Synthesis of Bioactive HEMA–MPS–CaCl$_2$ Hybrid Gels: Effects of Catalysts in the Sol-Gel Processing on Mechanical Properties and *in vitro* Hydroxyapatite Formation in a Simulated Body Fluid

TOMOHIRO UCHINO*, CHIKARA OHTSUKI

Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya

464-8603, Japan

MASANOBU KAMITAKAHARA

Graduate School of Environmental Studies, Tohoku University, 6-6-20 Aoba, Aramaki,

Aoba-ku, Sendai 980-8579, Japan

TOSHIKI MIYAZAKI

Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology,

2-4 Hibikino, Wakamatsu-ku, Kitakyushu 808-0196, Japan

SATOSHI HAYAKAWA, AKIYOSHI OSAKA

Graduate School of Natural Science and Technology, Okayama University, Tsushima,

Okayama 700-8530, Japan

Corresponding author:

TOMOHIRO UCHINO

**Phone: +81-52-789-3346, Fax +81-52-789-3182

***E-mail: h046106d@mbox.nagoya-u.ac.jp

* TOMOHIRO UCHINO

Affiliation: Faculty of Pharmacy, Research institute of Pharmaceutical Sciences, Musashino

University,1-1-20 Shinmachi, Nishitokyo-shi,Tokyo 202-8585, Japan

**Present numbers

Phone and Fax: +81-42-468-8679

***Present E-mail address

E-mail: t_uchino@musashino-u.ac.jp

Abstract

We investigated synthetic conditions for the fabrication of bioactive hybrid gels from monomers of 2-hydroxyethylmethacrylate (HEMA) and 3-methacryloxypropyltrimethoxysilane (MPS) in combination with $CaCl_2$, at a starting molar ratio of HEMA:MPS:$CaCl_2$ of 9:1:1. Hydroxyapatite formation, essential to show bone bonding, was observed on the HEMA–MPS–$CaCl_2$ hybrid gels with the added catalysts NH_3 or HCl with a molar ratio to MPS of 0.1, but not on the hybrid gel with HCl at a molar ratio to MPS of 1. The mechanical properties of the gels were dependent on the catalysts, which may affect the microstructures that develop during sol-gel processing.

KEY WORDS: bone bonding, sol-gel processing, hydroxyapatite, simulated body fluid, mechanical property

INTRODUCTION

Bioactive ceramics such as Bioglass[®] [1], glass-ceramic A–W [2], and sintered hydroxyapatite [3,4] are attractive as bone substitutes because they spontaneously bond to living bone, i.e., to show bone-bonding property after implantation in bony defects [5]. The direct bonding of the bioactive ceramics to living bone leads tight fixation of the implants after long-term implantation. Bone formation on bioactive ceramics is often called osteoconduction. Osteoconduction is a useful property for scaffolds for bone tissue regeneration. Therefore, bioactive ceramics have received much attention with regard to their application in bone-tissue engineering and as bone substitutes. However, their application remains limited because of their brittleness and low flexibility. Bone is regarded as an organic–inorganic hybrid composed of hydroxyapatite and collagen fibers. Therefore, one of the important design features required of bioactive bone substitutes is of an organic–inorganic hybrid showing not only bone-bonding properties but also flexibility so as to achieve a lower Young's modulus than conventional bioactive ceramics.

Recently, organic–inorganic hybrids with bone-bonding properties were proposed based on the fundamental understanding of the bone-bonding mechanisms of bioactive ceramics [6,7]. Previous reports have already revealed that the essential requirement for artificial

materials to show bone-bonding properties is the formation of a bone-like hydroxyapatite layer on their surface in the body environment [8,9]. Hydroxyapatite formation ability can be evaluated *in vitro* using a simulated body fluid (SBF) that has a composition similar in inorganic composition to body fluid, and formulated according to Kokubo *et al.* Previous research on the formation of hydroxyapatite layers on calcium silicate binary glasses in SBF has shown that hydroxyapatite formation was triggered not only by the release of calcium ions from the glass, but also by the formation of silanol (Si-OH) groups that may induce heterogeneous nucleation of hydroxyapatite on the glasses [10,11]. This means that organic modification of these chemical species, i.e., Si-OH and Ca^{2+}, may lead to bioactive organic–inorganic hybrids. Based on this idea, Miyazaki *et al.* developed hydrophilic organic–inorganic hybrid gels starting from 2-hydroxyethylmethacrylate (HEMA) by modification with 3-methacryloxypropyltrimethoxysilane (MPS) and calcium chloride ($CaCl_2$) as a model of bioactive organic–inorganic hybrid gels. In addition to their flexibility, the HEMA–MPS–$CaCl_2$ hybrid gels showed hydroxyapatite formation ability in SBF [12]. The design of HEMA–MPS–$CaCl_2$ hybrid gels is therefore a typical example of a hydrophilic polymer with bioactivity, i.e., bone-bonding properties. In the molecular structure of HEMA–MPS–$CaCl_2$ hybrid gels, the MPS leads to the formation of Si-OH groups through

hydrolysis after exposure to ambient atmosphere or to aqueous solutions such as SBF. Moreover, MPS acts as a cross-linker because of the formation of siloxane (Si-O-Si) bonds after hydrolysis and polycondensation. However the hydrolysis and polycondensations of alkoxysilane were markedly affected by catalysts used during the sol-gel process [13]. Therefore, the behavior of silanol groups is expected to have an important contributions to determining not only the apatite formation on the organic–inorganic hybrid but also their mechanical strength. Hydrolysis and polycondensation during the sol-gel processing are generally accelerated by the presence of catalysts such as HCl and NH_4OH. However, the effects of these catalysts during the sol-gel process on hydroxyapatite formation in SBF and mechanical properties have been not clarified in HEMA–MPS–$CaCl_2$ hybrid gels. In this study, HEMA–MPS–$CaCl_2$ hybrid gels were synthesized using different catalytic conditions in which aqueous hydrochloric acid (HCl) or ammonia (NH_3) solutions were used during the hydrolysis and polycondensation of the gels. The hydroxyapatite formation in SBF of the gels prepared with different catalysts was examined. Their mechanical properties were also evaluated by tensile test. The differences in mechanical properties were also examined with respect to the microstructure of the prepared gels after the measurement of ^{29}Si-NMR spectra.

MATERIALS AND METHODS

Preparation of HEMA–MPS–CaCl$_2$ Hybrid Gels

HEMA–MPS–CaCl$_2$ hybrid gels were prepared by following previous reports [12].

Reagent grade HEMA (CH$_2$=C(CH$_3$)COO(CH$_2$)$_2$OH, Wako Pure Chemical Industries, Ltd.,

Japan) and MPS (CH$_2$=C(CH$_3$)COO(CH$_2$)$_3$Si(OCH$_3$)$_3$, Chisso Corporation, Japan) were used

as starting reagents without further purification. HEMA and MPS in a molar ratio of 9:1 were

dissolved in ethanol, and 100 cm^3 of the solution at a total concentration of 1 mol·dm^{-3} was

prepared. The solution was heated at 75°C for three hours with 0.001 mol benzoylperoxide

(BPO) as an initiator of the polymerization of HEMA and MPS. The obtained polymer

solution was then mixed with 20 cm^3 of ethanol solution containing 0.01 mol calcium

chloride (CaCl$_2$, Nacalai Tesque, Inc., Japan). Some of the resultant solutions were cast in

polypropylene containers and dried until the weight loss became less than 2% in 24 hours.

These dried gels are referred to as Hybrid N (No-catalyst-added hybrid). In addition, aqueous

HCl or NH$_3$ solutions were added to some of the resultant solution as an acid or base catalyst,

respectively. The solutions with a catalyst were cast in sealed polypropylene containers until

the solutions gelled. After gelation, the containers were opened, and the solutions were dried

for more than one week until the weight loss became less than 2% in 24 hours. Hybrids were

prepared with 0.01 or 0.001 mol of HCl (acid catalyst) or 0.01 mol of NH$_3$ (base catalyst) and

applicable amount of water, that is, 1 mol·dm^{-3} and 0.1 mol·dm^{-3} HCl aqueous solution and 0.1

mol·dm^{-3} aqueous NH$_3$ solution were added, and are referred to as Hybrid A1, Hybrid A0.1,

and Hybrid B0.1, respectively. The starting compositions of these hybrids are given in Table

1.

Evaluation of Hydroxyapatite Formation in SBF

The hydroxyapatite formation on the hybrid gels was evaluated by *in vitro* examination

using SBF, as proposed by Kokubo *et al.* [14-16]. SBF was prepared by dissolving reagent

grade NaCl, NaHCO$_3$, K$_2$HPO$_4$·3H$_2$O, MgCl$_2$·6H$_2$O, CaCl$_2$, and Na$_2$SO$_4$ (Nacalai Tesque, Inc.,

Japan) in ultra-pure water, with the detailed composition given in Table 2. The solution was

buffered at pH 7.25 with an appropriate amount of tris(hydroxymethyl)aminomethane

((CH$_2$OH)$_3$CNH$_2$, Nacalai Tesque, Inc., Japan) and HCl at 36.5°C. A rectangular specimen

10×10×1 mm^3 in size was cut from the hybrids and soaked in 35 cm^3 of SBF at pH 7.25 at

36.5°C for various periods of up to seven days. After soaking for one or seven days, the

specimens were removed from the fluid, gently rinsed with ultra-pure water, and dried at

room temperature.

The surface of the hybrids, before and after soaking in SBF, was analyzed using thin-film

X-ray diffraction (TF-XRD: MXP3V, MAC Science Co., Ltd., Japan). They were also

observed under a scanning electron microscope (SEM: S-3500N, Hitachi Co., Ltd., Japan).

Measurement of Mechanical Properties

Mechanical properties of the prepared hybrid gels were examined by tensile test using a

universal testing machine (Model 5566, Instron, Co., USA) under ambient conditions.

Dumbbell-type specimens in conformity to the JIS K7113 (Type No. 1/small size) were

stamped out using a cutting die. The gauge length was 12.5 mm and the crosshead speed was

5 mm/min. More than seven specimens were tested for each hybrid gel.

Characterization of the HEMA–MPS–CaCl$_2$ Gels

The microstructures of the dried hybrid gels were characterized by Fourier transform

infrared (FT-IR: Spectrum GX, PerkinElmer, Japan) spectroscopy using KBr tablets. The

spectra were obtained at a resolution of 4.0 cm^{-1} with 16 scans. The samples of the prepared

hybrid gels were also characterized by solid-state ^{29}Si cross-polarization/magic-angle

spinning NMR (^{29}Si CP-MAS NMR: ^{UNITY}INOVA300, Varian, Inc., USA). The samples were

placed in a zirconia sample tube. The ^{29}Si CP-MAS NMR spectra (59.6 MHz) were obtained

using a sample spinning rate at the magic angle to the magnetic field of 3.5 kHz, 2.5 ms

contact time, 5.0 μs pulse length (pulse angle, π/2), 5 s recycle delays and 10 μs dead time.

The ^{29}Si NMR chemical shifts were reported in ppm, referenced to polydimethylsilane

(PDMS: δ =-34.0 ppm against tetramethylsilane: δ = 0 ppm) as the secondary external

reference.

RESULTS

Transparent monolithic bulk gels were obtained regardless of the addition of a catalyst

such as HCl and NH_3 aqueous solutions. The IR spectra of the prepared HEMA–MPS–$CaCl_2$

hybrid gels are shown in Figure 1. The peak at 950 cm^{-1}, assigned to Si-OH groups [17], was

detected in all the IR spectra, irrespective of the preparation conditions: all specimens had

similar formation of Si-OH groups. The samples prepared without a catalyst (Hybrid N) also

formed Si-OH groups, which seemed to result from reaction with water in the ambient

environment. From the IR spectra of the synthesized hybrid gels, there were no significant

detectable differences between the specimens, with the gels prepared through the sol-gel

process all having Si-OH groups and Si-O-Si bonds.

All specimens were soaked in SBF for periods of one or seven days. According to

previous studies, apatite formation can be observed from the formation of particles on the specimens [6,12,18,19]. Figure 2 shows SEM photographs of the hybrids before and after soaking in SBF for one and seven days. After soaking for one day, spherical particles had formed on the surface of Hybrid N, Hybrid A0.1, and Hybrid B0.1. The particles grew in size during the seven days of immersion. In contrast, no surface change was observed on the surface of Hybrid A1, with no spherical particles formed after soaking in SBF for seven days. This indicates that the Hybrid N, Hybrid A0.1, and Hybrid B0.1 specimens have the potential to deposit hydroxyapatite in SBF, indicating that these specimens have the potential bone-bonding property of osteoconduction, and that after implantation in bony defects the formation of a hydroxyapatite layer will occur. The formation of low-crystalline hydroxyapatite was confirmed by thin-film X-ray diffraction (TF-XRD), as shown in Figure 3. The TF-XRD patterns of the surfaces of the hybrids indicate diffraction about both 26° and 32°, assigned to hydroxyapatite, for the Hybrid N, Hybrid A0.1, and Hybrid B0.1 specimens after soaking in SBF for one and seven days. The peaks at $2\theta = 32°$ are broad and are regarded as indicating low-crystalline hydroxyapatite, which has characteristics similar to bone mineral. This means that so-called bone-like apatite was deposited on the Hybrid N, Hybrid A0.1, and Hybrid B0.1 specimens. In contrast, these peaks assigned to hydroxyapatite

were not detected for Hybrid A1 soaked in SBF for seven days. These results indicate that Hybrid N, Hybrid A0.1, and Hybrid B0.1 have higher potential for bone-like apatite formation in SBF than does Hybrid A1.

The three types of hybrid—Hybrid N, Hybrid A0.1, and Hybrid B0.1—that had the ability to form bone-like apatite in SBF within seven days were then mechanically tested. Typical stress-strain curves of the three types of the examined hybrid gels are shown in Figure 4. It is clear that Hybrid B0.1 has a higher tensile strength at breakage and a higher elastic modulus than do Hybrids N and A0.1. It is interesting that Hybrid A0.1 has a lower strength at breakage than Hybrid N with higher elongation. Therefore, the tensile strength results show the differences among these hybrid gels with the ability to form bone-like apatite. Table 3 summarizes the data of the mechanical properties, showing the average values and standard deviations of the tensile tests of the Hybrid N, Hybrid A0.1, and Hybrid B0.1 specimens. It was clearly shown that both the Young's modulus and tensile strength were increased in the sequence Hybrid A0.1 < Hybrid N < Hybrid B0.1. The strain at failure was increased in the sequence Hybrid B0.1 < Hybrid N < Hybrid A0.1. These evaluations clearly indicate that the addition of catalyst significantly influenced the mechanical properties of the hybrid gels. The catalysts in the sol-gel processing would affect the processes of hydrolysis and

13

polycondensation of the alkoxysilane. Therefore, it is important to evaluate the local structure of the Si in the gels. ^{29}Si CP-MAS NMR spectra of the Hybrid N, Hybrid A0.1, and Hybrid B0.1 samples are shown in Figure 5, in which the assignment of the each T^n unit is also illustrated. The T^n unit denotes a particular R′-Si(-OSi)$_n$(OCH$_3$, OH)$_{3-n}$ (n = 0, 1, 2, and 3) group [20], where R′ is the organic structure of MPS. Although the peak T^0 was reported to appear at -42.0 ppm for monomeric MPS [21], T^0 was not detected in the spectra of all the hybrid gels. Peaks at about -50, -58 and -66 ppm were assigned to units T^1, T^2, and T^3, respectively. The fraction of each T^n unit, obtained from the deconvolution of the peaks with a Gaussian function, is given in Table 4. The fraction of T^3 in Hybrid A0.1 was much higher than that in Hybrid N and the fraction of T^2 in Hybrid A0.1 was the same as that in Hybrid N. T^1 was not detected in Hybrid A0.1. The fraction of T^3 in Hybrid B0.1 was much higher than that in Hybrid N, while the fraction of T^1 in Hybrid B0.1 was a little smaller than that in Hybrid N. The number of ≡Si-O-Si≡ bridging bonds per Si atom was calculated according to the following equation:

$$((\text{Fraction of } T^1) \times 1) + ((\text{Fraction of } T^2) \times 2) + ((\text{Fraction of } T^3) \times 3) \qquad (1)$$

The average numbers of bridging bonds calculated for Hybrids N, A0.1, and B0.1 were 2.01, 2.39, and 2.28, respectively. There were no significant differences between the three types of

gel in the average number of bridging oxygens.

DISCUSSION

The prepared hybrid gels show similar characteristics in their IR spectra, which means that the overall chemical structures of the hybrid gels were similar. Hydrolysis and polycondensation progressed through reaction of the alkoxysilane even when no aqueous solution was added to the synthetic procedure. These reactions proceeded with water supplied from the ambient atmosphere. All the specimens therefore contain siloxane bonds and Si-OH groups. However, the hydroxyapatite formation ability of the prepared hybrid gels was lost following the addition of large amounts of HCl. The results from the evaluation of hydroxyapatite formation on the HEMA–MPS–$CaCl_2$ hybrid gels indicate that the addition of HCl catalysts did not suppress hydroxyapatite formation in SBF when the ratio of HCl to MPS was 0.1 or less. There were no special treatments used to remove residual compounds from the gels produced for this study. Therefore, the high remaining concentration of HCl may retard apatite formation in SBF because the low pH decreases the degree of supersaturation of the surrounding solution with respect to hydroxyapatite. Therefore, a low concentration of the acidic catalysts is preferred in the preparation of bioactive

15

organic–inorganic hybrids with hydroxyapatite formation ability and potential bone-bonding properties.

The tensile properties of the hybrid gels and their stress–strain curves varied with the addition of catalysts. The catalysts were added after polymerization of HEMA and MPS in ethanol solution. A copolymer consisting of HEMA and MPS was produced, followed by hydrolysis and polycondensation of the alkoxysilane groups in the copolymer with water supplied from the atmosphere or from the aqueous catalyst solution. The formation of silanol groups proceeded during the aging and drying processes [22,23]. The silanol groups condensed to form siloxane bonds ($\equiv$Si-O-Si$\equiv$), and to make the cross-links between the HEMA–MPS copolymers. These hydrolysis and condensation reactions were affected by the addition of the catalysts. As seen from the ^{29}Si CP-MAS NMR spectra, the fractions of the R-Si(-OSi)$_3$ structure assigned to T^3 in Hybrids A0.1 and B0.1 were much larger than that in Hybrid N. The average number of bridging bonds in Hybrids A0.1 and B0.1 was also larger than that in Hybrid N. These results indicate that the addition of the catalysts accelerated the hydrolysis and condensation reactions. No T^1 fraction was detected in Hybrid A0.1, while the T^1 fraction in Hybrid B0.1 was detected but was a little smaller than that in Hybrid N. The differences in the T^1 fraction of Hybrids A0.1 and B0.1 may be caused by a difference in their

hydrolysis and condensation reaction mechanisms [23]. In previous studies, it was shown that the mechanism of tetraethoxysilane (TEOS) polycondensation affords the formation of linear or three-dimensional siloxane networks or silica gels [24]. This study indicates that the distribution of cross-link points in the structure will be different between the hybrids with an added acidic or basic catalyst. The cross-link points of the hybrids with a basic catalyst added are considered to be heterogeneously distributed, while the cross-link points of the hybrid with an acid catalyst added are considered to be homogeneously distributed. If the cross-link points are heterogeneously distributed, parts with strong mechanical properties and parts with weak mechanical properties might be fabricated. The strong parts of Hybrid B0.1 were dominant, so the tensile strength and Young's modulus of Hybrid B0.1 were larger than those of Hybrid A0.1, and the strain at failure of Hybrid B0.1 was smaller than that of Hybrid A0.1. Therefore, the mechanical properties of these hybrids may differ when the distribution of cross-link points is different, even though the ratio of the T^n is the same. The addition of a catalyst controls the siloxane networks in the hybrids and controls the mechanical properties and hydroxyapatite-forming ability of the resulting hybrids.

These findings show that the osteoconductivity of hybrids derived from sol-gel is affected by catalysts such as HCl and NH_3. In particular, residual HCl catalyst may

remarkably reduce the potential for hydroxyapatite formation, while lower concentrations of

HCl do not significantly affect hydroxyapatite formation. The type of catalysts also affected

the mechanical performance of the hybrids, because of the routes of hydrolysis in forming the

silanol groups and the subsequent polycondensation to form siloxane networks being

determined by the catalysts. The selection of the catalyst type is now regarded as an important

condition in determining the mechanical properties of bioactive organic–inorganic hybrids

fabricated through sol-gel processes.

CONCLUSIONS

Homogeneous HEMA–MPS–CaCl$_2$ hybrids were prepared by the addition of different

catalysts, and the effects of the catalysts on their biological and mechanical properties were

evaluated. The addition of a catalyst during the synthesis of HEMA–MPS–CaCl$_2$ hybrids

influences the mechanical properties of the resulting organic–inorganic hybrids because of the

different siloxane networks produced. The type of catalyst used also affected the

hydroxyapatite-forming ability. These findings reveal that the addition of a catalyst governs

the mechanical and biological properties of the prepared hybrid because the microstructure of

the resulting hybrids is determined by catalyst.

ACKNOWLEDGMENTS

REFERENCES

1. Hench, L.L., Splinter, R.J., Allen, W.C. and Greenlee, T.K. (1971). Bonding Mechanisms at the Interface of Ceramic Prosthetic Materials, *J. Biomed. Mater. Res. Symp.,* **2** (Part 1): 117-141.

2. Kokubo, T., Shigematsu, M., Nagashima, Y., Tashiro, M., Nakamura, T., Yamamuro T. and Higashi, S. (1982). Apatite- and Wollastonite-Containing Glass-Ceramics for Prosthetic Application, *Bull. Inst. Chem. Res., Kyoto Univ.,* **60**: 260-268.

3. Jarcho, M., Kay, J.F., Gumaer, K.I., Doremus, R.H. and Drobeck, H.P. (1977). Tissue, Cellular and Subcellular Events at a Bone-Ceramic Hydroxyapatite Interface, *J. Bioeng.,* **1**: 79-92.

4. Aoki, H., Kato, K., Ogiso, M. and Tabata, T. (1977). Sintered Hydroxyapatite as a New Dental Implant Materials, *J. Dent. Outlook,* **49**: 567-575.

5. Hench, L.L. and Wilson, J. (1993). Introduction, In: Hench, L.L. and Wilson, J. (eds), *An Introduction to Bioceramics,* pp. 1-24, World Scientific, Singapore.

6. Ohtsuki, C., Miyazaki, T. and Tanihara, M. (2002). Development of Bioactive

Organic–inorganic Hybrid for Bone Substitutes, *Mater. Sci. Eng.: C*, **22**(1): 27-34.

7. Ohtsuki, C., Miyazaki, T., Kamitakahara, M. and Tanihara, M. (2007). Design of Novel Bioactive Materials through Organic Modification of Calcium Silicate, *J. Euro. Ceram. Soc.*, **27**: 1527–1533.

8. Hench, L.L. (1991). Bioceramics: From Concept to Clinic, *J. Am. Ceram. Soc.*, **74**(7): 1487-1510.

9. Neo, M., Nakamura, T, Ohtsuki, C., Kokubo, T. and Yamamuro, T. (1993). Apatite Formation on Three Kinds of Bioactive Material at an Early Stage in vivo: A Comparative Study by Transmission Electron Microscopy, *J. Biomed. Mater. Res.*, **27**: 999-1006.

10. Ohtsuki, C., Kokubo, T. and Yamamuro, T. (1992). Mechanism of Apatite Formation on CaO-SiO$_2$-P$_2$O$_5$ Glasses in a Simulated Body Fluid, *J. Non-Cryst. Solids*, **143**: 84-92.

11. Li, P.J., Ohtsuki, C., Kokubo, T., Nakanishi, K., Soga, N., Nakamura, T. and Yamamuro, T. (1992). Apatite Formation Induced by Silica Gel in a Simulated Body Fluid, *J. Am. Ceram. Soc.*, **75**: 2094-2097.

12. Miyazaki, T., Ohtsuki, C. and Tanihara, M. (2003). Synthesis of Bioactive Organic–inorganic Nanohybrid for Bone Repair through Sol-Gel Processing, *J. Nanosci. Nanotech.*, **3**(6): 511-515.

13. Brinker C.J., and Scherer G.W. (1990). Hydrolysis and condensation II: Silicates. In: *Sol–Gel science*, pp. 97-233, Academic Press, San Diego.

14. Kokubo, T., Kushitani, H., Sakka, S., Kitsugi, T. and Yamamuro, T. (1990). Solutions Able to Reproduce *in vivo* Surface-Structure Changes in Bioactive Glass-Ceramic A-W, *J. Biomed. Mater. Res.*, **24**: 721-734.

15. Cho, S.B., Nakanishi, K., Kokubo, T., Soga, N., Ohtsuki, C., Nakamura, T.,

Kitsugi, T. and Yamamuro, T. (1995). Dependence of Apatite Formation on Silica Gel on Its Structure: Effect of Heat Treatment, *J. Am. Ceram. Soc.*, **78**(7): 1769-1774.

16. Kokubo, T. and Takadama, H. (2006). How Useful is SBF in Predicting in vivo Bone Bioactivity?", *Biomaterials*, **27**(15): 2907-2915.

17. Ou, D.L. and Seddon A.B. (1997). Near- and Mid-Infrared Spectroscopy of Sol-Gel Derived Ormosils: Vinyl and Phenyl Silicates, *J. Non-Cryst. Solids*, **210**(2,3): 187-203.

18. Kim, I.Y., Kawachi, G., Kikuta, K., Cho, S.B, Kamitakahara, M. and Ohtsuki, C. (2008). Preparation of bioactive particles in CaO-SiO$_2$ system through sol-gel processing under coexistence of poly(ethylene glycol), *J. Euro. Ceram. Soc.*, 28, 1595-1602.

19. Ohtsuki, C., Kushitani, H., Kokubo, T., Kotani, S., Yamamuro, T. (1991). Apatite formation on the surface of ceravital-type glass-ceramic in the body, *J Biomed. Mater. Res.* 25, 1363-1370.

20. Delattre, L., Roy, M. and Babonneau, F. (1997). Design of Homogeneous Hybrid Materials through a Careful Control of the Synthetic Procedure, *J. Sol-Gel Sci. Tech.*, **8**: 567-570.

21. Yabuta, T., Tsuru, K., Hayakawa, S., Ohtsuki, C. and Osaka, A. (2000). Synthesis of Bioactive Organic–inorganic Hybrid with γ-Methacryloxypropyltrimethoxysilane, *J. Sol-Gel Sci. Tech.*, **19**: 745-748.

22. Plueddemann E.P. (1991). Chemistry of silane coupling agents, *Silane Coupling Agents*, 2, 31-54, Plenum, New York.

23. Keefeer K.D. (1984). The Effect of Hydrolysis Conditions on the Structure and Growth of Silicate Polymers, In: Brinker C.J., Clark D.E., and Ulrich D.R. (eds),

Better Ceramics through Chemistry, pp.15-25, Elsevier Science Publishing, New York.

24. Brinker C.J., and Scherer G.W. (1990). Structures of Porous Gels: Xerogels and Aerogels. *Sol–Gel Science*,pp.518-546, Academic Press, San Diego.

Table and Figure Captions

Table 1.   Starting compositions of the HEMA–MPS–CaCl$_2$ hybrid gels.

Table 2.   Ion concentrations of human blood plasma and the simulated body
             fluid (SBF) [14].

Table 3.   Mechanical properties of the prepared hybrid gels.

Table 4.   Chemical shift (ppm) and fraction (%) of the T^n siloxane structure in
             the prepared hybrid gels.

Figure 1.   IR spectra of HEMA–MPS hybrids prepared with different catalysts
              without soaking in SBF. (a) wide range (b) narrow range   ●:peaks
              assigned to silanol groups.

Figure 2.   SEM images of the surfaces of HEMA–MPS–CaCl$_2$ hybrid gels,
              before and after soaking in SBF for one and seven days.
              0d: before soaking in SBF.

Figure 3.   Thin-film X-ray diffraction (TF-XRD) patterns of the surfaces of the
              hybrid gels, before and after soaking in SBF for one and seven days.
              0d: before soaking in SBF.
              ○: peaks assigned to hydroxyapatite

Figure 4.   Typical stress–strain curves of the specimens of the prepared hybrid
              gels before soaking in SBF.

Figure 5. ^{29}Si CP-MAS NMR spectra and T^n structures in HEMA–MPS–CaCl$_2$
              hybrid gels.

Table 1. Starting compositions of the HEMA–MPS–CaCl$_2$ hybrid gels.

Sample	Constituents / molar ratio					
	HEMA	MPS	CaCl$_2$	HCl	NH$_3$	H$_2$O
Hybrid N	9	1	1	-	-	-
Hybrid A1	9	1	1	1	-	4
Hybrid A0.1	9	1	1	0.1	-	5
Hybrid B0.1	9	1	1	-	0.1	5

- : No addition

Table 1, UCHINO *et al.*

Table 2. Ion concentrations of human blood plasma and the simulated body fluid (SBF) [15].

Ion	Concentration / mol·m^{-3}	
	Plasma	SBF
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	103.0	147.8
HCO_3^-	27.0	4.2
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5

Table 3. Mechanical properties of the prepared hybrid gels.

Sample	Young's modulus / MPa	Tensile strength / MPa	Strain at failure / %
Hybrid N (n=9)	2.65 ± 0.38	0.68 ± 0.07	44.7 ± 5.4
Hybrid A0.1 (n=6)	1.07 ± 0.09	0.41 ± 0.03	54.2 ± 3.7
Hybrid B0.1 (n=7)	41.8 ± 6.45	2.15 ± 0.37	36.0 ± 10.6

Values: mean ± standard deviation
*: the range from value to value

Table 3, UCHINO *et al.*

Table 4. Chemical shift (ppm) and fraction (%) of T^n siloxane structure in the prepared hybrid gels.

Sample	T^1		T^2		T^3	
	chemical shift / ppm	fraction / %	chemical shift / ppm	fraction / %	chemical shift / ppm	fraction / %
Hybrid N	-49	19.0	-57	61.2	-67	19.8
Hybrid A0.1	No detection	0.0	-57	61.3	-65	38.7
Hybrid B0.1	-51	13.8	-58	44.6	-66	41.6

Figure 1. IR spectra of HEMA–MPS hybrids prepared with different catalysts without soaking in SBF. (a) wide range (b) narrow range ●:peaks assigned to silanol groups
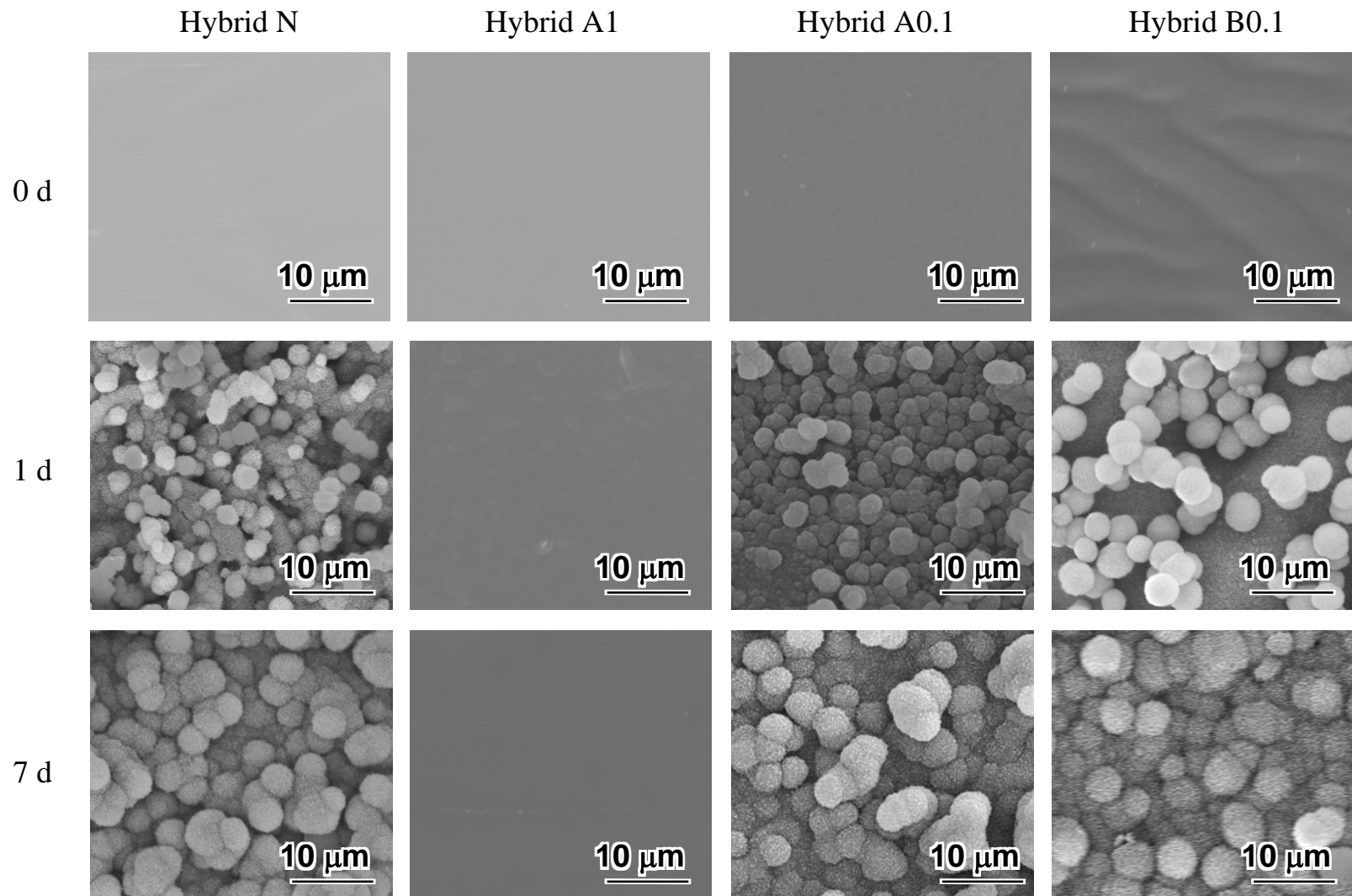
Fig. 1, UCHINO *et al.*

	Hybrid N	Hybrid A1	Hybrid A0.1	Hybrid B0.1

Figure 2. SEM images of the surfaces of HEMA–MPS–CaCl$_2$ hybrid gels, before and after soaking in SBF for 1 and 7 d.   0d: before soaking in SBF.
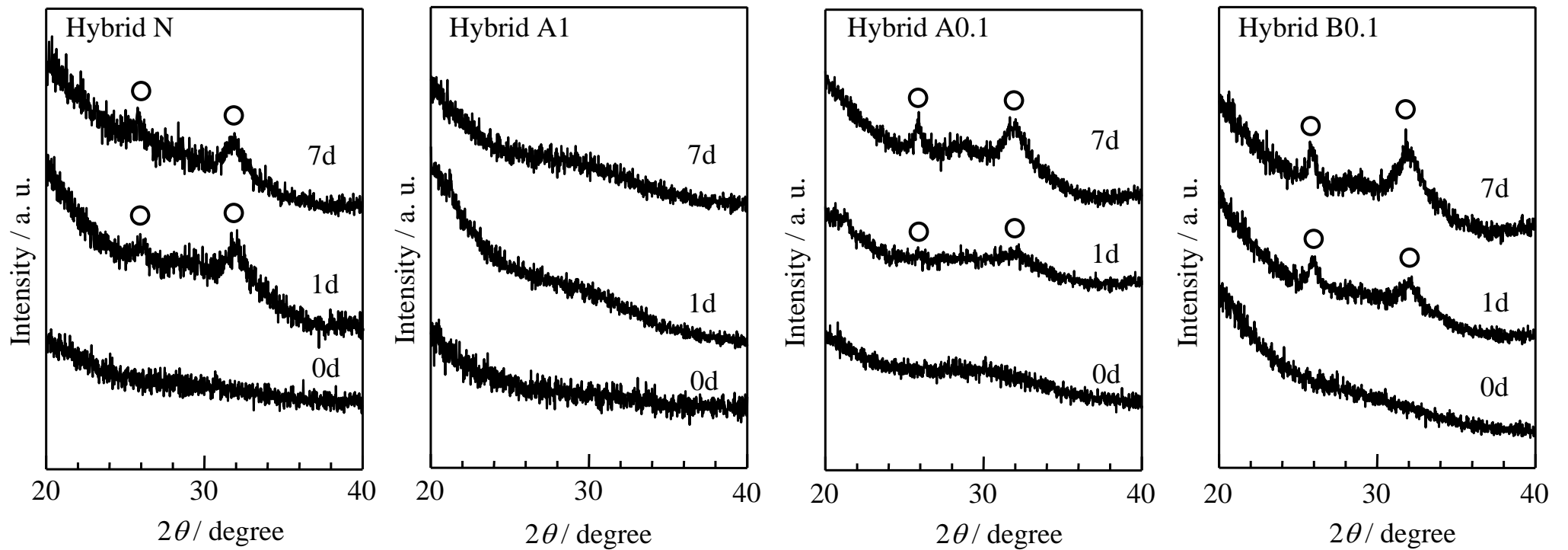
Figure 3. Thin-film X-ray diffraction (TF-XRD) patterns of the surfaces of the hybrid gels, before and after soaking in SBF for 1 and 7 d.
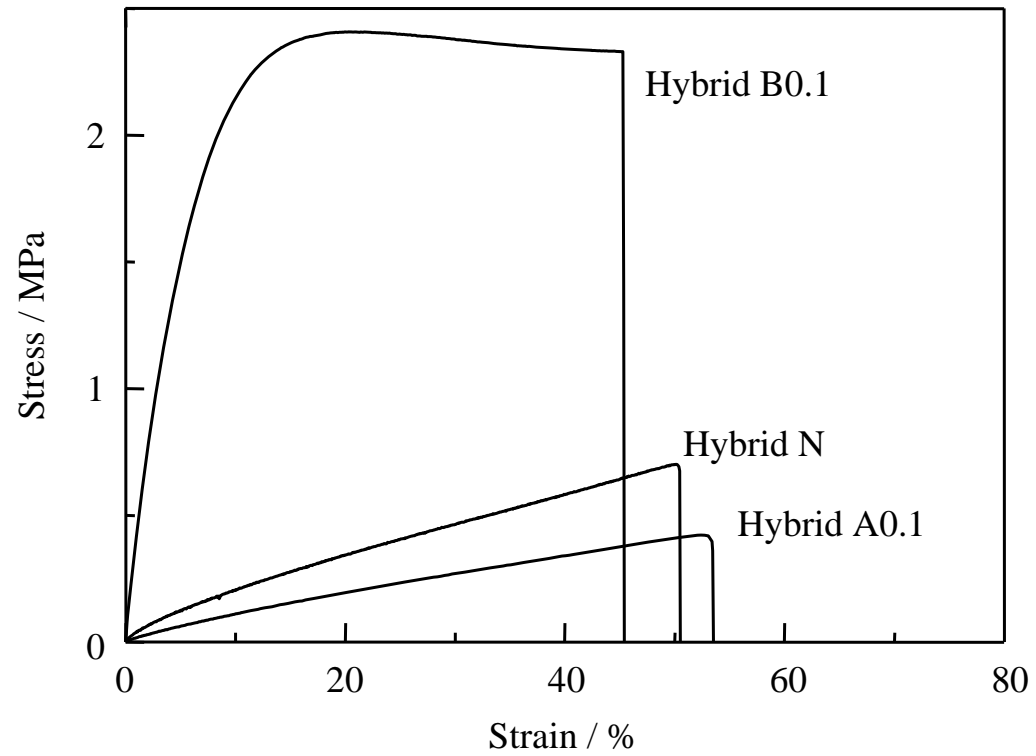0d: before soaking in SBF. ○: peaks assigned to hydroxyapatite

Fig. 3, UCHINO *et al.*

Figure 4. Typical stress-strain curves of the specimens of the prepared hybrid gels before soaking in SBF.
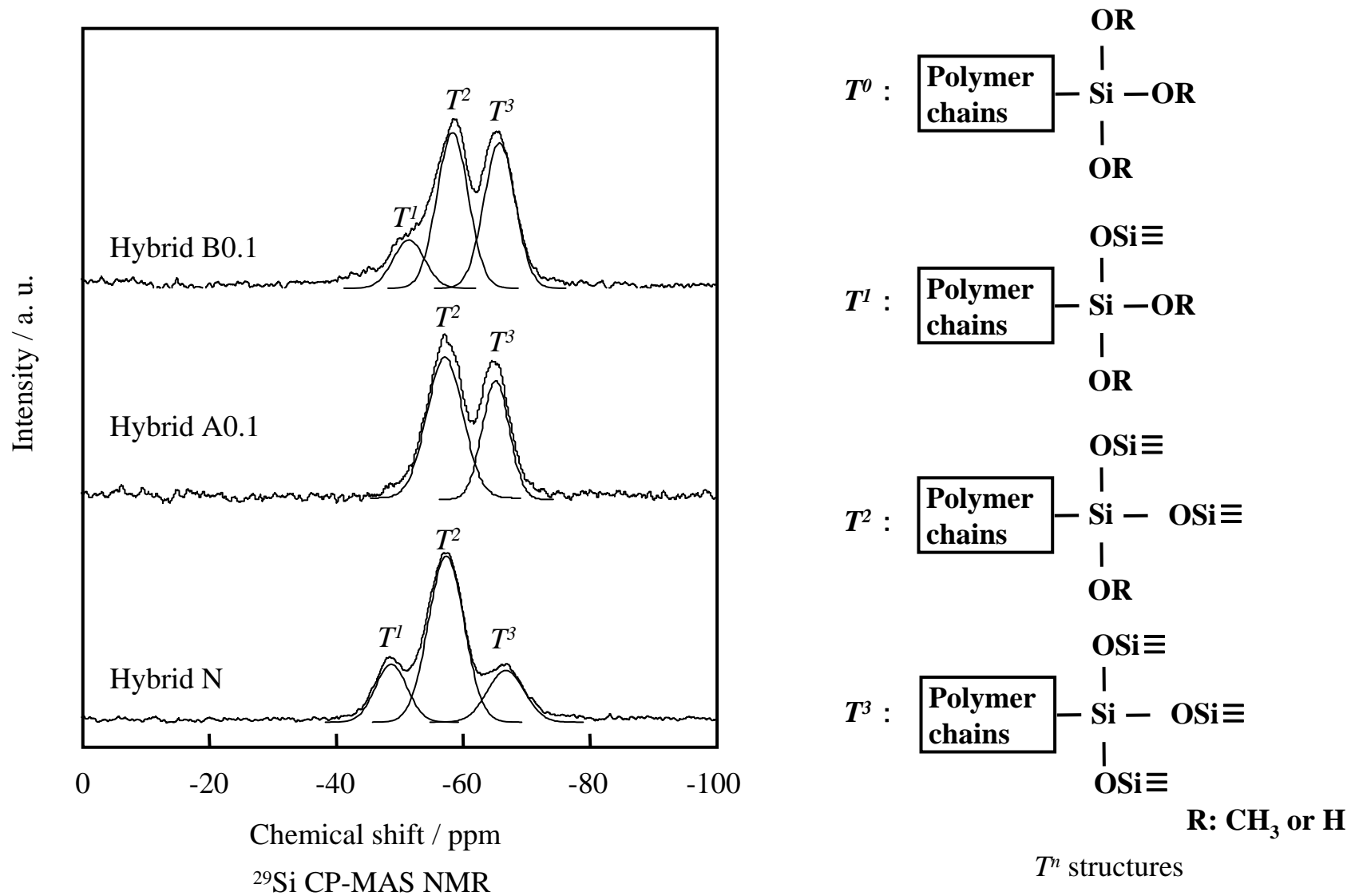
Figure 5. ^{29}Si CP-MAS NMR spectra and T^n structures in HEMA–MPS–CaCl$_2$ hybrid gels.

Fig.5, UCHINO *et al.*