Collagen Coating of Titanium Implants Using Nonthermal Plasma

Liam O’Neill, a, Barry Twomey, b, Fei Tan, b,c John O’Donoghue, b & John A. Hunt d,e

a TheraDep, Questum Acceleration Centre, Clonmel, Co. Tipperary, Ireland; b ENBIO, DCU Alpha Innovation Campus, Dublin, Ireland; c Shanghai East Hospital, and School of Medicine and Institute for Advanced Study, Tongji University, Shanghai, China; d Medical Technologies Innovation Facility, Nottingham Trent University, Nottingham, UK; e College of Biomedical Engineering, China Medical University, Taichung, Taiwan

*Address all correspondence to: Liam O’Neill, TheraDep, Questum Acceleration Centre, Clonmel, Co. Tipperary, Ireland, E-mail: liamoneill@theradep.com

ABSTRACT: Surface modification of implants is now an essential aspect of biocompatibility. A single-step process to attach biomolecules to implants represents a major advance, and plasma coating potentially achieves this. An aqueous solution of collagen was sprayed into a nonthermal plasma and deposited onto titanium surfaces. The plasma instantly transformed the liquid aerosol into a coagulated dry coating on the implant surface. Surface analysis confirmed the presence of a thin, conformal protein layer on the metal surface. Titanium fixation screws were coated first with hydroxyapatite and then with a layer of collagen and were implanted into the femurs of New Zealand white rabbits and compared to various control surfaces. Measurements of the rotational torque removal force indicated that the collagen coating enhanced bone fixation and created a more reproducible well-anchored, stable implant than the traditional plasma-sprayed hydroxyapatite coatings. Nonthermal plasma processing offers a single step route to the biological functionalization of implantable surfaces. The process requires no polymers, primers, or linkers and offers an opportunity to control biocompatibility and to tailor local response to the implant in vivo. This opens the door to a wide variety of novel biological surface coatings across all aspects of life sciences and medicine.

KEY WORDS: plasma, nonthermal, protein, coating, implant, hydroxyapatite

I. INTRODUCTION

Surface modification is now an essential component of medical implant manufacturing. An optimized surface can deliver improved quality, reproducibility, functionality, and biocompatibility, thereby reducing implant failure and producing enhanced performance in situ. This has driven development of a range of surface coatings to deliver specific functionality and to direct the host response. 1 For orthopaedic, spinal, and other bone-fixated tissue implants, this had lead the industry to introduce a range of roughened surfaces, three-dimensional surface structures, and the use of plasma-sprayed hydroxyapatite (HA) coatings to improve bone apposition, integration, and fixation of the implant to the bone. 1,2 HA coatings are used because apatite is a major constituent of natural bone and it is therefore well tolerated and promotes bone integration in vivo. However, there are problems with current high-temperature plasma deposition of HA
and current coatings can exhibit poor adhesion, variations in crystallinity, and may release particles which result in third-party wear.\textsuperscript{1} In addition, high temperatures during deposition can thermally degrade small metal structures and the thick coatings can obscure precision-machined features. This has led to significant research into alternative coating technologies.\textsuperscript{3–5}

Although HA provides up to 70% of the weight in bones, the other main structural constituent is collagen. This has attracted less attention as a coating material to date. However, some researchers have investigated collagen coatings and these have shown positive results in rat, goat, and sheep models.\textsuperscript{6–10} Various coatings based on mixtures of collagen with chondroitin sulphate, bone morphogenetic proteins, and calcium phosphates have also been evaluated and have been demonstrated to be beneficial for bone formation. This area has been reviewed by Junker et al.\textsuperscript{11} Further studies have shown considerable promise for calcium phosphate-collagen surfaces in orthopedic applications.\textsuperscript{12–14}

However, there are currently no approved collagen coatings for hard-tissue implants and this may be partly explained by the complexities in manufacturing, sterilizing, and storing a protein-based coating. It is noteworthy that all of the collagen coatings described above rely upon wet chemical processing to attach the protein to the surface. These processes typically involve fibrillogenesis followed by multiple cycles of dipping and air drying and can take several days. The resultant coatings lack any form of structural cross-linking and are poorly adhered.\textsuperscript{15} This has led several groups to attempt to improve this process by first activating the implant surface with a plasma treatment to improve biomolecule adhesion.\textsuperscript{16} This has been further developed by other groups, who have deposited a plasma polymerized primer coating to which collagen can be chemically bonded.\textsuperscript{17–19} The resultant coatings have been shown to perform well \textit{in vitro} and \textit{in vivo}.\textsuperscript{17} However, this still represents a multistep coating process in which the final coating is applied using wet chemical techniques.

These wet methods are slow, difficult to control and not production friendly. It is possible to produce cross-linked collagen substrates by exposing the collagen to chemicals such as glutaraldehyde, but the resulting materials are structurally stiff and the chemical residues in the cross-linked collagen have been shown to be cytotoxic.\textsuperscript{20,21} Recently, it was reported that gamma irradiation could be used to cross-link collagen on a titanium implant, though this method also involved an initial dip-coating of the implant.\textsuperscript{22}

Alternative developments have shown that atmospheric pressure plasma systems can be used to deposit biomolecules onto surfaces in a single step.\textsuperscript{23–28} Early experiments involved introducing a mixture of the biomolecule and a film-forming monomer into a nonthermal atmospheric pressure plasma in the presence of the target substrate. As the plasma converted the monomer into a coating, the biomolecule became entrapped in the growing polymer network. This resulted in the biomolecules being immobilized on the substrate surface while also retaining their biological activity. The coating is produced rapidly, effectively cross-linked, and bonded to the substrate in seconds or less. However, the requirement to entrap the biomolecule within a polymer network can still pose difficulties for coating applications on implantable devices. Polymer coatings
dilute concentration of the biomolecule on the surface and can limit the interaction of
the biomolecule with the local environment and therefore its activity. More significantly,
polymer-based coatings can have poor biocompatibility and have been shown to cause
negative reactions and even death in clinical use.\textsuperscript{29,30}

This approach has been further explored in a range of plasma coatings that directly
deposit pure biologics onto surfaces without polymers, linkers, or binders.\textsuperscript{31–33} Using a
low-energy nonthermal plasma, it has been shown that coagulated collagen coatings can
be applied to wounds and other surfaces while still retaining biological activity. Detailed
chemical, structural, and \textit{in vitro} studies have shown that plasma-deposited collagen
promotes cellular proliferation in an manner identical to that of collagen layers depos-
ited using wet chemical techniques.\textsuperscript{33–35} This can be achieved without the use of primers,
polymers, or linker chemistries and offers significant clinical benefits.

Having proven the efficacy \textit{in vitro}, the next step is to determine if similar effi-
cacy can be obtained in an \textit{in vivo} study. Our investigation aimed to determine if a
collagen coating can be successfully deposited on a titanium orthopedic implant using
atmospheric pressure plasma deposition. Collagen layers were applied on top of a hy-
droxyapatite coating because previous work showed that this combination mimics the
chemical composition of natural bone.\textsuperscript{11,12} The chemical properties of the resultant coat-
ing were evaluated using standardized surface science analysis and the efficacy of the
coating was then evaluated in an \textit{in vivo} device-specific femoral implant model to deter-
mine if the collagen induces an improved earlier and stronger implant-to-bone fixation.

\section*{II. MATERIALS AND METHODS}

\subsection*{A. Sample Preparation}

Coatings were deposited onto flat $15 \times 15 \times 1$-mm titanium coupons (Lisnabrin Engineering)
for surface analysis. For \textit{in vivo} examination, coatings were applied to 2.7-mm-diameter by
10-mm self-tapping cortex screws (Syntec Scientific Corporation, Dublin).

Three surfaces were chosen for surface characterization: (1) untreated titanium, (2)
titanium with HA applied by CoBlast, and (3) titanium with HA applied by CoBlast and
an additional collagen layer applied via cold plasma processing. The first two surfaces
were controls against which the third surface could be compared. For the \textit{in vivo} study,
an thermal plasma–sprayed hydroxyapatite coating was included as an additional reference
control.

CoBlast HA coatings were applied using the CoBlast process as described else-
where.\textsuperscript{3,36,37} CoBlast is a modified grit-blasting process developed for depositing ceramic
coatings onto metallic surfaces. In brief, the dopant, HA (S.A.I.) and the abrasive, MCD
(Himed) were supplied by corresponding powder feeders and simultaneously blasted
onto metallic substrates, resulting in formation of a thin HA layer on the implant surface.

The plasma-sprayed HA coatings were deposited onto the same titanium screws by
APS Materials, an independent commercial supplier of medical device coatings. The
up-to-date APS master file conforms to FDA guidance document \textit{510(K): Information}

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Needed for Hydroxyapatite Coated Orthopedic Implants. Samples underwent grit blasting, substrate cleaning, HA plasma spray, and removal of overspray as per standard implant-processing conditions.

Collagen coatings were applied on top of the CoBlast HA samples using a customized plasma device, which has been described in detail elsewhere, and is shown schematically in Fig. 1. In brief, the system was configured with a dielectric head housing two electrodes on either side of a pneumatic nebulizer through which the precursor was introduced. RF power was delivered to both electrodes from a modified PTI 100W RF power supply at a frequency of 15–25 kHz. Helium was introduced into the device at a flow of 5 L/min. Purecol collagen solution (Advanced Biomatrix) was diluted in deionized water to give a final concentration of 1 mg/mL. The liquid precursor was pumped from a remote syringe pump to the unit and converted into an aerosol using a T2100 nebulizer (Burgener Research). A precursor flow rate of 20 μL/min was used throughout the experiments. Approximately 1-L helium flowed through the nebulizer in addition to the process gas flows reported above. The resultant helium- aerosol mix exited the system through a 75-mm-long by 15-mm-diameter fluoropolymer tube. The substrates were placed adjacent to the plasma exit and the coatings were deposited for 1 min. For the screw samples, the implant was mounted on a rotary chuck and rotated at approximately 30 Hz to give a uniform coating.

B. Plasma Characterization

Electrical characterization of the device consisted of a Bergoz Instrumentation, CET-E5.0-B toroidal current transformer which was used to measure the plasma current (I_p), and a North Star PVM-5 high voltage probe which was used to determine the applied voltage (V_app). The current transformer toroid was positioned around the high voltage
lead connecting the plasma electrodes to the power supply. The high-voltage probe was applied at the output of the power supply. The outputs of both probes were captured on a Tektronix TDS 2024 four-channel digital storage oscilloscope with a 200 MHz bandwidth.

Optical emission spectroscopy (OES) data was collected using an Ocean Optics USB4000 fitted with a cosine corrector (CC-3-UV-S, Ocean Optics, Dunedin, FL). This probe was positioned under the plasma exit and pointed into the chamber to maximize light capture. The OES data were collected using the OceanView software package with parameter settings as follows: integration time, 3,500 ms; scans, average of 2; boxcar width, 5; electric dark, active; and trigger mode, continuous.

C. Surface Analysis

X-ray photoelectron spectroscopy (XPS) analysis was performed to study the chemical composition of the films using a Kratos Analytical Axis Ultra electron spectrometer equipped with a monochromated Al K$_\alpha$ X-ray source. XPS survey spectra were collected in the binding energy range of 0–1,200 eV. Photoelectrons were detected at a 90° take-off angle (TOA) and the corresponding depth of analysis was approximately 10 nm. Contact angle measurements were obtained via sessile drop using an OCA 20 video capture apparatus (Dataphysics Instruments, Charlotte, NC). Drop volumes of 1.5 μl were used and images were collected 30 s after placing the droplet on the surface. Scanning electron microscope (SEM) imaging was carried out using a JEOL 5410 electron microscope. Surface roughness was measured using a Wyko NT1100 profilometer (Veeco) operating in vertical scanning interferometry (VSI) mode at 10× magnification. Four measurements per four samples were used to determine average roughness (Ra). Fourier transform infrared (FTIR) spectroscopy was conducted on a Perkin Elmer Spectrum 2000 instrument operating in single-beam mode using 64 scans at 2-cm$^{-1}$ resolution.

D. In Vivo Testing

Local ethical and UK Home Office regulations were complied with throughout the animal trial. Transcortex femoral implantation was conducted using skeletally mature male New Zealand rabbits, which were housed and acclimated for 2 weeks before the surgery. The rabbits were anesthetized using pentobarbital 30 mg/kg intravenously. The anterolateral surface of the femur just distal to the level of lesser and third trochanter was exposed sufficiently through layers: skin, subcutaneous tissue, tensor fasciae latae, and intermuscular junction between vastus lateralis and biceps femoris. Two adjacent transcortex 2-mm diameter holes were drilled using sterile saline site flooding as the drill bit and bone site coolant during drilling. A handheld 2.5-mm Allen key was used to acquire a bite on the threads of the self-tapping screw in the cortical bone. Then an MTS 858 torque wrench (MTS Systems, Eden Prairie, MN) was employed to further insert the screws, stopping at an insertion torque load of 0.07 Nm, which usually corresponds to the tip of screw locating on the opposing endosteal surface of the femur and tightening up. The wound was closed.
in layers: fascia sutured with 5-0 Vicryl, and skin sutured with 4-0 Ethilon (Ethicon). All rabbits received postoperative analgesics and antibiotics. At two and eight weeks after the implantation, the rabbits were sacrificed and femurs exposed for biomechanical testing. The same digital handheld torque gauge used during screw insertion was applied as the torque drive extract device connected to the screw head. The maximum force encountered during removal of the screws was recorded and displayed on the built-in force meter.

III. RESULTS

Electrical diagnostics revealed a plasma operating at a frequency of 19 kHz with a distorted sinusoidal waveform, as shown in Fig 2. The peak-to-peak voltage was about 22 kV and the peak current was about 9 mA, which was in line with previously published detailed studies of this system. The curves show that most of the current was displacement with current about 90° out of phase with voltage. From the time-dependent applied voltage \( V_p \) and current \( I_d \), measurements of the delivered power averaged over one period \( T \) can be calculated from

\[
P_d = \frac{1}{T} \int_{t}^{t+T} V_p(t)I_d(t)dt
\]

(1)

The actual discharge power was calculated as the average over 10 periods of the current-voltage product and was found to be 6.7 W. This was in line with reported values for similar systems and consistent with the energy levels reported to generate controlled soft polymerization and to deliver biologically functional collagen coatings.

As shown in Fig. 3, the OES spectra were dominated by nitrogen-related features, as is typical of atmospheric helium plasma systems. The molecular nitrogen emissions were observed at 337, 358, 380, and 406 nm with the \( \text{N}_2^+ \) producing the most intense emission at 391 nm. The only helium-related feature detected was the weak emission at 707 nm. Interestingly, there were notable features at 316 and 777 nm, which can be attributed to the presence of hydroxyl and atomic oxygen species.

FIG. 2: Voltage and current measurements taken from the plasma device operating at equilibrium
These species would be expected to produce significant oxidation of the deposited coating and this would be expected to impair the functionality of the coating. The presence of these species may be due to the introduction of the collagen precursor as a water-based aerosol, which would provide a significant source of hydroxyl groups. Alternatively, these species may have been due to traces of atmospheric air entering the plasma system. There were weak indications of features below 300 nm, which may have been associated with NO, but these were not well resolved.

Following deposition, the samples were subjected to detailed surface analysis. Profilometry analysis confirmed that the CoBlast HA samples exhibited significantly higher surface roughness when compared to the untreated metal, in agreement with previous studies, as illustrated in Fig. 4. Based on a 2-sample $t$-test, the addition of the collagen layer on top of the CoBlast HA treatment did not significantly alter the

![Optical emission spectrum of helium plasma discharge with liquid aerosol](image1)

FIG. 3: Optical emission spectrum of helium plasma discharge with liquid aerosol

![Plot of surface roughness (Ra) comparison between untreated, CoBlast HA, and CoBlast HA plus Collagen coated parts](image2)

FIG. 4: Plot of surface roughness (Ra) comparison between untreated, CoBlast HA, and CoBlast HA plus Collagen coated parts
surface roughness, as consistent with the deposition of a conformal nanoscale coating of collagen.\textsuperscript{33}

The surface structure was further probed using SEM and the imaging confirmed that the deposition of the hydroxyapatite layer produced significant roughening of the substrate surface and a ceramic deposit was clearly present, as shown in Fig. 5. However, further deposition of a plasma-coagulated collagen layer had no detectable impact on the surface topography and there was no clear indication of a discernible protein layer, in agreement with the surface roughness measurements. Detailed surface chemistry studies were therefore undertaken to search for evidence of a collagen deposit, and the results shown in Table 1.

Water contact angle measurements indicated that a range of surface responses was produced from the various surface treatments, as reported in previous studies.\textsuperscript{31,36} Of particular interest was the change in the contact angle of the CoBlast HA surface after collagen deposition. The plasma treatment would be expected to reduce the contact angle of the surface significantly due to the activation and oxidation of the surface by the plasma, but the contact angle actually increased by 6°. Based on this observation, the

![](image)

**FIG. 5:** SEM imaging of unmodified titanium metal, CoBlast-deposited hydroxy, and collagen layer deposited over CoBlast HA surface (HA plus collagen)

**TABLE 1:** Surface analysis results from titanium surfaces

<table>
<thead>
<tr>
<th>Surface</th>
<th>XPS (atomic %)</th>
<th>Water contact angle (°)</th>
<th>Roughness (R\textsubscript{a})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ti</td>
<td>Al</td>
<td>C</td>
</tr>
<tr>
<td>Untreated Ti</td>
<td>4.7</td>
<td>0.7</td>
<td>78.3</td>
</tr>
<tr>
<td>CoBlast HA</td>
<td>2.1</td>
<td>0</td>
<td>20.1</td>
</tr>
<tr>
<td>Collagen</td>
<td>0</td>
<td>0</td>
<td>52.0</td>
</tr>
</tbody>
</table>
The presence of the OH and atomic oxygen species in the OES spectra did not translate into significant oxidation of the surface. Instead, the surface chemistry was dominated by the presence of the deposited protein-based coating, as on other surfaces reported.\textsuperscript{31,32} This effect was confirmed by XPS analysis (Table 1). While the untreated grade V titanium did exhibit the expected features arising from Ti and Al, the analysis was dominated by the C and O signals from the adsorbed hydrocarbon layers present on the surface of the sample. After HA deposition, the coated samples also revealed significant levels of calcium, phosphorous, and oxygen consistent with the presence of a calcium phosphate coating.\textsuperscript{3,36} The collagen sample was applied as a nanoscale coating over the CoBlast HA coating, and low levels of calcium and phosphorous were still observed in the spectra. This may suggest that the protein-derived coating is either thin or does not provide complete coverage due to the surface roughness of the HA coating. Given the micron-level roughness of the apatite surface, it is unlikely that the thin collagen coating could fully obscure the underlying surface and apply uniform coverage over both the peaks and troughs of the roughened surface. In addition to the features from the underlying substrate, the collagen-treated samples also contained high levels of carbon and nitrogen, which is indicative of the presence of amide or amino groups arising from the presence of a protein layer on the surface. It is notable that the oxygen level measured by XPS was significantly reduced after plasma treatment, which indicated that the surface did not undergo significant oxidation.

The chemistry of the collagen deposit was further investigated by plasma deposition of a collagen layer directly onto a KBr disc and then collection of the FTIR spectra. Comparing the spectra of the coated sample to that of an untreated liquid collagen solution showed significant similarities (Fig. 6). Both spectra had the expected amide features at 1,239 cm\(^{-1}\) (N-H bend), 1,554 cm\(^{-1}\) (N-H bend), 1,652 cm\(^{-1}\) (carbonyl stretch), and the broad N-H bend around 3,300 cm\(^{-1}\), which overlapped with the COOH and −OH peaks. The weaker peaks due to the aliphatic CH\(_x\) features at 2,850 and 2,923 cm\(^{-1}\) and the COO\(^-\) stretch at 1,454 cm\(^{-1}\) were also evident in both spectra. All of these features are

**FIG. 6:** FTIR spectra of collagen solution (lower) and plasma-deposited collagen layer (top) on KBr disc
typical of FTIR spectra produced by protein deposits. The only significant difference between the two samples was the higher intensity and broader range of the peak between 3,150 and 3,700 cm$^{-1}$ in the liquid sample, which was attributed to the presence of the aqueous acetic acid solution. The lack of acetic acid features in the FTIR spectra of the deposited coating suggested that the volatile acetic acid was not integrated into the coating in significant quantities and possibly that the solvent had largely evaporated during deposition. When coupled with the XPS analysis, this indicated that the chemistry of the collagen had been largely retained in the deposited coating.

The surface analysis data clearly demonstrated that the cold plasma process effectively deposited a collagen-derived layer onto the implant surface. The chemical and physical analysis indicated that a thin protein-like coating was effectively deposited onto the implant surface as a nanoscale film without evidence of delamination or flaking, in agreement with previous studies.$^{31-33}$

However, the chemical and microscopic analysis provided no information on the biological properties of the deposit. As it is well known that plasma processes can fragment and rearrange molecules, the biomolecule could have been denatured by the plasma process during deposition through physical rearrangement or minor chemical alterations that were below the limit of detection of the chemical analysis. The choice of plasma system was tailored to minimize such effects. Low-power, helium-based systems are known to deliver very low levels of thermal energy, operate at ambient pressure, and rely on inert gases which minimize oxidation.$^{38-40}$ This minimizes the physical damage that can be imparted by vacuum systems or oxidizing plasma environments. In addition, the protein was delivered as an aerosol, in which the protein was effectively wrapped in an aqueous droplet which might have been expected to shield the biomolecule from the more aggressive plasma species, in a process described by Heyse et al. as akin to a “protective shuttle.”$^{23}$ This approach has produced collagen coatings that enhance wound healing in both rabbit$^{31}$ and rodent$^{32}$ animal models, suggesting that the biological properties of the collagen had been preserved. In vitro studies have suggested that the protein layer behaves like established collagen coatings.$^{33}$ Despite these precautions, it is still possible that the collagen was denatured by the reactive species in the plasma either during deposition or by long-lived radicals present within the coating. Therefore, an in vivo study was undertaken to determine if the coating would improve the integration of metal surfaces in an orthopedic animal model. A series of titanium orthopedic fixation screws were processed to produce implants that were untreated, coated with CoBlast HA, or coated with CoBlast HA and a layer of collagen. In addition to the untreated titanium and the CoBlast HA controls, a thermal plasma-sprayed hydroxyapatite coating was included as a control. These various control surfaces had been extensively compared in previous studies.$^{3,36,37}$ As seen in Fig. 7 and reported elsewhere,$^{37}$ the application of the thermal plasma-sprayed HA significantly increased the roughness of the screw (Ra $\approx 7.39$ μm), above that of the HA deposited using CoBlast. An image of the CoBlast HA plus collagen is not included as there was no significant roughness difference between it and the CoBlast HA without collagen.

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The treated screws were implanted into the femurs of rabbits using an established transcortex model, and the removal force was measured after 2 and 8 weeks. The results are depicted in Figs. 8 and 9. Increased removal force is known to correlate with enhanced fixation of the implant to the host bone and can be used to measure osseointegration. After two weeks, the early fixation measurements clearly showed that both of the HA coatings (thermal plasma-sprayed and CoBlast-deposited) significantly outperformed the untreated Ti screw, as both hydroxyapatite surfaces exhibited significant increases in the torque force required to remove the screws, in agreement with previous results. Although the mean force required to extract the thermally sprayed HA sample was slightly higher than that for the CoBlast HA samples, the high variability in the thermal plasma-sprayed group meant that the differences were not statistically

![FIG. 7: Thread profile of screws: (a) untreated; (b) CoBlast HA; and (c) plasma HA](image)

![FIG. 8: Extraction torque values after two weeks of implantation](image)
significant based on a 2 sample t-test ($p < 0.05$). This variability in implant fixation, commonly reported for thermally sprayed HA coatings, has been attributed to both high surface roughness and high temperatures encountered in thermal plasma spraying to deposit the calcium phosphate coating. This is known to recrystallize the powder precursor and to produce a range of amorphous and semicrystalline phases that can produce a variety of responses \textit{in vivo} and in clinical settings.\textsuperscript{1,3} In line with previous studies, the CoBlast HA surface uses ambient-temperature HA deposition, thereby producing a highly crystalline HA deposit which was found to produce a more controlled and consistent response \textit{in vivo}.\textsuperscript{3,36}

While the HA surface treatments both improved the early-stage fixation of the titanium implant, the highest overall average torque-out values were produced by the plasma-polymerized collagen coating applied over the CoBlast HA surface. Based on a 2-sample t-test, the extraction forces of the CoBlast HA plus collagen were significantly higher than those of the CoBlast HA but not significantly different from the plasma HA. This indicated that the collagen treatment produced significantly enhanced bone fixation. The addition of the collagen layer on the CoBlast HA surface produced a statistically significant improvement in removal force ($p < 0.001$, $n = 4$) while maintaining the consistent \textit{in vivo} response of the CoBlast HA samples. When compared to the industry-standard thermal plasma-spayed HA coating, the average torque-out force was higher in the collagen samples and was also far more consistent and reproducible, confirming that the surface was highly stable and that cold plasma coating is reproducible and predictable. When compared to the other three standard control surfaces, it was evident that the plasma-deposited collagen clearly outperformed all of the other samples after two weeks. It is also noteworthy that the plasma-deposited collagen survived the physical insertion of the screw into the bone defect and was not removed by the mechanical pressure. This suggested that the coating was well adhered to the surface.

At the eight-week time point, the remaining animals were sacrificed and torque-out values were again determined. All samples showed an increase in osseointegration over the intervening six weeks, as shown in Fig. 9. As expected, both HA-based coatings

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{fig9}
\caption{Extraction torque values after eight weeks of implantation}
\end{figure}

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continued to outperform the titanium control. As at the two-week time point, the plasma-sprayed HA had a higher mean torque-out value than the CoBlast sample, but the high roughness and variability of the high-temperature plasma spray coating gave rise to a wide range of values, and the differences between the two HA coatings were again not statistically significant. In contrast, the addition of the collagen coating once more produced a statistically significant increase in bone fixation when compared to the CoBlast HA surface ($p < 0.05$, $n = 4$) and maintained the reproducibility of the CoBlast HA process, as seen in the 2-week test data. When compared to the thermal plasma-sprayed HA process, the collagen-loaded surface produced a mean value equivalent to that of the thermal spray coating, but with dramatically reduced variability. This confirmed that the collagen surface treatment produces a highly controlled and predictable osseointegration in vivo and offers a significant benefit overall.

IV. DISCUSSION

Taken as a whole, the data indicated that plasma deposition produced a nanoscale conformal protein layer on the surface of the implant. There was no evidence of chemical degradation of the protein and the plasma-deposited collagen appeared to remain biologically functional. The added plasma-deposited collagen layer produced a significant and more repeatable increase in the fixation of the implant into the bone than the other surface treatments evaluated. This suggested that the collagen deposit was biologically active and that it acted as a biocompatible coating that induced early and stable formation of new bone around the implant. Given that this result was similar to those reported in other studies of bone fixation,$^7,9,10–12,22$ the data showed that the plasma process had not damaged or denatured the collagen and that the plasma-deposited coating was biologically active, as reported in a recent in vitro study showing that a low-temperature plasma process can deposit intact collagen layers.$^33$ No toxic chemical residues were produced by the deposition process, and no additional chemicals were required to form the coating.

The findings of our study open the way for production of other coatings based on important biomolecules such as hyaluronic acid, chitosan, or heparin, and they greatly increase the potential for achieving biologically optimized implant surfaces for clinical applications. Separate studies have shown that these plasma coatings can also be used as drug delivery layers.$^{43}$ When combined with pharmaceutical materials, such coatings could combat infections, accelerate healing and tailor host-implant responses.

V. CONCLUSIONS

A collagen coating was deposited onto metal implants using a nonthermal plasma process without the use of additional coating materials. Nebulizing a solution of collagen and exposing the aerosol to a low-temperature atmospheric pressure plasma produced a thin biomaterial coating that was conformal and retained the chemistry of the starting material. This coating consisted of collagen only and did not require the use of cross-linkers,
primers, linkers, or polymeric encapsulation systems. As such, it contained no components that would be expected to provoke a negative host immune reaction.

Because the coating showed improved biocompatibility in an in vivo setting. It was applied to orthopedic screws and evaluated using a device-specific rabbit in vivo model. The deposited layer was found not only to be biocompatible but also to promote bone fixation throughout the trial period in a manner consistent with the deposition of a stable collagen coating. This suggested that the collagen was not degraded or denatured by the plasma deposition process. For this reason, it is now possible to create tailored biomolecule coatings on implant surfaces in a single step using an economical and effective plasma process.

REFERENCES


