JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Communication
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# A Large-Molecular-Weight Polyanion, Synthesized via Ring-Opening Metathesis Polymerization, as a Lubricant for Human Articular Cartilage

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Supporting Information

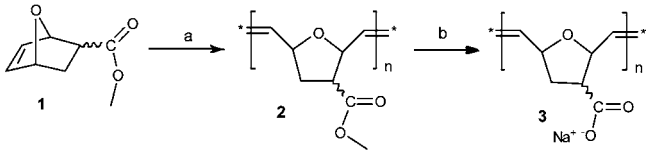
**ABSTRACT:** A large-molecular-weight polyanion is found to possess lubricating properties for cartilage. The polyanion, sodium poly(7-oxanorbornene-2-carboxylate), is synthesized by ring-opening metathesis polymerization of methyl 5-oxanorbornene-2-carboxylate. When dissolved in aqueous solution and applied to the surface of human cartilage it reduces the friction at the interface and acts as a lubricant. Its performance is similar to that of synovial fluid and superior to those of saline and Synvisc in an *ex vivo* human cartilage plug-on-plug model. The polymer is also not readily degraded by hyaluronidase or cytotoxic to human chondrocytes *in vitro*. As such, this polymer is a new type of viscosupplement, and the results provide insight into the design requirements for synthesizing highly efficacious synthetic biolubricants.

Ring-opening metathesis polymerization (ROMP) using ruthenium carbene catalysts is a reliable and efficient method to synthesize well-defined polymers with relatively low polydispersity indexes (PDIs).[1−13] These polymers, mostly synthesized from norbornenes and cyclopentadienes, are of widespread interest since diverse functionalities and architectures can be prepared. For pharmaceutical applications, chemotherapeutic or antibacterial agents can be attached to create bioactive polymers.[14−22] However, the macroscopic or bulk properties of these polymers may also provide opportunities to design and evaluate functional medical devices, an area less explored. During our preliminary research, we found that large-molecular-weight polymers of poly(7-oxanorbornene-2-carboxylic acid) in aqueous solution were lubricious. Building on this observation, we hypothesized that this polymer may have efficacy as a synthetic biolubricant for cartilage surfaces. Herein, we report the rheological and frictional properties, cytotoxicity, and lubrication performance in human *ex vivo* cartilage-on-cartilage surfaces of sodium poly(7-oxanorbornene-2-carboxylate).

Synovial fluid is a clear, viscous liquid that acts as a lubricant and shock absorber for the cartilage surfaces of the joint. Hyaluronic acid (HA) and lubricin, two components of the synovial fluid, are both responsible for lubricating joints, with HA contributing to the lubrication, shock absorption, and viscoelastic properties of the synovial fluid.[23−26] In functionally compromised joints, such as those afflicted with osteoarthritis, it is known that the concentration and molecular weight of HA have decreased. In fact, one of the current treatments for knee osteoarthritis is injection of HA or cross-linked HA to replenish the HA and afford better joint performance (i.e., viscosupplementation).[27,28] For example, Synvisc, a currently used viscosupplement, is a 0.8% cross-linked HA formulation of molecular weights ranging from 5 to 7 MDa. There is significant controversy surrounding the use of intra-articular injections of HA due to its limited clinical efficacy, susceptibility to enzymatic degradation, and short residence time ($t_{1/2}$ = 24 h) in the synovial joint.[29−31]

The cyclic, hydrophilic polymer containing pendant carboxylate groups, **3**, was synthesized via ROMP of methyl 5-oxanorbornene-2-carboxylate (**1**)[32] using the ruthenium catalyst, [1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]-dichloro(phenylmethylene)(tricyclohexylphosphine) (Scheme 1).[12,33] The monomer-to-catalyst ratio was varied to afford

**Scheme 1Synthesis of Sodium Poly(7-oxanorbornene-2 carboxylate)**[a]




[a]Key: (a) (i) $Ru(PPh_3)_2(Cl)_2(CHPh)$, benzene, $CH_2Cl_2$, 25 °C, 4 h, 80−95%; (ii) ethyl vinyl ether, quant. (b) THF, NaOH, 2 days, 90−90%.

polymers **3a**, **3b**, and **3c**, of molecular weights 2 500 000 (PDI = 1.2), 3 200 000 (PDI = 1.3), and 3 700 000 (PDI = 1.4) g/mol, respectively (Table 1). The polymers were subsequently saponified and dialyzed against a 3400 $M_w$ cutoff at pH 7.4 to afford the product as the sodium salt. As such, these polymers belong to a family of acidic oxanorbornene polymers containing pendant carboxylic acids.[34−36] This molecular weight range was chosen in order to retard diffusion through the synovial

**Received:** January 21, 2013
**Published:** March 15, 2013

   dx.doi.org/10.1021/ja400695h | *J. Am. Chem. Soc.* 2013, 135, 4930−4933

**Table 1. Rheological Properties of the Polymers (Average ± SD; $n$ = 3)**

| sample | $G'$ (Pa) | $G''$ (Pa) | delta |
|---|---|---|---|
| 2.5 MDa, 2% w/v (**3a**) | 14 ± 2 | 15 ± 2.3 | 47 ± 2 |
| 2.5 MDa, 1% w/v (**3a**) | 3 ± 0.8 | 5 ± 1.2 | 59 ± 2 |
| 3.2 MDa, 2% w/v (**3b**) | 10 ± 1.6 | 14 ± 2.1 | 45 ± 2 |
| 3.2 MDa, 1% w/v (**3b**) | 3 ± 0.9 | 4 ± 1.1 | 53 ± 2 |
| 3.7 MDa, 2% w/v (**3c**) | 25 ± 3.1 | 29 ± 3.3 | 49 ± 2 |
| 3.7 MDa, 1% w/v (**3c**) | 4 ± 1 | 7 ± 1.5 | 60 ± 2 |
| Synvisc | 80 ± 9 | 21 ± 3 | 15 ± 1 |
| synovial fluid[25] | 23 | 7 | |

membrane (>$M_w$ cutoff ≈ 500 kDa to 1 MDa) and, thus, increase residence time in the synovial space, which will be confirmed in future experiments.

To evaluate these polymers as synthetic biolubricants, we first assessed their rheological properties. The rheological properties of the 2.5 (**3a**), 3.2 (**3b**), and 3.7 (**3c**) MDa polymers as 1 and 2% w/v solutions were analyzed using a RA 1000 controlled strain rheometer (TA Instruments). The data were collected and the results are reported at a frequency of 1 Hz, since this represents a normal walking frequency in adults.[37] As expected, the rheological properties of the polymers were dependent on both %w/v and $M_w$, as shown in Table 1. Samples at a concentration of 2% w/v had higher storage ($G'$) and loss ($G''$) moduli than those at a concentration of 1% w/v. Likewise, increasing the polymer $M_w$ to 3.7 MDa from the lower values of 2.5 and 3.2 MDa resulted in a more viscous solution and larger $G'$ and $G''$ values. For comparison, Synvisc has $G'$ and $G''$ values of 80 and 21 Pa, respectively. These higher values are a result of HA being lightly cross-linked. Consequently, the delta for Synvisc is 15 compared to ∼50 for the polymers, indicating that the polymers are better able to dissipate energy away from the load-bearing surfaces. With regard to human synovial fluid, Balazs et al. reported that healthy knee synovial fluid has a $G'$ value of 23 Pa and a $G''$ value of 7 Pa.[27] Although there are some experimental differences in determining these values, it is important to recognize that these are reference values, and the values obtained with polymer **3** are within a factor of 2.

We then screened the lubricating ability of these polymers in a standard assay using metal surfaces. The coefficients of friction for the 2.5 (**3a**), 3.2 (**3b**), and 3.7 (**3c**) MDa polymers at 1 and 2% w/v solutions were determined using a RA 1000 controlled strain rheometer. A normal force of 5 N was applied to the polymer solutions, and an oscillatory frequency sweep with a controlled strain was used. The results are reported at a frequency of 1 Hz. This method measures the oscillation stress of the material, which can be converted into the coefficient of friction using the normal stress. As shown in Figure 1, the smallest-molecular-weight polymer, **3a**, gave the lowest coefficient of friction.

Next, we evaluated the lubricating properties of polymer **3a** (2.5 MDa) at 2% w/v in a more biologically relevant setup using *ex vivo* human cartilage and compared its performance to saline, bovine synovial fluid (BSF), and Synvisc (Figure 2). Briefly, a diamond-tipped cylindrical cutter was used to extract pairs of mated osteochondral plugs (7 mm diameter) from opposing articular surfaces of the patella–femoral joint of a cadaver. Each osteochondral plug pair was aligned collinearly and compressed to 18% of the total cartilage thickness for the pair at a displacement rate of 0.005 mm/s (Enduratec 3230,

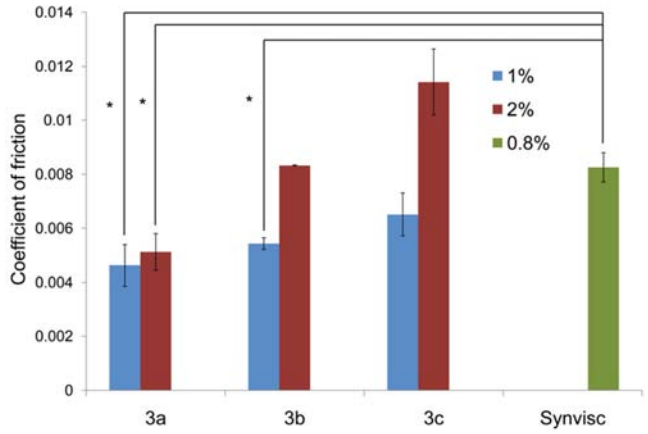



**Figure 1.** Coefficient of friction values of synthetic biolubricants at molecular weights of 2.5, 3.2, and 3.7 MDa (at 1 and 2% w/v) and Synvisc on metal surfaces ($n$ = 3, * $p$ < 0.05 vs Synvisc).

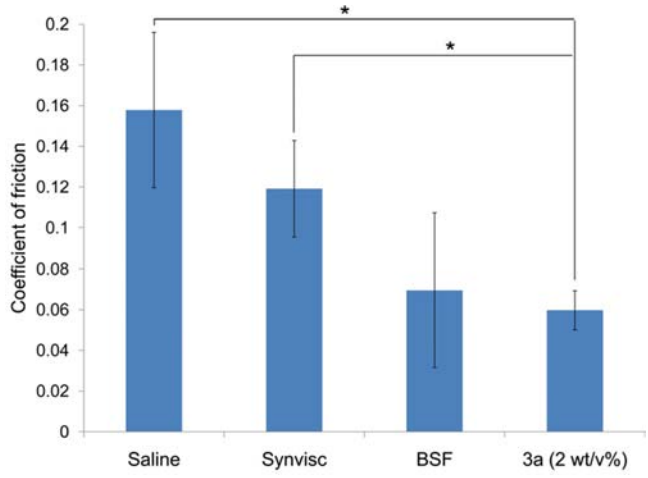



**Figure 2.** Coefficient of friction $\mu_{static}$ values for the lubricants (saline, bovine synovial fluid (BSF), **3a** 2% wt/vol, and Synvisc) on *ex vivo* human cartilage–cartilage plugs ($n$ = 6; * $p$ < 0.05 vs saline and Synvisc).

Bose). Following compression, the samples were allowed to relax for 60 min and then subjected to a relative rotation of +2 revolutions (720°) at 5 deg/s (an effective velocity of 0.3 mm/s).[38] Force, torque, displacement, and rotation data were collected. All the osteochondral cores were immersed in each of the respective lubricants before the lubrication test was performed. After the measurements, the plugs were immersed in saline for 12 h prior to exposure to the next lubricant sample. In order to minimize cartilage degradation, the samples were maintained at 4 °C and in a cocktail of antibiotics/protease inhibitors except for coefficient of friction evaluation. Each pair of osteochondral plugs was tested with all the test samples to minimize biological variation between plugs, and the pilot reproducibility studies indicated that there was no change in friction properties over 4 days of testing for cartilage plugs in saline. With regard to this experimental design, there are two caveats: (1) BSF is being used instead of human since human synovial fluid is not easily obtained, and (2) a rotational force is being applied to the cartilage surface following an established method for determining the coefficient of friction.[39] Specifically, the coefficient of friction was determined using the equation $\mu = T/RN$, where $\mu$ is the coefficient of friction, $T$ is


dx.doi.org/10.1021/ja400695h | J. Am. Chem. Soc. 2013, 135, 4930–4933

the torque, $N$ is the normal force, and $R$ is the radius of the cylindrical sample. It was found that **3a** (average $\mu_{static}$ = 0.059) provided statistically significant ($p < 0.05$) better lubrication compared to saline (average $\mu_{static}$ = 0.157) and Synvisc (average $\mu_{static}$ = 0.119). The mean $\mu_{static}$ for **3a** was 65% and 50% lower than those of saline and Synvisc, respectively. The lubrication provided by Synvisc was not statistically significant from that of saline. Similarly, BSF and **3a** were both equally adept at providing superior lubricating capabilities and were not statistically significant when compared with each other. Future studies will address the mechanism of lubrication with **3a**, but the current working hypothesis is boundary lubrication, as this large anionic polymer is not likely to penetrate into the negatively charged cartilage matrix.

As mentioned earlier, HA after intra-articular injection is degraded relatively quickly, and this may be one of the reasons that clinical studies reporting HA injection show mixed results or results similar to those obtained with the placebo saline intra-articular injection controls.[40−42] Consequently, we subjected HA, Synvisc, and **3a** to hyaluronidase. After 24 h, the samples were analyzed by gel electrophoresis. Both Synvisc and HA were degraded by hyaluronidase whereas **3a** was not degraded by the enzyme, consistent with its lack of glycosidic linkages (see SI). Finally, we evaluated the cytotoxicity of polymer **3a** against human chondrocytes *in vitro*. No significant toxicity was observed, as there was minimal difference (∼10%) between the treatment and control groups (see SI).

In summary, ROMP has been used to synthesize high-molecular-weight polyanions as biolubricants for cartilage surfaces. The polymers possess a range of rheological properties that can be modified by varying both molecular weight and concentration. Moreover, when polymer **3a** is used as a lubricant at the cartilage surface it reduces the friction at the interface, and it performs similarly to synovial fluid in an *ex vivo* human cartilage model. As such, this polymer may have utility as a new type of viscosupplement. Additional *ex vivo* and *in vivo* tests are planned to further evaluate its performance. These encouraging results further support the investigation of different polymer compositions and architectures to better understand the key characteristics required for lubricity as well as to synthesize an optimal structure for efficient lubrication of cartilage surfaces.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

Synthesis, characterization of polymers, cytotoxicity, and degradation. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare the following competing financial interest(s): M.W., B.D.S., and M.W.G. have a conflict of interest as a small company has licensed the BU technology around a synthetic biolubricant.

## ■ ACKNOWLEDGMENTS

This work was supported in part by Boston University and the Coulter Foundation.

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