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ORIGINAL ARTICLE

Novel microinjector for carrying bone substitutes for bone regeneration in periodontal diseases

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Background/Purpose: Traditionally, guide bone regeneration (GBR) was a widely used method for repairing bone lost from periodontal disease. There were some disadvantages associated with the GBR method, such as the need for a stable barrier membrane and a new creative cavity during the surgical process. To address these disadvantages, the purpose of this study was to evaluate a novel microinjector developed for dental applications. The microinjector was designed to carry bone graft substitutes to restore bone defects for bone regeneration in periodontal diseases. The device would be used to replace the GBR method.
Methods: In this study, the injected force and ejected volume of substitutes (including air, water, and ethanol) were defined by Hooke's law ($n = 3$). The optimal particle size of bone graft substitutes was determined by measuring the recycle ratio of bone graft substitutes from the microinjector ($n = 3$). Furthermore, a novel agarose gel model was used to evaluate the feasibility of the microinjector.
Results: The current study found that the injected force was less than 0.4 N for obtaining the ejected volume of approximately 2 mL, and when the particle size of tricalcium phosphate (TCP) was smaller than 0.5 mm, 80% TCP could be ejected from the microinjector. Furthermore, by using an agarose model to simulate the periodontal soft tissue, it was also found that bone graft substitutes could be easily injected into the gel.

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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Conclusion: The results confirmed the feasibility of this novel microinjector for dental applications to carry bone graft substitutes for the restoration of bone defects of periodontal disease.

Introduction

Although periodontal disease is an infectious disease of the gingival tissue, changes in the bone are crucial because bone destruction eventually leads to tooth loss. Periodontal disease results in different types of defects in the alveolar bone. These defects are called bone defects. Losing supporting bone around a tooth results in the movement and dislocation of that tooth, and systemic health is adversely affected.[1–4]

Traditionally, guide bone regeneration (GBR) was a widely used method for repairing bone lost from periodontal disease; it was based on barrier membranes with bone graft sealing off the cavity site of bone for patients with periodontal disease.[5] Although GBR has been used for the treatment of periodontal disease for 10 years, the method has some disadvantages, such as the need for a stable barrier membrane and a new creative space during the surgical process.[6–8] In addition, most patients do not like the wound cavity created by surgical processes. A microinjector with bone substitutes, therefore, was developed to replace the GBR surgical process for restoring bone defects in periodontal disease.

Although microinjection technology in general was widely used in orthopedic surgery, plastic surgery, and dermatology,[9–11] it has not yet been used in dentistry. In this study, a new microinjector was developed to carry bone graft substitutes to repair bone defects of periodontal disease; the use of the microinjector in dentistry is a new concept. The advantages of a small wound, low injective force, and minimal patient discomfort with microinjector use have been reported previously.[12–14] In this study, the novel microinjector for dental application was designed to carry bone graft substitutes. Compared with the traditional GBR method, the microinjector for dental application is very convenient because the bone defects could be filled by the substitutes carried by the microinjector via a one-step injection. The bone substitute used for testing was synthetic calcium phosphate bone substitutes with similar chemical components and structure to normal bone.[15,16] Previous studies have also shown that synthetic calcium phosphate substitute is osteoconductive and can be resorbed by the host body.[17] Synthetic substitutes are advantageous for applying for oral tissue engineering because they have uniform particles, and are biocompatible, cheap, and have no ethics-related issues.[18]

In this study, synthetic tricalcium phosphate (TCP) was used as the bone substitute carried by the microinjector. The injected force and ejected volume of substitutes from the microinjector were investigated; the optimal particle size of TCP was also determined. Furthermore, the feasibility of microinjector use was evaluated with an agarose gel model to observe the distribution of bone substitutes ejected from the microinjector.

Materials and methods

Design of the microinjector for dental applications

The novel microinjector for dental applications was designed according to the following steps. Commercially available syringe needles, 18G and 23G, were used in the design of the microinjector. The microinjector was a double-tube structure, and an 18G needle was used as the outer tube for filling with bone substitutes, and a peak-polished 23G needle was used as the inner tube to propel the bone substitutes out, as shown in Figure 1.

Analyzing the correlation between injected force and ejected volume by Hooke's law

The correlation between injected forces and ejected volumes of substitutes was evaluated by Hooke's law, which is

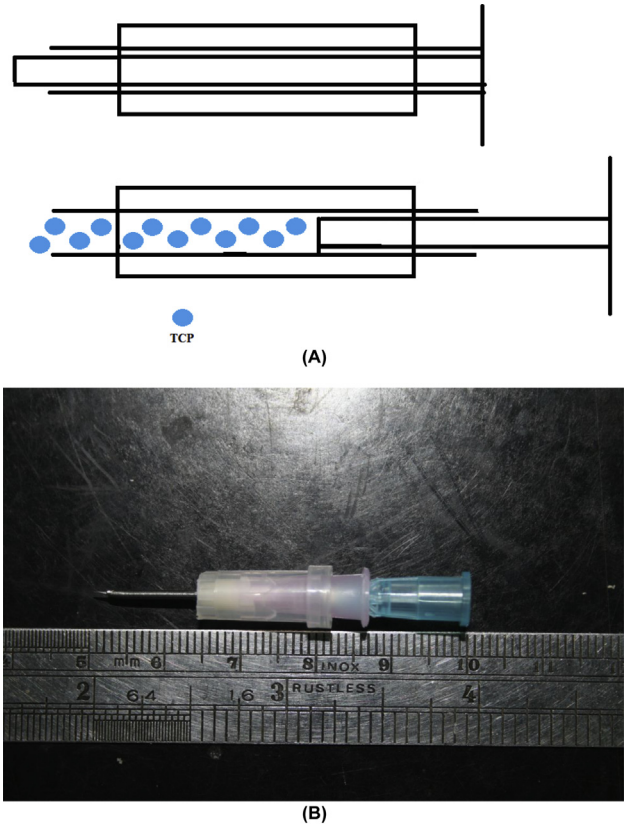

Figure 1 (A) The schematic diagram of a double-tube structure of a dental microinjector; (B) photograph of a novel dental microinjector.

stated mathematically as follows: $F = k\Delta x$.[19] In this study, F is the applied force (N), k is the spring constant of spring steel [SAE 1095, 0.0322 (N/mm)], and Δx is the strain of spring [i.e., $\Delta x = x_{extend} - x_{orignal}$, (mm)]. The force F was calculated by the strain of spring when exerting a force on the inner tube of the microinjector. Thus, the correlated injected forces with the ejected volumes of substitutes including air, water, and ethanol, respectively, were measured.

Optimal particle size of bone substitutes measured by recycling test

The optimal particle size of bone graft substitute, TCP, for the novel microinjector was evaluated using a recycling test. The particle sizes of bone graft substitutes from commercial products of TCP (Ostecera, Hsinchu, Taiwan) used for testing in this study are about 0.25—0.50 mm, 0.50—1.00 mm, 1.0—2.0 mm, and 2.0—3.0 mm in dimension, respectively. According to the clinical experiences of dentists, the general sizes of the oral cavities in periodontal disease range from 0.15 cm^3 to 0.25 cm^3, but only 0.2 g of bone graft substitute is the maximum amount required to fill these cavities. Thus, the outer tube of the microinjector was filled with 0.2 g of bone graft substitutes with different particle sizes, and then the inner tube was exerted by a force to extrude the bone graft substitutes. The recycle ratio was calculated by measuring the volume ratio of ejected and filled bone graft substitute. The results are shown as the ratio (%) (bone graft substitutes$_{extruded}$ over bone graft substitutes$_{filled}$). A high recycle ratio represents better efficiency. The optimal particle size of the bone graft substitutes could therefore be determined.

Feasibility of the microinjector evaluated by an agarose gel model

When evaluating the feasibility of the microinjector, it is important to observe whether the bone graft substance can be extruded easily and evenly distributed in the bone defects. For this purpose, a novel agarose gel model for evaluation was developed. In this study, 1% agarose gel was filled in a 1-mL Eppendorf tube to form a cone shape for simulating periodontal soft-tissue and bone defects. Bone graft substitutes with a 0.25-mm to 0.50-mm particle size were carried by the novel microinjector and were then injected 2—3 mm in depth, into the agarose gel. A camera (Canon, EOS 400D, Tokyo, Japan) was used to capture the distribution of bone graft substitutes. The injection without bone graft substitutes was used as a control. Because agarose is soft and translucent, it is easy to observe the distribution of extruded bone graft substitutes from the microinjector.

Statistical analysis

All statistical analysis was performed using one-way analysis of variance followed by a *post hoc* procedure (Duncan's test) with $p < 0.05$ considered significant for all tests.

Results

Correlation between injected forces and ejected volumes

Figure 2 shows the correlation between the injected forces and the injected syringe volumes by Hooke's Law. It was observed that when injected volumes of air, water, and ethanol were 2 mL and 4 mL, the injected forces are, respectively, 0.31 ± 0.04 N, 0.33 ± 0.07 N, and 0.34 ± 0.03 N and 0.62 ± 0.06 N, 0.53 ± 0.07 N, and 0.56 ± 0.08 N. These data indicate that injected forces have a linear correlation with injected volumes, but not with the type of substitutes.

Recycle ratio and optimal particle size of bone graft substitutes

In order to choose the optimal particle size of bone graft substitutes, the recycle ratio of TCP was estimated by the developed microinjector. Figure 3 shows the recycle ratio of TCP of different particle sizes 0.25—0.5 mm, 0.5—1 mm, 1—2 mm, and 2—3 mm were 83.3 ± 4.2%, 25.8 ± 1.4%, 0.0 ± 0.0%, and 0.0 ± 0.0%, respectively. It appeared that when particle size was larger than 1 mm, the recycle ratio of TCP is zero, which indicated that the TCP powder could not pass through the microinjector. When the particle size of TCP is in the range of 0.5—1 mm, the recycle ratio is lower than 30%. The acceptable recycle ratio should be larger than 80%; the optimal particle size of tested bone graft substitutes, therefore, is 0.25—0.5 mm.

Evaluation of the feasibility of the microinjector with an agarose gel model

The feasibility of the microinjector was evaluated with an agarose gel model to represent the soft-tissue and bone

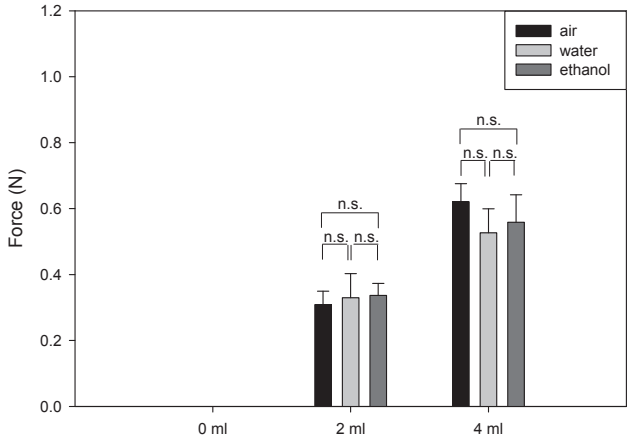

Figure 2 The correlation between injected force (N) and ejected volumes of air, water, or ethanol. Results represent the means ± standard deviations from three independent experiments for each condition ($n = 3$/condition). No significant differences (n.s.) were denoted compared with significant difference (*$p < 0.05$), determined using analysis of variance followed by a *post hoc* procedure (Duncan's test).

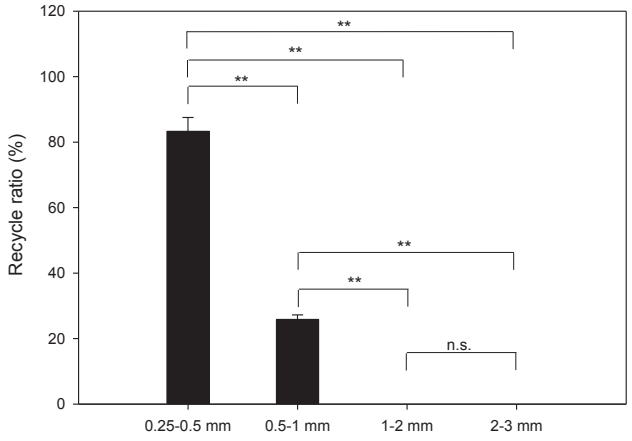

Figure 3 The recycle ratio of different particle sizes of tricalcium phosphate (TCP), ejected from the microinjector. Results represent the means ± standard deviations from three independent experiments for each condition ($n = 3$/condition). No significant differences (n.s.) were denoted compared with significant difference (**$p < 0.01$), determined using analysis of variance followed by a *post hoc* procedure (Duncan's test).

defects. By using the developed microinjector, bone graft substitutes TCP with a particle size of 0.25—0.5 mm was found to be ejected and distributed in agarose gel as shown in Figure 4. The agarose model demonstrated that bone graft substitutes can be carried by a microinjector and ejected into bone defects easily and suggested the feasibility of the microinjector for dental applications. Figure 5 is a schematic diagram showing the application of a microinjector to carry bone graft substitutes for injection with only a small invasion wound and avoiding a flap operation for the patients.

Discussion

In the current study, a novel dental microinjector was designed to carry bone graft substitutes for restoring bone defects for bone regeneration in periodontal diseases. Synthetic TCP was used as the bone substitute for testing in this study. Calcium phosphate has been widely used as a bone graft substitute since 1920.[15] It has been reported that calcium phosphate could supply nutrition for cells and then promote bone formation.[20,21] Recently, GBR with bone graft substitutes are applied to restore bone defects in periodontal diseases.[22] However, it is usually necessary to create a new and large wound in the patients receiving treatment with the GBR method.

The novel microinjector has double tubes and is easy to handle (Figure 1A). The total length of the dental microinjector was only 4.8 cm, which is smaller than a commercial clinical syringe (Figure 1B) and is easy to manipulate. The correlation between the injected force and ejected volume of substitutes was measured using Hooke's law. It appeared that, in the elastic range, the ejected volumes of substitutes had no significant differences among air, water, and ethanol. It was also found that the injected force is less than 0.4 N for obtaining the

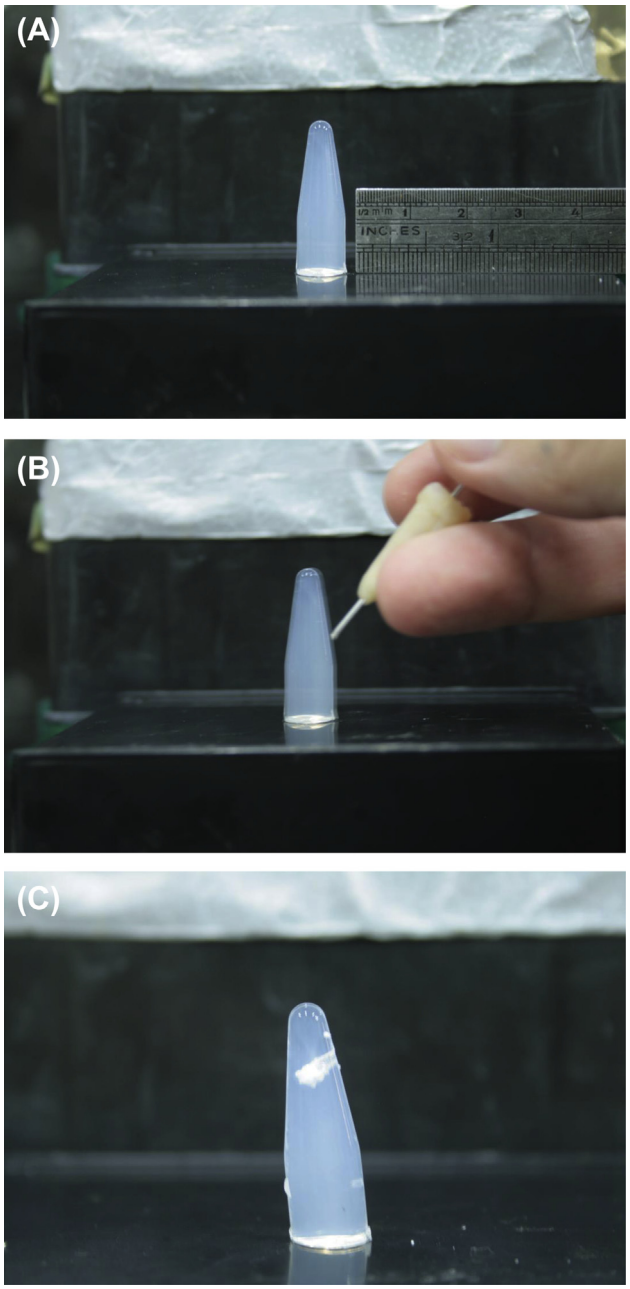

Figure 4 An agarose gel model used for observing the distribution of ejected bone substitutes from a microinjector to evaluate the feasibility of the microinjector. (A) The 1% agarose gel formed cone was used to stimulate alveolar soft tissue; (B) the morphology of agarose gel without microinjection; (C) agarose gel before microinjection and distribution of bone graft substitutes after microinjection of bone graft substitutes, tricalcium phosphate (TCP), into the agarose gel.

ejected volume of about 2 mL (Figure 2). This suggests that the device was easy to carry and saved effort. In this study, the recycle ratio of the bone graft substitute, TCP, with different particle sizes to estimate the optimal particle size of the bone substitutes was measured. According to the results of the recycle ratio, it was found that if the particle size of TCP was smaller than 0.5 mm, 80% TCP could be ejected (Figure 3). Previous studies reported the injection

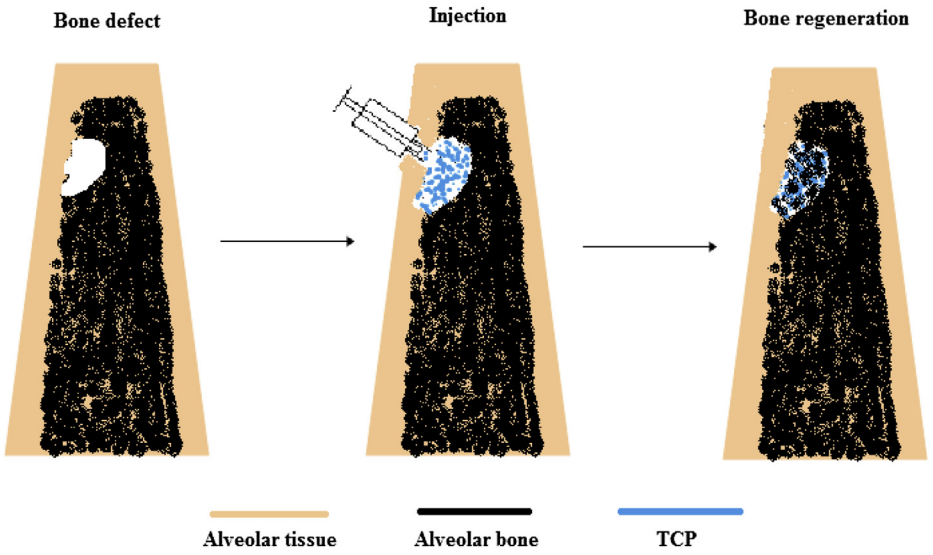

Figure 5 The schematic diagram of the TAIDA injector injecting tricalcium phosphate (TCP) into alveolar tissue.

of calcium hydroxyapatite gel and hyaluronic acid gel for the rejuvenation or treatment of infraorbital hollows and dark circles.[23,24] The materials used in these reports were in gel state. For dental applications of the novel microinjector, the bone graft substitutes for injection were TCP powder. Previous studies have shown that calcium phosphate powder has bioactivity and osteoconductivity.[25–27] This suggests that a powdered bone graft substitute is suitable for bone regeneration in periodontal disease.

Previous studies show that the stiffness of the artificial human oral mucosa stroma (HOMS) based on agarose is similar to human soft tissue; then, after transplantation, a HOMS based on agarose to be an engineering gingival tissue could profile the expression of cytokeratin of oral mucosa and reconstruct the oral cavity.[28,29] For these reasons, a novel agarose gel model was developed by using 1% agarose gel to mimic the soft-tissue and bone defects for measuring the volume and distribution of ejected bone graft substitutes for evaluation of the feasibility of the microinjector. The density of 1% agarose gel is similar to the soft-tissue and bone defects. The optical transparency of the agarose gel also provides an excellent view of the distribution of the bone graft substitutes. It was found that TCP could be ejected into agarose gel effectively through the microinjector (Figure 4). The feasibility of the dental microinjector indicated that this method can replace the traditional GBR method, which has the disadvantages of requiring a suitable barrier membrane and a two-stage surgical process.[28,29] Compared to the traditional GBR method, by using the novel microinjector, no barrier membrane is necessary, only a one-step procedure is necessary, and the injected wound is very small (Figure 4). The feasibility of the microinjector was demonstrated by the agarose gel model and suggests that it is worthy of further clinical applications.

In this study, the novel developed dental microinjector to carry bone graft substitutes for replacing the traditional GBR method for bone regeneration in the periodontal field was evaluated. It was demonstrated that the optimal size of the bone graft substitutes is 0.25—0.5 mm, and an ejected volume of the substitutes of about 2 mL only require an injected force of about 4 N, which is indicative of the ease of manipulation of the microinjector. The feasibility of the microinjector was also proven by the novel agarose gel model. It was also found that the agarose model is a good model for mimicking the soft-tissue and bone defects and helps investigate the distribution of the ejected bone graft substitutes. It has been shown that the dental microinjector serves as a new device that can carry bone graft substitutes when repairing the bone defects from periodontal diseases. The manufacturing process guarantees controllable, consistent, and reproducible dental treatment quality. These findings are very encouraging and worthy of further clinical applications.

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