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Research Article

The Investigation of Bioactivity and Mechanical Properties of Glass Ionomer Cements Prepared from Al_2O_3-SiO_2 Glass and Poly(γ-glutamic acid)

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The glass ionomer cement as one of the dental cements has been subjected to be widespread application in restoring tooth structure. Most of glass ionomer cements employ the poly(acrylic acid) (PAA) as the liquid phase, but the presence of PAA inhibits the apatite formation on the surface in the body environment, which is an essential requirement for exhibiting bone-bonding ability (bioactivity). In this study, poly(γ-glutamic acid) (γ-PGA), a kind of biopolymer, was utilized for cement preparation. The effort of preparation parameters including the glass powders/liquid ratio (P/L) and the concentration of γ-PGA on diametral tensile strength were investigated. A maximum diametral tensile strength value of 11.88 ± 1.43 MPa was obtained when the cement sample was prepared by P/L ratio of 1 : 1 and the γ-PGA concentration of 30% after aging for 3 days. The TF-XRD patterns, SEM images, and EDX spectra suggested that the cement induced a precipitation of calcite on the surface after 7 days of immersion in stimulated body fluid (SBF), although the apatite formation was not observed. The present results suggest that the cement has potential to show bioactivity *in vivo*, because calcite is also reported to be bioactive.

1. Introduction

Glass ionomer cements (GICs), one kind of restorative materials, have been successfully used in dentistry for more than three decades [1]. Recently, the application is extending to implant fixation [2] and reconstructive surgical procedures [3]. Their attributes in dental role include direct adhesion to tooth mineral and release of fluoride ions to defend against dental caries [4]. Compared with other restorative cements, GICs present ease of molding, fast setting reaction, no obvious shrinkage, no significant increase in temperature [5], and better biocompatibility without inflammatory response in mouth [6].

Commercial products for cement preparation consist of CaO-Al_2O_3-SiO_2-CaF_2 glass powders and about 40–50% m/m (mass per mass) PAA solution. GICs can bond chemically to the tooth structure by developing an ion enriched layer due to the reaction occured between carboxyl group (–COOH) of PAA and calcium from the dentine or enamel [7]. When implanted into the body, negatively charged Si-OH groups on the surface of glass particles and –COOH groups in PAA can attract Ca^{2+} ions easily [8]. The bonding between cements and bone is attributed to mechanical interlocking rather than a bioactive mineralized layer. Kamitakahara et al. revealed that the existence of PAA even in ppm grade inhibited the apatite formation on the GIC surface, which means that any PAA-containing GICs will lose their bioactivity in body environment [9]. If such cements are intended for orthopaedic use, a new substitution of polyalkenoic acid must be developed.

In order to provide GICs with bioactivity, a microbial γ-PGA will be adopted as an alternative acidic polymer to prepare cements. γ-PGA is a polypeptide in which the repetitive units of D- and L-glutamic acids are copolymerized

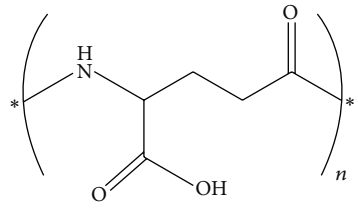

Figure 1: Chemical structure of poly(γ-glutamic acid).

through the chemical bond between the amino and the carboxylic groups to give the chemical structure shown in Figure 1. The polymer comes from a natural component of Natto, one kind of Japanese soybeans [10], owing water solubility, bioresorption, and nontoxicity to human beings and environment. Due to its rich –COOH groups, γ-PGA as a biomaterial has been applied in drug delivery [11] and water absorption hydrogels [12]. Apatite formation on Ca^{2+}-modified γ-PGA hydrogels in simulated body fluid (SBF) has been reported by the present authors [13]. The analysis of FT-IR spectra in the literature indicated that the formation process of cement prepared by γ-PGA is similar to that described for cement prepared by PAA [14], but the information related to the bioactivity of cement is not reported.

In the present study, the aim was to build bioactive glass ionomer cements with better mechanical strength. Besides the bioactivity testing, the preparation parameters in improving the mechanical properties of cements were also optimized.

2. Materials and Methods

2.1. Poly(γ-glutamic acid). The poly(γ-glutamic acid) (γ-PGA) used in this study was a food grade polymer supplied by Meiji Seika Kaisha, Japan. The range of molecular mass was from 800,000 to 1,200,000, and the concentrations (m/m) of the γ-PGA solutions were set as 10%, 20%, 30%, and 40%, respectively.

2.2. Glass Synthesis. Glass of the basic composition of (in wt%) 50 SiO_2, 50 $A1_2O_3$ was synthesized by sol-gel method [15]. The molar ratios of raw materials $Si(OC_2H_5)_4$ (Nacalai tesque, Inc., Kyoto, Japan), $Al(NO_3)_3{\cdot}9H_2O$ (Wako Pure Chemical Industries, Osaka, Japan), C_2H_5OH (Wako Pure Chemical Industries, Osaka, Japan), distilled water, and hydrochloric acid (HCl, Nacalai tesque, Inc., Kyoto, Japan) as a catalyst were maintained at 1 : 1.18 : 10 : 50 : 0.02. The initial sol solutions were divided into two parts. Solution A was the mixture of 0.1 kmol m^{-3} HCl solution, half of the C_2H_5OH, and $Al(NO_3)_3{\cdot}9H_2O$ dissolved in the distilled water. Solution B contained $Si(OC_2H_5)_4$ and the remaining C_2H_5OH and was stirred with a magnetic stirrer for 1 h at ambient temperature. Then, solution A was added dropwise to the continuous stirring solution B; the totally mixed solution was stirred for another hour and then moved into an 358 K drying oven standing for 3 days. The gel was grinded and calcined in an electrically heated furnace in an air atmosphere at 1073 K for 2 h, where the heating rate was controlled at 5 K/min. The glass powders passed through a <45 μm mesh sieve were adopted to prepare the filler of the cements.

2.3. Cement Preparation. Cement pastes were obtained by homogeneous mixing of glass powders with different concentration of γ-PGA solution and 10% m/m (+) tartaric acid (Wako Pure Chemical Industries, Osaka, Japan) solution on a glass slab with a spatula. The mixing ratios of powder/liquid (P/L, g/g) were increased from l : l to 2 : 1, 0.25 as an interval, and the liquid was the combination of γ-PGA and (+) tartaric acid solution. The pastes packed into the cylindrical poly(meth acrylic) molds were allowed to set and aged at 310 K in an incubator with a relative humidity (RH) of 98%.

2.4. Mechanical Strength Measurement. The mechanical strength of cements was assessed by the diametral tensile strength (DTS). The samples removed from the molds (8 mm in diameter, 4 mm in height) were applied to DTS measurement after 3 days of aging. Before the DTS testing, the diameter and length of each specimen need to be remeasured with a micrometer. The samples were crushed in diametrical direction at a crosshead speed of 1 mm/min using a computer-controlled Universal Testing Machine (Autograph AG-1, Shimadzu Co., Kyoto, Japan). The DTS values can be calculated by an equation: DTS = $2P/\pi DL$, where P is the maximum applied load recorded at the fracture and D and L are the diameter and length of the sample, respectively. The DTS shown in the figure were average values of 10 specimens, and the bars represented standard deviation.

2.5. Incubation in Simulated Body Fluid. The simulated body fluid (SBF) was prepared by dissolving reagents of NaCl, $NaHCO_3$, KCl, $K_2HPO_4{\cdot}3H_2O$, $MgCl_2{\cdot}6H_2O$, $CaCl_2$, and Na_2SO_4 in ultrapure water with stirring constantly and buffering at pH 7.40 with tris(hydroxymethyl)aminomethane ($(CH_2OH)_3CNH_2$) and an appropriate volume of 0.1 kmol m^{-3} HCl solution; all reagents were supplied by Nacalai tesque, Inc., Kyoto, Japan, and the details about SBF preparation were described in the literature [16]. The final composition was Na^+ 142.0, K^+ 5.0, Mg^{2+} 1.5, Ca^{2+} 2.5, Cl^- 147.8, HCO_3^- 4.2, HPO_4^{2-} 1.0 and SO_4^{2-} 0.5 in mol m^{-3}, which is nearly equal to that of human blood plasma [17].

The aged cements with the highest mechanical strength were chosen for SBF trial to evaluate the bioactivity in terms of the changes on surface structure and morphology. The cylindrical specimens with dimensions of ϕ8 mm × 4 mm stored in the plastic containers filled with 30 mL SBF were incubated at 310 K. After 7 days of immersion, the samples were removed, rinsed with distilled water, and dried at room temperature.

2.6. Characterization. The X-ray powder diffraction patterns were performed by thin-film X-ray diffractometer (TF-XRD; MXP3V, MAC Science Ltd., Yokohama, Japan) operated at 40 kV and 30 mA using CuKα as a radiation; the angle of the incident beam was anchored as 1° against the specimen

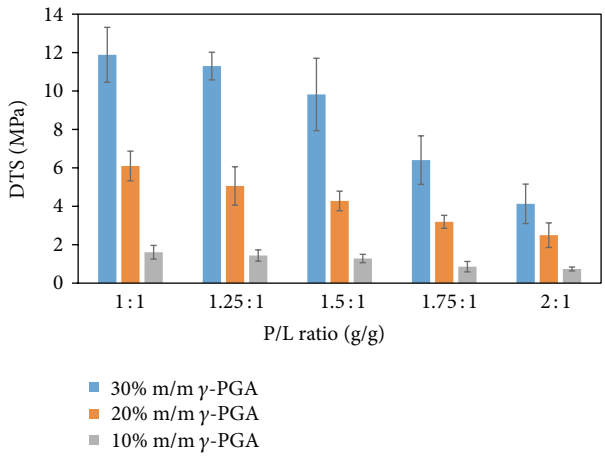

Figure 2: Diametral tensile strength as a function of preparation parameters of the mixing P/L ratio and the concentration of γ-PGA.

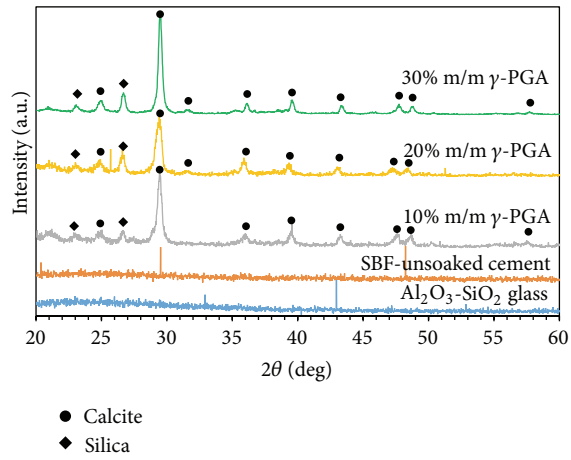

Figure 3: TF-XRD patterns of the surface of SBF-unsoaked cement prepared by the γ-PGA concentration of 10% m/m and P/L ratio of 1 : 1, the Al_2O_3-SiO_2 glass powders, and the surfaces of cements prepared by different concentration of γ-PGA solution using P/L ratio of 1 : 1, after soaking in SBF for 7 days.

surface, and the record was using a step scanning mode with steps at 0.02° steps and 1 s. All samples were scanned from 20° to 60° in 2θ (where θ is the Bragg angle). Surface morphological features of the SBF-soaked cements were examined by scanning electron microscope (SEM; S-3500N, Hitachi High-Technologies, Tokyo, Japan) using energy-dispersive X-ray microanalyzer (EDX; EMAX Energy, Horiba Ltd., Kyoto, Japan) after sputter coating a thin film of gold on them.

3. Results and Discussion

Solid specimens stable in SBF were obtained at 10 to 30% of γ-PGA. Rough setting time of the cements was about 1 hour. When the concentration was increased up to 40%, a tendency to gelation was found in this γ-PGA solution, and the high viscosity created difficulties in the stage of measuring the amount of liquid phase and mixing the cement paste.

Figure 2 summaries the DTS values of the cement specimens using P/L ratio of 1 : 1 to 2 : 1 and the γ-PGA concentration of 10% to 30% after 3 days of aging. The highest strength (11.88 ± 1.43 MPa) was obtained with the P/L ratio of 1 : 1 and the 30% m/m γ-PGA solution. It was clearly found that the preparation parameters produced significant variation on the DTS. The deterioration of DTS was following the increase of P/L ratio, and this change trend was consistent at various concentrations of the γ-PGA solution. In addition, the increase of the concentration of γ-PGA brought about apparent increase in DTS under the same P/L ratio.

When the glass powders are mixed together with the liquid, Al^{3+} ions are released from the surface of glass particles by acid attack and then leached into the aqueous medium. The leached ions bind with the polyanion chains via the carboxyl groups to precipitate a hard polycarboxylic salts gel [18–20]. The set cement consists of unreacted glass particles with a surrounding siliceous hydrogel bound together by a matrix of polyanions cross-linked by ionic bridges [21]. In the cement components, the hydrated salts composed of aluminum ion and polymer were the dominant phase in determining the mechanical strength. Enhancement of physical properties can be attributed to the increase in the amount of ionic cross-links between Al^{3+} and polymer chains [22].

In this Al_2O_3-SiO_2 glass/γ-PGA cement, increasing concentration of γ-PGA manifested the increase in the amount of polymer chains. In addition, boosting the acidity of liquid forced more Al^{3+} ions to be released from particles. The increased polymer chains and Al^{3+} ions were sources of ionic cross-links, which implied that more aluminum polymer salts would be formed to improve the mechanical properties. Similarly, in the case of a limited content of liquid, excessive powders did not produce more ionic cross-links. Consequently, they brought about the decline in the proportion of polymer salts which resulted in the deterioration of mechanical strength, as shown in the results of DTS.

Measured maximum DTS value of the present cements is about 70% of the commercially available GICs [23]. It is reported that mechanical properties can be improved by the addition of polymer with high molecular weight [24]. Enhancement of the mechanical properties should be attempted through control in component and composition in future research.

The TF-XRD patterns of the surface of SBF-unsoaked cement, the Al_2O_3-SiO_2 glass powders, and the surfaces of cements after soaking in SBF for 7 days are depicted in Figure 3. No crystalline peaks except a broad band centered at $2\theta = 22.8°$ which is the characteristic of amorphous SiO_2 (JCPDS Card no. 29-0085) were observed, meaning that the Al_2O_3-SiO_2 glass still maintained noncrystalline structure without forming any precipitations even after soaking in SBF. The SBF-unsoaked specimen was prepared by the γ-PGA concentration of 10% m/m and P/L ratio of 1 : 1; the TF-XRD pattern is similar to Al_2O_3-SiO_2 glass's, which indicated that the powders were the main component in the cement and no crystalline phase was created during the setting and

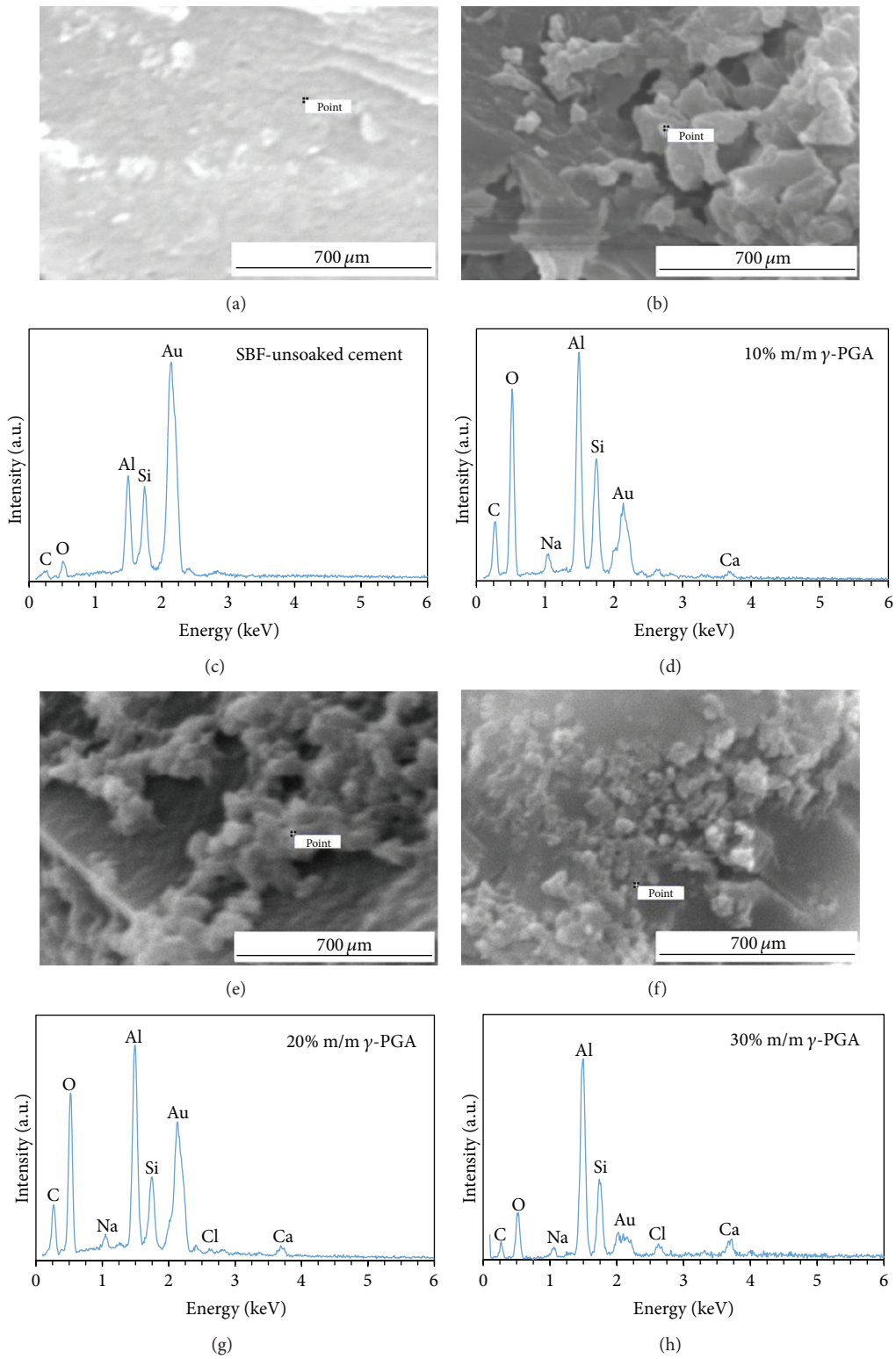

Figure 4: SEM micrographs and EDX spectra of the SBF-unsoaked cement surface, the deposits precipitating on the surfaces of cements, after soaking in SBF for 7 days. PGA concentration of the SBF-unsoaked cement is 10% m/m.

aging process. Moreover, the cements prepared by 20% m/m and 30% m/m γ-PGA solution had almost the same patterns before soaking in SBF. The peaks appearing at about 23.1°, 29.5°, 36.0°, 39.4°, 43.1°, 47.7°, and 48.6° in 2θ on the diffraction pattern of cements surfaces were assigned to a diffraction envelope of (102), (104), (110), (113), (202), (018), and (116) that resulted from the calcite (JCPDS Card no. 05-0586). Besides the calcite as main phase, the peaks assigned to the low-crystalline silica (JCPDS Card no. 33-1161) were also detected. The rest peaks were still unknown. The TF-XRD patterns of cements have illustrated that a chemical compound was deposited on the surfaces of cements irrespective of the concentration of γ-PGA, after soaking in SBF.

Figure 4 shows SEM micrographs combined with EDX spectra of the SBF-unsoaked cement surface and deposits. Except the elements of cement itself, no other substances were discovered on the surface of SBF-unsoaked cement according to the EDX spectra, which made it look flat and smooth in SEM micrograph. These deposits looked like spherical particles, and the range of size was from 0.5 μm and up, most of them agglomerated with each other into larger particles and precipitated on the surface of the cement. It was more obvious in the micrograph of the cement prepared by 20% m/m γ-PGA solution. The Ca peaks were detected in EDX spectra; it was an evidence that the deposits were calcium-containing compound, and the specific phase was confirmed by the TF-XRD results of cements. Besides, the amount and the size of deposits in micrographs and the intensity of calcium peak in spectra seemed not to increase with the increase in the concentration of γ-PGA.

The bioactive materials achieve the osteoconduction which is considered as a chemical attaching to bone by the formation of a biologically active apatite layer on their surfaces via chemical reactions with the surrounding body fluid [25]. This bioactive layer can prevent the materials being encapsulated by tissues then isolated from the bone [26]. The nucleation of the apatite layer is initialed by specific functional groups such as Si-OH [27], Ti-OH [28], carboxyl group (–COOH), and phosphate group [29, 30] derived from the surface of the materials. In this study, the Si-OH groups were the main constituents of a siliceous hydrogel surrounding the glass particles; the carboxyl groups may come from the unreacted γ-PGA; both of them would be ideal sites to induce the Ca^{2+} ions precipitating on the surface of the cements.

However, unlike the commercial bioactive ceramics, the precipitates were assigned as the calcite instead of the apatite. No precipitations were formed in the Al_2O_3-SiO_2 glass filler itself even after soaking in SBF (see Figure 3). This means that the combination of the glass with γ-PGA and tartaric acid would produce preferable condition for the calcite precipitation. It is known that γ-PGA has high potential to adsorb Ca^{2+}. It is therefore assumed that the mixture of γ-PGA and other components of the cements may adsorb a lot of Ca^{2+} to produce the surface able to favorably deposit the calcite, unlike the pure γ-PGA able to deposit the calcium phosphate. The detailed mechanism on this result should be investigated in the next research.

The calcite is also considered as bioresorbable biomaterial applied in drug delivery [31]. In addition, it is reported that not only the apatite, but also the calcite can bond to rabbit tibia, although apatite layer formation in the body is not observed unlike typical bioactive materials [32]. On the basis of the report, the prepared GIC may also exhibit bioactivity.

4. Conclusions

The glass ionomer cements have been successfully attempted by using glass powders of 50 wt% SiO_2-50 wt% Al_2O_3 composition mixed with γ-PGA solution. Increasing the concentration of γ-PGA or decreasing the P/L ratio can enhance the cross-linking degree of acidic polymers and the proportion of aluminum polymer salts in cements; both are key roles in determining the mechanical properties. The cement prepared by the P/L ratio (g/g) of 1 : 1 and the γ-PGA concentration of 30% m/m exhibited the highest diametral tensile strength (11.88 ± 1.43 MPa) after aging for 3 days. The calcite phase was deposited on the surface after 7 days of immersion in SBF, meaning that this Al_2O_3-SiO_2 glass/γ-PGA cement may own the bioactivity. Based on the diametral tensile strength and bioactivity testing result, the γ-PGA can be chosen as another alternative polyalkenoic acid in the preparation of glass ionomer cement.

Conflict of Interests

The present authors declare no conflict of interests related to this paper.

Acknowledgment

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References

[1] A. D. Wilson and B. E. Kent, "A new translucent cement for dentistry. The glass ionomer cement," *British Dental Journal*, vol. 132, no. 4, pp. 133–135, 1972.

[2] I. M. Brook and P. V. Hatton, "Glass-ionomers: bioactive implant materials," *Biomaterials*, vol. 19, no. 6, pp. 565–571, 1998.

[3] R. T. Ramsden, R. C. D. Herdman, and R. H. Lye, "Ionomeric bone cement in neuro-otological surgery," *Journal of Laryngology and Otology*, vol. 106, no. 11, pp. 949–953, 1992.

[4] J. W. McLean, "Glass-ionomer cements," *British Dental Journal*, vol. 164, no. 9, pp. 293–300, 1988.

[5] R. G. Hill and A. D. Wilson, "Some structural aspects of glasses used in ionomer cements," *Glass Technology*, vol. 29, no. 4, pp. 150–159, 1988.

[6] J. W. Nicholson, "Chemistry of glass-ionomer cements: a review," *Biomaterials*, vol. 19, no. 6, pp. 485–494, 1998.

[7] M. J. Tyas and M. F. Burrow, "Adhesive restorative materials: a review," *Australian Dental Journal*, vol. 49, no. 3, pp. 112–121, 2004.

[8] T. Kokubo, H. Kim, and M. Kawashita, "Novel bioactive materials with different mechanical properties," *Biomaterials*, vol. 24, no. 13, pp. 2161–2175, 2003.

[9] M. Kamitakahara, M. Kawashita, T. Kokubo, and T. Nakamura, "Effect of polyacrylic acid on the apatite formation of a bioactive ceramic in a simulated body fluid: fundamental examination of the possibility of obtaining bioactive glass-ionomer cements for orthopaedic use," *Biomaterials*, vol. 22, no. 23, pp. 3191–3196, 2001.

[10] M. Kunioka, "Biosynthesis and chemical reactions of poly(amino acid)s from microorganisms," *Applied Microbiology and Biotechnology*, vol. 47, no. 5, pp. 469–475, 1997.

[11] K. Hoste, E. Schacht, and L. Seymour, "New derivatives of polyglutamic acid as drug carrier systems," *Journal of Controlled Release*, vol. 64, no. 1–3, pp. 53–61, 2000.

[12] H. J. C. Hyuk Joon Choi and M. Kunioka, "Preparation conditions and swelling equilibria of hydrogel prepared by γ-irradiation from microbial poly(γ-glutamic acid)," *Radiation Physics and Chemistry*, vol. 46, no. 2, pp. 175–179, 1995.

[13] A. Sugino, T. Miyazaki, and C. Ohtsuki, "Apatite-forming ability of polyglutamic acid hydrogels in a body-simulating environment," *Journal of Materials Science: Materials in Medicine*, vol. 19, no. 6, pp. 2269–2274, 2008.

[14] A. S. Ledezma-Pérez, J. Romero-García, G. Vargas-Gutiérrez, and E. Arias-Marín, "Cement formation by microbial poly(γ-glutamic acid) and fluoroalumino-silicate glass," *Materials Letters*, vol. 59, no. 24-25, pp. 3188–3191, 2005.

[15] M. Taira and M. Yamaki, "Preparation of SiO_2,-Al_2O_3 glass powders by the sol-gel process for dental applications," *Journal of Materials Science: Materials in Medicine*, vol. 6, no. 4, pp. 197–200, 1995.

[16] T. Kokubo and H. Takadama, "How useful is SBF in predicting *in vivo* bone bioactivity?" *Biomaterials*, vol. 27, no. 15, pp. 2907–2915, 2006.

[17] T. Kokubo, S. Ito, Z. T. Huang et al., "Ca, P-rich layer formed on high-strength bioactive glass-ceramic A—W," *Journal of Biomedical Materials Research*, vol. 24, no. 3, pp. 331–343, 1990.

[18] S. Crisp and A. D. Wilson, "Reactions in glass ionomer cements: I. Decomposition of the powder," *Journal of Dental Research*, vol. 53, no. 6, pp. 1408–1413, 1974.

[19] S. Crisp, M. A. Pringuer, D. Wardleworth, and A. D. Wilson, "Reactions in glass ionomer cements: II. An infrared spectroscopic study," *Journal of Dental Research*, vol. 53, no. 6, pp. 1414–1419, 1974.

[20] S. Crisp and A. D. Wilson, "Reactions in glass ionomer cements: III. The precipitation reaction," *Journal of Dental Research*, vol. 53, no. 6, pp. 1420–1424, 1974.

[21] V. H. W. Khouw-Liu, H. M. Anstice, and G. J. Pearson, "An *in vitro* investigation of a poly(vinyl phosphonic acid) based cement with four conventional glass-ionomer cements. Part 1: flexural strength and fluoride release," *Journal of Dentistry*, vol. 27, no. 5, pp. 351–357, 1999.

[22] S. Crisp, B. G. Lewis, and A. D. Wilson, "Characterization of glass-ionomer cements 1. Long term hardness and compressive strength," *Journal of Dentistry*, vol. 4, no. 4, pp. 162–166, 1976.

[23] D. Xie, W. A. Brantley, B. M. Culbertson, and G. Wang, "Mechanical properties and microstructures of glass-ionomer cements," *Dental Materials*, vol. 16, no. 2, pp. 129–138, 2000.

[24] A. H. Dowling and G. J. P. Fleming, "Can poly(acrylic) acid molecular weight mixtures improve the compressive fracture strength and elastic modulus of a glass-ionomer restorative?" *Dental Materials*, vol. 27, no. 11, pp. 1170–1179, 2011.

[25] D. Arcos, I. Izquierdo-Barba, and M. Vallet-Regí, "Promising trends of bioceramics in the biomaterials field," *Journal of Materials Science: Materials in Medicine*, vol. 20, no. 2, pp. 447–455, 2009.

[26] L. L. Hench, "Bioceramics," *Journal of the American Ceramic Society*, vol. 81, no. 7, pp. 1705–1727, 1998.

[27] P. Li, C. Ohtsuki, T. Kokubo, K. Nakanishi, N. Soga, and K. de Groot, "The role of hydrated silica, titania, and alumina in inducing apatite on implants," *Journal of Biomedical Materials Research*, vol. 28, no. 1, pp. 7–15, 1994.

[28] M. Uchida, H. Kim, T. Kokubo, S. Fujibayashi, and T. Nakamura, "Structural dependence of apatite formation on titania gels in a simulated body fluid," *Journal of Biomedical Materials Research A*, vol. 64, no. 1, pp. 164–170, 2003.

[29] M. Tanahashi and T. Matsuda, "Surface functional group dependence on apatite formation on self-assembled monolayers in a simulated body fluid," *Journal of Biomedical Materials Research A*, vol. 24, no. 2, pp. 305–315, 1997.

[30] T. Miyazaki, C. Ohtsuki, Y. Akioka et al., "Apatite deposition on polyamide films containing carboxyl group in a biomimetic solution," *Journal of Materials Science: Materials in Medicine*, vol. 14, no. 7, pp. 569–574, 2003.

[31] J. Wang, J. Chen, J. Zong et al., "Calcium carbonate/carboxymethyl chitosan hybrid microspheres and nanospheres for drug delivery," *Journal of Physical Chemistry C*, vol. 114, no. 44, pp. 18940–18945, 2010.

[32] Y. Fujita, T. Yamamuro, T. Nakamura, S. Kotani, C. Ohtsuki, and T. Kokubo, "The bonding behavior of calcite to bone," *Journal of Biomedical Materials Research*, vol. 25, no. 8, pp. 991–1003, 1991.