Research Article

A Short Communication On Kinetics of Antimicrobial Agents Release from Porous Collagen Scaffolds

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ABSTRACT

Objective: Burst release of drugs occurs in a very short time compared to the entire sustained release process, yet it has not been specifically investigated in most of the work whereas our study aims the investigation of the burst release of antimicrobial agents from various collagen scaffolds and the release kinetics of antimicrobial agents from the collagen scaffolds. **Materials and Methods:** The extracted Type 1 Collagen from Bovine tendons is used for scaffold fabrication. The macro porous collagen scaffold is prepared by the CLRI protocol developed. The porous collagens scaffold is prepared by freeze drying method. Furthermore, the antimicrobial agents Triphala/Ciprofloxacin is physically conjugated and entrapment within the pores of the collagen scaffold. The in vitro release studies are carried out in Franz-type diffusion cells. The bacterial count in the granulation tissue is carried out in *In Vivo* studies. **Results:** The result concludes collagen dressings with burst release followed by controlled release effectively regenerate highly infected soft tissue. The collagen dressing provides burst release. **Conclusion:** To sum up, the collagen scaffold with burst release provides effective control of infection and better wound closure at the wound site. This investigation used to design the scaffold with burst release for eradication of infection and effective regeneration of skin at the wound site.

Keywords: Burst release, Controlled Release, Wound infections and Collagen scaffolds.

INTRODUCTION

The Collagen was used as protein based biomaterial for fabrication of scaffolds for regeneration of skin. Even though it has the capacity to regenerate tissue effectively, also to mimic the extracellular matrix at the site of injury, but to be degraded by enzymes such as collagenase in the infected wound site. Therefore, the antimicrobial agents incorporated with collagen biomaterials help to stabilize the collagen biomaterial from microbial degradation and also to control the infection at wound. The sustained release of antimicrobial agents at the wound surface provides a prolonged antimicrobial action to eradicate pathogens rapidly. Additionally, burst release is necessary to eradicate wound pathogens immediately and followed by controlled release of drug to eradicate wound pathogens as well as to inhibit the proteolytic enzymes secreted by pathogens and support for tissue regeneration.

Drug release refers to the process in which the physically or chemically encapsulated/ entrapped drug is released from the collagen scaffold to the wound environment, however, it is critical in the design of bioactive template for soft tissue repair. The protein nature of collagen makes it unsuitable scaffold material that necessitates the conjugation of anti-microbial agents into the collagens scaffold to avoid microbial degradation by eradicating and controlling bacterial infection at the wound site. Topical administration of antimicrobial or wound healing agents either in the form of solution or gel directly over the wound diffuses rapidly and eliminates pathogens from the site, which leads to delayed wound healing as local effective concentration of inhibiting wound pathogens cannot be maintained. Therefore, Controlled drug delivery application can either be sustained delivery or targeted delivery at one-time or in sustained basis. (1). In addition to that, Controlled release formulations reduce the amount of drug necessary to cause the same therapeutic effect in patients. In many of the controlled release formulations, immediately upon placement in the release

medium, an initial large bolus of drug is released before the release rate reaches a stable profile. This phenomenon is typically referred to as Burst release. (1,2). Burst release accomplishes high initial drug delivery at short duration and reduces the effective lifetime of device. The study of burst release can provide the highest possible rate of drug release achievable at the wound at initial stages and the inhibition and control of microbial infections expected. Though burst release is considered as a negative consequence in long- term release, it is crucial in infected dermal wound treatments.

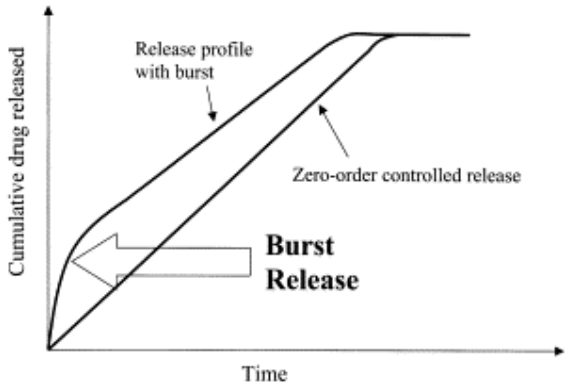

Fig. 1: Importance of Burst release in drug delivery devices

Release Kinetics

The order of drug release is predicted from the following equation

$$\frac{M_t}{M_\infty} = Kt^n$$

Where, M_t/M = fraction of drug released at time t, K = kinetic constant (when K increases, the release of drug increases), and n = exponential related to the mechanism of drug release.

Table 1: Characteristics of Burst Release

n	Release characteristics
0.5	Fick law of diffusion
1	Zero order release kinetics
0.5 <n<1	Non –ficken release.

The previously reported the collagen scaffold has certain limitations such as a variety of difficulties in releasing the antimicrobial agents into the wound exudates, cytotoxicity due to the extreme concentration of antimicrobial agent leading to delayed wound healing or development of antibiotic resistance by wound pathogens, over adherence causing damage to newly formed epithelium, additionally the non delivery of drug to the wound in a controlled fashion to arrest the infection. If the drug is released speedily, entire drug will be released before infection is arrested. If the release is delayed, infection may further set in, thus making it difficult to manage the wound environment. This work investigates kinetics of release of antimicrobial agents from various collagen scaffolds and how it does impact controlling infection at wound site and wound closure in Wister albino rats.

MATERIALS AND METHODS

The Type 1 collagen was extracted from bovine tendons based on the method developed by Bio products lab, Central Leather Research Institute, Chennai, India. The heterogeneous macro porous collagen scaffold was prepared and then conjugation with antimicrobial agents (Physical entrapment of antimicrobial agents with collagen scaffold) is preceded with the developed protocol. (3)

***In vitro* release of antimicrobial agents from the scaffold**

In vitro release of ciprofloxacin and triphala from various scaffold were carried out in Franz-type diffusion cells at 37◦C. The receiver compartment was filled with phosphate buffer solution (PBS, pH 7.4), which was stirred with a magnetic stirring bar. At regular time intervals, an aliquot (1 ml) was removed from the receiver compartment and replaced with an equal quantity of PBS to maintain a constant volume. In the case of Ciprofloxcin, its concentration was determined by high performance liquid chromatography (HPLC) (515 pump (Waters, USA) and SPD 10A Shimadzu UV-Visible detector (Shimadzu, Japan)). The stationary phase was a Phenomenex C18 column (5 µm, 250 × 4.6 mm) and the mobile phase was CH3OH/Na2HPO4 (30:60), pH 3.0. The flow rate was 1.0 ml/min and the detector was set at 276 nm. A ciprofloxacin standard curve ranging from 0.1 to 400 µg/ml was established and *p*-hydroxybenzoic acid was used as an internal standard. Ciprofloxacin released were collected in Phosphate buffered saline (PBS; pH 7.4) at predetermined time interval and measured at 278nm using a shimadzu UV-2100S spectrophotometer. Whereas, triphala being a phytopharmaceutical rich in polyphenol and ascorbic acid, the release of triphala were evaluated with Gallic acid as the

marker using modified colorimetric Folin-Ciocalteu method.

***In vivo studies* for Bacterial count at Wound Site**

The animal experiment was performed according to the Institute's ethical committee approval and guidelines (466/01/a/CPCSEA). Male Wister albino rats weighing 150 to 200 g were housed individually in standardized environmental conditions. A total of 36 animals were segregated into three groups (n = 6 per group) (treatment and two controls for this study). The animals were rehabilitated following experimentation.

Group 1	Open wound covered with gauze dressing
Group 2	Plain Collagen Scaffold
Group 3	Ciprofloxacin - incorporated collagen scaffold
Group 4	Ciprofloxacin loaded gelatin microspheres impregnated collagen scaffold
Group 5	Triphala incorporated collagen scaffold
Group 6	Collagen Bilayer Dressing Collagen Scaffold

Full thickness wounds (1.5 x 1.5 cm) were created on the shaved dorsal side of rats using sterile surgical blade and inoculated with the test organisms at 10^6 CFU (0.1 mL) between thin skin muscle and paraspinus muscle and allowed to infect for 24 h. All surgical procedures were carried out under thiopentone (40 mg/kg body weight, intramuscular). The infected wounds were covered with collagen dressings and outer covered with gauze dressings. An infected animal without dressings and an animal with dressings without drugs were also maintained in individual cages to be considered for control.

RESULTS AND DISCUSSION

The release of various antimicrobial agents from the collagen scaffold was studied. In the case of ciprofloxacin loaded gelatin microspheres impregnated collagen scaffold, the presence of various proteases secreted by wound pathogens degraded gelatin microsphere and produced burst release of ciprofloxacin and eradicate pathogens at the wound site and followed by controlled release of ciprofloxacin from the microsphere and also supported tissue regeneration. The release depends on the degradation of microspheres and collagens scaffold at wound environment. In this case, the wound closure > 99% in 16th Day was observed and effective removal of wound pathogens in 12th Day at the wound site.

In the case of ciprofloxacin incorporated collagen scaffold, ciprofloxacin physically entrapped into the pores of macro porous collagen scaffold. The drugs drastically impregnated in the pores and cause burst release when scaffold contacts the wound surface. Once the collagen started to degrade at wound site, it offers the controlled release to wound environment. Generally, infected wound has high amount of expression of MMPs (matrix metalloproteinases) at wound site and an elevation of MMP expression is due to presence of wound pathogens. The abnormal expression of MMPs degrades the collagen scaffold rapidly and as a consequence, it produces the burst and controlled release at the wound site.

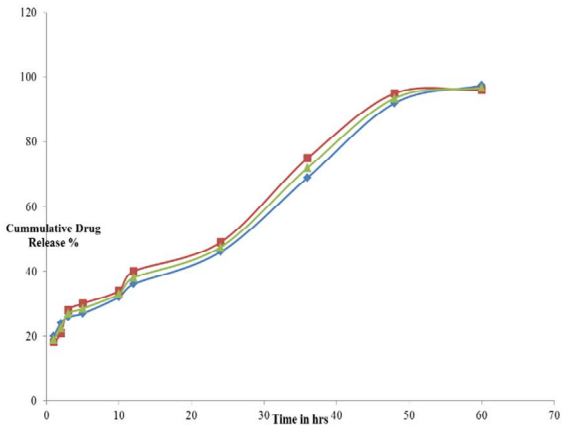

Fig. 1: Ciprofloxacin released from Ciprofloxacin loaded gelatin microsphere impregnated collagen scaffold.

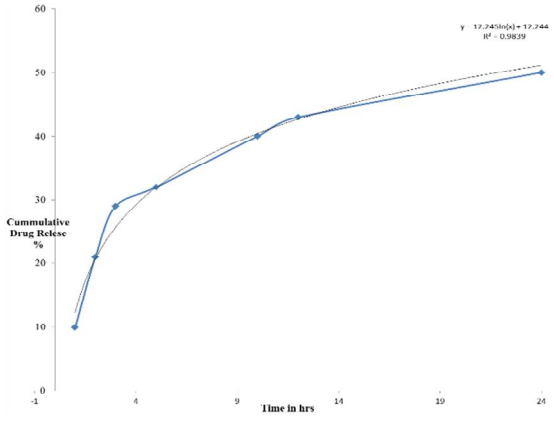

Fig. 2: Ciprofloxacin release from ciprofloxacin incorporated Collagen Scaffold

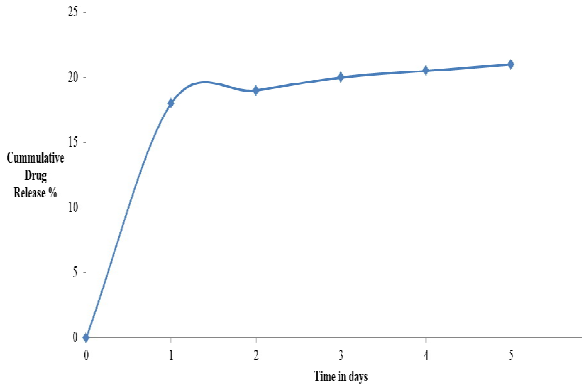

Fig. 3: Ciprofloxacin release from bilayer collagen dressing.

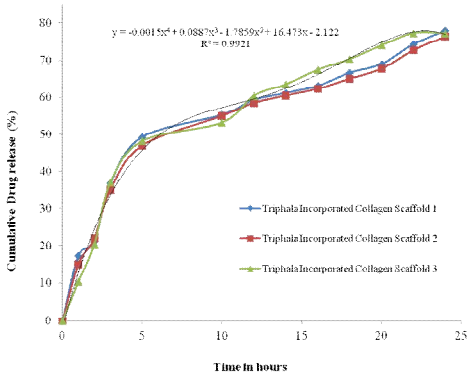

Fig. 4: Triphala released from the Triphala Incorporated Collagen Scaffold

In this scaffold case, the bilayer dressing consisting of collagen membrane (film) and collagen sponge has been designed to overcome certain limitations such as release of drug into the wound, and growth of wound pathogens. In case of infection, both immediate and time regulated release of antibiotic at the site is essential to control the proliferation rate. In the bilayer system the sponge is prepared in such a way that it will have smooth surface on one side and rough on the other. The sponge acts as a drug reservoir. It binds the drug ionically when wet and starts releasing the drug immediately but in a regulated fashion. The rough side of the sponge forms the bilayer system with the membrane, and the smooth surface comes in contact with the wound. Collagen used in sponge and membrane preparation has been modified by succinylation. Chemical modification of Collagen by succinylation offered advantages like better adherence of bilayer dressing to the wound surface, anionic collagen network at neutral pH and sharp isoionic pH. These properties are unavailable with unmodified collagen and can advantageously be used for the delivery of drug from the bilayer dressing system. The drug, after dispersing in poly (N-vinyl-2-pyrrolidione) (PVP) solution was allowed to spread in the bilayer system by diffusion. The release mechanism depends on polarity based controlled release system. (4)

In triphala incorporated collagen scaffold, the polyphenols from the triphala were abruptly released from the scaffold and eradicated wound pathogens at wound site. The burst release is caused by the degradation of surface of the collagen scaffolds when it contacts with wound surface. Additionally, the microbial enzymes and MMPs degrade the collagen scaffold at wound site. (4)

Table 3: Infection Control at Wound Site

Time in Days	Control Gauze Dressings	Plain Collagen Scaffold	Cipro Incorporated Collagen Scaffold	Bilayer Collagen Scaffold	Microspheres Incorporated Collagen Scaffolds	Triphala Incorporated Collagen Scaffold
4	1.00E+09	1.00E+08	1.00E+07	1.00E+05	1.00E+05	1.00E+06
8	1.00E+08	1.00E+07	1.00E+05	1.00E+03	1.00E+03	1.00E+04
12	1.00E+06	1.00E+06	1.00E+03	1.00E+01	Nil	Il

From table 3, it has been observed that a decreasing trend in bacterial population in all cases. Bilayer collagen scaffold, Microspheres based collagen scaffold and Triphala incorporated collagen scaffold showed 1000 colonies on day 8 and almost no colonies on day 12. These scaffolds not only provide the burst release of antimicrobial agents and also offer controlled relase for effective control of wound infection. As a result, the wound closure > 90% in the Wister rats treated by these collagen scaffolds. (4,5,6)

Table 4: Wound Closure for regeneration of epidermis and dermis at wound site

Time in Days	Control Gauze Dressings %	Plain Collagen Scaffold %	Cipro Incorporated Collagen Scaffold %	Bilayer Collagen Scaffold%	Microspheres Incorporated Collagen Scaffolds %	Triphala Incorporated Collagen Scaffold %
4	38±0.94	40±0.84	45±0.23	50±0.56	70±1.05	65±0.49
8	52±1.02	60±0.55	78±0.54	63±0.75	85±0.34	80±0.52
12	65±0.42	71±0.45	85±0.67	90±0.45	95±0.54	95±1.05
16	75±0.51	80±0.33	95±0.78	99±0.87	99±0.37	99±1.04

Form the table 4, Significant difference in the wound closure was observed in group treated by antimicrobial agents conjugated collagens scaffolds from day 4 onwards and also the rate of wound closure was much faster on later days when compared with control. Complete wound closure was observed in group treated by collagen scaffolds on day 16 whereas in untreated group it was about 30 days. (4,5,6).

CONCLUSION

The kinetics and mode of release of antimicrobial agents depends the design and architecture collagen scaffold, the binding/ conjugation of drugs with scaffold either physically or encapsulated in microspheres, and the mechanism of degradation of the scaffold at the wound environment. The burse release followed by sustained release of antimicrobial agents from the scaffold effectively eradicates the wound pathogens and promotes faster wound healing. These scaffolds also serve as a template to support tissue regeneration and acts as reservoirs for drugs. In contrast with scaffold without drugs, controlling of bacterial wound pathogens is significant in drugs incorporated collagen scaffold. Therefore, the conjugation of anti-microbial agents into the collagen scaffold yields better closure of wound in a short time and facilitates inhibition of microbial growth at the wound environment.

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