A Study on Bioelectret Collagen

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ABSTRACT: The aim of this paper is to study the properties of collagen, which is used as a promoting bioelectret for wound healing. So first of all, a bioelectret material based on collagen was prepared, then the processes of thermally stimulated discharge of collagen were measured under different conditions. Cell culture studies were used in the determination of the suitability of this kind of bioelectret as surfaces for the attachment and growth of cells. Some results show the potential use of bioelectret collagen in soft tissue wound healing. © 1997 John Wiley & Sons, Inc. J Appl Polym Sci **64:** 267–271, 1997

Key words: bioelectret; collagen; TSDC; cell culture

INTRODUCTION

Electric field may play an important role in the healing of a wound. Many papers have been published on its stimulating effects on tissue growth in bone, nerve, and special membranes.[1,2] However, it is not always convenient to apply an electric field in any treatment, so the use of electrets is considered. An electret is a piece of dielectric material exhibiting a quasipermanent electrical charge. Generally, bioelectret refers to those electrets applied in the biomedical field and those biomaterials possessing the electret effects. For biomedical applications, electrets have found many uses, such as stimulating tissue growth films, and in antithrombogenic surfaces, hearing aids[3] and electret dosimeters, etc. They have many advantages; they need no electrodes and have lasting effects and changeable forms. In fact, the fundamental macromolecules of biology, including collagen, hemoglobin, DNA, and chitin, have been widely applied as biomaterials and have seldom been used as bioelectrets; they not only exhibit the effect, but also may have various sources for polarization and charge storage. As the components of the biological body, they have excellent biocompatibility and play important roles in the living activities. Therefore, collagen was chosen as bioelectret material in this paper, and some properties related to the biomedical applications were studied.

EXPERIMENTAL

Preparation of Bioelectret Collagen

Collagen used in the present study was extracted from pig skin with pepsin treatment and purified by salt precipitation, which had been prepared as described in many papers.[4–5] After dissolution in a dilute acetic solution (pH = 3), collagen was made in the form of films by the solvent-cast method under ultraviolet radiation, cross-linked with 0.01% glutaraldehyde. Then the films were polarized in a high-intensity DC electric field with the thermal stimulated current technique.

To quantitatively determine the protein purity of collagen, collagen ultraviolet absorption band was measured, and its molecular weight was assayed by 8.0% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). The gels

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were stained with Coomassie Brilliant Blue for band visualization.

Methods for Thermal Stimulated Discharge Current

To eliminate the charging and discharging of bioelectret collagen, thermal stimulated discharge currents of collagen were measured before and after polarization. The measuring process was carried in vacuum to exclude atmospheric effects such as humidity. The thermal stimulated discharge current (TSDC) setups are shown in Figure 1.

In actual biomedical applications, bioelectret collagen has to make contact with body liquid and has to be sterilized for disinfection. The discharge spectra of the specimens were measured after being treated by ultraviolet radiation for 30 min and soaked in ethanol for 10 min, respectively. Additionally, TSDC of polarized collagen in Dulbecco's Modified Eagel's Medium (DMEM) at 37°C with time were tested as well. In these experiments, the heating rate is 2.5°C/min.

Cell Culture

Epithelial cells were kindly offered by Chinese Biophysics Institute before tests. Electret collagen films were cut into discs (0.5 mm thick and 20 mm in diameter) and were placed at the bottom of 24-well plates. Then, the cells were overlaid with 1 mL DMEM (GIBCO, New York). 10% fetal bovine serum were added into each well. All cells were cultured at 37°C in a 5% CO_2 atmosphere.

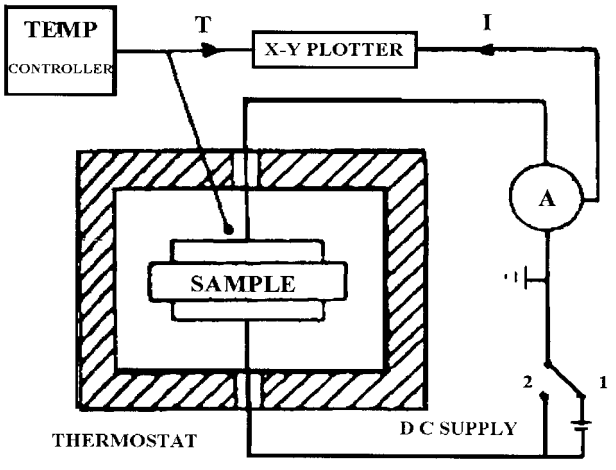

Figure 1 TSDC experimental setup.

The numbers of cells were recorded 24, 48, 72, and 96 h after plating.

RESULTS AND DISCUSSION

Characterization of Collagen

The method of extracting collagen from pig or bovine skin is easily obtained after being purified by acid and alkali for several times to get rid of carbohydrates and impurities. The spectrum of its ultraviolet absorbtion shows a very sharp single peak at 230 nm, which indicates that the protein production is rather pure (Fig. 2).

The chief components in pig epidermis are type I and type III collagen. Because our aim in this article is to test whether the bioelectret collagen has any effect on the cell growth, these two types of collagen were not seperated further. On the SDS-Page gel, the prepared collagen was separated into its constituent band α, β aggregates, of which the former molecular weight is 110 KD, and the later one is about 210 KD, (Fig. 3). This result is very similar to that of the type I collagen standard.[6]

TSDC Spectrum and the Related Factors

Since collagen is a kind of protein, a polymer of amino acid, it is known to possess dipolar groups and shows typical electret behavior even without polarization. However, the orders of TSDC peaks of nonpolarized samples are much lower than that of the polarized collagen. After being polarized in the high DC electric field, the discharged current can be increased by two orders. (Fig. 4). In fact, the TSDC spectrum has proven to be a very sensitive tool for the characterization of the denaturation state of collagen through the low-temperature electret bands. The more prominent peak in Figure 4 at higher temperature was shown to be due to a space charge effect, and the lower temperature peaks are due to a molecular dipole orientation mechanism[7]; but these are not the only source of electret behavior because, if the protein is hydrated, electret behavior can also be affected by so-called bound water or solvent.[8] Figure 5 shows the obvious influence of cell culture medium on electret collagen with time. The samples discharged very rapidly in DMEM liquid, which is the cell culture medium in our experiment. It may be due to microstructure change of collagen film

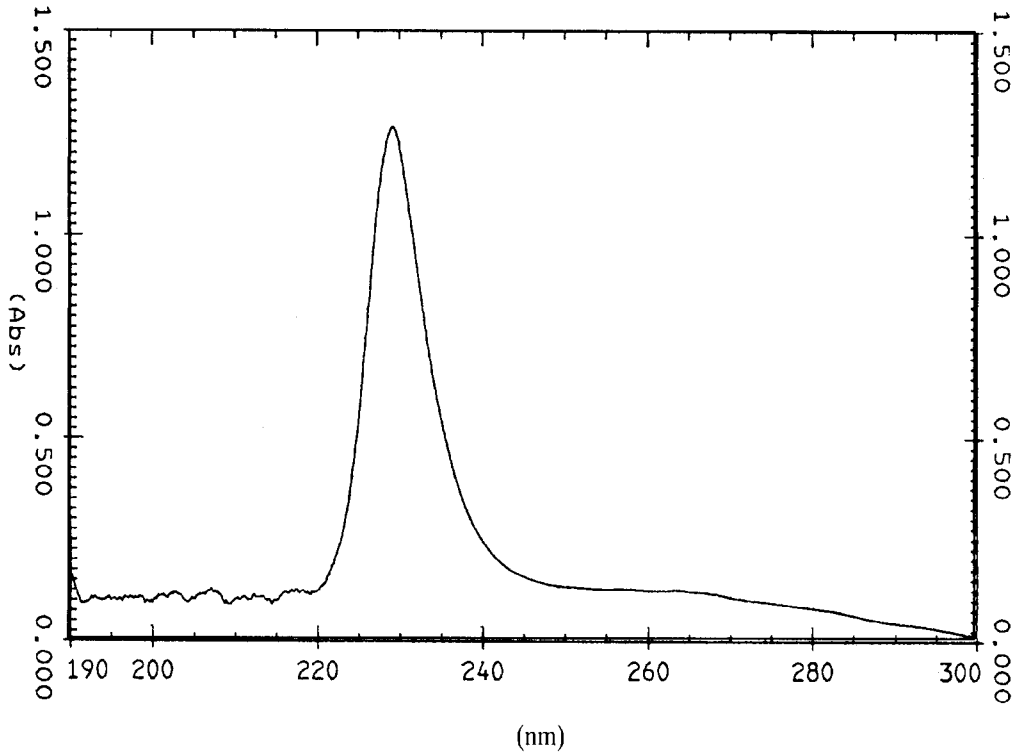

Figure 2 Ultraviolet absorption spectrum of collagen.

and its contact with molecules of the solvent. Since there are many ions in DMEM solution, such as Na^+, K^+, Cl^-, and PO_4^{-3}, they can easily affect the diploe orientation or neutralize the charges on the film. Three days later in DMEM, there is almost no current to be released.

Ultraviolet radiation and ethanol are two ordinary sterilizing methods for biomedical material. However, they have different effects on polarized collagen. As solvent, ethanol is able to affect not only bound water of collagen but also ion movements and dipole orientation; whereas ultraviolet rays as a source of energy very similar to heating can stimulate electret to discharge. By comparing the TSDC spectrum before and after sterilization, it can be seen in Figures 6 and 7 that both of

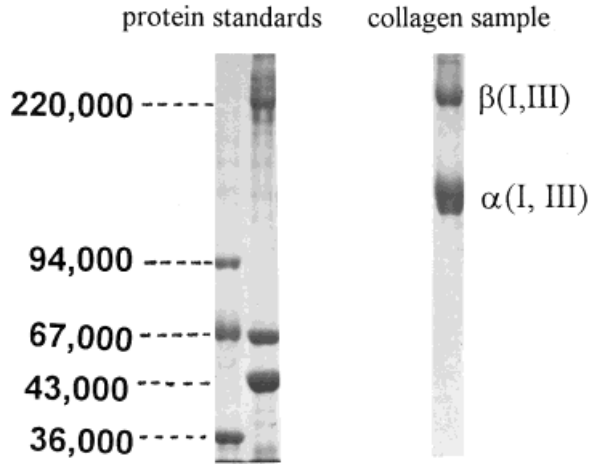

Figure 3 Results of SDS-PAGE electrophoresis.

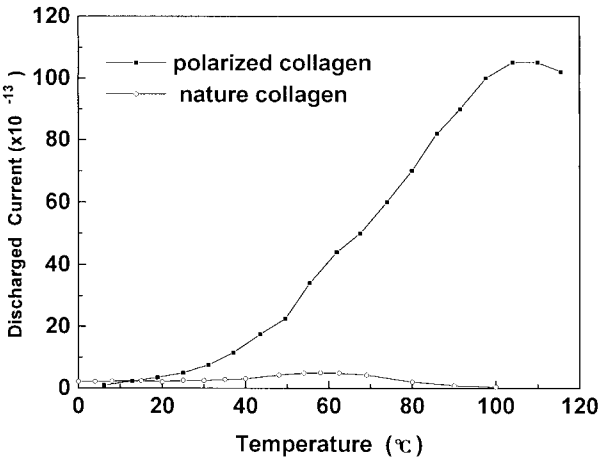

Figure 4 TSDC of polarized and nature collagen.

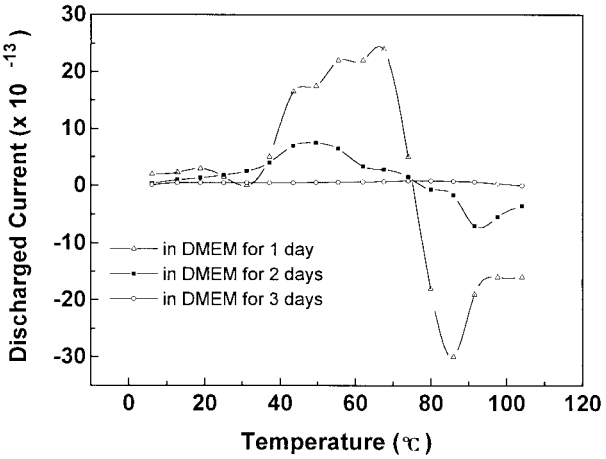

Figure 5 TSDC of polarized collagen in DMEM.

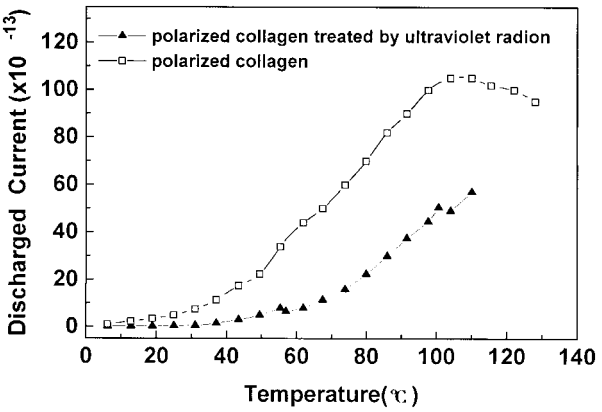

Figure 7 TSDC of polarized collagen treated by ultraviolet radiation.

the sterilizing methods can decrease the electret capacity, but the effect of ultraviolet radiation is a little smaller than that of the treatment in ethanol. Therefore, the electret materials for medical use had better be sterilized before polarization or be kept in a nonbacterial atmosphere during the whole preparation.

Cell Growth on the Bioelectret *In Vitro*

The purposes of this study were to observe the effects of bioelectret collagen on cell growth, so cell culture studies were used to test the suitability of this kind of bioelectret for cell growth. The comparative performance of the polarized and nonpolarized materials was tested with time using Hela cells. The cell growth data are plotted in Figure 8 as a function of time. In order to exclude the effect of serum on cells, we decreased its concentration as low as 5%; as a result of that, the proliferation of controlled cells is much slower than those on the collagen substrates. As time passed, the differences between the substrates before and after polarization became more and more obvious. Since the chemical components of the substrate are identical and only bulk electrical properties differ, we can draw the conclusion that for materials of the same constituents, the electric field of the bioelectrets may influence the cell growth. It is worthy to notice that the TSDC peak of charged collagen, which had been soaked in DMEM for three days, is very low; but the cell number increased greatly at the same time, which indicated that electret may influence a certain process in the cell growth cycle and can therefore accelerate the speed of the cell growth.

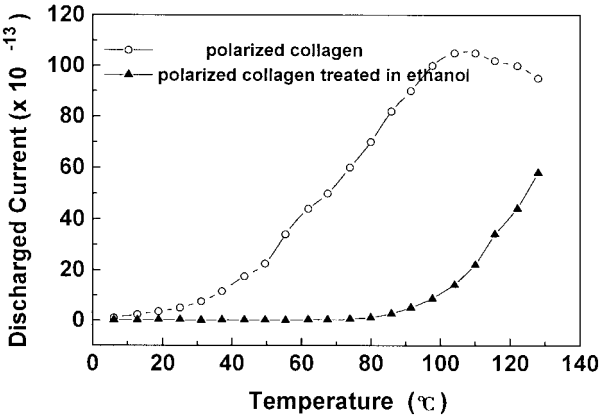

Figure 6 TSDC of polarized collagen treated in ethanol.

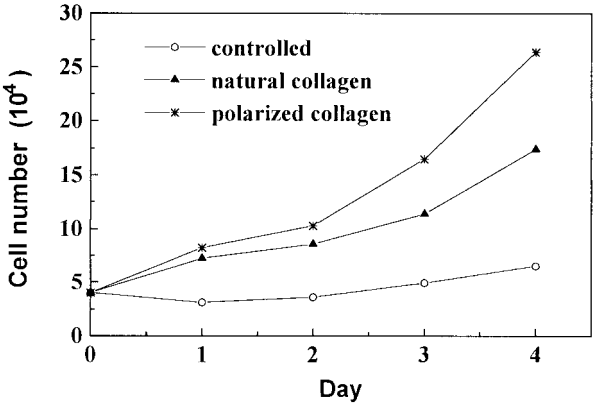

Figure 8 Cell culture on different collagen films.

CONCLUSION

This study demonstrates that polarized collagen has a significant influence on the cell growth *in vitro*. So bioelectret collagen appears very promising for enabling to enhance cell growth and wound healing in the future biomedical application.

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