Evidence-Based Complementary and Alternative Medicine

Traditional and Herbal Medicine in Oral Tissue Engineering

Lead Guest Editor: Hamid Tebyanian
Guest Editors: Karam Soliman, Mohsen Yazdanian, Mehrdad Moosazadeh Moghaddam, and Ali Mohammad Latifi

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Research Article

The Effect of Toothpastes Containing Natural Ingredients Such As Theobromine and Caffeine on Enamel Microhardness: An In Vitro Study

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The current study aimed to investigate the effect of biocompatible kinds of toothpastes containing natural ingredients such as theobromine and caffeine on the enamel microhardness after demineralization. 72 maxillary premolar teeth extracted for orthodontic purposes were used in this study. Primary enamel surface microhardness examinations were performed using a Digital Micro Vickers Hardness Tester following the Knoop technique (50 g load for 15 s with three indentations at various points). The specimens were immersed in lactic acid (pH = 5.4) for 7 days, washed with distilled water, dried, and then retested for microhardness. According to the type of toothpaste used for brushing, all specimens were categorized as follows: Group 1, Theodent classic® toothpaste (theobromine); Group 2, Power Energy toothpaste (caffeine); Group 3, Colgate toothpaste (fluoride); and Group 4, distilled water as the negative control. The specimens were retested for enamel microhardness after brushing 2 times a day for one month. After brushing with different types of toothpaste, for all experiment groups, the increase in microhardness values in the demineralized enamel surfaces was significant and there were significant differences between them (p value <0.05). The fluoride group had the highest microhardness and had a significant difference with the caffeine and distilled water groups, but there was no significant difference with the theobromine group (p value <0.05). In the theobromine group, the hardness was considerably higher than in the caffeine and distilled water groups. There was no significant difference between the caffeine and distilled water groups. Theobromine toothpaste had the same remineralization effect as that of fluoride toothpaste, while caffeine toothpaste had no positive effect on the remineralization process.

1. Introduction

The enamel has natural demineralization and remineralization cycles; enhanced demineralization of the tooth surface causes dental caries, which in turn results in continual mineral loss from the crown or root of teeth. This process is mainly caused by bacteria. There are five factors that can stimulate the occurrence of caries, including accumulation of plaques, high consumption of carbohydrate or acidic foods and beverages, natural ingredients like saliva and pellicle, and fluoride levels. It is worth noting that the first three factors are mentioned as the main causes of caries [1].

Scholars have been reporting on enamel remineralization for about one century, and it is noted that "the non-invasive treatment of early caries lesions by remineralization has the potential to be the major advance in the clinical management of the disease" [2]. Several techniques have been introduced to enhance the mechanical characteristics of enamel to avoid the early development of caries [3]. The

most successful caries-preventive agent is fluoride [4]. High consumption of fluoride may result in fluorosis, damage to the teeth, dark stripes on teeth, and tooth loss. In addition, it may result in decreased intelligence in children who receive low levels of fluoride as well as early aging, abortion, and brittle bones [5]. It has been reported that fluoride consumption at a level more than 5 mg F/kg body weight for children and adults could cause acute toxic effects, while the lethal dose for children is considered to be 16 mg F/kg body weight and it is 32 mg F/kg body weight for adults [6]. Due to the side effects of these chemical components, an increasing public interest in natural or herbal-based healthcare products, especially in different kinds of toothpaste, is being observed. This incline is not only observed in cosmetic markets and dental practices but also in the scientific world [7]. An increasing amount of clinical study is being performed to verify the effectiveness of these products; thus, in terms of dental hygiene products, attention nowadays is paid to some natural and biocompatible alternatives to fluoride, like theobromine [4]. Theobromine (3, 7-dimethylxanthine) is a primary alkaloid derived from the *Theobroma cacao* plant [8]. It can also be found in cacao leaves, although its concentration is significantly lower [3]. Theobromine is a water-soluble, crystalline, bitter powder that is available in chocolates along with tea and other foods [8]. It is differentiated from caffeine by only one methyl group [9]. The molecular formula for theobromine is $C_7H_8N_4O_2$ [4]. It is proven that theobromine compounds can improve the hardness of the tooth enamel surface through stimulating interstitial reactions between the HA crystals and theobromine on the enamel surface [3]. Recently developed kinds of toothpaste contain theobromine can trigger remineralization without causing any toxicity.

All around the world, coffee is served, mainly because of its taste and its effect on both mental and physical activities. Mixed messages from scientists about the advantages or disadvantages of coffee are almost a weekly event [10]. Caffeine (1, 3, 7-trimethylxanthine) is a bitter, white crystalline purine, a methylxanthine alkaloid. In addition, chemically, it belongs to the adenine and guanine bases of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) [11]. *Coffea canephora* is rich in polyphenols, which are mentioned as potential agents that can prevent oral diseases, especially those related to biofilms. According to Cowan, the potential reasons for phenolic toxicity to microorganisms are enzymatic inhibition by oxidized compounds, possibly through reactions with sulfhydryl groups or nonspecific interactions with proteins [12]. Of all the documented bioactivities of coffee, a few show antioxidant, anticarcinogenic, and antimutagenic activities [13]. Different compounds are reported as the cause of the chemoprotective effects of coffee, which are mainly polyphenols, such as chlorogenic acids and their degradation products, as well as caffeine, kahweol, cafestol, and other phenolics [13]. Coffee can influence *Streptococcus mutans*, the organism that causes the development of dental caries. In addition, it is reported that roasted coffee has antiadhesive characteristics. Hence, it can intervene in the adhesion of *S. mutans* and the rest of the detrimental compounds to the teeth. Those who drink coffee regularly not only have caries-free teeth, but also their teeth are whiter as compared to others [10]. Because of this proven effect, nowadays, manufacturers produce toothpaste containing caffeine to utilize its antibacterial and mental-stimulating effect. Since there have always been conflicting reports in the field of dentistry about the effect of caffeine on dental health and no study has been done on the effect of caffeine-containing toothpaste on tooth enamel, we designed this study. In the present study, the effect of different kinds of toothpaste containing theobromine, caffeine, and fluoride on enamel microhardness was evaluated after artificial demineralization.

2. Materials and Methods

2.1. Teeth Preparation. 72 maxillary premolar teeth with caries-free buccal surfaces that were extracted for orthodontic purposes were used in this study which was approved by the Ethical Committee of Shiraz University of Medical Sciences, Dental School with number 21804-37-01-98.

Then, all the teeth were rinsed with water and maintained in 70% ethanol until the experiment day. The teeth were mounted in a polyester mold (Figure 1). All the teeth were cleaned with a brush and pumice and stored in artificial saliva.

2.2. Enamel Microhardness Analysis. Primary enamel surface microhardness analyses were performed using a Digital Micro Vickers Hardness Tester machine following the Knoop technique (50 g load for 15 s with three indentations at various points) (SCTMC, model: MHV-1000Z, China). This machine can directly show the test mode, test force, indentation length, dwell time, test numbers, conversion scale, and date and time. It can use an optional Knoop indenter to measure Knoop hardness.

2.3. Demineralization Process. A solution of lactic acid (titration min. 90.0%) with pH 5.4 was used to demineralize the enamel surfaces. The specimens were immersed in the demineralized solution for 7 days, washed with distilled water, dried, and then retested for microhardness.

2.4. Application of Toothpastes. The samples were randomly categorized into four groups according to the type of toothpaste: Group 1, brushed with Theodent classic® toothpaste (Theodent classic™, Rennou, UK-853069003006); Group 2, brushed with Power Energy toothpaste (Power Energy, Denver, USA-86095200020); Group 3 (positive control) brushed with Colgate toothpaste (Colgate Regular and Colgate-Palmolive, Thailand); and Group 4 (negative control) treatment with distilled water. These kinds of toothpaste were chosen based on their similar excipients to exclude the effects of any dissimilar excipient and to only evaluate the effective component of each toothpaste.

The specimens were brushed twice a day for 1 month, and each brushing period lasted for 1 minute [1]; then, they

Figure 1: Mounting the specimens.

were immersed in a solution containing toothpaste for 2 minutes. The quantity of toothpaste added to the solution was managed at a ratio of 1 : 33, where 3 g of paste was added to 10 mL of distilled water. The samples were retested for enamel microhardness after treatment with different kinds of toothpaste using the same method as the previous steps.

2.5. Statistical Analysis. Data analyses were administered using SPSS version 22. The normality of the data was evaluated using the Shapiro–Wilk test. In addition, the Wilcoxon signed-ranks test was used to evaluate differences in microhardness values between the initial, demineralization, and remineralization steps in each group. Differences in mean values between the experimental groups were investigated by the Kruskal–Wallis and Mann–Whitney tests.

3. Results

According to the results of the Shapiro–Wilk test, data were not distributed normally ($p > 0.05$); hence, nonparametric tests (i.e., Kruskal–Wallis and Mann–Whitney tests) were used to investigate differences in mean values of hardness between the study groups following the demineralization process and treatment.

3.1. Enamel Microhardness in Initial and Demineralization Phase. Table 1 presents the mean values of microhardness of four experimental groups in the initial (T1) and demineralization (T2) phases and the differences between these two steps (T1-T2). Microhardness values were significantly decreased after the demineralization process in each treatment group with no statistically significant differences between them.

3.2. Enamel Microhardness after Brushing. According to Table 2, a considerable increase in microhardness values ($p < 0.05$) after brushing could be seen in all groups. A significant difference was found in mean values between the four groups ($p < 0.05$).

3.3. Comparison of Microhardness Values. A post hoc analysis was done using a Mann–Whitney test, which revealed no significant difference between the fluoride and theobromine groups, while the microhardness values were significantly higher in these groups than in the caffeine and distilled water groups. The caffeine and distilled water groups had no statistically significant differences in their microhardness values (Table 3).

4. Discussion

In recent years, many studies have investigated the impact of early noninvasive treatment of incipient lesions through remineralization of the enamel surface [2]. As a result, some novel enamel remineralization systems have been introduced, some of which are currently being used in the clinical setting [14]. The primary method recommended to either prevent or reverse initial lesions is to use different kinds of toothpaste, followed by mouthwashes and gels with good active biocompounds. Active compounds as well as other necessary components are present in different kinds of toothpaste. Different active compounds that contribute to the promotion of enamel remineralization are present in different kinds of toothpaste, the most common compound being fluoride [15]. To prevent the side effects of fluoride, studies have been conducted to find a natural and biocompatible alternative to fluoride. According to various studies, theobromine is a good alternative to fluoride [15]. In this study, changes in enamel microhardness after demineralization followed by treatment with toothpaste containing theobromine, caffeine, and fluoride were investigated. After exposure to different kinds of toothpaste, the increase in microhardness values in the demineralized enamel surfaces of all four experimental groups were significant, and there were significant differences between their values (p value <0.05). The findings revealed no significant difference between the fluoride and theobromine groups, while the microhardness values were significantly higher in these groups than in the caffeine and distilled water groups. The caffeine and distilled water groups had no statistically significant differences in their microhardness values. The ability of fluoride to remineralize caries is the gold standard against which other remineralization systems must compete, either alone or in combination with fluoride. Currently, several acidulated fluoride products are available. The most common product is mouth rinse of sodium fluoride with phosphoric acid at pH 3.0–4.0, gels, and foams [16]. In the present study, the fluoride group had the highest hardness and had a significant difference with the caffeine and distilled water groups, but there was no significant difference with the theobromine group. In an in vitro study conducted by Sulistianingsih et al., fluoride (solution of 1000 ppm fluoride for 15 minutes) could increase the harness of enamel samples after artificial demineralization [17]. Arnold et al. demineralized the enamel surface of 90 human premolars in a hydroxyethyl cellulose solution at pH 4.8, followed by immersion of teeth in toothpaste slurry. They concluded that amine fluoride compounds in different kinds of toothpaste result in marked remineralization of caries like enamel lesions followed by sodium fluoride and sodium monofluorophosphate formulations [18]. The result of our study is the same as the previous studies in the literature about the remineralization effect of fluoride. The main components of hydroxyapatite in tooth enamel are phosphate ions (PO_4^{3-}) and calcium ions (Ca^{2+}). There is a steady equilibrium

Table 1: Enamel microhardness in the initial and demineralization phases in each group.

Group	Hardness mean value ± SD (KHN)			p value
	Initial hardness ± SD	Demineralization hardness ± SD	Diff 1 ± SD	
1 (theobromine)	235.96 ± 87.09	151.09 ± 82.76	84.86 ± 47.49	0.001
2 (caffeine)	216.40 ± 66.75	151.20 ± 64.03	65.20 ± 58.74	0.001
3 (fluoride)	214.48 ± 64.14	146.29 ± 69.26	68.18 ± 37.42	0.001
4 (distilled water)	247.37 ± 65.44	149.98 ± 64.42	97.39 ± 64.52	0.001
	0.457	0.996	0.146	

Diff 1 = initial–demineralization; SD: standard deviation.

Table 2: Enamel microhardness values after brushing in each four groups.

Group	Hardness mean value ± SD (KHN)			p value
	Demineralization hardness ± SD	Remineralization hardness ± SD	Diff 2 ± SD	
1 (theobromine)	151.09 ± 82.76	289.34 ± 100.55	138.24 ± 110.89	0.001
2 (caffeine)	151.20 ± 64.03	231.05 ± 49.82	79.84 ± 56.86	0.001
3 (fluoride)	146.29 ± 69.26	291.82 ± 94.51	145.53 ± 84.43	0.001
4 (distilled water)	149.98 ± 64.42	209.15 ± 57.24	59.16 ± 53.38	0.001
	0.996	0.004	0.003	

Diff 2 = remineralization–demineralization; SD: standard deviation.

Table 3: Comparison of microhardness values in pairwise groups.

Group	Theobromine	Caffeine	Fluoride	Distilled water
Theobromine	—	0.035	0.780	0.005
Caffeine	0.035	—	0.017	0.460
Fluoride	0.780	0.017	—	0.002
Distilled water	0.005	0.460	0.002	—

among calcium and phosphate ions in saliva, in normal conditions, and in the crystalline hydroxyapatite that makes up 96% of tooth enamel. When pH is lower than the critical level (5.5 and 6.2 for enamel and dentin, respectively), it stimulates demineralization, that is, the dissolution of tooth mineral (hydroxyapatite). However, in cases where the natural buffer capacity of saliva results in increased pH, it causes remineralization. In situations where fluoride exists in oral fluids (i.e., saliva), the remineralization process results in the formation of fluorapatite, not hydroxyapatite. Fluoride ions (F^-) replace hydroxyl groups (OH–) in the formation of the apatite crystal lattice. In other words, fluoride stimulates further remineralization [19].

Recently, some studies have mentioned theobromine as an effective remineralizing agent and a potent alternative to fluorides [8]. Nakamoto et al. mentioned theobromine and fluoride as substances that can enhance apatite crystal size, which is related to enamel surface microhardness [20]. In the present study, the specimens brushed with theobromine showed an increase in microhardness values significantly. The results showed that there was no significant difference between fluoride and theobromine, while a significant difference exists between these two groups and the caffeine and distilled water groups. There are some conflicting reports in the literature regarding the remineralization effect of theobromine in comparison to fluoride. Nakamoto et al. in their study reported that theobromine and fluoride had the same remineralization effects [20]. The result of the Nasution et al. study showed that fluoride had a higher remineralization effect than theobromine [4], also Parvathy et al. indicated that theobromine had less remineralization potential in comparison to a dentifrice containing fluoride and with no significant difference between the two groups [8]. Pribadi et al. studies reported that the surface hardness of enamel following immersion in theobromine cacao rind extract seems to be significantly higher than in the fluoride group [3]. The tooth enamel surface hardness can be affected by the exchange of minerals on the surface of the enamel [21]. Amaechi et al. reported that theobromine can cause the growth of new enamel and stimulates calcium and phosphate growth from the saliva to integrate into a crystal unit that is larger (by 4-folds) than hydroxyapatite. The combination of mineral placement as new enamel growth may cause alternations in enamel hardness [22].

All around the world, coffee is widely served, mainly because of its taste and its effect on both mental and physical activities [23]. Conflicting results are reported regarding the advantages or disadvantages of coffee. According to the currently available evidence, it cannot be argued that drinking coffee either protects or damages the arteries, as it has several useful antioxidants, or may stimulate anything from cancer to bone loss [10]. In limited amounts, coffee makes teeth healthier and whiter. In addition, it has several other health benefits.

Moreover, apart from keeping one alert and awake, coffee contains several health benefits. Coffee is not only a morning jolt, as it has components called antioxidants, which are useful. In a study conducted several years ago in California, a professor of environmental toxicology reported that the amount of antioxidants available in freshly brewed coffee is equal to three oranges. Generally, antioxidants have several potential benefits, such as preventing cardiovascular diseases and liver and colon cancers (among different types of cancer), type 2 diabetes, and Parkinson's disease [10].

In dentistry, there are two opposite reports about the effect of caffeine on tooth enamel. On the one hand, scientists at two Italian universities conducted laboratory tests and reported that coffee molecules could intervene with the adhesion of *S. mutans* on tooth enamel. The first author, Gabriella Gazzani, a university professor at the Department of Druggist Chemistry of Pavia University, reported that all coffee solutions contain high antiadhesive characteristics, mainly because of naturally occurring as well as roasting-stimulated molecules. She and other scholars at the University of Ancona investigated sampled green and roasted *Arabica* and *Robusta* coffee collected from various countries. They reported that all analyzed samples could prevent *S. mutans* adsorption and revealed inhibitory properties, ranging from 40.5 to 98.1% [10]. Some of other studies reported that the acidity of caffeine may promote the demineralization of the enamel surface [12]. On the other hand, Falster et al. in their study reported the negative effect of consumption of caffeine during pregnancy on the enamel microhardness of newborns [24]. Because of caffeine's anti-*S. mutans* properties and emotional effects, nowadays some toothpaste manufacturers add caffeine as the main component. So far, we could not find a study to evaluate the effect of caffeinated kinds of toothpaste on enamel hardness; therefore, we designed this study.

Because there was no available toothpaste whose only active ingredient was caffeine, in this study, we used Power Energy toothpaste, whose active ingredients were caffeine and xylitol. The mechanism of action of xylitol has been studied in various research; different kinds of toothpaste that contain xylitol not only decrease the number of *S. mutans* colonies in saliva and the level of secreted saliva but can also increase the pH value. Increased flow of saliva with high levels of calcium and phosphate and shorter duration of low plaque pH may result in remineralization. The anticaries property of xylitol is mainly due to its impact on plaque and cariogenic microorganisms [25]. The presence of xylitol in the caffeinated toothpaste used for the experiment was the major limitation of our study because we could not differentiate the effects of caffeine and xylitol on enamel microhardness. In the present study, the microhardness was increased after brushing with caffeinated toothpaste, but this increase was the same as in distilled water groups. It could be concluded that the caffeine in toothpaste did not have the ability to demineralize nor it had a negative effect on enamel microhardness. Toothpaste containing xylitol and caffeine had the same effect as distilled water. In a study conducted by Tange et al., they concluded that xylitol itself had the same remineralization effects as that of fluoride [26], but in our study, we found that toothpaste containing xylitol and caffeine had a significantly lower remineralization effect than fluoride. One of the reasons for this difference might be the presence of caffeine. Caffeine might have reduced the remineralization effect of xylitol.

The present study demonstrated a significant increase in hardness values in the negative control group (group 4) following treatment with distilled water (p value <0.05). It is well proved that brushing can remove the demineralized soft enamel layer. In addition, it exposes the underlying hard enamel layer, which in turn results in increased hardness of the enamel [27].

The limitations of this study were the lack of toothpaste in which the only active ingredient was caffeine, and the presence of xylitol in the caffeinated toothpaste that was used for the experiment, because of which we could not differentiate the effects of caffeine and xylitol on enamel microhardness, laboratory study, and the impossibility of investigating the antibiotic properties of caffeine in the oral environment.

5. Conclusion

There was no significant difference between the fluoride and theobromine groups, while the microhardness values were significantly higher in these groups than in the caffeine and distilled water groups. Therefore, theobromine is a good alternative to fluoride to overcome the side effects of fluoride in high concentrations. The caffeine and distilled water groups had no statistically significant differences in their microhardness values.

Data Availability

All the data generated or analyzed during this study are included in this published article, and the datasets used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

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Research Article

Effect of Surfosept and Deconex® 53 Disinfectant Agents on the Accuracy and Dimensional Stability of Panasil Dental Impression Materials: An Experimental Study

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Aim and Objective. Impression materials and stone casts are the main sources of cross-contamination and transmission of dental infections between dental offices and laboratories. Both Surfosept (alcohol-based) and Deconex® (quaternary ammonium compound) are biocompatible solutions that have superior antimicrobial properties against bacteria, fungi, and viral envelope. Considering the influence of disinfection substances on the dimensions of impression materials, this study aimed to compare the effect of Surfosept and Deconex® 53 on the accuracy and dimensional stability of the Panasil® dental addition silicone material. *Materials and Methods*. This in vitro study was performed on 30 dental casts. The samples were divided into one control group and two experimental groups to be disinfected with Surfosept (1%) and Deconex® 53 (2%) using a sequential sampling method (10 per group). The impressions in the experimental groups (i.e., Surfosept and Deconex® 53) were rinsed and dried; then, the disinfectant was sprayed on the impressions and remained for 30 seconds before pouring with stone. In the control group, the impressions were only rinsed and dried and were poured in 10 minutes. Cast dimensions were measured by a profile projector device, and the mean values obtained from the experimental groups were compared with those of the control group. *Results*. There were no significant differences among the groups regarding the height of the resulting dies without undercut ($P = 0.62$). Moreover, there was no significant difference among the groups regarding the distance between the two dies ($P = 0.77$). However, the diameter of the dies with undercut and without undercut was different significantly among the control and experimental groups ($P < 0.005$). *Conclusion*. In general, no significant difference was encountered between dimensional stability and accuracy of the dental impressions using Surfosept and Deconex® 53 in this study.

1. Introduction

Dental materials are exposed to various pathogenic microorganisms which are potentially harmful [1]. The main source of cross-contamination especially between dental offices and laboratories is due to the contaminated impression trays, dental impression materials, and stone casts [2]. Several methods of disinfection such as chemical disinfection with the immersion method or spray method, microwave, and ultraviolet radiation are used to disinfect the dental impressions and dental casts [3]. Chemical disinfection has remained a common practical approach to eliminate microorganisms, since heat or steam sterilization of impressions and occlusal records cannot be performed due to the risk of distortion [3]. However, as all disinfectant solutions can have remarkable effects on the dimensional changes of impression materials, immersion duration is recommended to be short, i.e., less than 30 minutes [4].

There is invaluable evidence to support the transmission of microorganisms through impression materials [5, 6].

According to the literature, most of the materials used in dental laboratories contain various infectious microorganisms, such as *Streptococci* [7, 8]. Badaró et al. estimated the prevalence of *Streptococci, Staphylococcus aureus, Methicillin-resistant Staphylococcus*, and *Candida* in the impressions taken from the patients' mouths at 100%, 55.6%, 25%, and 9%, respectively [9]. In the current COVID-19 pandemic, proper care should be given to reduce possibility of coronavirus cross-contamination. According to Kampf et al., disinfection with 0.1% sodium hypochlorite or 62–71% ethanol can significantly reduce coronavirus load on surfaces within 1 min exposure time [10]. Therefore, disinfection of dental impressions can remarkably reduce the number of microorganisms.

It is of utmost importance to select the suitable material for disinfection and to identify the potential problems with each approach [11]. Shelf life, solidification, ease of application, low price, robustness, and resistance to different kinds of stress are among suitable characteristics of impression materials [12, 13]. On the other hand, dimensional changes of the impression materials, following the use of disinfectants, are among the main problems in the process of preparation of dental prostheses. Silicones are the most common impression materials used to fabricate fixed dental prosthesis [14]. Recently, the use of addition silicone impressions is escalated due to its high accuracy [3]. Addition-type silicone impression materials with enhanced hydrophilic properties have the potential to show larger dimensional changes after disinfection, compared to conventional condensation-type materials, due to the fact that disinfection solutions may promote water absorption from surrounding environment [15]; however, there is a dearth of research in this regard in the literature. Deconex® 53 is a disinfectant solution, which is commonly used in hospital settings for disinfecting flexible and rigid endoscopes. The recommended concentration of this substance is 1-2% depending on the expected effect, while a maximum of 4% concentration is used in certain circumstances [12]. This solution is composed of alkyl propylene diamine guanidinium diacetate and N-didecyl-N-methyl-poly (oxyethyl) ammonium propionate. Surfosept (Reza Rad Co., Iran) is another alcohol-based disinfectant material which is used for cleaning surfaces and objects. This substance is standardized by the European Medicines Agency (Standards EN 1040). It can also be utilized to disinfect dental instruments. It can be used for eliminating bacteria and viruses, such as influenza A virus subtype H1N1, hepatitis C virus, hepatitis B virus, and human immunodeficiency virus. This solution contains isopropanol, didecyldimethylammonium chloride, ethanol, and other additives [16].

As dental impression material such as elastomeric silicone carries the risk of microbial colonization and infection, of particular concern is the biocompatibility of the disinfection solutions applied on them. Alcohol-based solutions (Surfosept in this study) are found to act as bactericidal, fungicidal, and virucidal against enveloped viruses (e.g., HIV) and have low cytotoxicity to human cells [17].

Also, studies of bacterial resistance to antibacterial agents such as Deconex (a quaternary ammonium salt) have shown a loss of bacterial resistance upon application of them to the surface [18]. The effect of Deconex on the dimensional changes of impression materials has been investigated in some studies; however, there is no study assessing the dimensional changes in the casts disinfected by Surfosept material. As disinfecting dental impressions is necessary, there is a need to investigate different disinfection materials and their effects on the characteristics of impression materials, especially their dimensional changes. Therefore, this study aimed to determine the effect of two substances (i.e., Surfosept and Deconex® 53) on the accuracy and dimensional stability of dental impressions made of addition silicones (i.e., Panasil®).

2. Materials and Methods

2.1. Metal Bases and Dies. This in vitro experimental study was performed on 30 casts. Samples were divided into one control group and two experimental groups to be disinfected by Surfosept and Deconex® 53 using a sequential sampling method (10 per group). The study included two upper and lower sections simulated based on an intraoral dentate situation (Figure 1). The lower section had a metal base including two stainless steel dies with three degrees taper per each wall. One of the dies was trimmed in a horizontal direction at the cervical region (2 mm) to create an undercut with depth of 1.5 mm and 45 degrees angle. The metal base had four guide bars for placing the upper section in a specified direction. The die base consisted of a metal plate with dimensions of 30 mm width, 60 mm height, and 15 mm length. The upper section, which acted as a custom impression tray, was made of metal base with holes to provide retention for the impression material and to reduce intersection pressure. Additionally, it had 4 holes on 4 sides to hold the base bars. This section had the same dimensions as the lower section but differed in height (12 mm). The die base and the upper and lower sections were made of E.C.N, and the bars and bushes were made of B.O.Z.

2.2. Impression. Initially, using the 2-stage method, two units of putty were mixed with two units of accelerator. The mixture was placed in a horse-shoe shape plastic tray, and the impression was taken. Initially, the required space for the wash layer was provided with a 1.5 mm metal spacer. The initial setting time, working time, and total setting time lasted 120, 120, and 240 seconds, respectively, at 32°C.

After the solidification of the impression material, the upper and lower sections were separated and the spacer was removed from the putty material. Light body Panasil® (Kettenbach Co., Germany) was injected on the putty material and around the die, and the impression was taken again. After hardening, the material was separated from the tray following the manufacturer's recommendations. The initial setting time, working time, and the final setting time for the light body material were 150, 60–90, and 240 s, respectively.

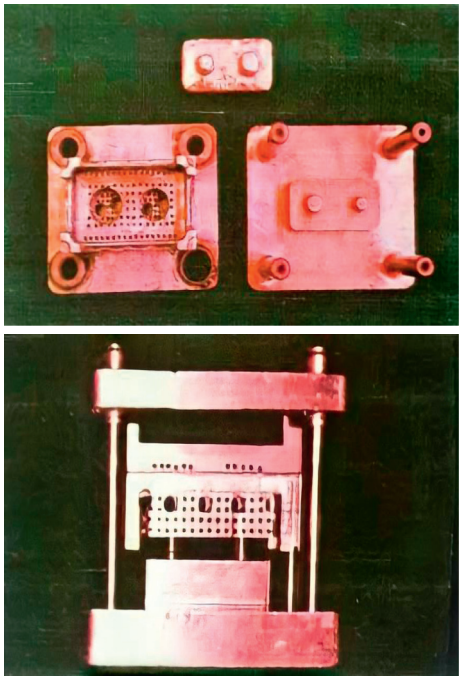


Figure 1: Upper and lower sections simulated based on an intraoral situation.

2.3. Disinfection Process. Deconex® 53 (1%) was sprayed on the impressions for 30 s. In the experimental groups (i.e., Surfosept and Deconex® 53), the impression materials were rinsed and dried after taking impressions. Subsequently, the disinfectant was sprayed on the impressions. The impressions were left in the room temperature for 30 min, for the rebound phenomenon to happen, before pouring them with stone. In the control group, the impressions were washed and dried.

2.4. Preparation of BegoStone Samples. In the next stage, the impressions were poured using BegoStone (type IV dental stone) (Wilhelm Herbst Bremen, Germany). According to the guidelines, 50 g of BegoStone was mixed with 10 cc water at 23°C for 30 s, and it was poured into the impressions in three minutes utilizing slow vibration using a dental laboratory vibrator (Silfradent, VIB24, Italy). The casts were separated from the impression after one hour (Figure 2).

2.5. A Profile Projector. A profile projector (Tesa Co., Switzerland) with 0.001 mm resolution was utilized to compare the dimensions and geometry of the samples. The profile projector, which is known as an optical comparator, is a shadowgraph device which uses principles of optics for accurate measurement and dimensional inspection of manufactured samples.

2.6. Dimensional Measurements. 5 variables were specified and examined on cast models. These factors which were measured by the profile projector include the height of the die without undercut (Figure 3(a)), the diameter of the die without undercut (Figure 3(b)), the distance between two dies (Figure 3(c)), the diameter of the die with undercut (Figure 3(d)), and the height of the die with undercut (Figure 3(e)) (Figure 3).

2.7. Statistical Analysis. The data were analyzed in the IBM SPSS software (version 21). The three groups were compared in terms of the mean of studied variables. One-way ANOVA was used to compare the groups, and in case of a significant difference, the post hoc test (Tukey test) was used to determine the differences among groups. A P value less than 0.05 was considered statistically significant.

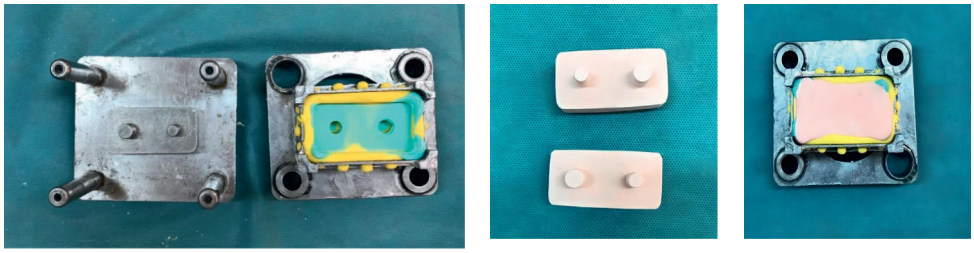
3. Results

3.1. The Mean Height and Diameter Evaluation. The mean height and diameter of the dies with and without undercut and distance between two dies are given in Table 1. The mean height of the dies without undercut was measured at 10.012 mm and 10.014 mm in the Surfosept and Deconex and 10.015 mm in the control group ($P = 0.62$). Also, measurements of the mean height of the dies with undercut were found to be the same ($P = 0.62$). The mean diameter of the dies without undercut was measured at 8.016 mm and 8.012 mm in the Surfosept and Deconex and 8.035 mm in the control group ($P < 0.005$). Also, the mean diameter of the dies with undercut was measured at 10.04 mm and 10.028 mm in the Surfosept and Deconex and 10.055 mm in the control group ($P < 0.005$). The mean distance between the two dies was measured at 21.739 mm and 21.749 mm in the Surfosept and Deconex and 21.75 mm in the control group ($P = 0.77$).

3.2. The Mean Difference of Height and Diameter Evaluation. As a result, the height of the dies with or without undercut was the same in the experimental and control groups. The distance between the dies was also calculated to be the similar between the experimental and control groups. Whereas, the diameter of the dies was found to be different, meaning the experimental groups had a lower diameter of the dies, with or without undercut, compared to the control group ($P < 0.005$). Table 2 summarizes the results obtained from the post hoc test.

4. Discussion

This study aimed to determine the effect of two disinfectant materials (i.e., Surfosept and Deconex® 53) on the accuracy and dimensional changes of impression materials and the resulting casts. Among disinfectants, quaternary ammonium salts (Deconex) and alcohol-based solutions (Surfosept) are potent candidates with superior antibacterial properties. Therefore, they are biocompatible materials, which have low cytotoxicity to human cells, and at the same time are environment friendly and may be further developed for home use [18]. The dimensional stability of the impression materials can be influenced by several factors, including the contraction during the polymerization and expansion after



(a) (b) (c)

Figure 2: Impression steps: impression obtained from dies (a); poured impression with BegoStone (b); resulting casts (c).

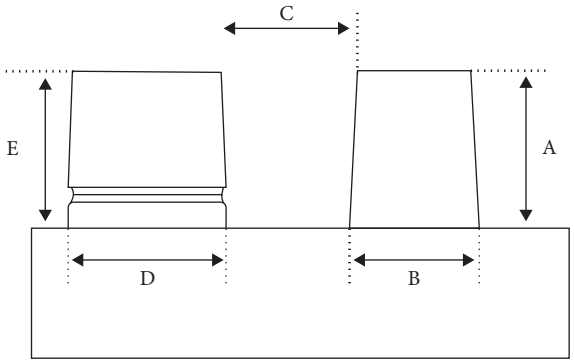


Figure 3: The height of the die without undercut (a), the diameter of the die without undercut (b), the distance between two dies (c), the diameter of the die with undercut (d), and the height of the die with undercut (e).

Table 1: The mean height and diameter of the die with and without undercut and distance between two dies.

Dimensions	Surfosept		Deconex		Control		F	P value
	Mean	SD	Mean	SD	Mean	SD		
Height of the die without undercut (mm)	10.012	0.0091	10.014	0.0069	10.015	0.0052	0.43	0.62
Height of the die with undercut (mm)	10.012		10.014		10.015			0.62
Diameter of the die without undercut (mm)	8.016	0.0051	8.012	0.0042	8.035	0.0052	62.723	>0.005
Diameter of the die with undercut (mm)	10.04	0.0047	10.028	0.0042	10.055	0.0085	48.921	>0.005
Distance between two dies	21.739	0.0325	21.749	0.0233	21.75	0.0551	0.258	0.77

Table 2: The mean difference of height and diameter of the die with and without undercut and distance between two dies.

Dimensions	Control-Surfosept		P value	Control-Deconex		P value	Surfosept-Deconex		P value
	Mean difference	SD		Mean difference	SD		Mean difference	SD	
Height of the die without undercut (mm)	0.003	0.0032	0.635	0.00100	0.00328	0.95	−0.002	0.0032	0.81
Diameter of the die without undercut (mm)	0.019	0.00219	>0.005	0.023	0.00219	>0.005	0.004	0.00219	0.18
Diameter of the die with undercut (mm)	0.015	0.00274	>0.005	0.027	0.00274	>0.005	0.012	0.00274	>0.005
Distance between two dies	0.0115	0.01759	0.792	0.0012	0.01759	0.99	−0.0103	0.01759	0.82

immersion in disinfectant solutions [19]. The thermal contraction of addition-type silicone rubber impression materials may lead to a 10–12 μm dimensional change in cylindrical stone casts for every 1 mm increase in impression thickness [20].

Addition silicones (i.e., Panasil®) are the impression materials made of polyvinyl siloxane or vinyl polysiloxane. They have maximum dimensional stability and minimum dimensional changes when exposed to disinfectant materials [19]. The polymerization reaction of these materials helps

obtain optimal dimensional stability [16]. Due to the novelty of the addition-type silicone, the type of disinfectant suitable for these materials is not properly specified in the literature. It seems that Deconex® 53 tends to be able to absorb water out of the air, which is consistent to the hydrophilic property of an addition silicone material. However, the use of Deconex® 53 with alginate and polyether should be performed with caution, since eliminating microorganisms in these materials could be compromised [12]. Hiraguchi et al. used addition-type silicones to assess the dimensional changes of impression materials after immersing in glutaraldehyde (2%) and orthophthalaldehyde (0.55%) for 30 min. According to the results of the aforementioned study, no remarkable dimensional changes were observed in casts [20]. This finding was in line with the results obtained from other similar studies [21], in which the use of addition silicone (i.e., Panasil®) was suggested as a suitable impression material.

Comparison of the experimental (i.e., Surfosept and Deconex® 53) and control groups in this study showed that the height of the dies with or without undercut were not significantly different ($P = 0.62$). In addition, no significant difference was observed among the groups in terms of the distance between two dies ($P = 0.77$). However, there was a significant difference among the groups regarding the diameter of the dies with undercut and the diameter of the dies without undercut ($P < 0.005$). Therefore, no significant difference was found between the experimental and control groups regarding the dimensional changes, except for the diameter of the dies with and without undercut. Based on the obtained results, both Surfosept and Deconex® 53 can be used without significant concern, on the impression materials made of Panasil®.

In a study conducted by Ghasemi et al., Deconex, sodium hypochlorite (25.5%) and Epimax were utilized to disinfect alginate, silicone, and polyether impression materials [12]. According to the results, no significant difference was observed among the impressions in terms of the dimensional changes after disinfection. This finding is consistent with the results obtained from the current study.

Similarly, a study was conducted by Sabouri et al. on 30 impressions to determine the effect of disinfectants on dimensional changes of impression materials. In this study, 20 impressions were disinfected by sodium hypochlorite and acid glutaraldehyde (10 impressions per group) and 10 impressions were considered as a control group. They rinsed impressions with cold water and stored them at room temperature for 30 and 20 min in the disinfected and control groups, respectively. Similar to our study, the height and diameters of the dies and the distance between the dies were measured in three groups. They showed no significant differences between the disinfected and control groups regarding the dimensional changes [22].

In our study, the disinfectant solutions were sprayed on impressions for 30 seconds. Nassar et al. performed a study to determine the effects of disinfectant solutions on the dimensional changes of the impression materials. Four elastomeric impression materials (i.e., Xantopren, Express, Permlastic, and Soft Impregum) were used to compare the effect of immersion time on the dimensional changes of casts made with each of these materials. Dimensional changes were reported in all materials over time, except for immersion periods lower than 20 min [23]. In another study, sodium hypochlorite (25.5%), Deconex, and Sanosil were employed for disinfecting impressions for 8–10 min periods, and the results were acceptable in terms of accuracy and dimensional stability of the material [12].

5. Conclusion

The results indicated that the height and the distance between two dies had higher accuracy and dimensional stability following the use of the Surfosept (1%) and Deconex® 53 (2%) disinfecting material. However, in general, no significant difference was noted in terms of influencing the dimensions or accuracy of the impression materials, whether disinfection solutions were used or not.

Data Availability

The data generated or analyzed are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Research Article

A Novel Developed Bioactive Composite Resin Containing Silver/Zinc Oxide (Ag/ZnO) Nanoparticles as an Antimicrobial Material against *Streptococcus mutans*, *Lactobacillus*, and *Candida albicans*

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Aim. The objectives of this study were to develop a new bioactive composite resin containing silver/zinc oxide (Ag/ZnO) nanoparticles and investigate the effects on mechanical, cytotoxic, biocompatibility, and antimicrobial properties. *Materials and Methods*. Disc-shaped specimens were prepared from composite with and without nanoparticles in separate culture media containing *Streptococcus mutans*, *Lactobacillus*, and *Candida albicans*. Bracket bonding evaluation was performed on composite without nanoparticles (O), composite containing ZnO (Z) nanoparticles, composite containing ZnO nanoparticles and silver ions (A&Z), and composite containing Ag/ZnO nanoparticles (AZ) synthesized using optical precipitation. *Results*. Composite resin with nanoparticles (AZ, A&Z, and Z) showed significant antimicrobial properties ($P < 0.05$). The mean shear bond strength of A&Z composite resin (13.61 ± 0.73 MPa) was significantly less than that of conventional composite resin (19.03 ± 4.12 MPa) ($P < 0.05$). In addition, the mean shear bond strength of AZ composite resin (20.49 ± 1.03 MPa) was significantly higher than that of Z (16.35 ± 1.03 MPa) and A&Z composite resins. *Conclusions*. Incorporation of ZnO nanoparticles and their compounds into orthodontic composite resins induced antibacterial properties against oral pathogens, and of all these nanoparticles, the AZ group exhibited the best antimicrobial activity and highest shear bond strength.

1. Introduction

Orthodontic treatment possesses many advantages for patients with functional and esthetic problems and results in the satisfaction of the majority of such patients. However, such treatment might have complications for these patients, including demineralization, caries, and tooth discoloration around brackets and the bonded areas in the form of white spot lesions (WSL), which are considered a major challenge for clinicians and a major challenge factor for dissatisfaction of patients. This problem makes the patients susceptible to more widespread caries, especially in patients with poor oral hygiene. In this context, patients receiving a full fixed treatment plan are more susceptible to caries and exhibit a

significant increase in *S. mutans* counts in their plaque and saliva [1, 2]. In the presence of an increased count of cariogenic bacteria in the saliva and plaque and poor oral hygiene, decalcification and carious lesions can occur in less than 4 weeks [3, 4].

Fluoride-containing materials, especially fluoride varnishes, are widely used to prevent caries. Still, they have two main problems: first, they need regular patient cooperation, and second, they have a moderate effect on prevention of WSL and caries [5]. Fluoride-containing bonding agents as well have the problem of rapid release during the first 24 hours, with a decrease in release over time [2]. Nanoparticles (NPs) have been broadly used as antimicrobial agents in medicine and dentistry. Nanoparticles are particles smaller than 100 nm in size [6]. Because of the greater surface-to-volume ratio, nanoparticles interact more closely with microbial membranes and present a considerably larger surface area for antimicrobial activity [7]. ZnO and ZnO-containing materials have significant antibacterial and antifungal activity and are used in different ways for the treatment of traumatic injuries, foot injuries, and burns [8]. Dental materials, including endodontic sealers and adhesive cements, use this property of ZnO for bonding of fixed restorations. The results of a study by Tavassoli Hojati et al. in relation to the effect of incorporating ZnO nanoparticles into flowable composite resins on the antibacterial, physical, and strength properties showed that an increase in the concentration of these nanoparticles resulted in a significant increase in the antibacterial properties of these composite resins [9].

Also, Ahn et al. improved the antibacterial properties of orthodontic composite resins as adhesive agents by adding silver nanoparticles to their structure. However, there was no significant difference between these experimental composite resins and the conventional composite resin in shear bond strength [10]. Although silver nanoparticles induce favorable antibacterial properties, they might result in a dark color in composite resin, creating esthetic problems. Ag/ZnO (AZ) nanoparticles are white, and incorporating them into composite resins with the same color does not lead to esthetic problems. On the other hand, it is expected that the use of these nanoparticles, which contain both silver and zinc oxide, will increase their antibacterial properties [10].

Application of low or nontoxic dental materials is essential in long-term usage to guarantee patient and staff health and safety. Biocompatible materials are compatible with pulp and other live tissues with minimal cytotoxic impacts [11].

Previous studies have confirmed that ZnO nanoparticles are safe and biocompatible and can be used in dental and medical applications [12]. Moreover, based on previous investigations, silver NPs showed a biocompatible behavior, which means not affecting cell metabolism and proliferation, or cause genotoxic damage to cells [7].

Considering the properties mentioned for AZ nanoparticles and the unfavorable properties of silver nanoparticles alone and since fixed orthodontic treatment requires a biocompatible material with antibacterial properties concomitant with preservation of mechanical properties, the present study was undertaken to evaluate the effect of incorporating ZnO and AZ nanoparticles into orthodontic composite resins on the antibacterial properties, biocompatibility, and shear bond strength of these composite resins for the first time.

2. Materials and Methods

2.1. Preparation of Nanoparticles. Zinc acetylacetonate, sodium hydroxide, $AgNO_3$, ethanol (absolute), and starch were purchased from Merck Company (Darmstadt, Germany). As described in our previous work [13], ZnO and Ag/ZnO nanoparticles were synthesized and named as Z and AZ. As a sample, 0.4 g zinc acetylacetonate was dissolved in 20 ml absolute ethanol and 20 ml of starch solution (30%) was added drop wise, and the mixture was kept stirring for 4 h. Then, the aqueous solution of $AgNO_3$ was dropped into the solution under stirring for 30 minutes and at last heated in a water bath. During the whole process, the system was continuously stirred. The solution gradually became milky gel with temperature rising up to 80°C. At last, the gel was dried at 100°C for 24 h, heated in a laboratory furnace at 400°C for 8 h to burn out the starch residues, and calcined at 550°C for 5 h. After synthesis of ZnO nanoparticles, the solution of $AgNO_3$ (0.2 μL) was added drop wise in the aqueous suspension of ZnO nanoparticles and named as A&Z.

Nanoparticles were mixed continuously with no-mix self-cure composite resin (Unite Bonding System; Reliance, USA) using a plastic spatula for 15 min at different concentrations of 0% (without nanoparticle), 5%, 10%, 15%, and 20% in weight. The samples were prepared in glass containers of 10 mm diameter and 1.5 mm height. An activator primer liquid was placed on the samples, and their setting time was completed. After that, the samples were polished with 600, 800, and 1200 grit SiC papers (991A Softlex, Berlin, Germany) to obtain highly polished samples with identical surface roughness (Ra) values. To confirm the Ra was homogenous, two samples from each group were observed randomly by microscope-assisted precision (MAP).

X-ray diffraction technique (XRD; Siemens D5000, Germany) was utilized in order to characterize the crystalline structure of Ag-doped ZnO nanoparticles. X-ray diffraction patterns (XRD) were collected using a Siemens D500 diffractometer with Cu kα radiation (=1.5418 Å and $2\theta = 4$–80°) at room temperature. A scanning electron microscope (Philips XL30) equipped with energy dispersive X-ray (EDX) facility was used to capture SEM images and to perform elemental analysis. The SEM sample was gold coated prior to examination, and SEM was operated at 5 kV while EDX analysis was performed at 15 kV. The TEM study was carried out on a Zeiss LEO 912 Omega instrument, operating at 100 kV.

2.2. Shear Bond Strength (SBS) Test. Different resin composites were prepared in four separate experimental groups. In group 1, composite resin was used for bonding of brackets without nanoparticles (O). In groups 2–4, composite resin with ZnO nanoparticles (Z), composite resin with ZnO nanoparticles and silver ions (A&Z), and composite resin

with Ag/ZnO nanoparticles (AZ) were synthesized, respectively. The powder of nanoparticles was added to the no-mix self-cure composite resin and consistently mixed for 15 minutes by means of a glass spatula.

120 extracted human maxillary first premolars were stored in 0.01% thymol solution (Thymol Mylan, Seiyaku, Japan) at 4°C to prevent bacterial growth and dehydration. The specimens were embedded in a self-cure acrylic (Ivoclar Vivadent, Naturno BZ, Italy) block up to cementoenamel junction in a way that the labial surface of tooth vertically crossed the horizontal line of block base and were stored in distilled water at 37°C for 24 h. They were then coded from 1 to 120 ($n = 30$).

The teeth surface was cleaned with fluoride-free pumice paste using a nylon brush attached to the low-speed handpiece for 5 seconds and washed for 10 seconds by running water. The midcoronal enamel surface was etched with 37% phosphoric acid (3M Unitek, Monrovia, USA) according to manufacturer's instruction and then was thoroughly washed by water spray for 15 seconds. The excess water was removed by gentle air flow from 2 cm distance for 10 seconds. When the white chalky surface of enamel was observed, a thin layer of autopolymerization adhesive (Unite Bonding System; Reliance, USA) was applied on etched section of teeth and bracket base (ortho-organizer, stainless steel). Finally, no-mix self-cure composite (Unite Bonding System; Reliance, USA) was applied to bracket base, which was seated by the application of moderate compressive force for 10 seconds in order to obtain smooth steady composite thickness on the enamel surface.

The blocks were placed in the Hounsfield Test Equipment (Surrey, UK) and fixed in lower grip of the machine. A steel rod with the cutting edge of 0.5 mm was attached to the crosshead of the machine. Each tooth labial surface was oriented to be parallel to the force during the SBS test. The tooth placement in the machine was examined by two operators. An occlusogingival load was applied to the bracket, producing a shear force at the bracket-tooth interface. The force was measured in Newton at a crosshead speed of 0.5 mm/min and divided by the surface area of the brackets pad to calculate the SBS in megapascals (MPa).

2.3. Zn and Ag Release. Composite discs containing nanoparticles (5 mm × 1 mm, $n = 5$) were prepared and stored at a dry place at 37°C for 24 hours. Then, they had been separately immersed in artificial saliva (Nik Ceram Razi corporation, Isfahan, Iran) buffered at a pH of 7 using HEPES (4-(2-hydroxyethyl) piperazine-1-ethane-sulfonic acid)) solution (50 mmol/L) with a constant ratio of 3 mm^3/mL between specimen volume and immersion medium. Specimens were kept immersed for a total of 30 days, and every 7 days, the immersing solution was totally replaced. The solutions were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OEP, 700, Agilent Technologies, Santa Clara, CA, USA) to determine Zn and Ag ionic concentrations released from the composite.

2.4. Antibacterial Activity Assay. Antimicrobial activity of the components was investigated by using the microbroth dilution (MIC) method. In this regard, *S. mutans* ATCC 35668, *Staphylococcus aureus* ATCC 25923, *Lactobacillus gasseri* ATCC 33323, *Escherichia coli* ATCC 25922, *and Candida albicans* ATCC 10231 as common pathogens were selected and purchased from Persian microbial collection (PTCC) related to the National Research Center for Science and Technology, Tehran, Iran. For MIC identification of components, all pathogens were cultured in Mueller-Hinton agar (Merck, Germany) overnight at 37°C (for 24 hours), and yeasts were cultured in Mueller-Hinton agar plus 1% glucose under aerobic condition. Exponential growth phase of pathogens was provided by culture in Mueller-Hinton broth (Merck, Germany) at 37°C, and a concentration equal to 0.5 McFarland of the pathogens was used for study. MIC experience was done according to Clinical Laboratory Standard Institute (CLSI) protocol for microbroth dilution in 98-well polystyrene plates. Composite resins with different concentrations were exposed with pathogens. For anaerobic experience, GasPak Grade A (Merck, Germany) was used. Test groups included nanocomposite resin with different concentrations of silver and zinc oxide nanoparticles, namely, ZnO at 5%, ZnO at 10%, Ag/ZnO with 0.1% Ag and 10% ZnO, Ag/ZnO with 0.05% Ag and 10% ZnO, Ag/ZnO with 0.1% Ag and 5% ZnO, and Ag/ZnO with 0.05% Ag and 5% of ZnO. Negative controls were wells with composite resin without any nanoparticles and a well without any material to investigate any possible contamination. Positive control was a well with pathogen and culture media. One-way analysis of variance [4] was run to determine any significant differences in width of inhibitory zone of the study groups, followed by high significant difference Tukey test (HSD Tukey) for pair-wise comparisons.

2.5. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium Bromide) Assay for Cell Viability. HGF (human gingival fibroblast) cells (7×10^4 cells/well) were incubated in 96-well plates, each containing 200 μL of supplemented cell culture media, for 24 hours at 37°C and 5% CO_2. The cells were divided into 4 groups in triplicates, blank, Z, AZ, and A&Z nanoparticles (different concentrations: 1, 2, 5, 10, and 25 μg/ml), and were treated. After an incubation period of 24 h, the spent media were removed and the plate wells were washed with phosphate-buffered solution. In brief, 50 μL of 2 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) and 150 μL of culture medium was added to each well. The cells were incubated at 37°C and 5% CO_2 for 4 hours, and then the media were discarded. Dimethyl sulfoxide and Sorensen buffer were added to each well as solubilizer buffer. Finally, absorbance was read using an ELISA plate reader (BioTek, Bad Friedrichshall, Germany) at 570 nm wavelength.

2.6. Wettability Measurements. The wettability of the samples was assessed by measuring the contact angles of distilled water on composite resins with Adobe Photoshop® software. The contact angle was defined as the angle at which the liquid

interface met the solid surface of the composite disc at four points on each sample, and the mean of the points was reported as the contact angle of each sample. The surface of the drop was continuously monitored, and the contact angle was measured just after 20 seconds when the droplet was stabilized.

2.7. Statistical Analysis. Data were analyzed using Anderson–Darling and Levine tests to check the homoscedasticity and normality, thereof. Standard deviations were calculated for all repeat contact angle measurements and averaged for each series. A one-way ANOVA test was used for data comparison. Tukey's test was used to check the significance of differences between pairs of means. All statistical tests were run at 5% significance level ($P < 0.05$).

3. Results

3.1. Characteristics Analysis. Figure 1 shows the powder XRD patterns of as-prepared ZnO and Ag/ZnO recorded in the range of 30–70° with a scanning step of 0.02°. The observed diffraction peaks of the pure ZnO catalyst can be indexed to those of hexagonal wurtzite ZnO (PCPDF79-0207). No characteristic peaks of impurity phases such as Zn, $Zn(OH)_2$, Ag, or Ag(OH) were observed.

Figure 2 indicates the distribution of nanoparticles in composite resins which demonstrates homogenous disposition of the NPs in the resin matrix.

For the release test, after 30 days' period for composites containing AZ, A&Z, Z nanoparticles, no significant release of silver or zinc ions was detected and the values were zero for both named ions at time intervals of 7, 14, and 30 days.

3.2. Shear Bond Strength Test. In this study, 4 different types of orthodontic composite resins were evaluated. In group 1, conventional composite resin (O) was used, and in the other 3 groups, composite resins with ZnO nanoparticles (Z), ZnO and silver nanoparticle solution (A&Z), and Ag/ZnO nanoparticles (AZ) synthesized with optical precipitation were used.

Table 1 presents the descriptive data of shear bond strength tests in each study group separately. Based on these data, the mean shear bond strength value in the A&Z group was lower than that in other groups, with the highest shear bond strength in the AZ group (Figure 3).

The Kolmogorov–Smirnov test showed normal distribution of data in the present study ($P > 0.05$). In addition, Levene's test showed that the variances of the groups were the same ($P > 0.05$). Therefore, one-way ANOVA was used to compare the mean shear bond strength values between the study groups, which revealed significant differences in mean shear bond strength values between the different study groups ($P = 0.0001$). The Tukey test showed that the mean shear bond strength of A&Z composite resin was significantly less than that of conventional composite resin ($P < 0.05$).

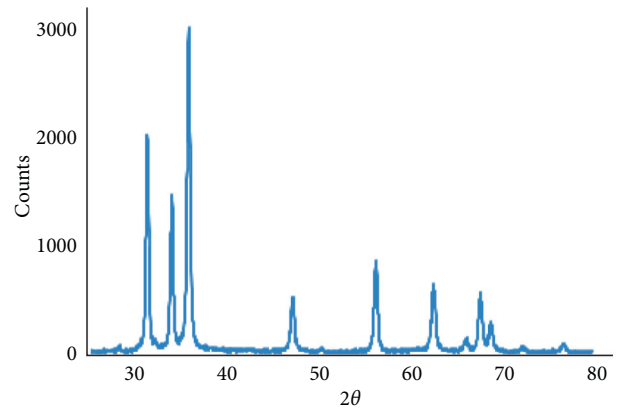

Figure 1: XRD pattern of Ag/ZnO nanoparticles. The existence of high-grade peaks proves the creation of a ZnO system.

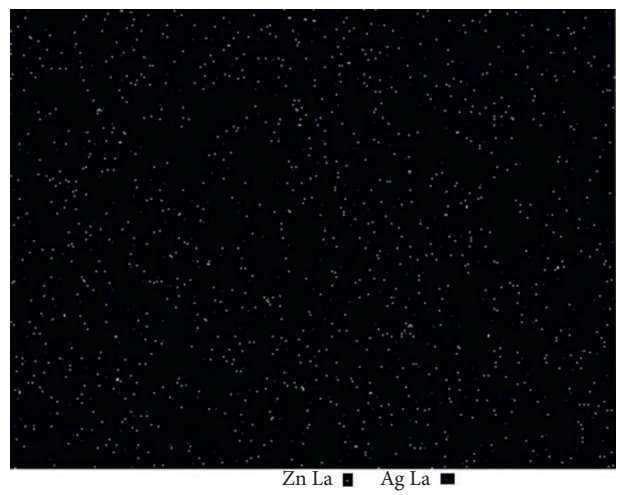

Figure 2: Zn and Ag map of the resin composite containing 10 wt % Ag/ZnO. Light yellow spots represent Zn, and blue ones represent Ag element.

3.3. Antimicrobial Properties. The results of antimicrobial properties are presented in Table 2. These results include different concentrations of composite with concentrations including ZnO at 5%, ZnO at 10%, Ag/ZnO with 0.1% Ag and 10% ZnO, Ag/ZnO with 0.05% Ag and 10% ZnO, Ag/ZnO with 0.1% Ag and 5% ZnO, and Ag/ZnO with 0.05% Ag and 5% of ZnO, which shows no growth for all Gram-positive pathogens ($P < 0.05$) and slight antimicrobial properties against Gram-negative (*E. coli*) pathogen. In all wells of *C. albicans*, growth was observed. ZnO with 10% concentration had higher antimicrobial properties than 5% concentration.

3.4. Viability Test. According to the results obtained from the MTT test, the cytotoxicity of AZ and ZnO nanoparticles indicated that certainly no major and significant damaging effect is expected to the cells up to 0.1 mg/ml of ZnO and AZ nanoparticles. Data are summarized via Figure 4.

Table 1: The descriptive data of shear bond strength in each study group.

Group	Number	Mean of shear bond strength (MPa)	Std. deviation	Minimum	Maximum
A (O)	30	19.03	4.12	13.42	28.95
B (Z)	30	16.35	1.11	7.90	32.63
C (A&Z)	30	13.61	0.73	8.64	22.61
D (AZ)	30	20.49	1.03	10.48	32.17
Total	120	17.37	0.51	7.90	32.63

Different capital letters indicate different types of orthodontic composite resins, where A (O) stands for conventional composite resin, B (Z) for ZnO nanoparticles, C (A&Z) for ZnO and silver nanoparticle solution, and D (AZ) for Ag/ZnO nanoparticles ($P < 0.05$).

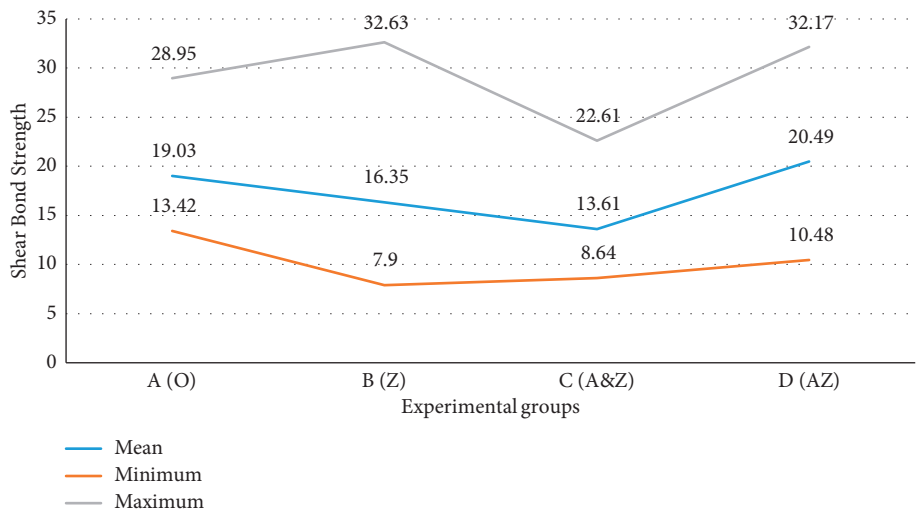

Figure 3: Comparison of shear bond strength in each study group.

Table 2: Antimicrobial effects of the test nanoparticles.

Test groups containing Ag and ZnO (wt %)	Bacteria group (colony-forming unit)				
	S. mutans ATCC 35668	*S. aureus* ATCC 25923	*E. coli* ATCC 25922	*C. albicans* ATCC 10231	*Lactobacillus gasseri*
A. ZnO [10%]	0	0	0	1500	0
B. ZnO [5%]	0	0	500	50000	0
C.Ag [0.1%]/*ZnO* [10%]	0	0	0	10000	0
D. Ag [0.05%]/*ZnO* [10%]	0	0	0	20000	0
E. Ag [0.1%]/*ZnO* [5%]	0	0	7	50000	0
F. Ag [0.05%]/*ZnO* [5%]	0	0	32	>10000	0
Control (−)	0	0	0	0	0
Control (+)	>10000	>10000	>10000	>10000	>10000

3.5. Wettability Measurements. The means and standard deviations of the contact angles of the studied groups are summarized in Table 3. The contact angle was not significantly different between the O group and other groups (Z, AZ, and A&Z). Since the contact angles of all groups are less than 90°, both of them are hydrophilic.

4. Discussion

Patients' cooperation to observe the oral hygiene has always been a challenge during orthodontic treatment. Many clinicians prefer methods that do not require patient cooperation. Although fluoride-releasing materials are appropriate for patients susceptible to caries, they are predominantly used in the dental office and there are also limitations in relation to the number of times they can be used [14].

The present study was undertaken to evaluate the effect of incorporating nanoparticles into orthodontic composite resins on the antibacterial properties and shear bond strength of these composite resins, which indicated that composite resin with nanoparticles (AZ, A&Z, and Z) had antibacterial properties against oral pathogens. Of all these nanoparticles, AZ nanoparticles synthesized using optical precipitation (AZ composite resin) exhibited antibacterial activity even at lower concentrations (5%), but ZnO nanoparticles (10%) and ZnO nanoparticles containing silver ions exhibited antibacterial activity at higher concentrations (15–20%).

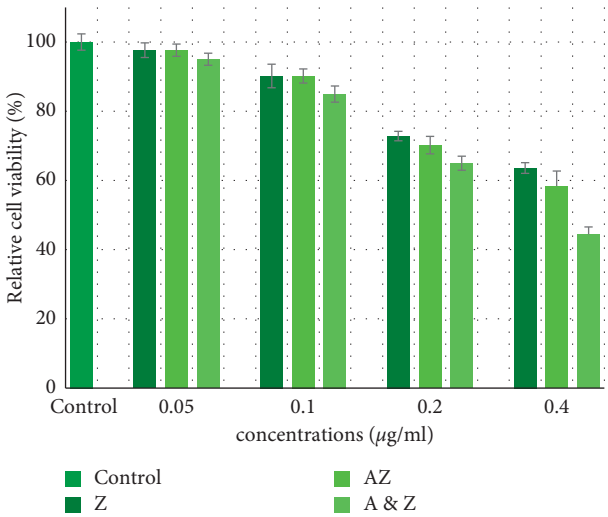

Figure 4: Effects of the Ag/ZnO and ZnO nanoparticles on cell viability in HGF cells. Data are expressed as the mean of percent cell viability compared to control after exposure for 24 hours ± standard deviation ($n = 3$) ($P = 0.05$).

Table 3: The contact angles of the composites in degrees (mean ± SD).

Composite	Contact angle
Conventional composite resin (O)	48.40 ± 2.24
ZnO nanoparticles composite resin (Z)	51.08 ± 1.86
ZnO nanoparticles and silver ions composite resin (A&Z)	54.32 ± 3.54
Ag/ZnO nanoparticles composite resin (AZ)	55.74 ± 5.38

These results indicated the strong antimicrobial properties for these nanocomposites. Most of the nanocomposite resins have better antimicrobial properties for Gram-positive pathogens. The results of the present study showed that these composites had significant antimicrobial properties against *S. mutans*, *S. aureus*, and *L. gasseri* but less power against *E. coli* and no effect against *C. albicans*.

A number of studies have assessed the antibacterial properties of silver nanoparticles [15]. A study by Alt et al. demonstrated the antibacterial activity of silver nanoparticles against resistant pathogens [16]. However, silver nanoparticles induce dark gray discoloration in composite resins, creating problems for dental applications [17]. Eslamian et al. evaluated the effect of Ag nanoparticle (50 nm, 0.3% w/w) incorporation to the conventional orthodontic adhesive to form an orthodontic nanoadhesive. According to results, this nanoadhesive is associated with significant antibacterial properties, which endured for 30 days. Moreover, incorporating AgNPs caused a significant reduction of the mean SBS in the nanoadhesive group [18]. On the other hand, the results of a study by Tavassoli Hojati et al. on the effect of adding ZnO nanoparticles to flowable composite resins on their antibacterial and physical properties and strength showed that an increase in these nanoparticles resulted in a significant increase in their antibacterial activity. In addition, they showed that incorporation of nanoparticles into composite resins resulted in a significant increase in the compressive strength and shear bond strength of composite resins, with no changes in their flexural strength [9]. In our study, although the shear bond strength of AZ nanoparticle-containing composite resins, synthesized using optical precipitation, was higher than that in the control group, the difference was not significant statistically. The average shear bond strength for the different groups in our study ranged from 13.61 to 20.49 MPa (Table 1). An important factor is whether the bond strength of media is within a clinically acceptable range. However, there is no clear consensus regarding what the minimum shear bond strength should be, with some reports suggesting a range of 13–21 MPa and others, 6–8 MPa. The average shear strength of all composites tested in this study was >6 MPa, which is considered by studies to be appropriate for routine clinical use [19]. Therefore, incorporation of nanoparticles to orthodontic composite resins does not result in a change in the mechanical properties of these composite resins.

Moreover, Garcia-Contreras et al. evaluated the effect of incorporating titanium nanoparticles into glass ionomer and concluded that incorporation of these particles increases the compressive and flexural strengths of glass ionomers, in addition to conferring antibacterial properties. In their study, as well, no changes were detected in the shear bond strength to enamel [20]. Poosti et al. showed in another study that incorporation of titanium nanoparticles into orthodontic composite resins confers antibacterial properties, with no changes in the shear bond strength [17]. In another study by Cheng et al. in 2012, incorporation of silver nanoparticles into composite resins improved the mechanical properties of these composite resins and they exhibited antibacterial properties but silver nanoparticles create a dark gray color change in composites, which defies the esthetic purposes [21]. The antibacterial mechanism of ZnO involves its activity as an activator for enzymes. It is toxic to bacteria at a concentration of 0.5 ppm, and concentrations of 4, 6, and 16 ppm can inhibit bacterial growth [13].

Finally, Argueta-Figueroa et al. in 2015 showed that the shear bond strength of orthodontic adhesives, containing copper nanoparticles, was reported to be higher than that in the control group, with no changes in color and other properties [22].

Clinical trials are the ideal methodology for biocompatibility evaluation. Nevertheless, this approach is restricted by ethical considerations. Dental materials must be assessed through several toxicity and biocompatibility steps before being used in the clinic. The definition of biocompatible dental material is to have no or infrequent harmful effects on oral tissue [11]. According to the results obtained from the MTT test, the cytotoxicity of AZ and ZnO nanoparticles indicated that certainly no major and significant damaging effect is expected to the cells up to 0.1 mg/ml of ZnO and AZ nanoparticles. Based on the results of this study, it can be seen that, in the presence of

all nanocomposites in the current study, the major bacteria in dental and oral caries (*Streptococcus mutans*, *Lactobacillus*, and *Candida albicans*) cannot grow sufficiently; due to the long presence of orthodontic brackets in the mouth, the formation of plaque and dental caries can be reduced. It should be noted that the release of nanoparticles from polymerized nanocomposites is zero in all three periods of time. In fact, these nanocomposites retain their properties for a long time without delaying the nanoparticles, and because of these unique properties, using these nanoparticles in orthodontic composite resins is suggested. Meanwhile, since the nanocomposites studied are hydrophobic, and as a result, the absorption of bacterial plaques is also reduced, which is a significant advantage in orthodontic use. Lower toxicity and similar tooth coloring of nanoparticles containing zinc oxide with a slight difference in bond strength compared to the control group tend to be more productive for nanocomposites containing this material.

5. Conclusion

The present study found that incorporation of different nanoparticles (ZnO, ZnO and silver ions, and Ag/ZnO synthesized) into orthodontic composite resins induced antibacterial properties against oral pathogens. Of all these nanoparticles, AZ exhibited antibacterial activity even at lower concentrations (5%). Based on the MTT cell viability test, the concentration of AZ and ZnO nanoparticles up to 0.1 mg/ml was biocompatible and had no major and significant damaging effect to the human cells. Also, incorporation of AZ into orthodontic composite resins did not change mechanical properties; however, incorporation of ZnO nanoparticles containing silver ions decreased the shear bond strength, but this reduction probably is not concerned clinically.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no known conflicts of interest or personal relationships that could have appeared to influence the work reported in this paper.

Authors' Contributions

Mojgan Kachoei and Baharak Divband have contributed equally to this study.

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Review Article

The Role of Biomaterials and Biocompatible Materials in Implant-Supported Dental Prosthesis

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The dental implant is one of the appropriate instances of the different dental materials and their application, which is the combined procedure of technology and science in physics, biomechanics, and surface chemistry from macroscale to nanoscale surface engineering and manufactured technologies. In recent decades, biomaterials in implant therapy promote bone response and biomechanical ability, which is long-term from surgical equipment to final prosthetic restoration. Biomaterials have a crucial role in rehabilitating the damaged structure of the tooth and supplying acceptable outcomes correlated with clinical performance. There are some challenges in implantation such as bleeding, mobility, peri-implant infections, and the solution associated with modern strategies which are regarded to biomaterials. Various materials have been known as promising candidates for coatings of dental implants which contain polyhydroxyalkanoates, calcium phosphate, carbon, bisphosphonates, hydroxyapatite, bone stimulating factors, bioactive glass, bioactive ceramics, collagen, chitosan, metal and their alloys, fluoride, and titanium/titanium nitride. It is pivotal that biomaterials should be biodegradable; for example, polyhydroxyalkanoates are biodegradable; also, they do not have bad effects on tissues and cells. Despite this, biomaterials have important roles in prosthetic conditions such as dental pulp regeneration, the healing process, and antibacterial and anti-inflammatory effects. In this review study, the role of biocompatible materials in dental implants is investigated in *in vitro* and *in vivo* studies.

1. Introduction

Nowadays, one of the dependable methods for repairing missing dentition is dental implant therapy which is broadly recognized. Bothe et al. in the 1940s explained the fundamental conception of metallic instrument implantation in bone for the first time. Then, Leventhal et al. represented that titanium has potential as a biocompatible material for surgical implantation 60 years ago [1]. The favorable outcome and purposes of the dental implants are crucially correlated with tissue response which is essential to find out the basic features of the tissue response to dental implantation [2]. Early failures of implants have been generally based on modified or inadequate wound healing which inhibits osseointegration [3]. Some other factors such as insufficient quality of bone, diversity of surgical technique, occlusal overload, and postoperative inflammation and infection have been involved in early failures of implantation. Delayed implant failures are usually the consequence of dissection in osseointegration, typically after the practical loading of implant-supported prostheses. Also, delayed failures of the implant have been commonly related to occlusal overload which is a biomechanical failure and peri-implantitis [4].

The restoring response to biocompatible materials is fibrous or fibrosis encapsulation. The repairmen after implantation generally include 2 procedures which are regeneration and replacement of connective tissue (contains fibrous capsule). The procedures are under the control of tissue framework persistence of implant location and cell proliferative capacity. The compatibility of dental implants is better with osseous tissue than soft tissue. Also, the bone-

bonding power of dental implants is significant although the bonding characteristics with soft tissue are not acceptable and lead to the encapsulation of fibrous [2]. Many different types of materials are used in dentistry, which contains filling materials of intracanal, liners, medicaments of intracanal, subgingival implants, restorative materials, mouth rinses, and prosthetic materials [5]. As abundant dental devices and materials, vital necessities are resulting from systems of dental implants, since dental implant surface is straightly connected with critical soft/hard tissue and accustomed to chemical as well as mechanical features. At least, some necessities might involve morphological compatibility, mechanical compatibility, and biological compatibility to setting critical tissues [6]. Biomaterials have been also known as the biological or synthetic materials that are utilized to rehabilitate a segment of living structure to sustain involvement with tissues that are living. The systemic and local tissue responses state crucial characteristics of biocompatibility [2]. Furthermore, biomaterials containing dental implants and correlated components may be identified as any substances, synthetic or natural, that can be utilized for any time duration that deals with biological systems to improve the quality of life of each person of the society [7]. Any type of biocompatible materials can be prepared as nanoparticles that can beneficially enhance material properties in comparison with their same bulk ones. Also, research findings indicate that nanoparticles can be used as particle coatings to the surface of the dental implant to develop the integration of soft tissue and improve dental implant results [8]. After biomaterial implantation, the basic features of tissue response include acute and chronic inflammation, injury, interactions of blood material, formation of the provisional matrix, granulation tissue formation, progression of the fibrous capsule, and foreign body responses [2]. Classically, in biocompatibility terms, materials of bone graft are categorized as bioactive, biotolerant, or bioinert. Implant materials that are biotolerant are maintained in the body with encapsulation of fibrous due to the reaction of the tissue. Bioinert materials that are used as implants are associated with the bone tissue adjoining without chemical reactions. Bioactive implants set up chemical bonds with the bone tissue which causes straight deposition of bone matrix on the implant material. This theoretical categorization is established on histopathological observations of local influences after implantation into bone tissue [9]. Bioinert materials (e.g., stainless steel, stabilized zirconia, titanium, ultrahigh molecular weight polyethylene, and alumina) have minimum bonding with the encompassing tissue. Other materials have demonstrated the capability of biochemical and biophysical responses with around tissues. An actual chemical interaction capability with soft tissues has been displayed in several bioactive ceramics such as bioactive glasses of specific constitutions. Nevertheless, bioactive ceramics (bulk form) are inadvisable for load-bearing utilization because their strain-to-failure, the strength of flexuous, and toughness of fracture are less than those of bone, and their elasticity is more than that of bone [10]. Calcium phosphates ceramics are regarded to be osteoconductive and bioactive. The responses of ion exchange between the encompassing body fluids and bioactive implant set an active carbonate hydroxyapatite layer upon the implant that is the same as the mineral phase in bone. Bioresorbable bioactive materials also start to resorb upon the position of the human body and are steadily replaced by tissue that is progressing. Also, polylactic–polyglycolic acid copolymers and tricalcium phosphate [$Ca_3(PO_4)_2$] are instances of this kind of biomaterials [11]. This review discussed the biocompatible material effects and their role in supporting dental implant prostheses.

2. The Role of Biomaterial in Prosthesis Implant

Using dental implants has been widely accepted as a common method of repairing dentition defects over the past decades. Dental implants are simply infected because of the oral pathogenic bacteria, although the rate of implant survival is enhanced to about 95 ± 2 percent over a 10-year follow-up duration. Occlusal overload and oral biofilms are two principle etiologies of peri-implantitis, and oral biofilms that have progress on dental prostheses have a vital role in the pathogenesis of peri-implantitis. In the absence of therapy and prevention, implant loss occurred due to peri-implantitis. The implant can be connected with oral bacterial cells, blood, and saliva during and after the surgery of implantation, and bacterial cells are linked to the abutment harm of encompassing gingiva [12]. The chemical and physical features of dental prostheses and materials are restorative and can affect pellicle coating, formation of biofilm, and adhesion of initial bacteria. Dental material application is progressing and has shown accelerating necessity to better find out the responses between the surface material and biofilm in the oral cavity. A higher range of biofilm density and viable microbial cells on the structure of prostheses based on Co–Cr (cobalt-chromium) alloys was detected, when contrasted with prostheses attributed to titanium, for the base-metal alloys [13]. One of the gold standards for oral implants is titanium implant screws because of their facility and exceeding biocompatibility to achieve osseointegration. The anticipated hypothesis is attaining a straight connection between the implant and living bone to ensure the long-standing action of the fixed prosthetic instrument [14].

3. The Role of Biomaterial in Surface Modifications and Coatings

Dental implant surface modification has been named as a valuable approach to support osseointegration and also facilitate the correlation between cells and biological fluids to accelerate the regeneration of peri-implant bone. Recently, different surface modification methods have been suggested and examined to develop the osseointegration of implants. One of the most common surface modifications, used in modern dental prostheses, is microroughness. Moreover, it has been known as a crucial function in linking to nearby tissues and anchoring cells which are appropriate for peri-implant osteogenesis. Various physicochemical approaches have been progressed to identify the roughness of implant

surface, for example, acid-etching, grit-blasting, or combinations. Grit-blasting is usually conducted by hydroxyapatite, TiO_2 particles, silica, and alumina. Acid-etching is performing as a homogenizer of the implant surface microprofile and eliminating the remaining blasting particles. Sulfuric acid, nitric, hydrofluoric, or combinations are the chemical agents which are used for acid-etching [15].

4. The Role of Biocompatibility in Healing of Dental Prosthesis

Nonosteogenic cells contribute to the early stage of healing procedures and are called to prescribe definite following phases in process of healing which is regulated by osteogenic cells. Macrophage cells are conducted as an important character in the initial phases of the process of bone healing implants due to macrophage-controlled immunoinflammatory reaction to the implanted materials and their effect on the result of healing response of the osteogenic cells. Therefore, the primary function of macrophage cells after implantation influences the result of bone healing and identifies the quality of tissue healing procedures. Regenerative macrophage phenotype is expressed dominantly and also crucially correlated with the healing of favor bone through osteogenic cell differentiation and producing different types of growth factors and cytokines [16]. In recent years, most studies are concerned with the healing of soft tissue surrounding implants and the integration of hard tissue of dental implants because of their central character in long-term maintenance. Also, it has been displayed that concentrates of platelet have a particularly pronounced impact on wound healing of soft tissue in comparison with hard tissue because of their attributes with different growth factors containing TGF-β1 (transforming growth factor-β1), VEGF (vascular endothelial growth factor), and PDGF (platelet-derived growth factor). Marx et al. use PRP (platelet-rich plasma) in dental cases; afterward, the utilization of PRP is broadly approved in various fields of orthopedics, esthetics, and dentistry in tissue regeneration due to their potential in enhancing angiogenesis. Moreover, they have limitations because of their contribution with anticoagulants which are called suppressors of regeneration in tissues. Therefore, PRF (platelet-rich fibrin) was developed to eliminate anticoagulant in 2001, and also PRF was used as a three-dimensional scaffold for tissue regeneration that has various advantages such as quick tissues angiogenesis, faster wound healing, and complete immune-biocompatibility [17].

5. The Role of Materials in Antibacterial and Anti-Inflammatory Effects

Various types of dental implant surface coating including fluoride, copper, chlorhexidine, zinc, silver, and antibiotics (e.g., amoxicillin, gentamycin) have been examined to supply antibacterial effects [18]. Infections surrounding implant instruments are related to biofilm and microbial infections that are linked to a solid surface. These microbial infections are exceedingly complex to treat bacteria that are adsorbed and resistant to immune system mechanisms and antimicrobials. Biomaterial surface has various properties such as hydrophobicity, roughness, charge, and micro- and nanostructure which have an important role in preventing biofilm infection on an implant surface. Lesser biofilm susceptibility to antimicrobial agents with diverse resistance of antibiotics for many bacteria strains requires study development on novel options for antibacterial approaches [19]. Some implant surfaces have anti-inflammatory and antibacterial effects with immobilization of bioactive molecules such as peptides, proteins, and growth factors. Nevertheless, using bioactive molecules has some disadvantages like short half-life, deficient stability, exorbitant cost, and side effects. To provide appropriate bioactive surfaces that may be easily converted to clinical applications, ZnO and Ag nanoparticles as metal nanoparticles have been examined because of their ability as anti-inflammatory and antibacterial agents [20]. Titanium bacterial colonization leads to the loss of the implant due to a formation of biofilm which is considered to help bacteria escape antibiotics and the host defense mechanism. Pathogens cause loss of bone surrounding implant; thus, it is necessary to do surgery; either the influenced bone is damaged or infected implants are removed or replaced. The infected locations are also treated with systemic antibiotics to eliminate the existence of bacteria. Consequently, antimicrobial agents such as sliver and fluorine ions are necessary to contribute to the dental implant to bind to the inside proteins of the bacteria for inhibiting the activities. Also, these ions are integrated on the titanium surface and have been exhibited to be useful against the formation of bacterial biofilm. Coatings of antibiotic releasing, made of fixed antimicrobial oligopeptides, are helpful in a short duration and may not prevent peri-implantitis after years of implantation. Using polymer-based materials with antibacterial features is another choice. Antimicrobial features are based on their structure as a consequence of organic or inorganic antimicrobial agents' introduction, as a consequence of the chemical modification. Chitosan is one of the biocompatible and antibacterial materials that also prevent the action of pathogens. Although the antibacterial mechanism of chitosan is not clear, it is considered the positive charge of amines' captivate negative charge of bacteria cell walls due to cell membrane disruption or the cell dynamics [21].

6. The Role of Materials in Osseointegration

Osseointegration has also been known as a dynamic process in which initial stability is replaced by secondary stability. Instantly after installation of implants, primary stability prepares mechanical stabilization by direct connection between the dental implant surface and the surface of the bony wall of the implant bed. It has been shown that the surface properties of biomaterials such as titanium dental implants have a decisive effect on the rate of osseointegration. Recently, dental implants made of zirconia and titanium alloys have been examined as an alternative biomaterial to replace missing teeth. Titanium alloys such as TiZr (titanium-zirconium) and Ti6Al4V (titanium-6aluminum-4vanadium)

have better mechanical features than zirconia, and pure grade 4 titanium or ceramics compounds possess more advantages than titanium alloys and titanium. Surface modifications of these biomaterials eventually have effects on the procedures of osseointegration [22]. As a result of the chemical and physical connection between the surface of implants and the bone tissue, osseointegration occurred. The bone-implant interface has an exceedingly dynamic structure in which oxidative stress resulting from implant surgical insertion to the bone causes the surface TiO_2 layer thickening which integrates phosphorus and calcium ions from the bone matrix [23]. Bioactivity has displayed automatic carbonated HA (hydroxyapatite) layer formation on the surface of biomaterial after its absorbance to the body fluid. Conversely, the HA layer that is formed can promote powerful bonding to the bone as a result of osseointegration [24].

7. The Role of Materials in Dental Pulp Regeneration

Dental pulp regeneration has been named as a challenging and complicated system that depends on vascularization and reinforced tissue. Endodontic regeneration is composed of regeneration of connective tissue and pulp, revascularization, and dentin formation [25]. Biomaterial designing is also related to the controlled release of molecular signaling of bioactive materials and induces mesenchymal stem cell differentiation to odontoblasts as a high-potential method in comparison with conventional endodontic therapy [26]. Chitosan scaffolds were loaded with biomolecules and growth factors because of increment of odontoblastic marker expression like alkaline phosphate, dentin matrix acidic phosphoprotein, and dentin sialophosphoprotein, preparing an extracellular matrix-like environment for differentiation and proliferation of dental pulp cells to the odontoblasts with biomineralization capacity [27]. Moreover, chitosan-based scaffolds as a novel biomaterial including molecular signaling such as mineral contents, BMPs (bone morphogenetic proteins), and drugs (i.e., metformin and simvastatin) have been stated to induce adhesion of cells, proliferation, and differentiation of dental pulp stem cells. TGF-β1 is an important biomolecule concerned with critical pulp therapy since it may be conducted as a regulator of the activity of alkaline phosphatase, the induction of the odontoblast-like cell proliferation, expression of OCN gene/protein, and mineral deposition [28]. Rehabilitation of dentinopulpal defect is one of the long-term complications in dentistry. Various restrictions of biomaterials are utilized as scaffolds for complicated regeneration of dentin pulp or used in restorative dentistry to stimulate dentin to seal the exposed pulp chamber. Also, the procedure of regeneration or reparation sometimes might be uncompleted [29].

8. Common Biocompatible Material in Supported Dental Implants

Biocompatibility has been determined as the compatibility of the material based on the biological environment. A long-standing connection between particular functions and tissues has an important role in dental implantation. Furthermore, biocompatibility has been defined with responses between implants and tissue examination which are examined in studies (*in vivo* or *in vitro*) [11]. Additionally, biocompatible materials that are utilized as dental implants may be categorized as bioactive, bioinert, and biotolerant [30]. The common biocompatible materials are mentioned in Table 1.

8.1. Bioactive Glass. Bioactive glass (BG) is one of the biomaterials which are used currently. Bioactive materials are associated with the biological conditions to evoke a particular response like the hydroxyapatite layer forming with a formation of the bond between biomaterials and tissues. Dentin, enamel, teeth, and bone are mainly composed of hard mineral tissue in the structure of crystalline calcium phosphate and hydroxyapatite. On the contrary, bioinert materials repress any reactions or communication of the biological environment. Nevertheless, these biomaterials affect environmental responses and fibrous capsule formation. The fibrous capsule can cause the prosthesis to micromovement and failures. Also, bioactive materials can be osteoinductive or osteoconductive, and their abilities indicate BG applications in abundant clinical situations comprising hard tissue regeneration in dentistry and medicine. BG is commonly used as coating material of dental implants, mineralizing agents, treatment of root canal, pulp capping, air-abrasion, and dental rehabilitation materials in dentistry. In medicine, it uses several applications from the restoration of soft tissue to orthopedics [40].

8.2. Collagen. One of the most common biomaterials used in the process of implantation and dental therapy is collagen. Collagen is also utilized in the different pathways as being prepared to linked cross-link or utilized in films, composites, three-dimensional matrix, and lattice-like gel. Also, collagen can improve restoration and granulation of tissues, protects wounds and tissues from infections mechanically, and has analgesic effects [41, 42]. Hence, hydrolyzed collagen can be utilized as a healing process booster, binding tissue fluids, and is a compatible biomaterial in dental therapy. Moreover, collagen not only has an important impact on the rejuvenation of epithelial cells but also is nontoxic, biodegradable, and well absorbed. For example, collagen membrane-scaffold graft in combination with recombinant human platelet-derived growth factor facilitates both regeneration and fibroblast adhesion to connective tissue. Therefore, collagen is often combined with other biocompatible materials to enhance the quality and the rate of treatment of defects in dental implantation [43].

8.3. Chitosan. Chitin deacetylation provides a biomaterial called chitosan which has been examined with incorporative procedures for its applications. Chitosan has individual characteristics such as adhesion to mucose, nontoxicity, biodegradability, antifungal activity, antibacterial effects,

Table 1: The common biocompatible materials as supportive substances in implantation of dental prosthesis.

Type	Design	Method	Result	Ref/ year
Carbon-reinforced polyether ether ketone (CRF-PEEK)	*In vitro*	3D (three-dimensional) model of implants in the first mandibular molar using a combination of lithium disilicate, Ti, and CRF-PEEK for abutment/implant materials	Replacement of Ti implant with PEEK implant does not prepare significant advantages to lead to improved stress distribution to the bone with peri-implant	[31]/ (2019)
Hydroxyapatite (HA)	*In vivo*	Ti implants with HA coatings and grit-blasted surfaces (Al_2O_3) as control were embedded in rabbit tibiae	Coating with HA can improve the physical and chemical properties of osseointegration in comparison with the grit-blasted implant	[32]/ (2019)
Polyetheretherketone (PEEK)	*In vitro*	Investigation of bioactivity in PEEK sample coated with pure Ti by electron beam deposition technique and unmodified PEEK sample	Dental implants with PEEK and electron beam deposition of Ti as surface modification have increased bioactivity in comparison with unmodified PEEK implants	[33]/ (2020)
Chitosan	*In vitro*	Evaluation of the cell viability, morphology, and osteogenic capacity of chitosan in dental implants	The combination of chitosan and laser surface increases the healing process and osseointegration of dental implants	[34]/ (2020)
Polymethylmethacrylate (PMMA)	*In vivo*	Investigation of PMMA-based material in patients with compromised dentitions as immobilized dental prostheses	PMMA-based material can be utilized with 3- to 4-unit FDPs for long-term temporization at least more than a year	[35]/ (2014)
Ceramic	*In vivo*	Investigation of MC (metal-based ceramic) and ZC (zirconia-based ceramic) in posterior immobilized dental prostheses in patients	MC and ZC posterior immobilized dental prostheses have the same results as the most results measured in 10 years	[36]/ (2018)
Keratinized tissues (KT)	*In vivo*	Evaluation of the influence of the width of KT on peri-implant tissues by investigation of peri-implant clinical and inflammatory parameters	Free gingival graft around KT causes major improvements in inflammatory parameters and peri-implant clinical features	[37]/ (2013)
Titanium (Ti)/Zirconia (Zr)	*In vivo*	Evaluation of proinflammatory cytokines and mediators of bone metabolism in patients who received Ti and Zr as abutments fixed dental implants	Ti and Zr as abutment biomaterials have no remarkable difference in mediator profiles of bone metabolism and proinflammatory cytokines	[38]/ (2015)
Chitosan-enriched fibrin hydrogel	*In vitro*	Investigation of antimicrobial influences (with growth analysis of *Enterococcus faecalis*), DP-MSC (dental pulp-mesenchymal stem/stromal cell) viability, proliferation, production of collagen, and deposition of extracellular matrix	Chitosan-enriched fibrin hydrogel can stimulate neoformation of human dental pulp tissue due to the antimicrobial effects of chitosan and influence on the morphology of dental pulp cell, proliferation, viability, and production of collagenous matrix	[39]/ (2019)

and biocompatibility. Chitosan degradation, especially by lysosomes, does not make toxic compounds, and chitosan implantation does not promote the activity of the immune system. Chitosan-based scaffolds are commonly utilized for dentin pulp, periodontal, and bone regeneration in dentistry. Chitosan lacks mechanical application and bioactivity is necessary for cartilage and bone tissue engineering, although it is compatible with matching membrane characteristics. Moreover, chitosan scaffolds loaded with bioactive components, growth factors, and synthetic polymers have been examined to fix a composite material with increased mechanical features and facilitate osteogenesis [28]. Chitosan has an application in the bone generation of surrounding dental implants. Chitosan loaded with hDPSCs (human dental pulp stem cells) is implanted in rabbit models, and the findings demonstrated the osseointegration and healing of bone in comparison with xenografts in the animal models. Also, chitosan loaded with hDPSCs displayed its potential in the regeneration of bone formation surrounding dental implants and ameliorate osseointegration [44].

8.4. Polyhydroxyalkanoates. Various bacteria synthesize different biopolyesters like PHAs (polyhydroxyalkanoates) as storage materials for energy and intracellular carbon which have several applications in studies containing medical implants. These findings indicated that PHAs have many characteristics for appropriate implantation like compatibility of tissues, biodegradable properties, and adequate mechanical properties. Due to the hydrophobic features of PHAs, it is a favorable choice for encapsulating nanostructure or microparticle hydrophobic drugs [45].

8.5. Polyetheretherketone. PEEK (polyetheretherketone) has been commonly used in orthopedics and in clinical dentistry as a synthetic material in the coloring of teeth and other applications. For instance, PEEK has shown lower shielding of stress in comparison with titanium as dental implants because of suitable interactions between bone and PEEK. Thus, PEEK has the potential to use in immobilized and removable prostheses. Recently, researchers have also investigated the bioactivity of PEEK implants at the nanostructure. Regarding physical and mechanical features of PEEK that are similar to bone, PEEK has promising usage in various properties of dentistry. One of the important complications in utilizing PEEK as dental implants is improving the PEEK bioactivity without changing mechanical properties. Moreover, upgrading the properties of materials and modifications can increase its ability in dentistry [46].

8.6. Calcium Phosphate-Based. CaP (calcium phosphate) has been named due to the mineral including calcium ions with different types of phosphate and hydrogen or hydroxide ions. 100 years ago, CaP- (calcium phosphate-) based materials have been generally utilized in craniofacial surgery, and due to their outline potential, it is a candidate as a drug delivery system and bone substitutes. CPC (calcium phosphate cement) as a particular CaP biomaterial has favorable properties to use in coating implants that can facilitate the healing of bone surrounding implants. CaP-based materials also play an important role in dental implants because of their similarity to bone composition, their bioactive ability, and their osteoconductive properties. Also, CPC has various excellent capabilities to use as biomedical materials in clinical dentistry applications, which have great properties of bone repairing and tremendous biocompatibility [47].

8.7. Titanium. Ti is an element from transition metal (atomic number = 22) with lustrous silver color, high strength, and low density. Ti has high resistance to corrosion under different circumstances, and it is claimed that Ti has biocompatible and nontoxicity properties in humans. Therefore, Ti and Ti alloys have promising applications in several medical situations (i.e., implants and surgical implements) and clinical dentistry such as prostheses, abutment, and wires of orthodontic. Various materials such as stainless steel and Vitallium (cobalt-chromium) are candidates as an implant of the misplaced tooth, but the progression of technology and science of materials accepted that Ti becomes the pioneer and the most common biocompatible material because of its properties. Ti is broadly successful in the process of implantation because of its abundant advantages. Ti is a bioinert material that can bind to osteoblasts with its great biocompatibility. Ti oxides have high stability and suitable resistance against corrosion as film and may divide the bulk titanium from the surrounding parts [48]. Because of the compact Ti oxide thin layer in the surface, Ti and its alloys have excellent biocompatibility and favorable metallic selection for subgingival implants and are also utilized as coatings and surface modification to stimulate osseointegration. Titanium and its alloys have tremendous abilities to bind to bone and living tissue. Usually, metal ions such as Co, Hg, Cr, Cu, Zn, and Sn that are located on a culture medium can induce inhibited zone of different organisms which demonstrated damage to cells and cytotoxicity. Also, these metal ions and their alloys are used in clinical dentistry as components and constituents for dental amalgams. Ti alloys, such as TiMo and TiNi, are utilized as materials in the wire of orthodontics because of their memory characteristics and particular spring [5].

8.8. Au. Au (gold) has been known as dental prostheses since a long time ago, because it has resistance to corrosion, has an appropriate melting point, and might attain suitable mechanical features by alloying. Gold like copper and silver fixes Ti b-phase to a lower temperature by the diagram phase of binary equilibrium. Ti_3Au forms as an intermetallic compound contain gold with a concentration of about 15%. Thus, gold is a favorable alloying element for Ti with positive influences on the grinding capability and mechanical characteristics. Passive layers of Ti-alloy include metal defect, outer, intermediate, and inner layers. The Ti-Au outer layer makes primary galvanic corrosion. The current density of initial galvanic corrosion is decreased due to the porous and thin outermost layer which is formed [48, 49].

8.9. Ceramic. Ceramic is originally based on "keramos" which is a Greek word and is definite as burnt or pottery article. Nowadays, ceramic has various wide definitions and contains cement systems, advanced ceramics, and glass. Also, ceramics such as nonmetallic and inorganic solids are commonly produced with adequate heat approaches and then cooling, which are seldom regarded as covalent combinations and ionic metallic bonding. Materials of ceramics can be noncrystalline, partly crystalline, and crystalline which consisted of glass or pure ceramics. YSTZP (Yttria-stabilized tetragonal polycrystals of zirconia) is one of the most frequent ceramics which is used as the dental implant due to its toughness formation and appropriate biocompatibility property. Nevertheless, the stability of YSTZP induced by degradation of temperature and stress can be useful in protection and improve the strength of integration between tissue and implant. During production, many fabrication procedures in the industry can lead to final microstructures of YSTZP material that commonly have different stability [1]. ZBC (ZrO2-based ceramics) is conducted as an important material in medical instrument applications. Recently, utilization of ZBC has been significantly increased as a biomaterial of dental crowns and implants because of ZBC's excellent mechanical properties such as biocompatibility, strengths, and great resistance to friction and abrasion. Zirconia has been named as a promising ceramic material for medical device application particularly in clinical dentistry because of corrosion resistance, effective biocompatibility, and low weight features in comparison with other ceramics. One of the explicit advantages of ZBC over Ti or the other metallic

implants is being a natural choice to generate immobilized teeth substitutes [50].

8.10. Polymer-Based Implants. Polymer-based implants are comprised of polymeric materials such as PET (polyethylene terephthalate), PEEK (polyether ether ketone), PTFE (polytetrafluoroethylene), PU (polyurethane), PMMA (polymethylmethacrylate), PE (polyethylene), UHMW-PE (ultra-high-molecular-weight polyethylene), and PDS (polydimethylsiloxane) which have been used for replacement of missing teeth and dental roots [11]. Materials with major similarity to the color of teeth and bone, for instance, elasticity modulus, are examined to be used in implantation such as a composite of fiber-reinforced PEEK and alternative to Ti. PEEK is also used in dental abutments, implants, and mobilized and fixed prostheses. The hypothetical benefits of PEEK in Young's modulus indicated that PEEK is similar to bone in comparison with Ti, particularly ceramic implants, but these trials just investigated in silico simulation, and long-standing clinical studies might be examined and confirmed [1]. Generally, polymer-based materials prepare properties in dental implant and root progressions such as excellent porosity, electrical and thermal apathy, biocompatibility, elastic modulus that is like soft tissue, simple controlling, low-priced fabrication, and suitable stretching in contrast to other biocompatible materials. Nevertheless, polymer-based implants are more complicated for sterilization with autoclave or ethylene oxide. Electrostatic responses of surfaces of polymers and the cleaning condition of oral environments may collect micro- and macro-particulates. The open zones of tissues that lead to growth are closed by deforming porous polymers which depend on elasticity [11].

9. Future Challenges

Recently, the knowledge about biocompatible materials and biomolecular-related procedures has been developed and displayed the progression in repairmen and regeneration of tissues that are linked to teeth, but several experiments have remained to find out the potential properties of biocompatible materials. Also, functional models of biomaterials can prepare favorable esthetics, inhibit the formation of biofilm, and stimulate remineralization and some prospective challenges for clinical approaches. Accelerating clinical translation procedures and preparing powerful fabrication, approvable testing data and methods are accessible. Thus, these abilities can help in the findings of new biomaterials which are needed [51].

10. Conclusion

In recent decades, many biocompatible materials are used in dental implants and prostheses due to their ability to have anti-inflammatory properties. Physicochemical modifications of dental prostheses also reduce the adhesion of microorganisms but cannot inhibit peri-implantitis. Biocompatible materials have been commonly used as antimicrobial agents to sustain the treatment and prevention of peri-implantitis and mucositis of peri-implant. Also, some types of biocompatible materials such as polymer-based implants, metal implants, and natural bioactive materials indicate their role in the wound and bone healing process, stimulating dental pulp regeneration and surface modification of dental implants. Further researches are needed to achieve the ideal dental implant.

Data Availability

This article is a review and does not contain any studies with humans or animals performed by any of the authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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