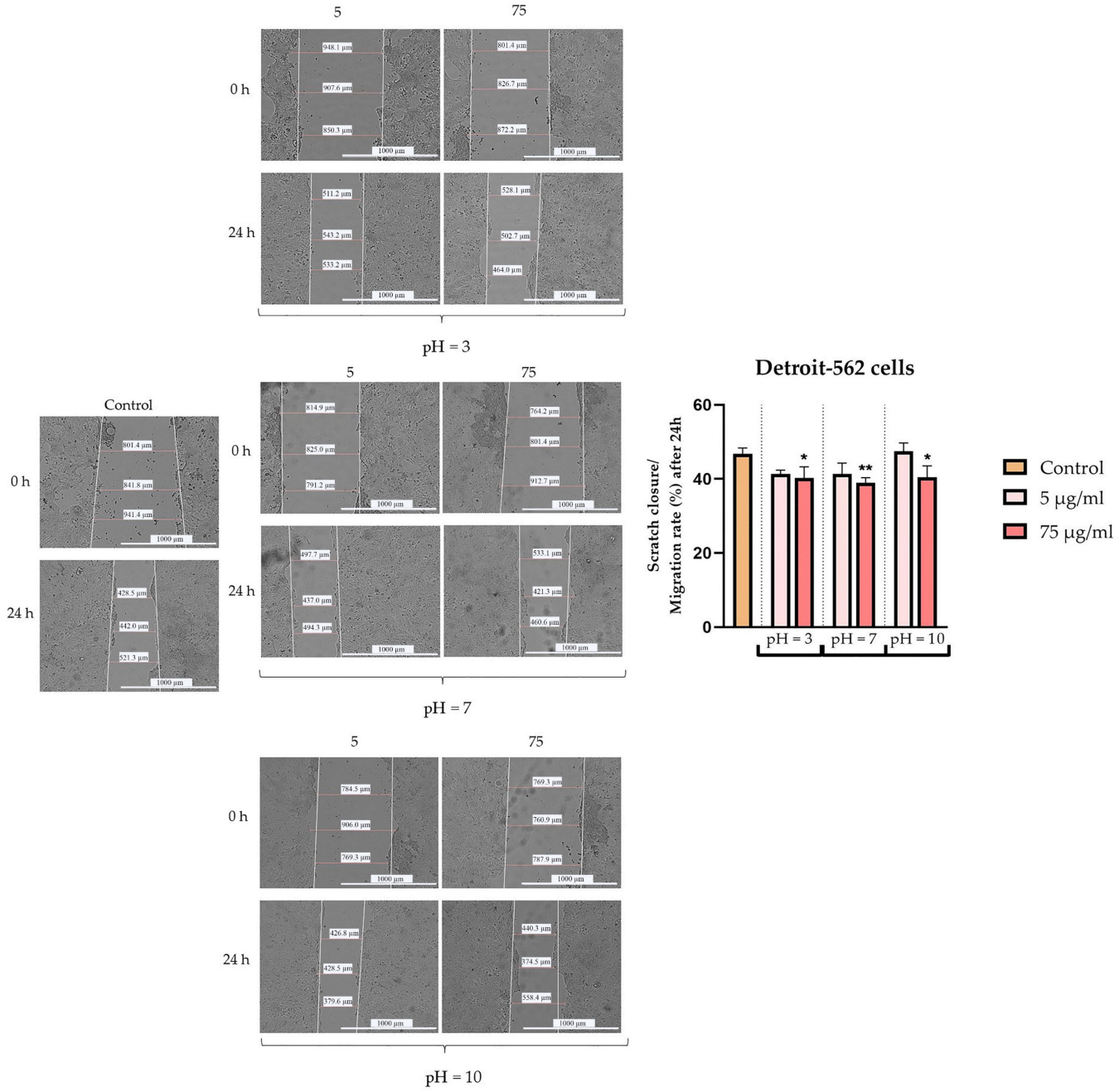

Figure 5. Scratch closure rate of Detroit-562 cells following P1 treatment (5 and 75 µg/mL) in artificial saliva with three types of pH (acid, neutral, and basic). The bar graphs are presented as wound closure percentage after 24 h compared to the initial surface. The data are expressed as mean values ± SD of three independent experiments performed in triplicate. The statistical differences between the control and the P1-treated groups were identified by applying one-way ANOVA followed by the Dunnett's multiple comparison post-test (* $p < 0.05$; ** $p < 0.01$).

P2 also inhibited of migration, but this inhibition was less pronounced compared to P1. Therefore, even in this case, the strongest inhibition of cell migration was observed at a concentration of 75 ug/mL in saliva with neutral pH. In this case, the rate of approach of the cells was about 42%, whereas the rate of approach of the unstimulated control cells was nearly 45% (Figure 6).

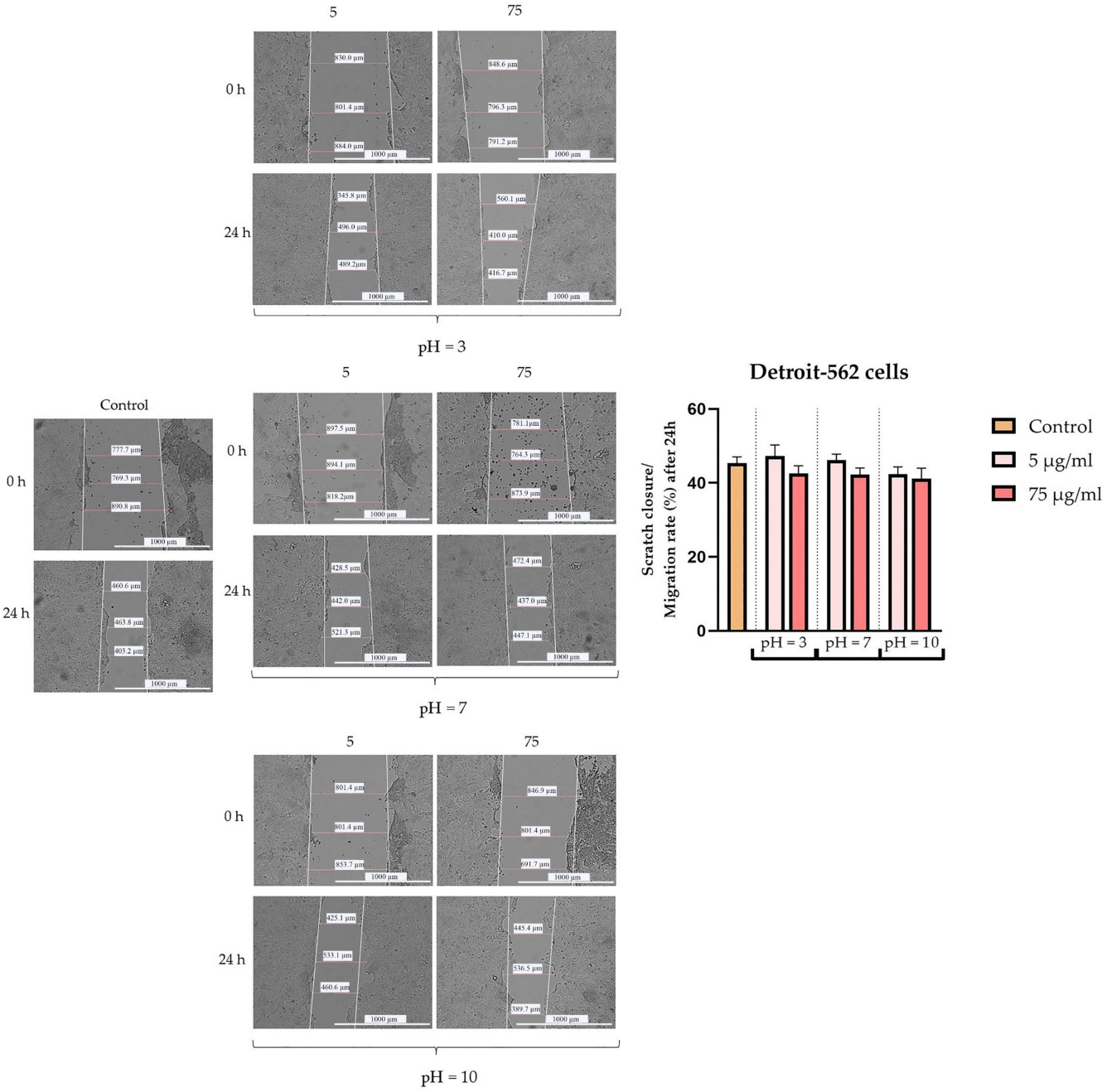

Figure 6. Scratch closure rate of Detroit-562 cells following P2 treatment (5 and 75 µg/mL) in artificial saliva with three types of pH (acid, neutral, and basic). The bar graphs are presented as wound closure percentage after 24 h compared to the initial surface. The data are expressed as mean values ± SD of three independent experiments performed in triplicate. The statistical differences between the control and the P2-treated groups were identified by applying one-way ANOVA followed by the Dunnett's multiple comparisons post-test.

3.4. Fluorescence Immunocytochemistry

To obtain a clearer picture of the effect of the two ceramic materials on pharyngeal carcinoma cells, immunofluorescence staining was performed to examine the cell morphology.

The results shown in Figure 7 indicate that the highest concentration (75 µg/mL) of artificial saliva in which P1 and P2 were immersed produced no changes to the nucleus or cytoskeleton in comparison to control cells, which were not stimulated. As far as structure and number of nuclei are concerned, they were not affected by the treatment. Furthermore,

the structure of the microtubes remained unchanged, being distributed evenly both in control cells and in tested samples.

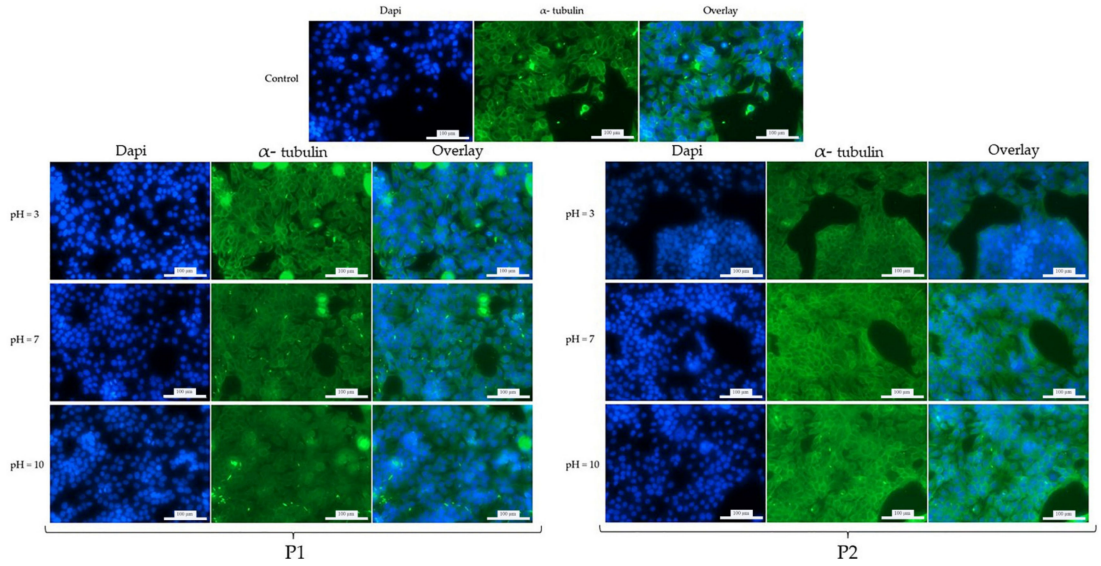

Figure 7. Detroit cells visualized by fluorescence microscopy after 72 h of treatment with P1 and P2 (75 μg/mL) in artificial saliva with three types of pH (acid, neutral, and basic). Nuclear and microtubule staining are expressed both separately (DAPI and α-tubulin, respectively) and combined (overlay). Both the structure of the nuclei and the distribution of the cytoskeleton were uniform in the cells treated with P1 and P2 and in the control cells (unstimulated).

4. Discussion

Malignancies of the head and neck account for approximately 5% of all cancers. About half of these tumors affect the oral cavity. In general, people older than 60 years who drink alcohol or smoke are most susceptible, but recent statistics have shown an increase in the incidence of the disease among children and adolescents [7]. Radiation therapy is the most employed therapeutic approach for head and neck cancers, including pharyngeal cancer. Despite the effectiveness of radiation therapy, side effects, such as tooth erosion, tooth root fracture, and dysphagia, adversely affect patients' quality of life [20]. As a result of radiotherapy, edentulous patients require oral rehabilitation protocols that include the placement of dental implants. Such implants can be placed either before or following radiotherapy [21].

In the present study, we investigated of two types of ceramic materials as scaffolds, which were developed using the foam replication technique with a polymeric sponge forming a template for a porous scaffold—a technique that provides the scaffolds with suitable chemical and thermal properties [22]. In the case of P1, the ceramic material was obtained from Ceramco iC Natural Dentin, Dentsply Sirona, whereas in the case of P2, the ceramic material was obtained from Ceramco iC Natural Enamel, Dentsply Sirona. The use of calcium silicate coatings is currently a favored method for attainment of dental materials due to its increased biocompatibility. In a study conducted by Qin et al., the bioactivity of a bio-nanocomposite of wollastonite-hydroxyapatite was evaluated by adding magnetic nanoparticles and single-wall carbon nanotubes. The results showed that the bio-nanocomposite contains bioactive magnetic nanoparticles, and with the addition of magnetic nanoparticles and single-walled carbon nanotubes, the dissolution rate of the bio-nanocomposite coatings decreases. Furthermore, preparation of the coating structure at a temperature of 550 C led to a decrease in the appearance of microcracks [23]. Li et al. used a 3D printer to construct porous bone scaffolds using polylactic acid, which were then covered with alginate composed of varying amounts of hydroxyapatite through a lyophilization process. Based on an analysis of mechanical resistance and biological behavior, it was

observed that the elastic modulus and compressive strength increased, whereas the porosity percentages decreased. Furthermore, the composites were found to possess antibacterial properties and to be compatible with blood pH [24]. The electrophoretic detection technique is another method of obtaining nanocomposite coatings. Through this method, Khandan and collaborators prepared a nanocomposite coating with hydroxyapatite and bovine diopside, which was then evaluated in terms of physical properties, such as wettability and hardness. A percentage of 30% of bovine diopside produced the best results in terms of bioactivity, wettability, and hardness [25].

Previously, the two materials have been characterized in terms of their physical, chemical, and biological characteristics. The method for obtaining the two samples was fully described, and they were subjected to analysis in a healthy cell line of primary human gingival fibroblasts, i.e., HGF cells. According to the results of the study, neither of the materials showed cytotoxic effects on gingival fibroblasts. In addition, after applying the in ovo method, HET-CAM, to determine irritative effects, it was found that the test samples did not produce irritating effects on the vascular plexus [26]. As a continuation of the aforementioned study, pharyngeal carcinoma cells were treated with two ceramic materials suspended in artificial saliva at three different pH values (3, 7, and 10). Consequently, the effect on cell viability, cell morphology, and nucleus and cytoskeleton structure, as well as cell migration capacity, was assessed. Both ceramic materials were finely ground with a mortar and pestle and then suspended in artificial saliva with acidic, neutral, and basic pH for 21 days to determine their biological effects. In the next step, Detroit-562 cells were exposed for 72 h to five different concentrations of artificial saliva (5, 10, 25, and 75 µg/mL). Neither sample showed evidence of cytotoxicity according to ISO Standard 10993-5:2009 regarding the Biological Evaluation of Medical Devices [27], and the viability of the cells was comparable to that of the control cells. Additionally, the morphology of the cells was not affected by the three types of artificial saliva in which P1 and P2 were suspended, and the structure and number of nuclei remained similar to those found in control cells. Furthermore, the distribution of tubulin fibers was similar to that observed in control cells without stimulation, suggesting that the cytoskeleton of the treated cells was not affected.

In order to assess the biocompatibility of dental materials and their biological effects, it is necessary to create conditions that mimic, as closely as possible, the physiological conditions that exist in the oral cavity. Saliva in the oral cavity plays a vital role in the maintenance of optimal oral and systemic health. The first mention in the literature of the use of artificial saliva was in a publication by W. Souder and W.T. Sweeney, who studied the release of mercury from various types of amalgam kept in artificial saliva [28]. The literature presents various methods for obtaining artificial saliva, depending on the study purpose. There is a heavy emphasis on evaluating the properties of dental ceramics [29,30], as well as the properties of other materials, such as composite materials [31,32] or metal alloys used in the dental industry [33]. Salivary pH is physiologically neutral; however, under certain pathological conditions, salivary pH can become acidic or basic, so three pH values (3, 7, and 10) were chosen to create an environment as similar as possible to that of biological saliva [34]. Several studies have examined the impact of pH on the physicochemical characteristics of different dental materials. Accordingly, it has been found that glass-phase ceramics are more prone to dissolution when compared to oxide ceramics [35]. In addition, glass-phase ceramics immersed in acidic media release ions in greater amounts than in neutral media [36]. A study conducted by Tomozawa et al., on the other hand, revealed that an alkaline environment had unfavorable effects on the strength of the glass materials, which led to cracks in the material [37]. An investigation was carried out by Pinto and colleagues on the impact of pH on dental ceramics. According to their findings, the effect of pH of the immersion medium on corrosion susceptibility differs according to the type of ceramic used [38]. The ceramics examined in the present study consist of sodium potassium aluminosilicates in an amount of 80 to 100% and tin oxides in an amount of 0 to 5% obtained from Ceramco iC Natural Dentine, Dentsply Sirona (P1) and synthesized from Ceramco iC Natural Enamel, Dentsply Sirona (P2) [26]. Fahmy et al.

evaluated the hardness, cracking, and breaking strength of a glass-ceramic material after immersion in artificial saliva in a similar manner to the present study. As a result of storage in artificial saliva for 21 days, an important influence on the microhardness, cracks, and breaking hardness was found [39]. Additionally, Lakhloufi and colleagues examined the impact of immersion time in artificial saliva on the chemical composition and physical properties of dental materials. Glass ceramics and zirconia were immersed in artificial saliva for 7, 14, and 21 days. According to the obtained results, artificial saliva significantly affected the studied materials, as ionic compound release or dissolution was observed as a degradation phenomenon [40]. Furthermore, Mohammed and Alwahab evaluated the impact of artificial saliva with varying pH values (3.50, 7, and 10) on the strong resistance of each veneering ceramic to metal and zirconia substructures after immersion for 21 days. An influence of artificial saliva with varying pH values on the harsh shear strength both metal-ceramic groups and zirconium ceramics was observed [41].

Significant global health problems are associated with oral and pharyngeal cancers. These diseases account for 2% of all cancer cases and have a survival rate of approximately 50%–60% [42]. Oral cancer is largely attributed to improperly fitting prostheses, according to an epidemiological study [43]. The degradation of dental ceramics is predominantly caused by mechanical forces and chemical agents. The ceramic surface is susceptible to degradation when exposed to an acidic environment for a prolonged period of time. There is a particular risk associated with ceramic prostheses for patients with cancer of the head and neck [44]. Based on the foregoing premises, in the present study, we evaluated the biological effects of two types of ceramic materials. Most often, physical and mechanical properties are comprehensively studied, whereas biological properties are neglected. Therefore, the primary objective of this study was to assess the effects of ceramic materials suspended in saliva at different pH levels on the proliferation and migration of pharyngeal carcinoma cells. A common practice involves the use of in vitro techniques to determine the biocompatibility and biological properties of dental materials. The accuracy of laboratory tests is one of the most important features of in vitro evaluations. MTT has advantageous properties with respect to determination of the viability and proliferation of cells. One of the main advantages of the MTT technique is its rapid reaction time, as well as the ability to see the results visually, which makes it useful when rapid qualitative results are needed [45]. One such study was conducted by Sjögren et al., who evaluated the cytotoxic effects of several types of dental materials on a human fibroblast cell line using the MTT method. According to their findings, none of the materials exhibited a toxic potential in vitro [46]. Similarly, MTT was used in a comparative analysis of the biocompatibility of new and older generations of ceramic materials. The results indicated that the biocompatibility and safety of ceramics varied depending on the type of material used. Only 1 Li-disilicate material (Empress-2) demonstrated cytotoxic effects in vitro, whereas other materials showed only a slight suppression of cell function [47].

Cell migration is another important characteristic of tumor cells, which facilitates the spread of the tumor from its primary site to secondary sites, initiating metastatic spread. In light of the fact that the cytoskeleton plays a significant role in metastasis, in the present study, we investigated the effect of ceramic materials at the level of tubulin fibers [48]. According to the results, none of the three types of saliva in which the ceramics were immersed caused significant changes in the nucleus or cytoskeleton. Tassin et al. evaluated new types of dental materials obtained by infiltrating resin into a ceramic network at high pressures and temperatures. By using immunofluorescence staining to evaluate the changes produced by these materials in the cytoskeleton of oral stem cells, the researchers observed that the structure of tubulin fibers remained unchanged [49]. Martins et al. investigated the influence of bioactive glass-based surfaces on the cytoskeleton by analyzing the qualitative epifluorescence of actin and tubulin. Their findings indicate that the expression of actin and tubulin mRNA is influenced by the culture of osteogenic cells on these types of materials. The cells grown on bioactive materials exhibited rounded surfaces and lacked fluorescence [50].

5. Conclusions

According to the present study, the two ceramic materials tested (P1 and P2) displayed no obvious protumor effects on the pharyngeal cancer cell line Detroit-562. In addition to the results obtained from cell viability studies, morphology and immunofluorescence analyses also showed that these types did not affect cell proliferation, migration, or nuclei and cytoskeleton morphology. Additionally, the materials displayed good stability under conditions that were similar to those found in biological environments. As a result, immersion in artificial saliva with acidic, neutral, and basic pH followed by saliva testing in pharyngeal carcinoma cells did not alter the cellular characteristics. In conclusion, both types of dental ceramic materials tested in the present study have properties that ensure their safe use in patients with cancer. However, it is important to conduct further studies to confirm the safety, biocompatibility, and interaction of these types of materials with possible oncological treatments.

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