A novel ultra high molecular weight polyethylene–hyaluronan microcomposite for use in total joint replacements. I. Synthesis and physical/chemical characterization

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Abstract: A novel microcomposite between ultra high molecular weight polyethylene (UHMWPE) and hyaluronan (HA) was developed to create a hydrophilic and lubricious UHMWPE surface for total joint replacement and other biomedical load-bearing applications. Preforms with interconnected micropores were used as the UHMWPE starting material to form a microcomposite with HA, rather than starting with fully dense, bulk UHMWPE. HA was silylated first to increase its hydrophobicity and compatibility with UHMWPE. The silylated groups were removed through hydrolysis prior to final compression molding. A uniform and enzymatic degradation resistant HA film layer was produced on the microcomposite surface, which quickly hydrated in water, forming a lubricious surface film that was fully wetted by water drops during contact angle measurements. Presence of HA film on the composite surface was also demonstrated through X-ray photoelectron spectroscopy analysis and Toluidine Blue O dye assay. The mechanical and tribological properties evaluation of the novel microcomposites are presented in Part II. © 2006 Wiley Periodicals, Inc. J Biomed Mater Res 78A: 86–96, 2006

Key words: hyaluronan; polyethylene; microcomposite; surface modification; total joint replacements

INTRODUCTION

Although clinical successes have been achieved with ultra high molecular weight polyethylene (UHMWPE) as the weight-bearing surface in total joint replacements (TJRs), wear debris generated from UHMWPE components remains a major cause of implant loosening and failure, limiting the longevity of current TJRs.[1] Many research efforts to reduce friction and wear of UHMWPE are currently underway, seeking either to improve the properties of the bulk UHMWPE material or to modify its surface. Highly crosslinked UHMWPE is the most developed of these modifications. It was introduced ~5 years ago and has captured a significant portion of the UHMWPE bearing market. Crosslinking does markedly improve wear resistance, but at the sacrifice of the toughness and mechanical properties of conventional UHMWPE.[2] Some researchers have attempted modification of the UHMWPE surface structure without changing the bulk material properties, such as subsurface crosslinking of UHMWPE with plasma[3] or ion implantation.[4–6] With appropriately tailored treatments, the wear resistance of UHMWPE was significantly improved.

In the above bulk material or surface structure modifications, the effect of UHMWPE surface chemistry was not considered. UHMWPE is extremely hydrophobic, while the natural joint surface, articular cartilage, is very hydrophilic and negatively charged. It is the interaction of articular cartilage and synovial fluid that plays a key role in the very low friction and wear of synovial joints.[7] Therefore, some efforts have been made to modify the surface chemistry of UHMWPE. Oxygen-plasma was used to attach hydrophilic groups (e.g. —OH and —COOH) on the UHMWPE surface, resulting in a significant decrease in friction.[8] This treatment, however, is relatively short-lived. Beauregard modified UHMWPE by introducing a synthetic polypeptide (poly-L-lysine) into the surface to form a semiinterpenetrating polymer network be-

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tween polypeptide and UHMWPE. However, the decrease in the coefficient of friction was not very significant, and no wear data were available.[9] A more promising method to improve lubrication and reduce wear of UHMWPE with hyaluronan (HA) was explored in this study.

As a natural lubricant present in all vertebrate tissues and body fluid,[10] HA can impart both biocompatibility and lubrication to the surface of hydrophobic, synthetic biomaterials. It has been used to coat various biomedical devices, such as artificial valves, intraocular lenses, vascular grafts,[11] contact lenses,[12–15] and catheters.[16] However, these coatings were not meant for load-bearing surface applications. To withstand the severe stress conditions on articular surfaces, HA molecules must be firmly anchored on and within the UHMWPE matrix.

In this study, a novel microcomposite surface was created to fix HA molecules within the UHMWPE matrix, to enhance the lubrication, and to improve the tribological properties of UHMWPE. However, HA cannot be directly introduced into UHMWPE due to its extreme hydrophilicity. A silylated derivative of HA was synthesized first to increase its compatibility with UHMWPE.[17] Instead of starting with a solid bulk material, an UHMWPE preform with a porous surface layer was used as a starting point in the present study to easily combine the HA with the UHMWPE. The pores in UHMWPE preforms are interconnected and tens of microns in size and so the finally molded material is called a microcomposite. The main purpose of this article (Part I) is to present the synthesis conditions that lead to a uniform, durable HA film layer on the surface of UHMWPE and to characterize the chemical and physical properties of the new material. The mechanical and tribological (which are improved compared to conventional UHMWPE) properties of this new material will be reported in Part II.

MATERIALS AND METHODS

Materials

Sodium hyaluronate (HyluMed®, medical grade, MW: 1.36×10^6 Da) was purchased from Genzyme (Cambridge, MA). Cetyltrimethylammonium bromide (CTAB), hexamethyldisilazane (HMDS, 99.9%), Toluidine Blue O (TBO), and urea were obtained from Aldrich (Milwaukee, WI). Desmodur N 3200 (1,6-hexamethylene diisocyanate based polyisocyanate) was provided by Bayer (Pittsburgh, PA). Hyaluronidase lyophilized powder (Type I-S from bovine testes, 608 units/mg solid), monobasic sodium phosphate (cell culture tested), dibasic sodium phosphate (cell culture tested), and sodium chloride solid (cell culture tested) were purchased from Sigma (St. Louis, MO). Silylation grade dimethylsulfoxide (DMSO) was purchased from Pierce (Rockford, IL). Xylene and acetone were purchased from Fisher (Pittsburgh, PA), dried by refluxing over Na and anhydrous $CaSO_4$, respectively, and distilled just before use. Ethanol (ACS/USP grade) was obtained from Pharmco (Brookfield, CT). All chemicals were used as received unless specified. UHMWPE porous disk preforms (Φ 1.5″ and 4″) with different porosities (20 and 40%) were made from GUR 1020 resin via a porogen or sintering process,[18] and were provided by DePuy Orthopaedics (Warsaw, Indiana). The interconnected pore size of the samples was ~1–20 μm (for measurement method of pore size, refer to Ref. 18).

Silylation of HA

The details of HA silylation reaction have been reported elsewhere.[17] Only a brief description is given here. Sodium hyaluronate was precipitated with CTAB from aqueous solution. The white precipitate (HA-CTA) was dried, and then silylated with HMDS. The reaction was carried out in DMSO under N_2 flow at 70°C for 24 h. In the resulting two-phases of the solution, the upper HMDS layer contained silylated HA-CTA, which was separated from the bottom DMSO layer, and dried under vacuum to remove HMDS, yielding light yellow silyl HA-CTA powder.

Preparation of crosslinked silyl HA-CTA powder

Silyl HA-CTA xylene solution (50 mg/mL, 5 mL) was charged into a small vial, and 2 mL of 2% (w/v) Desmodur N 3200 acetone solution was added under dry N_2. The solution mixture was shaken for 5–10 min, and left to stand at room temperature for 1 day. The precipitate was filtered from the solution, washed with acetone several times, and then vacuum dried at 50°C for 24 h. The resulting powder was used for FTIR and thermal gravimetric analysis (TGA) as described in the Characterization section.

Fabrication of UHMWPE-HA microcomposites

The general procedure for HA-UHMWPE microcomposite formation is shown in Figure 1. All the UHMWPE preforms were washed with acetone and ethanol, and then dried under vacuum before treatment. In general, the treatment included soaking in a silyl HA-CTA xylene solution, crosslinking of the silyl HA-CTA to itself, hydrolysis of silyl HA-CTA back to HA, coating with an HA water solution, and remolding (i.e., first molding is creation of porous preform structure, remolding results in fully dense structure). All the treated preforms were remolded at DePuy Orthopaedics within a high vacuum environment under a pressure of ~2200 psi at 155–160°C. All the hydrolysis was performed at 45°C for 24 h in a 0.2*M* NaCl solution of water and ethanol (v/v 1:1), unless otherwise specified (as in Treatment-3).

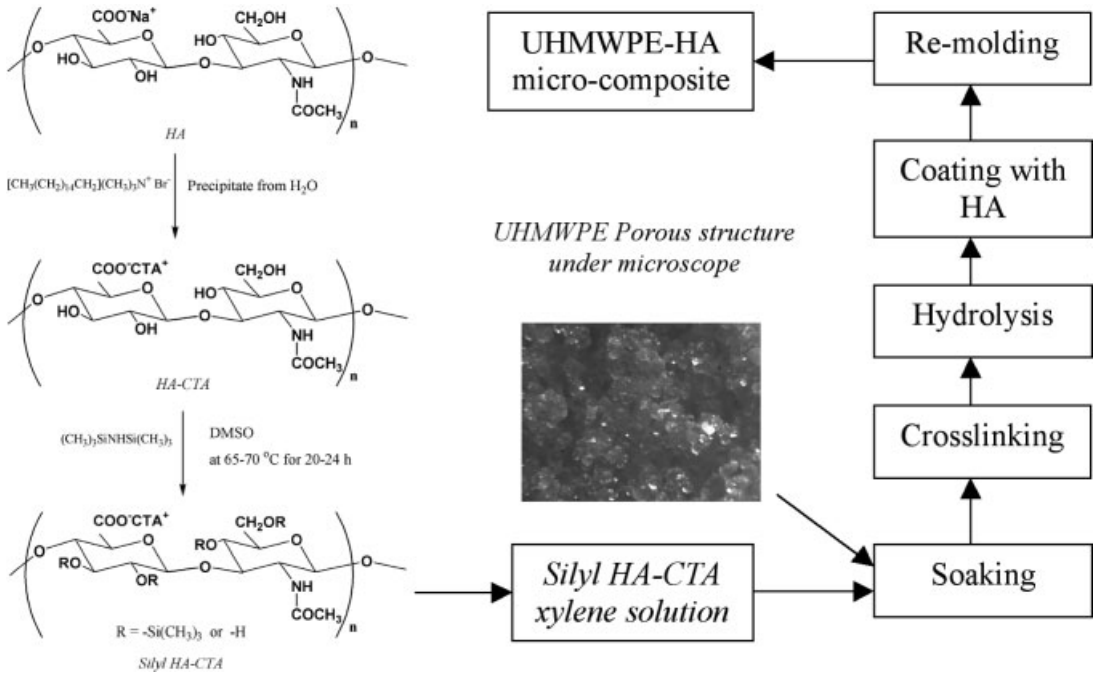

Figure 1. Formation of UHMWPE-HA microcomposite (with exception of those in Treatments-1A and -1B-see Table I).

Three different types of treatment (details summarized in Table I) were used to determine the effect of different process parameters. In Treatment-1, the UHMWPE preforms were sequentially soaked in 25, 50, and 75 mg/mL silyl HA-CTA solutions for about 10 min. Between soakings, each sample was vacuum-dried at 50°C for 1 h. After soaking and drying steps, all preforms were dipped in Desmodur solution, and then crosslinked in a 50°C vacuum oven for 2 h. The soaking and crosslinking procedure was then repeated once. All preforms were submitted to the above protocol, but differed in the following hydrolysis and remolding processes. Preforms in Treatment-1A were remolded and then hydrolyzed, but in Treatments-1B and -1C, preforms were hydrolyzed prior to remolding, and those in Treatment-1C were coated with an aqueous 0.5% HA solution for 10 min after hydrolysis, dried and crosslinked with Desmodur solution as previously described.

In Treatment-2, the preforms were first soaked in a 25 mg/mL silyl HA-CTA solution, and then crosslinked. The soaking and crosslinking operations were then repeated in 50 and 75 mg/mL silyl HA-CTA solutions. After hydrolysis, but before remolding, all samples were coated with an aqueous 1% HA solution and crosslinked with Desmodur solution.

In Treatment-3, the preforms were soaked in a 50 mg/mL silyl HA-CTA solution, and then crosslinked. Hydrolysis was carried out in a 0.2*M* NaCl solution of water and ethanol (v/v, 1:1) for 40 h, and the solution was changed every 10 h. An ultrasonic water bath was employed to assist hydrolysis, but the total time was not greater than 2 h. The hydrolyzed samples were coated with an aqueous 1% HA solution twice and then crosslinked.

The weight change of UHMWPE preforms during microcomposite fabrication was recorded (shown in Table IV). Immediately after soaking with silyl HA-CTA and crosslinking with Desmodur, hydrolysis, and final coating with HA, all the preforms were dried in a 50°C vacuum oven and weighed. The weight gain after coating with HA was considered an approximation to the HA content on the surface of microcomposites. The HA aqueous solution was very viscous and so most HA obtained through coating was concentrated on the surface.

TABLE I
Summary of Treatment Methods

	Conc. of Silyl HA-CTA	Conc. of Desmodur	Soaking and Crosslinking	Hydrolysis	Coating with HA	Conc. of HA
Treatment-1						
A	I – 25 mg/mL	1%	I → II → III	After molding	No	N/A
B	II – 50 mg/mL	1%	→ Desm.	Before molding	No	N/A
C	III – 75mg/mL	1%	2 cycles	Before molding	Yes	0.5%
Treatment-2	I – 25 mg/mL	5%	I→Desm. →	Before molding	Yes	1%
	II – 50 mg/mL		II→Desm.→			
	III – 75mg/mL		III→Desm.			
Treatment-3	I – 50 mg/mL	2%	I→Desm.	Before molding	Yes	1%

Enzyme degradation experiment

Two levels of hyaluronidase solutions, 15 and 150 units/mL, were prepared with phosphate buffer saline (PBS, prepared based on the method described in U.S. Pharmacopeia for hyaluronidase injection[19]). The buffer solution, glassware, and all supplies used for enzyme solution preparation were autoclave-sterilized.

The enzyme experiment was first performed on treated porous preforms. Twelve small squares (2.0×1.0 cm^2) were cut from 20% porosity preforms (same as those used in Treatment-1). They were divided into four groups and treated with the process of Treatment-1C. However, in this case, preforms were not consolidated (i.e. remolded) to create a worse case scenario in terms of allowing maximum access of the enzyme to the HA. Four different Desmodur concentrations (0.2, 1, 2, and 5%) (w/v) were used for each group to test the relationship between crosslinking concentration and enzymatic stability. In each group, one sample was dyed with 0.1% TBO solution (in 8*M* urea) for control, while the other two samples were exposed, respectively, to the two enzyme solutions at 37°C for 1 month, and then dyed with TBO solution.

Microcomposites (Treatments-1C and -2) were also investigated for enzymatic stability. They were dyed with 0.1% TBO solution (in 8*M* urea), and then cut into small squares (2.0×1.0 cm^2). TBO dye bound to HA on the sample surfaces was eluted with a 0.15*M* NaCl solution (in 8*M* urea) to determine the surface density of HA before degradation. Elution was performed dropwise, and continued until no more dye was visible in the runoff. The eluant was collected, and measured in volume. Before enzyme degradation, all microcomposite samples were soaked in a 0.4*M* NaCl solution for 5 days (to remove any TBO residual molecules), and then rinsed with distilled water and sterilized with ethanol.

Falcon® sterile polypropylene tubes (15 mL) were used to contain the test samples and 10 mL of enzyme solutions. All the tubes were tightly capped, and placed in a (37 ± 0.5)°C water bath for the desired exposure intervals. After washing with water and soaking in ethanol, the enzymatically degraded samples were redyed with TBO to determine the surface density of HA after degradation.

The activity of the hyaluronidase during the experiment was checked by viscosity reduction of a freshly prepared 0.1% HA aqueous solution as described in the literatures.[20,21]

Characterization

Fourier transform infrared spectroscopy

A Nicolet Magna-IR 760 spectrometer (Nicolet Instrument Corporation, WI) was used to record FTIR spectra. The crosslinked silyl HA-CTA and other sample powder (1%, w/w) was ground with KBr, and pressed into pellets for analysis. Transmission absorption spectra were collected over a range of 600–4000 cm^{-1} at a resolution of 4 cm^{-1} with 128 scans.

Thermal gravimetric analysis

The thermal gravimetric properties of the crosslinked HA-CTA and other samples were determined using a Seiko TG SCC 5200 instrument at a heating rate of 5°C/min in air.

X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) analyses were performed on a PHI 5800 spectrometer (Physical Electronics, MN). Measurements were taken at an electron takeoff angle of 45° from the surface normal (sampling depth ~50 Å). Surface elemental compositions were determined from 0–1000 eV survey scans acquired with a pass energy of 100 eV. High resolution spectra (C1s, N1s) were obtained at a pass energy of 25 eV. Small pieces were cut from the surface layer of UHMWPE porous preform and microcomposite for analysis, while HA, HA-CTA, and silyl HA-CTA were dissolved in water, DMSO, and xylene, respectively and cast into films before analysis.

Surface density of HA

The surface density of HA was determined as the amount of TBO dye over the nominal surface area of the microcomposites. The TBO molecules bound to HA were eluted with the method described in Enzyme degradation experiment section earlier. The visible absorbance of the elutant at 632 nm was measured with a Cary 500 UV–vis–NIR spectrometer (Varian Analytical Instruments, CA). A series of TBO standard solutions (10, 20, 30, 40, and 50 μ*M*) in a solution of 8*M* urea and 0.15*M* NaCl were prepared to obtain a standard calibration curve for calculating the TBO concentration in the elutant.

Aqueous contact angle analysis

Static water contact angles were measured using the sessile drop method with a Krüss DSA 10 goniometer (KRŰSS GmbH, Hamburg). Before measurement, all microcomposites were conditioned in distilled water for 4 h. At ambient temperature, a water drop (1 μL) was applied to the sample surface through the automatic dosing feature. The contact angles were determined with the circle fitting profile feature. At least five different locations on each sample surface were tested. This method was used for the contact angle measurement of microcomposites in both Treatments-1 and -2. The Treatment-3 microcomposites exhibited full hydration or a zero degree contact angle. Thus, to observe how the dry, uniform HA film on Treatment-3 samples hydrated with time, 3-μL water drops were used, and the contact angles were recorded at 1-min intervals until no data could be extracted from the system. At least three different locations were tested for each microcomposite.

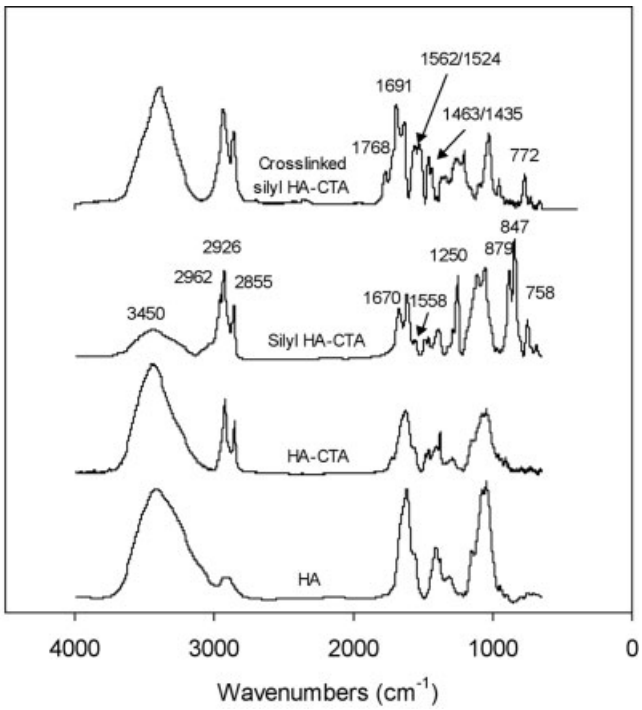

Figure 2. FTIR spectra of crosslinked and original silyl HA-CTA, HA-CTA, and HA.

RESULTS AND DISCUSSION

Silylation of HA

Each disaccharide unit of HA carries one carboxylate group (—COO^-), four hydroxyl groups (—OH), and one amide group (—CONH—) (Fig. 1) making the HA molecules extremely hydrophilic and thus, incompatible with highly hydrophobic UHMWPE. Thus, it is impossible to directly introduce native HA into the surface of UHMWPE. Those hydrophilic groups on HA molecules must be modified or masked to achieve hydrophobicity. Silylation is a typical method to impart hydrophobicity to hydrophilic polymer and organic compounds,[22,23] but HA cannot be silylated directly. By complexing the —COO^- groups on HA with long aliphatic chain quaternary ammonium cations [—$N^+(CH_3)_3CH_2$ $(CH_2)_{14}CH_3$, —CTA^+], the hydrophobicity of HA was improved. The HA-CTA complex was soluble in DMSO, making it an effective starting material for silylation. The silylated HA-CTA was soluble in xylene and completely compatible with UHMWPE, easily diffusing into the UHMWPE porous structures in solution. In Figure 1, from HA, HA-CTA, to silyl HA-CTA, the hydrophobicity increased continually with masking of the —COO^- groups first by —CTA^+, and then the —OH groups by —$Si(CH_3)_3$ until complete hydrophobicity was obtained.

To recover the hydrophilic and lubricious properties of HA, those masking groups, including —CTA and —$Si(CH_3)_3$, must be removed. It has been demonstrated that hydrolysis is an easy way to do this, and the structures of HA regenerated from HA-CTA and silyl HA-CTA were the same as that of native HA. The hydrolysis reactions are discussed in detail elsewhere.[17]

Crosslinking of silyl HA-CTA

In the UHMWPE-HA microcomposite formation process shown in Figure 1, the silyl HA-CTA was immobilized *in situ* within the UHMWPE porous preforms via crosslinking. The FTIR spectrum of crosslinked silyl HA-CTA is shown in Figure 2, and in comparison with HA, HA-CTA, and silyl HA-CTA (uncrosslinked). All peaks related with —$Si(CH_3)_3$ vibrations (758, 847, 879, and 1250 cm^{-1})[17,24,25] disappear or decrease, indicating the removal of $Si(CH_3)_3$ groups during crosslinking. Increases in the intensity of peaks at 3450, 2926/2855 and 1463/1435 cm^{-1} respectively are related to the —NH— stretching of urethane, the stretching and bending of —CH_2 groups introduced with Desmodur N 3200. The absence of a strong peak near 2260 cm^{-1} demonstrates the complete conversion of —N═C═O into urethane during crosslinking.[24] The new peaks at 1768, 1524 and 772 cm^{-1} respectively are due to the C═O stretching, —NH— in-plane and out-of-plane bending of secondary urethanes (—NH—COO—).[26,27] The amide I and II (—NHC═O) peaks of silyl HA-CTA shift to 1691 and 1562 cm^{-1} from 1670 and 1558 cm^{-1} after crosslinking.

Desmodur N 3200 is an aliphatic polyisocyanate resin based on hexamethylene diisocyanate (HDI). The reaction between Desmodur N 3200 and the unsilylated —OH residues of silyl HA-CTA should follow the regular reaction mechanism of isocyanates and alcohols to form urethane linkages, illustrated in Figure 3(a).[28] Gonda and Antalová[29] have verified that the silylated N-containing nucleophiles could undergo nucleophilic addition to isocyanates similar to their nonsilylated counterparts. The reactivity of the silylated nucleophiles depends on the stability of the Si—N bonds, and in some cases, they are more reactive than their nonsilylated counterparts. Along simi-

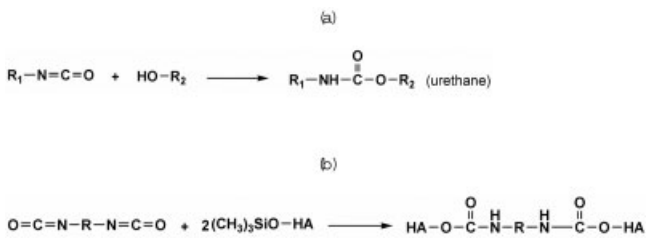

Figure 3. Reaction between isocyanates and (a) alcohols; (b) silylated hydroxyl groups of silyl HA-CTA.

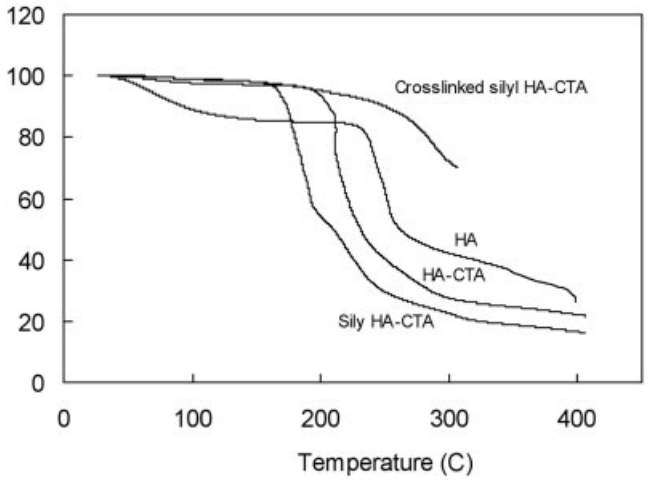

Figure 4. TGA of crosslinked and original silyl HA-CTA, HA-CTA, and HA.

lar lines of thought, the silylated O-containing nucleophiles, O—$Si(CH_3)_3$ of silyl HA-CTA, may also react with isocyanates. The disappearance or decrease of —$Si(CH_3)_3$ vibration related peaks and the appearance of urethane vibration related peaks in the FTIR spectrum of crosslinked silyl HA-CTA demonstrates that the crosslinking reaction occurring between O—$Si(CH_3)_3$ groups and isocyanates is as shown in Figure 3(b).

The TGA curve of crosslinked silyl HA-CTA is shown in Figure 4. Compared with silyl HA-CTA, HA-CTA, and HA, the thermal stability of crosslinked silyl HA-CTA is significantly increased. In the uncrosslinked form, derivatization of the HA reduces its thermal stability. Thus, after hydrolysis and removal of —CTA groups, the crosslinked HA should be even more heat resistant, and thus able to withstand the remolding temperatures used on the UHMWPE preforms.

UHMWPE-HA microcomposites

The existence of HA on the surface of UHMWPE-HA microcomposites was confirmed with XPS analysis and TBO dye assay. Table II shows the XPS elemental analysis results of the UHMWPE control, the various intermediates, and one of the microcomposites (T1C-20). Nitrogen, at similar levels to that found in HA, was found at the surface of the microcomposite, but none was detected in either the UHMWPE preform or molded control disk, demonstrating the presence of a thin layer of HA on the microcomposite surface. The amount of nitrogen beyond that found in HA might come from urethane crosslinking linkage or from the —CTA residue, which was not completely removed during hydrolysis. The presence of —CTA residue was demonstrated by the N1s spectrum of this microcomposite as shown in Figure 5(a). Compared to the native HA [Fig. 5(b)], a new peak component appeared at 402.8 eV, which was due to ammonium salt N^+ introduced with the —CTA.[30] However, the intensity of this peak was very weak, indicating the removal of most —CTA groups during hydrolysis. To completely remove —CTA groups, the hydrolysis conditions should be improved, for example, using ultrasound as done in Treatment-3. The silicon detected in the microcomposite might possibly come from both contamination and unhydrolyzed —$Si(CH_3)_3$ residue. However, it is most likely to be from laboratory contamination, such as vacuum grease and elastomer gloves, because even in the UHMWPE solid control, a significant amount of silicon was found.

The C1s spectrum of the UHMWPE preforms [Fig. 6(a)] can only be split into two components: C0 (CH_x, C—C; 285.0 eV, reference) and C1 (C—O, C—N; 826.5 eV).[31] C0 of the preforms is very strong, meaning the $(CH_2—CH_2)_n$ component still predominates. The weak C1 peak may be due to residue of the pore-forming agent (porogen), for example polyethylene oxide, used by DePuy to make the porous preforms. In the C1s spectrum of the microcomposite [Fig. 6(b)], two new components, C2 (O—C—O, C═O; 287.8 eV) and C3 (—O—C═O, —HN—C═O; 289.2 eV),[31] are observed. They are respectively assigned to O—C—O and —O—C═O/—HN—C═O of HA, indicating the presence of HA on the surface of microcomposite.

The incorporation of HA on the microcomposite surface was also demonstrated by dye assay. TBO is a cationic dye, which can bind negatively charged

TABLE II
XPS Elemental Analysis Results

	C (%)	O (%)	N (%)	Si (%)	Na (%)	Cl (%)	F(%)	Br (%)
HA	57.8	35.9	3.2	0	3.0	0	0	0
HA-CTA	80.8	12.9	4.3	0	0	0.4	0	1.7
Silyl HA-CTA	65.6	20.4	2.7	11.4	0	0	0	0
UHMWPE preform	97.2	2.8	0	0	0	0	0	0
Molded UHMWPE (control)	77.9	14.0	0	6.6	0	1.0	0.5	0
Microcomposite (T1C-20)	71.8	22.4	3.7	2.1	0	0	0	0

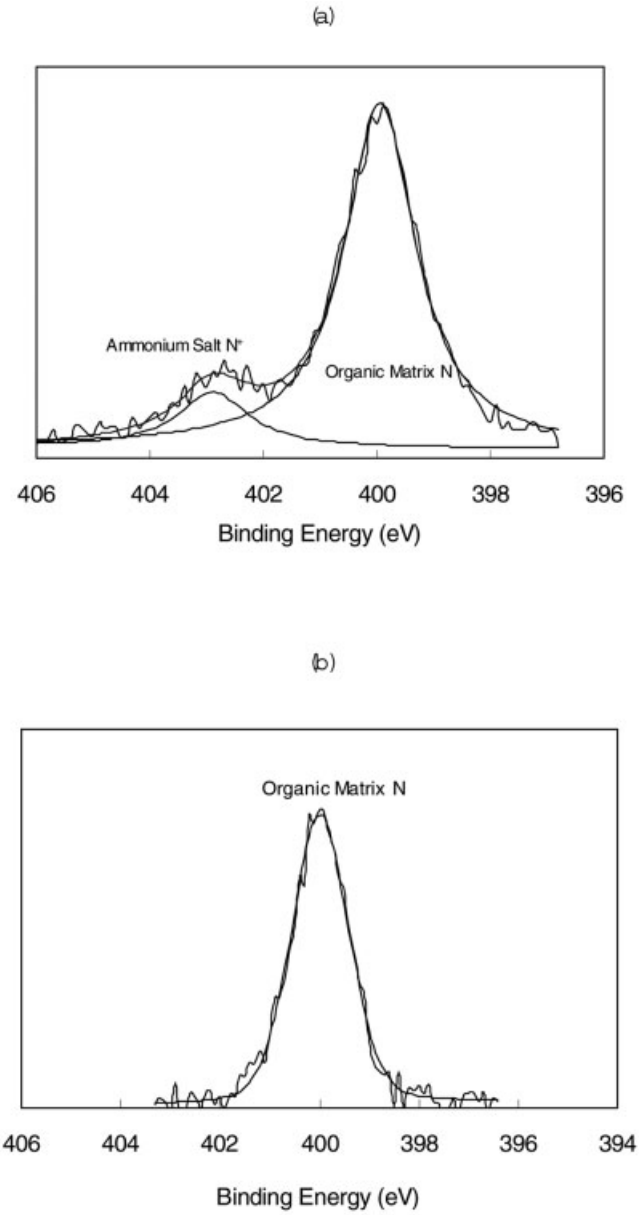

Figure 5. XPS N1s spectra of (a) microcomposite surface (T1C-20); (b) native HA.

groups on the polymer, such as sulfate groups on heparin and carboxyl groups on HA and so it is often used to visualize or quantify polysaccharide in coatings and tissue sections.[32] After soaking in TBO solution for several minutes, the UHMWPE-HA microcomposite surface exhibited a dark to light purple color depending on the surface density of HA, which will be discussed in detail. However, the UHMWPE preforms and solid control were not dyed by the TBO solution. Although they have significant amounts of oxygen on their surfaces (Table II), they remained undyed, indicating that their oxygen is not that in carboxylate groups (—COO^-).

The effect of treatment conditions on the surface properties of microcomposites

Aqueous contact angles, the surface density of bound HA and enzyme resistance were used to characterize the quality of the microcomposite surface and screen the treatment parameters. The amount of TBO eluted from the dyed microcomposite surface represented that of bound HA. In a 8*M* urea solution, TBO binds a carboxyl group at a 1:1 molar ratio,[32] while each HA repeat disaccharide unit has just one carboxyl group and so the TBO amount can be directly used to calculate the surface density (nmol/cm^2) of bound HA. The binding between TBO and HA is a simple ion exchange equilibrium process and so

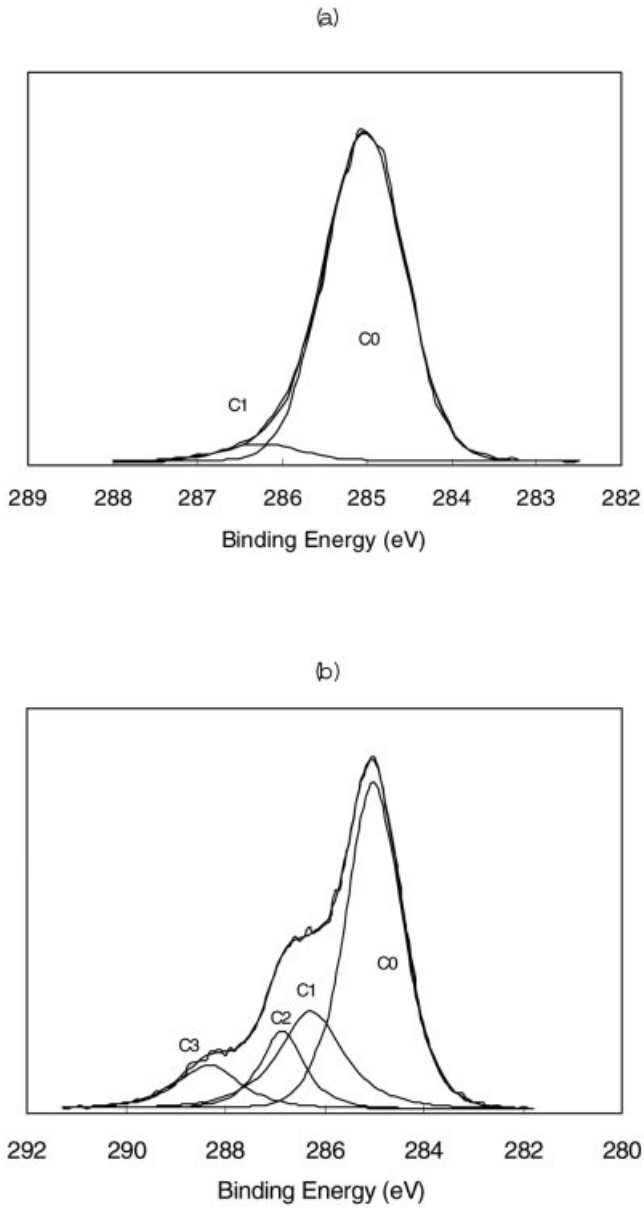

Figure 6. XPS C1s spectra of (a) UHMWPE preform and (b) microcomposite surface (T1C-20).

TABLE III
Surface Properties of Microcomposite in Treatment-1 and -2

	Surface Density of HA (nmol/cm^2)	Aqueous Contact Angle (°)
Control	0	91.20 ± 1.64
T1A-20	6.31	46.15 ± 5.53
T1B-20	34.01	32.44 ± 3.18*
T1C-20	36.17	26.77 ± 6.28*
T2–20-1	N/A	51.66 ± 7.95**
T2–20-2	N/A	59.01 ± 7.98**
T2–40-1	N/A	50.02 ± 26.86**
T2–40-2	N/A	55.59 ± 16.87**

[a]* or ** groups are not significantly different ($p \geq 0.05$).
[b]Nomenclature: T1A-20 for the microcomposite with treatment-1A and 20% porosity preform. T2–40-1 for the microcomposite with treatment-2 and 40% porosity preform.

strong salts, such as NaCl, can be used to remove TBO from the dyed microcomposite surface. TBO has a strong absorption at 632 nm in a 8*M* urea solution and shows a linear relationship with its concentration within 50 μ*M* and so the urea solution was used with NaCl to elute bound TBO.

For total joint replacement applications, HA film formed on the microcomposite surface should be durable in a physiological environment. Hyaluronidases are enzymes that degrade HA. The presence of hyaluronidases in synovial fluid and its activity against HA have been demonstrated,[20] although significant HA degradation in synovial joints has not been observed.[33] The enzyme concentrations used in this study are the same as those used by Lowry and Beavers[21] for their HA coatings. The two enzyme levels, 15 and 150 units/mL, were respectively six and 60 times the hyaluronidase concentration present in human serum.[34] The enzyme degradation environment used here should be much more severe than the human synovial fluid environment, because hyaluronidase activity in synovial fluid is much lower than that in human serum.[20]

In Treatment-1, the effects of hydrolysis timing (i.e. before or after remolding) and coating with native HA solution after hydrolysis on the microcomposite surface properties were investigated. The results of HA surface density and contact angle of the microcomposites with this treatment are listed in Table III. The relationship between the contact angle and HA surface density is apparent: with increasing HA surface density, contact angles decrease. Microcomposites T1B-20 and T1C-20, hydrolyzed before remolding, exhibited significantly lower contact angles than microcomposite T1A-20, hydrolyzed after remolding. The regenerated, crosslinked HA within T1B-20 and T1C-20 preform pores after hydrolysis had good high temperature resistance and high hydrophilicity. This prevented HA from degrading and UHMWPE from overflowing and masking HA on the surface during remolding. Thus, the HA surface density of T1B-20 and T1C-20 was much higher than that of T1A-20. Furthermore, the trimethylsilyl residues were easily removed from the microcomposites hydrolyzed before remolding. Although microcomposite T1C-20 is not significantly lower in contact angle than T1B-20, it appears that soaking with an HA solution after hydrolysis, and subsequent crosslinking of the coating, increases the surface density of HA. If an HA solution with higher concentration is used, the effect may become significant.

However, visual observation and TBO dye assay of the HA film on the surface of microcomposites T1B-20 and T1C-20 indicated that the films were not uniform and so process modification was necessary to improve the surface HA film uniformity. The possible methods include crosslinking after each soaking with silyl HA-CTA solution, increasing the HA concentration of the final soaking, and using a higher porosity preform. The effects of these methods were examined in Treatments-2 and -3.

Compared to Treatment-1, process changes in Treatment-2 included: (1) use of preforms with higher porosity (40%), (2) crosslinking silyl HA-CTA after each soaking, (3) crosslinking with 5% Desmodur solution, and (4) final soaking with 1% HA solution. The 5% Desmodur concentration was selected based on the enzyme degradation experiment performed on porous preforms. It was found that the porous preform crosslinked with 2 and 5% Desmodur solution still had a uniform HA film after degradation for 1 month in both hyaluronidase solutions, which was comparable to that of their controls. However, for those preforms crosslinked with 0.2 and 1% Desmodur solution, the HA film was peeled off in small local regions after degradation.

Aqueous contact angles for Treatment-2 microcomposites are also listed in Table III. No significant differences are found between all the microcomposites through ANOVA. The results for this batch of microcomposites are not desirable, and are inferior to the results obtained in Treatment-1. One possible reason was the high crosslinker concentration, which might consume too many —OH groups of HA, decreasing the surface density of polar groups. The multiple soaking and crosslinking operations also resulted in a very high silyl HA-CTA content in the porous preforms (Table IV), especially in the 40% porosity preforms. It was very difficult to completely hydrolyze so much silyl HA-CTA within very small pores. The molecular weight of the silyl HA-CTA repeat unit (951.6) is more than two times that of the HA repeat unit (401.3) and so after complete hydrolysis, the weight change of the samples should decrease to less than half of that after soaking and crosslinking. However, data in Table IV show that the weight after hydrolysis for these sam-

TABLE IV
Weight Gain ($\Delta w/w_0$, %) of UHMWPE Preforms During Treatment

	After Soaking and Crosslinking	After Hydrolysis	After Coating with HA
Treatment-1			
T1A-20	5.55		
T1B-20	5.06	0.35	
T1C-20	5.00	0.40	1.54
Treatment-2			
T2–20-1	2.97	2.27	2.68
T2–20-2	1.98	1.46	1.64
T2–40-1	8.50	6.35	8.00
T2–40-2	15.94	11.02	13.6
Treatment-3			
T3–40-1	6.36	1.10	3.31
T3–40-2	3.88	1.12	2.51
T3–40-3	5.60	0.83	1.80
T3–40-4	8.66	1.63	5.03
T3–40-5	6.10	0.81	3.39

w_0, Original weight of UHMWPE preforms; Δw, weight change of treated preforms in comparison with w_0.

ples is much larger than expected, indicating an incomplete hydrolysis. The silyl HA-CTA residue was compatible with UHMWPE, and was easily covered by flowing UHMWPE during remolding. This may be the reason that the 40% porosity samples have more scattered contact angles, while the 20% porosity samples have a more uniform film of HA. In addition, the unhydrolyzed silyl HA-CTA easily decomposed at the molding temperature. This is demonstrated by the much more severe localized discoloration in the 40% porosity samples. Too much HA might also prevent the full consolidation of UHMWPE, resulting in a poorly consolidated final product. Based on the above analysis, it can be seen that lower silyl HA-CTA content within the porous preform and high HA content on the surface are necessary to obtain a material with good comprehensive properties.

To solve the above problems, the following changes were performed in Treatment-3: (1) soaking and crosslinking just once, (2) crosslinking with 2% Desmodur solution, (3) application of ultrasound during hydrolysis, (4) coating with HA solution twice and crosslinking. Selection of 2% Desmodur concentration was based on the enzyme experiment of both preforms and microcomposites. The HA surface density for the microcomposites before and after degradation is listed in Table V. No significant decreases in HA density were found for all the tested microcomposites after degradation, including the T1C-20, crosslinked with 1% Desmodur. They were still lubricious, water-wettable, and showed no staining difference when compared to the surfaces before degradation. Therefore, 2% Desmodur concentration should be sufficient to generate an enzyme resistant film.

TABLE V
HA Surface Density (nmol/cm^2) of Microcomposites before and after Degradation by Hyaluronidase

	Enzyme Conc.	10-day degradation		30-day degradation	
Microcomposite	(units/mL)	Before	After	Before	After
T1C-20	15	32.0	41.6	39.5	41.5
	150	37.4	32.5	41.8	44.5
T2–20-2	15	21.7	27.4	33.4	22.3
	150	38.4	33.3	36.3	31.1

The microcomposites made with Treatment-3 have a moderate silyl HA-CTA (i.e., that ends up as crosslinked HA within the microcomposite) and high surface HA content (Table IV). After soaking in water, the surfaces of all samples were completely wetted by water, a uniform water-swollen layer formed at the surface. The water drop immediately spread on the surface, indicating the formation of a uniform layer of HA film on the microcomposite surface. The wetted surface could not be used for contact angle measurements due to the rapid spreading of the water drops and so a dry surface was used to observe the hydration of the surface HA film. One 40% porosity disk preform (same as those used in Treatment-2 and Treatment-3) which was remolded without any treatment was used as a control. The water drop used in this contact angle testing was 3 μL in volume. It is observed that with the hydration of HA film, the contact angles (shown in Fig. 7) of the microcomposites rapidly decrease from the initial almost hydrophobic state until the water drop evaporated. From the trend of the curves, it may be inferred that within 15–20 min, water drops should completely spread in a saturated water-vapor environment, and the HA film on the microcomposite can be completely hydrated. However, for the control sample, the decrease of contact angles with time was very slow, and equilibrated around 65°.

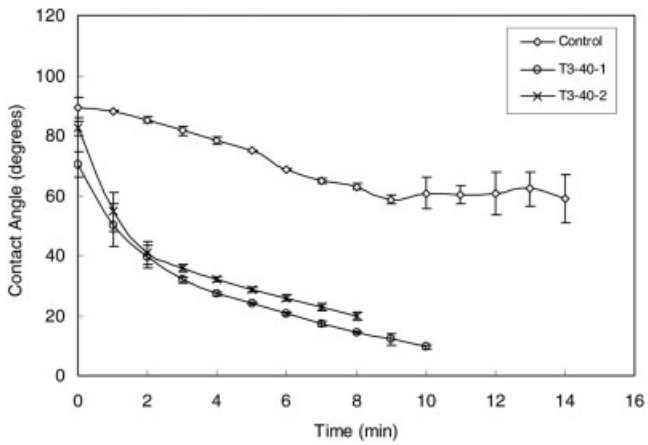

Figure 7. Kinetics of aqueous hydration of microcomposite surface.

CONCLUSIONS

A new microcomposite between HA and UHMWPE was synthesized at the surface of UHMWPE. With properly tailored processes, a layer of uniform and enzyme-resistant HA film formed on the surface that was hydrophilic and completely water-wetted. The following conclusions can be drawn from this study.

XPS analysis and TBO dye assay demonstrated the presence of HA on the surface of the microcomposite. In comparison with the control, the contact angles of the appropriately treated microcomposites significantly decreased, and the degree of decrease was related to the surface density of HA. The higher the surface density of HA, the lower the contact angles of the microcomposites. The Treatment-3 groups were completely water-wetted.

Hydrolysis before remolding was necessary to completely remove all modification groups (e.g. —CTA and —$Si(CH_3)_3$), and to effectively prevent the overflow of UHMWPE and keep the HA exposed at the microcomposite surface. Furthermore, soaking with a native HA solution after hydrolysis and before remolding helped to improve the HA surface density.

The concentration of the Desmodur crosslinking solution must be moderate to obtain a lubricious and stable HA film. High crosslinker concentration consumed too many polar groups, causing higher contact angles, while too low crosslinker concentration led to a sparse HA network, which was not strong enough to resist enzymatic-degradation. The 2% Desmodur solution seemed a good compromise between these extremes.

A small to moderate silyl HA-CTA content within UHMWPE preforms and high surface HA content (obtained through soaking with HA solution after hydrolysis) were desirable to generate a uniform layer of HA film on the microcomposites. Short periods of ultrasonic treatment were helpful in completely removing the modification groups of silyl HA-CTA during hydrolysis.

The mechanical and tribological properties of the microcomposites are presented in Part II. This study confirms mechanical integration between the microcomposite surface layer and the solid UHMWPE substrate, and demonstrated that the addition of HA reduces the wear of UHMWPE by ~40%.

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