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Potential of Gallic acid loaded Polysaccharide-Protein (Agar-Gelatin) Co-Hydrogels in Wound Healing

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ABSTRACT

We report on the wound healing potential of gallic acid (3, 4, 5-trihydroxy benzoic acid) encapsulated in agar-gelatin (polysaccharide-protein) binary and ternary co-hydrogel matrices. Gallic acid is found in red wine, green tea, nutgall and other plants; it has a wide range of biological activities, including anti-oxidant, anti-inflammatory, anti-microbial, anti-cancer and radical scavenging abilities. Release profiles of gallic acid from agar, and its binary and ternary gels, AgarGelB and AgarGelAB, with gelatin A and B were found to be biphasic with an initial fast burst release followed by very slow release phase (zeroth order kinetics). The burst release phase was of short duration and within 30 minutes, 77.0%, 76.4% and 74.9% gallic acid was released from Agar, AgarGelB and AgarGelAB gels respectively. Second phase was governed by slow release kinetics persisting for 10 hrs where only 83%, 81.9, and 80.6% of the loaded amount could be released. Antimicrobial studies revealed the bactericidal property of gallic acid, where a correlation between the release profile and antimicrobial property could be ascertained. Sample showing higher release resulted in larger zone of inhibition.

Key words: *Gallic acid, Antimicrobial, Release Kinetics, Co-Hydrogels.*

INTRODUCTION

Wounds, the physical injuries to skin, disrupt the continuity of tissues. Healing of wound, a natural biological process starts right from the moment of injury and depending upon the extent of wound, continues for different time periods. However it can be speeded up with the use of healing agents. Since early ages, medicinal plants have been used by mankind for treatment of wounds. Even today in rural and tribal areas, where medical facilities are scarce, various parts of plants are being used for wound healing purposes [1-7]. Chemical compounds like tannins, flavonoids, polyphenols, alkaloids, terpenes and terpenoids, ascorbic and phenolic acids are known to be responsible for wound healing properties of medicinal plants. Healing processes are influenced by factors like infections, nutritional status, drug, hormones, type and sites of wound. Healing is severely hampered by microbial infection and reactive oxygen species (ROS). Open wounds are particularly prone to infection, by microbial pathogens like *Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus faecalis, Escherichia coli, Clostridium perfringens, Clostridium tetani, Coliform bacilli and enterococcus* especially and provide an entry point for systemic infections [8]. These pathogens proliferate in a wound, causing tissue damage and elicit a host inflammatory response [9]. These infections are major cause of morbidity and mortality in wound patients. Infected wounds heal less rapidly and often result in the formation of unpleasant exudates, and the toxins produced kill nascent cells. Apart from infection reactive oxygen species are deleterious to wound healing due to their harmful effects on cells and tissues [10] therefore application of antimicrobial and antioxidant healing agents especially derived from plant extract will result in early recovery. Gallic acid (3, 4, 5-trihydroxy benzoic acid) is one such compound found abundantly in processed beverages such as red wine, green tea [11], nutgall and other plants, which has a wide range of biological activities, including anti-oxidant, anti-inflammatory, anti-microbial, anti-cancer and radical scavenging activities [12-14]. It is also used as an astringent in veterinary science and in internal hemorrhage treatment. It is not only a non-toxic substance to man but also known to have health-promoting effects. Therefore importance of gallic acid as wound healing agent necessitates comprehensive understanding.

The use of hydrogels in the healing of wounds dates back to late seventies or early eighties. Hydrogel is a crosslinked polymer matrix with a three dimensional network structure capable of holding varying amount of water depending on the type of polymers used and also has the ability to absorb more water or exudates from wounds. The high water content makes them highly biocompatible. For routine healing of different kinds of wounds, mainly burn wounds, ulcers, bedsores, etc. hydrogels have specific benefits than textile dressings, calcium alginates and hydrocolloids as they soothe pain, give pleasant cooling sensation and cushioning effect, absorb wound exudates alongwith bacteria, cause barrier to micro organisms from outside and also deliver wound healing drugs. They are not sticky to wound and are removable/replaceable without any damage to new epidermis, gel transparency allows instant monitoring of healing process, not antigenic and not allergenic, easy in storing and usage. Hydrogels have the ability to donate water molecules to dehydrated tissue while allowing the passage of water vapour and oxygen to the wound surface [15, 16]. This helps to increase the phagocytic activity of leucocytes and enzymatic activity of damaged cells. This, in turn, removes devitalized tissues during the destructive phase of healing of a wound – autolysis [17-19].

Thus the advantages of hydrogels and various biological properties of gallic acid motivated us to study the potential of gallic acid loaded hydrogel as effective wound dressing material. This work presents the antimicrobial and in-vitro release kinetic studies of gallic acid from binary and ternary agar-gelatin co-hydrogel films.

MATERIALS AND METHODS

Agar was a gift from Central Salt and Marine Research Institute, Bhavnagar, Gujrat. It has been extracted from the red seaweed Gracilaria dura collected from the Gulf of Mannar at the southeast coast of India, employing a patented method [20]. Gelatin type A (from porcine skin, bloom number 175) and type B (from bovine skin, bloom number 225) gelatin were purchased from Sigma-Aldrich and used as received. The two types of gelatins are

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characterized by their mode of manufacture. Type A gelatin (pH 3.8–6.0; isoelectric point 6–8) is derived by acidic hydrolysis of pork skin and contributes plasticity and elasticity to the blend. Type B gelatin (pH 5.0–7.4; isoelectric point 4.7–5.3) is derived by basic hydrolysis of bones and animal skin and contributes high gel strength to the blend. Gelatin used in the pharmaceutical industries is a blend of these two types, although sometimes only Type A or Type B was used. Gallic acid was purchased from Merck.

Agar and gelatin solutions were prepared separately. Agar solution was prepared by autoclaving agar powder in water at 120°C and both gelatin A and B were prepared by dissolving weighed amount of protein in water at 40 °C followed by continuous stirring until an optically clear solution was obtained. Two compositions of co-hydrogels were prepared, one binary, AgarGelB (1:1), and one ternary AgarGelAB (2:1:1) designated as Sample 1 and 2 respectively. For making gallic acid loaded thin films, weighed amount of gallic acid (5 mg) was placed in number of circular grooves of 2cm diameter and 2mm depth on a white teflon plate. After thoroughly mixing the agar and gelatin warm solutions at 40°C in the above ratio, 1 ml was taken out and poured in each groove and immediately mixed with already placed gallic acid powder. The whole plate was allowed to cool till the films started separating from the plate. Along with gallic acid loaded films, unloaded films were also prepared.

In vitro gallic acid release experiments were performed at physiological temperature 37°C in 100 ml of phosphate buffer (pH 7.4) simulating in vivo environments. Drug release was monitored by taking out 3 ml of the sample at definite time intervals and measuring the absorbance at 260 nm using a UV-VIS Spectrophotometer, (Model CE 7200, Cecil Instruments Ltd., England)., Japan). An equal volume of phosphate buffer was added to keep the volume constant.

The antimicrobial activity was performed by filter paper disc plate method [21, 22].

Antimicrobial activity was performed by using the Kirby-Bauer single disk susceptibility test. Whatman No. 1 filter paper disk of 3 mm diameter were sterilized by autoclaving for 15 min at 121°C. The sterile filter paper disks were impregnated with gallic acid (GA) loaded films. Agar hydrogel films were used as the control and placed on the surface of the agar plates seeded with *Escherichia coli* and incubated at 37 °C over night. The compound diffuses from the filter paper into the agar. For positive control we used gentamycin 2mg/ml and for negative control DMSO (dimethyl sulphoxide). If the test materials have any antimicrobial activity, it will inhibit the growth of the microorganisms giving the clear distinct zone around the disk called "Zone of Inhibition". The results were recorded by measuring the zone of inhibition surrounding the disk.

RESULTS AND DISCUSSION

a) Release kinetics studies

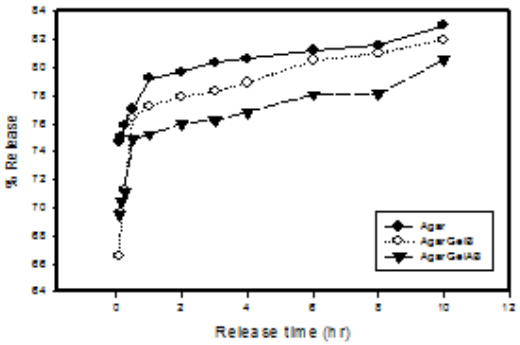

Fig. 1: Release profile of gallic acid from Agar and its co-gels

Release profiles of gallic acid from pure agar and its binary and ternary gels with gelatin A and B are presented in **Fig. 1**. A biphasic release pattern was seen in all three gels, an initial fast burst release phase followed by very slow release phase. The burst release phase was of short duration with very high release rate. Within 30 minutes, 77.0%, 76.4% and 74.9% gallic acid was released from Agar, AgarGelB and AgarGelAB respectively. Second phase release was very slow releasing only 83%, 81.9, and 80.6% of the loaded amount till 10 hours. Such biphasic release is beneficial for wound treatment as initial burst provides immediate relief and thereafter prolonged release promotes gradual healing. A number of factors viz. surface characteristics of host material, sample geometry, host/drug interaction, surface adsorption, morphology and porous structure of dry material, uneven distribution of drug during drying are responsible for this burst release. In our samples as the gels were naturally dried over a teflon plate for making thin films, the chances of uneven distribution of drug may not be ignored. The migration of drug with water to the gel surfaces during drying process results in higher concentrations at the surface which is immediately released when placed in the release medium. The amount released in the second phase is the amount bound to functional groups of agar-gelatin co-gels or entrapped within the gel structure. Gallic acid has three-OH groups and one carboxylic group. Similarly Agar has numerous OH groups while both gelatins have NH_2 and COOH groups. Since during the process of forming gallic acid loaded films, the polymers were mixed first and then poured on teflon plate having weighed gallic acid powder, gallic acid groups may interact with the remaining functional groups of binary and ternary co-gels or it may get entrapped within the co-gel network. Various interactions between agar and gelatin A, B and therefore the strength of network structure (AgarGelAB> AgarGelB> Agar) were described well in our previous paper.[23] The decreasing trend of gallic acid release, Agar>AgarGelB>AgarGelAB is in accordance with the increasing strength of the gels. Stronger the gel, lesser is the release of drug. Both release phases could be fitted to zero order release equation $Q_t=Q_0+kt$, where Q_t = amount released at time t, Q_0= initial amount and k = release rate constant.

b) Antimicrobial studies

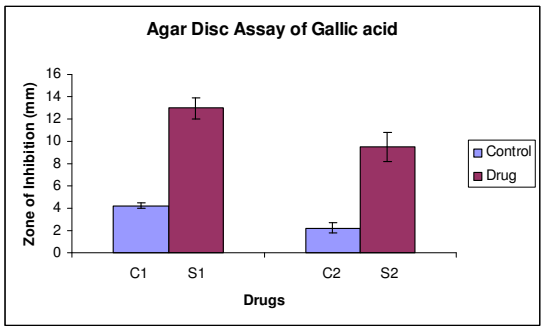

Fig. 2: Graphical representation of antimicrobial activity of gallic acid against *Escherichia coli*. Antimicrobial activity was observed for both samples 1 and 2 of gallic acid. Sample 1 showed more zone of inhibition (13 mm) as compared to Sample 2 (9 mm).

E. coli is Gram-negative, facultative anaerobic and non-sporulating prokaryote. It is a rod-shaped bacterium propelled by long, rapidly rotating flagella. *E. coli* is used as a model organism to explore the potential causes and treatments for human disease when human experimentation would be unfeasible or unethical. Gallic acid is a bacteriocidal agent inhibiting the growth of *E.coli*. With increase in time and concentration of this phenolic compound there was an increase in its inhibition activity. Spectrophotometric and electrophoretic studies (Bradford assay and SDS-PAGE) at protein level revealed that Gallic acid exhibited very little effect at translation, while it effects to a greater extent at DNA replication level [unpublished data]. It can be postulated that chelation of the metal co-factor to the compound can be partly responsible for inhibiting enzymatic activity followed by the inhibition of microbial activity and also the unavailability of substrate by binding to the polysaccharide and protein might be responsible for the anti-microbial activity of phenolic compound: gallic acid. Pro-oxidative activity of gallic acid can also be responsible for its cytotoxic effects. Structure activity relationship analysis showed that the o-dihydroxy group of gallic acid is important for the inhibitory activity *in vitro*.

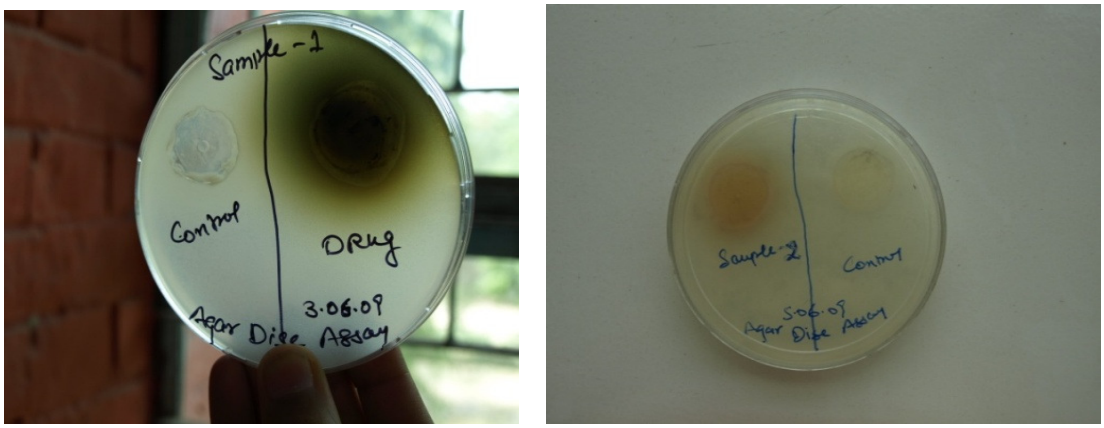

Fig. 3: The zone of inhibition of Sample 1, 2 of gallic acid against *Escherichia coli.*

CONCLUSION

***I**n-vitro* release kinetics of gallic acid from agar and its co-gel thin films showed zero-order biphasic pattern and is better controlled by cogels. Release from Sample 1(AgarGelB) due to its looser network structure is higher than Sample 2 (AgarGelAB). Antimicrobial studies revealed the bactericidal property of Gallic acid. Sample 1 showed more bactericidal property as compared to Sample 2. A correlation was seen in release and antimicrobial studies. Sample showing higher release resulted in larger zone of inhibition. The size of the zone of inhibition depends on the diffusion rate of the drug, the degree of sensitivity and the growth rate of the microorganism. The last two factors being same for both samples, the diffusion rate of the gallic acid is dominant. The results obtained demonstrate a direct relationship between the release rate and the antimicrobial effect and the potential of gallic acid in healing of wounds. However, the gallic acid dressing can be tailored according to the wound type and type of microorganism.

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