The Preparation, the Physicochemical Assessment, and the Antimicrobial Action of Nanocurcumin-Loaded Dental Temporary Restorative Material

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Introduction. The aim of this study was to prepare a temporary restorative material containing curcumin nanocrystals and to evaluate the physicochemical properties and the antimicrobial action against Enterococcus faecalis (E. faecalis), Staphylococcus aureus (S. aureus), and Escherichia coli (E. coli). Materials and Methods. Dental temporary restorative material was physically mixed with curcumin nanocrystals at weight percentages of 80/20 and 70/30. The prepared materials were then subjected to the physicochemical tests: Fourier transmission infrared spectroscopy (FTIR) to investigate possible bonds, X-ray diffraction (XRD) method to test the crystallinity pattern, and scanning electron microscopy (SEM) method to study the morphology of the prepared materials. Besides, the release pattern of nanocurcumin from the temporary material was evaluated using drug dissolution USP apparatus II and UV spectrophotometer. For microbial evaluation, the disk diffusion method was used against the mentioned bacteria. Results. The results showed the homogenous mixing of nanocurcumin with the temporary restorative material. Nanocurcumin showed a two-stage release pattern from the temporary material, and both types of samples containing 20% and 30% nanocurcumin had antimicrobial action against all selected bacteria. Conclusion. Extensive cellular, animal, and clinical studies are needed to demonstrate the use of temporary restorative material containing antimicrobial nanoparticles.

1. Introduction

The success of endodontic therapy is closely related to the elimination of the microorganisms in the root canal system, through a correct biomechanical preparation. For the maintenance of the aseptic chain created during the treatment, coronal sealing becomes paramount, avoiding marginal percolation of oral fluids and microorganisms into the system. Several studies indicate a direct relationship between the quality of coronal sealing and the success of endodontic treatment. The coronal leakage by restorative material (temporary or permanent) may be responsible for contamination of the dental canal and the appearance of periapical complications during the transoperative period [1]. The temporary restorative material must have properties that prevent leakage, allow adhesion to substrates, and show good dimensional stability, mechanical strength, and antimicrobial activity. Intraradicular posts are commonly used to repair endodontically treated teeth whose crowns do not provide sufficient retention and support for restoration. Placement of the intraradicular post in the filled canal maintains the strength of the remaining tooth structure and also restores the lost tooth structure [2]. Therefore, the canal is exposed to microorganisms in the interval between cleaning and
obturation to the preparation and cementing of the post, as a result of improper temporary dressing which increases the possibility of contamination of the canal [3]. The addition of the antibacterial agents to the temporary restorative material can enhance coronal sealing and prevent the growth of residual microorganisms in inaccessible areas [1].

Nanotechnology demonstrates the ability to image, manipulate, and model functions at the nanometer scale [4–6]. This field includes the study of nanoparticles, which can be defined as particles smaller than 100 nanometers [7, 8]. The properties of nanoparticles such as their active surface, chemical reactivity, and biological activity are different and sometimes more desirable than larger particles [9, 10]. Various nanoparticles have been widely used as antibacterial drugs in medicine and dentistry. The small size of these particles increases their surface-to-volume ratio. The higher surface-to-volume ratio and the charge density cause these materials to interact more with the environment and thus increase their antibacterial properties than conventional drugs [11–13].

Curcumin as a polyphenolic compound is a natural yellow pigment known as the main ingredient in the turmeric plants. It is known for its numerous biological activities including anti-inflammatory, antiviral, and antimicrobial properties. Despite the good biological activity of curcumin, this substance has low solubility and fast metabolism which leads to low stability and low bioavailability of curcumin [14]. The conversion of curcumin to nanoparticles has improved the inherent limitations of this substance. The dissolution rate of curcumin nanoparticles in water is higher due to the large surface-to-volume ratio. It has also greater physical and chemical stability in the nanoform [15, 16].

The aim of this study was to prepare a temporary restorative material containing curcumin nanocrystals and to evaluate the physicochemical properties and the antimicrobial action against Enterococcus faecalis (E. faecalis), Staphylococcus aureus (S. aureus), and Escherichia coli (E. coli).

2. Materials and Methods

2.1. Preparation of Temporary Restoration Containing Curcumin Nanoparticles. Temporary dressing paste (Coltosol, Coltene/Whaledent AG, Switzerland) was physically mixed with curcumin nanocrystal powder (Alborz Company, Tehran, Iran) with an average particle size of 95 nm, with weight percentages of 80 to 20 and 70 to 30.

2.2. Physicochemical Evaluation

2.2.1. Identifying the Functional Groups. Fourier transmission infrared spectroscopy (FTIR, Shimadzu, Japan) was used to investigate the possible connections and identify the functional groups. For this purpose, 4 micrograms of sample was placed in a pan, and the wavelength of 400 to 4000 (cm\(^{-1}\)) was adjusted.

2.2.2. Morphological Study. Scanning electron microscopy (SEM, TESCAN, Warrendale) was used to examine the morphology and the mixing pattern of the prepared materials. Samples were placed on a scanning electron microscope (SEM) screen and coated with gold. After setting the device and selecting the appropriate magnification, the image was taken from the appropriate area under the microscope.

2.2.3. Evaluation of the Curcumin Release Pattern. To determine the release pattern of curcumin from the temporary material, drug dissolution USP apparatus II was used. Phosphate buffer (300 ml) was poured into each of the 6 wells. A small piece of the prepared materials with a specified weight was placed inside each of the 6 wells of the device, and the pH was adjusted to 7.4. The temperature of the device was...
set at 37 degrees. The mixing speed was set to 100 rpm. Samples were taken from the wells every day (1 ml), and the absorbance was read using a UV spectrophotometer (Landa Max of 350 nm). The sample taken from the wells was replaced with 1 ml of a new buffer medium. The absorbance was converted to concentration, and the concentration versus time graph, which is the diagram of the drug release pattern, was plotted to find out what the drug release pattern was.

2.2.4. Determination of the Crystallinity Pattern. X-ray diffraction (XRD) patterns were measured at room temperature for the samples. The samples were exposed to an X-ray diffraction device (Siemens Germany, Model D5000) and irradiated with a wavelength of 1.5405 Å, a voltage of 40 kV, and a current of 30 mA, and their patterns were recorded by the device in 2 theta degree range of 0-60°.

2.2.5. Antimicrobial Action. Bacterial strains of Enterococcus faecalis (ATCC: 6538), Staphylococcus aureus (ATCC: 6538), Escherichia coli (ATCC: 25922) were prepared as standard strains from Pasteur Institute of Iran (Tehran, Iran). The disc diffusion method was used for microbial evaluation of temporary restoration containing curcumin nanocrystals. The temporary restoration composition containing curcumin nanocrystals and the temporary restoration composition without curcumin (as a negative control) were prepared as discs with a diameter of 6 mm. Vancomycin was used as the
positive control group for the gram-positive bacteria and rifampicin as the positive control group for gram-negative bacteria. Müller Hinton agar culture medium and the microbial suspension with a concentration of half McFarland \((1.5 \times 10^8)\) were used in the microbiological test. The prepared disks were placed on the culture medium for 24 hours of incubation at 35°C. The plates were then examined in terms of the growth inhibition zone diameter.

2.3. Statistical Analysis. To compare the findings of the average inhibition zone across microorganisms and among the investigated groups, a one-way analysis of variance was employed (GraphPad Prism software, version 9). A P value less than 0.05 was considered statistically significant.

3. Results

3.1. Identifying the Functional Groups. The results of FTIR are shown in Figure 1: temporary restoration containing 20% nanocurcumin (a), temporary restoration containing 30% nanocurcumin (b), temporary restoration without curcumin (c), and curcumin nanocrystals (d). There was no sign of a new interaction between the two substances.

3.2. Morphology Determination. The results of electron microscope morphological evaluations showed that the temporary restoration and nanocurcumin were uniformly mixed (Figure 2).

3.3. Curcumin Release Pattern. Figure 3 shows the pattern of drug release from a temporary restoration containing nanocurcumin (20% and 30%). The pattern of curcumin release from the restoration in both types of restoration was a two-stage release, 40% release in the first two days and the rest slowly until the 12th day.

3.4. Determination of the Crystallinity Pattern. Figure 4 shows the X-ray diffraction pattern for the prepared materials. No new interactions were observed in the XRD spectra of the studied materials, which is the reason for the physical mixing of these two materials.

3.5. Microbial Results. The results of antimicrobial evaluations (Figures 5(a)–5(c)) showed that both types of temporary restorations with 20% and 30% curcumin had antimicrobial properties. These two substances were able to stop the growth of all three selected bacteria. The mean variable diameter of the growth inhibition zone for \(S.\) aureus was higher than the mean in both other bacteria (significant, \(P\) value < 0.05). Also, the growth inhibition zone for temporary restoration containing 50% of curcumin nanoparticles was larger than the temporary restoration inhibition zone containing 20% of curcumin nanoparticles (nonsignificant, \(P\) value > 0.05).

4. Discussion

The temporary dressing paste used in this study was Colyene (AriaDent, Coltosol), which contains zinc oxide, hydrated
zinc sulfate, calcium sulfate, polyvinyl acetate, and peppermint aroma extract. The peppermint aroma extract is often used as flavoring agents in dental materials [17]. According to FTIR spectroscopy, the vibration peaks in the range of 600-1000 cm\(^{-1}\) are related to zinc oxide. A tensile peak at 562 cm\(^{-1}\) is also related to zinc oxide [18]. The tensile band in the range of 3000-4000 cm\(^{-1}\) belongs to the OH group of water, which either is related to hydrated zinc oxide or indicates the absorption of water by the sample or nanoparticles at the dressing surface. Also, the four basic peaks observed at 758 cm\(^{-1}\), 981 cm\(^{-1}\), 613 cm\(^{-1}\), and 1004 cm\(^{-1}\) are related to zinc sulfate [19]. On the other hand, the four basic peaks observed at 669 cm\(^{-1}\), 672 cm\(^{-1}\), 1005 cm\(^{-1}\), and 1014 cm\(^{-1}\) belong to the calcium sulfate group [20]. The index peak at 1730 cm\(^{-1}\) belongs to the carbonyl polyvinyl acetate group [21]. Also, the adsorption peak at 1720 cm\(^{-1}\) is for the C=O fraction of the ester group, the adsorption at 1300 cm\(^{-1}\) is related to the ether group, and the adsorption at 3200-3500 cm\(^{-1}\) is related to the OH curcumin group [22].

Due to the crystalline nature of curcumin and the XRD peaks recorded from curcumin in the sources, the curcumin nanoparticles used in this study showed lower peak intensities in XRD. According to the reports, this lower intensity indicates lower crystallization (amorphous and crystalline mixture) and smaller sizes compared to curcumin [23]. Also, due to the presence of different materials in the temporary restorative material, different peaks related to these materials can be seen in the XRD spectrum, which indicates the crystalline nature of its ingredients.

The addition of the antibacterial agents to the temporary restorative material can enhance coronal sealing and prevent the growth of residual microorganisms in inaccessible areas [1]. It has been reported that the antimicrobial properties are an extremely important factor for a temporary restorative material, but according to the reports, the temporary restorative materials that are currently available in the market do not have such properties [24, 25].

The pharmaceutical effects of curcumin include anti-inflammatory, antioxidant, anticancer, antidiabetic, and antibacterial activity [26]. The use of nanotechnology can improve the bioavailability of curcumin and its high stability in the bloodstream and reduce its toxic effects. On the other hand, due to the drug resistance of bacteria to antibiotics, the construction and optimization of drug delivery nanosystems to target tissues have been considered by researchers [27, 28]. Also, the synthesis of curcumin in the form of nanocrystals due to its small size has different properties compared to its bulk state [29]. Depending on the particle size as well as the type of bacteria, the nanoparticles exert their antibacterial effects on bacteria through various mechanisms different from what has been reported about the mechanism of action of conventional antibiotics. Nanoparticles generally in the range below 100 nm, compared to larger nanoparticles, are able to disrupt the function of the cell membrane by binding to the surface of the cell membrane with a high affinity. Such an effect is more prevalent in smaller nanoparticles due to their larger surface area. Membrane-nanoparticle interaction causes localized cavities in the membrane and further damage to the bacterium by entering nanoparticles into the bacterium and interacting with intracellular proteins (especially sulfur-rich proteins) and DNA. Another possible mechanism for the antibacterial effects of antimicrobial nanoparticles is their binding to bacterial membranes and their gradual entry into the cytoplasm and disruption of bacterial function. Some drug nanocarriers are also able to mix with the bacterial cell wall and release their antimicrobial agent into the bacterium [30, 31].

In this study, the mean diameter of the growth inhibition zone for S. aureus was higher than the mean in both other bacteria (significant, \(P\) value < 0.05). Many studies have revealed that nanomaterials show better antimicrobial action against gram-positive bacteria than against gram-negative bacteria, due to the existence of lipoproteins and phospholipids in the cell wall of gram-negative bacteria [32].

Also, the growth inhibition zone for temporary restoration containing 30% of curcumin nanoparticles was larger than the temporary restoration inhibition zone containing 20% of curcumin nanoparticles (nonsignificant, \(P\) value > 0.05). The results of Mofazzal Jahromi et al. showed that the use of nanoparticles increased the bactericidal effect of curcumin in inhibiting Staphylococcus aureus in the infected skin of mice. In fact, the gradual release of curcumin from
nanoparticles occurs at the site of infection and well inhibits bacterial infection [33, 34]. Singh et al. also reported the similar results, and the antimicrobial properties of curcumin were improved following the use of its nanoparticles in infectious therapies [35]. In a study by Leonardo et al., a zinc oxide temporary restoration (containing 0.05% triphenyl tetrazolium chloride) inhibited S. aureus and E. coli while it did not inhibit E. faecalis [36]. The antimicrobial effect of a temporary restorative containing various agents (triclosan, chloramine T, and their combination) was studied by Mushashe et al. All materials showed inhibitory effects against E. coli and S. aureus. However, there was no statistically significant difference between the study groups and the control group (without the antimicrobial agent) [1].

It has been recently reported that the metallic compounds used as restorative materials can be also harmful by means of serious adverse effects [37, 38]. Both metallic restorative compounds and nanoparticles may show numerous types of toxicities [37, 38]. One of the main toxicity mechanisms is oxidative damage, and since curcumin (with confirmed antioxidant capacity [39], also in nanoformulation [40]), has been employed in this study, it appears reasonable to potentiate the beneficial role of curcumin in the prevention of those adverse effects.

5. Conclusion
The results of this study showed that the use of new herbal antimicrobials in nanoformulation form can be effective in controlling dental bacterial infections. The cellular, animal, and clinical studies are also needed to demonstrate the function of dental temporary materials containing nanocurcumin.

Data Availability
The raw/processed data required to reproduce these findings can be shared after publication by requesting from the corresponding author.

Ethical Approval
This study was approved by the Ethic Committee of the Tabriz University of Medical Sciences (ethical code: IR.TBZMED.VCR.REC.1398.453).

Disclosure
This article was written based on a dataset from a thesis registered at Tabriz University of Medical Sciences (number 64014).

Conflicts of Interest
The authors state that there are no conflicts of interest.

Authors’ Contributions
Amir Reza Jamei Khosroshahi and Solmaz Maleki Dizaj contributed equally to this work.

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