Colloids and Surfaces B: Biointerfaces 113 (2014) 207–212

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Fish scale collagen sponge incorporated with *Macrotyloma uniflorum* plant extract as a possible wound/burn dressing material

Thangavelu Muthukumar[a], P. Prabu[a], Kausik Ghosh[b], Thotapalli Parvathaleswara Sastry[a,*]

[a] Bio-Products Laboratory, Central Leather Research Institute, Adyar, Chennai 600 020, Tamilnadu, India
[b] FDDI International Testing Centre, Ministry of Commerce & Industry, Chennai, Tamilnadu, India

ARTICLE INFO

Article history:
Received 3 June 2013
Received in revised form 16 August 2013
Accepted 6 September 2013
Available online 17 September 2013

Keywords:
Fish scale collagen
Macrotyloma uniflorum
Collagenase
Wound dressing
Mupirocin and sponge

ABSTRACT

Application of plant extracts for the burn/wound treatment is followed over the decades as a common practice and it is an important aspect in clinical management. In this study porous collagen sponges (CS) were prepared using fish scales and were incorporated with mupirocin (CSM) and extracts of *Macrotyloma uniflorum* (CSPE) separately to impart antimicrobial activity to the sponges. The results showed that the addition of plant extract increased the tensile strength of CSPE and stability against collagenase enzyme. FTIR studies have shown the incorporation of plant extract in CSPE, SEM studies have revealed the porous nature of the sponges and XRD patterns have shown the retention of collagen triple helical structure even after the addition of plant extract. CSPE and CSM have exhibited antimicrobial properties. The sponges prepared were analysed for their *in vitro* biocompatibility studies using fibroblasts and keratinocyte cell lines and the results have shown their biocompatible nature. Based on the results obtained, CS, CSM and CSPE may be tried as a burn/wound dressing materials, initially, in small animals *in vivo*.

1. Introduction

It is well known that the wound healing process is a complex physiological response to injury which is composed of several overlapping phases containing inflammation, new tissue formation and remodelling [1]. The main objective of using wound dressing is to accelerate wound healing by preventing bacterial infection and the quickening of tissue regeneration. Wound dressing materials should possess essential properties like flexibility, durability, gas permeability, and ability to prevent water loss.

Many collagen related products have been developed in the past few years for the wound healing purposes; some of them are approved by drug controlling authorities and are now commercially available [2]. Selecting collagen as base material offers several advantages, which has well-documented structural, physical and chemical properties. Moreover, collagen has low antigenicity, low inflammatory, good biocompatibility and has the ability to promote cell attachment and proliferation. Collagen or collagen based matrix materials are the most commonly used biomaterials in the skin, connective tissue, and nerve tissue engineering [3–5]. In addition to its physiological properties as a natural extracellular matrix (ECM) component, the stability and microstructure also plays a vital role in cell–cell and cell–matrix interaction. Collagen is now available in different forms *i.e.*, gels, sheet or films, sponges, and spray etc. Collagen sponges differ in their stability and porosity. Sponge form of collagen is more useful in the treatment of different types of wounds, such as donor sites, leg ulcers, pressure sores, and decubitus ulcers, because of its special properties to adhere well to wet wounds, absorb large quantities of tissue exudates, preserve moist environment, and encourage the formation of new granulation tissue and epithelium on the wound [6,7]. Collagen alone cannot assist the healing of infected wounds because it is protein in nature and bacteria can use collagen as a substrate. Because of the imbalance between host resistance and bacterial growth, infection of the wound occurs. Treating of the wounds by systemic administration of drugs may lead to insufficient drug release to the site of infection, systemic toxicity, and drug associated side effect. This can be overcome successfully by topical application of drugs, and collagen dressings with antibiotics. Bacteria are developing resistance towards antibiotics, due to increase in the application of new antimicrobial agents [8].

Plant extracts possessing active ingredients are incorporated into biomaterials for better performance against bacterial infections [9]. Traditionally villagers use different crude extracts for preparing poultice to treat a variety of skin ailments including wounds. *Macrotyloma uniflorum* (MU) (Family: Fabaceae) is one of the lesser known plant found mainly in Asia and Africa and is commonly growing twining plant all over India [10]. MU leaf extracts

* Corresponding author. Tel.: +9144 24420709; fax: +914424911589.
E-mail addresses: sastrytp@hotmail.com, sastrytp56@gmail.com (T.P. Sastry).

0927-7765/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.colsurfb.2013.09.019

are reported to possess antibacterial, antifungal, and antioxidant activity [11].

In the present study, collagen sponge (CS) and CS incorporated separately with antibacterial drug, mupirocin (M) and MU extract were prepared and evaluated for their physiochemical properties. *In vitro* cell culture studies were carried out using embryonic fibroblasts cells and human keratinocyte cell lines for evaluating biocompatibilities of the scaffolds and cell viabilities. These cell lines were chosen as they are found to be key cellular components of wound healing cascade and repair process [12,13].

2. Materials

2.1. Isolation of fish scale collagen (FSC)

Fish scales were collected from nearby fish market (*Lates calcarifer*), cleaned and washed twice in 10 wt% of NaCl solutions to remove residual proteins on the surface. Later demineralization of the scales were carried out using with 0.5 M EDTA (pH 7.4) solution for 48 h. The demineralized scales were washed three times with distilled water and collagen was extracted using slightly modified method by Pati et al. [14]. Briefly, demineralized fish scales were treated with 0.5 M acetic acid solution (pH 2.5) for a period of 48 h under magnetic stirring in cold room (4 °C), the insoluble part of the scales were filtered out. 0.9 M NaCl was used to salt out the collagen from the supernatant by keeping them undisturbed for 24 h at 4 °C. Next day the suspension was centrifuged at 8000 rpm for 1 h at 4 °C and the precipitate was re-solubilized in 0.5 M acetic acid. The final content was then dialyzed against 0.1 M acetic acid and distilled water respectively for 24 h each and the samples (CS) were later freeze-dried.

2.2. Isolation of Macrotyloma uniflorum plant extract (MPE)

Fresh leaves of *M. uniflorum* plants were washed and cleaned with water, shade dried and coarsely powdered with help of a domestic mixer. Dried powder weighing 10 g was subjected to extraction (Soxhlet apparatus) using 100% ethanol; the extraction was continued until the powder was free of extracts. The ethanol was removed by using rotary evaporator apparatus under reduced pressure at 40 °C and the dried extract was stored at 4 °C until further use.

2.3. Preparation of collagen scaffold impregnated with mupirocin/Macrotyloma uniflorum

The collagen scaffolds were prepared using the slight modification of the method explained in reference [15]. Briefly, the collagen solution of 1% wt/vol in 0.5 M acetic acid (20 ml) was prepared, this solution was continuously stirred under IKA T25 Homogenizer at 13,500 rpm to generate uniform foam. A drop of Triton X-100 was added to the mixture as a frothing agent and 0.25% glutaraldehyde (0.25 ml) solution was added as a cross linking agent. The formed collagen foam was poured in to teflon trays and frozen at 80 °C for 24 h followed by freeze drying for 48 h lyophilizer using (Operon Co., Korea). The completely dried scaffolds were stored 4 °C in airtight plastic containers and denoted as collagen sponge (CS). To prepare CSM, 20 mg M was added to 20 mg collagen solution (1% wt/vol in 0.5 M acetic acid) and other steps were followed as in the case of preparing CS. 2 ml (23 mg dry weight) of MPE were added to 20 ml collagen solution (1% wt/vol in 0.5 M acetic acid) and further steps were followed as in the case of preparation of CS.

2.4. Bacterial culture and antimicrobial activity

Four bacterial strains were used including two gram positive (*Bacillus subtilis* (NCIM 2718) and *Staphylococcus aureus* (NCIM 5021) and two gram negative bacteria (*Proteus vulgaris* (NCIM 2813) and *Escherichia coli* (NCIM 293)). All bacterial cultures were sub-cultured periodically and maintained on nutrient agar (NA) medium at room temperature (30 ± 2 °C) for further experiments. The antimicrobial activity of the prepared leaf extract was found my modified agar well diffusion method [10,16]. About 100 μl [10^8 CFU (colony forming units)] of each bacterial culture was spread on the agar surface (Müller-Hinton agars) using sterile glass spreader. Wells of 10 mm diameter was made using sterile cork borer on the agar media. About 100 μl (0.1–0.6 mg) of the plant extract were added to the well and they were kept in refrigerator for 20 min for diffusion. The prepared scaffolds (CS, CSM and CSPE) were cut into 0.5 cm diameter discs and they were also analysed in same manner. Then, the plates were incubated for 24 h at 37 °C. Ethanol was used as a control. The antibacterial activity was evaluated by measuring the diameter of zone of inhibition against the test organisms.

2.5. Characterization

2.5.1. Infrared spectroscopy (FTIR)

Fourier transform infra red (FTIR) spectral studies were carried out to determine the formation and changes in the functional groups of prepared CS, CSM and CSPE. The spectra were measured at a resolution of 4 cm^{-1} in the frequency range of 4000–500 cm^{-1} using Nicolet 360 Fourier Transform Infrared (FTIR) Spectroscope.

2.5.2. X-ray diffraction

Crystal structure and phase composition of CS, CSM and CSPE were detected using X-ray diffractometer. (Bruker D8 ADVANCE) using Cu $K\alpha$ (λ = 0.1548 nm) radiation in the 2θ scan range of 2° to 90° at a scan rate of 1°/min.

2.5.3. Morphological study

Surface morphology of the samples was visualized by scanning electron microscope (SEM Model LEICA stereo scan 440). The samples were coated with gold ions using an ion coater (Fisons sputter coater) 0. 1 Torr pressure, 20 mA current, and 70 s coating time, using a 15 kV accelerating voltage.

2.5.4. Porosity and density measurements

Scaffold porosity and density were determined via liquid displacement method using ethanol as the displacement liquid because of its easy penetration through the pores of the scaffolds and which will not induce shrinking or swelling as a nonsolvent of the polymers [17]. A known weight (W) of the sample was immersed in a graduated cylinder containing a known volume (V_1) of ethanol. The sample were kept in ethanol for 5 min, and then a series of brief evacuation–repressurization cycles were conducted to force the ethanol in to the pores of the scaffold. The process was repeated until the air bubbles stops. The total volume of the ethanol and the ethanol-impregnated scaffolds was then recorded as V_2. The difference in the volume was calculated by (V_2–V_1). The scaffolds impregnated in ethanol were removed from the cylinder, and the residual ethanol volume was recorded as V_3. Thus, total volume of the composite scaffolds was

$$V = (V_2 - V_1) + (V_1 - V_3) = V_2 - V_3$$

The density of the scaffold (not the real density), ρ, was expressed as

$$\rho = \frac{W}{(V_2 - V_3)}$$