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Biodegradation and In Vivo Biocompatibility of Rosin: a Natural Film-Forming Polymer

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ABSTRACT

The specific aim of the present study was to investigate the biodegradation and biocompatibility characteristics of rosin, a natural film-forming polymer. Both in vitro as well as in vivo methods were used for assessment of the same. The in vitro degradation of rosin films was followed in pH 7.4 phosphate buffered saline at 37°C and in vivo by subdermal implantation in rats for up to 90 days. Initial biocompatibility was followed on post-operative days 7, 14, 21, and 28 by histological observations of the surrounding tissues around the implanted films. Poly (DL-lactic-co-glycolic acid) (PLGA) (50:50) was used as reference material for biocompatibility. Rate and extent of degradation were followed in terms of dry film weight loss, molecular weight (MW) decline, and surface morphological changes. Although the rate of in vitro degradation was slow, rosin-free films showed complete degradation between 60 and 90 days following subdermal implantation in rats. The films degraded following different rates, in vitro and in vivo, but the mechanism followed was primarily bulk degradation. Rosin films demonstrated inflammatory reactions similar to PLGA, indicative of good biocompatibility. Good biocompatibility comparable to PLGA is demonstrated by the absence of necrosis or abscess formation in the surrounding tissues. The study provides valuable insight, which may lead to new applications of rosin in the field of drug delivery.

KEYWORDS: biodegradation, biocompatibility, rosin

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INTRODUCTION

Biomaterials are considered to be any nonviable materials that become a part of the body either temporarily or permanently to restore, augment, or replace the natural functions of the living tissues or organs in the body.[1] A number of biomaterials have been used for medical applications including controlled drug delivery,[2,3] orthopedic devices,[4] sutures, cardiac pacemakers, and vascular grafts.[5] Ideally, the biomaterials should not elicit any systemic, immunologic, cytotoxic, mutagenic, carcinogenic, or teratogenic reactions when introduced in vivo[6] (by injection, insertion, or surgical implantation). The use of natural polymers and their semi synthetic derivatives in drug delivery continues to be an area of active research despite the advent of synthetic polymers. Natural polymers remain attractive primarily because they are economical, readily available, capable of chemical modifications, and potentially degradable and compatible due to their origin. Rosin, a film-forming biopolymer, and its derivatives have been extensively evaluated pharmaceutically as film-coating[7,8] and microencapsulating[9,10] materials to achieve sustained/controlled drug release. They are also used in cosmetics, chewing gums, and dental varnishes.[11,12] Rosin is a natural product obtained from the oleoresin of pine trees *viz Pinus soxburghi* and *Pinus toeda*. It is primarily composed of abietic and pimaric acid, which contain 2 reactive centers: the carboxylic group and the double bonds. The increasing use of rosin biopolymers as matrices in drug delivery systems requires testing of their biodegradability and tissue compatibility. In this context, it seems particularly desirable to elucidate the biodegradable and compatible characteristics of rosin. More specifically this work is focused on investigating the in vitro – in vivo degradation and biocompatibility of free films of rosin. Free films of poly(DL-lactic-co-glycolic acid) (PLGA) (50:50) (Alkermes, Cincinnati, OH) used as control for biocompatibility were prepared as previously described.[13]

MATERIALS AND METHODS

Rosin (N grade) was received as a gift sample from Derives Resiniques Terpeniques (DRT) (Gambetta, France). Other reagents and chemicals were of analytical or pharmacopoeial grade.

Fabrication of Rosin Films

Neat films of rosin (plasticizer free) were fabricated by solvent evaporation technique using a mercury substrate. Thirty percent wt/vol solution in methylene chloride was utilized for film casting (area of casting, 19.5 cm^2), allowing the solvent to evaporate for 48 hours. Films were stored in desiccators at ambient temperature for 12 hours before use.

In Vitro Degradation

Free films of rosin (2 cm × 1 cm × 0.4 mm, 120 mg) were subjected to in vitro degradation by placing them in 10.0 mL of 0.2M phosphate buffered saline (PBS) (pH 7.4, 37°C) and maintained on a rotating shaker.[14] The PBS was changed every 8 hours for the first day, every day for the first week, and weekly thereafter to keep the pH relatively constant.[15] Films were withdrawn at intervals of 30, 60, and 90 days, washed with distilled water, dried, and subjected to analysis.

In Vivo Degradation

To monitor the in vivo degradation, films were subcutaneously implanted on the backs of male wistar rats (200-300 g). Anesthesia was induced by intraperitoneal injection of a mixture of ketamine HCl (85 mg/kg body weight) and xylazine (12 mg/kg body weight). Tetracycline, 10 mg/kg dose, was given at the time of surgery. An incision (2.5 cm) was inflicted laterally about the midportion of the back. Subcutaneous pockets were formed around each incision, free film was inserted, and the wounds were closed by intermittent nylon sutures, 0.5 cm apart. Films were explanted at 30, 60, and 90 days for analysis.[13,16] The animals were housed individually with free access to water and movement within their cages. The animal experimental and care protocols followed the guidelines accredited by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and protocols were approved by the Institute Animal Ethics Committee. For both in vitro and in vivo degradation, 4 samples per time point were used for weight change, MW decline, and surface morphological analysis.

In Vivo Biocompatibility

For biocompatibility investigation, implant procedures were performed as described earlier in this text. Animals were euthanized (if necessary) with diethyl ether at specific time points (7, 14, 21, and 28 days) after surgery. Tissues surrounding the implanted rosin and PLGA films were sectioned, stained, and examined under a light microscope to follow the inflammatory responses.[17,18] The responses observed with rosin films were compared with those with PLGA.

Analysis of Degradation

The weight average MW of initial and degraded films was analyzed by gel permeation chromatography (GPC) (Perkin-Elmer, Newton Centre, MA) equipped with a refractive index (RI) detector. Samples dissolved in tetrahydrofuran were eluted through PL (Polymer Laboratories, Amherst, MA) gel 3 μ mixed column, at a flow rate of 1 mL/min. Polystyrene standards were used for calibration. Surface morphology of films was followed under a scanning electron microscope (Stereoscan-250-MK-III, London, UK). Samples were gold coated with sputter coater (Jeol JX-A-840A, London, UK) for 120 seconds under argon atmosphere before analysis under the microscope.

RESULTS AND DISCUSSION

Rosin is a low molecular weight (MW = 400) polymer exhibiting excellent film-forming property. The weight decline of the free films of rosin following in vitro and in vivo degradation indicates a faster decline when implanted in rats (**Figure 1**). Rosin films maintained nearly 77.0% of day 0 value after 90 days of degradation in PBS. In the in vivo study, free films could not be recovered at the end of 90 days with the weight decline being rapid during the first phase (15 and 30 days) maintaining about 40.0% day 0 weight at the end of 1 month. The films showed complete degradation by the end of 75 days. In the in vitro study, the solution was changed frequently to ensure that the pH value never went below 7.0 with any of the sample tubes. Although the amount of degradation was small in vitro, it showed significant sequelae when reproduced in vivo.[19] Few reports document loss of the polymer character even after 1% degradation, while the integrity is completely lost after 10% degradation in most cases.[20] The pattern of degradation was more or less similar following the MW loss (**Figure 2**). After placement in

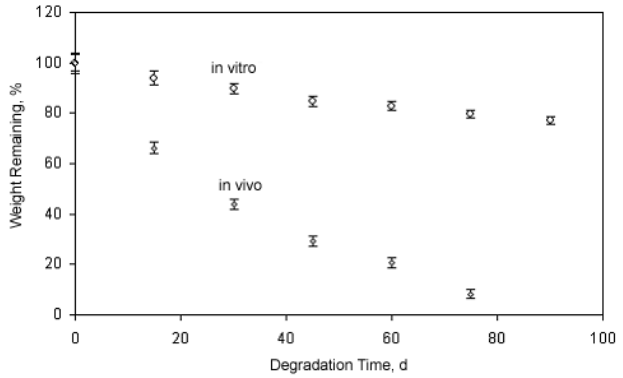

Figure 1. Percentage weight remaining as a function of the degradation of rosin-free films. Error bars indicate mean ± SD (n = 4).

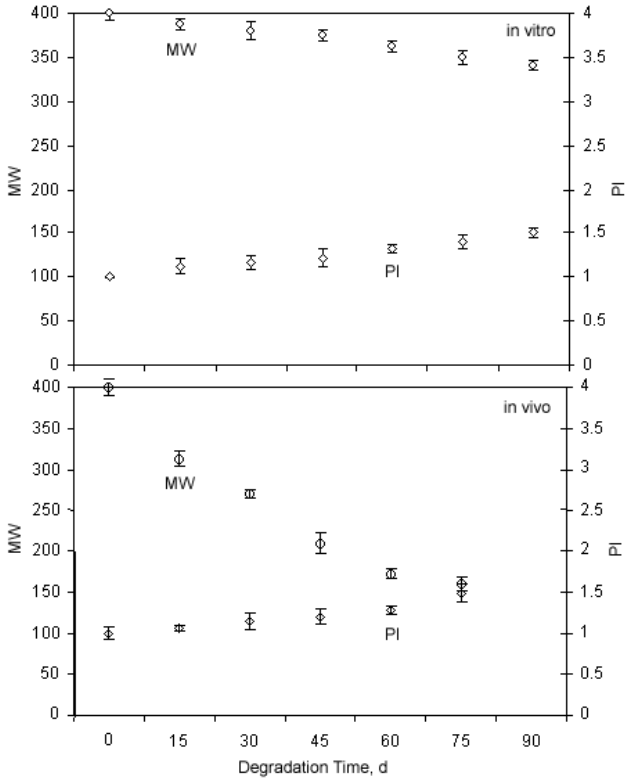

Figure 2. Change in weight average MW as a function of the degradation of rosin-free films. PI indicates polydispersity index.

PBS, the rosin films showed MW loss of 14.7%, with the films being recovered at the end of 90 days. After in vivo implantation in rats, the free films showed MW loss of 60.0% at around day 75 and complete loss at the end of 90 days. The MW loss profiles again indicate faster degradation in vivo as compared with in vitro. This finding may be due, in part, to the foreign body response.[21,22] As a result of the in vivo implantation, the typical response results in the accumulation of cells such as macrophages around the foreign body leading to a walling off of the region. Free radicals, acidic products, or enzymes produced by these cells during the foreign body response may accelerate degradation.[23] The rates of degradation observed in vitro and in vivo in terms of weight and MW decline were not parallel, but the mechanism seems identical (ie, heterogenous bulk degradation). This finding is further supplemented by the scanning electron micrographs (SEM) (**Figure 3**) of the initial and degraded films with a uniformly distributed bulk erosion of the film surfaces. As previously understood, the free films of rosin showed complete degradation at the end of 90 days with few fragments being recovered at the end of 75 days. Hence the SEM of the films recovered at the end of 60 days is presented for comparison. The micrographs reveal bulk degradation with the pores remaining evenly distributed throughout.

When biomaterials are placed inside the body, the compatibility responses involve both the time-dependent effects of the host on the material and that of the material on the host.[24] Inflammation, wound healing, and foreign body responses are generally considered as parts of the tissue or cellular host responses to injury. Normally, placement of a biomaterial in the in vivo environment involves injection, insertion, or surgical implantation, all of which injure the tissues involved. The degree to which the homeostatic mechanisms are perturbed and the extent of the pathophysiological responses and their resolutions are measures of the host reactions to the biomaterial. The in vivo biocompatibility studies commonly utilize subcutaneous implantation with serial analysis of the sequence of events of the inflammatory and wound healing response.[25] Such responses are dependent on the material characteristics and properties and the safety is governed in part by them.[26] On a quantitative basis, the cage implant system is used to determine the dynamic nature of cell function at the implant site. This system provides a simple means by which the inflammatory exudate is monitored serially without sacrificing the animal.[27]

Tissues surrounding the implanted rosin films were removed at specific postoperative points (7, 14, 21, and 28 days) and analyzed histopathologically for the compatibility response. The tissue in contact with rosin films evoked a moderate inflammatory response at the end of 7 days postimplantation (**Figure 4A**). The implant site contained a thin, fibrous layer with evidence of new blood vessels. The inflammation was characterized by the presence of polymorphonuclear leucocytes.

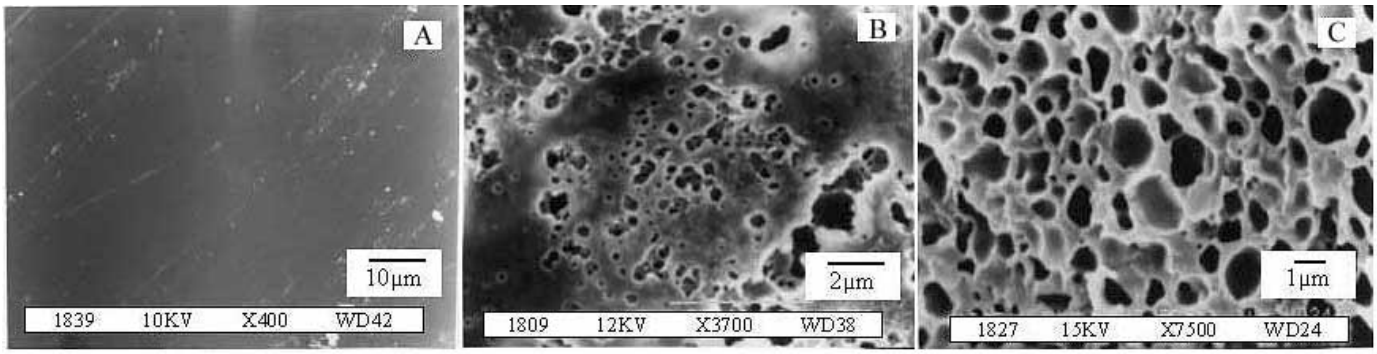

Figure 3. Scanning electron micrographs of (A) rosin-free film (original magnification ×400), (B) rosin film after 90 days of in vitro degradation (original magnification ×3700), and (C) rosin film after 60 days of in vivo degradation (original magnification ×7500).

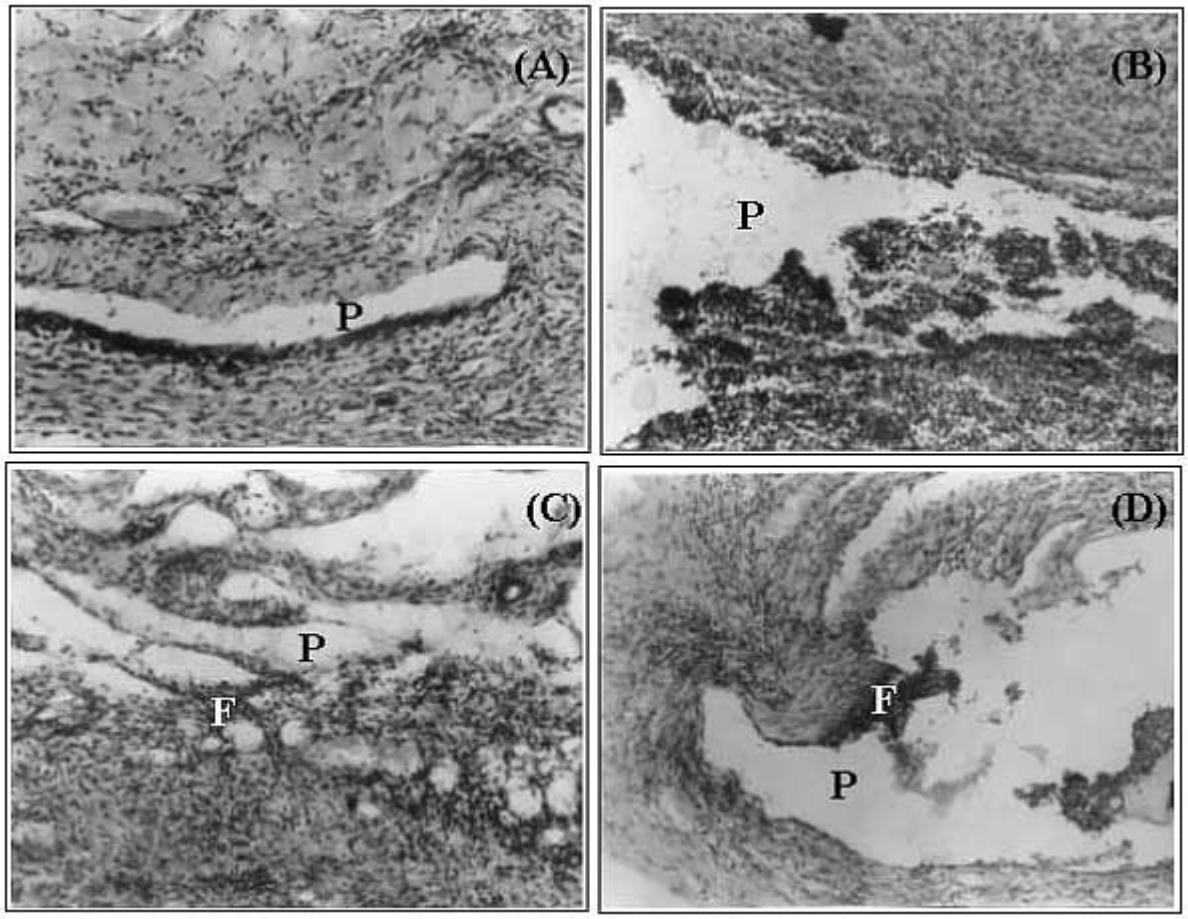

Figure 4. Photomicrographs of rat subcutaneous tissue response to rosin film implantation (original magnification ×110): (A) day 7, (B) day 14, (C) day 21, (D) day 28. P indicates rosin-free film and F, fibrous tissue.

After 14 days postimplantation, the intensity of the inflammatory reaction continued with the invasion of inflammatory cells in polymer (**Figure 4B**). Denser fibrosis with new blood vessels was evident. The inflammatory reaction gradually declined by days 21 and 28 (**Figures 4C** and **4D**), relative to day 14 observations. The response, however, showed few hemorrhages with dense fibrosis. In comparison, the tissue in contact with PLGA films evoked an intense inflammatory response with prominent infiltration of polymor-

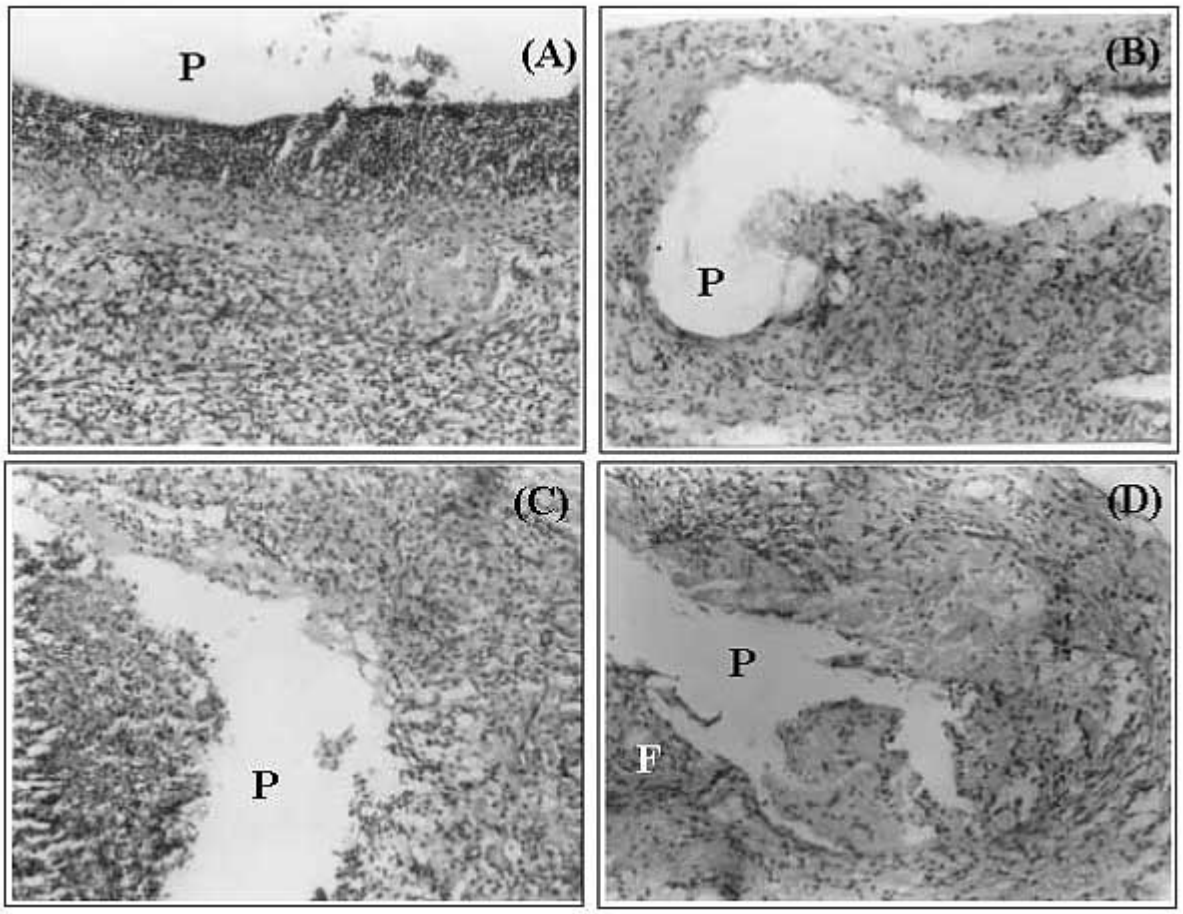

Figure 5. Photomicrographs of rat subcutaneous tissue response to PLGA film implantation (original magnification ×110): (A) day 7, (B) day 14, (C) day 21, and (D) day 28. P indicates PLGA-free film and F, fibrous tissue.

phonculear neutrophils and lymphocytes at day 7 (**Figure 5A**). There was evidence of angiogenesis with prominent mononucleated cells. The inflammatory reaction reduced by day 14 (**Figure 5B**) with mild fibrosis. The overall characteristics remained the same at day 21 with further reduction in the inflammatory response (**Figure 5C**). Mild inflammation was evident at day 28 with fibrosis around the implant (**Figure 5D**). Although the reaction was intense initially, it gradually subsided and demonstrated good compatibility at the end of 28 days. The observations are consistent with the typical wound-healing response to biomaterial implantation.

So far, in the field of biomaterials, the ideal combination of biodegradability and biocompatibility has been seldom achieved. Polyesters represent one of the extensively investigated classes of biodegradable and biocompatible polymers approved for human use worldwide.[28,29] One of the major problems faced by researchers is the lack of sufficient biocompatibility over extended periods.

CONCLUSION

This study investigates a natural polymer, rosin, for its degradability and compatibility in and with the physiological environment. Rosin has shown faster degradation in vivo as compared with in vitro. After placement in PBS, the rosin films showed MW loss of 14.7%, with the films being recovered at the end of 90 days. After in vivo implantation in rats, the free films showed MW loss of 60% at around day 75 and complete loss at the end of 90 days. Bulk degradation is evident both in vitro and in vivo. Although rosin degrades over a period of 2 to 3 months, it provides good compatibility compared with PLGA to the extent investigated in this article. This finding presumably will lead to new applications of rosin in the field of drug delivery. In the fu-

ture, this material may provide a relatively economical and readily available matrix for drug delivery.

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