

Erythropoietin Implantable Dosage forms for the Treatment of Anaemia Associated with Renal Failure Induced in Rabbits

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Received 25/9/2024, Accepted 21/5/2025, Published 28/9/2026



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Abstract

This study aimed to develop, optimize, and evaluate implantable film-based dosage forms of Erythropoietin (EPO) for the sustained treatment of anemia in patients with chronic renal failure. The designed system offers controlled EPO release over six months from a single subcutaneous implantation, with the flexibility for dose adjustment based on individual therapeutic needs. Two types of implantable films were formulated: one by 3D printing technology and the other via a casting method using a polyvinyl alcohol (PVA)/glycerol matrix. Each film contained a combination of sustained-release EPO (100,000 IU) and pure EPO powder (10,000 IU). In vitro evaluation demonstrated that both formulations exhibited acceptable mechanical and release characteristics. The casting films showed superior mechanical strength, with a folding endurance 66 times greater than that of the 3D-printed films. Drug release from both systems followed a biphasic pattern: an initial release of approximately 10% within the first week, followed by extended release over six months, reaching 98.73% for the casting film and 88.79% for the 3D-printed film. In vivo studies in animal models revealed normalization of hemoglobin (Hb) and EPO levels within the first two weeks post-implantation for both film types, with levels remaining stable throughout the six-month evaluation period. In contrast, conventional subcutaneous EPO injections maintained Hb within normal limits but resulted in elevated serum EPO levels, which may increase the risk of cardiovascular complications. The findings confirm that both implantable film formulations provide a promising alternative to routine EPO injections, ensuring long-term therapeutic efficacy, enhanced patient compliance, and reduced potential for adverse effects. Furthermore, strong correlation between in vitro and in vivo data supports the reliability and performance of these sustained-release systems.

Keywords: 3D printing, Erythropoietin, Haemoglobin, Implantable films, long-acting.

Introduction

Making implantable films under the skin is one of the traditional methods used to increase the acceptability of the drug by the patient, especially drugs that require continuous administration of drug doses by intramuscular or subcutaneous injection ⁽¹⁾. There are several ways to make implantable films; the traditional ways, include casting, molding, or compression methods ⁽²⁾. The method of using 3D printing in the manufacture of implantable films is considered one of the modern methods that guarantee several benefits in the manufacture of these films, including ensuring that the active ingredient is spread equally on all parts of the films while ensuring the manufacture of films of different shapes and sizes, such as using 3D printing in implantable film is the 3D printing PLGA-Doxorubicin films provide an effective localized chemotherapy approach, reducing tumor progression while minimizing systemic side

effect⁽³⁾. Another example is 3D-printing PCL-Vancomycin films offer a promising localized antimicrobial therapy, reducing bacterial infection rates and supporting bone healing ⁽⁴⁾. In addition, the use of 3D printing in industry assists in manufacturing a film that contains more than one method for releasing profiles. As well as the 3D printing technique can also be used to manufacture materials that are affected by heat or affected by the solvent that was used in traditional manufacturing methods. On the other hand, the manufacturing method using 3D printing ensures the ability to manufacture pharmaceutical forms according to personal needs ^(5,6).

Erythropoietin is also called epoetin (EPO), and it is a glycoprotein with a molecular weight (MW) of 30.4 kDa. It can be considered a cytokine precursor of red blood cells and controls the formation of those cells.

EPO which is used as a treatment in anemia associated with CRD is synthesized by recombinant DNA technology ⁽⁷⁾. EPO is administered subcutaneously in a dose of 25-150 IU every other day for continuous treatment ⁽⁸⁾.

The aim of the study is to manufacture subcutaneous implantable films using a 3D printed method containing epoetin and compare their results with subcutaneous implantable films which is prepared using the traditional casting method, and evaluate their results *in vitro* and *in vivo*. Through this study, we hope to create a new dosage form with a long period effect and maybe replace the existing pharmaceutical dosage forms on the market that include frequent injections.

Materials and Methods

Materials

Erythropoietin alpha (EPO) was purchased from Provizer, India. Glycerol and Polyvinyl alcohol (PVA 0588 (MW = 27000-28000 D)) from GCC, U.K. NaCl from Alpha chemical – India. Aluminum chloride and anhydrous calcium chloride were purchased from Laboratory Chemical, India. Benzalkonium chloride was purchased from Sinopharm Chemical Reagent, China. Potassium phosphate is monobasic and was purchased from Himeda – India. Sodium azide was purchased from Panreac AAG, Spain.

Statistical analysis

The statistical analysis was performed using

an independent sample t-test and a single-variable analysis method (ANOVA) and similarity factor (F1 to F2). The statistical differences were taken when ($P < 0.05$). Data analysis was done by using the SPSS Statistics base 16 program.

Preparation of the base of the implantable film

The base of the implantable film was prepared using the solvent evaporation method, where 3 g of PVA was dissolved in (15mL) hot distilled water (80-90 °C), and then 1g glycerol was added with continuous stirring on a magnetic stirrer for 30 min on 1000 rpm leaving the solution for 24 hrs. aside ⁽⁹⁾.

Preparation of the implantable film by the casting method

In this method, 2.5 mL of the prepared base film solution was added to 2 mL of distilled water in which a certain amount of EPO microcapsules previously prepared by double emulsion method in the laboratory ⁽¹⁰⁾ (equivalent to 95,000 IU) was suspended, and then 0.5 mL of aqueous solution of pure uncoated EPO (5000 IU) was added. A Magnate stirrer under 1500 rpm and for 15 min was used to homogenize the mixture, and then the mixture was cast in a circular glass mold with a dimension of 2 cm in diameter. The mold was dried at 40 °C for 24 hrs ⁽¹¹⁾, to get a solid implantable film containing micro-encapsulated EPO (equivalent to 95000 IU) in addition to 5000 IU uncoated pure EPO (Figure 1).

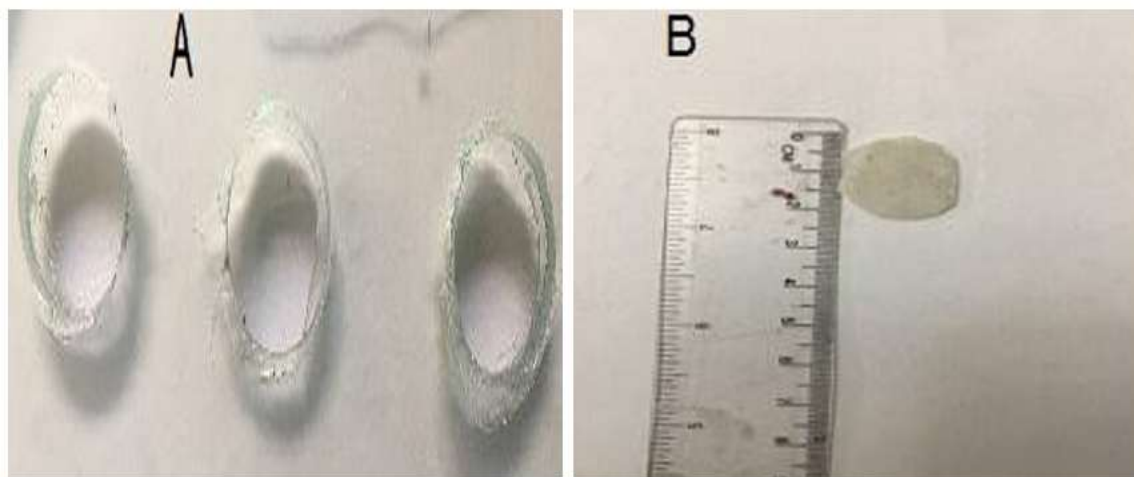


Figure 1. (A) Mold used for casting and (B) dried film of EPO prepared by the casting method

Preparation of implantable film by 3D printing technology

Seven grams of Polyvinyl Alcohol (PVA) were mixed with a certain amount of EPO microcapsules (equivalent to 95000 IU of EPO) previously prepared in laboratory using mortar and pestle for 2 min, and then extruded at 150 °C with 100 rpm screw speed to obtain filaments (the extrusion finished within 5 min). The obtained filaments were soft and directly passed through a cold metal nozzle ^(12, 13). In order to choose a

suitable final shape for the implantable EPO dosage form, a digital file of the selected 3D shape was designed using computer-aided design BRL-CAD software. After choosing the suitable design, the prepared EPO filaments were printed and assembled using a Tevo 3D printer and a perforated implantable dosage form shape with 2cm x 1cm x 0.5cm in dimensions was obtained ⁽¹⁴⁾. A temperature of about 160 °C temperature was used without platform heating in 3D printing, standard mode with 0.2 mm layer thicknesses and 100%

infill, which required 3 ± 0.5 min to get the final dosage form. EPO pure drug powder (5000 IU) was forced with microneedles to fill the micropores available on the surface of the perforated dosage

form. The surface of the prepared implantable dosage form was covered with pure PVA filaments using the same 3D printer (Figure 2).



Figure 2. (A) 3D printer, (B) Extruder, (C) 3D printing films of EPO

In vitro evaluation of the prepared EPO implantable films'

Three prepared films (prepared by each method) were individually weighed by using a digital balance and calculating the average weight for the films⁽¹⁵⁾. The thickness of the prepared film was measured from five different points (the center and edges), and it was applied to three prepared films (from each method)⁽¹⁶⁾. This test was performed on three films for each manufacturing method. The surface of each manufactured film was wetted with 1 ml of distilled water, and then the surface pH was measured using the electrode of a pH meter⁽¹⁷⁾. This test was conducted in three patches for each preparation method (3D printing and casting methods). The test includes re-folding the implantable film in the same place several times to measure the flexibility of the manufactured film⁽¹⁸⁾.

In-Vitro release study

A modified Franz cell was used to measure the extent of material released from films prepared for implantation and manufactured using both methods (casting method and 3D printing method). The modified Franz cell contains two sides, a donor side and a recipient side. The two sides are separated by a dialysis membrane (MWCO300Kd) and each side can accommodate 10 ml of phosphate buffer with a pH of 7.4⁽¹⁹⁾. The film is placed on the donor side in a modified Franz cell, and the extent of the permeability of the substance is measured by measuring the concentration of epoetin on the receiving side. The release from the films was monitored for 6 months using three modified Franz cells. The temperature was maintained at 37°C, and the rotation speed was set between 30-40 rpm. Additionally, the sampling schedule was initially set for daily withdrawals that continued for 1 month, then changed to weekly withdrawals until the end of the experiment.

The release process continued for 6 months, which was followed by the withdrawal of samples from the receiving side of the Franz cell at pre-calculated times, and (RP-HPLC) analysis (reversible high-performance liquid chromatography) was performed using an ultraviolet detector to estimate the concentration of epoetin. RP-HPLC analysis was carried out using a mobile phase consisting of 0.1% trifluoroacetic acid (TFA) with MilliQ water and acetonitrile using a gradient program (T(min)% mobile phase) (0/65,4/65, 12/50,14/50,15/40,16/65,20/65) and at a flow rate of 1.5 ml per minute. A system equipped with an injection valve (quaternary), an ultraviolet detector, and chemstation software was used. A reverse-phase C18 column (4.6 mm ID-250 mm L, porosity 3001 A, particle size 5 mm) with a guard column (reverse-phase C18 column of 4.6 mm ID-35 mm L, porosity 3001 A, particle size 5 mm) was used for separation⁽²⁰⁾.

In vivo test animals used

The work was done according to the ethical committee in the Iraqi center for Cancer Research, Mustansiriyah University, using New Zealand white rabbits. The rabbits were kept in the animal house under natural light-dark cycles & environmental temperature of 23 – 25 °C. The rabbits had free access to tap water & food⁽²¹⁾.

Grouping of the animals

The total number of animals used was 20 rabbits, divided into 4 groups as shown in Table 1. The dose for EPO to be used for animals was calculated according to the following equation⁽²⁰⁾.

$$\text{Human equivalent dose (HED)} = \text{Animal dose} \times \left(\frac{\text{animal weight}}{\text{human weight}} \right)^{0.33} \quad \text{Eq. 1}^{(22)}$$

The dose required for the rabbits weighing 1.5-2 Kg was approximately equal to 10,000 IU in each implantable film. Each film for animals contains

EPO microcapsules equivalent to 9,500 IU as well as 500 IU pure EPO (untreated).

Table 1. The groups of animals used during the *in vivo* evaluation of the prepared EPO implantable films.

Group no.	The name of the group	Number animals
Group 1*	Control group	5
Group 2**	The group received EPO S.C. injections every other day after induction of renal failure and anaemia.	5
Group 3***	Casting group (group implanted with film prepared by casting method) after induction of renal failure and anemia.	5
Group 4****	3D group (group implanted with film prepared by 3D printing method) After the induction of renal failure and anaemia.	5

*The animal used in group 1 received 0.9% NaCl and they were used to see normal values of creatinine, Hb and EPO levels.

**The animal used in group 2 was treated with EPO injection (50 IU/kg) as SC injection every other day for 6 months after induction of renal failure using cisplatin IP injection (10 mg/kg) and anemia signal was demonstrated.

***The animal used in group 3 was implanted with film prepared by casting method after the induction of renal failure using cisplatin IP injection 10 mg/kg and anemia signal was demonstrated.

**** The animal used in group 4 was implanted with film prepared by 3D printing after induction of renal failure using cisplatin IP injection 10 mg/kg and anemia signal was demonstrated.

After induction of renal failure using cisplatin IP injection 10 mg/kg and anemia was demonstrated, then the film was implanted in the dorsal part of the animal subcutaneously (Figure-3). The process of sterilizing implantable films manufactured using two methods (3D printing method and traditional casting method) was done by sterilizing the solutions in each step by sterile filtration and performing all film manufacturing steps inside a sterile laminar

cabinet. The process of testing the sterility of each manufactured film was carried out through the direct inoculation method, where the films were incubated in a special culture medium. Soybean – Casein digest medium was used as a culture medium which is suitable culture for both aerobic bacteria and fungi. After incubation for 14 days at 25 °C the sample was visualized for any sign of turbidity^(23, 24). Figure 3 shows the implantation procedure.



Figure 3. Implantation of film Sc in rabbits

Induction of renal failure

All animals were fed and had free access to water during the test. Experimental rabbits received 10 mg cisplatin/kg body weight to induce renal failure before treating them with SC injection, or an implanted film prepared by casting or 3D printing method, while the control group received an equal volume of 0.9% NaCl. The first blood samples were taken 3 days after cisplatin induction to measure the levels of hemoglobin and creatinine, in

addition to the level of epoetin within the blood. The ELISA method was used to measure the above factors inside the blood⁽²⁵⁾. The process of measuring blood factors continued every week until signs of kidney failure appeared in the animal, including high creatinine levels of more than 1.4 mg/dL, which usually occurs 3-4 days after cisplatin injection. Also, signs of anaemia were observed two weeks after cisplatin injection. Hemoglobin levels appeared to be less than 9

gm/dL, which is one of the expected symptoms of kidney failure. Cisplatin is considered a toxic substance to human cells, as it causes ischemia of kidney cells and ureteral obstruction, which in turn leads to a significant and sharp decrease in the rate of glomerular filtration, which in turn leads to an increase in the creatinine level which lead to decrease in Hb levels ⁽²⁶⁾.

Sampling

After inducing renal failure accompanied by anaemia in each rabbit (groups 2, 3, and 4), each rabbit in each group received its dosage form as treatment. The rabbit's auricular artery was used to withdraw blood samples every week, and the drawings continued for 6 months, during which the levels of creatine and hemoglobin were monitored, as well as the level of epoetin in the blood. The ELISA method was used to monitor the levels of hemoglobin and creatinine, as well as epoetin. The Rabbits ELISA KIT was used to monitor the level of epoetin in the blood, as the level of epoetin is supposed to rise after its release from doses implanted under the skin ⁽²⁷⁾. The same blood sampling was applied for each rabbit in the control group and analysed for creatinine, Hb level and EPO level for 6 months.

Results And Discussion

In vitro evaluation of the prepared EPO implantable films'

The results showed that the weight variation between the prepared implantable films was less

than 5% , with a weight range from 755 ± 8.5 mg for casting EPO films, while the films prepared by 3D printing gave a weight range from 1003 ± 4.6 mg.

This indicated that all the prepared films had weight variation within the acceptable limit ⁽²⁸⁾. The average thickness for each implantable film was found to be uniform, equal to 2.02 ± 0.05 mm for casting EPO films, while for 3D printing films, equal to 4.5 ± 0.02 mm, which indicated that these films had a homogenous thickness and agreed with the reported requirements ^(29,30). Surface pH is an important test to ensure that the film will not irritate the implanted area and should have a surface pH near normal (pH 7.2–7.4) ⁽³¹⁾. The results (Table 2) showed that all the prepared films by casting and 3D printing methods had an average pH of 7.13 ± 0.07 and 7.27 ± 0.03 , respectively, which are not significantly different from body pH; therefore, no irritation or changes are expected in the implanted area ⁽³²⁾. The results showed that the folding endurance of the films manufactured using the traditional casting method was more than $66.34 + 1.08$ times, and this indicates that they have acceptable flexibility and good ability for packaging and storage ⁽³³⁾, as shown in Table 2. While the films made using the 3D printing method did not show any folding, due to the thickness of the films made, which showed resistance to folding.

Table 2. In Vitro evaluation of 3D printing EPO film and casting EPO film.

Test	Films prepared by Casting	Films prepared by 3D printing
Weight variation	755 ± 8.5 mg	1003 ± 4.6 mg
Thickness	2.02 ± 0.05 mm	4.5 ± 0.02 mm
Surface pH	7.13 ± 0.07	7.27 ± 0.03
Folding endurance	66.34 ± 1.08	N.A

In-Vitro Release

Figure 4 shows that the liberation of the active substance from the film manufactured by the casting method during the first week reached 18.91%, while the film manufactured by 3D printing showed 13.42% drug release in the first week. The first week's release was mainly due to the pure untreated drug (equivalent to 10000 IU of EPO) incorporated in the films. The release continued gradually from the included microcapsules to reach 89.98% and 81.46% (no

significant difference, $P \geq 0.05$) from casting and 3D printing films, respectively, within 6 months. The release of the drug from the casting film was higher than from the 3D film with a similarity factor (f_2) equal to 55.927, which is acceptable according to the FDA ⁽³⁴⁾ since the 3D film was much thicker than the casting film, so the drug required longer time to release from microcapsules included within the film. Same results observed with 3D implant for an antimicrobial polylactide material ⁽³⁵⁾.

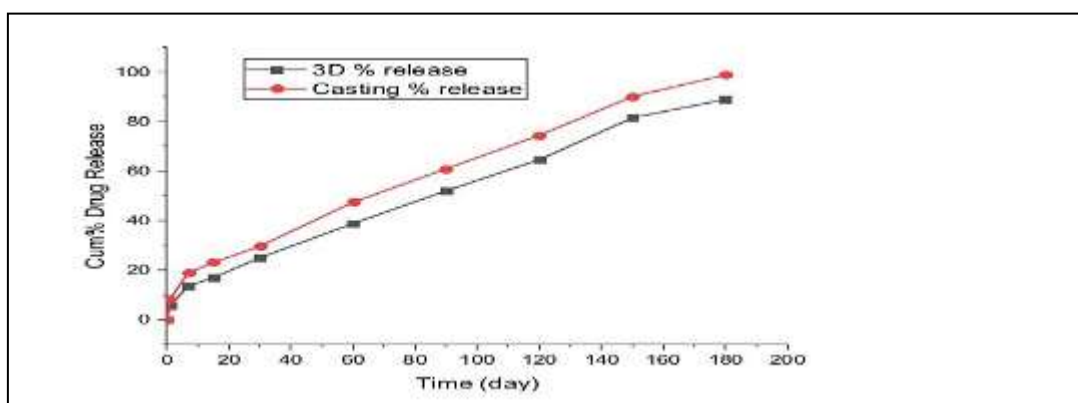


Figure 4. *In vitro* EPO release profile from the films prepared by casting and 3Dprinting in phosphate buffer pH 7.4 at 37 °C.

In-vivo test

The PVA implants are biodegradable with high biocompatibility and biosafety. The results demonstrated that there was no redness, exudation, suppuration, or other infections in the implanted area of the tested animals during the study (6 months) as well as the results of the sterility test (Figure 5 A and B) which indicated no growth of bacteria or fungi after 14 days of culturing the prepared implantable films. The results presented in Table 3 show that creatinine levels for rabbits started to increase within the first 3 days of implantation and continued increasing during the experiment because kidney failure continued to the end of the experiment. This indicated that a single cisplatin IP injection (10mg/kg) was enough to induce kidney failure (where serum creatinine was increased ≥ 3 mg/dl) within the first 3 days of cisplatin injection and continued to increase to the end of the experiment, while the Hb level started to decrease (< 9 mg/dl) within two weeks. Group 1 (control group) showed normal values for Hb, creatinine level, and EPO (12.06 ± 0.24 gm/dl, 0.67 ± 0.07 , and 11.724 Mu/ml, respectively), indicating that healthy rabbits were used in the experiment^(36, 37). Group 2 (rabbits treated with repeated S.C. injection group) showed an increase in Hb level from the first month, and EPO level increased to 5-6-fold higher than the control indicating the effectiveness of repeated Sc EPO

injection which is similar to the treatment regimen applied for human, the increase exceeds the normal physiological level, potentially leading to undesirable side effects that could negatively impact the cardiovascular system^(38,39). Group 3 demonstrated more acceptable Hb levels one month after implantation of therapeutic films, with EPO levels increasing to twice the normal range. This can be attributed to the slow release of EPO from the implantable films compared to Sc injections. Group 4, which received a therapeutic film prepared by 3D printing technology, showed stable and acceptable Hb levels from the first month. Meanwhile, EPO level remained within the normal range, similar to healthy rabbits. This observation suggested that the drug release from 3D printing films was slower than that of casting films, enabling better control over EPO release and maintaining level close to physiological norms. The controlled elevation of EPO subsequently led to increased Hb levels. Notably, preventing excessive EPO elevation helps to minimize potential side effects commonly associated with Sc EPO injection for treating anaemia induced by kidney failure. The results in groups 2, 3 and 4 indicated an Hb increase of approximately 2 g/dl after 2-3 weeks, which aligns with meta-analysis studies that have evaluated the effects of EPO on Hb levels in patients with kidney failure.^(40,41).



Figure 5. A: Implantable films prepared by casting and 3D printing at A: firstday of sterility test, B: at day 14 of sterility test

Table 3. Blood parameters for 4 groups of rabbits for 6 months.

	3D printing (G4)			Casting (G3)			Sc inj (G2)			Control (G1)		
	Hb g/dl	Cr level mg\dl	EPO Mu/ml	Hb g/dl	Cr level mg/dl	EPO Mu/ml	Hb g/dl	Cr level mg/dl	EPO Mu\ml	Hb g/dl	Cr level mg/dl	EPO Mu/ml
W1	9.4±0.37	5.02±0.4	11.788±0.66	9.24±0.26	5.6±0.46	21.428±0.86	9.18±0.34	5.16±0.4	61.106±3.24	12±0.23	0.4±0.08	11.834±1.038
W2	9.64±0.298	6.02±0.39	11.698±0.578	9.84±0.23	6.38±0.44	21.704±0.883	9.58±0.32	5.98±0.34	62.382±2.69	11.96±0.2	0.5±0.04	11.788±1.11
W3	10.94±0.24	6.58±0.27	11.704±0.515	10.26±0.12	7.22±0.4	21.256±0.826	10.14±0.33	6.88±0.23	62.188±2.55	12±0.22	0.54±0.07	12.172±1.068
W4	11.18±0.16	7.32±0.26	11.638±0.42	11.36±0.2	8.2±0.3	20.76±0.874	11.34±0.3	7.94±0.29	62.53±2.72	12.06±0.26	0.5±0.05	11.902±1.073
W8	11.34±0.143	8.48±0.5	11.432±0.403	11.6±0.15	9.1±0.23	20.148±0.757	11.5±0.14	9.06±0.31	62.17±2.42	12.06±0.25	0.5±0.08	12.02±1.07
W12	11.38±0.14	9.76±0.4	11.18±0.386	11.6±0.2	10.16±0.21	19.266±0.837	12.78±0.15	10.04±0.18	61.658±2.37	12.08±0.23	0.54±0.06	11.944±1.09
W16	11.35±0.15	9.92±0.3	11.036±0.408	11.5±0.14	10.21±0.25	18.766±0.73	12.67±0.16	10.15±0.17	60.18±2.76	12.06±0.24	0.67±0.07	11.724±1.07
W20	11.32±0.16	9.84±0.4	11.32±0.306	11.4±0.21	9.78±0.23	18.45±0.87	12.76±0.14	10.22±0.12	62.34±2.54	12.05±0.32	0.61±0.06	11.871±1.02
W24	11.54±0.18	9.67±0.5	11.45±0.45	11.4±0.25	9.89±0.35	19.46±0.92	12.92±0.25	9.78±0.27	65.67±3.25	12.08±0.31	0.59±0.08	11.94±1.05

Hb =Hemoglobin, Cr= Creatinine, EPO= Erythropoietin, Sc inj.= subcutaneous injection.

Conclusions

The results of this study demonstrated that the preparation of an implantable therapeutic film using 3D printing technology, containing EPO provides a long-term release lasting up to six months with a single implantation procedure. This approach maintains EPO levels in the body close to normal levels just two weeks after implantation. The implantation of these films resulted a significant improvement in Hb levels, despite the reduction in natural erythropoietin in rabbits due to induced kidney failure. From these findings, Epoetin (EPO) loaded implanted films could serve as a promising alternative to the conventional use of frequent Sc injection of EPO for treating anemia in patient with renal failure. Additionally, the use of 3D printing technology in manufacturing films may allow for greater customization based on individual patient needs, which is difficult to achieve with traditional pharmaceutical manufacturing methods.

Acknowledgment

The authors would like to thank Al-Mustansiriyah University, for their support of the present work and thanks are extended to Al-Farahidi University, Baghdad/Iraq for supporting this work.

Conflicts Of Interest

The authors stated no conflict of interest in the manuscript.

Funding

There is no financial support for this work.

Ethics Statements

The authors declared the Iraqi Centre for Cancer Research/Mustansiriyah University's animal ethical council (approval no. ICCMGR2020-016).

Author Contribution

All the authors have contributed equally.

References

- MR MR, Bandarapalle K, Rajasekhar KK, Bhagyamma T. A Comprehensive Review on Parenterals, Int. J.Exp. Biomed. Res. 2022; 1(4): 136-147.
- Stewart S, Domínguez RJ, Donnelly R, Larrañeta E. Implantable Polymeric Drug Delivery Devices: Classification, Manufacture, Materials, and Clinical Applications. *Polymers*, 2018; 10(12), 1379.
- Zheng X, Wang F, Wang Y, Ma Z, Guo W, Li X, et al. 3D printed polydimethylsiloxane prosthesis with paclitaxel and doxorubicin microspheres for breast cancer therapy. *Front bioeng Biotechnol.*2020;8:590010.
- Lopez-Gonzalez I, Hernandez-Heredia AB, Rodriguez-Lopez MI, Aunon-Calles D, Boudifa M, Gabaldon JA, et al. Evaluation of the in vitro antimicrobial efficacy against staphylococcus aureus and epidermidis of novel 3D- printed degradable drug delivery system based on polycaprolactone/chitosan / vancomycin-preclinical study. *Front Bioeng Biotechnol.*2023;11:1042128.
- Hwang HH, Zhu W, Victorine G et al. 3D-Printing of Functional Biomedical Microdevices via Light- and Extrusion-Based Approaches. *Small Methods*, 2017; 1700277.
- Azad MA, Olawuni D, Kimbell G et al. Polymers for extrusion-based 3D printing of pharmaceuticals: A holistic materials–process perspective. *Pharmaceutics*, 2020;12(2), 124.
- Aoun M, Sleilaty G, Boueri C et al. Erythropoietin in Acute Kidney Injury (EAKI): a pragmatic randomized clinical trial. *BMC nephrology*, 2022; 23(1), 100.
- Santos EJJ, Dias RSC, Lima JFDB et al. Erythropoietin resistance in patients with chronic kidney disease: current perspectives. *International journal of nephrology and renovascular disease*, 2020; 231-237.
- Mohamed W, Nidhal KM. Preparation and evaluation of microcapsule dexamethasone sodium phosphate using double emulsion method, Al mustinsiria journal of pharmaceutical science, 2019; 19(1): 1-11.
- Anmar AI, Nidhal KM. Fabrication and optimization of prolong release microcapsules containing protein drug (Erythropioten). *International journal of drug delivery technology* , 4 (2);2022.
- Utomo E, Domínguez RJ, Moreno CN et al. Development of intranasal implantable devices for schizophrenia treatment. *International journal of pharmaceutics*, 2022;624, 122061.
- Jamróz W, Kurek M, Lyszcza E et al. 3D printed orodispersible films with Aripiprazole. *International Journal of Pharmaceutics*, 2017;533(2), 413–420.
- Velykodniy. Reengineering of open software system of 3d modeling brl-cad. *Innovative technologies and scientific solutions for industries*. 2019; No. 3 (9).
- Chakka LJ, Chede S. 3D printing of pharmaceuticals for disease treatment. *Frontiers in Medical Technology*, 2023; 4, 1040052..
- Muhammad I, Sumeira R, Quratulain B et al. Orally disintegrating films: A modern expansion in drug delivery system. *Saudi Pharmaceutical Journal*. 2016; 24: 537–546.
- Yoshifumi M, Kyoko K, Chieko Maida .Control of Drug Dissolution Rate from Film Dosage Forms Containing Valsartan. *International Scholarly Research Notices*.2016; 5: 112-119.
- Behbahani SB, Kiridena SD, Wijayarathna, UN et al. pH variation in medical implant biofilms: Causes, measurements, and its

- implications for antibiotic resistance. *Frontiers in Microbiology*. 2022 ;13, 1028560.
18. Neamah FW, Maraie NK.. Factors affecting preparation and evaluation of Kitorolac tromethamine ocular microsponges. *Al Mustansiriyah Journal of Pharmaceutical Sciences*, 2020; 20(2)
 19. Neamah WF, Maraie NK, Al-Timmemi HA. Intravitreal Implantable Film Containing Ketorolac Tromethamine Microsponges In Vitro/In Vivo Correlation. *Systematic Reviews in Pharmacy*. 2020;11(5):313-321.
 20. Rane SS, Ajameri A, Mody R, Padmaja P. Development and validation of RP-HPLC and RP-UPLC methods for quantification of erythropoietin formulated with human serum albumin. *Journal of pharmaceutical analysis*. 2012;2(2):160-165.
 21. Levine JK, Seixas JN, Ritter JM et al. Effects of Exogenous Erythropoietin on Rabbit (*Oryctolagus cuniculus*) Hematological and Biochemical Parameters. *Comparative Medicine*. 2023; 73(6), 439-445.
 22. Ye K, Ke Y, Keshava N, Shanks J et al. Opium alkaloid noscapine is an antitumor agent that arrests metaphase and induces apoptosis in dividing cells. *Proceedings of the National Academy of Sciences*, (1998); 95(4), 1601– 1606.
 23. Kaiser SJ, Mutters NT, Backhaus J et al. Sterility Testing of Injectable Products: Evaluation of the Growth- based BacT/ALERT(R) 3D Dual T Culture System. *PDA Journal of Pharmaceutical Science and Technology*, 2016; 70(6), 568–576.
 24. Zeki Y; Sadik S, Ersan O, Mustafa I et al. Oral erdosteine administration attenuates cisplatin-induced renal tubular damage in rats, 2003; 47(2), 149–156.
 25. Hassan SMA, Saeed AK, Rahim OO. Alleviation of cisplatin-induced hepatotoxicity and nephrotoxicity by L-carnitine. *Iranian Journal of Basic Medical Sciences*, 2022;25(7), 897.
 26. Barakat LA, Barakat N, Zakaria MM et al. Protective role of zinc oxide nanoparticles in kidney injury induced by cisplatin in rats. *Life Sciences*, 2020;262, 118503.
 27. Kumar BS, Chandra DS. Implantable microchip: the futuristic controlled drug delivery system, *DrugDelivery*.2016; 23(1): 1-11.
 28. Jiajie F,Tao Z, Jie S et al. Thickness dependent effective viscosity of a polymer solution near an interface probed by a quartz crystal microbalance with dissipation method. *Journal of Pharmaceutical Research*. 2015; 3:126-133.
 29. Wang X, Burgess DJ. Drug release from in situ forming implants and advances in release testing. *Advanced Drug Delivery Reviews*. 2021;178, 113912.
 30. Suh MS, Kastellorizios M, Tipnis N et al. Effect of implant formation on drug release kinetics of in situ forming implants. *International Journal of Pharmaceutics*. 2021;592, 120105.
 31. Kheder W, Al Kawas S, Khalaf K et al. Impact of tribocorrosion and titanium particles release on dental implant complications—A narrative review. *Japanese Dental Science Review*. 2021; 57, 182-189.
 32. Mayur KAB. A brief review on sustained release matrix type drug delivery system. *Journal of pharmaceutical research* .2017; 2:282-289.
 33. Dolete G, Chircov C, Motelica L, Ficai D et al. Magneto-mechanically triggered thick films for drug delivery micropumps. *Nanomaterials*. 2022;12(20), 3598.
 34. Elbadawi M, Gustaffson T, Gaisford S et al. 3D printing tablets: Predicting printability and drug dissolution from rheological data. *International Journal of Pharmaceutics*. 2020;590, 119868.
 35. Water JJ, Bohr A, Boetker J et al. Three-Dimensional Printing of Drug-Eluting Implants: Preparation of an Antimicrobial Polylactide Feedstock Material. *Journal of Pharmaceutical Sciences*, 2015; 104(3), 1099-1107.
 36. Pogány SM, Chrastinová L, Lauková A. Autochthonous strain *Enterococcus faecium* EF2019 (CCM7420), its bacteriocin and their beneficial effects in broiler rabbits—A review. *Animals*. 2020; 10(7), 1188.
 37. Robach P, Gammella E, Recalcatti S et al. Induction of erythroferrone in healthy humans by micro-dose recombinant erythropoietin or high-altitude exposure. *haematologica*, 2021;106(2), 384.
 38. Cox HD, Miller GD, Manandhar A et al. Tracking immature reticulocyte proteins for improved detection of recombinant human erythropoietin (rhEPO) abuse. *American Journal of Hematology*, 2021;96(12), 1621-1629.
 39. Touraj E, Marwan A, Yamir I et al. The Application of 3D Printing in the Formulation of Multilayered Fast Dissolving Oral Films. *Journal of Pharmaceutical Sciences* .2017; 1-10.

40. Shahab M, Saifullah Khan S . Erythropoietin Administration for Anemia Due to Chronic Kidney Disease - Subcutaneous OR Intravenous, What Do We Know So Far. Cureus. 2020; 12(9): e10358.
41. Fishbane S, Pollock CA, El-Shahawy M et al.

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شكل دوائي قابل للزرع الاريثروبايوتين لعلاج فقر الدم المصاحب للقصور الكلوي المستحدث في نموذج الارانب.

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الخلاصة

يتضمن هذا العمل التصنيع والتحسين والتقييم في المختبر وفي الجسم الحي للاريثروبايوتين على شكل افلام قابلة للزرع والتي تستخدم بشكل مفيد لعلاج فقر الدم المرتبط بالفشل الكلوي المزمن لمدة ستة أشهر عند زرعه مرة واحدة تحت الجلد ويمكن تعديله حسب الحاجة الشخصية. من خلال هذه الدراسة تم تحضير نوعين من الافلام القابلة للزراعة، أحد هذه الافلام تم تحضيره بواسطة فيلم الطباعة ثلاثية الأبعاد والافلام الأخرى تم تحضيرها بطريقة الصب باستخدام فيلم أساس بولي فينيل الكحول/ كلبيسول. يحتوي كل فيلم على كمية معينة من ايبوتين ذات مفعول مديد (أي ما يعادل ١٠٠٠٠٠ وحدة دولية) بالإضافة إلى مسحوق الارثروبايوتين النقي (١٠٠٠٠ وحدة دولية). تم إجراء التقييم في المختبر والتقييم داخل الجسم الحي لكلا الفيلمين بالمقارنة مع العلاج المطبق بشكل روتيني. نجح كلا الفيلمين في اجتياز التقييم المعمل، وكانت قدرة تحمل طي الافلام المصبوبة أكبر بـ ٦٦ مرة من افلام الطباعة ثلاثية الأبعاد. بالإضافة إلى ذلك، أعطى كلا الفيلمين إطلاق دواء ثنائي الطور حيث تم إطلاق ١٠٪ من الارثروبايوتين خلال الأسبوع الأول واستمر لمدة ٦ أشهر ليصل إلى ٩٨,٧٣٪ لفيلم الصب و ٨٨,٧٩٪ لفيلم الطباعة ثلاثية الأبعاد. أظهر التقييم داخل الجسم الحي لكلا النوعين من الافلام مستوى خضاب الدم الطبيعي خلال الأسبوعين الأولين من الزرع المرتبط بتصحيح مستوى الارثروبايوتين إلى المستوى الطبيعي في كلا الفيلمين، واستمر مستوى الارثروبايوتين/ خضاب الدم طبيعياً لمدة ٦ أشهر مقارنةً بـ الحقن المتكرر للارثروبايوتين والذي أظهر مستوى لخضاب الدم الطبيعي مع ارتفاع الارثروبايوتين (أكثر بكثير من الطبيعي) مما قد يزيد من حدوث اضطرابات القلب. نجح هذا العمل في تحضير أفلام قابلة للزرع من الايبوتين يمكنها ضبط كل من خضاب الدم و الايبوتين إلى المستوى الطبيعي وتستمر بشكل طبيعي لمدة ستة أشهر عند زرع واحدة بسيطة تحت الجلد مع وجود علاقة جيدة بين التقييم في المختبر وكذلك في الجسم الحي. يمكن استخدام هذا المستحضر كبديل للحقن المتكرر تحت الجلد بشكل روتيني لتحسين امتثال المريض مع إمكانية أقل لإنتاج آثار جانبية ملموسة.

الكلمات المفتاحية: طباعة ثلاثية الأبعاد، ارثروبايوتين، خضاب الدم، افلام قابلة للزرع , طويل الامد.