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Phosphorylcholine-based polymer coatings for stent drug delivery

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Phosphorylcholine-based polymers have been used commercially to improve the biocompatibility of coronary stents. In this study, one particular polymer is assessed for its suitability as a drug delivery vehicle. Membranes of the material are characterized in terms of water content and molecular weight cut-off, and the presence of hydrophilic and hydrophobic domains investigated by use of the hydrophobic probe pyrene. The *in vitro* loading and elution of a variety of drugs was assessed using stents coated with the polymer. The rate of a drug's release was shown not to be simply a function of its water solubility, but rather more closely related to the drug oil/water partition coefficient. This finding was explained in terms of the more hydrophobic drugs partitioning into, and interacting with, the hydrophobic domains of the polymer coating. The suitability of the coated stent as a drug delivery vehicle was assessed *in vivo* using a radiolabeled analog of one of the more rapidly eluting drugs, angiopeptin. Autoradiography showed that the drug was released locally to the wall of the stented artery, and could be detected up to 28 days after implantation.

1. Introduction

Coronary stents are small expandable scaffolds that are placed within coronary arteries following angioplasty (a procedure for widening diseased vessels in order to restore normal blood flow). While stenting is a very effective method for holding the vessel open, there are still a significant number of patients that suffer from a long-term re-narrowing of the stented area of the artery, a process known as in-stent restenosis. The use of drug therapies in an attempt to control this over-exuberant healing process has been largely unsuccessful, the vast majority of the active agent being lost systemically, even when administered using an infusion balloon directly at the site of the angioplasty. Recently, there has been a great deal of interest in the use of stents as vehicles for site-specific drug delivery in order to circumvent restenosis. The disease however, is very complex and not well understood and consequently there are a variety of potential targets and a wide range of drugs that might be of use in its treatment.

We have reported in detail on the use of phosphorylcholine (PC)-based polymers as biocompatible coatings for a variety of medical devices [1, 2]. As a component of their structure, these polymers contain an exact chemical copy of the predominant phospholipid headgroup (PC) found in the outer leaflet of the red blood cell membrane. This affords the materials exceptional bio- and hemocompatibility. Unlike many other polymer systems [3], stents coated with these materials have been shown to be non-thrombogenic, non-inflammatory and stable *in vivo* for over six months [4]. Moreover, it has been reported that these essentially hydrogel-like materials are capable of sustained delivery of biologically active molecules [5]. Here we discuss the characterization of a PC coating with respect to its use as a drug delivery vehicle and describe how it is an ideal platform for the delivery of a range of therapeutics for the treatment of restenosis.

2. Materials and methods

The phosphorylcholine-based polymer system of interest in this study has been described by us in great detail in a recent publication [6]. It is a four-component random copolymer formally named Poly(2-(methacryloyloxyethyl)-2′-(trimethylammoniumethyl) phosphate, inner salt)-*co*-(*n*-dodecylmethacrylate)-*co*-(hydroxypropylmethacrylate)-*co*-(3-(trimethoxysilyl)propyl-methacrylate) (23 : 47 : 25 : 5 mole %) or referred to by the trivial names PC1036 or PC100B [7]. Characterization of cast membranes of this material included water content studies, molecular weight cut-off assessment and the use of fluorescent spectroscopy to determine whether formal hydrophobic domains were present. Drug delivery studies involved *in vitro* assessment of the loading and release of a variety of drugs from coated stents, supported by an *in vivo* study using a radiolabeled

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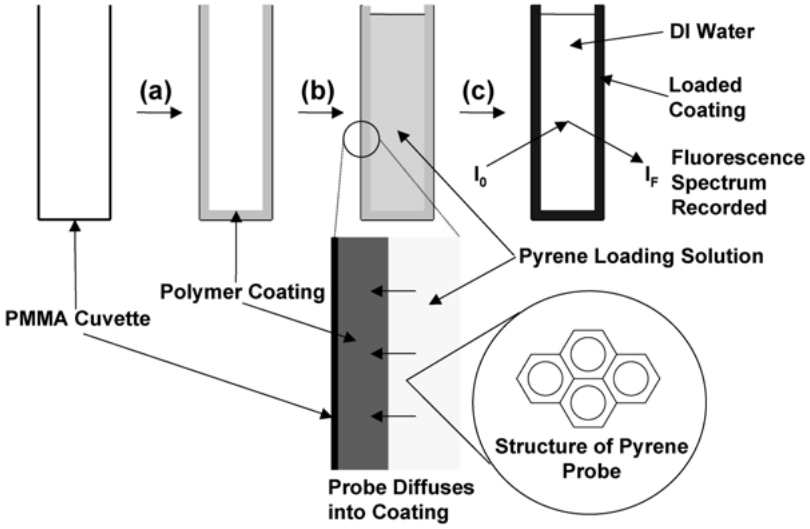

Figure 1 Method for probing hydrophobic domains in polymer coatings: (a) PMMA cuvette is coated with polymer and dried/cured; (b) cuvette is filled with pyrene solution and left to allow diffusion into coating; (c) pyrene solution is replaced with pure water and the fluorescence spectrum recorded.

drug to determine the site and period of delivery to the vessel.

2.1. Water content determination

Concentrated ethanolic solutions of the polymer (10% w/v) were cast into membranes and crosslinked using various curing temperatures and/or regimes involving thermal or irradiation methods. The resulting water contents of the various membranes were determined gravimetrically or by differential scanning calorimerty (DSC) using a Perkin Elmer DSC-7 equipped with intercooler, using a method described by us more fully elsewhere [8]. This technique also allowed the calculation of bound and free water in the membranes.

2.2. Molecular weight cut-off (MWCO) determination

The fully crosslinked polymer membrane was subjected to a mixed dextran challenge in order to estimate its approximate molecular weight cut off (MWCO). A portion of pre-hydrated membrane was placed between two halves of a Franz cell, in which the upper resorvoir consisted of a solution of dextrans of mixed molecular weight (180, 360, 1200 and 4400 daltons, low polydispersity, SPS Polymer). After 48 h, a sample of permeate from the lower half of the cell was analyzed by gel permeation chromatography (GPC) and compared to that of the feed in the upper cell, in order to determine the maximum dextran molecular weight that could diffuse across the membrane. This was termed as the nominal MWCO of the membrane.

2.3. Fluorescent probe analysis

The presence of hydrophobic domains within this amphiphilic hydrogel was investigated using the hydrophobic probe pyrene. PMMA cuvettes were coated with various polymers, allowed to hydrate in a pyrene solution (0.08 g/50 ml) and the fluorescence spectrum of the pyrene partitioned within the coating recorded on a Perkin Elmer LS 50B luminescence spectrometer using a 350–500 nm scan with excitation at 336 nm (Fig. 1). Information about the environment of the probe was gained by examination of the intensity of the vibronic bands at 373 nm (I1) and 383 nm (I3), usually expressed in terms of the I3/I1 ratio [9]. Others have used this technique to investigate PC-polymers in solution [10] but this is the first time to our knowledge that it has been used on a coating of such materials.

2.4. *In vitro* drug delivery studies

Coated stents (15 mm Bio*divYsio*® Matrix LO (Biocompatibles Ltd), $\sim$ 1 µm outer PC-coating thickness) were loaded with a variety of drugs by simple immersion in an alcoholic or aqueous solution of the drug (depending upon its solubility) for 5 min, followed by an evaporation step at room temperature to dry the stent. The amount of drug loaded onto a stent could be controlled by varying the concentration of the drug loading solution. The maximum loading was thus limited somewhat by the solubility of the drug in a particular solvent system. The total loading achieved was determined by sonication of the loaded stent in solvent to completely remove all of the drug, followed by appropriate spectroscopic or chromatographic analysis of the eluant. The release of the drug from the stents was monitored *in vitro* by sampling over time by UV, fluorescence or HPLC methods dependent upon the compound under study. The elution model employed involved a simple release from stents into a large volume of phosphate buffered saline (PBS), which although unrepresentative of release of the drug into the vessel wall, could provide information on how rapidly the drug may be lost from the stent in the blood stream during its journey through the vasculature to the site of the stenosis. This was expressed as a percentage of drug remaining on the stent to allow a simple comparison between drugs of differing total loading. A minimum of five stents were used per loading and elution study.

2.5. *In vivo* drug delivery studies

An example of *in vivo* release into tissue was performed at the Northern General Hospital (Sheffield) by use of a radiolabeled version of the drug angiopeptin (Amersham), loaded onto the stents and implanted for up to 28 days in porcine coronary arteries [11]. Stents were loaded with the radiolabeled drug by simple dipping in a 1 mg/ml solution (2 mg/ml was the maximum solubility of the drug in PBS); this resulted in a total loading of $\sim 8 \pm 0.8\,\mu g$/stent $(n = 10)$. Autoradiography of histological sections was used to show the location of the drug in the tissue with respect to the stent. Analysis of tissue from the vessel surrounding the stent measured drug penetration and systemic loss at set time points throughout the study in order to determine the extent of the delivery period.

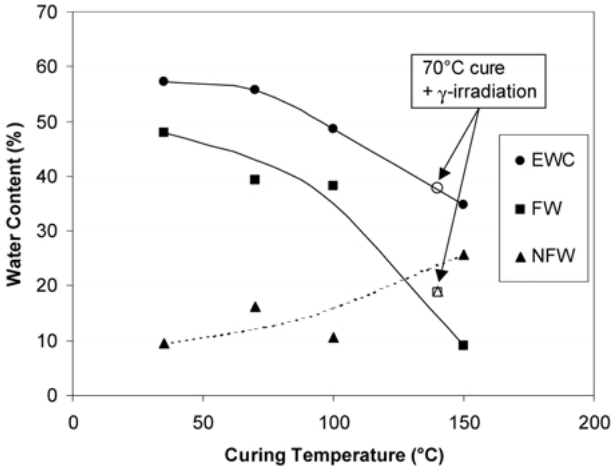

Figure 2 Effect of curing regime on water contents of PC1036 membranes. (EWC = equilibrium water content; FW = freezing (free) water; NFW = non-freezing (bound) water).

3. Results and discussion

3.1. PC-polymer membrane/coating characterization

Membrane studies on PC1036 showed that the equilibrium water content decreased as the curing temperature was increased (Fig. 2). Furthermore, the ratio of bound (non-freezing) to free (freezing) water in the polymer increased with increasing cure temperature. These observations are entirely consistent with other hydrogel-type polymer systems [12], the degree of crosslinking being increased at higher temperatures, as more energy is required to make all three silyl groups in the polymer react due to steric considerations. The polymer was also completely cured by using a 70 °C treatment followed by a 25 kGy dose of γ-radiation. The fully-cured system had a water content in the region of around 30–35%, a value confirmed by swelling studies on coatings of this material using spectroscopic ellipsometry on 0.5 μm thick films [13]. The amount of non-bound water in this system was still sufficient ($\sim$ 20%) to indicate that water-soluble drugs could be loaded into the coating from an aqueous solution, as described previously for other PC polymers [5].

Fig. 3 shows a comparison of the GPC traces for both the feed and permeate from a challenge solution of a mixture of molecular weight dextrans. This result indicated that the 70 °C cure + γ-irradiated membrane had a MWCO in the region of 1200 daltons; this was further confirmed for the coating on the stents used in the loading and release studies, as drugs $>$ 1200 daltons failed to load in appreciable amounts into the coating. There are a vast number of drugs in the molecular weight range of 0–1200 daltons that are of potential interest in the treatment of restenosis. This is therefore only a minor limitation, and indeed, a new PC coating capable of delivering high molecular weight biologically active compounds has been developed and approved for use on coronary stents to compliment this system [14].

The coatings were probed for hydrophobic domains by use of a pyrene aqueous solution. The I3/I1 ratio obtained for pyrene partitioned into the coating (I3/I1 = 0.98) was more akin to that obtained for pyrene in pure lauryl methacrylate (the hydrophobic component of the PC1036 polymer, I3/I1 = 1.03; Fig. 4(A)). Alternatively, for classical hydrogels such as polyHEMA, the I3/I1 ratio was the same as that for pyrene in water ($\sim$ 0.63 [9]; Fig. 4(B)), suggesting no formal hydrophobic areas exist within its structure into which the pyrene can partition. This showed there was indeed a hydrophobic environment within the PC1036 polymer into which pyrene, and presumably other hydrophobic compounds can partition.

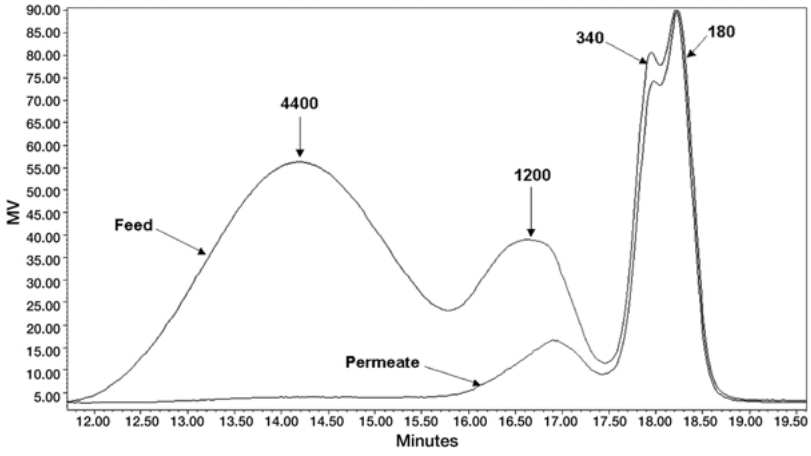

Figure 3 GPC traces for mixed dextran feed and permeate solutions.

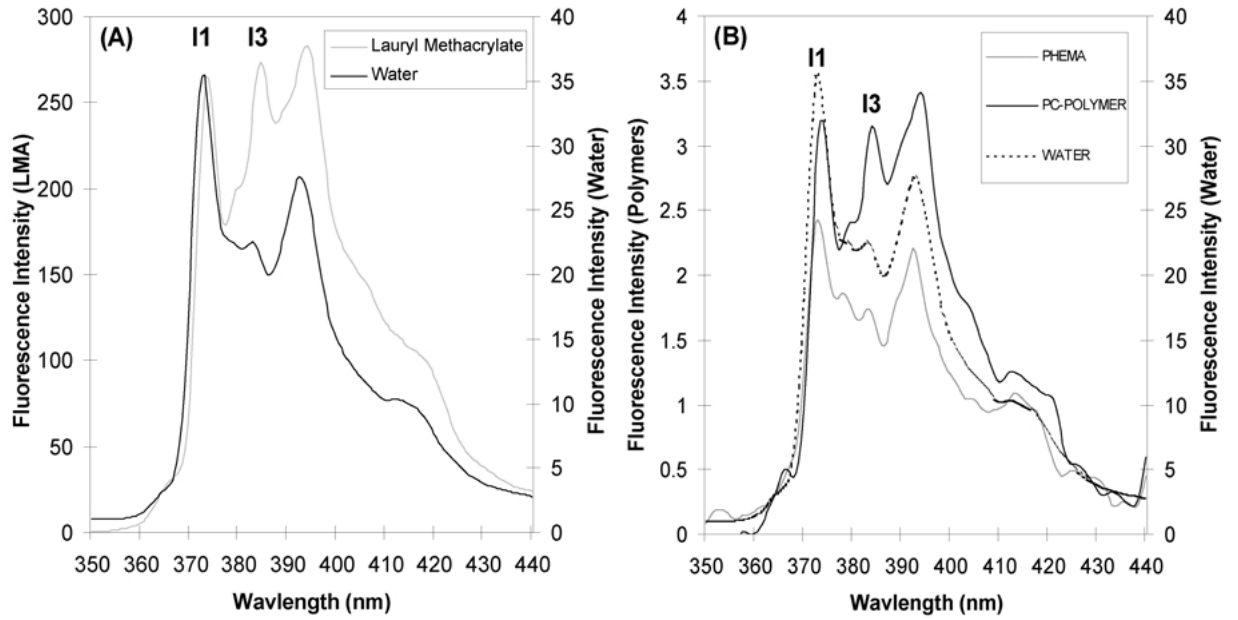

Figure 4 (A) Fluorescent spectra of pyrene in water and lauryl methacrylate; (B) Fluorescent spectra of pyrene in water, PHEMA and a PC-polymer.

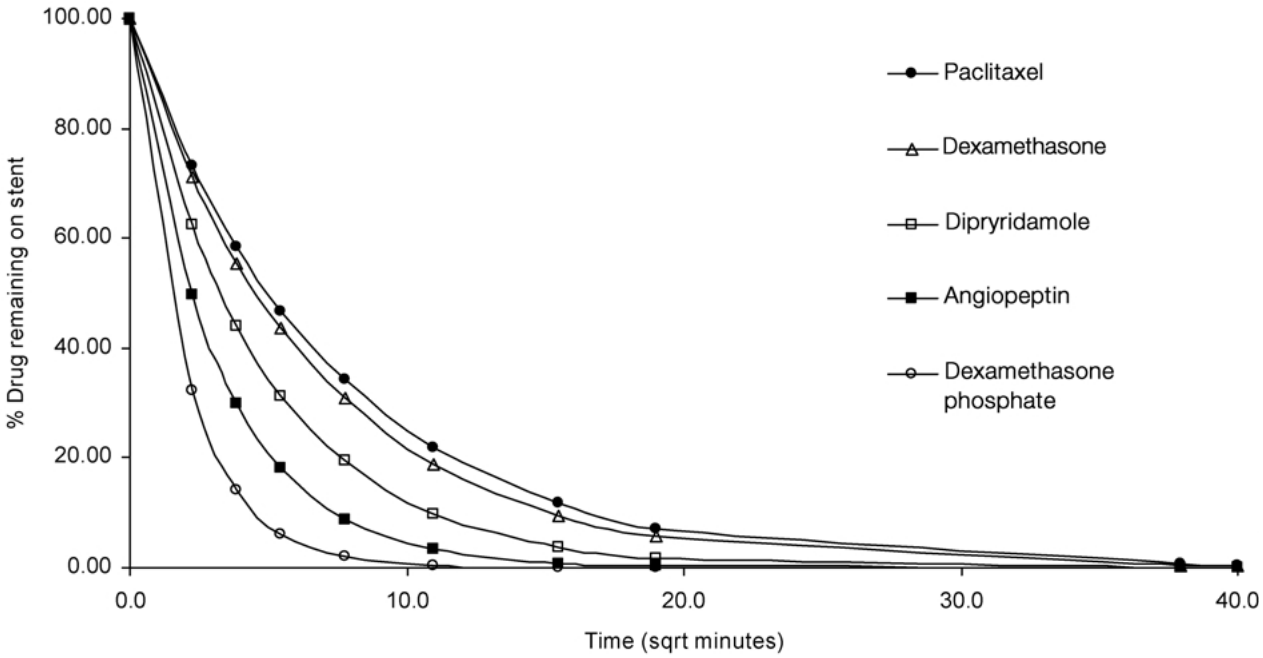

Figure 5 *In vitro* release profiles of a number of drugs from PC-coated stents.

3.2. Drug delivery studies

Loading of a variety of drug compounds onto stents was shown to be dependent upon the concentration of drug in the loading solution. Spectroscopic ellipsometry studies on the coating have shown that the polymer hydrates in an initial rapid stage to $\sim 80\%$ its final value within a few minutes, and that it swells even more in alcoholic solvents [13]. Thus, significant drug loadings could be obtained by just 5 min soaking in an aqueous or alcoholic solution. *In vitro* elution studies on these compounds showed that there were significant differences between the release rates. In general, the more water soluble drugs tended to elute more rapidly from the stent than the more hydrophobic counterparts (cf. dexamethasone and dexamethasone phosphate in Fig. 5). This was not, however, the complete explanation, as a plot of T_{90} (a convenient measure which was simply the time taken for 90% of the drug to have been eluted from the stent) did not correlate well with the drug solubility alone.

The log*P* (oil/water partition coefficient) of the drug is an indirect measure of the compound's hydrophobicity. It is a function of the chemical structure of the compound. Fig. 6 shows a reasonable correlation between this characteristic and the T_{90} of drug elution from the stent into PBS. By plotting the theoretical release based upon drug solubility and comparing it with actual release, it can be shown that the more hydrophobic drugs interact with the hydrophobic polymer domains and retard their release. Fig. 7 illustrates this point for the corticosteroid dexamethasone as an example. Perhaps it is not overly surprising that this type of compound is able to interact with the lipid-like structure of the polymer, just as

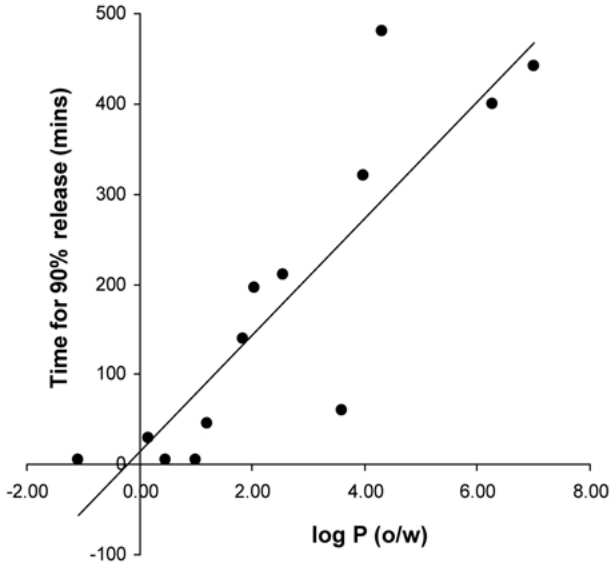

Figure 6 Plot of T_{90} vs. log$P_{(o/w)}$ for a range of different drugs.

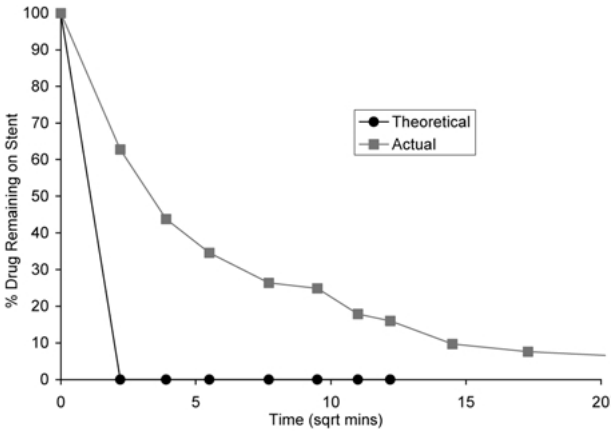

Figure 7 Theoretical release profile for dexamethasone based on solubility compared to actual release. The disparity between the two curves indicates the interaction with the polymer.

cholesterol is capable of insertion within the naturally occurring lipid bilayers of cell membranes.

Angiopeptin has been used previously in studies investigating treatments for restenosis [15], but as yet not using stent-based delivery. This relatively water-soluble compound was seen to elute fairly rapidly *in vitro*. The *in vivo* release of a radiolabeled version of the drug was therefore performed in a porcine coronary model to see if compounds with this rapid elution profile could be delivered successfully to the artery. The findings from this study were very encouraging. The levels of angiopeptin within the left coronary artery were 300-fold those, for example, in the circumflex artery showing the specificity of the delivery of angiopeptin to the stented region of the artery. After 28 days it was impossible to separate the stent from the vessel, and thus, in order to determine the distribution of the drug, autoradiography and stent cross-sectioning were used. The cross-sectioning showed the stent position within the vessel (Fig. 8A) while the autoradiograph showed the distribution of angiopeptin (Fig. 8B). By superimposing the two, the local distribution of the radiolabeled compound in the stented section could be confirmed (Fig. 8C). This

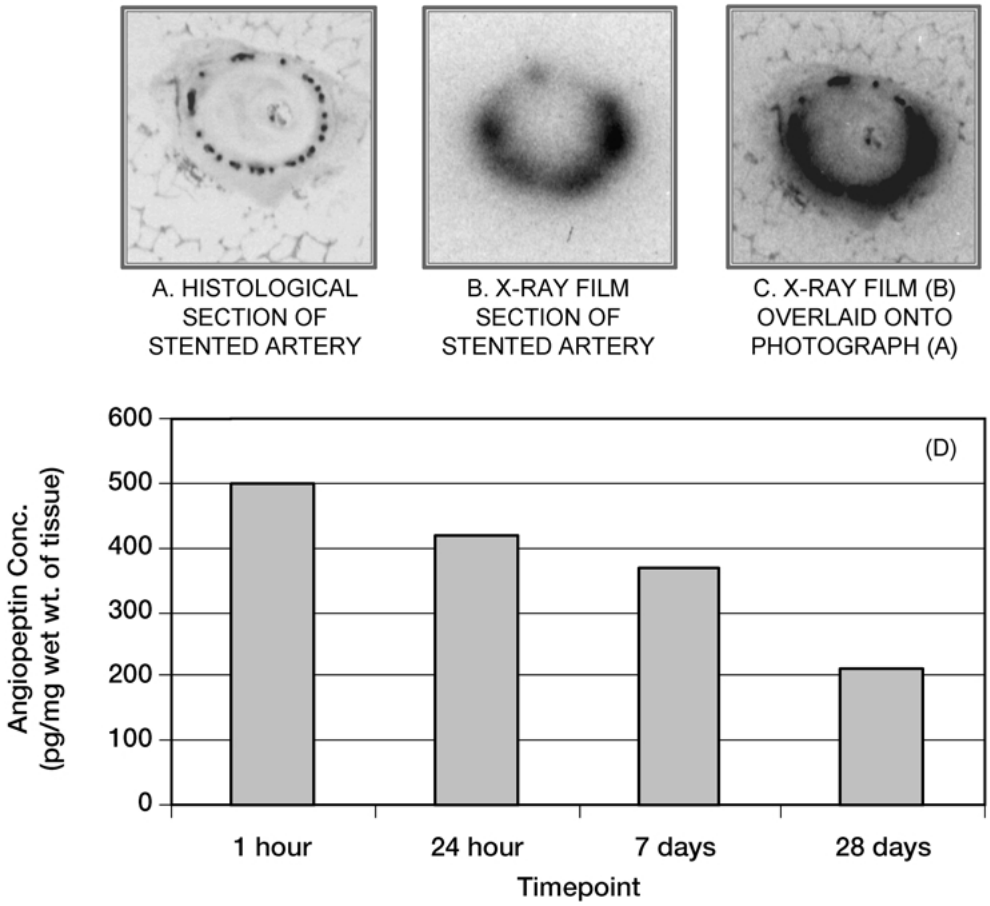

Figure 8 (A–C) Histological and autoradiographical images of the stented artery showing distribution of the radiolabeled compound; (D) Graph depicting amount of radiolabeled drug found in tissue over a 28-day period.

rapidly eluting drug could be detected in the tissue even up to 28 days after implantation (Fig. 8D).

After deployment of the stents, angiopeptin was detected in the blood at 1 and 24 h but after seven days level was reduced to zero. The maximum blood level of the drug was after 1 h (0.5 ng/ml). Angiopeptin was detected in the urine at 1 h (0.6 ng/ml) and up to 28 days (0.2 ng/ml). Negligible amounts of the drug were detected in tissues outside the heart. These figures indicate the locality of the angiopeptin delivered from the coated stent and demonstrate the very low systemic dose administered by this method.

4. Conclusions

Stents coated with PC-polymers are an effective way to deliver a range of therapeutics locally to the artery wall. The interstitial spaces inside the polymer coating can be controled by the degree of crosslinking, and ordinarily the stent coating has a drug molecular weight limit of 1200 daltons for the final product. The release kinetics are influenced by interactions between the drugs and hydrophobic domains that are present within the polymer structure, the $\log P_{(o/w)}$ of the drug thus being of use as a first indicator of the likely expected elution profile. Targeted local delivery of therapeutics can be achieved *in vivo* with minimal systemic loss; the release is seen to be sustainable over a number of weeks, even for compounds that would appear to elute rapidly *in vitro*.

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