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# Enhanced Lubrication on Tissue and Biomaterial Surfaces through Peptide-mediated Binding of Hyaluronic Acid

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## Abstract

Lubrication is key for the efficient function of devices and tissues with moving surfaces, such as articulating joints, ocular surfaces and the lungs. Indeed, lubrication dysfunction leads to increased friction and degeneration of these systems. Here, we present a polymer-peptide surface coating platform to non-covalently bind hyaluronic acid (HA), a natural lubricant in the body. Tissue surfaces treated with the HA-binding system exhibited higher lubricity values and *in vivo* were able to retain HA in the articular joint and to bind ocular tissue surfaces. Biomaterials-mediated strategies that locally bind and concentrate HA could provide physical and biological benefits when used to treat tissue-lubricating dysfunction and coat medical devices.

Lubrication, a process that reduces the resistive force between two opposing surfaces and decreases friction and wear[1], is key to function of a number of industrial technologies, including vehicular engines, wind turbines and hard drives[2]. Here, chemists join with engineers to design surfaces that work together with liquid lubricants to achieve low-friction systems. Lubrication between tissues is also important to maintain low-friction movement within a number of biological systems, including the pleural cavity, the surface of the eye, visceral organs, and diarthroidal joints[3]. In diarthroidal joints, healthy, painless movement is facilitated both by molecules at the tissue surface and in the lubricating synovial fluid. Synovial fluid bathes the joint surface with several molecules that contribute to lubrication, including lubricin[4,5], surface active phospholipids[6–8] and hyaluronic acid (HA)[9]. The role of each of these components has been supported and challenged on the basis of various *in vitro* studies on cartilage lubrication[9]; however, in a healthy joint, these molecules work synergistically and with the tissue surface to reduce friction coefficients in lubrication to achieve normal physiological performance[9–11]. Similarly, insufficient lubrication on the ocular surface is a key component of dry eye disease, where the ocular surface and tear fluid



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**Author contributions.** The experiments were designed by A.S., M.C., S.A.U, P.M. and J.H.E, and carried out by A.S., M.C., S.A.U. Preliminary *in vitro* studies were performed by S.A.U. and K.A.W. Data analyses were performed by A.S., M.C. and J.H.E. The manuscript was written by A.S. and J.H.E.

should work synergistically[12]. Medical devices employed in certain tissues, such as contact lenses for ocular surfaces, generally lack lubrication, hampering their integration with the body and their function. Today, therapeutic options to enhance tissue and device lubrication focus only on replacing or enhancing the lubricant in the fluid phase, severely limiting functional capability and longevity. Taking cues from industrial applications and the normal tissue-fluid interface, we designed a biomimetic system for tissue and biomaterial surfaces to work synergistically with fluid phase biological lubricants.

To develop a biomimetic surface modification, HA-binding peptides (HABpep), discovered through phage display[13–17], were covalently or non-covalently bound to surfaces via a heterobifunctional poly(ethylene glycol) (PEG) chain. The HABpep-polymer system noncovalently binds HA, either endogenously available in the local fluid environment or provided exogenously, to the modified surface. We hypothesized that localizing HA to tissue surfaces via HABpep would provide the physical benefits of increasing cartilage lubrication to the cartilage and increase retention of HA in the articular joint. Binding HA to the cartilage tissue mimics one of the functions of the lubricin protein[18] that is normally found on the surface of cartilage and that is altered during disease[19].

In addition to its lubrication role, HA has a number of potential biological functions that would be ideal to concentrate at material and tissue surfaces, including reducing inflammation, mediating matrix metalloproteinase expression and protecting cells from free-radical damage[20–22]. Coating surfaces with HA may also physically protect the surfaces from cytokines and degrading enzymes that are frequently found in a diseased, post-traumatic or surgical environment. Finally, and most critically, the presence of the polymer-HA binding modification provides a biomimetic mechanism to concentrate HA on the surface. Numerous endogenous enzymes and reactive oxygen species can degrade HA and its fluid concentrations can quickly decrease with normal turnover via local transport process[22]. The HABpep can capture HA that is found in low concentrations in a diseased environment or lost through a physical or biological mechanism, and provide the stable anchor on the tissue surface that is necessary to dynamically bind and concentrate HA where it is needed.

## HA-binding to cartilage

The HA-binding strategy was first applied to treat the surfaces of osteoarthritic cartilage (Figure 1a). Because osteoarthritis is so common and debilitating, there is a significant interest in understanding and treating the disease. Joint lubrication is one of the key disease components that may be addressed to improve overall joint health and reduce the progression of cartilage degeneration[6]. Healthy articular cartilage provides low-friction properties to the synovial joint through a combination of lubrication mechanisms, including interstitial fluid pressurization and boundary lubrication[9]. Pressurized fluid, within the tissue and between the surfaces, can bear significant levels of load. In addition, lubricant molecules, such as HA, lubricin and phospholipids on the articular cartilage surface provide functions that include boundary lubrication and mediating load bearing[8,9]. Osteoarthritic knees are challenged by altered and abnormal structural and compositional changes that include depleted and disrupted boundary lubricants[23,24]. Unless addressed, these changes

can further increase surface friction, accelerate degeneration of cartilage, and lead to abnormal joint motion.

HA is a component of the native cartilage tissue matrix and is present in the synovial fluid. HA in the synovial fluid works synergistically with the tissue surface and molecules such as lubricin to provide wear protection and improve joint lubrication in addition to biological functions[18,25,26]. For example, high molecular weight HA reduced cartilage degeneration in a rabbit model, possibly through restoration of the viscoelastic properties of synovial fluid[27–29]. In addition, researchers have suggested potential biological functions of HA in the joint in *ex vivo* models, such as protecting chondrocytes from oxidative stress, reducing inflammation and promoting chondrocyte cell survival[30–32]. As a result, one common clinical treatment for OA is injection of HA directly into the joint to improve synovial lubrication. Despite the importance of HA in the normal joint and its attributes, clinical results of HA injections, also known as viscosupplementation, have been controversial with some clinical studies showing benefits while others not achieving statistically significant efficacy[33–36]. This lack in efficacy of injectable HAs may be due in part to the clearly observable rapid turnover of HA molecules within the joint after injection, use of nonphysiological crosslinked HA, and the limited ability to target the regions of tissue where increased lubrication is needed. While we have previously targeted cartilage reconstruction[37,38], engineered cartilage lacks normal lubrication properties[6] and the tissue surface is an ideal focus for therapeutic intervention to prevent the development of OA and treat existing disease by protecting the underlying tissue from exposure to physical and biological elements.

To create a HABpep coating, a bifunctional PEG (N-hydroxysuccinimide-PEG-maleimide, or NHS-PEG-MAL) was attached to the amine functionality-rich tissue surface via an amine-NHS reaction, which was further linked to HABpep via a MAL-thiol reaction (Fig. 1b). PEGylation of the cartilage surface coating was confirmed by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. Polymer-modified surfaces produced spectra that indicated a large peak at ~1066 $cm^{-1}$, consistent with the ether bonds of the PEG spacer that was not present on unmodified cartilage (Fig. 1c). Additionally, X-ray photoelectron spectroscopy (XPS) spectra of the PEGylated cartilage compared to native cartilage demonstrated a significant decrease in nitrogen content and a drop in the carbon to oxygen ratio (closer to 2, the ratio in PEG), indicating that a synthetic, lower-nitrogen containing layer had been successfully grafted to the cartilage surface (Fig. 1d). HABpep functionalization on the tissue explant and its ability to bind HA was confirmed by positive staining on incubation with biotin hydrazine-labeled HA and streptavidin-conjugated horseradish peroxidase (Fig. 1e). Direct covalent binding, while useful for device coatings, will be difficult to implement clinically.

## Non-covalent tissue binding

Considering the translation of the materials strategies to *in vivo* applications, we developed an additional chemical strategy to introduce the surface coating in a single-step application that can be employed alone or with existing viscosupplementation technologies. In contrast to reacting amine groups of the cartilage tissue with a reactive functionality (*e.g.,* NHS), we

targeted the collagen molecules of the tissue surface as an anchor for the HABpep coating. A collagen binding peptide (ColBpep) was employed to non-covalently bind to collagen in tissues, creating a coating of HABpep on the surface. A thiolated PEG spacer was linked to HA binding peptide (Fig. 2a) followed by the Michael addition reaction of thiol functional groups with vinyl dimethyl azlactone. PEG with HABpep and azlactone functionality was further conjugated to a peptide that non-covalently binds to the molecules present in the target tissue, *e.g.*, collagen II present in the articular cartilage. $^{1}$H-NMR and FTIR-ATR spectra (Supplementary Figs. S1a,b) confirmed the functionalization of PEG with azlactone (~2.9 ppm of $CH_2$ of thioether and~1750 $cm^{-1}$ of FTIR-ATR spectrum) and collagen binding peptide (6.5–7.5 ppm of $^{1}$H-NMR spectrum). This methodology is based on a specific non-covalent interaction that does not undergo hydrolysis; therefore, HA can be mixed with HABpep-PEG-ColBpep prior to application. The azlactone-based HABpep system can also be used for *in vivo* covalent reactions with amine functionalities of biomaterial or tissue surfaces and does not release any side products on ring opening of lactone by amines. Furthermore, azlactone's superior hydrolytic stability[39] compared to NHS allows mixing of the polymer-HABpep with an aqueous solution prior to injection.

## In vivo targeting

Translation of the tissue surface modification strategy to the complex *in vivo*, and, specifically, the joint environment, is required for therapeutic application. The half-life time of HA retention after intra-articular joint injection can vary from a few hours for ~$10^6$ Da (~17 h) to ~9 days (cross linked HA with ~mol wt 23 × $10^6$ Da), depending upon the molecular weight of injected HA and the injection site[36,40,41]. However, purported HA-induced positive effects in joint pain and joint function are reported to last for months, highlighting the potential multiple mechanisms of action[41]. We applied the HABpep polymer to rat joints to evaluate *in vivo* efficacy of HA binding and retention in a complex environment. In a single step, a mixture of HABpep polymer (50 μL) designed to target Type II collagen (10.0 mg/mL) and fluorescently labeled HA (20.0 mg/mL) was injected into rat knees. Time course imaging of fluorescence demonstrated that the HABpep-polymer coating improved HA retention in rat joints compared to controls with no surface modification. Surface treatment in combination with the HA injection increased longevity of linear HA in the joint over 12-fold (6 h in controls to 72 h in surface treated joints, Fig. 2b). Through specific, noncovalent interactions, the HA was anchored to the tissue surfaces of the rat knee via Col IIBpep and the HABpep bridge. While lubricin is present in the normal joints tested, these *in vivo* experiments (Fig. 2b) demonstrated that bound HA via HABpep was retained for longer times in normal rat knees (up to 72 h) compared to control without HABpep (6 h). Therefore even in the complex and harsh environment of the knee, the polymer-peptide binding system increased retention of linear HA in the joint, prolonging the potential physical and biological benefits.

## Frictional properties

Since the physical lubrication properties of HA are a key therapeutic modality of function in the joint, we further investigated the impact of surface modification. In particular, we compared this to the lubricity ($\langle\mu\rangle_{\text{in PBS}}$ - $\langle\mu\rangle_{\text{in HA}}$) of cartilage treated with the surface

modification (surface-bound HA only) versus untreated cartilage in a bath of HA. Cartilage samples were then coated with HABpep-PEG-Col IIBpep polymer, pretreated with HA, thoroughly washed to remove unbound HA, and mechanically tested in phosphate buffered saline (PBS) (Fig. 3a & experimental setup pictured in Supplementary Fig. S2c). The static and kinetic total friction values for normal cartilage tissue decreased significantly (35% and 72 %, respectively, Figs. 3b,c) when tested in an HA bath ($<\mu_s>$ of 0.018 and $<\mu_k>$ of 0.008) compared to a PBS bath ($<\mu_s>$ of 0.028 and $<\mu_k>$ of 0.028). With application of the HABpep coating and HA pretreatment, the cartilage samples tested in PBS recorded an $<\mu_s>$ of just 0.014 and an $<\mu_k>$ of 0.008, levels similar to the HA bath (Figs. 3b,c). Normal cartilage treated with HA-binding coatings and pre-incubated in HA was able to replicate the low friction characteristics of native cartilage tested in an HA-rich environment. This pivotal result suggests that most of the lubrication effects of HA on the tissue can be replicated by surface-bound HA alone, without the need for large concentrations of HA in the local environment. HABpep technology consistently reduced total friction values which include physiologically relevant and reproducible elements of interstitial fluid depressurization and boundary layer lubrication mechanisms (Supplementary Information & Fig. S3).

Diseased tissue represents a further challenge, as it is characterized by a rough, fibrillated surface with very different frictional properties. Multiple pathogenic mechanisms can lead to cartilage deterioration and not necessarily correlate to higher friction values[42], including inflammatory and other biochemical pathways. The results from our mechanical testing however suggested that *in vitro* degenerated cartilage exhibited higher friction values compared to normal samples using the cartilage-on-cartilage mechanical testing protocols (Supplementary Information & Fig. S2). A number of recent studies also found a similar correlation of increasing osteoarthritis stage and increasing frictional response[43,44]; however, the increased potential for OA cartilage pressurization may be considered a factor. Tribological studies of diseased human cartilage (OA) explants, categorized by severity of cartilage damage, suggested that HA improved the lubrication properties of the diseased tissue to an even greater degree than normal tissue. An increasing HA lubrication effect was observed proportional to the severity of OA cartilage (Figs. S2u,v). The static and kinetic lubricity values for the most severely damaged OA samples were approximately 5 times higher (Figs. S2w,x) than those of the normal “healthy” cartilage samples. These values directly relate to the surface damage and low lubrication values in OA cartilage samples and highlight the need and increased benefit for enhanced lubrication and delivery of HA to the diseased environment. Similar to the results from the normal cartilage surfaces, the OA cartilage samples treated with HABpep polymer coating that produces surface-bound HA produced static and kinetic friction values nearly equal to those found with testing in an HA bath (Figs. 3d,e). OA cartilage samples with the HABpep polymer coating and bound HA had higher static and kinetic lubricity values compared to normal modified tissue (Figs. S4a&b), which suggest that the HABpep and bound HA have a greater effect on improving the lubrication of rough OA surface compared to their effect in normal tissues. The practical implication is that even in a pathological environment, where low HA levels are present in the synovial fluid[31], the HA-binding coating can concentrate the limited HA available at the tissue surface to improve lubrication. Both normal and arthritic cartilage tissue benefited from the application of the HA binding technology with respect to lubrication and HA

retention in the articular joint, suggesting that the technology is useful even in the presence of lubricin or could be synergistically applied with lubricin[25,26]. To further evaluate functional capabilities, the HA-binding technology was applied to normal and arthritic cartilage tissues that were treated to remove lubricin. Surface-bound HA via the HABpep-polymer coating system significantly reduced friction on normal ($<\mu_s>$ of 0.23 to 0.12 and $<\mu_k>$ of 0.12 to 0.047) and OA tissue ($<\mu_s>$ of 0.24 to 0.13 and $<\mu_k>$ of 0.12 to 0.051) confirming that the technology functions both in the presence and absence of lubricin (Figs. 3f–i). As HA bound to the tissue surface coating was washed vigorously before testing (Fig. S2a), the improved lubrication implies that a relatively stable surface coating of HA is generated on the tissue that will not be quickly flushed from the joint. Fluorescence imaging of the cartilage explants pre- and post-mechanical testing also verified the retention of HA-rhodamine onto the surface (Fig. S4c). Semi-quantitative analysis of surface fluorescence found that HABpep increased binding of HA compared to no treatment. HABpep increased surface binding on normal tissue and tissue treated to remove lubricin (Supplementary Information and Fig. S5). Prophylactic treatment with HA-binding coatings during trauma treatment may also be able to enhance local surface lubrication and prevent or reduce the onset of joint degeneration.

## Ocular applications

HA is a key molecule in many tissues and its therapeutic application is extending to other fields, including ophthalmology[45]. HA is an important component of artificial tears to treat dry eye and in eye drops that accelerate healing after surgery or trauma[45,46]. Many ophthalmic products, including multipurpose contact lens care solutions take advantage of HA's ability to enhance wettability and water retention[46,47], which is much needed in treating dry eye disorders. Furthermore, HA provides several biological benefits[45] to ocular tissues, such as improving corneal epithelial cell migration and wound healing[48], reducing inflammation[49] and protecting cells from free-radical damage[20]. Therefore, we investigated the application of the HA-binding strategy to ocular tissues, such as sclera, conjunctiva and cornea of an eye, and to medical devices, such as contact lenses. We applied HA bound to HABpep polymer as an eye-drop with the polymer-peptide system anchored onto Type I collagen of the sclera, conjunctiva and cornea of an eye via collagen I binding peptide (Fig. 4a). Fluorescently labeled HA exposed to the treated ocular surface tissues demonstrated stronger binding compared to a scrambled peptide and control untreated tissues, with the sclera showing the highest levels of binding (Fig. 4b). This eye-drop methodology can recruit and retain HA via HABpep functionalization on eye surfaces with a damaged epithelial layer. Extending the technology to a synthetic device, a contact lens surface was covalently modified with the HABpep polymer system (Fig. 4c). Fluorescently labeled HA via HABpep was visualized bound to the lens, confirming the presence of the surface coating (Fig. 4d). As a functional test for HA binding on contact lenses, the rate of water evaporation from lenses was evaluated. The water evaporation rate decreased significantly on the coated contact lens ($0.23 \pm 0.026$ μL/min) compared to a bare contact lens ($0.50 \pm 0.017$ μL/min) and a control contact lens with only physically adsorbed HA ($0.39 \pm 0.033$ μL/min).

## Materials and Methods

### Synthesis of hyaluronic acid binding peptide

Thiolated hyaluronic acid binding peptide (C-HABpep; sequence CRRDDGAHWQFNALTVR) was synthesized using standard Fmoc-mediated solid phase peptide synthesis on a Symphony Quartet peptide synthesizer (Protein Technologies). Following synthesis, peptides were cleaved using a solution of trifluoroacetic acid, triisopropylsilane and water in a 95:2.5:2.5 ratio. Crude product was purified using reverse-phase high performance liquid chromatography (HPLC, C18 Grace-Vydac column) on a water/acetonitrile gradient. Purified peptides were frozen and lyophilized; identity of purified peptides was confirmed using matrix assisted laser-desorption ionization time of flight (MALDI-TOF) mass spectroscopy.

### Preparation of HA-binding coatings

C-HABpep was conjugated to articular cartilage through a heterobifunctional poly(ethylene glycol) (PEG) spacer. MAL-PEG-NHS (3.5 kDa,), which has functionalities that are thiol- and amine-reactive, was dissolved to 5 mM in 50 mM sodium bicarbonate, pH 7.5, and added to the articular surface. The NHS groups were allowed to react with endogenous amines on the cartilage surface for 30 minutes. PEGylation was confirmed by ATR-FTIR. Following thorough washes in buffer to remove unreacted crosslinker, a 1.5 mM solution of C-HABpep was added to the surface to react with maleimide groups for an additional 30 minutes. Surfaces were carefully washed to remove unreacted peptide, yielding a cartilage surface with covalently attached HA-binding functionality. In another methodology for one-step application, thiol-PEG-succinimidyl glutaramide (SGA) (3.5 kDa,) was reacted with HABpep (GAHWQFNALTVR) (dissolved in dimethyl sulfoxide) in a PBS buffer (pH 7.4) for 4 h. After dialysis and lyophilization, thiol functionality of the product was reacted overnight with vinyl functional groups of vinyl dimethyl azlactone using a Michael addition reaction in the presence of dimethylphenylphosphine in dimethyl sulfoxide at room temperature. The product was dissolved in water and washed multiple times with cold ether and dried *in vacuo*. The resultant product was added to a sodium bicarbonate solution (pH 8.3) of collagen binding peptide (Col II-WRYGRLC, Col I-YSFYSESLQ)[50–52]. After 4 h of reaction time, the solution was dialyzed against water (MWCO 2000 Da) and lyophilized to yield a white fluffy powder.

### X-ray photoelectron spectroscopy

XPS was performed to verify the presence of the HA-binding coating on articular cartilage. Lyophilized cartilage samples were adhered to the specimen stage and loaded into a PHI 5400 XPS instrument at ultrahigh vacuum. The samples were analyzed using Mg Kα X-rays (1253.6 eV), and spectra were acquired at a take-off angle of 45°. Atomic concentrations were determined by integration of the relevant photoelectron peaks using commercially available software (CasaXPS).

### Visualization of the HA-bound layer

Cartilage was conjugated with C-HABpep and incubated with biotinylated HA synthesized as previously described. Briefly, HA (975 kDa, LifeCore Biomedical) was dissolved in 50 mM boric acid, pH 5.2, at 2.0 mg/mL. This was combined with biotin hydrazide in a 20:1 weight ratio. N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC, was added to a final concentration of 100 mM). The reaction was allowed to proceed at room temperature for 16 h, after which the product was dialyzed to remove unreacted biotin hydrazide and EDC. HA-biotin was lyophilized and stored at −20 °C for later use. Following incubation with 5 mg/mL HA-biotin, HABpep-functionalized cartilage samples were washed vigorously to remove unbound HA-biotin. Presence of biotin was visualized using streptavidin and horseradish peroxidase based on the Histostain SP kit (Life Technologies Inc., Grand Island, NY).

### Cartilage sample preparation for lubrication testing

Bovine (12 to 14 weeks old from Research87 Inc, MA) and human articular cartilage samples (National Disease Resource Institution, Philadelphia) harvested from femoral condyles of cartilage were prepared for lubrication testing as a modification of previously published protocols[9,53]. Covalent modification of cartilage surface by HABpep (Fig. 1) was performed on bovine articular cartilage samples. All non-covalent HABpep modification and *in vitro* lubrication studies were performed using human articular cartilage samples (Fig. 3). Normal human tissue was isolated from cadavers with ages 51 (F) and 78 (M) years. Human OA cartilage samples were isolated from the patients with ages, 58 (F), 60 (F), 61 (M), 65 (M), 69 (M), 71 (F), who underwent total knee arthroplasty. Care was taken to avoid damaging the articular surface during dissection. Samples (outer diameter = 8.0 mm, inner diameter = 3.0 mm) were microtomed and evenly cut to obtain a flat surface. The superficial layer was maintained intact and only the deep layer of cartilage was cut to obtain a flat layer to glue to the metal counter-surface while friction measurements. Cartilage was used fresh without freezing or the addition of protease inhibitors so as not to change the surface lubrication properties. Samples were washed vigorously in PBS overnight to deplete the cartilage surface of any residual synovial fluid, after which they were functionalized with an HA-binding layer as needed and incubated at 4 °C for ~24 h in the test lubricant. HABpep modified cartilage samples were soaked overnight in HA bath (5.0 mg/mL, 975 kDa) followed by washing them vigorously in PBS overnight to remove unbound HA. These samples were incubated in PBS for 1 h and lubrication testing was performed. Cartilage samples with no HABpep modification were incubated in either PBS or HA (5.0 mg/mL, 975 kDa) for 1 h, after 24 h PBS wash, and lubrication testing was performed. Further experimental information is provided in the Supplemental Information and Figure S3. The friction values were recorded as mean and standard deviation. Please see Supplementary Tables S1a&b for examples of raw data and calculations.

### Friction measurements on the human cartilage samples treated to remove lubricin

Lubricin was removed from the human cartilage samples in accordance to a published procedure by *Jones et al*[54]. In brief, endogenous lubricin was extracted from cartilage discs on incubation at room temperature for 20 min in PBS containing 1.5 M NaCl followed by an

additional 20 min incubation in pH 6.2 of 4M Guanidine-HCl solution. The cartilage discs in each solution were shaken throughout the experiment. Friction measurements on these samples were performed by a procedure as described above.

### In vivo imaging for HA retention

*In vivo* imaging was conducted on 6 to 8 weeks-old male Sprague Dawley Rats ($n = 4$ for each group; total = 8). The rats were anesthetized with isoflurane under a pre-established protocol (The Johns Hopkins University Animal Care and Use Committee approved the animal procedures, protocol#RA12A136). Each rat was injected with 50 μL solution mixture of HA-rhodamine (20 mg/mL, CreativePEGworks) with HABpep polymer (10 mg/mL) under sterile conditions penetrating the joint capsule and bursa. After injection, rats were imaged and kept under isoflurane anesthesia with an IVIS Spectrum In-Vivo Imaging System (Rats were imaged at different time points: immediately after surgery (<2h), 6 h, 24 h and 72 h after surgery). All images were taken at the same excitation (570 nm) and emission (620 nm). Rats were anesthetized before each imaging time point. A method for semi-quantitative analysis of HA bound on the cartilage surface is described in supplementary information.

### Ocular surfaces and contact lens modification

Rabbit eyes (8 weeks to 12 weeks old rabbits purchased from Pel-Freez Inc, AK) were processed *ex vivo* to separate epithelial layers from the sclera, conjunctiva and cornea. Each of these tissues was cut into small pieces using a 3-mm diameter biopsy punch, and washed rigorously with PBS. To these tissues, a solution of HA-fluorescein (975 kDa, CreativePEGworks), mixed with HABpep-PEG-Col IBpep polymer was added (final concentration 5.0 and 1.0 mg/mL, respectively), kept on a shaker for 2 h and washed with PBS before taking images by Zeiss Discovery V2 imaging microscope. The ability of the HABPep technology to bind HA to multiple different external ocular tissues was tested and compared to controls for each tissue using fluorescein labeled HA. Tissue samples were cut from biopsy punches and separated into three equal pieces and treated with HA alone (negative control for nonspecific HA binding), HA with scramble peptide (control for nonspecific peptide binding) and HA with col IBpep). All ocular tissues were imaged at the same exposure time and magnification. Contact lens (PuriVision from Bausch & Lomb was treated with diazirine-based photo-leucine (5.0 mg/mL) under UV light (365 nm) for 30 minutes at an approximate distance of 3.0 cm, followed by reacting the amine groups with azlactone functionality of the PEG-HABpep (1.0 mg/mL) in a sodium bicarbonate solution (pH 8.3) for 4 h. HABpep-modified contact lenses were added to a solution of HA-rhodamine (1.0 mg/mL) and kept on a shaker for 2 h. After washing with PBS, fluorescence images were taken by Zeiss Discovery V2 imaging microscope and processed with ImageJ. To measure water evaporation rate from the contact lens, an evaporation cell was designed by cutting the cap and hinge off a 1.5-mL SealRite® microcentrifuge tube (USA Scientific, Inc.). After filling the cell with 1.2 mL of Hank's buffer solution (HBSS), the contact lens was glued with instant Krazy Glue® (Elmer's Products) to the rim of the cell. The cell was gently placed on its side, keeping the contact lens inside completely hydrated, into an analytical balance and the weight of the cell was recorded at the start and every 5 min for 50 min ($n$=3).

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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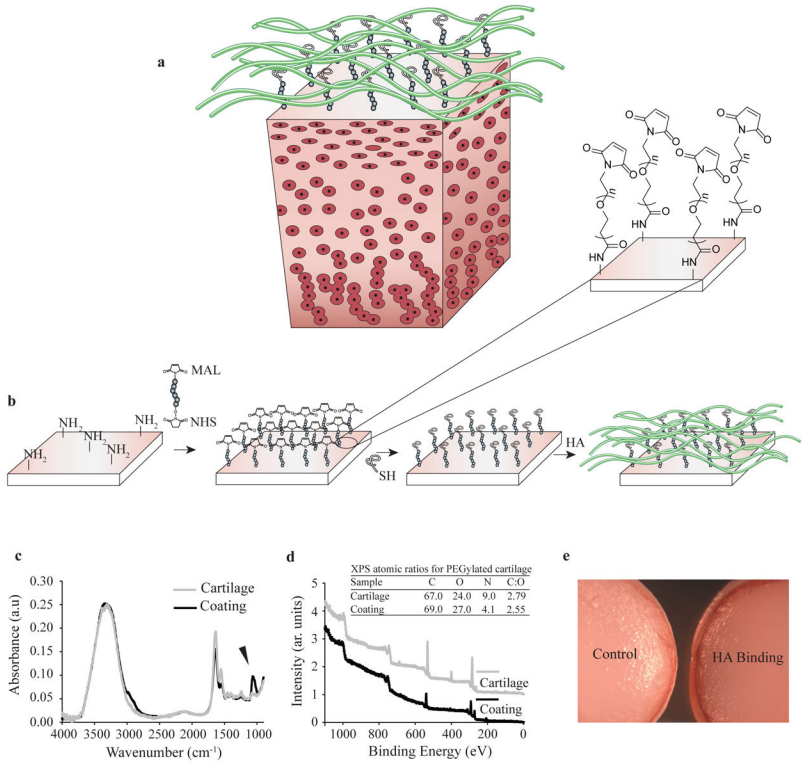


**Figure 1. Tissue surface modification with an HABpep-polymer system**

**a,** Schematic of cartilage surface modified with an HABpep designed to interact with and bind HA in surrounding fluid. **b,** An *in vitro* covalent strategy for coating the cartilage surface with MAL-PEG-NHS crosslinker, which on reaction with primary amines of the cartilage surface, creates an exposed thiol-reactive surface. Subsequently, a thiolated HABpep is reacted to the maleimide functionality. On exposure to an HA solution, the HA binds to the peptide-polymer coating on the cartilage surface. **c,** The PEG crosslinker reaction to articular cartilage was confirmed by ATR-FTIR spectroscopy that validated the presence of the ether-rich PEG coating with a large ether peak at ~1066 $cm^{-1}$. **d,** PEGylation was further verified by XPS atomic ratios. Compared to unmodified cartilage, coated samples had a carbon to oxygen ratio closer to 2, the ratio in PEG, and significantly lower nitrogen content. **e,** HA-binding functionality of the peptide-conjugated cartilage was visualized using a biotinylated HA. Biotinylated HA was synthesized and applied to unmodified cartilage and cartilage modified with the HA-binding polymer system. After thorough washing, the biotinylated HA was treated with streptavidin and horseradish peroxidase for visualization. The tissue surfaces treated with the HA-binding polymer coating stained darker than the untreated native cartilage.

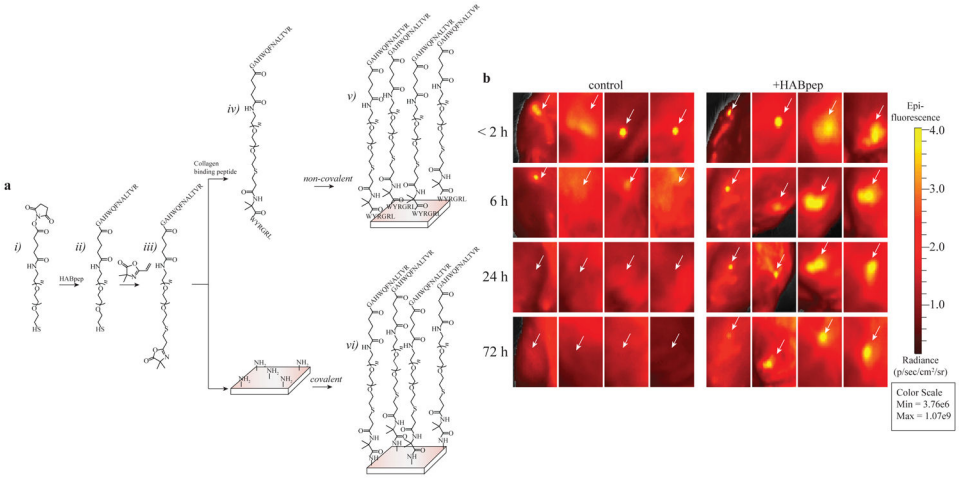


**Figure 2. Single-step strategy for application of HABpep-polymer system to a tissue surface and functional translation to a joint environment**

**a,** Schematic of synthesis of a PEG bifunctional linker with one end group as an HABpep (GAHWQFNALTVR) and another end that either reacts with the amine groups or binds to a tissue surface via an extracellular matrix (ECM) binding peptide, such as collagen II binding peptide, WYRGRL. First, an HA-binding peptide is linked to a thiol-PEG-SGA linker *(i)* via amine-SGA conjugation reaction *(ii)* followed by the Michael-addition reaction of thiol functionality and vinyl dimethyl azlactone *(iii)*. On a tissue surface, this amine-reactive azlactone functionality can be conjugated with either a peptide *(iv)* that non-covalently binds to ECM components *(v)*, or covalently reacts with the amine functionality present in the tissue *(vi)*. Both (*iii)* and *(iv)*, with or without HA, can be applied on a tissue surface in a single-step application. **b**, HA-rhodamine together with HABpep-PEG-Col IIBpep polymer was injected into healthy rat knees in a single step and HA retention was monitored over time using an IVIS spectrum *in vivo* imager. HA-rhodamine (white arrows) via the HABpep polymer system was retained in rat knees even 72 h post-injection, compared to only 6 h without HABpep coating.

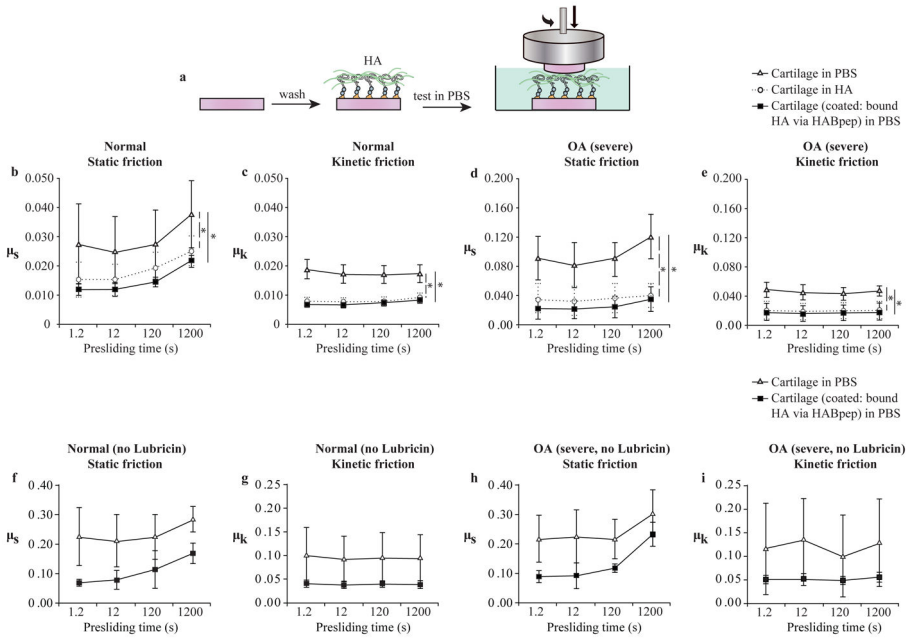


**Figure 3. Cartilage surface-bound HA via the HABpep-polymer coating system in the absence of an exogenous lubricant can recapitulate the friction coefficients of high concentration HA lubricants**

**a,** Representative schematic for the preparation and incubation of HABpep coated samples in test solution PBS. Lubrication properties of normal cartilage and severely damaged cartilage coated with the polymer-peptide system were tested in the presence of saline, and compared with uncoated surfaces in either saline or HA. Representative graphs of static friction and kinetic friction *vs.* pre-sliding time (s) for the normal cartilage sample (**b & c**) and severely damaged cartilage sample, OA stage 3–4 (**d & e**). (For statistical analyses: dashed lines represent cartilage samples (no HABpep modification) in PBS *vs.* HA bath and solid lines represent cartilage samples in PBS *vs.* cartilage samples coated with bound HA via HABpep in PBS.) Cartilage surface-bound HA via the HABpep-polymer coating system reduced friction values when lubricin is extracted from the tissue. Lubrication properties of normal cartilage and severely damaged cartilage (lubricin removed) coated with the polymer-peptide system was measured in PBS and compared to controls. Representative graphs of static friction and kinetic friction *vs.* pre-sliding time (s) for the normal cartilage sample (**f & g**) and severely damaged cartilage sample, OA stage 3–4 (**h & i**).

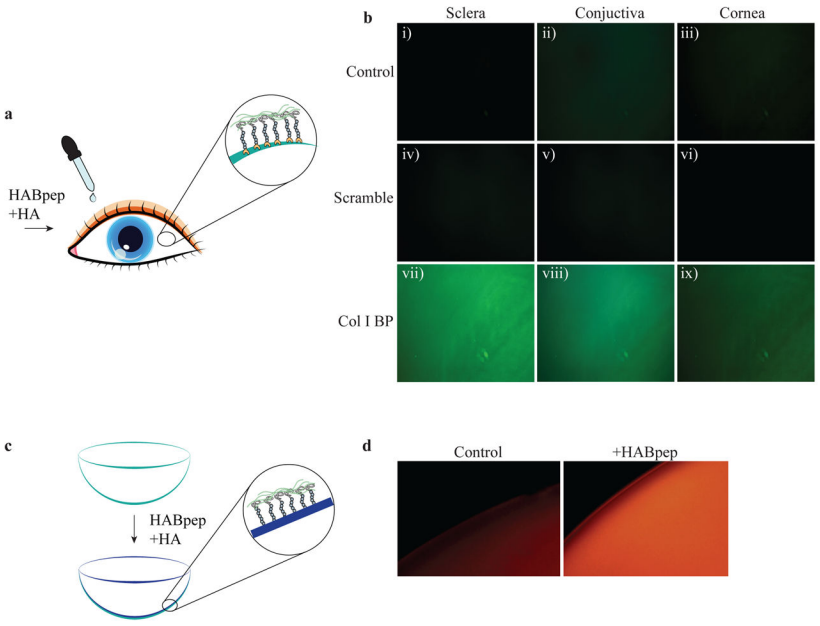


**Figure 4. Ocular surface application of HABpep-polymer system**

**a,** HABpep polymer system as an eye-drop solution can be used to retain HA on the eye surface. Collagen I-abundant eye tissues without epithelial layers, such as sclera, conjunctiva and cornea, act as anchors for the HABpep polymer system. **b,** Fluorescence images of HA retention on untreated and treated eye tissues: sclera, conjunctiva and cornea of untreated eye, *i)* to *iii)*; treated with scrambled collagen I binding peptide (YFDEYSLSQS), *iv)* to *vi)*; and treated with collagen I binding peptide, *vii* to *ix)*. **c,** Contact lens modification with the HABpep polymer system was performed by the covalent reaction methodology. **d,** Fluorescence images for HA-rhodamine retention on modified contact lens showed relatively darker staining compared to the control.