Functional Polymer Hydrogels for Water Purification Applications

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ABSTRACT

In this paper, we utilized cross-linked polymer hydrogels to encapsulate microbes for water purification applications. We encapsulated the microbe (*Pseudomonas fluorescens)* in the polymer fibers via electrospinning. The presence of bacteria inside the fibers was confirmed by scanning electron microscopy (SEM). We are also exploring the use of other hydrogels, such as gelatin to encapsulate microbes (*Alcanivorax borkumens)*. Here, we can easily immobilize the microbes within the hydrogel without introducing toxic cross-linking agents. Proliferation test showed that while bacteria proliferated in the presence of the cross-linker or the gelatin solution, they did not proliferate once the cross-linking reaction occurs. These results have significant implications of microbes for water purification applications.

Keywords: hydrogel, cross-linking, immobilization, microbe, water filtration

1 INTRODUCTION

Filtration is necessary in many engineering fields. Polymeric membranes have been used in filtration application for more than a decade. However, some of the contaminants, e.g. radionuclides and toxic metals, present in soluble form in the waste water and therefore can not be removed by general methods. Although microbes can convert radionuclides and toxic metals into an insoluble form, successful immobilization of microbes while maintaining their usefulness for any desired application had been elusive [1, 2].

We describe the use of cross-linked polymer hydrogels to encapsulate microbes of water purification genera, such as *Alcanivorax borkumens*, which can digest oil, and *Pseudomonas fluorescens (P. fluorescens)* for purification of radionuclides. We use (polyethylene oxide)$_{99}$-(polypropylene oxide)$_{67}$-(polyethylene oxide)$_{99}$ dimethacrylate (FDMA) triblock polymer fibers, created by electrospinning, to encapsulate microbes. The presence of bacteria inside the fibers was confirmed by scanning electron microscopy (SEM). We are also exploring the use of other hydrogels, such as gelatin, to encapsulate microbes. We found that 82% of the bacteria survived the hydrogel cross-linking process. On the other hand, we noticed that while bacteria proliferated in the presence of cross-linker or the gelatin solution, they did not proliferate once the cross-linking reaction had occurred.

2 MATERIALS AND METHODS

2.1 Materials

The synthesis and characterization of FDMA was described in an earlier article [3]. The weight average (M_w) and number average (M_n) molecular weight of FDMA were determined to be M_w*=21,900* Da and M_n*=12,600* Da. Gelatin (type A from Porcine) was obtained from Sigma-Aldrich (Milwaukee, WI). The calcium-independent microbial transglutaminase (mTG) was obtained from Ajinomoto (Chicago, IL) and was used without further purification. This enzyme is reported by the manufacturer to have a specific activity of 100 U/g. Distilled phosphate-buffered saline (1×dPBS, PH 7.4) was prepared in the lab according to a standard protocol.

2.2 Bacterial Cultures

P. fluorescens (ATCC 55241) were cultured in a medium, 1 liter of which was consisted of citric acid, 2.0g; $MgSO_4.7H_2O$, 0.2g; NH_4Cl, 1.0g; KH_2PO_4, 1.0g; K_2HPO_4, 1.0g; NaCl, 5.0g; pH 6.1 adjusted using NaOH. *Alcanivorax borkumens* were cultured in an agar gel in an incubator at 27^{o}C. Typically, cultures at the end of the log phase of growth were used for testing.

2.3 Electrospun FDMA Matrix Preparation

Electrospinnng solution was prepared by dissolving FDMA powder in deionized (DI) water at a concentration of 13 wt%. The solution was kept at 4^{o}C until the solution became clear. For the encapsulation experiment, a predetermined amount of the bacteria, as required, was dispersed homogeneously in the FDMA solution before electrospinning.

The experimental set-up of the electrospinning stage was as described elsewhere [4], except for using stationary alumina plate as the collector. The fibers were electrospun and collected on a sterile silicon wafer for about 30 min.

To cross-link the electrospun FDMA fibers, ammonium persulfate (APS), ascorbic acid (AsA), and ferrous sulfate solutions were prepared freshly in DI water and used as the catalysts. The electrospun fibers (along with the silicon wafer support) were placed into a glass vial containing catalysts solution and were allowed stand overnight at room temperature. The cross-linked membrane then was washed 3 times with DI water to remove unreacted monomers and catalyst.

2.4 Gelatin Hydrogel Preparation

Gelatin solution (20% w/w) in dPBS buffer was prepared and maintained at room temperature before use. The mTG (20% w/w) stock solution in dPBS buffer was prepared, distributed into vials and stored at -18^{o}C. The enzyme solution was thawed at room temperature (RT) before use. Immediately before applying, the hydrogel was prepared by mixing the warm gelatin (37^{o}C) and mTG solution (RT) in different ratio. To prepare the microbe-encapsulating hydrogel, the procedure is similar as described above, but mix microbes with warm gelatin solution (37^{o}C) before adding mTG solution.

2.5 Characterization of Microbes

For SEM (LEO 1550) imaging, the cross-linked FDMA fibers were freeze dried (Consol 1.5, Virtis Inc.) at -40^{o}C, followed by lyophilization. Samples were sputter-coated with gold for 15 seconds twice.

The viability of the microbes was assessed using the LIVE/DEAD BacLight bacterial viability kits (Molecular Probes). Live microbes (intact cell membranes) stain fluorescent green, whereas dead microbes (damaged cell membrane) stain fluorescent red. Live and dead bacteria later were viewed simultaneously by Leica TCS SP2 laser-scanning confocal microscopy (LSCM) (Leica Microsystem Inc.).

The viability of the bacteria, before and after hydrogel cross-linking, was evaluated. The encapsulated microbes were stained with LIVE/DEAD BacLight Bacterial viability kit and observed under LSCM. Photomicrographs of the stained bacteria were obtained. The number of live bacteria was averaged over several views of the same condition. Microbial counts at each time point was performed in triplicate.

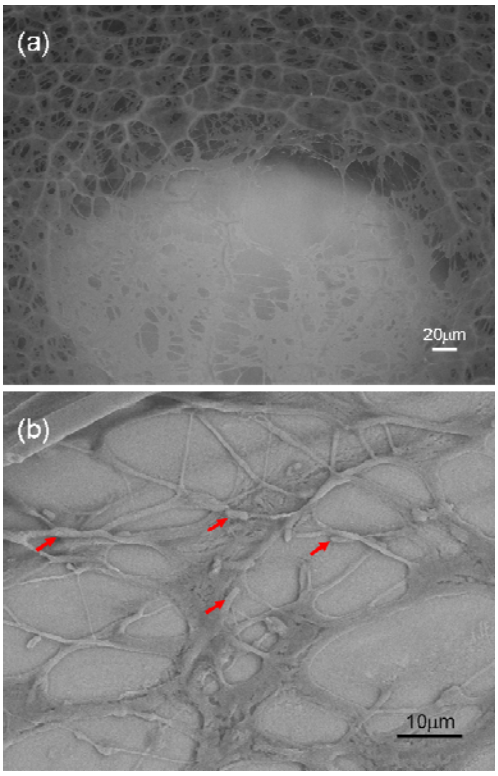
3 RESULTS AND DISCUSSION

3.1 Microbes Immobilized in FDMA Fibers

Here we use the electrospinninig technique to encapsulate rod-shaped bacteria in a polymer matrix, which forms a composite fiber during electrospinning. However, the water-soluble nature of FDMA presents a great challenge, because any contact with water can destroy the fibrous structure immediately, thereby severely limiting the application of this polymer. Cross-linking the fibers after electrospinning creates hydrogel fibers with improved resistance to water. Such cross-linked fibers with encapsulated microbes can be used in various applications.

SEM image in figure 1a shows that the cross-linked membrane still maintained the three-dimensional (3D) porous structure after the cross-linking process. The porous structure was seen not only on the surface of the electrospun samples, but through the whole thickness of the sample.

To verify encapsulation of *P. fluorescens* by the FDMA fiber, freeze-dried cross-linked FDMA fibers encapsulating the bacteria were examined by SEM. Normally, the cross-linked FDMA fibers are multilayered. A single layer of FDMA fibers encapsulating *P. fluorescens* cells is shown here, because it provides better contrast between the microorganism and fiber. A lower- magnification image (figure 1b) shows that the microbes were distributed over the entire area of the cross-linked fiber. Figure 1c shows a representative cell of *P. fluorescens* bacterium inside the FDMA fiber. The bacteria were aligned along the direction of the fibers and the cellular integrity of the microorganism seemed to be well preserved, regardless of the location.



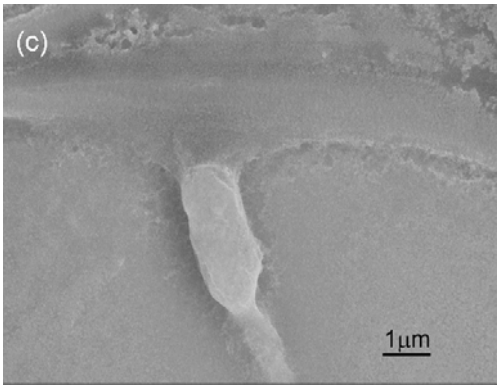


Figure 1: (a) Surface image of the FDMA fibrous membrane obtained by lyophilization. (b) and (c) are SEM images of bacteria in the cross-linked FDMA fibers. Arrows in (a) indicate the location of bacteria. (b) indicates a microbe encapsulated in a single fiber.

3.2 Nutrients Exchange in Polymer Network

Although the cross-linking treatment improved the water resistance of the FDMA membranes, such treatment could inhibit nutrient exchange of bacteria encapsulated in the fiber. As an indication of the rate of nutrient exchange, it was observed that complete exchange of glucose at concentration of 80g/L occurred in about 2 h.

Our previous results also showed that there was no proliferation of bacteria within the fibers [5]. The sketch shown in figure 2 illustrates the conditions of cross-linked fiber. The fibers discussed here are formed from a uniform hydrophilic polymer solution. When immersed in water, the cross-linked prevent the fibers from dissolving and lead to the formation of a mesh-like network of the swollen polymer within a short time. When the gel fibers are fully cross-linked, we can estimate that the greatest distance between cross-links is the stretched end-to-end distance of the polymer, which is less than 100nm. This distance is large enough to allow the exchange of nutrients and microbial metabolic products between the microbe and the environment, but it is much smaller than the size of the bacteria, thereby immobilizing the microbes inside the open mesh-like enclosure.

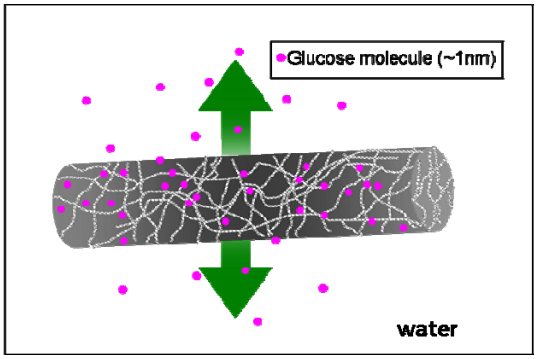


Figure 2: Illustrate the exchange of glucose between the cross-linked FDMA fiber and DI water.

3.3 Microbes Immobilized in Gelatin Hydrogel

As another example of bacteria encapsulation, we are also exploring the use of other hydrogels, such as gelatin, to encapsulate microbes. Here, we can easily encapsulate the bacteria within the cross-linked hydrogels without introducing toxic cross-linking agents. The fluorescent image in figure 3 shows a homogeneous distribution of the bacteria, *Alcanivorax borkumen*, in the cross-linked hydrogel. Quantitative analysis shows that about 82% of the bacteria were still alive after the cross-linking process, which shows the low toxicity of the chosen catalyst system allowed the microbes to survive.

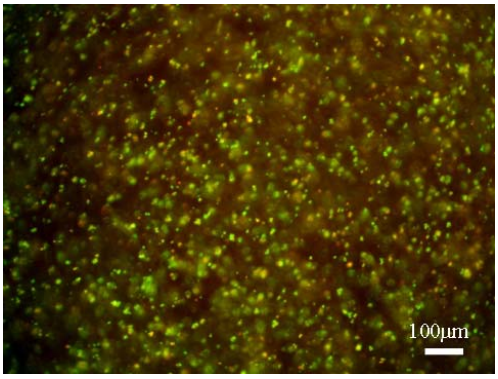


Figure 3: Fluorescent microscopy image of the bacteria encapsulated in the cross-linked gelatin hydrogels. Bacteria were stained by LIVE/DEAD BacLight bacteria viability kits. Live bacteria stained fluorescent green, while dead microbes stained fluorescent red.

3.4 Microbes Proliferation in Gelatin Hydrogel

To determine whether the bacteria proliferate within the gelatin bulk hydrogel, without undergoing electrospinning, we monitored the total number of live and dead bacteria cells in cross-linked gelatin hydrogels for 3 days. The results indicate that the total number of live and dead cells remained almost constant, and there was no proliferation.

On the other hand, we noticed that while bacteria proliferated in the process of the cross-linker or the gelatin solutions, they did not proliferate once the cross-linking reaction had occurs (data not shown here). This indicated that the chemicals in themselves were not toxic, rather the conformation of the cross-linked gel which prevented the proliferation. A similar effect was observed in the hydrogel fibers. Initially we believed that the confinement was due to the fiber geometry. Since the radius of the fiber is much larger than the distance between cross-links, these results confirmed that it is not the confinement in the fiber, but

rather the contact inhibition due to the actual cross-links in the gel. This inhibition is advantageous, since the doubling time of these bacteria is less than 60 minutes, continual proliferation would very quickly have consumed that available volume in the gel, causing rapture and release of the bacteria within days.

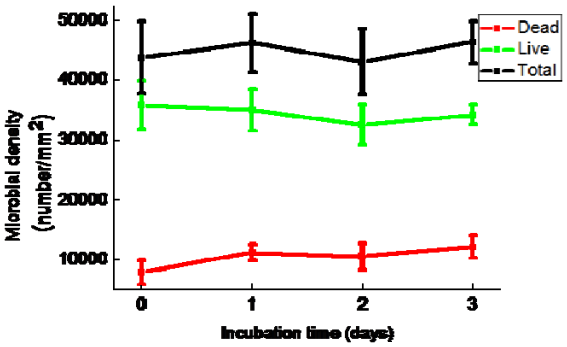


Figure 4: Counting of bacteria immobilized in the hydrogel at different days.

4 CONCLUSION

This study describes the application of cross-linked polymer hydrogel to encapsulate microbes of water purification genera. We first described the development and formation, via electrospinning, of an FDMA fibrous hydrogel material with encapsulated microbes. The integrity of the bacteria was maintained through the cross-linking process. The mesh-like network of the polymer effectively immobilized the microbes while allowing the exchange of nutrients and metabolic products between the microbes and the environment. We then also encapsulated the bacteria in the gelatin bulk hydrogels. We noticed that while bacteria proliferated in the process of the cross-linker or the gelatin solutions, they did not proliferate once the cross-linking reaction had occurs. These results confirmed that it is not the confinement in the fiber, but rather the contact inhibition due to the actual cross-links inhibits bacteria proliferation.

5 ACKNOWLEDGMENTS

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