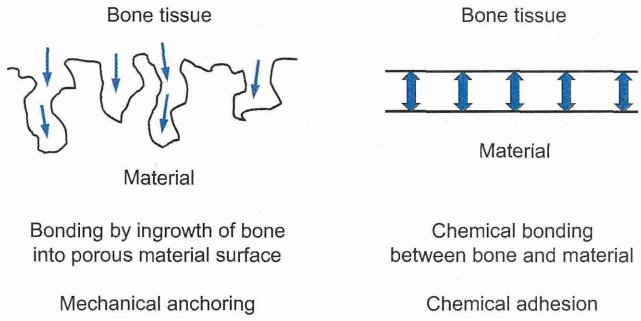

Figure 14. Schematics of chemical bonding and mechanical anchoring connection between bone and implanted material.

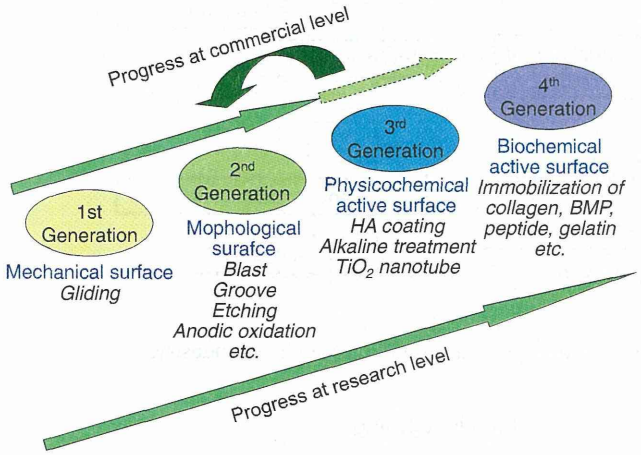

Figure 15. History of surface modification techniques to improve hard-tissue compatibility at the research level and estrangement in surface modification techniques between research and commercialization.

8.1.2. Mechanical anchoring and chemical bonding. In the stems of artificial hip joints and dental implants, the chemical bonding of metal surfaces with bone is not expected. In other words, it is impossible for metals as typical artificial materials to chemically and naturally bond with bone as living tissue, especially in the human body with body fluid. Therefore, the surface morphology is sometimes controlled, and rough and porous surface is formed in biomaterials. Living tissue, such as bone, is expected to grow into the rough porous surface, and the materials and bone are strongly connected as a result of the so-called anchoring effect. Figure 14 shows chemical bonding and a mechanical anchoring connection between bone and material. The current surface modification techniques for the improvement of hard tissue compatibility focus on the effect of chemical bonding and/or mechanical anchoring.

8.1.3. Gap in surface modification techniques between research and commercialization. Figure 15 shows the evolution of surface modification techniques to improve hard tissue compatibility at the research level, with reference to the stems of artificial hip joints and the fixtures of dental implants:

First generation: grind machining of the surface.

Second generation: grooving, blast, acid etching, anodic oxidation and laser abrasion. Nanostructured titanium oxide and titanium oxide nanotube are also categorized in this group.

Third generation: chemical treatment and hydroxyapatite coating.

Fourth generation: Immobilization of biofunctional molecules (collagen, bone morphogenetic protein and peptide).

The bone formation of the materials surface is accelerated when biomolecules concerning bone formation are immobilized on the material surface, such as in the fourth generation above. Therefore, many studies have achieved good results in this direction. However, to increase the popularity of the immobilization of biofunctional molecules, it is necessary to ensure the safety, quality maintenance during storage and dry-conditioned durability of the immobilized layer. Therefore, it is difficult for manufacturers to commercialize those research results.

Most of commercialized goods are categorized into the second generation, a few belong to the third generation, and there is no prospect for the commercialization of the fourth generation, at present. The commercialization went faster for the second than third generation possibly because materials employing mechanical anchoring are more practical than materials employing chemical bonding with bone.

8.2. Inhibition of assimilation of titanium alloy

When Ti alloys are used for bone fixators, such as bone screws and bone nails implanted in bone marrow, Ti alloys form callus on their surfaces and sometimes assimilate with bone. Therefore, bone may be refractured when the fixators are retrieved after bone healing because Ti easily forms calcium phosphate on itself [58, 59]. Stainless steel is used for complete retrieval after healing. Therefore, surface treatments that do not cause callus formation are necessary for the safe utilization of Ti alloy devices. It has been reported that Zr forms zirconium phosphate but not calcium phosphate [60, 61].

The coating of Zr inhibits the formation of calcium phosphate on Ti [62]. Figure 16 shows the relative concentration of elements detected in Zr-coated Ti, Zr and Ti. Calcium was not detected in Zr-coated Ti and Zr. According to the binding energies of the Ca $2p_{3/2}$ and P 2p electrons, Zr-coated Ti and Zr surfaces precipitate zirconium phosphate but not calcium phosphate. Therefore, Zr coating is a useful technique to inhibit the assimilation of Ti alloys with bone. Another approach is the development of a Zr-based alloy, which is being actively studied in Japan [63, 64].

9. Biofunctionalization—immobilization of biofunctional molecules

9.1. Purpose

Immobilization of biofunctional molecules on metal surfaces is effective for the biofunctionalization of the surfaces. Bone formation and soft tissue compatibility of metallic materials are sometimes required where the materials are used as

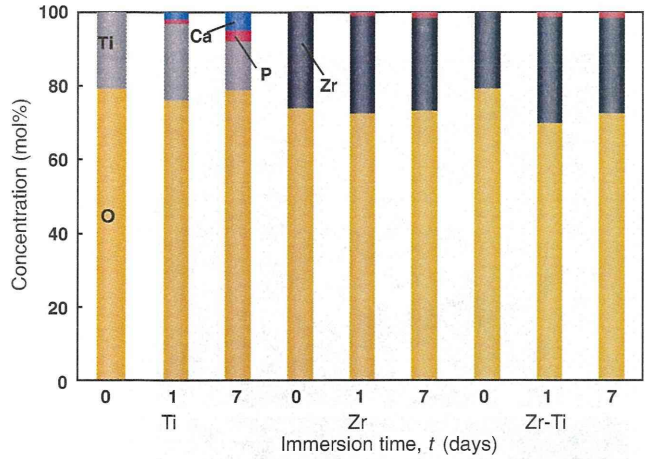

Figure 16. Relative concentration of elements in Zr-coated Ti, Zr and Ti determined by x-ray photoelectron spectroscopy [62].

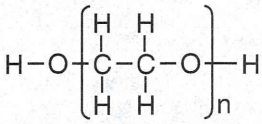

Figure 17. Chemical structure of poly(ethylene glycol).

parts of implant devices. Stents are placed in stenotic blood vessels for dilatation, and blood compatibility or prevention of adhesion of platelets is necessary. In addition, lubrication of the blood vessels is necessary for proper sliding and insertion of guide wires and catheters. When metals are used as sensing devices, cell adhesion must be controlled. The major cause of the retrieval of implants is infection due to biofilm formation. What is required is a biofilm-inhibiting surface, the fundamental property of which is to control the adsorption of proteins, cells, platelets and bacteria. This functional surface may be created by the immobilization of biofunctional molecules. These techniques make it possible to apply metals to scaffolds in tissue engineering. Some metal-polymer composites are reviewed in textbooks [65–67].

9.2. Poly(ethylene glycol)

Poly(ethylene glycol) (PEG) is an oligomer or polymer of ethylene oxide, but historically PEG referred to oligomers and polymers with a molecular weight below 20 000. PEG has the structure shown in figure 17. It is soluble in water, methanol, benzene and dichloromethane and is insoluble in diethyl ether and hexane. It is coupled to hydrophobic molecules to produce non-ionic surfactants. This property, combined with the availability of PEGs with a wide range of end-functions, contributes to the wide use of PEGs in biomedical research, particularly in drug delivery, tissue engineering scaffolds, surface functionalization and many other applications [68]. On the other hand, PEG is a biofunctional molecule on which adsorption of proteins is inhibited. Therefore, immobilization of PEG on metal surface is important for biofunctionalization of the metal.

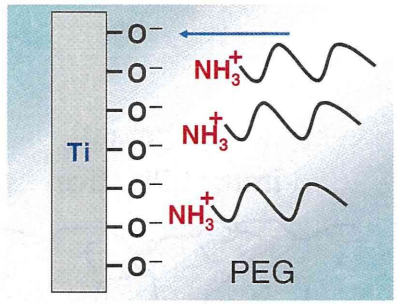

Figure 18. The cathodic potential was applied to Ti; during charging, the terminated PEGs electrically migrate to and are deposited on the Ti cathode.

9.2.1. Chemical immobilization. Biofunctional polymers are usually immobilized on noble metals, such as Au, via the –SH or –SS– group; however, this technique can only be used for noble metals. The adhesion of platelets and adsorption of proteins, peptides, antibodies and DNA are controlled by modifications of the above technique. A class of copolymers based on poly(L-lysine)-g-poly(ethylene glycol), PLL-g-PEG, has been found to spontaneously adsorb from aqueous solutions onto TiO_2, $Si_{0.4}Ti_{0.6}O_2$ and Nb_2O_5 to develop blood-contacting materials and biosensors [69]. In another case, TiO_2 and Au surfaces were functionalized by the attachment of poly(ethylene glycol)-poly(DL-lactic acid) (PEG-PLA) copolymeric micelles. The micelle layer can enhance the resistance to protein adsorption to the surfaces up to 70% [70]. Stainless steel (SS) surface was modified by a silane-coupling agent (SCA), (3-mercaptopropyl)trimethoxysilane. This surface was subsequently activated by argon plasma and subjected to UV-induced graft polymerization of poly(ethylene glycol)methacrylate, PEGMA. The PEGMA graft-polymerized stainless-steel coupon, PEGMA-g-SCA-SS, with a high graft concentration and, thus, a high PEG content was found to be very effective in preventing the absorption of bovine serum albumin and γ-globulin [71]. These processes require several steps, but are effective for immobilization; however, no promising technique for the immobilization of PEG to a metal surface has been developed so far. Photoreactive PEG was photoimmobilized on Ti [72].

9.2.2. Electrodeposition. Electrodeposition is useful for immobilizing PEG on a metal surface, because metals are usually electrically conductive. In one immobilization method both ends of PEG molecule ($M_W = 1000$) are terminated with $-NH_2$ (NH_2–PEG–NH_2). The cathodic potential is applied to Ti; during the charging, the terminated PEGs electrically migrate to and are deposited on the Ti cathode, as shown in figure 18. Terminated amines combine with Ti oxide as an NH–O bond by electrodeposition [73, 74]. The amount of the PEG immobilized onto the metals is governed by the concentrations of the active hydroxyl groups on each surface oxide [75]. The PEG-immobilized surface inhibits the adsorption of proteins and cells as well as the adhesion of platelets [76] and bacteria [77] (figure 19),

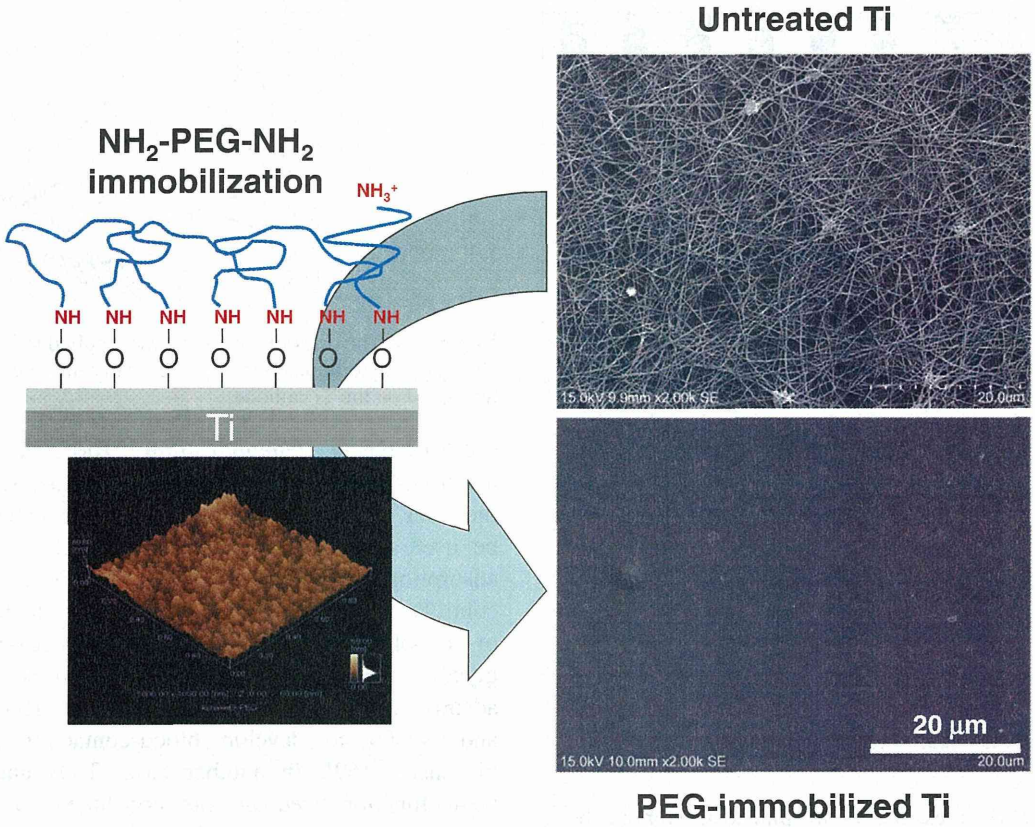

Figure 19. PEG immobilization by electrodeposition on the Ti surface and inhibition of platelet adhesion by immobilization [100].

indicating that this electrodeposition technique is useful for the biofunctionalization of metal surfaces. It can be applied to all electrically conductive materials and materials having complex surface topography.

9.3. Biomolecules

9.3.1. Concept. Since the natural environment around the implant is aqueous, while the surface of the implant is either bare or oxidized metal, specific demands are imposed on the coating to mediate between these different structural entities. The purpose of these demands is to obtain the native conformation of all proteins and cells that are in contact with the coating and to avoid all forms of aggregation and other conformational changes that might lead to protein denaturalization or cell death.

One approach is the immobilization of biological molecules (growth factors, adhesive proteins) on the implant surface to induce a specific cellular response and promote osseointegration. The application of large extracellular matrix proteins, however, can be impractical due to their low chemical stability, solubility in biological fluids, and high cost. In addition, entire extracellular matrix molecules are usually of allogenetic or xenogenetic origin and, thus, are associated with the risk of immune reaction and pathogen transfer.

Self-assembled monolayers provide chemically and structurally well-defined surfaces that can often be manipulated using standard synthetic methodologies [78]. Thiols on self-assembled monolayers [79, 80] and siloxane-anchored self-assembled monolayers [81] have been thoroughly studied. A problem related to the application of immobilized biomolecules via silanization techniques is the hydrolysis of siloxane films when exposed to aqueous (physiological) conditions [82]. More recently, alkyl phosphate films that remain robust under physiological conditions [83] have been used to provide an ordered monolayer on tantalum oxide surfaces [84, 85], and alkaliphosphonic acids have been applied to coat the native oxide surfaces of metals and their alloys including iron [86], steel [87] and Ti [88].

9.3.2. Peptides. In a living tissue, the most important role played by the extracellular matrix has been highlighted to favor cell adhesion [89]. Interactions occur between cell membrane receptors and adhesion proteins (or synthetic peptides) derived from the bone matrix, such as type I collagen or fibronectin [90]. These proteins contain the RGD (Arg–Gly–Asp) motif which specially connects transmembrane between the actin cytoskeleton and the RGD motif, and the whole system can activate several intracellular signaling pathways modulating cell behavior (e.g. proliferation, apoptosis, shape, mobility, gene expression and differentiation) [91].

Owing to the main role of the RGD sequence in cell adhesion, several research groups developed biofunctionalized surfaces by immobilization of RGD peptides. Grafting of RGD peptides has been performed

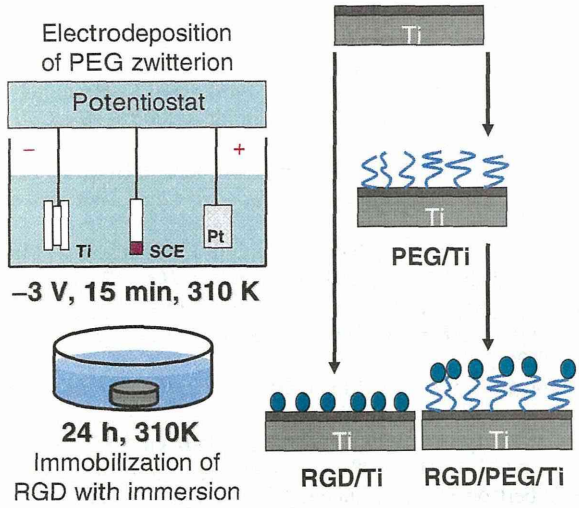

Figure 20. Schematic of the immobilization of RGD on PEG electrodeposited on Ti surface. To immobilize RGD, PEG with an $-NH_2$ group and a –COOH group (NH_2–PEG–COOH) must be employed. The $-NH_2$ group is required to bind with the metal oxide on the metal surface, whereas the –COOH group binds RGD.

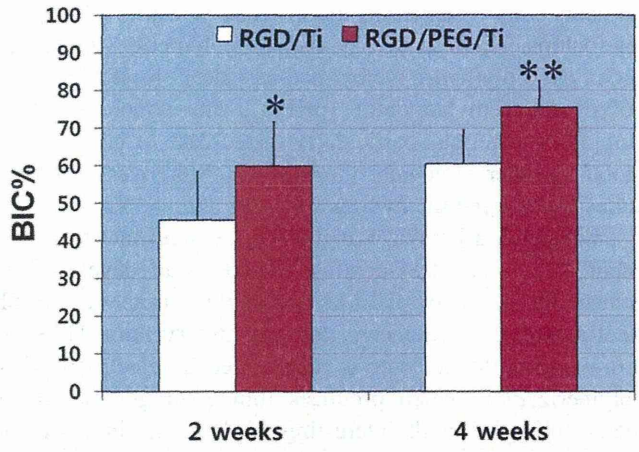

Figure 21. Mean percentage of the bone-to-implant contact (BIC%) over all threads of implants 2 and 4 weeks after implantation ($*p < 0.05$, $**p < 0.01$) [102]. RGD/PEG/Ti implants displayed significantly higher BIC% values in all threads and in the total lateral length compared with RGD/Ti implants at 2 and 4 weeks of healing.

on different biomaterials, such as Ti [92–94], and has been shown to improve osteoconduction *in vitro*. Methodologies differ by the conformation of RGD (cyclic or linear) and by the technique used for the immobilization [89, 90, 93–95]. Since the graft of an RGD peptide is known to be efficient in bone reconstruction [96], the challenge is to develop simple and cheap methods to favor cell attachment to biomaterial surfaces [94, 95].

Self-assembled molecular monolayers bearing RGD moieties have been grafted to various surfaces using either silanes [97], phosphonates on oxidized surfaces [94], or thiols on Au [95], but have revealed some application problems for large-scale production. Phosphonates are known to adsorb on Ti. To be mechanically and physiologically stable, phosphonate layers have to be covalently bound to the material surface by using drastic conditions [88, 98], such as anhydrous organic solvents or high temperature, which are not compatible with bimolecular stability. Monolayers of RGD phosphonates have been formed using a complex multistep process that requires tethering a primer onto a Ti surface, then a linker, and finally the peptide [99]. To immobilize RGD to the electrodeposited PEG on Ti, PEG with an $-NH_2$ group and a –COOH group (NH_2–PEG–COOH) must be employed. One terminal group, $-NH_2$, is required to bind stably with a surface oxide on a metal. The other terminal group, –COOH, is useful to bind biofunctional molecules such as RGD, as shown in figure 20 [100]. This RGD/PEG/Ti surface accelerates calcification by the MC3T3-E1 cell [101]. The calcification and bone formation are most pronounced on the RGD/PEG/Ti surface (figure 21) [102].

The glycine (G)-arginine (R)-glycine (G)-asparaginic acid (D)-serine (S) sequence peptide, the GRGDS peptide, is coated using a chloride activation technique to enhance the adhesion and migration of osteoblastic cells [103]. The expression levels of many genes in MC3T3-E1 cells are altered.

9.3.3. Protein and collagen. Among the relevant molecules involved in biochemical modification of bone-contacting surfaces, growth factors, such as bone morphogenetic protein-2 (BMP-2), are of primary interest. BMP-2 has been known to play an important role in bone healing processes and to enhance therapeutic efficiency. Ectopic bone formation by BMP-2 in animals has been well established following the first reports of BMP-2 [104–106]. A synthetic receptor-binding motif mimicking BMP-2 is covalently linked to Ti surfaces through a chemical conjunction process [107]. A complete and homogeneous peptide layer covers the Ti surfaces; the content is further measured by gamma counting. Biological evaluations show that the biochemically modified Ti was active in terms of cell attachment behavior. The rate of bone healing is higher on treated than untreated Ti surfaces. BMP-4 is immobilized on a Ti–6Al–4V alloy through lysozyme to improve the hard tissue response [108]. Proteins are silane-coupled to the oxidized surfaces of the Co–Cr–Mo alloy, the Ti–6Al–4V alloy, Ti and the Ni–Ti alloy to improve tissue compatibility [109].

Type I collagen is immobilized by immersion in a collagen solution [110]. The production of type I collagen increases with modification by ethane-1,1,2-triphosphonic acid and methylenediphosphonic acid grafted onto Ti [111]. Type I collagen is grafted through glutaraldehyde as a crosslinking agent [112]. For electrodeposition, the current alternating at 1 Hz between −1 V and +1 V versus SCE is effective to immobilize type I collagen on Ti, and the durability in water is high [113].

Fibronectin was immobilized directly on Ti using the tresyl chloride activation technique [114]. L-threonine and O-phospho-L-threonine were immobilized on an acid-etched Ti surface [115].

9.3.4. Hydrogel. Immobilization or coating of hydrogel on the metal surface is currently used in an attempt to add a drug delivery ability to orthopedic

implants and stents or fluorescent sensing ability to microchips. Currently, synthetic polymeric hydrogels, such as poly(hydroxyethylmethacrylate) (pHEMA) and poly(hydroxyethylacrylate) (pHEA), are widely used as compliant materials, particularly in the case of contact with blood or other biological fluids [116]. Moreover, when the adhesion between the hydrogel coating and the metal surface is inadequate, a breakage at the coating-steel interface may occur [117]. A spray-coating method was developed to control the coating by pHEMA of complex surfaces of a 316L steel stent for percutaneous coronary intervention [118]. A promising synthetic route is represented by electrochemical polymerization, which produces thin coatings directly on metal substrates with interesting applications in corrosion protection and development of bioactive films [119–122]. As far as the field of orthopedics is concerned, in recent years, many procedures based on surface modification have been suggested to improve the biocompatibility and biofunctionality of Ti-based implants [123]. HEMA, a macromer poly(ethylene-glycol diacrylate) (PEGDE), and PEGDE copolymerized with acrylic acid were used to obtain hydrogels. A model protein and a model drug were entrapped in the hydrogel and released according to the pH change [124].

10. Conclusions

Metallic materials are widely used in medicine for not only orthopedic implants but also cardiovascular devices and other purposes. Their physical properties, such as mechanical, biodegradable and magnetic properties, have been improved by redesigning alloys and manufacturing processes. On the other hand, the metal surface may be biofunctionalized by various techniques, such as dry and wet processes, the immobilization of biofunctional molecules, and the creation of metal-polymer composites. In particular, the electrodeposition technique is useful for all electroconductive and morphological materials. These techniques make it possible to apply metals to scaffolds in tissue engineering.

References

[1] Semlitsch M, Staub F and Weber H 1985 *Biomed. Tech.* **30** 334
[2] Wang K, Gustavson L and Dumbleton J 1993 *Titanium '92: Science and Technology* (Warrenda: TMS) 2697
[3] Zardiackas L D, Mitchell D W and Disegi J A 1996 *Medical Applications of Titanium and its Alloys* (West Conshohoken, PA: ASTM) 60
[4] Hanawa T 2010 *Metals for Biomedical Devices* ed M Niinomi (Oxford: Woodhead Publishing) p 3
[5] Kuroda D, Niinomi M, Morinaga M, Kato Y and Yashiro T 1998 *Mater. Sci. Eng.* A **243** 244
[6] Ozaki T, Matsumoto H, Watanabe S and Hanada S 2004 *Mater. Trans.* **45** 2776
[7] Nomura N, Sakamoto K, Takahashi K, Kato S, Abe Y, Doi H, Tsutsumi Y, Kobayashi M, Kobayashi E, Kim W J, Kim K H and Hanawa T 2010 *Mater. Trans.* **51** 1449
[8] Nomura N, Baba Y, Kawamura A, Fujinuma S, Chiba A, Masahashi N and Hanada S 2007 *Mater. Sci. Forum* **539–543** 1033
[9] Nakai M, Niinomi M, Akahori T, Yamanoi H, Itsuno S, Haraguchi N, Itoh Y, Ogasawara T, Onishi T and Shindo T 2008 *J. Japan. Inst. Metal.* **72** 839
[10] Scheinert D, Scheinert S, Sax J, Piorkowski C, Braunlich S, Ulrich M, Biamino G and Schmidt A 2005 *J. Am. Coll. Cardiol.* **45** 312
[11] Sianos G, Hofma S, Lighthart J M R, Saia F, Hoye A, Lemos P A and Serruys P W 2004 *Catheter. Cardiovasc. Interv.* **61** 111
[12] Schlager O, Dick P, Sabeti S, Amighi J, Mlekusch W, Minar E and Schillinger M 2005 *J. Endovasc. Ther.* **12** 676
[13] Chowdhury P and Ramos R G 2002 *N. Engl. J. Med.* **347** 581
[14] Kang W Y, Kim W, Kim H G and Kim W 2007 *Int. J. Cardiol.* **120** 273
[15] Robertson S W and Ritchie R O 2008 *J. Biomed. Mater. Res. Appl. Biomater.* B **84** 26
[16] Gall K, Tyber J, Wilkesanders G, Robertson S W, Ritchie R O and Maier H J 2008 *Mater. Sci. Eng.* A **486** 389
[17] Robertson S W and Ritchie R O 2007 *Biomaterials* **28** 700
[18] Heintz C, Riepe G, Birken L, Kaiser E, Chakfe N, Morlock M, Delling G and Imig H 2001 *J. Endovasc. Ther.* **8** 248
[19] Guidoin R *et al* 2000 *J. Endovasc. Ther.* **7** 105
[20] Kong H, Wilkinson J L, Coe J Y, Gu X, Urness M, Kim T H and Bass J L 2002 *Cardiol. Young.* **12** 260
[21] Takahashi E, Sakurai T, Watanabe S, Masahashi N and Hanada S 2002 *Mater. Trans.* **43** 2978
[22] Hosoda H, Fukui Y, Inamura T, Wakashima K, Miyazaki S and Inoue K 2003 *Mater. Sci. Forum* **426–432** 3121
[23] Kim H Y, Ohmatsu Y, Kim J I, Hosoda H and Miyazaki S 2004 *Mater. Trans.* **45** 1090
[24] Maeshima T and Nishida M 2004 *Mater. Trans.* **45** 1096
[25] Kim J I, Kim H Y, Hosoda H and Miyazaki S 2004 *Mater. Trans.* **45** 852
[26] Kim J I, Kim H Y, Inamura T, Hosoda H and Miyazaki S 2005 *Mater. Sci. Eng.* A **403** 334
[27] Heublein B, Rohde R, Kaese V, Niemeyer M, Hartung W and Haverich A 2003 *Heart* **89** 651
[28] Waksman R *et al* 2006 *Catheter. Cardiovasc. Interv.* **68** 607
[29] Peuster M, Wohlsein P, Brugmann M, Ehlerding Seidler K, Fink C, Brauer H, Fischer A and Hausdorf G 2001 *Heart* **86** 563
[30] Witte F, Kaese V, Haferkamp H, Switzer E, Meyer-Lindenberg A, Wirth C J and Windhagen H 2005 *Biomaterials* **26** 3557
[31] Ornhagen C, Nilsson J O and Vannevik H 1996 *J. Biomed. Mater. Res.* **31** 97
[32] Baba H, Kodama T and Katada Y 2002 *Corros. Sci.* **44** 2393
[33] Sumita M, Hanawa T and Teoh S H 2004 *Mater. Sci. Eng.* C **24** 753
[34] Gebau R C and Brown R S 2001 *Adv. Mater. Process.* **159** 46
[35] Menzel J, Kirschner W and Stein G 1996 *ISIJ Int.* **36** 893
[36] Uggowitzer P J, Magdowski R and Speidel M O 1996 *ISIJ Int.* **36** 893
[37] Kuroda D, Hanawa D, Hibaru T, Kuroda S, Kobayashi M and Kobayashi T 2003 *Mater. Trans.* **44** 414
[38] Olsrud J, Lätt J, Brockstedt S, Romner B and Björkman-Burtscher I 2005 *J. Magn. Reson. Imaging* **22** 433
[39] Shafiei F, Honda E, Takahashi H and Sasaki T 2003 *J. Dent. Res.* **82** 602
[40] Matsuura H, Inoue T, Konno H, Sasaki M, Ogasawara K and Ogawa A 2002 *J. Neurosurg.* **97** 1472
[41] Matsuura H, Inoue T, Ogasawara K, Sasaki M, Konno H, Kuzu Y, Nishimoto H and Ogawa A 2005 *Neurol. Med. Chir.* **45** 395
[42] Ernstberger T, Heidrich G, Bruening T, Krefft S, Buchhorn G and Klinger H M 2007 *Eur. Spine J.* **16** 179
[43] Usami E, Hamada K and Asaoka K 2010 *J. Japan. Soc. Dent. Mater. Dev.* **29** 415
[44] Chauvel B, Cathelineau G, Balac S, Lecerf J and de Certaines J D 1996 *J. Magn. Reson. Imaging* **6** 936

[45] Popowski Y, Hiltbrand E, Joliat D and Rouzaud M 2000 *Int. J. Radiat. Oncol. Biol. Phys.* **47** 759
[46] Nomura N, Tanaka Y, Suyalatu, Kondo R, Doi H, Tsutsumi Y and Hanawa T 2009 *Mater. Trans.* **50** 2466
[47] Suyalatu, Nomura N, Oya K, Tanaka Y, Kondo R, Doi H, Tsutsumi Y and Hanawa T 2010 *Acta Biomater.* **6** 1033
[48] Kondo R, Nomura N, Suyalatu, Tsutsumi Y, Doi H and Hanawa T 2011 *Acta Biomater.* **7** 4278
[49] Fontaine D D 1988 *Metall. Mater. Trans.* A **19** 169
[50] Sikka S K, Vohra Y K and Chidambaram R 1982 *Prog. Mater. Sci.* **27** 245
[51] Buenconsejo P J S, Kim H Y and Miyazaki S 2011 *Scr. Mater.* **64** 1114
[52] Balazic M, Kopac J, Jackson M J and Ahmed W 2007 *Int. J. Nano Biomater.* **1** 3
[53] Hanada S and Izumi O 1986 *J. Mater. Sci.* **21** 4131
[54] Hanada S, Takemura A and Izumi O 1982 *Trans. Japan. Inst. Met.* **23** 507
[55] Kuan T S, Ahrens R R and Sass S L 1975 *Metall. Mater. Trans.* A **6** 1767
[56] Hanawa T 2009 *J. R. Soc. Interface* **6** S361
[57] Hanawa T 2010 *Japan. J. Dent. Sci. Rev.* **46** 93
[58] Hanawa T and Ota M 1991 *Biomaterials* **12** 767
[59] Hiromoto S, Hanawa T and Asami K 2004 *Biomaterials* **25** 979
[60] Hanawa T, Okuno O and Hamanaka H 1992 *J. Japan. Inst. Met.* **56** 1168 (in Japanese)
[61] Hanawa T, Hiromoto S, Asami K, Okuno O and Asaoka K 2002 *Mater. Trans.* **43** 3000
[62] Kobayashi E, Ando M, Tsutsumi Y, Doi H, Yoneyama T, Kobayashi M and Hanawa T 2007 *Mater. Trans.* **48** 301
[63] Ikarashi Y, Toyoda K, Kobayashi E, Doi H, Yoneyama T, Hamanaka H and Tsuchiya T 2005 *Mater. Trans.* **46** 2260
[64] Kobayashi E, Matsumoto S, Doi H, Yoneyama T and Hamanaka H 1995 *J. Biomed. Mater. Res.* **29** 943
[65] Possart W 1998 *Metals as Biomaterials* (New York: Wiley) 197
[66] Worch H 1998 *Metals as Biomaterials* (New York: Wiley) 177
[67] Xiao S J, Kenausis G and Textor M 2001 *Titanium in Medicine* (Amsterdam, the Netherlands: Springer) 417
[68] Mahato R I 2005 *Biomaterials for Delivery and Targeting of Proteins and Nucleic Acids* (Warrenda, NY: CRC Press)
[69] Kenausis G L, Voros J, Elbert D L, Huang N, Hofer R, Ruiz-Taylor L, Textor M, Hubbell J A and Spencer N D 2000 *J. Phys. Chem.* B **104** 3298
[70] Huang N P, Csucs G, Emoto K, Nagasaki Y, Kataoka K, Textor M and Spencer N D 2002 *Langmuir* **18** 252
[71] Zhang F, Kang E T, Neoh K G, Wang P and Tan K L 2001 *Biomaterials* **22** 1541
[72] To Y, Hasuda H, Sakuragi M and Tsuzuki S 2007 *Acta Biomater.* **3** 1024
[73] Tanaka Y, Doi H, Iwasaki Y, Hiromoto S, Yoneyama T, Asami K, Imai H and Hanawa H 2007 *Mater. Sci. Eng.* C **27** 206
[74] Tanaka Y, Doi H, Kobayashi E, Yoneyama T and Hanawa T 2007 *Mater. Trans.* **48** 287
[75] Tanaka Y, Saito H, Tsutsumi Y, Doi H, Imai H and Hanawa T 2008 *Mater. Trans.* **49** 805
[76] Tanaka Y, Matsuo Y, Komiya T, Tsutsumi Y, Doi H, Yoneyama T and Hanawa T 2010 *J. Biomed. Mater. Res.* A **92** 350
[77] Tanaka Y, Matin K, Gyo M, Okada A, Tsutsumi Y, Doi H, Nomura N, Tagami J and Hanawa T 2010 *J. Biomed. Mater. Res.* A **95** 1105
[78] Balachander N and Sukenik C N 1990 *Langmuir* **6** 1621
[79] Bain C D, Troughton Y, Tao Y T, Evall J, Whitesides G M and Nuzzo R G 1989 *J. Am. Chem. Soc.* **111** 437
[80] Dubois L H and Nuzzo R G 1992 *Annu. Rev. Phys. Chem.* **43** 437
[81] Ulman A 1996 *Chem. Rev.* **96** 1533
[82] Xiao S J, Textor M and Spencer N D 1998 *Langmuir* **14** 5507
[83] Gawalt E S, Avaltroni M J, Danahy M P, Silverman B M, Hanson E L, Midwood K S, Schwarzbauer J E and Schwartz J 2003 *Langmuir* **19** 200
[84] Brovelli D, Hahner G, Ruis L, Hofer R, Kraus G, Waldner A, Schlosser J, Oroszlan P, Ehart M and Spencer N D 1999 *Langmuir* **15** 4324
[85] Textor M, Ruiz L, Hofer R, Rossi K, Feldman K, Hahner G and Spencer N D 2000 *Langmuir* **16** 3257
[86] Fang J L, Wu N J, Wang Z W and Li Y 1991 *Corrosion* **47** 169
[87] Van Alsten J G 1999 *Langmuir* **15** 7605
[88] Gawalt E S, Avaltroni M J, Koch N and Schwartz J 2001 *Langmuir* **17** 5736
[89] Verrier S, Pallu S, Bareille R, Jonczyk A, Meyer J, Dard M and Amedee J 2002 *Biomaterials* **23** 585
[90] Reyes C D, Petrie T A, Burns K L, Schwartz Z and Garcia A J 2007 *Biomaterials* **28** 3228
[91] Hynes R O 2002 *Cell* **110** 673
[92] Bagno A, Piovan A, Dettin M, Chiarion A, Brun P, Gambaretto R, Fontana G, Di Bello C, Palu G and Castagliuolo I 2007 *Bone* **40** 693
[93] Elmengaard B, Bechtold J E and Soballe K 2005 *Biomaterials* **26** 3521
[94] Rammelt S, Illert T, Bierbaum S, Scharnweber D, Zwipp H and Schneiders W 2006 *Biomaterials* **27** 5561
[95] Auernheimer J, Zukowski D, Dahmen C, Kantlehner M, Enderle A, Goodman S L and Kessker H 2005 *Chem. Biochem.* **6** 2034
[96] Ferris D M, Moodie G D, Dimond P M, Gioranni C W, Ehrlich M G and Valentini R F 1999 *Biomaterials* **20** 2323
[97] Xiao S J, Textor M, Spencer N D, Wieland M, Keller B and Sigrist H 1997 *J. Mater. Sci. Mater. Med.* **8** 867
[98] Silverman B M, Wieghaus K A and Schwartz J 2005 *Langmuir* **21** 225
[99] Schwartz J, Avaltroni M J, Danahy M P, Silverman B M, Hanson E L, Schwarzbauer J E, Midwood K S and Gawalt E S 2003 *Mater. Sci. Eng.* C **23** 395
[100] Tanaka Y, Saito H, Tsutsumi Y, Doi H, Nomura N, Imai H and Hanawa T 2009 *J. Colloid Interface Sci.* **330** 138
[101] Oya K, Tanaka Y, Saito H, Kurashima K, Nogi K, Tsutsumi H, Tsutsumi Y, Doi H, Nomura N and Hanawa T 2009 *Biomaterials* **30** 1281
[102] Park J W, Kurashima K, Tustusmi Y, An C H, Suh J Y, Doi H, Nomura N, Noda K and Hanawa T 2011 *Acta Biomater.* **7** 3222
[103] Yamanouchi N, Pugdee K, Chang W J, Lee S Y, Yoshinari M, Hayakawa T and Abiko Y 2008 *Dent. Mater. J.* **27** 744
[104] Urist M R 1965 *Science* **150** 893
[105] Lee Y M, Nam S H, Seol Y J, Kim T I, Lee S J, Ku Y, Rhyu I C, Chung C P, Han S B and Choi S M 2003 *J. Periodontol.* **74** 865
[106] Wikesjo U M, Lim W H, Thomson R C, Cook A D, Wozney J M and Hardwick W R 2003 *J. Periodontol.* **74** 635
[107] Seol Y J *et al* 2006 *J. Biomed. Mater. Res.* A **77** 599
[108] Puleo D A, Kissling R A and Sheu M S 2002 *Biomaterials* **23** 2079
[109] Nanci A, Wuest J D, Peru L, Brunet P, Sharma V, Zalzal S and McKee M D 1998 *J. Biomed. Mater. Res.* **40** 324
[110] Nagai M, Hayakawa T, Fukatsu A, Yamamoto M, Fukumoto M, Nagahama F, Mishima H, Yoshinari M, Nemoto K and Kato T 2002 *Dent. Mater. J.* **21** 250
[111] Viornery C, Guenther H L, Aronsson B O, Pechy P, Descouts P and Gratzel M 2002 *J. Biomed. Mater. Res.* **62** 149
[112] Chang W J, Qu K L, Lee S Y, Chen J Y, Abiko Y, Lin C T and Huang H M 2008 *Dent. Mater. J.* **27** 340
[113] Kamata H, Suzuki S, Tanaka Y, Tsutsumi Y, Doi H, Nomura N, Hanawa T and Moriyama K 2011 *Mater. Trans.* **52** 81
[114] Pugdee K, Shibata Y, Yamamichi N, Tsutsumi H, Yoshinari M, Abiko Y and Hayakawa T 2007 *Dent. Mater. J.* **26** 647

[115] Abe Y, Hiasa K, Takeuchi M, Yoshida Y, Suzuki K and Akagawa Y 2005 *Dent. Mater. J.* **24** 536
[116] Cadotte A J and DeMarse T B 2005 *J. Neural. Eng.* **2** 114
[117] Belkasm J S, Munro C A, Shoichet M S, Johnston M and Midha R 2005 *Biomaterials* **26** 1741
[118] Indolfi L, Causa F and Netti P A 2009 *J. Mater. Sci. Mater. Med.* **20** 1541
[119] Fenelon A M and Breslin C B 2003 *Corros. Sci.* **45** 2837
[120] Mengoli G 1979 *Adv. Polym. Sci.* **33** 1
[121] De Giglio E, Guascito M R, Sabbatini L and Zambonin G 2001 *Biomaterials* **22** 2609
[122] Rammelt U, Nguyen P T and Plieth W 2003 *Electrochem. Acta* **48** 1257
[123] De Giglio E, Gennaro I, Sabbatini L and Zambonin G 2001 *J. Biomater. Sci. Polym. Ed.* **12** 63
[124] De Giglio E, Cometa S, Satriano C, Sabbatini L and Zambonin G 2009 *J. Biomed. Mater. Res.* A **88** 1048

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RESEARCH ARTICLE

Influence of synovia constituents on tribological behaviors of articular cartilage

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Abstract: The extremely low friction and minimal wear in natural synovial joints appear to be established by effective lubrication mechanisms based on appropriate combination of articular cartilage and synovial fluid. The complex structure of cartilage composed of collagen and proteoglycan with high water content contributes to high load-carrying capacity as biphasic materials and the various constituents of synovial fluid play important roles in various lubrication mechanisms. However, the detailed differences in functions of the intact and damaged cartilage tissues, and the interaction or synergistic action of synovia constituents with articular cartilage have not yet been clarified. In this study, to examine the roles of synovia constituents and the importance of cartilage surface conditions, the changes in friction were observed in the reciprocating tests of intact and damaged articular cartilage specimens against glass plate lubricated with lubricants containing phospholipid, protein and/or hyaluronic acid as main constituents in synovial fluid. The effectiveness of lubricant constituents and the influence of cartilage surface conditions on friction are discussed. In addition, the protectiveness by synovia constituents for intact articular cartilage surfaces is evaluated.

Keywords: articular cartilage; synovial fluid; synovial joint; lubrication; biotribology

1 Introduction

In various biotribological systems, it is widely known that the healthy synovial joints maintain superior load-carrying capacity and lubricating properties with extremely low friction and minimal wear even in heavily loaded hip, knee and ankle joints. The synovial joints are prominent natural bearings different in geometric congruity depending on joint positions/movements and are in general covered with soft layers of biphasic articular cartilage lubricated with synovial fluid containing appropriate lubricating constituents. The superior tribological properties of synovial joints appear to be established by a well-suited combination of articular cartilage and synovial fluid. However, the detailed cooperative and/or interactive behaviors between articular cartilage and synovial fluid under various rubbing conditions have not yet been clarified. In this paper, we will focus on the influence of main synovia constituents such as phospholipid, protein and hyaluronic acid on tribological behaviors of articular cartilage different in surface conditions particularly as related to lubrication mechanism.

The operating conditions in human synovial joints change under variable loading and motions including sliding and rolling depending on joint types in various daily activities. Therefore, the superior lubricating performance of natural synovial joints is likely to be actualized not by a single lubrication mode but by the synergistic combination of various modes from fluid film lubrication to boundary lubrication [1, 2].

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Other specific lubrication mechanisms such as weeping lubrication [3], boosted lubrication [4], biphasic lubrication [5], micro-elastohydrodynamic lubrication (micro-EHL) [6] and so on have been proposed. The ingenious lubrication mechanism as the synergistic combination of various modes depending on the severity of operating conditions was called the adaptive multimode lubrication mechanism [7, 8]. For example, during normal walking, fluid film lubrication mechanisms such as soft-EHL and/or micro-EHL play major roles to maintain low friction and minimize wear. In contrast, in thin film conditions such as at slow motion or at movement after standing for a long time, it is expected that adsorbed films [9–12], surface gel films [13], hydration lubrication [14] and polymeric brush-like layers [15, 16] contribute to keep friction low and protect rubbing surfaces.

Another new development in lubrication theory is the elucidation of the biphasic lubrication mechanism. Since an experimental finding [17] and a proposal of boundary friction model based on biphasic lubrication by Ateshian [18], the important phenomena on the effectiveness of biphasic lubrication with interstitial fluid pressurization have been demonstrated on the basis of the biphasic finite element (FE) analyses and experimental observations [19, 20]. The articular cartilage has high water content from 70% to 80% in tissue as porous media composed of type II collagen, proteoglycan and chondrocytes, and thus exhibits a time-dependent biphasic behavior due to the simultaneous coexistence of solid and liquid phases [21]. When articular cartilage as biphasic material with low permeability is applied by compressive load, the fluid content in the tissue is trapped within contact area and the collagen matrix network resists interstitial fluid pressure in aggregate solid matrix. Thus, the interstitial fluid pressure supports significant proportion of total load in contact area and this situation consequently causes the reduction of contact force of solid phase for a considerable time. The time-dependent change in load support by interstitial fluid pressure in biphasic cartilage depends on the extent of exudation from cartilage tissue and rehydration of cartilage. If the fluid load support is maintained at high level for a long time, the low friction is maintained because of low level for solid-to-solid contact [20].

For reciprocating sliding under constant load, Pawaskar et al. [22] introduced sliding motion into their FE model and indicated the importance of migrating contact area for the sustainability of the biphasic lubrication in their biphasic FE analysis. Sufficient stroke for rehydration of cartilage tissue in reciprocating motion maintained the high level of load support by interstitial fluid pressure. Sakai et al. [23] examined the compressive response of the articular cartilage by high precision testing machine with a feedback-controlled servomotor and estimated material properties in physiological condition for the biphasic FE model, which included (1) the depth-dependence of apparent Young's modulus of solid phase, (2) strain-dependent permeability as compaction effect, and (3) collagen reinforcement in tensile strain. These properties (parameters) were estimated by the curve fitting between the experimental time-dependent compressive behavior and simulation in indentation tests for cartilage specimens with cylindrical rigid indenter of 5 mm radius. In the reciprocating test, the load of 0.5 N/mm was applied at the center of the cylindrical indenter in 1 s and then the reciprocating motion was introduced with the speed of 4 mm/s over a stroke length of 8 mm. FE analyses using commercial package ABAQUS (6.8-4) showed that the tensile reinforcement by spring elements representing the collagen network and the depth-dependent elastic properties improved the proportion of the fluid load support especially in the sliding condition. The compaction effect on permeability of solid phase was functional in a condition without the migrating contact area, whereas under sliding condition the compaction effect showed a little effect in terms of the proportion of the fluid load support.

In the next stage, the influence of operating conditions on the effectiveness of biphasic lubrication in reciprocating sliding was examined. The differences in frictional behaviors between the reciprocation with migration of contact zone, i.e., at on-off loading on articular cartilage (model A) as described above, and without migration of contact zone, i.e., at continuous loading on cartilage (model B), shown in Fig. 1 were compared in FE analysis [24]. In this simulation of reciprocating test with similar method to the previous study [23], the load of 0.5 N/mm was applied by

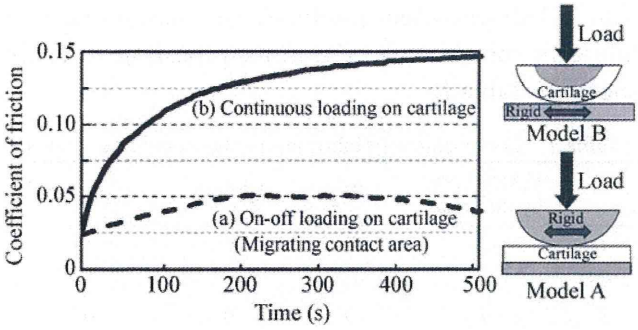

Fig. 1 Time-dependent frictional behaviors estimated by biphasic theory for cartilage.

the rigid cylindrical indenter against flat cartilage specimen or by the rigid flat plate against cylindrical cartilage specimen with a ramp time of 1 s and then the load was held constant during reciprocation. The reciprocation of rigid cylinder or flat plate at 4 mm/s was started immediately after loading and continued for 508 s, 127 cycles at period of 4 s. The initial fluid load support percentages are very high as 90% and 91% for models A and B, respectively. After 127 cycles, it is noticed that the high percentage of fluid load support (83%) was maintained even after 508 s in model A, but the percentage of fluid load support was remarkably decreased to 27% in the model B. The time-depending changes in friction coefficient μ_{eff} were estimated for μ_{eq} as coefficient of friction for solid-to-solid contact using the following formula by Ateshian et al. [20, 25].

$$\mu_{eff} = \mu_{eq}\,(1 - (1 - \Psi)\,W_p/W) \tag{1}$$

where W is the total load support, W_p the load support by fluid pressure and Ψ the fraction of contact area of solid phase.

In Fig. 1, the time-dependent changes in friction estimated from total traction force in biphasic FE analysis for assumption of μ_{eq} = 0.2 [24] are shown. It is worth noting that the lower friction level is maintained due to the sustainability of interstitial fluid pressure in the reciprocating sliding for model A. In contrast, significant gradual increase to high level in friction is observed in reciprocation for model B. It is supposed that the tribological problems are more likely to occur for model B with high friction level and thus the method to suppress friction increase is required.

In this study, the combination of cartilage-on-glass was used to simplify the frictional condition, although articular cartilage is rubbed against cartilage or meniscus in natural synovial joints. The glass plate has very smooth, hard and non-porous/impermeable surface compared with articular cartilage but hydrophilic surface with negatively charged property similar to proteoglycan on superficial cartilage layer in wet condition [12]. The adsorption of synovia constituents on glass plate appears to be considerably similar to boundary film formation on intact cartilage as shown by *in situ* observation for fluorescent images of adsorbed molecules during reciprocating rubbing process [26], while the interaction to the smooth, hard and non-porous/impermeable glass surface may induce certain different behaviors. Smooth glass surface minimizes ploughing resistance, but may enhance the adhesive resistance by interaction with adsorbed protein molecules at intimate contacts in very thin film condition. However, the intrinsic tribological properties of compliant and biphasic articular cartilage are expected to be reflected appropriately in the effectiveness of lubricant constituents even in sliding pair of articular cartilage and glass plate. As a matter of course, the difference in tribological behaviors between for cartilage-cartilage and cartilage-glass combinations should be explored. The influence of glass plate on frictional behaviors is discussed in Section 4. Thus, the frictional behaviors in a sliding pair of ellipsoidal articular cartilage specimens and reciprocating glass plate were examined in the sliding condition for model B without migration of contact zone for cartilage.

2 Materials and methods

The reciprocating test for the sliding pair of the upper stationary ellipsoidal articular cartilage specimen and the lower reciprocating flat glass plate was conducted in the reciprocating tester shown in Fig. 2. The continuous loading condition without migration of contact zone for articular cartilage corresponds to the severe operating condition for cartilage (model B) as described above in related to the biphasic FE analysis.