The H\textsubscript{2}O\textsubscript{2} treated agarose compositing with gelatin for skin repair

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Abstract. This paper presented that the H\textsubscript{2}O\textsubscript{2} treated agarose (abbr. AG) was composit with gelatin (abbr. Gel) as gel-like polymeric mixture for skin cut repair. The low molecular weight AG was prepared by H\textsubscript{2}O\textsubscript{2} treatment for certain intervals. The time of AG gelling might be tested by scattered light turbidimeter and its mechanical properties were evaluated. The degraded AG mixing with Gel was sprayed to cover rabbit lesion site. AG/Gel mixing gel had good biocompatibility and could repair rabbit skin wound without liquid exudation and bacterium infection. H&E staining results disclosed that type I collagen and other extracellular matrix in the repaired site have same with the original skin. The degraded AG mixing with Gel gels have potential as wound dressings.

1. Introduction
AG is extracted from agar, which is one of natural polysaccharides from red algae, and can gel in room temperature with certain molecular weight and has favourable biocompatibility for biomedical applications. Agar or AG was widely used as food additive, culture matrix and electrophoresis gel, and at present, many efforts were contributed to constitute AG-based sponge for tissue reconstruction or drug delivering vector. Hydrophilic agar or AG easily forms fragile gel without crosslinking in water, so it needs cross-linking for its structural stability in liquid medium [1]. At the same time, for agar or AG was not easily hydrolysed in vivo and could not fix cells, it needs mixing with degradable polymers, for example hyaluronic acid, type I collagen or Gel to accelerate degradation and to increase cell attachment [2]. Gel [3] might be composit with AG and crosslinked by EDC-NHS ((1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride and N-Hydroxysuccinimide) or aldehyde compounds for entrapping cells. It was found the Ag/Gel mixture promoting the seeded cells entrapment in vitro. Nowadays, to obtain materials from ocean algae draws considerable interests, which may bring multiple benefits as well as biomedical ones[4]. Meanwhile, cut dressing with functions of rapid and exact covering and healing, for example burning or traumatic cuts, chronic diabetic one, or decubitus, is urgently needed in medical treatment. Fortunately, sprayable gel-like dressings might provide a new way to treat these clinical cases. We try to demonstrate structures and properties of the degraded AG compositing with Gel as sprayable skin dressing.
2. Material preparation and characterization

2.1. Degradation of AG and preparation of composite gel

The degraded AG was prepared according to the reference [5]. Briefly, 2 gram AG was totally dissolved in 98 gram water heated at 95°C, after dissolution, the AG solution was cooled down 55°C, and 7 millilitre 30% H₂O₂ was poured into the AG solution for 2-28 hours degradation. After most part of water vaporizing in the solution, the degraded AG was washed with alcohol 3 times, aldehyde groups in the degraded AG were reduced by NaBO₃ and then freeze-drying. The degraded AG was abbreviated and named Dx (x means time of degradation). The gelling time of Dx in 2% weight percentage was tested by a WZG-20B portable turbidimeter. Dx and Gel composite gel consisting of 3% weight precent Dx or Gel at certain ratio (2:1, 1:1, and 1:2) was dissolved and mixed in heated water, and sterilized by 0.22 µm filter membrane. The gel mixture was sprayed onto different surface including human being skin, wood or glass for testing film forming of the gel mixture. The images of gel on the surface were observed under an Mshot M152-N inverted microscopy.

2.2. Structures and biocomptability and degradation of Dx

AG, Dx or the NaBO₃ treated Dx was pressed with the milled KBr. Their FT-IR spectra were obtained through a Bruker VERTEX 70 infrared spectroscopy. 90 KM rats were grouped into six. Dx, Gel and their mixtures were sterilized and implanted into rat back. rats died by neck lacing with 3d or 1- 4wk implanting time. The remained gel in the implanted lesion site were taken out, and cleaned, dried and weighed respectively. The gel degradation rate was calculated through the remaining gel weight divided by the implanted one. Biocomptability of the gel was evaluated by weight losses of rats, and changes of the rat’s tissues around the implanted gel. All animal tests were approved by Animal Care Committee of Medical College, Jinan Univ..

2.3 Implantation evaluation

All implanting gels were filtrated with 0.22µm Millipore or sterilized at 130°C, 1.5atom for 30min with YXQ-LS-50 SII High Pressure Steam Sterilizer. 30 white rabbits were grouped into five. The D8 and Gel were two control groups and mixtures of D8 and Gel with ratios of 2:1, 1:1 and 1:2 were experimental ones. Over 1.5 cm diameter circle was sheared on rabbit back, and tincture of iodine was besmeared in the circle. Rabbit was anesthetized with 3% barbital sodium by ear vein. When rabbit was fallen into a coma, a 1.5cm circle wound in rabbit back was made. D8 and Gel were sprayed into the cut area as control groups, and in experimental ones, D8 and Gel mixture with ratio of 2:1, 1:1 and 1:2 were sprayed into the wound area respectively. All lesions were covered with gauze. During implanting process, about 0.2mL barbital sodium was injected if rabbit slightly regained consciousness or cornea reflex took place. With different repairing time, the images of the lesion sites were taken by a ZEISS ZX1 camera. In certain intervals, some rabbits were executed. The lesion skin was cut and fixed with 10% paraformaldehyde solution, and dehydrated by 50-100%(v/v) alcohol solution and 100% xylene, and then embedded into paraffin and micromoted into slices with 3-5µm thickness, was analyzed with HE staining, and the stained slices were observed under an Mshot ML11 optical microscopy.

All data were shown as the mean ± SD from at least three independent experiments and the statistical significance of their differences was checked by the student’s test (ANOVA). p <0.05 was taken into account statistically significant. Statistical analysis was performed using SPSS v.13.0.0 (SPSS, Inc., Chicago, IL)

3. Results and discussion

3.1 Structures and gelling properties of Dx

AG could be degraded in H₂O₂ solution by oxidation reaction. After degradation, the stretching vibration peaks of aldehyde groups appeared in D8 (figure1b), and vanished after treating with NaBO₃ (Figure 1c). This meant H₂O₂ solution could oxidize hydroxyl groups into aldehyde ones in AG,
aldehyde groups were reduced by NaBO$_4$. At meantime, the FT-IR spectrum of D8 was similar to one of the original AG (figure 1a). This meant the degradation process did not change chemical structure of AG. Dx slowly gelled with degradation time (figure 2). It was found that when AG was degraded over 6h, Dx gelling needed over 100minutes, but of AG just 10 minutes. The longer gelling time of Dx was disadvantageous for mixture of Dx and Gel to quickly gel, which led to gel covering wound in longer time. So in this research, D4-D10 was suitable for compositing with Gel to achieve quick gelling. After Dx mixing with Gel, gel could be sprayed to biological skin, wood or glass and the gel was quickly formed on these surfaces. At the same time, after gel drying, there were a lot of fine and even particles in the gel. It proved that Dx and Gel gel easily adhered on the surface of amphiphilic material.

Fig. 1 FT-IR spectra of AG (a), D8(b) and D8 treated by NaBO$_4$ (c)

Fig. 2 NTU of Dx with time
3.2. **In vivo degradation and toxicity**

AG degraded slowly in vivo because of no agarose enzyme in animal body. Even if AG was degraded into lower molecular weight polysaccharide, the weight loss percent of AG was between 23% and 27% in 4 weeks and it was still slowly degraded in vivo with different degraded time. After composting with Gel at weight ratio of 1:2, the mixture could lose its weight rising to 80% because Gel could be quickly degraded in vivo. It proved that Gel in the composite could accelerate gel degradation and loss of AG in gel. After the gel injected into mice, mice lived very well over next few days. It was found that the Dx could not cause inflammatory reaction in the surrounding tissues of the implanting area, and there were a lot of blood vessels growing in Dx gel, however, in AG gel, no blood vessel were found (figure 3). This meant that Dx still had good compatibility and no toxicity.

![Fig. 3 The morphologies of AG (left) and D8 (right)](image)

3.3. **Skin repair**

The mixture of D8 and gelatin with ratio of D8/gelatin being 2:1, 1:1 and 1:2 was sprayed on rabbit wound skin. In 2 weeks, the composite gel and gelatin group could be degraded and the wound circle apparently reduced. However in D8 group, the remained AG was found. This meant Gel containing gel could be degraded in 2 weeks, and the degraded AG could not. H&E analysis results disclosed that there were some cavities in the Gel one because of Gel disappearing before skin tissues recovering. After 4-week repair, gross examination results showed the best repaired group was the gel of D8/Gel with 1/2 weight ratio, and histological analyses also proved that tissue structures and collagen content in the lesion site were homogenenous and similar to normal. In D8 group, part of D8 was still left in the repaired tissue after 4 weeks. In general, the repair processes of wound skin are roughly divided into 3 processes, contracting, scarring and regenerating though wound regeneration are complicated. Wound repair included a series of biological processes: restoring blood vessels, swallowing the debris, replacing and filling vacant spaces, and remodeling the regenerative skin tissues. For adult mammal, perfect skin regeneration is hardly achieved, and most part of repaired tissues are consisted of scar and contracting one. Gel, fibrous membrane or sponge-like cover might retard wound contraction and provide vacant spaces for skin tissue recovering, and it needs gel, fibrous membrane or sponge-like cover degrading at a rate matching with the wound repair one. We found that mixture of D8 and Gel with ratio being 1:2 had suitable degradation rate and matched one of wound repair. This sprayable gel had a potential for wound repair.

4. **Conclusion**

A D8/Gel gel consisting of D8 mixing with Gel at different ratio was prepared for wound regeneration. The D8/Gel composite could be degraded, and conveniently sprayed, and could successfully repair rabbit skin cut. The results proved that D8 and Gel mixing gel had a potential as wound cut dressing.

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