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Tuning model drug release and soft-tissue bioadhesion of polyester films by plasma post- treatment.

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ABSTRACT

Plasma treatments are investigated as a post-production method of tuning drug release and bioadhesion of poly-(lactic-co-glycolic acid) (PLGA) thin-films. PLGA films were treated under varying conditions by controlling gas flow-rate, composition, treatment time and radio-frequency (RF) power. *In-vitro* release of the drug-like molecule fluorescein diacetate (FDAC) from plasma-treated PLGA was tunable by controlling RF power; an increase of 65% cumulative release is reported compared to controls. Bioadhesion was sensitive to RF power and treatment time, assessed using *ex-vivo* shear-stress tests with wetted swine aorta. We report a maximum bioadhesion ~6-fold that of controls and 5-fold that of DOPA-based mussel adhesives tested to swine skin.¹ The novelty of this post-treatment is the activation of a hydrophobic polyester film for bioadhesion, which can be quenched, while simultaneously tuning drug-release kinetics. This exemplifies the promise of plasma post-treatment for in-clinic bioadhesive activation, along with technological advancements; i.e. atmospheric plasma and hand-held ‘plasma-pencils’.

INTRODUCTION

Over the last two decades, bioadhesion has become of interest for its potential to improve and optimize localised drug delivery. Bioadhesives enable an increased residence time at the administration site, leading to substantial enhancements in local drug therapy as well as significant increases in bioavailability for some drugs (vasopressin, oxytocin, insulin etc.).²⁻³ Drug delivery is optimized either by retaining a dosage form at the site of action (e.g. longer residence in the gastrointestinal tract for prolonged systemic delivery) or by retaining a formulation in intimate contact with the absorption site (e.g. the nasal cavity for prolonged local delivery).⁴⁻⁵

Most bioadhesive drug delivery systems are based on the mucoadhesion mechanism, which arises from non-covalent bonds, such as electrostatic, ionic or hydrogen bonding, or a combination thereof. However, these adhesion mechanisms are inadequate for other soft tissues, such as organs or intra-vascular surfaces where stronger, covalent bonds are required. Synthetic glues, such as cyanoacrylates, offer high adhesion strength and cure sufficiently fast upon contact with moisture. However, cyanoacrylates exhibit local toxicity caused by degradation by-products (i.e. release of pro-inflammatory aldehydes) and lack local drug delivery properties.⁶ Fibrin glues are widely used as biological tissue adhesives owing to their excellent biocompatibility, yet they suffer from poor mechanical and soft-tissue bioadhesive properties, and can be pro-inflammatory.⁷⁻⁸ Materials manufactured from biodegradable polyesters are hydrophobic in nature and lack soft-tissue bioadhesive properties.⁹ The identification of surface treatments that enable bioadhesion in wet environments will significantly advance the development of bioadhesives.

54
55 Owing to its established clinical use, favourable degradation characteristics and possibilities for
56 sustained drug delivery, poly-(lactic-co-glycolic acid) (PLGA) is one of the most popular
57 biodegradable polymers.¹⁰ PLGA is particularly attractive as a bioadhesive material as its bulk
58 properties and biodegradation rates can be controlled by the lactic acid/glycolic acid ratio,¹¹⁻¹⁴
59 and is an ideal material for biomedical applications.¹⁵ However, like other synthetic polymers,
60 PLGA is hydrophobic in nature with low surface energy and surface charge. The absence of
61 natural recognition sites for cells on the surface of PLGA compromises cell attachment¹⁶ and
62 tissue adhesive properties. As such, there is an increased tendency to employ surface
63 modification techniques (eg. plasma treatment) to improve the surface properties of PLGA.⁹

64
65 Often referred as the fourth state of matter, plasma is a mixture of charged and neutral particles,
66 such as atoms, molecules, ions, electrons, radicals, photons, etc. There are two main categories
67 of plasma: thermal and non-thermal.^{12, 15} Whilst the high gas temperatures of the former render
68 it unsuitable for the treatment of plastic thin-films, the ambient gas temperatures of the latter
69 render it suitable for such heat sensitive materials. The relatively high electron temperatures in
70 non-thermal plasmas give rise to reactive species which can modify the surface of polymers both
71 chemically and physically.¹⁷⁻¹⁸ Plasma treatment can be used to introduce different functional
72 groups onto inert surfaces either directly or indirectly. Examples of direct modification include:
73 ammonia (NH₃) plasma, which introduces amines; O₂ plasma, which introduces a mixture
74 consisting mainly of hydroxyl and peroxy radicals that react with air to form hydroxyl and
75 peroxide groups, respectively,^{9, 15, 19} and; argon plasma, which also introduces free radicals, the
76 reactivity of which is influenced by the immediate environment. The incorporated functional

groups also allow further polymerization or immobilization of additional molecules onto the treated surface.^{9, 15, 20} Examples of physical changes caused by plasma treatment include cross-linking of polymer chains, polymer degradation, and the temporary formation of free radicals.²¹

It has been reported that cell culture devices, petri dishes and various membranes can be modified by plasma treatment to improve cell adhesion and growth.²² Plasma treatment has also been employed on PLGA scaffolds,²³ films^{12, 23-24} and nanoparticles,²⁵⁻²⁶ either to improve surface properties or as a preliminary step for further modification, however the main focus has been on cell adhesion and cell affinity to the substrates. There are, however, few reports on the surface morphology of PLGA after plasma treatment and the effect of surface morphology on cell adhesion.⁹ Also, there exists scant information on surface functionalized plasma treatments specifically for bioadhesion on *ex-vivo* soft tissues. Apart from studies into the effect of plasma treatment on incorporation and subsequent release of Ketoprofen²⁷ and caffeine²⁸ from polyamide 6.6 fibers, no other studies into the effect of plasma treatment on tuning drug release have been conducted to our knowledge, although there exists plasma-based techniques to form tunable drug delivery nano-coatings.²⁹

In this work, we aim to identify post-manufacturing, plasma-based surface treatments to activate hydrophobic biodegradable polyesters for bioadhesion, while maintaining or increasing the drug release kinetics of the pretreated film. To our knowledge, such plasma-based treatments have yet to be employed for soft-tissue bioadhesion and tuning of drug release kinetics. Surface free-radicals are known to have protein immobilizing capabilities³⁰ and are capable of

cross-linking or even degrading the surface of thin-films. Therefore, we hypothesized that plasma-treating thin-films would produce free-radical-rich surfaces that exhibit improved soft-tissue bioadhesion and allow tuning of the drug release kinetics. The drug-like fluorescent molecule, fluorescein diacetate (FDAC), is a model system for the hydrophobic drug paclitaxel (PCTX). We employ FDAC in this work as a model for hydrophobic drug release, as it can be converted to the fluorescent molecule fluorescein after release and evaluated optically.³¹ Bioadhesion is assessed by shear adhesion measurements of PLGA films to porcine aorta tissue. We investigate the influence of plasma treatment parameters, including treatment time, gas flow rate, oxygen to argon ratio and radio frequency (RF) power towards FDAC release and bioadhesion.

1. EXPERIMENTAL SECTION

Materials

PLGA 50/50 (50/50 lactide-glycolide ratio), with inherent viscosity 1.03 dL/g and molecular weight ~100 kDa, was purchased from Purac (Netherlands). HPLC grade dichloromethane (Tedia, Singapore), Fluorescein Diacetate (FDAC) (TCI Japan, Singapore), and Polysorbate 80 (Tween 80) (Sigma-Aldrich, Singapore) reagents were used as received.

Preparation of PLGA films

Two sets of PLGA films were prepared, the first with 10% FDAC (w/w of PLGA) and the second without. For both, a 10 % (w/v) solution of PLGA was prepared by dissolution in DCM, which was left to stir overnight. The viscous solutions were cast onto flat substrates using an 80 mm wide knife applicator, with a wet thickness of 500 μ m. Cast PLGA films were dried at room temperature overnight (to prevent foaming), followed by a week at 40° C in a vacuum oven (for further solvent removal). All PLGA was taken from a single Purac batch and thin-films were cast in multiple batches, depending on the experiment. The overall dry thickness across casting batches was measured to be approximately 30 ± 7 μ m.

For in vitro FDAC release studies, 6-mm-discs were punched from PLGA films post-plasma-treatment. For ex vivo bioadhesion studies, PLGA films were cleaved into squares with dimensions of 2 cm and mounted onto glass slides using double-sided tape (Star Brand, Singapore) prior to plasma treatment. For contact-angle measurements, PLGA films were cleaved into 4 cm x 1 cm strips prior to plasma treatment. For molar mass analysis, PLGA films were cleaved into 1 cm² samples prior to plasma treatment.

Plasma treatment

Plasma surface treatment of PLGA films was carried out in a Covance multi-purpose plasma system (FEMTO SCIENCE, South Korea). Control samples were not plasma treated. All samples were placed between two parallel-plate electrodes enclosed in a rectangular stainless steel chamber with dimensions 193 mm x 143 mm x 210 mm. The base pressure was 3.00 e⁻¹ Torr. Plasma was created with an inductively coupled RF generator operating at a frequency of 50 kHz with a power range of 10-300 W. Flow rates of argon and oxygen were independently controlled to establish fixed gas ratios. The plasma treatment parameters investigated are summarised in **Table 1**.

Table 1

Contact angle measurements

The surface properties of plasma-treated and control films were analyzed by employing a contact angle goniometer (Analytical Technologies, Singapore) using a static sessile drop technique. Under ambient conditions, distilled water was pumped out of a syringe at a rate of 5 µL/s and allowed to relax for 15 seconds before the image was captured. Images were analyzed with FTA32 software, version 2.0, build 276.2. Averages of 5 measurements were taken for each film.

Atomic Force Microscopy (AFM)

The surface morphology of plasma treated PLGA films was examined by atomic force microscopy (AFM) using a Nanoscope IIIa microscope (Digital instruments Inc., Santa Barbara, CA). AFM images were recorded in tapping mode under ambient conditions employing NCH-50 Point Probe made from monolithic silicon (NanoWorld AG) (Force constant equal to 42 N/m,

resonance frequency equal to 320 kHz and tip radius equal to 10-15 nm). The films were imaged at a scan rate of 1 Hz with a resolution of 512x512 pixels. The scan size was fixed at either 1 μm x 1 μm or 5 μm x 5 μm . Three-dimensional images of surface topography and roughness data were acquired using Nanoscope image processing software (version 5.12r5). Consistent roughness data was observed for multiple scans of the same region, indicating minimal damage to the surface by the probe.

X-ray Photon Spectroscopy (XPS)

XPS was performed using a PHOIBOS 100 X-ray photoelectron spectrometer (SPECS GmbH, Germany) with an Al K α X-ray source ($h\nu=1486.6$ eV), operating at or below 10^{-10} Torr. Fixed analyzer transmission analysis mode was employed with a 7 mm x 20 mm entrance slit, leading to a resolution of 0.1 eV. Pass energies for the survey scan and the small regions equal to 100 eV and 20 eV were applied, respectively. The spectra were fitted using Casa XPS software (version 2.3.13, Casa Software, UK).

Ultraviolet absorbance/Size Exclusion Chromatography (UV/SEC)

PLGA films (FDAc free) were dissolved in 1 mL of chloroform. An Agilent 1100 series HPLC pump complete with degasser and PLGel aqueous 50 (Polymer Standards Service, Mainz, Germany) in a 35° C temperature-controlled oven was connected in-line with an Agilent 1100 refractive index and UV absorbance ($\lambda=270$ nm) detectors. A CHCl₃ elution buffer was used at a flow rate of 1.0 mL/min and the PLGA injection volumes were typically 50 μL . Molar mass

analyses were performed relative to polyethylene glycol standards of known molecular weight (MW) (Polymer Standards Service, Mainz, Germany).

In-vitro FDAC release studies

Each of the punched discs was immersed in 200 μ L of phosphate buffered saline (PBS)/2% Tween 80 solution, in a Corning 96 well black flat-bottom polystyrene plate. Samples were assayed in quintuplicate and stored in an incubator at 37 °C between sampling events. For each sampling event, 20 μ L of the aliquot was withdrawn and diluted into 180 μ L of 0.1M NaOH in the read plate. The resulting fluorescein (ex/em 490/520) was immediately read on a Tecan M200 plate reader (i-control™ – Microplate Reader Software) under ambient conditions. The remaining was solution carefully drawn out of the release plate and replaced with 200 μ L of PBS/2% Tween 80. Total FDAC concentration was determined as the sum of released FDAC and the amount of FDAC remaining in the film at the final time point. The latter was determined by dissolving the 6-mm-discs in acetone and diluting in release buffer before analyzing in the plate reader.

Ex-vivo bioadhesion studies

Porcine aortic vessels were freshly harvested within 8 h of slaughtering from Primary Industries Pte Ltd (Singapore) and stored in isotonic saline at 4° C. Excess fat was removed from excised porcine aorta and the aorta samples were mounted on glass slides using cyanoacrylate superglue, endothelium side up. PLGA films were plasma-treated directly on glass slides, according to the process parameters in **Table 1**. Immediately after treatment, films were pressed against the tissue using a paper clamp that applied an average force equal to 1375 ± 250 mN over an area of 4 cm² for 2 min to approximate the pressure sensitive bioadhesion principle. The glass slides were

attached to a modified tensile tester designed to measure shear tensile strength with a linear speed of 3 mm min⁻¹ employing a 10 N load cell (0.25% accuracy, TLC-0002, Chatillon, USA) at room temperature. For each film parameter, five replicates were measured.

Statistical analysis

Data are presented as means $\pm$ standard deviation across samples. Statistical analysis was performed using one-way ANOVA. A p-value <0.05 was considered statistically significant.

3. RESULTS

Plasma treatment alters the surface wettability and introduces reactive free-radicals

It can be seen from **Figure 1** that water contact angles on dry PLGA films were reduced by exposure to plasma. A minimum reduction of 30 % was observed on plasma-treated PLGA surfaces ($53.8 \pm 0.9^\circ$) compared to pristine PLGA surfaces ($78.4 \pm 1.7^\circ$), indicating an increase in hydrophilicity, which has been well documented elsewhere.^{12, 15, 32} Water contact angles on PLGA films treated under varying plasma conditions were not significantly different, although PLGA films treated with argon plasma exhibited consistently lower water contact angles than PLGA films treated with oxygen plasma.

Figure 1

Increased surface area and roughness owing to plasma etching

The average surface roughness, R_a , was calculated for each sample from AFM images which are given in **Figure 2**.

Figure 2

Average surface roughness, R_a , and root-mean-squared (rms) roughness, R_q , values are given in **Table 2**, along with surface area difference (the increase in surface area arising from plasma treatment), expressed as a percentage of flat geometric surface area. Minimal etching was observed for plasma-treatment-times of 1 minute and 5 minutes, with R_q values of 0.531 nm, 0.607 nm and 2.17 nm for control, 1 minute and 5 minutes treatment times, respectively.

However, films treated for 7 minutes and 10 minutes were etched significantly more, resulting in the appearance of valleys and peaks, and correspondingly high roughness values. The appearance of pits is attributed to degradation of polymer chains on the surface.¹²

Table 2

Increased distribution of C-O groups on the PLGA surface by plasma treatment

PLGA samples were analyzed by XPS prior to and after 10 min oxygen plasma treatment at an RF power of 100 W with an oxygen flow rate of 50 sccm. In both cases, the spectra show the presence of oxygen and carbon. The C1s spectra are given in **Figure 3**. Three contributions are present, C-C, C-O, and C=O at ~285, 287, and 289 eV, respectively.³³ The atomic percentages deduced from the integrated area are given in **Table 3**. For plasma treated samples, the amount of C-O and C=O were very similar, while the amount of C-C was ~50 % lower, as expected from the PLGA structure. For the untreated sample, the C-C peak was very high, indicating sample contamination, thus the first effect of plasma treatment would be surface cleaning. The O1s spectra are also shown in **Figure 3**. These spectra were fitted considering two contributions at 532.2 and 533.7 eV, which are attributed to C=O and C-O, respectively. The photopeak shape was modified by the plasma treatment showing a higher contribution of simple bound oxygen.

Figure 3

The calculated atomic percentages, given in **Table 3**, show a considerable increase of oxygen percentage at the surface after plasma treatment, arising mostly from the removal of organic contamination; the ratio of oxygen to carbon increased by a factor of 3. More significantly, the

ratio O/(C-O, C=O) increased by approximately 30%, indicating the presence of additional oxygen not bound to carbon, probably arising from C-O-O groups. Very few studies exist on the XPS analysis of peroxide, however the binding energy of these oxygen species is expected to be in the same region as O-C, at around 533.7 eV.³³

Table 3

Increased PLGA polydispersity with treatment time and argon flow rate.

Previous SEC experiments (data not shown) have shown that encapsulated FDAc elutes in the same region as small MW PLGA at 9-10 mL elution volume. Therefore, a set of films was prepared without FDAc specifically for UV/SEC analysis. Upon plasma treatment under the conditions outlined in **Table 1**, no significant shifts in peak maxima were observed (~0.04 RIUs at 6.8 mL and 0.01 RIUs at 9.4 mL), as can be seen in **Figure 4A**.

Figure 4

If plasma degradation occurred and produced molar mass fragments < 20 kDa, a ratio change at the 6.8 and 9.4 mL peaks would be expected—however ratio changes did not exceed 10 %, suggesting no significant degradation of the main PLGA peak at 6.8 mL. However, 6.8 mL peaks for plasma-treated films are slightly broader than non-treated control films, indicating an increase in polydispersity. Carbonyl species (C=O) and peroxide groups (C-O-O), products of PLGA degradation, are known to have small absorbance extinction coefficients in the range of

260-280 nm.³⁴ We sought to observe an increase in C=O and C-O-O groups at 270 nm to verify degradation of PLGA but, to our surprise, the absorbance of the 6.8 and 9.4 mL peaks decreased with increasing plasma treatment time, as shown in **Figure 4B**. **Figure 4C** summarizes the results of the SEC analysis, noting that the polydispersity of the PLGA peak at 6.8mL changed with plasma treatment time and argon flow rate, indicative of degradation or cross-linking. However, as SEC typically has an error of $\pm 10\%$ in molar mass, no significant changes in molar mass were seen compared to non-plasma treated control.

FDAc release can be controlled by treatment time.

To study the influence of plasma parameters on FDAc release, *in vitro* release studies of PLGA films containing 10% (w/w) FDAc were conducted over a span of 24 days. The effects of plasma treatment time, RF power, gas flow rate and oxygen to argon ratio on cumulative FDAc release were studied in detail to identify the process parameters critical to FDAc release properties.

The release profiles of control and plasma-treated PLGA films are plotted in **Figure 5**. It can be seen that all plasma treatments significantly increase cumulative FDAc release from PLGA films. We attribute this to the improved wettability and increased polydispersity of plasma-treated PLGA films.

Figure 5

Among the parameters studied, plasma treatment time had the most significant effect on the cumulative FDAc release. There was a significant increase in cumulative FDAc release with increasing plasma treatment time up to 7 minutes. Plasma treating PLGA films for 1, 5, 7 and

10 minutes resulted in cumulative FDAC releases of $11.2 \pm 0.4 \%$ and $13.7 \pm 1.0 \%$, $17.0 \pm 1.0 \%$ and $17.0 \pm 1.3 \%$, respectively, compared to $10.3 \pm 0.3 \%$ for control samples, as seen in **Figure 5A**. Put another way, the relative FDAC release increased by $\sim 9 \%$ and 33% relative to controls for plasma-treatment times of 1 and 5 minutes, respectively. As such, we consider 7 minutes to be the maximum threshold for plasma treatment for controlling FDAC release.

The cumulative FDAC release from films that have been plasma treated under various argon flow rates displayed an inverse trend from 10 sccm to 40 sccm. Although these films exhibited a higher FDAC release rate than controls, the cumulative FDAC release decreased with increasing flow rate, as shown in **Figure 5B**. Films subjected to argon plasma at a flow of 10 sccm and 40 sccm displayed cumulative FDAC releases of $14.7 \pm 0.5 \%$ (43 % higher than control films) and $12.1 \pm 0.9 \%$ (17 % higher than control films), respectively. We speculate that an increase in argon flow caused increased surface cross-linking, owing to an increased density of free radicals. The only differences between plasma treatments with oxygen and argon plasmas are the free radicals that are formed during the plasma treatment. Based on the known mechanisms of argon and oxygen plasmas, argon plasma would give rise to more polymer backbone cross-linking.³⁴

Neither power (in the limited range tested of 60 to 180 watts), nor the ratio of argon to oxygen gas had a significant effect on FDAC release, as shown in **Figure 5C and D**. Even pure oxygen and argon plasmas resulted in similar release rates (data not shown). This is likely owing to similar pathways for cross-linking or degradation of film surfaces in each case, which we consider the main parameters influencing cumulative FDAC release.

Bioadhesion is dependent on the free-radical generation

The effect of plasma treatment on bioadhesion was studied by evaluating the shear tensile strength between plasma-treated films and porcine aorta tissues, as outlined in **Figure 6A**. The shear tensile strength was augmented from 47.7 ± 14.4 mN/cm² to 156.9 ± 24 mN/cm² by increasing the oxygen plasma treatment time from 1 minute to 5 minutes, respectively, at an RF power of 100 W, as shown in **Figure 6B**. To identify the mechanisms responsible for the improved bioadhesion, i.e. change of surface roughness or free-radical cross-linking, the films were incubated for 24 h in water, so that all free-radicals were quenched. **Figure 6B** shows that bioadhesion strength reduced to that of the non-plasma-treated controls, indicating that free-radical cross-linking is responsible for the improved bioadhesion.

Figure 6

Bioadhesion strengths for oxygen-plasma-treated films were found to be ~2-fold and 6-fold higher than controls for a treatment time of 5 minutes at RF powers of 20 W and 100 W, respectively. Interestingly, oxygen: argon ratio did not have a significant effect on shear tensile strength, further supporting the role of free-radicals in improving bioadhesion.

4. DISCUSSION

Novel bioadhesive materials have the potential to transform localised drug delivery by increasing the residence time and bioavailability of drugs at specific tissue sites. Key considerations are the bioadhesive and mechanical properties of the material, as well as cytotoxicity, biodegradation time and drug release profile. Mucoadhesive materials have seen application in nasal and oesophageal drug delivery however, the non-covalent bonding of such materials to the mucosal membrane does not provide adequate adhesion to other biological surfaces such as intra-vascular surfaces. Furthermore, PLGA is commonly employed in drug delivery systems and there is a growing need to tune drug release rates to cater for various biomedical applications. Previously, our group has been able to tune release rates of FAc/paclitaxel from PLGA by controlling the concentrations of acidic terminal end-groups.³⁵ While these strategies allow tuning of release rates prior to production, the ability to tune release rates after production is limited. Here we have investigated the use of free-radicals generated by non-thermal plasma to improve bioadhesion and the effect of such plasma treatment on the release of the model drug FAc from PLGA films.

Chemical changes in the PLGA matrix

Non-thermal plasmas contain highly energetic reactive species, which abstract the proton from tertiary carbon of PLGA. The free radical is formed at the tertiary carbon $R^{\cdot}$ and the tendency of radical formation decreases from tertiary to primary carbon. Alkyl radical on exposure to air forms peroxy radicals ($RCOO^{\cdot}$), which abstract hydrogen from the environment or polymer to form the peroxide $RCOOH$ (**Figure 7**). The alkyl radicals react with molecular oxygen

practically without activation energy, i.e. the reaction occurs at approximately the same rate independently of temperature.³⁶

Figure 7

XPS characterization of plasma-treated and control films demonstrated an increased ratio of O/(C-O, C=O), arising from C-O-O-H, or peroxide, groups. The formation of peroxide groups resulted in the lower water contact angles measured on plasma-treated PLGA films relative to controls. Interestingly, the water contact angle does not appear to depend on plasma treatment parameters, instead displaying an ‘on/off’ behaviour. In addition, we noticed an increase in the oxygen to carbon ratio after plasma treatment, indicating the removal of surface contaminants. We speculate this organic contamination arose during drying of PLGA films in a vacuum oven.

AFM studies highlighted significant etching of PLGA surfaces after more than 5 minutes plasma treatment, with average surface roughness values reaching 16.1 nm after 10 minutes, in contrast to nm-roughness after 5 minutes and sub-nm-roughness after 1 minute, which suggests degradation of the polymer. UV/SEC data showed an increased polydispersity of PLGA, with negligible change in molar mass and a reduction in absorbance at 270 nm with increasing plasma treatment time. This, along with AFM data, suggests that plasma treatment causes a pyrolytic degradation of PLGA. This is in contrast to hydrolytic or trans-esterification degradation, where no drop in absorbance at 270 nm and a large reduction in molar mass would be expected owing to random chain scission.

Pyrolytic or thermal oxidative degradation mechanisms have previously been characterized for PLLA,³⁷⁻³⁸ a polyester with very similar chemical reactivity to PLGA. Under pyrolytic

degradation, a red shift in absorbance maxima has been reported during the formation of conjugated double bonds in PLLA under high stress, free-radical forming conditions.^{37, 39} A red-shift of absorbance maxima would cause a corresponding reduction in absorbance at 270 nm, as observed in our work. Additionally, pyrolytic degradation progresses as an unzipping de-polymerization, starting at the terminal hydroxyl end of the PLLA or PLGA chain.⁴⁰⁻⁴¹ The pyrolytic reaction kinetics are too slow in the bulk material to significantly affect the molar mass, which is consistent with our results. However, at the surface of the film we expect the reaction kinetics to be exponentially higher, owing to a lack of diffusion lag of argon or oxygen ions, which is consistent with the etched surfaces that we observed by AFM.

Effect of plasma treatment on bioadhesion

We have tested the shear adhesion force between plasma-treated thin films and non-plasma-treated controls and our results show a vast improvement in shear tensile strength to porcine aorta after plasma treatment. We observed a strong positive correlation between shear tensile strength and plasma treatment time, with a 6-fold increase in the shear tensile strength relative to controls after 5 minutes treatment in oxygen plasma at 100 W. We also noted a strong positive correlation between shear tensile strength and RF power. The maximum measured shear tensile strength was 156.9 ± 24 mN/cm².

To qualify the role of free-radicals in promoting bioadhesion, free-radicals were quenched by incubation in water for 24 hours after plasma treatment. After quenching, the shear tensile strength was comparable to controls, demonstrating that the generation of free-radicals is the dominant mechanism for adhesion promotion arising from plasma treatment. This is in contrast

to an increased surface area and mechanical interlocking arising from increased surface roughness.

The shear tensile strengths achieved in this work compare favourably to established bioadhesive materials. Cyanoacrylates currently offer the highest adhesion strengths, with a reported adhesion strength to porcine aorta equal to 5 N/cm².⁴² However, as discussed earlier, cyanoacrylates tend to be brittle and pro-inflammatory, whereas PLGA films exhibit excellent biocompatibility. Commercial fibrin glue, Tissucol, has been tested on porcine aorta and an adhesive strength of 800 mN/cm² has been reported,⁴² although this was conducted in a dry environment. The adhesion strength of DOPA-based adhesives to swine skin has also been tested and was found to be 30 mN/cm².¹ Plasma-treated PLGA films offer a five-fold increase in adhesion strength over DOPA-based adhesives and the mechanical properties are favourable. A recent hydrophobic light-activated bioadhesive has been reported with impressive adhesion strengths of 0.5-2.0 N/cm².⁴³ Despite the initial high adhesion values, the authors suggest entanglement is the major mechanism of adhesion, which, along with the use of photo-initiators, may prove detrimental for long term bioadhesion.

Through comparison of reported *in-vivo* flow measurements, we speculate that the shear tensile strengths achieved in this work can withstand typical blood flows from large millimeter-sized femoral arteries to micrometer-sized arterioles that have shear stresses of 0.03 to 0.5 mN/cm², respectively.⁴⁴ However, film edge effects, which tend to peel films under normal forces, need to be considered. Large capillary forces between our thin films and the swine tissue prevented determination of normal forces.

We speculate that these PLGA bioadhesive thin films may be applicable to other soft tissue ailments wherever adhesive tape, localized drug delivery, or both maybe necessary or advantageous. Thoracic surgeries are one example where a biocompatible adhesive tape is in great need. Intraoperative air leaks during lung surgery have a high incidence close to 70% and are one of the principal causes of chest tube drainage lasting longer than 7 days⁴⁵⁻⁴⁶. A resorbable synthetic sealant has yet to be developed that allows sealing of the punctured lung with the option of sustained antibiotic drug delivery. Similar sealants are needed for other soft tissue membranes including dura membranes involved in head, neck, and spine surgeries.

Effect of plasma treatment on FDAC release

We investigated the effect of cumulative FDAC release over 24 days from plasma-treated and control PLGA films. Cumulative release was found to increase with increasing treatment time, saturating at 7 minutes, with a maximum cumulative release 65 % higher than controls. No effect on cumulative release was observed for either RF power or plasma composition, however a reduction in cumulative release was observed with increasing flow rate for argon plasma.

We have previously reported a threshold ratio of 1:1000 acidic end-groups to give rise to increased drug release from PLGA.³⁵ Pyrolytic degradation will expose acidic end-groups and we postulate that this threshold is exceeded after a certain treatment time, giving rise to the positive correlation observed in this work. Plasma composition is not expected to affect the exposure of acidic end-groups since the degradation pathways of PLGA are similar for oxygen and argon plasmas. However, argon plasmas induce more polymer backbone cross-linking than oxygen plasmas, which would reduce the diffusion of FDAC through the polymer. This is a

443 possible explanation for the observed reduction in cumulative release with increasing argon flow
444 rate.

445

446

5. CONCLUSION

The adhesion of plasma-treated PLGA films to porcine aorta was investigated by determining the shear tensile strength as a function of plasma composition, treatment time, RF power and gas flow-rate. The effect of plasma treatment on the release of the model drug FDAc from PLGA films was also investigated as a means to control both drug release and bioadhesion post-fabrication.

Positive correlations have been established between shear adhesion strength and both plasma treatment time and RF power, allowing one to tune bioadhesion by appropriate choice of plasma-treatment parameters. We report a 6-fold increase in shear tensile strength to $156.9 \pm 24 \text{ mN/cm}^2$, relative to non-plasma-treated controls. From free-radical quenching studies, we can conclude that plasma-enhanced bioadhesion arises from the formation of surface free-radicals, which allow the formation of covalent bonds with tissue, consistent with our hypothesis. The novelty of this method is that the intrinsic physicochemical properties of the substrate are retained, while allowing control over the bioadhesive properties.

Plasma treatment significantly increased the cumulative release of the model drug, FDAc. Our results demonstrate that plasma treatment time is the critical parameter for controlling FDAc release, and we report a 65 % increase in maximum release at 24 days after only 7 minutes plasma treatment. We attribute the increased release to the pyrolytic degradation of PLGA by the plasma and the corresponding increase in exposed acidic end-groups. Notably, no correlation

was observed between FDAC release and RF power, highlighting the ability to independently tune drug release and bioadhesion by plasma-treatment.

These results highlight the control over FDAC release and bioadhesion afforded by non-thermal plasma treatment, while retaining the bulk material properties. Free-radicals offer a promising strategy for improving drug release and bioadhesion, although we recognize that vacuum plasmas are impractical for the simplified requirements needed in hospital operating rooms. Advancements in atmospheric plasma technology and hand-held ‘plasma-pencils’ could allow clinicians to activate a hydrophobic thin-film formulation on site or during surgery.⁴⁷ As such, we are exploring atmospheric plasma technology and other event-activated methods of surface free-radical formation towards soft-tissue bioadhesion.⁴⁸

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Figure captions

Figure 1. Contact angles of PLGA films are plotted as a function of: (A) plasma treatment time in a 1:1 plasma at 100 W; (B) gas flow rate at 100 W for 2 mins; (C) plasma power in a 1:1 plasma for 2 mins, and (D) oxygen:argon ratio at 100 W for 2 mins compared to literature.¹²
* $p < 0.05$; significant against non-plasma treated control PLGA films

Figure 2. Height AFM images and average roughness values of (A) control PLGA film and PLGA films after plasma treatment of 1:1 plasma at 100W for (B) 1min, (C) 5min, (D) 7min and (E) 10min respectively. Height Scale Range: 100nm. Scan size: 1 μ m (A, B) and 5 μ m (C, D, E).

Figure 3. X-ray photon spectroscopy (XPS) spectra of PLGA films before treatment (top), and after treatment (bottom) in a 1:1 plasma at 100 W for 10 min.

Figure 4. Plots of refractive index (A) and 270 nm absorption (B) as a function of elution volume for PLGA films treated in a 1:1 plasma at 100 W for 3, 5, 7 and 10 minutes, along with controls. Molar mass and polydispersity is plotted (c) as a function of plasma treatment parameters.

Figure 5. Cumulative FDAc release from PLGA films is plotted as a function of time as the following parameters are varied: (A) treatment time for a 1:1 plasma at 100 W; (B) argon flow rate for a treatment time of 2 mins at 100 W; (C) RF power for a treatment time of 2 mins in a 1:1 plasma, and (D) oxygen:argon ratio for a treatment time of 2 mins at an RF power of 100 W. Insignificant differences are indicated by an x. Cumulative FDAc release is also plotted as a function of (E) treatment time and (F) argon flow rate, evaluated after 24 days.

Figure 6. (A) Schematic of sample preparation for uniaxial tensile testing. Shear bioadhesion forces of PLGA films to porcine aorta are plotted as a function of: (B) treatment time in oxygen plasma at 100 W for immediate application and application after 24 hours immersion of PLGA

648 in water; (C) RF power in oxygen plasma for a treatment time of 5 minutes, and (D)
649 oxygen:argon ratio for a treatment time of 3 mins at 50 W. Significant differences, determined
650 by a p-value less than 0.05 are indicated by an asterix.

651 **Figure 7.** Illustration of the reaction scheme for plasma-treated PLGA. A pyrolytic degradation
652 is illustrated, proceeding from the –OH end-terminal functional groups.

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TABLES

Table 1: Parameters Varied for Plasma Treatment PLGA

Parameters varied	Time (mins)	Power (W)	Oxygen flow rate (sccm)	Argon flow rate (sccm)	O ₂ :Ar
For <i>in vitro</i> release studies					
Time	1	100	10	10	1 : 1
	5	100	10	10	1 : 1
	7	100	10	10	1 : 1
	10	100	10	10	1 : 1
Argon Flow	2	100	-	10	-
	2	100	-	20	-
	2	100	-	40	-
Oxygen Flow	2	100	10	-	-
	2	100	20	-	-
	2	100	40	-	-
O ₂ : Ar	2	100	8	12	2 : 3
	2	100	10	10	1 : 1
	2	100	12	8	3 : 2
Power	2	60	10	10	1 : 1
	2	120	10	10	1 : 1
	2	180	10	10	1 : 1
Control	-	-	-	-	-
For <i>ex vivo</i> bioadhesion studies					
O ₂ : Ar	3	50	5	15	1:3
	3	50	10	10	1:1
	3	50	15	5	3:1
	3	50	20	-	1:0
Time	1	100	50	-	-
	3	100	50	-	-
	5	100	50	-	-
Power	5	20	50	-	-
	5	60	50	-	-
	5	100	50	-	-
Control	-	-	-	-	-

Table 2: Surface Roughness data for pristine PLGA films and PLGA films that were plasma treated for different durations.

	Roughness Average, R_a (nm)	Roughness Parameter, RMS (nm)	Average max height(nm)	Average max depth(nm)	Surface Area difference (%)
Control	0.404±0.06	0.531±0.07			
1 minute	0.420±0.08	0.607±0.11	0.0828±0.02	0.0491±0.01	0.184±0.01
5 minutes	1.29±0.31 ^{*a}	2.17±0.47 ^{*a}	1.1±0.67 ^{*a}	0.219±0.11 ^{*a}	0.123±0.06
7 minutes	9.05±0.43 ^{*b}	10.9±0.51 ^{*b}	17.3±3.23 ^{*b}	8.52±1.33 ^{*b}	2.78±0.20 ^{*b}
10 minutes	16.1±0.80 ^{*c}	19.1±1.03 ^{*c}	36.2±3.40 ^{*c}	15.9±4.64 ^{*c}	4.73±0.58 ^{*c}

^{*}p<0.05; significant against PLGA films treated for ^a1 minute, ^b5 minutes, ^c7 minutes.

670 **Table 3: XPS data for pristine PLGA and PLGA films that were plasma treated for 10 min**

	C1s		O1s		Ratio		
	C-C	C-O	C=O	O=C	O-C	$\frac{O}{C}$	$\frac{O}{[C-O, C=O]}$
Untreated							
BE (eV)	284.7	286.9	289.1	532.2	533.7		
% at	43.6	16.3	13.6	14.3	11.6	0.35	0.86
Plasma treated							
BE (eV)	285.0	287.0	289.2	532.3	533.7		
% at	10.2	19.6	22.3	20.9	26.8	0.92	1.14

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A)

Ra = 0.40nm

B)

Ra = 0.43nm

C)

Ra = 1.29nm

D)

Ra = 9.05nm

E)

Ra = 16.1nm

Before treatment

After treatment

PLGA before Plasma Treatment

PLGA with Free-Radicals

PLGA Pyrolytic Degradation

PLGA with Peroxy Radical