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Short Communication

Cytotoxicity and Acute Gastrointestinal Toxicity of Bacterial Cellulose-Poly (acrylamide-sodium acrylate) Hydrogel: A Carrier for Oral Drug Delivery

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ABSTRACT

Background: Preliminary safety evaluation of polymer intended to use as drug delivery carrier is essential.
Methods: In this study polyacrylamide grafted bacterial cellulose (BC/AM) hydrogel was prepared by microwave irradiation initiated free radical polymerization. The synthesized hydrogel was subjected to *in vitro* cytotoxicity and acute gastrointestinal toxicity studies to evaluate its biological safety as potential oral drug delivery carrier.
Results: The results indicate that hydrogel was non cytotoxic and did not show any histopathological changes in GI tract after a high dose of oral administration.
Conclusion: The results revealed that hydrogel composed of bacterial cellulose and polyacrylamide is safe as oral drug delivery carrier.

Introduction

Oral route is the main portal of drug delivery along with pharmaceutical carrier and other excipients. Owing to the principal site of ingestion, gastrointestinal (GI) system is highly susceptible to toxic substances. GI tract wall is highly innervated structure which includes multi layers such as mucosa, sub-mucosa and muscularis externa. On exposure to a potentially toxic substance, each of these strata shows histopathological changes and clinical signs that vary from mild to severe toxicity.[1] In order to reduce the consequent toxicity of different chemicals and drugs through oral ingestion, different organisations like Organisation for Economic Co-operation and Development (OECD) and International Organization for Standardization guidelines (ISO/EN10993) are developing regulations.[2] The preliminary studies of bacterial cellulose-poly(acrylamide-sodium acrylate) hydrogel (BC/PAM) showed a great potential as oral drug delivery carrier due to well-studied pH sensitivity.[3] Both polyacrylamide and bacterial cellulose are biocompatible, nontoxic and not absorbed by GI tract due to greater particle size of polymer.[4] This study evaluates the *in vitro* cytotoxicity and oral toxicological profile of hydrogel especially in GI tract.

Materials and Methods

Synthesis of hydrogels

The 2% w/v BC solution was prepared by dispersing pulverized BC obtained from *nata de coco* in 8% NaOH and 4% urea concentrations as described by Chang et al.[5] The BC/PAM hydrogel was synthesized by addition of acrylamide (AM) (1.5g) into 20ml of BC solution followed by potassium persulfate (7.4×10^{-4} mole) as initiator and *N,N′*-methylenebisacrylamide (1.29×10^{-3} mole) as crosslinker. Next, the reaction mixture was irradiated with microwave for 40 sec at 170W using microwave irradiator (LG, model MS2388K, Korea). Removal of the unreacted monomers was facilitated by soaking the fabricated hydrogels in distilled water for 5 days to remove and dried in an oven for 24 h at 60ºC.[3]

In vitro cytotoxicity study

The cytotoxicity of hydrogel was evaluated by both direct and indirect contact methods. Lung fibroblast (V79) cell line from the Chinese hamster, procured from American Type Culture Collection (ATCC, Rockville, MD) was used for both methods. V79 is well established in toxicology studies. Stability of karyotype and morphology is making them suitable for general toxicity assays. Culturing of the cell line was performed in Dulbecco's modified Eagle's medium (DMEM) which included FBS (10%) and

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penicillin-streptomycin (1%). A temperature of 37°C with a humidity of 5% CO_2/95% air atmosphere was maintained for the cultures. Prior to study hydrogel samples were sterilized by autoclave.

Indirect contact method

Cultured cells were incubated for 24 h after seeding at a density of 3×10^4 cells per well in a 96-well culture plate. In this method hydrogel was incubated with DMEM for 48 h at the concentration ranges from 4.000mg/ml to 0.250mg/ml.

Cultures with identical conditions were used for incubation for the hydrogel extract for another 24 h and 48 h. Subsequently, Alamar Blue® (Invitrogen, Carlsbad, CA, USA) reagent (20 µl) was added to the treated cells and were analysed after a 4 h incubation period.[6] A microplate reader (Varioskan Flash, Thermo Scientific, Waltham, MA, USA) measured the absorbance of the samples at 570 nm. The following equation determined the cell viability:

$$Cell\ viability(\%) = (A_{570}\ of\ treated\ cells / A_{570}\ of\ control\ cells) \times 100 \quad \text{Eq.(1)}$$

Direct Contact method

The hydrogel sample (0.250mg/ml) was directly added onto the cells seed with V79 cells with 1×10^5 cells per well in a 24-well plate. Moreover, the cell morphology was inspected by inverted compound microscope (Olympus CK 30) with camera.

Acute GI toxicity test

To investigate GI toxicity of hydrogel, acute oral toxicity was tested according to Organization of Economic Co-operation and Development (OECD) Guidelines for Test of Chemical 425. Maximum Tolerance Dose method was adopted by keeping significant biocompatibility of hydrogel in view.

The Animal Ethics Committee of UKM approved the study protocol (FF/2013/CAIRUL/15-MAY/522-MAY-2013-DEC-2013). Adult female ICR mice (25-28 g) were maintained in a controlled temperature at 20–22°C with 50–60% relative humidity and dark-light cycles of 12 h.

All mice were in quarantine for a week before treatment. Twelve female mice were divided into control and test group. The test group was orally administered with hydrogel suspension at the dose of 2000 mg/kg while the control group fed with normal saline. Thereafter, the mice were observed for 14 days, twice daily for any mortal and toxic clinical signs. Organs were collected from the sacrificed mice on completion of the study period for examining gross pathological changes.

Statistical Analysis

The observed data are tabulated as mean ± standard deviation. Analysis of the data was with Student's *t*-test for pairwise difference between treatment and control group. Multiple groups were compared with analysis of variance (ANOVA), with an additional post hoc Tukey test using SPSS 19.0 (IBM corporation, Armonk, New York, USA). The level of significance was considered at $P < 0.05$.

Results and Discussion

Semi interpenetration pH sensitive network of bacterial cellulose-poly(acrylamide-sodium acrylate) hydrogel was synthesized by microwave irradiation method. These free radicals produce active center on acrylamide for polymerization and BC for grafting. Subsequently, grafted and polymerized chains get cross linked and produce hydrogel in the presence of crosslinker.

In vitro cytotoxicity study

In vitro cytotoxicity test is the first step to evaluate the biocompatibility of polymer intended to be used as drug carrier. Reduction in cell viability, if any, was usually owing to unreacted monomers and other chemical leaching during their application.

Cytotoxicity of hydrogel was evaluated by extract dilution (indirect contact) and direct contact methods as recommended by ISO guidelines. A dose dependent cell viability assay using Alamar Blue® (indirect method) on fibroblast cell revealed that cell viability decreased with increased hydrogel amount but not significantly ($P< 0.05$). Moreover, the cell viability was higher than 87.43% even when the hydrogel concentration was 4mg/ml (Figure 1).

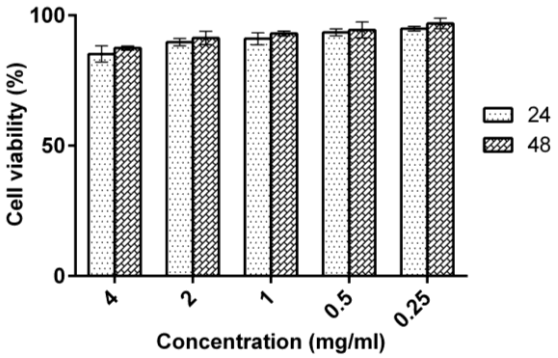

Figure 1. Mean Cell viability results evaluated by extract dilution (indirect contact) method after incubation for 24 h and 48 h.

Likewise Mandal et al. (2009) observed the composite of fibroin and PAM was nontoxic after assessing the cytotoxicity by indirect method on AH927 fibroblast cells.[7] On the other hand, optical microscopic image of cell (Figure 2) incubated with hydrogel for 24 and 48 h (direct contact method) illustrated that the cells possess normal

morphology even after 48h. Generally direct contact method has various advantages like they mimic physiological condition, no need of extract preparation etc. However, decrease in cell count may be attributed to size and viscosity of hydrogel in direct contact method which would hinder cell growth.

Acute GI toxicity

pH sensitive hydrogel, a self-regulated carrier, has been used frequently for oral delivery of lifesaving drugs exploiting response to variation in pH from stomach to intestine.[8] GI system is the preferred route for ingestion of all drugs, nutrient and chemicals makes it highly susceptible for toxicity. This study was conducted for safety assessment of the hydrogel as drug carrier through oral route.

In this case Mandal et al. (2009) and Vijan et al. (2012) already reported that PAM and its composite showed no signs of acute oral toxicity. On the other hand, BC which was extracted from *nata de coco* was a food grade material. So, limit test was performed according to OECD guidelines.[7,9]

All mice survived throughout the study period. There were no episodes of vomit, salivation and diarrhea as well as the feces was normal without pus and blood. Food and water consumption of hydrogel treated group was found to be similar ($P< 0.05$) with control group throughout the experiment (Figure 3).

Oesophagus, stomach, intestine and pancreas were collected for histopathological observation to investigate GI toxicity of hydrogel. It was clear from Figures 4a &4b, oesophagus lined by keratinized stratified squamous epithelium without any erosion and ulceration. Similarly, stomach mucosa is intact and muscular layer is normal (Figure 4c & 4d). Normal tissue structure of small intestine without any ulceration was observed (Figure 4e & 4f).

From the light microscopic image, it was clear that pancreas acini ducks and islets were normal without any ulceration and inflammation (Figure 4g & 4h). Throughout the micrographs, no neutrophilic infiltration is observed which may indicate an acute inflammation. There were no significant histopathological changes found in other vital organs.[10]

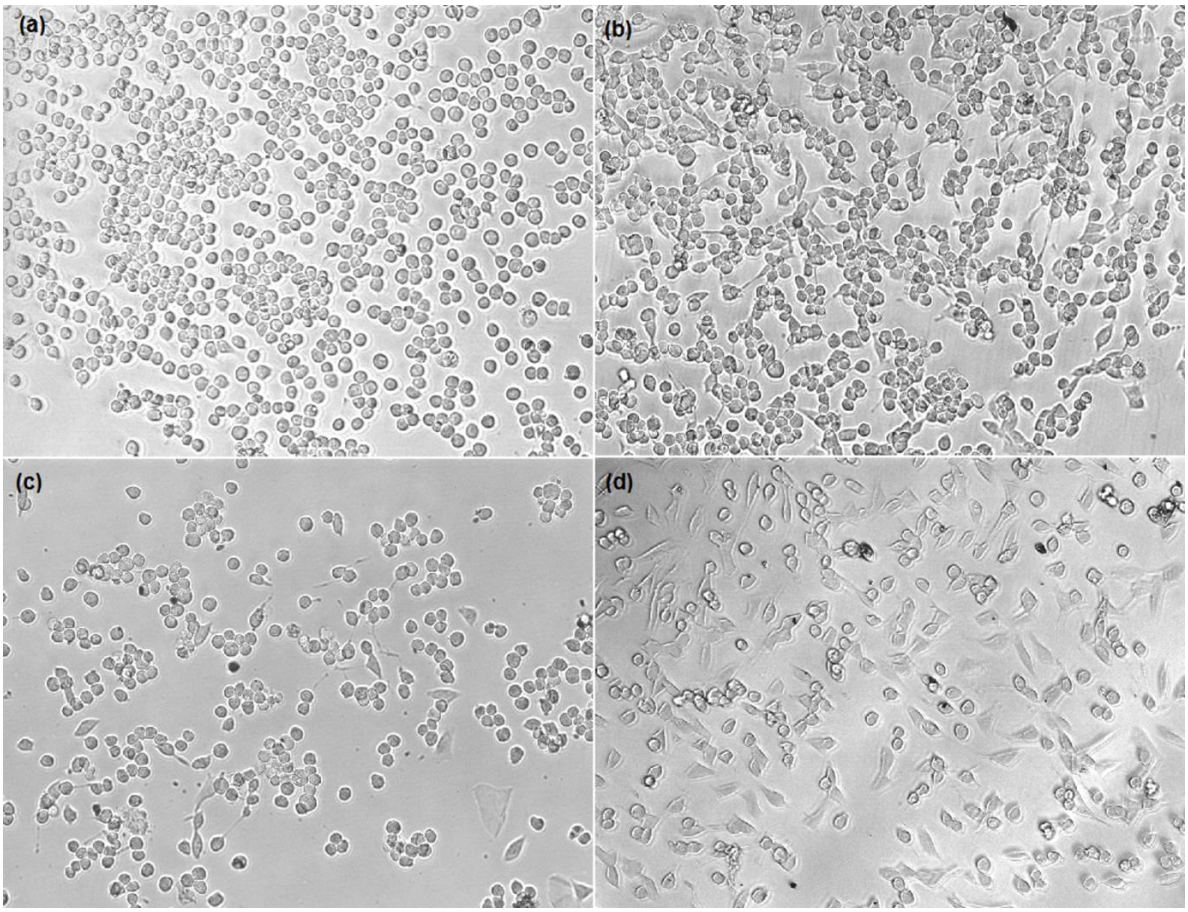

Figure 2. Optical microphotographs obtained after 24 h and 48 h incubation of V79 fibroblasts cell in DMEM only (a & b), cells in direct contact with hydrogel (c & d).

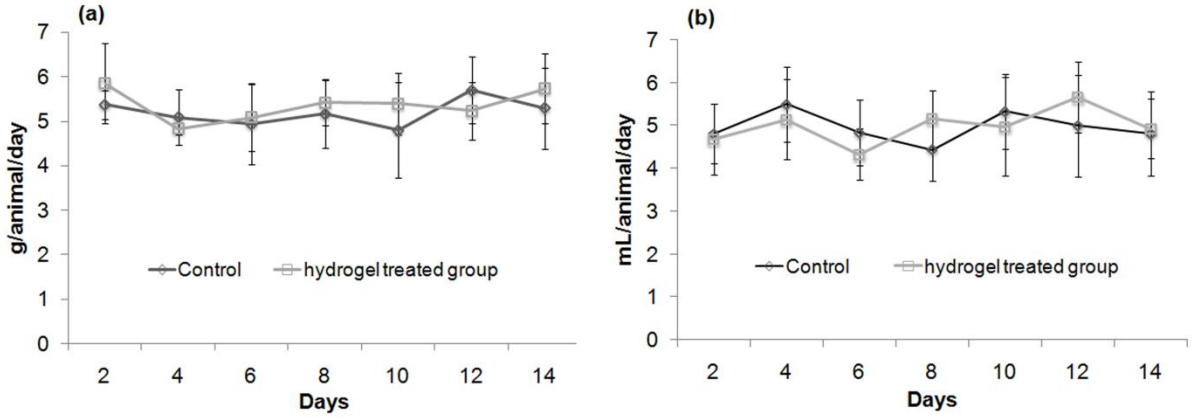

Figure 3. Mean food consumption (a) and mean water consumption (b) for mice dosed once with hydrogel (treated group) and control group throughout the experimental period.

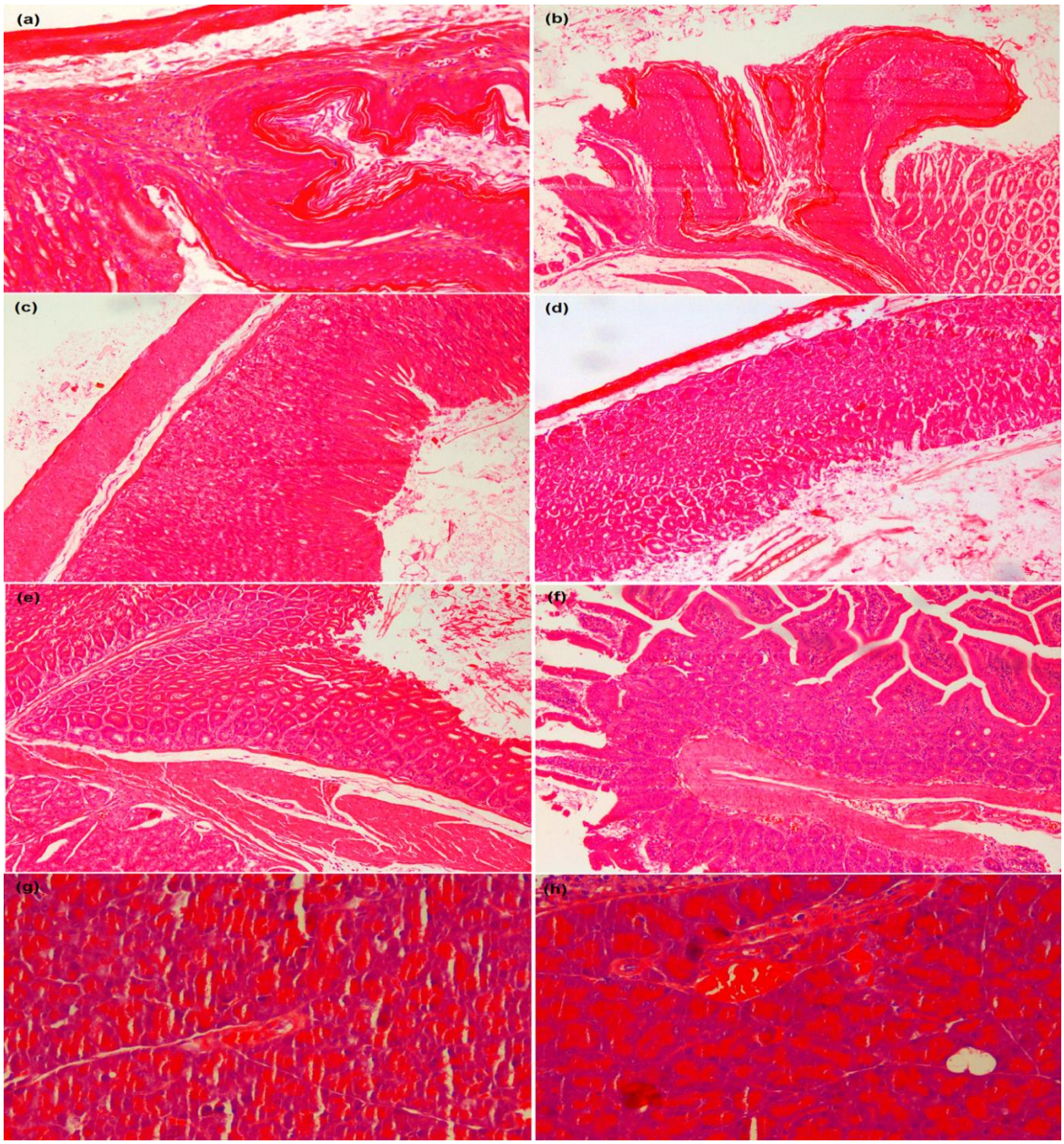

Figure 4. Photograph of collected organs of mice oesophagus, stomach, intestine and pancreas of control group (a, c, e, g) and BC/Am hydrogel treated group (b, d, f, h), respectively.

Conclusion

In-vitro cytotoxicity and GI toxicity test indicated that BC/AM hydrogel is non-cytotoxic, safe and may emerge as a better choice for oral drug delivery.

Conflict of interests

The authors claim that there is no conflict of interest.

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