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SYMPOSIUM

A Review of the Development of a Vehicle for Localized and Controlled Drug Delivery for Implantable Biosensors

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Abstract

A major obstacle to the development of implantable biosensors is the foreign body response (FBR) that results from tissue trauma during implantation and the continuous presence of the implant in the body. The *in vivo* stability and functionality of biosensors are compromised by damage to sensor components and decreased analyte transport to the sensor. This paper summarizes research undertaken by our group since 2001 to control the FBR toward implanted sensors. Localized and sustained delivery of the anti-inflammatory drug, dexamethasone, and the angiogenic growth factor, vascular endothelial growth factor (VEGF), was utilized to inhibit inflammation as well as fibrosis and provide a stable tissue–device interface without producing systemic adverse effects. The drug-loaded polylactic-co-glycolic acid (PLGA) microspheres were embedded in a polyvinyl alcohol (PVA) hydrogel composite to fabricate a drug-eluting, permeable external coating for implantable devices. The composites were fabricated using the freeze–thaw cycle method and had mechanical properties similar to soft body tissue. Dexamethasone-loaded microsphere/hydrogel composites were able to provide anti-inflammatory protection, preventing the FBR. Moreover, concurrent release of dexamethasone with VEGF induced neoangiogenesis in addition to providing anti-inflammatory protection. Sustained release of dexamethasone is required for the entire sensor lifetime, as a delayed inflammatory response developed after depletion of the drug from the composites. These studies have shown the potential of PLGA microsphere/PVA hydrogel-based composites as drug-eluting external coatings for implantable biosensors.

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Introduction

Technological advances in biomedical sciences have led to the development of various implantable devices such as heart valves, pacemakers, catheters, stents, defibrillators, scaffolds, and prostheses.[1–5] Implantable biosensors for metabolic monitoring constitute a very important category of implantable biomedical devices. These biosensors will improve the management of various chronic disease conditions (for example, glucose biosensors for continuous blood glucose monitoring of diabetes patients).[5] Continuous monitoring provides the

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Abbreviations: (AUC) area under the curve, (C_{max}) maximum concentration, (FBR) foreign body response, (H&E) hematoxylin and eosin, (HA) humic acid, (IV) intravenous, (MW) molecular weight, (PAA) polyacrylic acid, (PBS) phosphate-buffered saline, (PLGA) polylactic-co-glycolic acid, (PVA) polyvinyl alcohol, (RSA) rat serum albumin, (s.c.) subcutaneous, (t_{max}) time required to achieve the maximum concentration, (VEGF) vascular endothelial growth factor

Keywords: biosensor, continuous release, dexamethasone, foreign body reaction, neoangiogenesis, implants, localized delivery, microspheres

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rate and direction of change in glucose levels, which is important for early detection of hypo/hyperglycemic states.[6] The Diabetes Control and Complications Trial[7] and the United Kingdom Prospective Diabetes Study[8] have demonstrated that intensive insulin therapy, guided by frequent blood glucose monitoring, effectively delays the onset and slows the progression of diabetes complications. The concept of enzymatic electrochemical monitoring of glucose was first established by Clark and Lyons in 1962.[9] Although a lot of progress has since been made toward the development of totally implantable glucose biosensors, *in vivo* stability and long-term functionality of such sensors remains a problem. There is a progressive loss in sensitivity and accuracy, and within a few days following *in vivo* implantation, the sensors fail.[5,10–12] This biological instability poses a great challenge toward clinical adaptation of biosensor technology.

Most implanted biomaterials encounter an immune tissue response due to tissue trauma during implantation and the presence of foreign material in the body tissue.[13,14] This leads to the development of a foreign body response (FBR) that comprises an initial acute inflammatory phase and a subsequent chronic inflammatory phase, if materials are nondegradable. The acute phase lasts from hours to days and is marked by fluid and protein exudation and a neutrophilic reaction. The chronic phase is characterized by an influx of macrophages, the formation of multinucleate giant cells, neovascularization, and the deposition of collagen with subsequent formation of an avascular fibrotic capsule around the implant (i.e., scarring) and lasts from days to years.[13,15] Inflammatory cells, their secretions (such as proteolytic enzymes and reactive oxygen species), and change in the pH of the tissue surrounding the implanted biosensor cause damage to sensor components.[16] Another associated problem is biofouling, which is the adhesion of proteins and cells onto foreign material after implantation. Biofouling and the avascular fibrous capsule greatly decrease the transport of analyte to the sensor, compromising sensor functionality.[17–19] This *in vivo* instability poses a great challenge to the development of a totally implantable glucose biosensor. At present, six minimally invasive blood glucose monitoring systems[20,21] are approved by the Food and Drug Administration (FDA). These biosensors improve diabetes care; however, there is still a need for improvement in sensor lifetime and patient comfort. The longest *in vivo* functional time of a marketed system is 7 days, and frequent calibration is required with handheld glucose meters.[22] Therefore, controlling FBR to implantable sensors is very important for their clinical adaptation. The FBR can be prevented or suppressed by delivering anti-inflammatory drugs such as the glucocorticoid drug, dexamethasone.[23–27] Glucocorticoids suppress the immune response by inhibiting the formation and secretion of inflammatory mediators such as prostaglandins and leukotrienes, diminishing the release of inflammatory cells to the injured site, decreasing capillary permeability, and suppressing fibroblast proliferation.[28]

Another aspect of improving sensor function is to increase analyte transport to the sensor via angiogenesis. This can be achieved by inducing new blood vessel formation in the vicinity of the sensor with growth factors such as the vascular endothelial growth factor (VEGF).[27] The presence of well-vascularized tissue at the implant site is also critical for healing the trauma caused during implantation and the diffusion of reaction products from the sensor to surroundings.[27] Another issue is that corticosteroid drugs, which are used to treat inflammation, block angiogenesis by inhibiting or down-regulating endogenous VEGF.[29,30] Therefore, prevention of the inflammatory response, along with a method of inducing neoangiogenesis, may be important to achieve proper sensor functionality.[27]

As the subcutaneous (s.c.) tissue is the preferred site for biosensor implantation, localized delivery of drugs offer more advantages over systemic delivery. Therapeutic levels in the s.c. tissue might not be achieved at the implant site after systemic administration.[31,32] Moreover, long-term systemic use of corticosteroids produces a number of adverse and toxic effects (e.g., withdrawal symptoms, suppression of hypothalamus–pituitary axis, electrolyte imbalance, peptic ulcer, myopathy, behavioral changes, and osteoporosis) and may complicate disease states.[28] For protein growth factors, systemic administration may lead to loss of activity and short half-life.[33]

Our group is engaged in the development of an implantable biosensor with long-term *in vivo* sensitivity and functionality.[34,35] Apart from designing a robust miniaturized microelectronic platform to develop a successful device, we also focused on controlling and preventing inflammation and fibrous encapsulation through use of a drug-delivery multifunctional coating for the sustained and localized release of tissue-response modifying drugs and agents. This article reviews the salient features of the work undertaken by our group since 2001 to achieve a stable tissue–device interface by developing a vehicle for the localized delivery of both small and large molecule drugs to the s.c. tissue.

Details of the work discussed here can be found in their original articles.[23–27,33,36–39] It is important to mention that various approaches for the delivery of tissue-response modifying drugs have been reported in the literature.[3,17,19,40–51] For example, Voskerician *et al*.[49] used a microelectromechanical system for drug delivery to the tissue surrounding implantable devices, whereas Norton *et al*.[44–46] utilized hydrogels as a drug-delivery vehicle.

Microspheres as Delivery Vehicles

Microspheres are solid, spherical particles in the micrometer size range, usually made from synthetic or natural polymers such as polyesters (polylactic-co-glycolic acid [PLGA]), polyanhydrides, and chitosan.[52] PLGAs are the most popular synthetic polymers for clinical use, as they are biodegradable, resorbable (through natural pathways), and relatively biocompatible.[52,53] PLGA degradation monomers, lactice and glycolic acid, are metabolized via Kerb's cycle, and hence they are considered nontoxic.[53,54] A number of FDA-approved PLGA-based drug-delivery systems, biomedical implants, sutures, and grafts are already on the market. Due to its proven safety record, PLGA is a preferred polymer. PLGA microspheres have been utilized for localized and controlled delivery of both small and large molecule drugs. Efficient drug delivery from microspheres enables a reduction in dose size and thus decreases the possibility of adverse effects. Drug release from PLGA microspheres is a combination of polymer erosion and drug diffusion through the polymer matrix.[55] This is influenced by polymer properties (copolymer ratio, molecular weight [MW], and glass transition temperature), experimental conditions (pH, temperature, and medium), and the physicochemical properties of the drug (hydrophobic versus hydrophilic).[52] For a particular drug, controlled release from days to months can be easily obtained by changing the polymer properties. For example, an increase in polymer MW and/or lactide content will decrease the drug release rate.[38,39,52] Accordingly, our group investigated the use of PLGA microspheres to deliver the potent anti-inflammatory drug, dexamethasone, at the implant site to prevent FBR.[25,36] Similarly, PLGA microspheres containing VEGF were developed and investigated for the induction of neoangiogenesis.[33,37]

Dexamethasone-Loaded Microspheres

Initial studies were conducted with microspheres prepared using PLGA with a copolymer ratio of 50:50 and an average MW of 40–70 kDa.[25,36] These microspheres gave a triphasic *in vitro* release profile for dexamethasone with an initial burst release (1 day) followed by a lag phase and a second zero-order release phase (2 weeks to 1 month) over a 30-day study period (**Figure 1A**). The initial burst release is controlled by the diffusion of the surface-associated drug, whereas the lag phase and secondary release phases are controlled by a combination of diffusion and polymer erosion.[55] During the second release phase, the diffusion of the drug increases due to increased porosity following polymer degradation. Even though controlled release over a 30-day period could be achieved, dexamethasone release during the lag phase was very minimal. In order to obtain a zero-order release (approximate) after the initial burst phase, a system of predegraded and freshly prepared microspheres was developed. The predegraded microspheres were prepared by putting the microspheres in phosphate buffer (pH 7.4 at 37 °C) and allowing the degradation/release process to proceed for periods of 1 and 2 weeks. Microspheres degraded for 1 and 2 weeks were mixed with freshly prepared microspheres in a 1:1:1 ratio. An initial burst release followed by an approximately zero-order release of dexamethasone was observed over 35 days from this mixed microsphere system (**Figure 1B**). This release profile suggested that the mixed microsphere system may be useful for the localized delivery of dexamethasone in the s.c. tissue surrounding implantable devices to control FBR.

The *in vivo* efficacy of this mixed microsphere system was evaluated in a subsequent study using a suture-type inflammation model (cotton thread, 1 cm long and 0.5 mm in diameter, placed in the s.c. tissue of Sprague Dawley rats).[25] An initial dose-determining study was undertaken by injecting dexamethasone saline solution into the s.c. tissue (using a saline injection as the control) in the vicinity of the suture. Histopathological examination of the tissue, after staining with hematoxylin and eosin (H&E) in the vicinity of the implant, was carried out to investigate the pharmacodynamic effect. An intense acute inflammatory reaction was observed in the adjoining tissue one day after the implantation of the cotton thread suture in control animals (receiving normal saline but no microspheres). On the other hand, the injection of dexamethasone solution (in normal saline) at the suture site prevented the inflammatory response in a dose-dependent manner, with higher doses of 0.5 and 0.8 mg being more effective than lower doses of 0.1 and 0.3 mg.

Based on these results, a 0.8 mg dexamethasone dose (solution) was used as a loading dose together with 100 mg of dexamethasone-loaded microspheres as a maintenance dose for an *in vivo* pharmacodynamic

study using microspheres.[25] Histopathology was used to investigate any tissue reaction to microspheres alone. Rats were injected with blank PLGA microspheres in normal saline. High concentrations of blank microspheres produced a pronounced tissue reaction that progressed from an acute neutrophilic phase (1 day) to a chronic phase (1 month) with giant cells surrounding the microspheres. A similar FBR has been observed with other drug-delivery systems and polymeric materials such as polyesters, polyethylene, polyethylene glycol, polycaprolactone, and polydimethylsiloxane.[4,56–58] A subsequent *in vivo* study using a mixed population of predegraded and freshly prepared dexamethasone PLGA microspheres established the efficacy of this system to suppress the inflammatory response to implanted sutures for a 1-month time period (**Figure 2**). An acute inflammatory reaction was observed in the tissue surrounding the suture when only blank microspheres were injected. In the group of rats that received dexamethasone microspheres (with loading dose), no inflammation was observed at 1 day. Similar results were observed at 1 day in the group of animals that received empty microspheres with dexamethasone saline solution as a control. At 1 week, the inflammatory response to the sutures in rats treated with dexamethasone-containing microspheres was significantly lower compared to that in control rats treated with empty microspheres (**Figure 2**) or empty microspheres in dexamethasone saline solution. This indicated that a dexamethasone bolus injection did not provide protection for 1 week. Similar results were obtained at the 1-month time period, where minimal inflammatory response was observed in the treatment group (**Figure 2**), whereas in the control groups, inflammation had progressed to chronic phase (**Figure 2**). The suture was surrounded by fibrous encapsulation with inflammatory cells infiltrating the thread. These studies demonstrated the potential of the dexamethasone PLGA-microsphere delivery system to control both the acute and chronic phases of inflammation for a period of 1 month.[25,36]

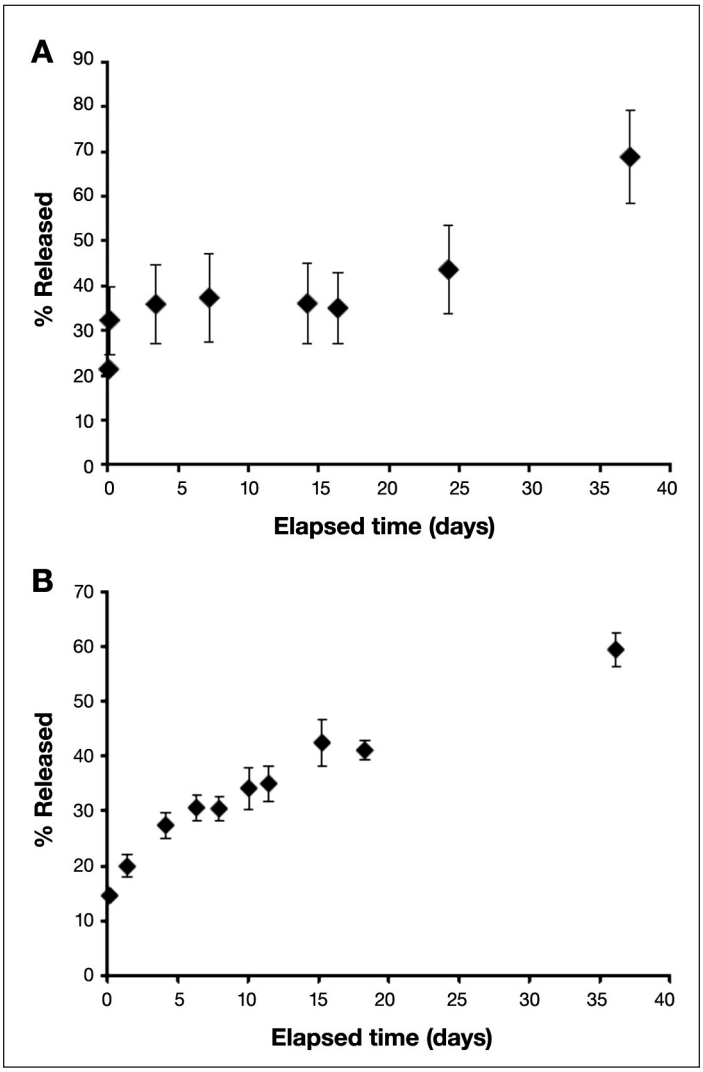

Figure 1. Cumulative dexamethasone released from PLGA microspheres during in vitro release studies in PBS (pH 7–7.4) containing sodium azide (bacterial growth inhibitor) at 37 °C (n = 3): **(A)** standard PLGA microspheres and **(B)** mixed predegraded microspheres system. (Reproduced from Reference 36 with the permission of Elsevier Ltd.)

The work presented in these studies focused only on PLGA microspheres prepared using intermediate MW

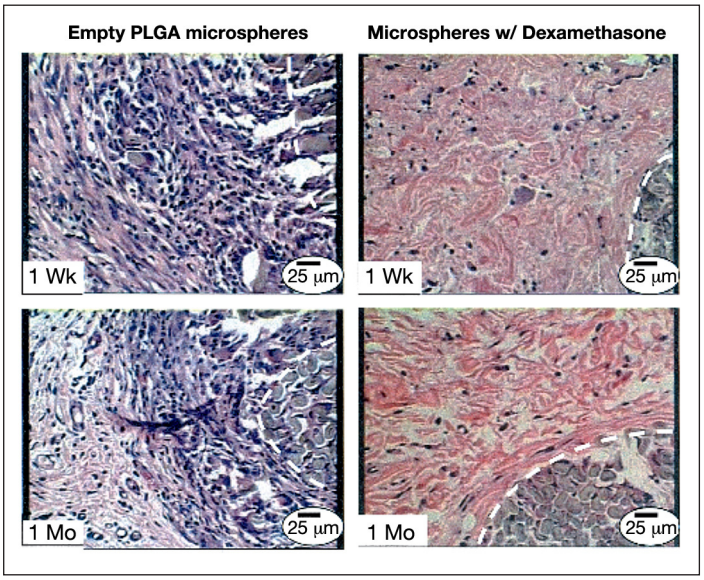

Figure 2. Evaluation of efficacy of a dexamethasone/PLGA microsphere system in suppressing inflammation to a thread suture used as a model sensor: magnified 200 times. The photomicrographs on the left are sites with thread and empty PLGA microspheres that have been implanted for 1 week and 1 month, respectively. The photomicrographs on the right are sites with thread and dexamethasone-loaded PLGA microspheres that have been implanted for 1 week and 1 month, respectively, stained with H&E. (Reproduced from Reference 25 with the permission of John Wiley & Sons, Inc.)

PLGA (40–70 kDa). Different types of PLGA microspheres were later developed using various grades of PLGA polymer to release drugs over periods ranging from days to 3 months.[38,39] In addition to the biophysical characterization of these different microspheres, accelerated *in vitro*-released testing methods were developed for rapid quality control of microsphere batches. Four different formulations of dexamethasone-loaded microspheres were prepared with the parameters in **Table 1**, where LAGA refers to lactic acid and glycolic acid ratio and encapsulation efficiency measures the fraction of total drug encapsulated.[39] The *in vitro* release from these microsphere formulations was analyzed using a modified Sotax® USP 4 dissolution apparatus using 0.1 M phosphate-buffered saline (PBS) at 37 °C. The mechanism of release changed with change in polymer MW. The low-MW (5 kDa) formulation released most of the contents within 24 h, which indicated diffusion-controlled release (**Figure 3**).[39] Microsphere formulations prepared using 25, 28, and 70 kDa PLGA exhibited typical triphasic release with the initial burst, lag, and secondary release phases (**Figure 3**). The 25 and 28 kDa MW PLGA formulations had similar physicochemical properties (**Table 1**) and showed similar release (**Figure 3**). The high-MW (70 kDa) formulation exhibited reduced initial burst effect (5%) and an extended lag phase (up to 46 days). However, dexamethasone release was extended to 85 days. The difference in dexamethasone release profiles from these formulations was due to difference in MW and copolymer ratio. PLGA degradation is a first-order hydrolysis process.[39] Therefore, degradation of high-MW PLGA takes more time than low-MW PLGA. This in turn affects microsphere porosity and the subsequent diffusion of the drug out of the microspheres, giving a slower, extended release from the high-MW PLGA microspheres. These results were corroborated by change in the morphology of the microsphere formulations.[39] The microsphere formulations developed in this study were utilized during the development of PLGA microsphere/polyvinyl alcohol (PVA) hydrogel-based composite coatings for the implantable biosensors (see the following section on coatings). The effect of pH on release from microspheres was also investigated in another study[38] but is not discussed in this review. However, pH might play a significant role during *in vivo* release, depending on the pH at the site of administration.

Table 1.
Microsphere Formulation and Characteristics[a]

Formulation	Ratio of LAGA	Mw (kDA)	Tg (°C)	Drug Loading (%)	Encapsulation Efficiency (%)
5K	50:50	5	37.6	7.68 ± 0.3	75.6 ± 4.0
25K	50:50	25	44.2	7.36 ± 1.3	70.2 ± 9.2
28K	50:50	28	45.6	8.25 ± 0.3	78.8 ± 4.4
70K	65:35	70	47.7	7.49 ± 1.1	73.0 ± 8.6

[a] Reproduced from Reference 39 with the permission of Journal of Controlled Release, Elsevier Ltd.

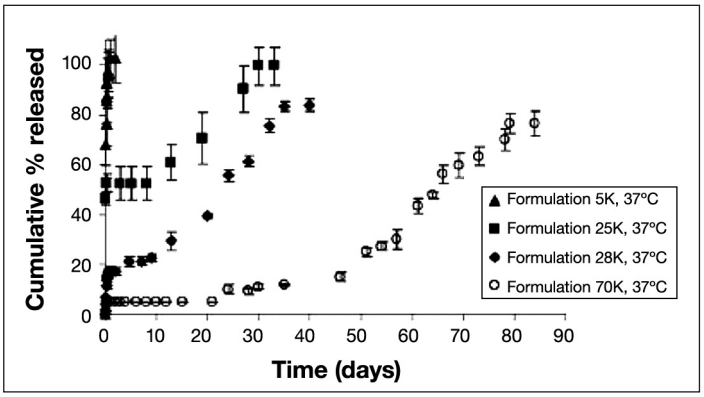

Figure 3. Dexamethasone release from four PLGA microspheres (formulations 5, 25, 28, and 70 K at 37 °C in PBS buffer [pH 7.4], using Sotax USP 4). For details of the formulations, refer to **Table 1**. (Reproduced from Reference 39 with the permission of Elsevier Ltd.)

Protein-Loaded Microspheres for Growth Factors

The presence of well-vascularized tissue surrounding the biosensor is essential for adequate analyte and gas exchange, maintaining the supply of nutrients for rapid healing after tissue trauma and improving localized drug delivery.[27,33] VEGF is a potent, critical angiogenic growth factor for endothelial cells.[42] Controlled delivery of VEGF has been utilized to induce neoangiogenesis for tissue engineering, wound healing, and improving circulation in ischemic tissue.[42,43,59] However, s.c. injections of proteins have low efficiency due to their rapid removal from the site of injection and protein stability issues. In addition, they may induce an immune response.[33] The encapsulation of proteins in delivery vehicles, such as microspheres, liposomes, and emulsions, has been shown to resolve these issues and provide sustained release.[60–62] However, the encapsulation efficiency of hydrophilic proteins in polymeric delivery systems, such as PLGA microspheres, is very low. In order to improve the encapsulation efficiency of proteins and increase stability, a model protein, rat serum albumin (RSA), was chemically modified[37] by (1) self-cross-linking, (2) conjugation with heparin, and (3) propylation. Chemical modification was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis and size exclusion chromatography, which showed changes in MW after conjugation. Approximately 5% of amino groups were nonconjugated after self-cross-linking and heparin conjugation of RSA, whereas for

propylated RSA, approximately 10% of amino groups remained nonconjugated. The hydrophilicity of modified proteins was also less than native RSA, as indicated by decreased retention times compared to native RSA in reversed-phase high-performance liquid chromatography analysis. The cross-linked and propylated RSAs were more hydrophobic than the RSA–heparin conjugate. PLGA microspheres were prepared using these modified proteins and characterized for *in vitro* release. Higher encapsulation in PLGA microspheres was achieved after chemical modification of RSA (70–75%) compared to native RSA (4%). This was attributed to the increase in the hydrophobicity of modified RSA conjugates and the better interaction with PLGA polymer compared to native RSA. The *in vitro* release of RSA and conjugated RSA from PLGA microspheres showed a small burst release followed by a slow release phase (up to 9 days) and then a rapid release phase (from 10–30 days) (**Figure 4**). The conjugation of RSA led to slower release from microspheres when compared to native RSA release. The RSA–heparin conjugate showed the slowest release rate, followed by the propylated and cross-linked RSA. The slow release for conjugated RSA correlated well with PLGA microsphere degradation (as measured using size-exclusion chromatography), as it was determined that the PLGA degradation rate was slowest for RSA–heparin and propylated RSA microspheres. This may have been due to a buffering effect of RSA–heparin conjugate decreasing the autocatalysis of PLGA and/or reduced water uptake due to increased hydrophobicity. In addition, accelerated microsphere degradation was observed at 9 days and coincided with a second fast-release phase. It is worth mentioning that the PLGA used in this study was a high-MW (60 kDa) Resomer® (Ingelheim, Germany) RG 504 (50:50). This study provided a strategy to improve encapsulation efficiency of protein drugs in PLGA microspheres and to decrease release rates. The propylated RSA was used as a carrier protein for potent VEGF in the preparation of VEGF-loaded PLGA microspheres in the subsequent pharmacokinetic study.

VEGF and RSA were radiolabelled with 14C and 3H, respectively, for pharmacokinetic studies following intravenous (IV) or s.c. injection of protein solutions and s.c. injection of protein-loaded PLGA microspheres.[33] The two-compartment model resulted in a better fit to the pharmacokinetic data than the one-compartment model after IV administration of VEGF solution. However, IV administration of protein (VEGF and RSA) solutions resulted in short half-lives, with most of the protein eliminated during the first 24 h. Similarly, the s.c. administration of VEGF and RSA solutions resulted in rapid clearance (4 h for VEGF and 3 h for RSA) from the target s.c. tissue (to plasma), with high plasma concentrations. On the other hand, the s.c. administration of VEGF microspheres resulted in sustained release of proteins with low plasma concentrations and high s.c. concentrations (63 times that in blood) over a period of 7 weeks. The area under the curve (AUC), the time required to achieve the maximum concentration (t_{max}), and the maximum concentration (C_{max}) in blood samples at the s.c. tissue site were selected to compare the pharmacokinetic characterization of VEGF microspheres with that of protein solutions (**Table 2**). Lower AUC and C_{max} with a higher t_{max} showed that microspheres provide sustained and localized s.c. delivery compared to s.c. injection of solutions (**Table 2**).

The *in vivo* release profile of the VEGF was slower than the *in vitro* release profile in PBS at 37 °C. The *in vitro*

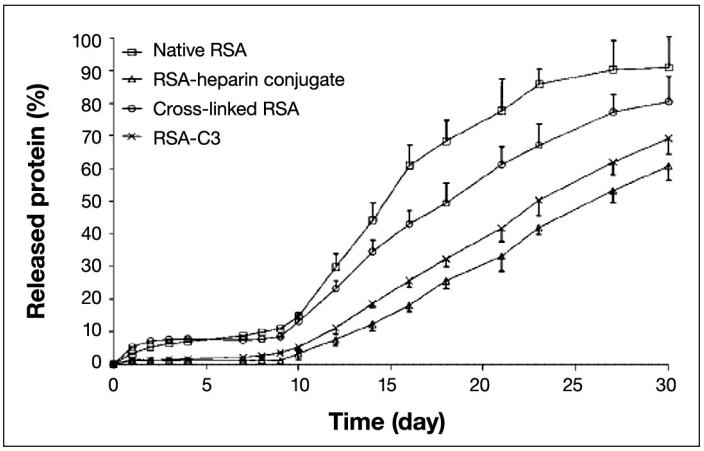

Figure 4. Normalized release profile of each modified protein from PLGA microspheres (37 °C, PBS pH 7.4, and stirring at 100 rev/min^{-1}). (Reproduced from Reference 37 with the permission of Pharmaceutical Press.)

Table 2.
Comparison of the Pharmacokinetic Parameters in Blood Following Subcutaneous Injection of Protein Solution and Their Microspheres Dosage Forms[a]

	$AUC_{0-\infty}$ (ng h ml^{-1})	t_{max} (h)	C_{max} (ng)
VEGF solution	2229	4.135	169.46
RSA solution	1287[b]	3.002	143.38[c]
VEGF microspheres	72.6	143	0.120
RSA microspheres	83.5[b]	136	0.165[c]

[a] Reproduced from Reference 33 with the permission of Journal of Pharmacy and Pharmacology, Pharmaceutical Press.
[b] Unit is 1 μg h ml^{-1}
[c] Unit is 1 μg

release increased after 9 days to a total of 65% at 30 days after an initial slow release phase. The *in vivo* profile was initially slow (less than 7.4±2.3% at 2 weeks) but increased thereafter (**Figure 5**). A comparison of release profiles with PLGA degradation kinetics showed that the *in vitro* and *in vivo* release followed the same rank order as *in vitro* and *in vivo* degradation rates (**Figure 5**). The *in vivo* degradation of PLGA was slower than the *in vitro* degradation. This suggested that PLGA degradation was the rate-determining step for VEGF release from the microspheres. Moreover, the release of encapsulated drugs from microspheres at a less perfused s.c. site might be affected by the body's immune response, lack of sink conditions, and type of drug (small versus large molecule). In this study, the immune response (protein fouling, inflammation, and fibrous encapsulation) as well as the lack of sink conditions at the s.c. site, which is exacerbated by the presence of fibrotic tissue, may explain the slower *in vivo* release of large VEGF molecules from microspheres. In the case of the dexamethasone microspheres mentioned earlier, the release of dexamethasone prevents the immune response, eliminating any potential effect this could have on drug release. The *in vivo* pharmacokinetic study on PLGA microspheres containing VEGF[33] established the potential for sustained and localized delivery of protein therapeutics at the s.c. site. The pharmacodynamic efficacy of VEGF PLGA microspheres to induce neoangiogenesis was investigated in subsequent studies with PVA hydrogel based coatings (see the following section).

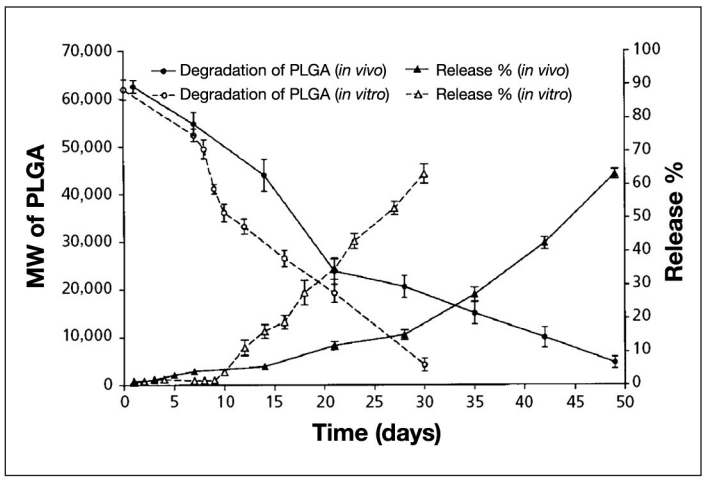

Figure 5. Degradation of PLGA microspheres and percent release of VEGF from the microspheres in vitro and in vivo (in vitro release conditions: PBS pH 7.4 at 37 °C and 100 rev/min^{-1}). The degradation of PLGA was determined using gel permeability chromatography (error bars represent standard deviation). (Reproduced from Reference 33 with the permission of Pharmaceutical Press.)

Polylactic-Co-Glycolic Acid Microsphere/ Polyvinyl Alcohol Hydrogel-Based Coatings

Although microspheres can be used for localized and controlled delivery systems, their utility for implantable biosensors is hindered by many factors. Multiple injections of microspheres carrying different drugs in the vicinity of the implant are difficult and induce additional tissue trauma. In addition, a constant zero-order release might be more desirable than the typical triphasic release profile from microspheres. These problems were overcome by designing a drug-eluting external coating that releases drugs in the immediate vicinity of the implant.[23,24,26,27] Incorporation of different drugs in the same coating was used to eliminate the need for separate injections. Drug-eluting coatings have been used in drug-eluting stent systems to decrease the incidence of restenosis after coronary angioplasty.[31,63–66] The stents are coated with biopolymers containing immunosuppressive drugs, which are released in a controlled and localized manner and decrease the incidence of restenosis. However, for biosensor applications, such a coating should be permeable to allow the transport of glucose and other analytes across the membrane. PLGA and other polymers used for stent coatings might not provide the required permeability. Therefore, a PVA hydrogel-based composite coating containing drug-loaded PLGA microspheres was designed, combining the advantages of two polymer systems.[23,24,26,27]

Hydrogels are three-dimensional polymeric networks that absorb and retain large amounts of water, have high permeability to small molecules, and have good mechanical properties. PVA hydrogel and blends of PVA with natural polymers, such as collagen, hyaluronic acid, chitosan, and dextran, have been evaluated as polymeric matrices for drug delivery.[67,68] PVA can be physically cross-linked by a freeze–thaw cycling process to form a hydrogel. This eliminates the need of chemical cross-linkers, residual amounts of which might be toxic to the body and damage the sensor enzyme and protein drugs.[69,70] In addition, the PVA hydrogel is permeable and would not inhibit the transport of glucose and other analytes across the coating. These properties render the PVA hydrogel suitable for biosensor coating to provide a hydrophilic interface with body tissue. However, drug release from the PVA hydrogels follow rapid first-order kinetics and ranges from minutes to weeks, depending on the extent of cross-linking.[40] This problem was resolved by embedding PLGA microspheres in a PVA hydrogel matrix, where the hydrogel provides structural and mechanical support, and the microspheres control drug

release (**Figure 6**).[24] The feasibility of these composites as potential external drug-eluting coatings for biosensors was investigated *in vitro* and after *in vivo* implantation in rats.[23,24,26,27]

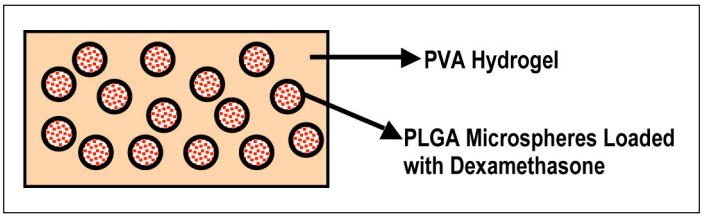

Figure 6. Schematic representation of PLGA microsphere/PVA hydrogel composite (not drawn to scale).

In vitro Characterization

The first step in the development of composite coatings involved the investigation of the mechanical properties after different freeze–thaw cycles (freezing at -20 °C and thawing at room temperature).[24] The hydrogels were formed by subjecting 5% w/w PVA (average MW of 133 kDa) to freeze–thaw cycles. For composite coatings, microspheres were added to PVA or a PVA/polyacid solution and then subjected to freeze–thaw cycles. Physicochemical characterization of hydrogel coatings was performed to optimize the number of freeze–thaw cycles required to form a stable hydrogel. Water uptake or degree of swelling decreased with an increase in the number of freeze–thaw cycles and reached a plateau after four cycles (500% swelling after one cycle versus 350% after five cycles) due to increased cross-linking and decreased porosity. Moreover, water uptake was less in high ionic strength PBS media than in water alone. At 37 °C, the fully hydrated state was reached within 30 min of immersion in PBS for PVA hydrogels that had undergone three freeze–thaw cycles. This meant that equilibrium swelling could be achieved rapidly at body temperature. The tensile strength of PVA hydrogel, measured by Young's modulus, increased with the number of freeze–thaw cycles, reaching an asymptotic value after three cycles for hydrogels with or without incorporated microspheres in both the dry and hydrated state. This was attributed to the gradual ordering and crystallization of macromolecular chains that serve as stress transfer points.[69] In the hydrated state, the Young's modulus was of an order of magnitude smaller than that in the dry state but was comparable to that of soft human tissue (0.1 to 4 MPa).[24] The number of freeze–thaw cycles inversely affected the *in vitro* release of dexamethasone from PVA hydrogels. The complete release of dexamethasone from the hydrogel was observed in only 10–14 days, which restricts their utility for long-term drug delivery.

In order to extend the release of dexamethasone, PLGA microspheres (MW 60 kDa, 50:50) were embedded in a PVA hydrogel matrix. A typical triphasic release of dexamethasone was observed from the microspheres alone in PBS at 37 °C (**Figure 7**). However, an extremely slow release was obtained when microspheres were embedded in the hydrogel (**Figure 7**). It was speculated that slow release was a result of the retardation of autocatalysis of microspheres due to the partition of acidic byproducts of PLGA degradation in the more hydrophilic hydrogel matrix. Another possible explanation for slow release could be that microcrystalline PVA domains formed at the PLGA microsphere surfaces significantly decreased water penetration into the microspheres and therefore decreased polymer degradation and drug release. In order to enhance PLGA degradation and dexamethasone release, surface active polyacids, such as polyacrylic acid (PAA), humic acids (HAs) and Nafion, were added to hydrogel matrix. It was hypothesized that the presence of these polyacids on the PLGA surface would increase the localized acidity and water penetration. The addition of polyacids significantly increased the release of dexamethasone, with HAs producing the most pronounced effect (**Figure 7**). Release from PAA- and Nafion-modified gels was similar but slower than from HA-modified gels. Increase in release from polyacid-modified hydrogels was explained on the basis of changes in the surface properties such as surface tension, zeta potential (surface electric potential), and contact angle. The addition of polyacids decreased the surface tension of water and PBS, decreased the zeta potential of microspheres significantly, and increased

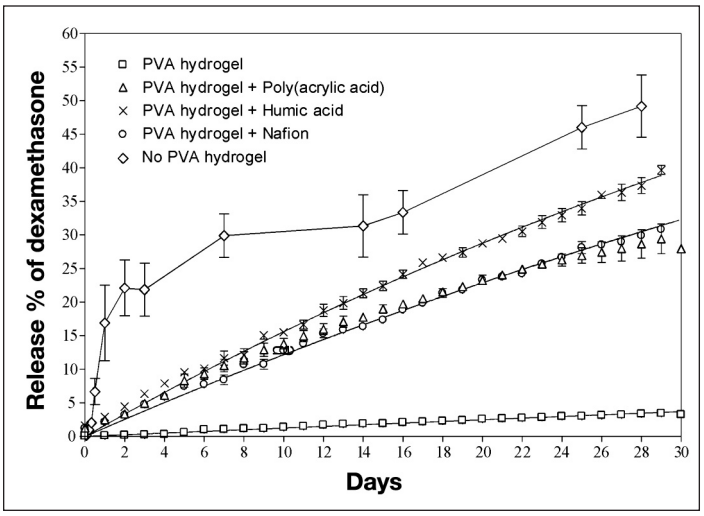

Figure 7. Cumulative release of dexamethasone from PLGA microspheres, microspheres incorporated into the PVA hydrogel matrix with no additives, and in presence of PAA, HA, and Nafion ($N_{f/t}$ = 3; PBS buffer pH 7.4, 37 °C; n = 3) (Reproduced from Reference 24 with the permission of AAPS Journal.)

the contact angle, suggesting a preferential surface adsorption on microspheres. Morphological studies to investigate the effect of HAs on microsphere degradation further corroborated the surface activity of polyacids. It was observed that the PLGA microspheres embedded in PVA hydrogels degraded slowly as compared to a PLGA microsphere alone after 4 weeks of incubation in PBS. However, degradation was faster in HA-modified hydrogels with increased porosity on the microspheres surface. These *in vitro* characterization studies showed the potential of HA-modified PLGA microsphere/PVA hydrogel composite coating for localized and sustained drug delivery. The *in vivo* efficacy was investigated after implantation in the s.c. tissue of rats.

In vivo Pharmacodynamic Studies

For *in vivo* evaluation, composite coatings were fabricated inside 18-gauge needles to match the dimensions of the miniaturized glucose biosensor they were designed to coat. The aim of the first study was to investigate the efficacy of coatings to release dexamethasone *in vivo* and control the FBR.[26] For the fabrication of coatings, 100 mg microspheres were dispersed into 10 ml of PVA solution (5% w/w) containing HA (2% w/w). The solution was injected inside needles and subjected to three freeze–thaw cycles. The same needles were used for *in vivo* implantation. ^{3}H dexamethasone was used in preparation of PLGA microspheres to determine the *in vivo* release after implantation into the intrascapular s.c. tissue of Sprague Dawley rats. Both *in vitro* and *in vivo* release profiles were approximately zero-order for the 28-day study period, similar to the previous *in vitro* characterization study (**Figure 7**).[24] After 1 day, the *in vitro* dexamethasone release rate was 0.13 μg/day, while the *in vivo* release rate was 0.17 μg/day, indicating a faster *in vivo* release. This release rate was considered sufficient for the pharmacodynamic study based on the earlier study with dexamethasone-loaded microspheres alone.[25]

Pharmacodynamic evaluation of the excised tissue surrounding the implanted coatings was performed after staining with H&E. Coatings containing blank PLGA microspheres and normal s.c. connective tissue were used as controls (**Figure 8**). A typical FBR was developed in the tissue around the control coatings. At 7 days, a strong acute inflammatory phase marked by the presence of neutrophils was observed in the tissue surrounding the control coatings. At 21 and 28 days, the inflammation progressed to the chronic phase characterized by macrophages and a thick fibrotic band around the control implant (**Figures 8A–8D**). On the other hand, no inflammation was observed in tissue surrounding the dexamethasone eluting coatings until 28 days (**Figures 8E–8H**). The tissue histology appeared similar to normal s.c. tissue histology. This indicated that localized elution of dexamethasone from coatings controlled the FBR for the 4-week study period. This was further confirmed by counting the number of inflammatory cells inside a fixed area around the implant. The number of inflammatory cells around dexamethasone-eluting coatings was similar to that in normal s.c. tissue, whereas this number was much higher in the tissue around the control coatings for the entire study time period. These results demonstrated that the PLGA microsphere/PVA hydrogel-based composite coatings could control both acute and chronic phases of the inflammatory response to the implant over 28 days and has potential for use as an external coating for glucose biosensors and other implantable devices.

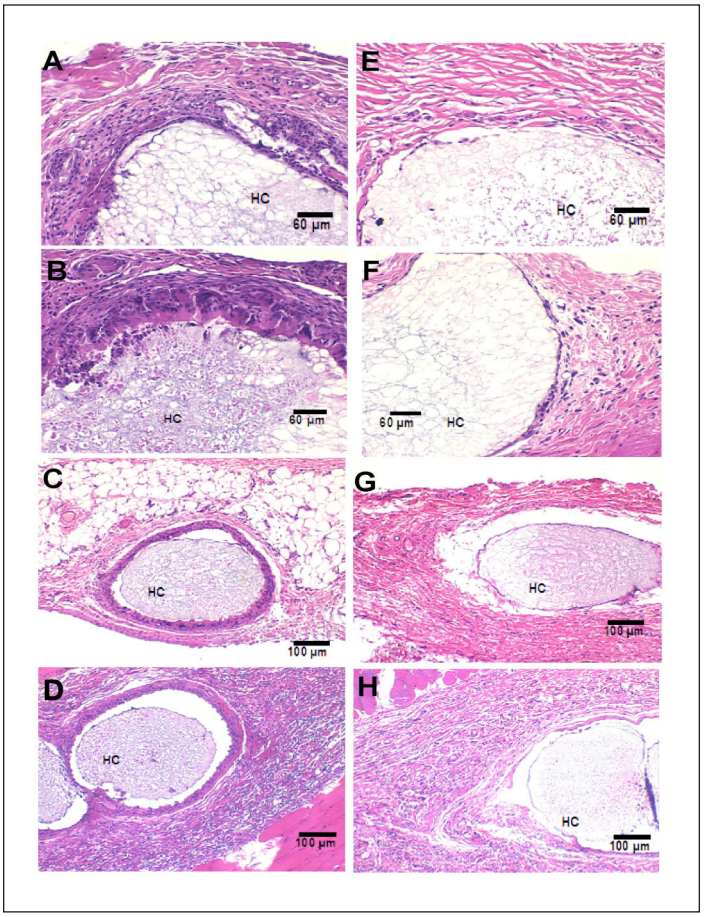

Figure 8. Pharmacodynamic changes in representative tissue sections from s.c. tissue of rats implanted with PLGA microsphere/PVA hydrogel composites (HC): **(A–D)** without dexamethasone and **(E–H)** with dexamethasone. Inflammation-mediating cells and normal cells are stained purple and pink, respectively, with H&E. (Reproduced with modifications from Reference 26 with the permission of Diabetes Technology and Therapeutics).

The focus of the next *in vivo* study was to control inflammation and induce neoangiogenesis by concurrent delivery of dexamethasone and VEGF from composite coatings at the tissue–implant interface.[27] It has been reported that dexamethasone blocks the angiogenic effect of endogenous VEGF.[29,30] Moreover, VEGF is a protein, and exogenous administration could lead to immune response (due to denaturation, aggregation, or use of a hetrologous analogue). Therefore, the objectives of this study were to determine (1) whether exogenous VEGF could induce neoangiogenesis and (2) whether dexamethasone could prevent any immune response to VEGF when delivered simultaneously. Dexamethasone- and VEGF-loaded microspheres (PLGA 50:50 and MW 26 kDa) were incorporated in PVA/HA composite coatings either alone or in combination. These microspheres release their contents over a period of 30–35 days as reported in an earlier study.[39] The *in vitro* release of dexamethasone and VEGF from composite coatings was approximately zero-order after an initial burst release (approximately 20%) over a 4-week study period. The dexamethasone release rate was 123 ng/day, and the VEGF release rate was 21 pg/day with an approximately zero-order fit.

The PLGA microsphere/PVA hydrogel coatings containing dexamethasone, VEGF, and a dexamethasone/VEGF combination were implanted in the s.c. tissue of rats, and histological evaluation of the surrounding tissue was performed to investigate the anti-inflammatory and angiogenic efficacy. Coatings containing blank microspheres were used as controls. A typical FBR was observed in the tissue surrounding the control coating implants over a 28-day study period (**Figures 9A–9D**).[26] The dexamethasone-eluting coatings were able to suppress the inflammation from 1 to 4 weeks (**Figures 9E–9H**). No fibrosis or chronic inflammation was observed, and these results are also in agreement with our previous study (**Figure 8**).[26] However, the incorporation of only VEGF microspheres in the implant coatings led to an intense immune response in the vicinity of the implant with a significantly higher number of inflammatory cells than even the control coatings during the acute phase (**Figures 9I–9L**). This reaction progressed to the chronic phase by 28 days, but interestingly a fibrotic capsule was not observed even though the tissue was in a highly inflamed state (**Figures 9I–9L**). This was attributed to the complex cellular functions of VEGF and its role in different biochemical pathways.[27] On the other hand, the simultaneous release of dexamethasone with VEGF suppressed both acute and chronic inflammatory phases over a 4-week period (**Figures 9M–9P**) without any inflammation or fibrosis. These results indicated that the simultaneous release of dexamethasone and VEGF from PLGA microsphere/PVA hydrogel composite coatings could control both the FBR to the implant and the immune response to VEGF.

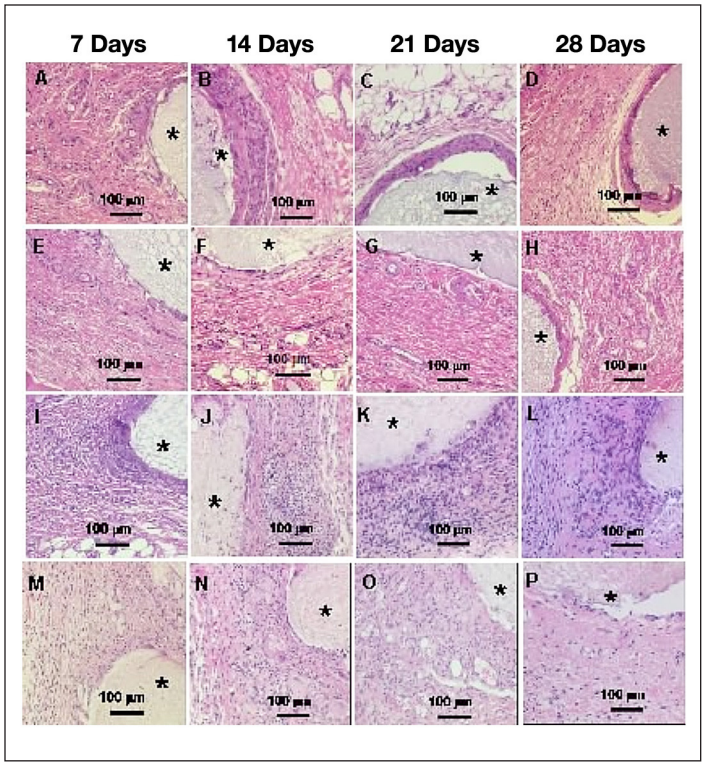

Figure 9. Pharmacodynamic changes in representative s.c. tissue sections of rats implanted with PLGA microsphere/PVA hydrogel composites (HC): **(A–D)** control coatings with drugs, **(E–H)** composites containing dexamethasone alone, **(I–L)** composites containing VEGF alone, and **(M–P)** composites containing a dexamethasone and VEGF combination. Inflammation-mediating cells and normal cells are stained purple and pink, respectively, with H&E. (Reproduced with modifications from Reference 27 with the permission of Elsevier Ltd.)

The ability of exogenous VEGF to induce neoangiogenesis in the s.c. tissue was evaluated after staining the blood vessels with α-smooth muscle actin (immunohistochemistry). Composites eluting VEGF alone or in combination with dexamethasone induced neoangiogenesis (**Figure 10**).[27] Nascent blood vessels in the surrounding tissue could be observed in both groups 1 week after implantation. Sustained release of VEGF led to the formation of a distinct vasculature by 4 weeks, which comprised small-, medium-, and large-size vessels (**Figure 10**). Counting the number of blood vessels in a fixed area around the implant demonstrated that composite coatings containing VEGF and a dexamethasone/VEGF combination were capable of a progressive increase in vessel density. In fact, vessel density was even lower in tissue surrounding dexamethasone-only eluting coatings as compared to

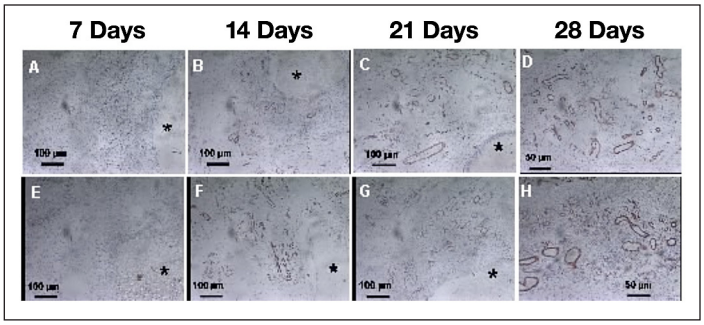

Figure 10. Pharmacodynamic changes in representative s.c. tissue sections of rats implanted with PLGA microsphere/PVA hydrogel composites containing **(A–D)** VEGF alone and **(E–H)** a dexamethasone and VEGF combination. Cells containing markers for angiogenesis are stained (smooth muscle actin staining). (Reproduced with modifications from Reference 27 with the permission of Elsevier Ltd.)

control blank coatings. Therefore, the strategy to deliver dexamethasone and VEGF simultaneously successfully induced neoangiogenesis and circumvented the antiangiogenic effect of corticosteroids.

The PLGA microspheres incorporated in coatings for previous pharmacodynamic studies released dexamethasone for approximately a 1 month period. Both acute and chronic inflammatory phases could be successfully controlled with these coatings for 4 weeks. However, the chronic inflammatory phase is a progression of an unresolved acute inflammatory phase through a cascade of events. Therefore, our group set out to investigate whether controlling only the acute phase with the hydrophilic PVA composite coatings would provide extended protection against the chronic phase (i.e., time period of drug release required). In order to determine this, composites containing fast-releasing (within 1 week *in vitro*) low-MW (5 kDa; 50:50) PLGA microspheres containing dexamethasone were fabricated and evaluated *in vivo* in the next pharmacodynamic study.[23] Dexamethasone release from these coatings successfully controlled the acute inflammation, as no inflammatory cells were observed in the tissue surrounding the drug-containing coatings at 3 and 8 days after implantation (**Figures 11D–11F**). However, in tissue surrounding control coatings (blank microspheres), an intense neutrophilic acute inflammatory reaction developed, which progressed to the chronic phase by 8 days (**Figures 11A–11B**). Another interesting feature is the development of numerous new leaky blood vessels around the control coatings (**Figure 11B, blue circles**), whereas in dexamethasone-containing coating, only mature preexisting blood vessels were observed (**Figure 11D, white circles**). The generation of new blood vessels are a part of the inflammatory process, and these resolve as inflammation progresses. At 30 days, multinucleated giant cells were observed in the tissue surrounding the control implant. A delayed chronic inflammatory reaction was observed in the dexamethasone group at 30 days with a large number of macrophages and fibrosis around the implant (**Figure 11C**). This indicated that the body recognized the implant as foreign after dexamethasone was depleted from the coatings and produced a delayed chronic response. This study indicated that controlling the acute phase of inflammation did not provide complete protection, and sustained release of anti-inflammatory drugs is necessary to control both acute and chronic phases.

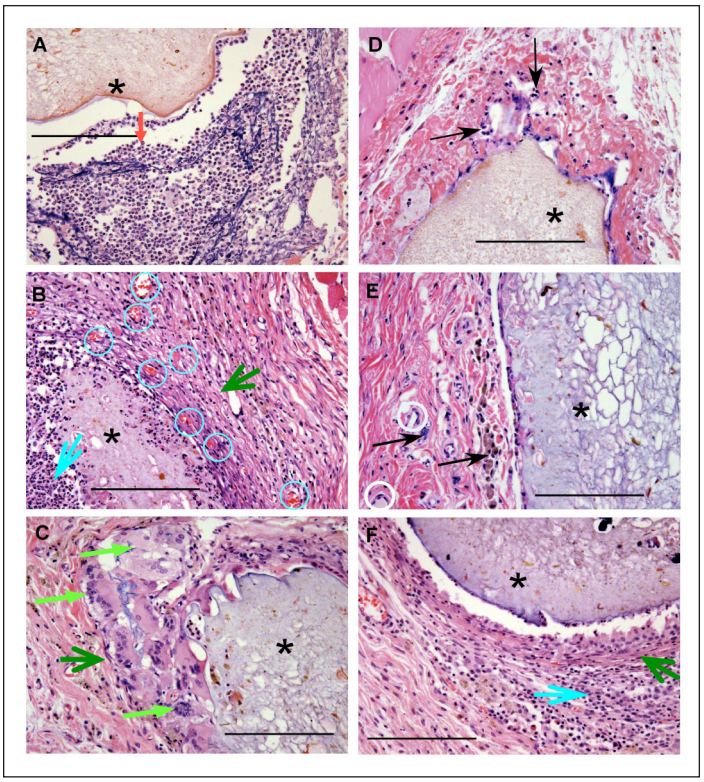

Figure 11. Pharmacodynamic changes in representative tissue sections taken from the s.c. tissue of rats implanted with fast-releasing (5 kDa) PLGA microspheres/PVA hydrogel composites **(A–C)** without dexamethasone and **(D–F)** with dexamethasone, magnified 200 times. The asterisk shows the implant; the red arrow shows neutrophils; the dark green arrow shows fibrous tissue; the light blue arrow shows macrophages; the light blue circle shows new blood vessels; the white circles show mature blood vessels; the light green arrow shows giant cells; the black arrow shows aggregates of mixed inflammatory cells; and the bar represents 100 μm. (Reproduced with modifications from Reference 23 with the permission of Journal of Diabetes Science and Technology.)

Conclusions

Localized and sustained delivery of dexamethasone and VEGF to the tissue surrounding a s.c. implant can prevent the FBR and induce neoangiogenesis. The PLGA microsphere/PVA hydrogel-based composite has the

potential for use as an external drug-eluting coating for implantable devices. Drug release from these composite coatings can be easily tuned by incorporating different types of PLGA microspheres. In addition, two or more drugs can be simultaneously delivered by embedding microspheres containing different drugs in the same composite coating. Development of this composite coating provides an exciting strategy for the localized delivery of tissue-response modifying drugs; however, some issues need to be addressed for the development of an optimized coating composition. These include the temporal aspect for the delivery of different drugs, dose optimization for different drugs, the sequence of drugs release, and extending drug delivery for long-term pharmacodynamic efficacy. Such an optimized composite coating is envisioned as an integral part of our glucose sensor, and this will aid in the development of long-term totally implantable glucose biosensors to improve the clinical management of diabetes.

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