Available online at www.sciencedirect.com

SciVerse ScienceDirect

Ceramics International 38 (2012) 2845–2850

CERAMICS INTERNATIONAL

www.elsevier.com/locate/ceramint

Cytotoxicity and degradation behavior of potassium sodium niobate piezoelectric ceramics

Sheng-Wen Yu [a], Shu-Ting Kuo [a], Wei-Hsing Tuan [a,*], Yu-Yu Tsai [b], Sea-Fu Wang [b]

[a] Department of Materials Science & Engineering, National Taiwan University, Taipei 10617, Taiwan

[b] Graduate Institute of Engineering Technology-Doctoral, National Taipei University of Technology, Taipei 10608, Taiwan

Received 3 September 2011; received in revised form 21 November 2011; accepted 21 November 2011

Available online 28 November 2011

Abstract

In the present study, two lead-free piezoelectric ceramics, potassium sodium niobate ($K_{0.5}Na_{0.5}NbO_3$, KNN) and lithium-doped potassium sodium niobate ($Li_{0.06}K_{0.47}Na_{0.47}NbO_3$, LKNN), were prepared by a solid-state reaction process. The cytotoxicity evaluation indicated that the cytotoxicity of KNN is low. However, a strength decrease was noted after soaking in saline solution for 7 days. The addition of 6 mol% Li into the KNN improves its density; the strength and piezoelectric coefficient are enhanced consequently. Nevertheless, the cytotoxicity of LKNN is slightly higher than that of KNN. The higher cytotoxicity is related to the release of Li ions. The release of Li ion also induces the degradation of piezoelectric performance.

Keywords: Piezoelectric; Cytotoxicity; Degradation; Potassium sodium niobate

1. Introduction

The use of bio-active materials may stimulate the growth of tissue; and consequently reduce the recovery time. The investigations on hard tissue replacements have thus moved their focus to the development of bio-active materials recently. Among these materials, the piezoelectric ceramics are unique for their mechanism to stimulate cell growth [1]. Electrical charges are produced by a piezoelectric under external loads. Several studies had demonstrated that the electrical charges were able to stimulate osteogenesis and cell proliferation [2–6]. The presence of charges may thus help the re-generation of bone. The use of piezoelectric ceramics as implants has therefore been proposed. Some *in vitro* and *in vivo* studies indicated a higher piezoelectricity had induced a faster cell growth [7]. Nevertheless, the benefit on cell proliferation had not been observed in some studies [8,9].

Piezoelectricity has been found in human bone [2–4]. However, the piezoelectricity of bone is very small (Table 1). The piezoelectricity has also been found in many synthetic ceramics, such as hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$; HAp), barium titanate ($BaTiO_3$, BT) and lead zirconate titanate ($Pb(Zr,Ti)O_3$; PZT) [7,10]. Baxter et al. has reviewed piezo-materials for bio applications as well as hydroxyapatite-barium titanate materials [11,12]. The piezoelectric coefficient of BT is higher than that of HAp. Feng et al. had applied BT as hard implant in small animals [7], the benefit on bone regeneration was observed. As indicated by a comprehensive evaluation on the cytotoxicity of various metallic salts [13], the toxicity of the metallic salts had demonstrated a trend as following: $Pb(NO_3)_2 > TiCl_4 > BaCl_2 > NbCl_5 > LiCl$. Since the toxicity of Pb salt is high, PZT cannot be used as the hard replacement. The lead-free alkali niobates piezoelectric ceramics, such as potassium sodium niobate ($K_{0.5}Na_{0.5}NbO_3$, frequently denoted as KNN), has been developed recently [14,15]. The piezoelectric charge coefficient, d_{33}, of dense KNN can reach a value of 80 pC/N. Due to the low melting point of KNN (around 1140 °C), and the high vapor pressure of Na_2O and K_2O, the sinterability of KNN is relatively poor [16,17]. The common approach to solve the problem is through the addition of sintering aids. For example, an amount of 5–7 mol% lithium oxide has been added into KNN to enhance its sinterability [18]. The piezoelectricity for KNN is also enhanced as Li is added [18]; it has been related to the

* Corresponding author. Tel.: +886 2 33663899; fax: +886 2 23659800.
E-mail address: tuan@ntu.edu.tw (W.-H. Tuan).

0272-8842/$36.00
doi:10.1016/j.ceramint.2011.11.056

Table 1
Physical and piezoelectric characteristics of KNN and LKNN specimens. The values for human bone are also provided for comparison.

	Bone	KNN	LKNN
Relative density (%)	–	94 ± 0.4	96 ± 0.4
Elastic modulus (GPa)	17–20[a] 6–13[b]	100	100
Biaxial strength (MPa)	–	161 ± 6	200 ± 8
Piezoelectric coefficient, d_{33} (pC/N)	0.7 [10]	63	98

[a] Elastic modulus in longitudinal direction [26,27].
[b] Elastic modulus in transversal direction [26,27].

formation of morphotropic phase boundary (MPB). A recent study indicated that the toxicity of KNN is low [19]. However, the degradation behavior of KNN and LKNN in water solution has not been investigated yet. In the present study, the KNN and LKNN specimens are fabricated by using conventional powder mixing and pressureless sintering techniques. The cytotoxicity of the KNN and LKNN is evaluated; then their degradation behavior in saline solution is investigated.

2. Experimental

In the present study, the potassium sodium niobate ($K_{0.5}Na_{0.5}NbO_3$, KNN) and 6 mol% Li doped KNN ($Li_{0.06}K_{0.47}Na_{0.47}NbO_3$, LKNN) specimens were prepared by using a solid-state synthesis process. The process started by mixing suitable amounts of potassium carbonate (K_2CO_3, J.T. Baker Co., USA), sodium carbonate (Na_2CO_3, J.T. Baker Co., USA), niobium oxide (Nb_2O_5, Sigma–Aldrich Co., Germany) and/or lithium carbonate (Li_2CO_3, Sigma–Aldrich Co., Germany) powders together in a ball mill. The mixing was carried out in ethyl alcohol for 4 h using yttria-stabilized zirconia milling media. The slurry was dried in a rotary evaporator to remove the alcohol, and then sieved through a #150 plastic mesh. The powders were consolidated into discs with 10 mm in diameter and 1 mm in thickness by die-pressing. The specimens were fired pressurelessly in a temperature range from 1065 °C to 1120 °C in air for 1 h, with the heating and cooling rates of 5 °C/min.

Specimen density was determined by applying the Archimedes method. The relative density was estimated by taking 4.51 g/cm^3 and 4.50 g/cm^3 as the theoretical densities for KNN and LKNN, respectively [16,18]. Phase analysis was conducted by using the X-ray diffractometry (XRD, X'pert diffractometer, Philips Co., Netherlands) technique. Scanning electron microscopy (SEM, XL30, Philips Co., Netherlands) was used for microstructure observation. The biaxial strength of the specimen discs was measured with a one-ball-on-three-balls fixture. During the measurement of biaxial strength, the edges of the discs were outside the loading area. The edge effects, such as chippings at edge, would not affect the strength of the specimen [20]. The method was a better technique to determine the strength of brittle ceramics. The elastic modulus of the specimens was determined by using the ultrasonic echo pulse method. The piezoelectric charge coefficient, d_{33}, was measured with a d_{33} meter (PM3001, KCF Co., USA). Before the piezoelectric measurement, silver electrodes were printed onto the top and bottom surfaces of the discs. The polarization was carried out in a silicon oil bath under a DC electric field at 3 kV/mm. After the polarization, the Ag electrodes were removed for further tests.

L929 mouse fibroblast cells were used for cytotoxicity test. The culture medium was minimum essential medium (MEM, Gibco BRL, USA) containing 3.7 g/L of sodium bicarbonate, 10 vol% horse serum (HS) and 1 vol% penicillin–streptomycin solution. The pH value of the MEM solution was adjusted to 7.2 by using HCl solution. The medium was then filtered by aseptic 0.22 mm filtering membrane in sterilized laminar flow and kept at 4 °C. The cells were cultured at 37 ± 1 °C in a 5 ± 1% CO_2 humidified atmosphere. Before the cytotoxicity test, the extracts from the specimens were prepared by following the ISO 10993-5 protocol. The KNN and LKNN specimens were first crushed and ground to produce powders. The powders were then sterilized by γ-ray radiation. A powder mass of 2 g was then soaked in 10 mL MEM solution inside a vessel at 37 ± 1 °C for 24 h in 5 ± 1% CO_2 humidified atmosphere. The vessel was then centrifuged for 5 min at 1000 rpm, and the supernatant solution, extract, was collected. For some tests, the pH value of extracts was adjusted to 7.2 by adding HCl solution. The cells were seeded inside 96 well plates with cell density of 10^4 cells/100 μL in each well. The cells were incubated with the supernatant of the release products for 24 h. The MTT agent (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was then added; the cell density was measured by using a cell counter (Countess, Invitrogen Co., USA). For comparison purpose, the MEM was used as the negative control group; the MEM with 10 vol% dimethyl sulfloxide (DMSO, Merck Co., Germany) was used as the positive control group. The cell viability was calculated by using the following equation:

$$\text{Cell viability} = \frac{\text{mean cell density of sample}}{\text{mean cell density of negative control}} \times 100\% \quad (1)$$

Six measurements had been conducted for each group. The average value and its standard deviation were then calculated.

For the degradation study, the poled specimens were soaked in normal saline (sodium chloride solution at 9 mg/L) at 37.5 °C. The ratio of specimen to saline solution was kept at 1 g to 10 mL. The pH value of the saline solution was measured after soaking with the specimens for 24 h. In order to accelerate the dissolution, if any, the solution was replaced with the fresh saline every day. The soaking test was repeated for 28th days. The d_{33} and biaxial strength of the discs after soaking for 7, 14 and 28 days were also measured. The ionic concentration in the saline solution was analyzed by using an inductively coupled plasma-atomic emission spectrum (ICP-AES, Model 3000DV, Perkin Elmer, Optima, USA). Surface morphology of the disc after soaking was also observed with SEM.

3. Results

Fig. 1 shows the relative density of the KNN and LKNN specimens as a function of sintering temperature. By adding

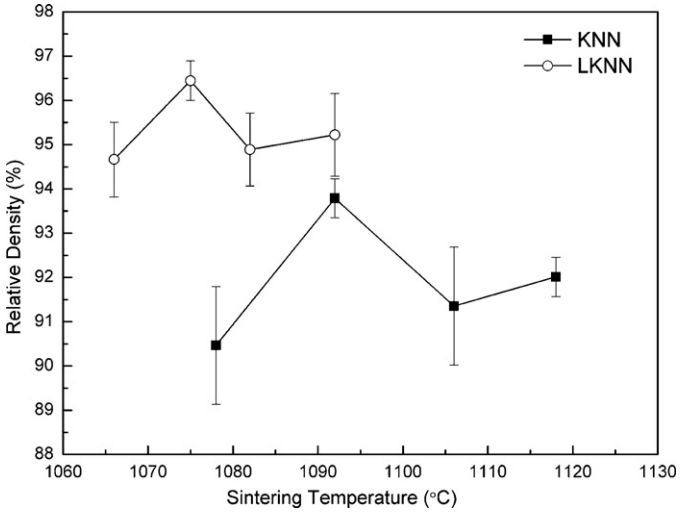

Fig. 1. Relative density of KNN and LKNN specimens as a function of sintering temperature.

6 mol% Li into KNN, the densification of KNN was enhanced. The densities of KNN and LKNN specimens reach their highest values as they were sintered at 1092 °C and 1075 °C for 1 h, respectively. Since the density affect the piezoelectric performance [21], the KNN and LKNN specimens were respectively prepared by sintering at 1092 °C and 1075 °C for subsequent evaluation. The microstructures of the KNN and LKNN specimens are shown in Fig. 2. The SEM micrographs demonstrate that the porosity in the KNN specimen is slightly higher than that in the LKNN specimen. Furthermore, the grain size of the LKNN specimen is larger than that of the KNN specimen, indicating that the Li addition enhances both the densification and coarsening of KNN.

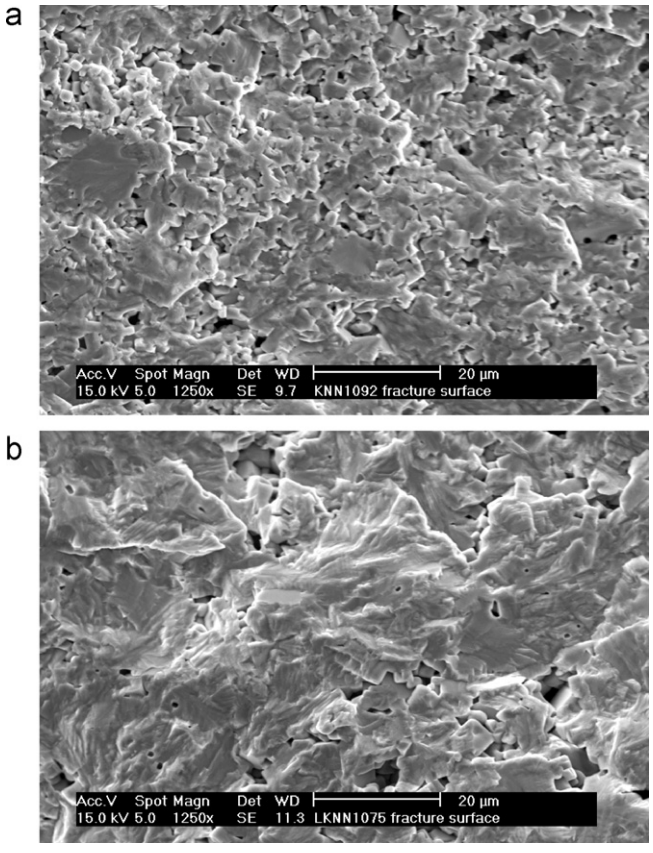

Fig. 2. Fracture surfaces of (a) KNN and (b) LKNN specimens after sintering at 1092 °C and 1075 °C respectively for 1 h.

The XRD patterns of sintered KNN and LKNN specimens are shown in Fig. 3(a). There was no starting material found in the patterns, indicting that solid-state reactions between the starting materials were completed after sintering. By looking into the details within the 2θ range from 44 to 47, Fig. 3(b), the shift of peaks is resulted from the presence of both the orthorhombic phase one and orthorhombic phase two due to the addition of 6 mol% Li [18].

The toxicity of DMSO is high. The cell viability in the MEM + DMSO solution (positive control group) was very low; only around 5% cells were survived (Fig. 4). The viability of the cells in the extract prepared from KNN powder was relatively high, ~84%, indicating that the toxicity of KNN specimen was low. The cell viability in the extract prepared from the LKNN powder was around 58%. However, the cell viability may be affected by the pH value [22]. To avoid the influence from the pH value, the pH values of KNN and LKNN extracts were

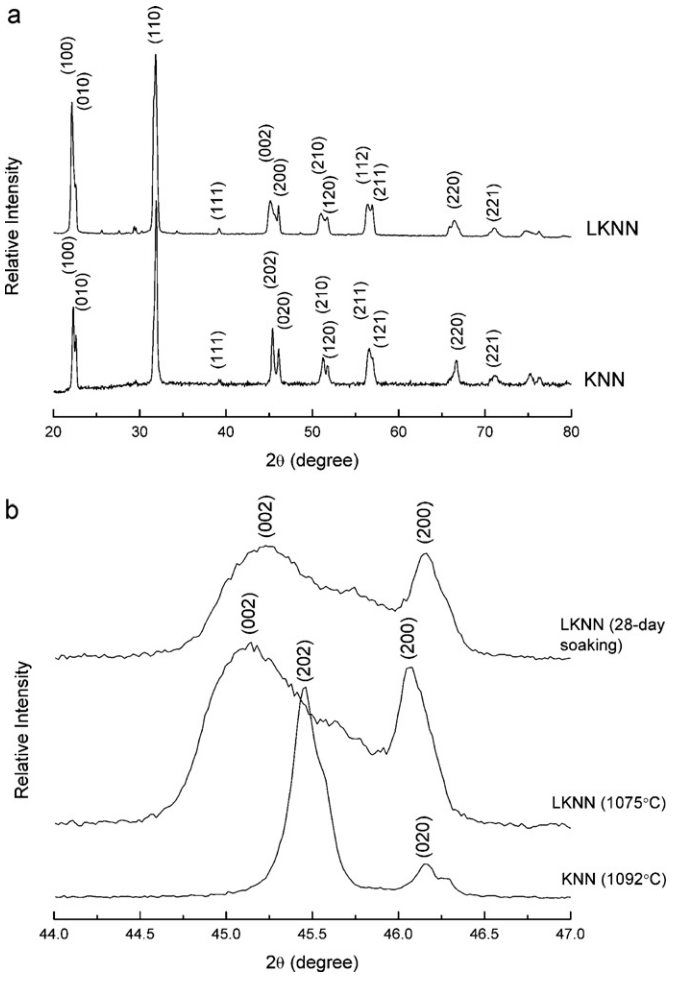

Fig. 3. XRD patterns for (a) KNN and LKNN specimens after sintering and (b) LKNN specimens after soaking in saline for 28 days.

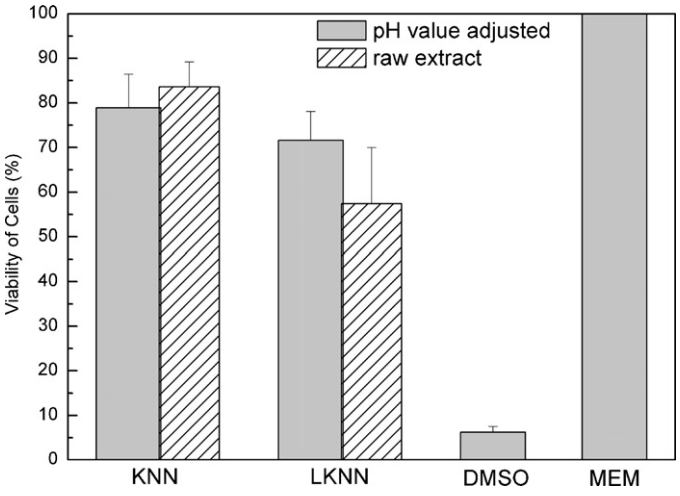

Fig. 4. Viability of cells after treated with the extracts from KNN and LKNN specimens. The filled columns are the data for the extracts with pH value of 7.2; the striped columns are the data for the extracts without pH adjustment. The cell viability of control groups is shown for comparison.

adjusted to 7.2 by adding HCl. This pH value was the same as that of the MEM (negative control group). After the adjustment of pH value, the cell viability in the KNN extract remains high. The cell viability in the LKNN extract increased to a value of 72%, confirming that a higher pH value is harmful to the cell viability. By taking the pH effect into account, the toxicity of the LKNN extract is also low.

In order to evaluate the degradation behavior of the piezoelectrics prepared in the present study, the KNN and LKNN specimens were soaked in normal saline solution up to 28 days. The pH value of the saline solution was monitored every day; the biaxial strength and piezoelectric coefficient after soaking for a fixed time frame were also measured. The pH value of a control saline solution (without soaking with specimen) was also measured for comparison purpose. The change of pH values as a function of soaking time is shown in Fig. 5. The measurement was conducted after soaking with or without the specimen for 24 h. After the opening of a bottle of

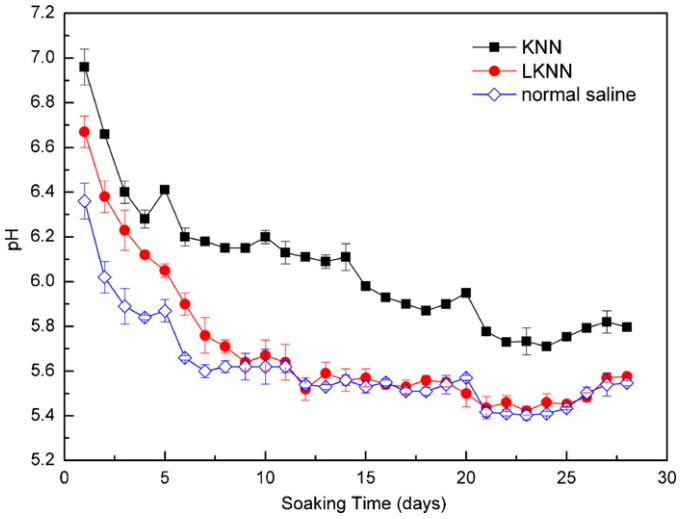

Fig. 5. pH values of normal saline after soaking KNN and LKNN specimens at 37.5 °C for various times.

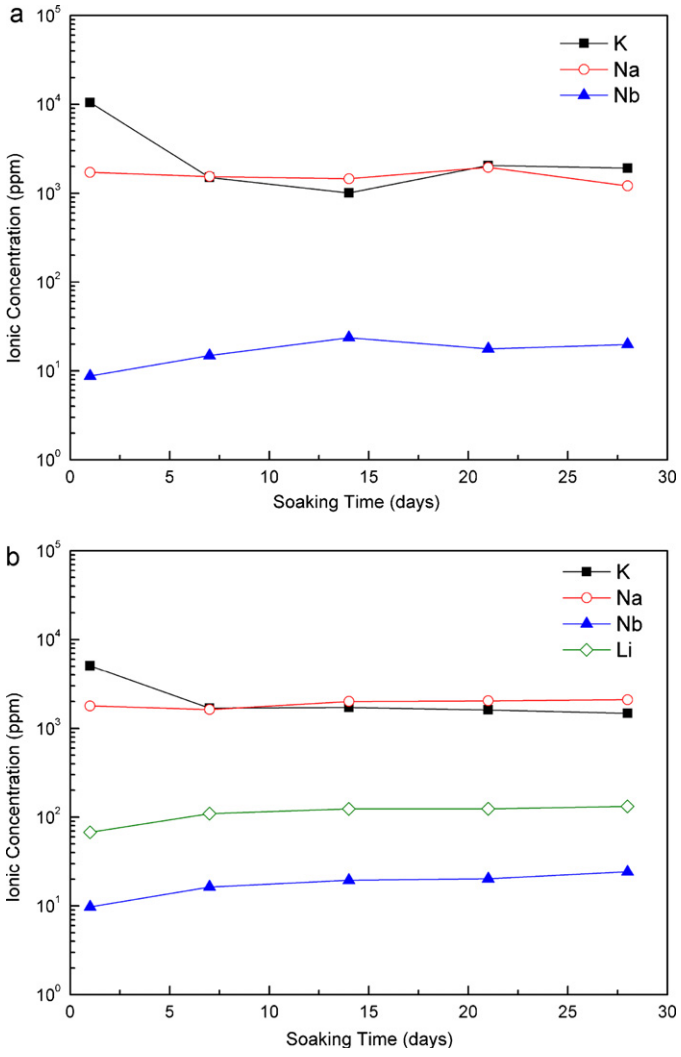

Fig. 6. Ion concentrations detected in the normal saline solution after soaking with (a) KNN and (b) LKNN specimens for various times.

fresh saline solution, the CO_2 in air starts to dissolve into the normal saline solution. The pH values of the control saline solution thus decrease with the increase of time. The pH values of the solution containing LKNN specimen were close to those of the control solution. However, the pH values of the solution containing KNN specimen were slightly higher than those of the solution containing LKNN specimen.

The concentrations of ions in the normal saline after soaking with the specimens for 7, 14, 21 and 28 days are shown in Fig. 6. A relatively large amount of potassium and sodium ions were released from the KNN and LKNN specimen. The concentration of K^+ ions was relatively high the first day, and then droped to a saturated value. The concentration of K^+ and Na^+ ions from the KNN specimen was slightly higher than that from the LKNN specimen. The concentration of Nb was very low, around 10 ppm. Even though the amount of Li in LKNN was only 6 mol%; the concentration of Li^+ ions in the solution after soaking with LKNN specimen was one order of magnitude higher than that of Nb^{5+} ions.

The biaxial strength of LKNN specimen before soaking in saline is higher than that of KNN specimen, Table 1. The

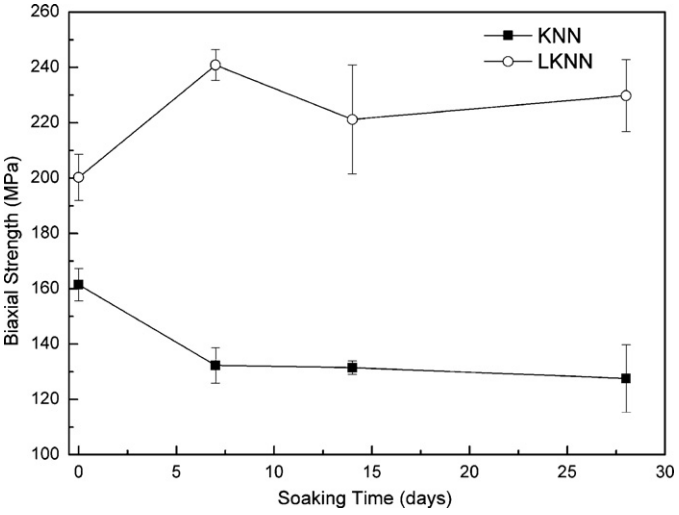

Fig. 7. Biaxial strength of KNN and LKNN specimens as a function of soaking time.

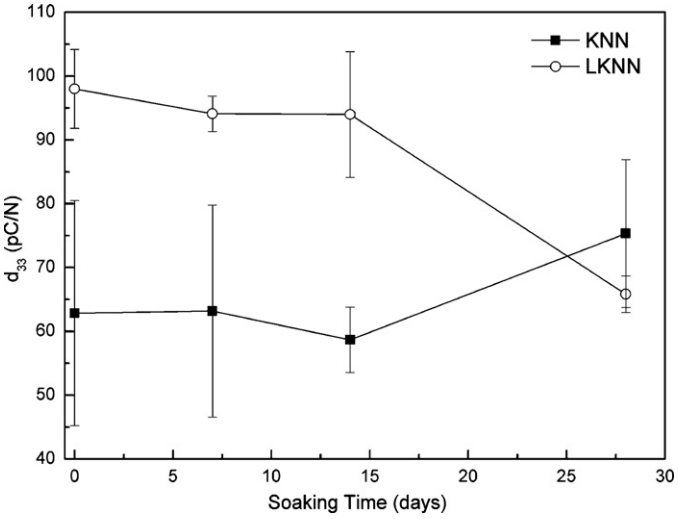

Fig. 9. Piezoelectric charge coefficient (d_{33}) of KNN and LKNN specimens as a function of soaking time.

strength of the LKNN specimen after soaking varies within a relatively narrow range from 200 MPa to 240 MPa, Fig. 7. For the KNN specimens, the strength drops by 20% after soaking in normal saline for 7 days. For the KNN specimen, cracks can be found on the surface. One typical example is shown in Fig. 8. There is no crack found for the LKNN specimen after soaking.

Fig. 9 shows the variation of d_{33} value as a function of soaking time. Differ from the strength degradation, the d_{33} values of KNN is more or less the same after soaking; however, the d_{33} value of LKNN specimens decreases by 30% after soaking for 28 days.

4. Discussion

Piezoelectric behavior depends on the arrangement of cations and anions in crystal. The piezoelectric coefficient thus depends strongly on the crystalline structure [2–4]. The addition of 6 mol% Li into KNN moves the structure closer the morphotropic phase boundary (MPB) [18,23], as indicated by the XRD pattern shown in Fig. 3(b). Since two ferroelectric phases are present in the sintered LKNN specimen; many directions for the dipoles to move around are available. The piezoelectric coefficient of the LKNN is therefore higher than that of KNN.

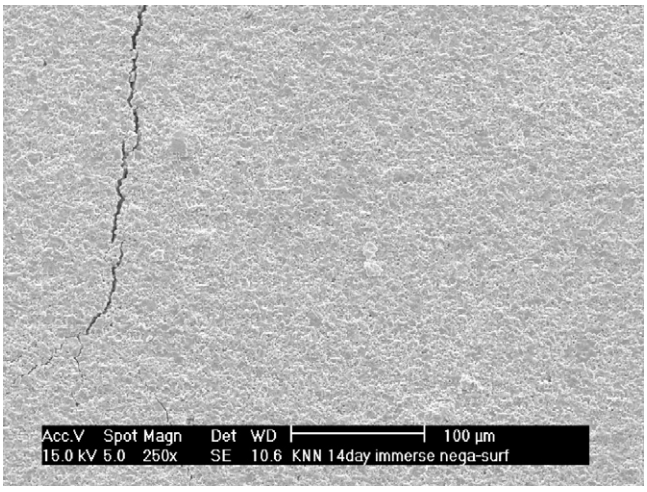

Fig. 8. Surface of a KNN specimen after soaking in normal saline for 14 days.

The ion release does take place from both KNN and LKNN specimens. The release of ions from the crystal induces structure distortion. The structure distortion consequently induces stress. However, as the pore volume is low enough and the strength is high enough, no cracks are formed. A high strength gains higher importance if the ion release is not avoidable. The strength values of KNN and LKNN are higher than that of HAp [24], suggesting that the potential of using KNN or LKNN as hard replacement is high. Though the strength of LKNN is higher than that of KNN, the piezoelectric coefficient decreases significantly after soaking in normal saline. The phase analysis for the LKNN specimen shows no significant difference after soaking in normal saline for 28 days (Fig. 3(b)). The decrease of d_{33} value is thus not resulted from the structure change, but affected by the release of Li^+ ions.

The cell viability is affected by the pH value [22]; therefore, it is necessary to compare the cell viability at a constant pH value. The ion release also affects the cell viability, Fig. 4. The release of K^+ and Na^+ ions imposes little risk to the cells. It is worth noting that the porosity in the KNN specimen is higher than that in the LKNN specimens. Therefore, more K^+ ions were released from KNN specimen in the first day after soaking in normal saline. The strength decrease of ceramic exhibits an exponential relationship with the amount of porosity [25]. The increase of density of KNN may enhance its strength significantly. A denser KNN specimen may survive after the release of ions from the specimen surface. Though the addition of Li^+ ions enhances the density and piezoelectric coefficient of KNN, the presence of Li imposes minor toxicity on surrounding cells. Furthermore, the piezoelectric coefficient is degrades as Li^+ ions are released from LKNN. Therefore, it implies that the KNN with higher density and higher strength is favorable as hard replacement for bone. Further study on the density

improvement of KNN through the addition of bio-compatible sintering additive is still needed.

5. Conclusions

In the present study, two piezoelectric potassium sodium niobates were prepared by solid-state reaction and pressureless sintering techniques. The cytotoxicity investigation has been conducted. The cytotoxicity of KNN is low. The degradation study indicates that a higher strength is preferred for biomedical applications. The addition of 6 mol% Li into KNN can produce dense LKNN, which fulfills the above requirement. However, the release of Li increases slightly the cytotoxicity of LKNN. It implies that the KNN with higher density and higher strength is a potential piezoelectric biological ceramic.

Acknowledgement

The present study was supported by the National Science Council, Taiwan, through the contract number of NSC98-2221-E-002-057.

References

[1] J.R. Woodard, A.J. Hilldore, S.K. Lan, C.J. Park, A.W. Morgan, J.A.C. Eurell, S.G. Clark, M.B. Wheeler, R.D. Jamison, A.J.W. Johnson, Mechanical properties and osteoconductivity of hydroxyapatite bone scaffolds with multi-scale porosity, Biomaterials 28 (2007) 45–54.
[2] E. Fukada, I.J. Yasuda, On the piezoelectric effect of bone, J. Phys. Soc. Jpn. 12 (1957) 1158–1162.
[3] C.A.L. Bassett, R.O. Becker, Generation of electric potentials by bone in response to mechanical stress, Science 137 (1962) 1063–1064.
[4] M.H. Shamos, L.S. Lavine, Piezoelectricity as a fundamental property of biological tissues, Nature 213 (1967) 267–269.
[5] M. Steiner, W.K. Ramp, Electrical stimulation of bone and its implication for endosseous dental implantation, J. Oral Implantol. 16 (1990) 20–27.
[6] P.R. Supronowicz, P.M. Ajayan, K.R. Ullmann, B.P. Arulanandam, D.W. Metzger, R. Bizios, Novel current-conducting composite substrates for exposing osteoblasts to alternating current stimulation, J. Biomed. Mater. Res. 59 (2002) 499–506.
[7] J.Q. Feng, H.P. Yuan, X.D. Zhang, Promotion of osteogenesis by a piezoelectric biological ceramic, Biomaterials 18 (1997) 1531–1534.
[8] J.B. Park, B.J. Kelly, G.H. Kenner, A.F. von Recum, M.F. Grether, W.W. Coffeen, Piezoelectric ceramic implants: *in vivo* results, J. Biomed. Mater. Res. 15 (1981) 103–110.
[9] J.B. Park, A.F. von Recum, G.H. Kenner, B.J. Kelly, W.W. Coffeen, M.F. Grether, Piezoelectric ceramic implants: a feasibility study, J. Biomed. Mater. Res. 14 (1980) 269–277.
[10] C.C. Silva, D. Thomazini, A.G. Pinherio, N. Aranha, S.D. Figueiró, J.C. Góes, A.S.B. Sombra, Collagen-hydroxyapatite films: piezoelectric properties, Mater. Sci. Eng. B 86 (2001) 210–218.
[11] F.R. Baxter, C.R. Bowen, I.G. Turner, A.C.E. Dent, Electrically active bioceramics: a review of interfacial response, Annal. Biomed. Eng. 38 (2010) 2079–2092.
[12] C.R. Bowen, J. Gitting, I.G. Turner, F. Baxter, J.B. Chaudhuri, Dielectric active piezoelectric properties of hydroxyapatite-$BaTiO_3$ composites, Appl. Phys. Lett. 89 (2006) 132906.
[13] A. Yamamoto, R. Homma, M. Sumita, Cytotoxicity evaluation of 43 metal salts using murine fibroblasts and osteoblastic cells, J. Biomed. Mater. Res. 38 (1998) 331–340.
[14] M.D. Maeder, D. Damjanovic, N. Setter, Lead free piezoelectric materials, J. Electroceram. 13 (2004) 385–392.
[15] Q. Wang, Q. Chen, J.G. Zhu, C.P. Huang, B.W. Darvell, Z.Q. Chen, Effects of pore shape and porosity on the properties of porous LNKN ceramics as bone substitute, Mater. Chem. Phys. 109 (2008) 488–491.
[16] R.Z. Zuo, J. Rödel, R.Z. Chang, L.T. Li, Sintering and electrical properties of lead-free $Na_{0.5}K_{0.5}NbO_3$ piezoelectric ceramics, J. Am. Ceram. Soc. 89 (2006) 2010–2015.
[17] H.L. Du, Z.M. Li, F.S. Tang, S.B. Qu, Z.B. Pei, W.C. Zhou, Preparation and piezoelectric properties of $(K_{0.5}Na_{0.5})NbO_3$ lead-free piezoelectric ceramics with pressure-less sintering, Mater. Sci. Eng. B 131 (2006) 83–87.
[18] Y.P. Guo, K. Kakimoto, H. Ohsato, Phase transitional behavior and piezoelectric properties of $Na_{0.5}K_{0.5}NbO_3$–$LiNbO_3$ ceramics, Appl. Phys. Lett. 85 (2004) 4121–4123.
[19] S.W. Yu, S.T. Kuo, W.H. Tuan, Y.Y. Tsai, C.H. Su, Ion release from three lead-free piezoelectric ceramics and their physical and cytotoxicity characteristics, Mater. Lett. 65 (2011) 3522–3524.
[20] W.H. Tuan, S.M. Liu, C.J. Ho, T.J. Yang, Biaxial strength of ZrO_2/(Ni + Al_2O_3) nanocomposite, J. Am. Ceram. Soc. 89 (2006) 754–758.
[21] M. Matsubara, T. Yamaguchi, K. Kikuta, S. Hirano, Sinterability and piezoelectric properties of (K,Na)NbO_3 ceramics with novel sintering aid, Jpn. J. Appl. Phys. 43 (2004) 7159–7163.
[22] M. Uo, M. Mizuno, Y. Kuboki, A. Makishima, F. Watari, Properties and cytotoxicity of water soluble Na_2O–CaO–P_2O_5 glasses, Biomaterials 19 (1998) 2277–2284.
[23] H.L. Du, F.S. Tang, F. Luo, D.M. Zhu, S.B. Qu, Z.B. Pei, W.C. Zhou, Influence of sintering temperature on piezoelectric properties of $(K_{0.5}Na_{0.5})NbO_3$–$LiNbO_3$ lead-free piezoelectric ceramics, Mater. Res. Bull. 42 (2007) 1594–1602.
[24] C.Y. Chiu, H.C. Hsu, W.H. Tuan, Effect of zirconia addition on the microstructural evolution of porous hydroxyapatite, Ceram. Int. 33 (2007) 715–718.
[25] R.W. Rice, Microstructure dependence of mechanical behavior of ceramics, Treatise Mater. Sci. Technol. 11 (1977) 197–381.
[26] S.V. Dorozhkin, Calcium orthophosphates in nature, biology and medicine, Materials 2 (2009) 399–498.
[27] K.A. Athanasiou, C.F. Zhu, D.R. Lanctot, C.M. Agrawal, X. Wang, Fundamentals of biomechanics in tissue engineering of bone, Tissue Eng. 6 (2000) 361–381.