Materials Science Forum *Online: 2009-01-02*
ISSN: 1662-9752, Vols. 610-613, pp 1192-1197
doi:10.4028/www.scientific.net/MSF.610-613.1192

Fabrication and Antibacterial Performance of Bio-graded Nano-composite Materials by Using Inkjet Printing Method

Dongbin ZHU[a], Anping XU[b,*], Yunxia QU[c], Zhimei MI[d], Jianqiang LI[e]

School of Mechanical Engineering, Hebei University of Technology, Tianjin 300130, China

[a]zhu_dongbin@163.com, [b]xap@hebut.edu.cn, [c]quyunxia69@163.com, [d]mzm2006start@126.com, [e]skyljq@126.com

Keywords: Fabrication, Biomaterial, Antibacterial, Graded Distribution, Inkjet Printing

Abstract. The graded composite materials were fabricated by direct inkjet printing of nano-electropolar mineral and nano-hydroxyapatite powders for the study of inhibiting function on streptococcus mutans, which is a primary pathogen for human dental caries. The electropolar mineral can be utilized in many fields due to its spontaneous permanent polarity and far infrared emission properties. In this study, the graded distribution of the streptococcus mutans on the surface of graded composite materials was investigated by energy dispersion spectrometry, and the mechanism of which is that the streptococcus mutans was remained in the culture of pH at about 8.5 self-adjustment induced by the electropolar mineral. This culture inhibits growth and acid production of streptococcus mutans. Therefore, this composite materials have inhibitory effects on streptococcus mutans, and can be used as a promising biomaterial for preventing human teeth from caries.

1. Introduction

Solid freeform fabrication (SFF), also termed as layered manufacturing, produces parts directly from a computer model without part-specific tooling and human intervention. It has great potential to fabricate functionally graded material. Through selective slurry extrusion (SSE) based technique of SFF, Xu et al. successfully fabricated multi-material dental crown [1] and further proposed a novel approach, equal distance offset (EDO), of representation and processing methods for modeling functionally graded material so as to meet the requirement of modeling and fabrication of 3D complex shaped functionally graded material [2]. In recent years, the inkjet color printing based SFF technology has been of great interests in tissue engineering [3, 4], as it can fabricate 3D complex shaped graded material with smooth gradients [5].

Human teeth have some very good properties such as high hardness and wearability, good heat insulation, high strength, etc. These properties are related to its graded structure. However, dental caries is one of the most important problems in human oral diseases [6]. It is known that *streptococcus mutans* play one of most important roles in dental caries [7]. During metabolizing carbohydrates, *streptococcus mutans* produces organic acid, which can induce the demineralization of tooth surface and results in dental caries [8]. Some natural products are reported to be candidates of new anticariogenic substances [9, 10]. However, there is not scientific evidence about the effect of natural tourmaline on the *streptococcus mutans*.

Tourmaline is a kind of electropolar mineral belonging to the trigonal space group of R3m, whose general chemical formula can be written as $XY_3Z_6Si_6O_{18}(BO_3)_3W_4$, where X is Na^+, Ca^{2+}, K^+, or vacancy; Y is Mg^{2+}, Fe^{2+}, Mn^{2+}, Al^{3+}, Fe^{3+}, Mn^{3+}, Cr^{3+}, Ti^{4+} or Li^+; Z is Al^{3+}, V^{3+}, Cr^{3+}, or Mg^{2+}; and W is OH^-, F^-, or O^{2-} [11]. The most important feature among the electric properties of the tourmaline is the possession of spontaneous and permanent poles, which would produce an electric dipole, especially in a small powder with a diameter of several microns or less [12]. Therefore, a strong electric field exists on the surface of a tourmaline powder [13, 14]. The electric field effect of tourmaline powders can influence the redox potential of water and regulated the pH value of solution [15]. The maintenance of pH value is very important for the growth and metabolism of bacteria [16].

Therefore, the graded composite materials were fabricated with nano-tourmaline and nano-hydroxyapatite powders by direct inkjet color printing for the study of inhibiting function on *streptococcus mutans*. Moreover, the mechanism by which tourmaline acts on the inhibiting the growth and acid production of *streptococcus mutans* was also explained from the view of pH value.

2. Printing setup

The printer consists of a printing table and two piezoelectric XJ126 monochromatic print head (Xaarjet Ltd. Cambridge, UK), linking a $ME1^+$ continuous ink supply system (Epson, JP) for the different inks. A PC104 Programmable Multi-Axis Controller (Delta Tau Ltd. California, USA) for the sliding table and print head movement, and a XaarJet HPC for print head operation were incorporated. The control program was XUSB application in windows XP. The XJ126 print head is a 126 nozzle, piezoelectric drop-on demand array of print width 17.14 mm and nozzle diameter 50 μm. The sliding table moved at 50 mm/s for printing. The gap between the nozzle plate and the printing surface was maintained at 1 mm by z displacement. The graded color pattern was writtern using Adobe Photoshop software and converted to monochrome binary image files, in device-independent bitmap format, for printing pattern on the substrate.

3. Materials and methods

Nano-tourmaline powders from the Inner Mongolia Autonomous Region of China, whose chemical compositions were analyzed by EDAX Phoenix energy dispersion spectroscope (EDS) and given in the mass ratio as follows: Al_2O_3, 34.98%; B_2O_3, 10.94%; K_2O, 0.036%; Na_2O, 0.91%; CaO, 2.534%; MgO, 0.2%; SiO_2, 34.6%; Fe_2O_3, 15.8%. The preparation of nano-hydroxyapatite powders was reported in Ref. [17]. The *streptococcus mutans* was purchased from Tianjin medical university. Brain heart infusion (BHI) broth, mitis salivarius agar (MSA) and phenol red broth were purchased from Hangzhou Tianhe Microorganism Reagent Co., Ltd, China. Other reagents were analytical.

These tourmaline and hydroxyapatite inks with ethyl alcohol as the carrier and oleic acid as the dispersant had a solid loading of 15 and 20 wt%, respectively. After mixing the powders in ethyl alcohol, the suspensions were kept in the ultrasonicator for 1 h. Then, sedimentation experiments for more than 24 h confirmed that this was indeed the case. To provide the visual appreciation of the graded materials fabricated, the tourmaline suspension was mixed with 20 wt% of the commercial XaarJet magenta ink, whereas the hydroxyapatite suspension was mixed with 20 wt% of the XaarJet cyan ink. The *streptococcus mutans* can be cultured on the graded materials. The compositional profiles were determined using a scanning electron microscope (Philips XL30, Amsterdam, Holland) equipped with energy dispersive spectrometry (EDS).

The growth of streptococcus mutans was examined at 37 ℃ in 0.95 ml of BHI broth containing 1% glucose and various concentrations of tourmaline. These tubes were inoculated with 0.1 ml of an overnight culture grown in the BHI broth, and incubated at 37 ℃ for 1 day. The optical density (OD) of cells was measured spectrophotometrically at 600 nm.

The filter-sterilized tourmaline was added to 0.95 ml of the phenol red broth containing 1% glucose, which was then inoculated with 0.05 ml of the seed culture of streptococcus mutans. The cultures were incubated at 37 ℃ for 1 day, and the pH of the cultures were determined using a precise pH meter (PHS-3B, Shanghai precision & scientific instrument Co., LTD, China).

Fifteen grams of tourmaline were added with 100 ml of bacterial suspensions. The initial pH of the medium was changed between 5.0 and 11.0, whereas the temperature was constant at 37 ℃. The pH of the bacterial suspension at different time was measured with precise pH meter mentioned above.

Ten replicates were made for each test. Data were analyzed using the statistical package for social sciences (SPSS). The data were expressed by the mean ± S.D. Differences between means of the experimental and control groups were evaluated by the Student's t-test. The level of significance for statistical analyses was 0.05.

4. Results and discussion

Graded materials were printed using 10 overprinted layers at a printer defined resolution of 185 dot-per-inch (dpi). As shown in Figure 1(a), the tourmaline in the two graded materials of dimensions 10 mm×10 mm increases with a linear gradient from the left to the right. And in Figure 1(b), the tourmaline in the elliptical two graded object increases with a linear gradient from the center to the edges. These compositional profiles of graded materials match that of the design very well.

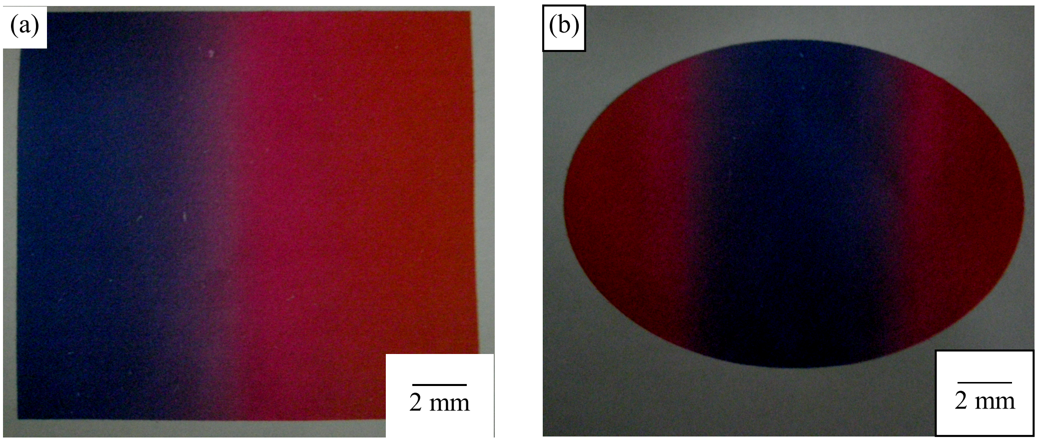

Fig. 1 Graded materials with a linear gradient of increasing tourmaline (a) from the left to the right and (b) from the center to the edges.

The *streptococcus mutans* was cultured on the graded materials in Figure 1(a). The compositional profiles of the graded materials before and after culturing *streptococcus mutans* determined by using an energy-dispersive spectrometer are shown in Figure 2.

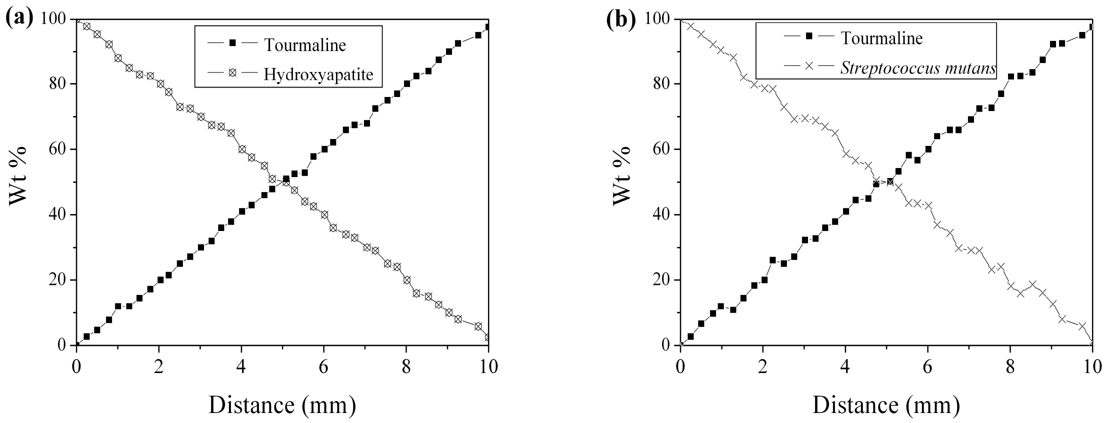

Fig. 2 Graded materials with a linear graded distribution (a) before and (b) after culturing *streptococcus mutans.*

As shown in Figure 2(a), before culturing *streptococcus mutans*, the graded compositional profiles vary in a linear distribution and match the designed composition. After cultured on the graded materials, the *streptococcus mutans* shows a reverse distribution to that of tourmaline (Figure 2(b)). To further investigate the effect of tourmaline on *streptococcus mutans*, the bacteria were exposed to 10, 30, 70, 100, 150, and 200 mg/ml of tourmaline culture. Figure 3 shows the effect of tourmaline on the growth of *streptococcus mutans*.

The bacteria were inoculated to BHI broth with each concentration of tourmaline and anaerobically incubated for 1 day at 37℃. The optical density of A_{600} was read by a spectrophotometer. From Figure 3, it can be seen that the tourmaline has an antibacterial activity against *streptococcus mutans* in a dose dependent manner, and exhibits significant inhibition at concentrations higher than 30 mg/ml compared to the control group ($p < 0.05$).

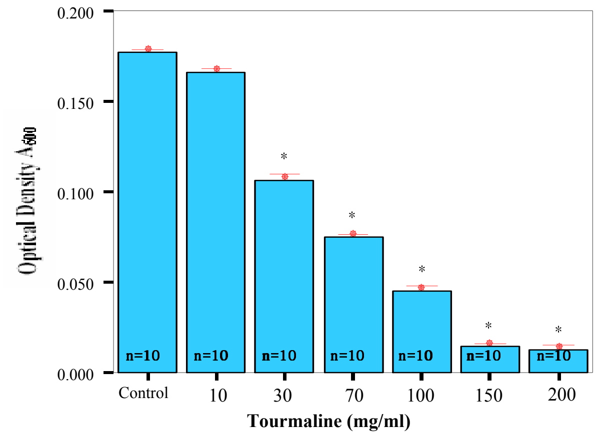

Fig. 3 Effect of tourmaline on the growth of *streptococcus mutans*. *p<0.05 is statistically significant as determined by the Student's *t*-test for the mean values different from the control group.

Streptococcus mutans grow in plaque, and releases various organic acids during metabolizing carbohydrates. The organic acids demineralize tooth surfaces and initiate the dental caries. The effect of tourmaline on acid production of *streptococcus mutans* is shown in Table 1.

The decrease of pH was significantly inhibited in the presence of tourmaline (30–200 mg/ml) compared to the control group. Except antibacterial activity of tourmaline, the reason may be that the strong electric field of tourmaline would affect the water molecule in the culture and produce plenty of negative ions, which can neutralize the organic acid in the culture.

Table 1. Effect of tourmaline on acid production of *streptococcus mutans*.

Concentration (mg/ml)	n	pH
Control	10	5.14 ± 0.023
10	10	5.15 ± 0.028
30	10	5.90 ± 0.072*
70	10	6.87 ± 0.018*
100	10	7.97 ± 0.017*
150	10	8.50 ± 0.202*
200	10	8.52 ± 0.130*

Note: *p<0.05 was statistically significant as determined by the Student's *t*-test for the mean values different from the control group.

The comparison of Figure 1 and Table 1 clearly shows that the antibacterial performance of tourmaline has the similar tendency of increasing pH value of the bacterial suspensions. This result gives the support to the suggestion given by Dashper et al [18] that *streptococcus mutans* exhibit significantly lower maximum culture OD at pH 7.1 compared with at pH 6.0 and 6.3.

The pH value is very important for the growth and metabolism of bacteria [15][10]. In what follows, we will give the mechanism by which tourmaline acts on the inhibition of *streptococcus mutans* from the view of pH value.

Figure 4 shows the effect of tourmaline on the different pH values of the bacterial suspensions. In Figure 4, 15 grams tourmaline were added with 100 ml of bacterial suspensions. The initial pH of the medium was changed between 5.0 and 11.0, whereas the temperature was constant at 37℃.

It can be seen that the pH value was shifted toward 8.5 irrespective of whether it was acidic or alkaline. The increase rate of pH value in acidic solution was than the decrease rate in basic solution. The electrode characteristics of tourmaline particles influenced the redox potential of water and regulated the pH value of solution to about 8.5, which was consistent with the results of Yoshitake et al.[19] and Xia et al.[15] The mechanism of which is that the *streptococcus mutans* was remained in the culture of pH at about 8.5 self-adjustment induced by tourmaline. This culture inhibits growth and acid production of *streptococcus mutans*.

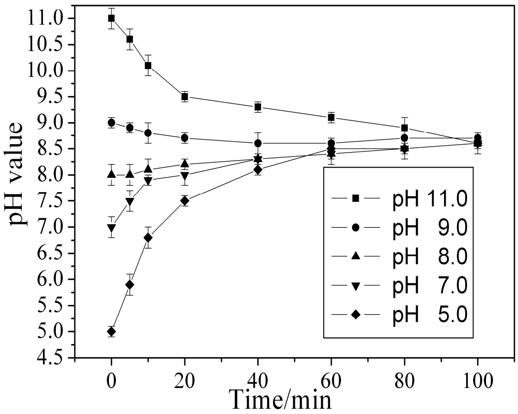

Fig. 4 Effect of tourmaline on the different pH values of the bacterial suspensions.

5. Conclusions

In this paper, the biomaterial graded materials can be fabricated by inkjet printing. Moreover, to the best of our knowledge, this study is the first to use self-adjustment pH value property of tourmaline for inhibiting the growth and acid production of *streptococcus mutans*. As the results show, tourmaline can inhibit the growth and acid production of *streptococcus mutans*, and exhibits significant inhibition at concentrations 30~200 mg/ml, resulting from pH at about 8.5 self-adjustment induced by tourmaline. Therefore, this material has an inhibitory effect on *streptococcus mutans*, and can be used as a promising biomaterial for preventing human teeth from caries.

Acknowledgements

The authors would like to thank the "211" Project of Hebei University of Technology, and the Natural Science Foundation of Hebei Province under Grant Numbers: E2004000052 and E2006000039, for the financial support.

References

[1] A.P Xu, Y.X. Qu, J.W. Wang, L. L. Shaw, Design for Solid Freeform Fabrication of Dental Restoration. Proceedings of the International Conference on Mechanical Engineering and Mechanics in Nanjing, China, October 26-28, 2005, p.1216

[2] A.P Xu, L.L. Shaw, Comput. Aided Design, Vol. 37 (2005), p. 1308

[3] P. Calvert, Science Vol. 318 (2007), p. 208

[4] T. Xu, C. A. Gregory, P. Molnar, X.F. Cui, S. Jalota, S.B. Bhaduri, T. Boland, Biomaterials Vol. 27 (2006), p. 3580

[5] J.W. Wang, L.L. Shaw, J. Am. Ceram. Soc. Vol. 89 (2006), p. 3285

[6] T. Namba, M. Tsunezuka, M. Hattori, Planta. Medica. Vol. 44 (1982), p. 100

[7] W.N. Jessica, S. Gunnel, Appl. Environ. Microbiol. Vol. 73 (2007), p. 5633

[8] T. Ooshima, Y. Osaka, H. Sasaki, K. Osawa, H. Yasuda, M. Matsumura, S. Sobue, M. Matsumoto, Arch. Oral Biol. Vol. 45 (2000), p. 639

[9] N. Shouji, K. Takada, K. Fukushima, M. Hirasawa, Caries Res. Vol. 34 (2000), p. 94

[10] M. Matsumoto, T. Minami, H. Sasaki, S. Sobue, S. Hamada, T. Ooshima, Caries Res. Vol. 33 (1999), p. 441

[11] C. Castañeda, S.G. Eeckhout, G.M. Costa, N.F. Botelho and E.D. Grave, Phys. Chem. Miner.Vol. 33 (2006), p. 207

[12] Z.Z. Jin, Z.J. Ji, J.S. Liang, J.Wang, T.B. Sui, Chin. Phys. Vol. 12 (2003), p. 222

[13] T. Nakamura, K. Fujishiro, T. Kubo, M. Iida, Ferroelectrics Vol. 155 (1994), p. 207

[14] D.B. Zhu, J.S. Liang, Y. Ding, G. Xue, L.H. Liu, J. Am. Ceram. Soc. Vol. 91 (2008), p. 2588

[15] M.S. Xia, C.H. Hu, H.M. Zhang. Process Biochem. Vol. 41 (2006), p. 221

[16] M.E. Esgalhado, J.C. Roseiro, M.T.A. Collaco, Process Biochem. Vol. 30 (1995), p. 667

[17] W. J. Shih, Y. F. Chen, M. C. Wang, M. H. Hon, J. Cryst. Growth Vol. 270 (2004), p. 211

[18] S.G. Dashper, E.C. Reynolds, J. Dent. Res. Vol. 79 (2000), p. 90

[19] N. Yoshitake, Y. Ayumu, O. Kazuya, J. Intelligent Mater. Syst. Structures Vol. 7 (1996), p. 260