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Original Article

In vitro evaluation of calcium alginate gels as matrix for iontophoresis electrodes

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Calcium alginate gel has some unique properties, such as the capability to keep the drugs, bioadhesiveness, safety, and low cost. The purpose of this study is to determine whether calcium alginate gel can be used as a matrix of electrodes for iontophoresis (IOP). We measured the concentration of lidocaine transported from calcium alginate gels with various concentrations of alginic acid using an *in vitro* experimental cell with square-wave alternating current (AC) application. Temperature and pH changes were also determined during AC-IOP. The results revealed that lidocaine was released from calcium alginate gels at concentrations nearly 1.71-fold larger at 5 V, 60 min after AC application than in the case of passive diffusion. Lidocaine transport depended on the alginic acid concentration in the gels. Although there were slight increases in temperature and pH, chemical and thermal burns were not severe enough to be a concern. In conclusion, the calcium alginate gel can be used as a possible matrix for IOP electrodes.

Key words: Drug delivery system (DDS), Iontophoresis (IOP), Calcium alginate gel, Alternating Current (AC), Electrode

1.Introduction

Iontophoresis (IOP) is one of the techniques used in a drug delivery system (DDS) to enhance the delivery of compounds through the skin or a mucous membrane via the application of an electric current. IOP is classified either as direct current (DC) IOP or alternating current (AC) IOP. DC has been widely used because its efficiency to transport is higher than that of AC. However, it has some side effects such as electrical burns, chemical burns, and erythema owing to electrode polarization during electrolysis. Using AC-IOP, we have made safer and more effective modifications associated with fewer side effects[1-6].

Although there are many studies of IOP, there are very few studies of matrices of electrodes. Bioadhesiveness to the skin, adhesiveness between matrices and metals, biocompatibility, capability to keep drugs, easy handling, and low cost are required for these electrodes.

Presently, the materials used for electrodes do not

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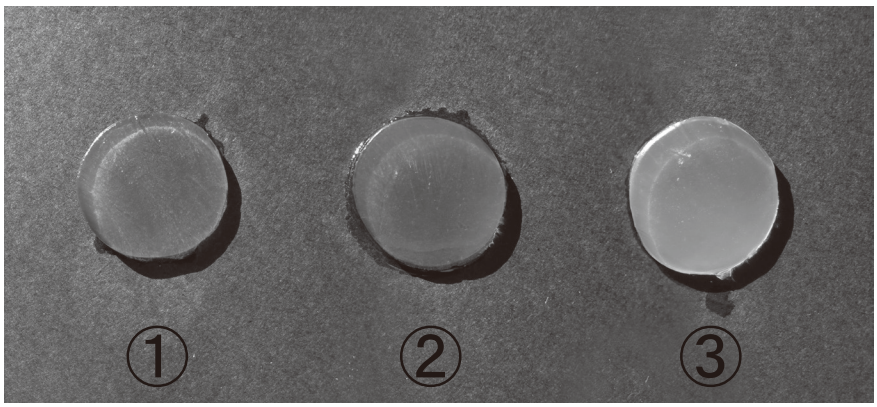

Figure 1 : Calcium alginate gels (the alginic acid concentrations are ① 0.5, ② 1.0, and ③ 1.5%). According to the concentrations of alginic acid, the gel was hardened and the color turned white.

satisfy these requirements. Saito et al. introduced the use of alginic acid for the electrodes on the basis of the results of their basic studies showing that the acid may be suitable for the purposes[7].

Alginic acid is a polysaccharide extracted from seaweed and forms a gel in the presence of cations such as calcium ions[8]. Calcium alginate gel tightly adheres to the skin and mucous membrane, can keep a drug inside its structure[9], and is clinically safe because of its natural origin. Alginic acid gel is frequently used as an impression material in dentistry. The goal of our study was to determine whether calcium alginate gel can be used for electrodes in IOP by examining lidocaine transport and the correlation between alginic acid concentration and the voltage used in IOP.

2. Materials and Methods

2.1 Materials

2.1.1 Calcium alginate gels

Sodium alginate (300-400 cP, Wako Pure Chemicals Industries Co., Ltd., Osaka, Japan) was used in all experiments. Distilled water (resistivity > 18 MΩ·cm) was obtained using Milli-Q SP TOC (Nihon Millipore K.K., Tokyo, Japan). Alginate aqueous solutions of 1, 2, and 3% were prepared using distilled water to obtain 0.5, 1.0, and 1.5% calcium alginate gels respectively. Fifty milliliters of 0.01 M $CaCl_2$ (FW,110.99; Sigma-Aldrich Co., Ltd., St. Louis, USA) aqueous solution was gradually poured into 50 ml alginate solutions with stirring. The resulting viscous alginate solutions were stored at 4 ℃ for 12 h. Then, another 100 ml of 0.01 M $CaCl_2$ aqueous solution was slowly added to the viscous solutions. The $CaCl_2$ solution and the viscous alginate solution were completely separated. The 0.01 M $CaCl_2$ aqueous solution was exchanged for 0.1 M $CaCl_2$ aqueous solution until it completely gelled at 4 ℃. The obtained calcium alginate gels were cut into a discus shape (20 mm diameter, 2 mm thickness, Fig. 1). The discus-shaped gels were washed with distilled water and stored in the distilled water until the experiments. In the experiments, 3 groups were prepared according to the alginic acid concentration of the gels, namely, 0.5, 1.0, and 1.5%.

2.1.2 Lidocaine hydrochloride solution

Lidocaine hydrochloride ($C_{14}H_{22}N_2O$·HCl: FW, 270.8: H_2O content, 1 mol/mol) was purchased from Sigma-Aldrich Co., Ltd. (St. Louis, USA). One percent lidocaine hydrochloride was prepared using distilled water for experiments. The pH of 1% lidocaine hydrochloride solution was 5.4.

2.1.3 Structure and setup of experimental cell

A cylindrical acryl drug delivery cell (Fig. 2) consisting of two chambers (one was the donor chamber and the other, the receptor chamber) was originally fabricated. Platinum plate electrodes with a diameter of 20 mm and a thickness of 0.5 mm were attached at opposite ends of the two chambers of the cell. The lengths of the chambers were 10 and 2 mm. A cellophane membrane (Futamura Chemical Co., Ltd., Nagoya, Japan) with many pores was sandwiched between the two chambers. The distance from a platinum plate electrode of the receptor side was 10 mm and the distance from a platinum plate electrode of the donor side was 2 mm. The pores were about 2-3 nm in diameter.

The donor chamber was filled with calcium alginate gel soaked in 1% lidocaine hydrochloride solution for 1 h, whereas the receptor chamber with a capacity of 3.1 cm^3 was filled with 3.0 ml distilled water. The donor chamber filled with 1% lidocaine hydrochloride solution

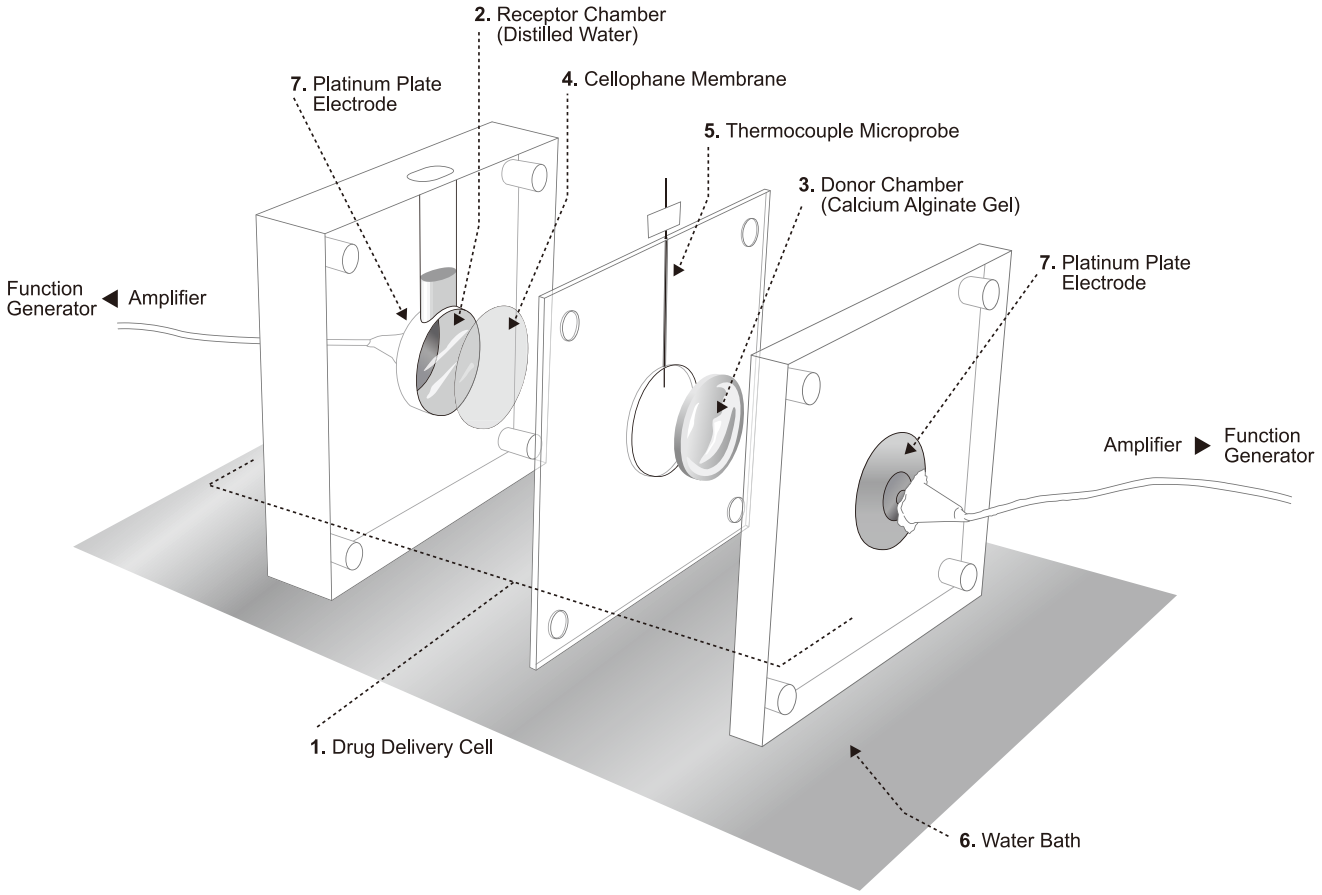

Figure 2 : Setup of the experimental system. It consisted of a drug delivery cell with two chambers (donor and receptor), a thermocouple microprobe, a thermostat, a function generator, and a high-speed power amplifier.

(0.63 ml) was prepared as the control group.

Acryl cells were set in the thermostat (KH-808, Sakura Finetek Japan Co., Ltd., Tokyo, Japan), the temperature of which was maintained at 36.0 ℃.

2.2 Methods

2.2.1 Electric current application

A bipolar square wave with an 80% duty cycle (Fig. 3) was continuously applied at 1 kHz between the parallel platinum electrodes of the drug delivery cell for 60 min using a function/arbitrary waveform generator (Agilent 33250A, Agilent Technologies, Colorado, USA) and a high-speed power amplifier (4025, NF Electric Instruments, Kanagawa, Japan). The frequency of the wave was 1 kHz and the voltages applied were 2.5 and 5 V. For the passive diffusion group, no current was applied. The waveform and voltage of output were monitored with a digitizing oscilloscope (HP54503A, Hewlett Packard, Tokyo, Japan) throughout the experiment.

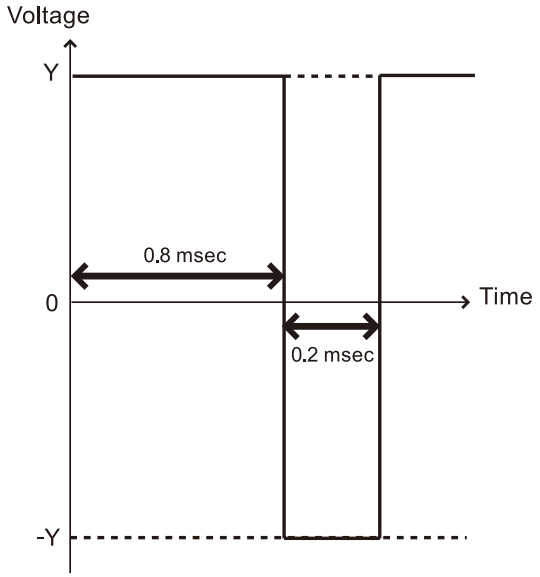

Figure 3 : Diagram of bipolar square wave (AC) with 80% duty cycle at 1 kHz. The duty cycle is the ratio of the positive cycle to the full cycle. Y represents applied voltage.

2.3 Measurement

2.3.1 Lidocaine concentration

Twenty microliters of lidocaine hydrochloride solution was sampled every 10 min during a 60-min AC application using a micropipette (20-200 μl, Nichiryo, Tokyo, Japan) placed at the center of the receptor chamber.

The samples were diluted 25 times with distilled water. The concentration of lidocaine in the receptor chamber was determined using a spectrophotometer (U-3310; absorbance range, − 2～4 Abs; precision, ± 0.002 Abs: Hitachi, Ltd., Tokyo, Japan) at room temperature. The absorbance of the samples was measured at a wavelength of 262 nm and an optical path of 10 mm. Lidocaine concentration was quantified using a calibration curve. The detection limit was 11.6 μg/ml.

2.3.2 Temperature

A thermocouple microprobe (BAT-12, Physitemp, NJ, USA) was inserted directly into the center of the donor chamber to measure the temperature of the gel. Temperature was monitored 30 and 60 min after AC application.

2.3.3 pH

pH was measured in the receptor chamber using a pH meter (TPX-999i, Toko Chemical Laboratories Co., Ltd., Tokyo, Japan). The probe was inserted directly into the receptor chamber before and 60 min after AC application.

2.4 Statistical analyses

All values are shown as mean ± standard deviation (S.D.). A two-way ANOVA test was used to analyze the voltage- and concentration of alginic acid- dependences of the transport efficiency of lidocaine. In addition, the Tukey test was used to analyze the dependence of the transport efficiency of lidocaine, temperature changes, and pH changes on the voltage and concentration of alginic acid in the gel. Statistical significance was assumed at $p < 0.05$.

3. Results

3.1 Lidocaine concentration

Figures 4a, 4b, and 4c show the changes in lidocaine concentration in the receptor chamber with (2.5 and 5 V) or without AC application (passive diffusion) for 0.5, 1.0, and 1.5% alginic acid gels, respectively. The amount of lidocaine that passively diffused and was transported from calcium alginate gels to the receptor chamber increased significantly in a time-dependent manner in all the groups (0.5, 1.0, and 1.5% alginic acid gels). Moreover, the amount of lidocaine transported increased during AC application. When the applied voltage was increased, a significant amount of lidocaine was transported in all groups from 40 to 60 min, as compared with the passive diffusion group ($p < 0.05$).

The correlations between the concentration of alginic acid and the amount of lidocaine transported are shown in Figs. 4d, 4e, and 4f. The lidocaine concentration 60 min after AC application in the 0.5% alginic acid gel group was higher than those in the 1.0 and 1.5% groups. A lower concentration of alginic acid enhanced the diffusion and transport of lidocaine ($p < 0.05$). In the 0.5% alginic acid gel group, the lidocaine concentration at 60 min of 5 V AC application was about 1.71-fold higher than that in passive diffusion. In the control group without calcium alginate gel, the lidocaine concentration was the higher than those in the calcium-alginate-gel-containing groups.

3.2 Temperature changes

The temperatures ranged from 34.26 to 35.92 ℃, which were close to 36.0 ℃. There were no significant differences in temperature among the passive diffusion, 2.5 V, and 5 V groups. Moreover, there were no significant differences in temperature among the 0.5, 1.0, and 1.5% alginic acid gel groups ($p < 0.05$). Thus, the voltage applied and the concentrations of alginic acid in the gels did not affect the temperature of the gel.

There were no significant differences in temperature between the calcium alginate gel groups and the control groups, which indicated that alginic acid itself did not alter temperature.

3.3 pH changes

The pH of the passive diffusion groups decreased after 60-min application ($p < 0.05$). When 2.5 V was applied, although pH increased slightly, the differences were insignificant. The pH 60 min after 5 V application increased significantly compared with that at the start of the AC application (Table Ⅰ). At higher concentrations of alginic acid in the gels, pH increased significantly. Thus, the highest pH was observed in 1.5% alginic acid gels at 5 V application (10.76 ± 0.11).

The pH of the control group was 7.23 ± 0.19 after 60 min of 5 V application. The pH changes in the control groups were smaller than those in the calcium alginate gel groups.

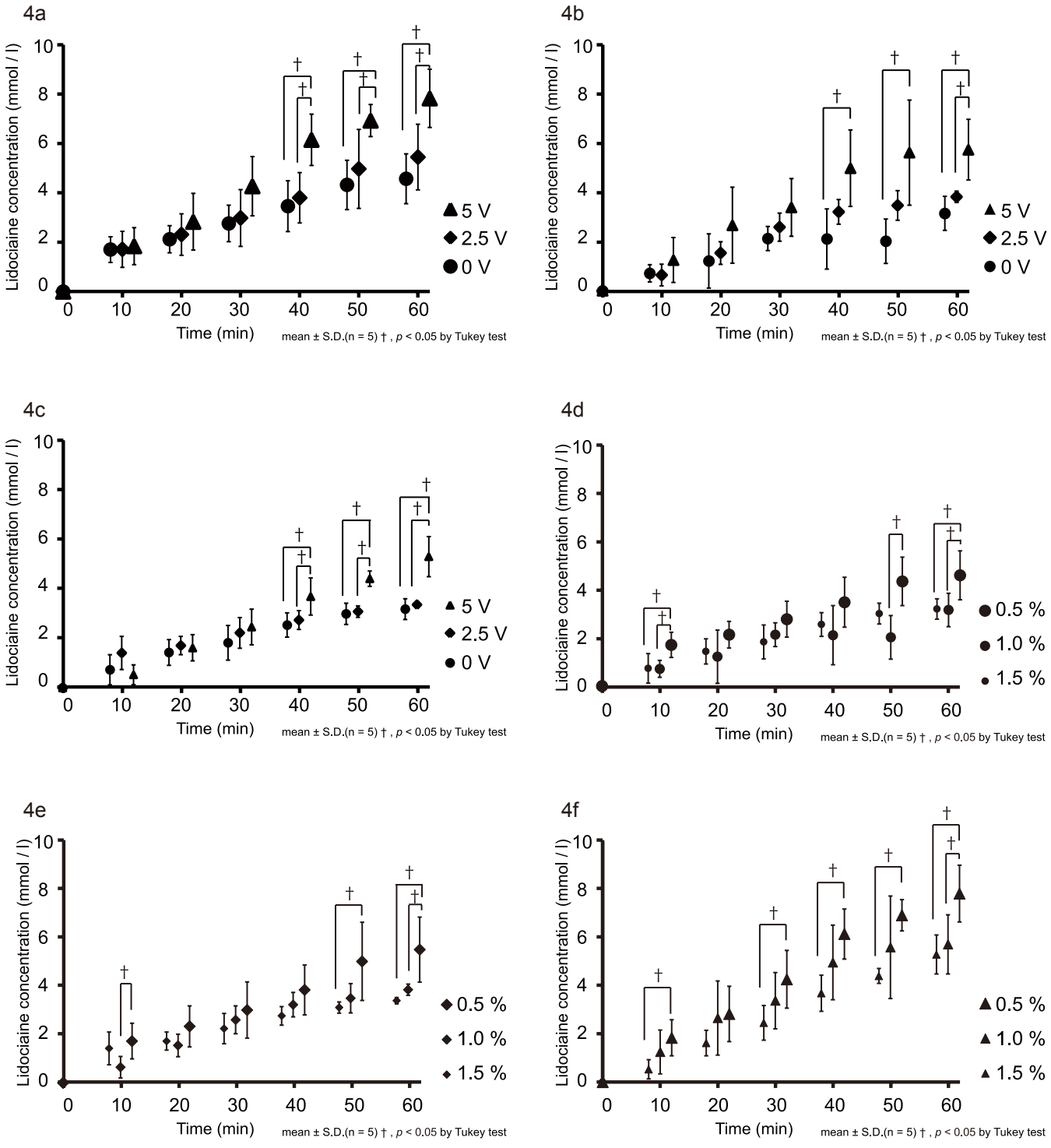

Figure 4 : Changes in lidocaine concentration in 0.5% (4a), 1.0% (4b) and 1.5% (4c) alginic acid gel groups with AC square-wave application with 80% duty cycle. Comparison of lidocaine concentration among 0.5%, 1.0%, and 1.5% alginic acid gel groups with AC square-wave application with 80% duty cycle at 0 V (4d: passive diffusion), 2.5 V (4e), and 5 V (4f) at 1 kHz for 60 min. The factor of two-way ANOVA test of Figures a through c was voltage and those of Figures d through f was concentration of alginic acid, respectively. The error bars represent the standard deviations from the mean. † indicates a significant difference among the conditions.

Table I. pH changes in receptor chamber. pH decreased at 0 V (passive diffusion) and increased significantly at 5 V at all concentrations of alginic acid. ¶ indicates a significant difference among the conditions.

Voltage (V)	Concentration of alginic acid (%)	0.5		1.0		1.5	
	Time (min)	0	60	0	60	0	60
0 (passive diffusion)		7.41 ± 1.08	5.96 ± 0.31	7.20 ± 0.77	6.07 ± 0.26	7.10 ± 0.80	5.80 ± 0.15
2.5		7.26 ± 0.80	7.32 ± 0.10	6.86 ± 0.05	7.03 ± 0.27	7.08 ± 0.33	7.31 ± 0.08
5		7.25 ± 0.74	8.27 ± 0.16 ¶	7.88 ± 0.53	10.22 ± 0.41 ¶	7.48 ± 0.85	10.76 ± 0.11 ¶

mean ± S.D. (n = 5). ¶ : $p < 0.05$ versus 0 min.

4. Discussion

4.1 Calcium alginate gel as electrodes for AC-IOP

Generally, the electrodes for IOP require tight bioadhesiveness to the skin or mucous membrane, should be able to contain a certain amount of drugs, should not be harmful, and should be easy to handle. When IOP is applied, many materials as electrodes are used, such as lint cloth, felt, paper towels, gauze, and nonwoven fabric. Such reservoirs keeping drugs are connected to a current generator by a soft metal or an alloy electrode[9]. However, these systems are very complicated, because the drugs need to be poured precisely into the reservoirs each time to prevent leakage from the electrodes. In addition, there are some disadvantages in using such materials as reservoirs: the electrodes are inhomogeneously contacted, causing an insufficient IOP effect. These reservoirs cannot adhere to the skin or mucous membrane because of their lack of bioadhesiveness, nor can they be applied to uneven, difficult-to-adhere surfaces; thus, they can only be used for flat surfaces.

Other materials have been developed as matrices for IOP electrodes; they are hydrophilic polymers, such as polyacrylic acid (PAA), polyethylox-azoline (PEO), and polymethacrylic acid (PMAA). These matrices can hold drugs but they have neither biocompatibility nor biodegradability. In addition, inflammation of the skin and mucous membrane may be caused by their materials of PAA, PEO and PMAA, poly (*N*-isopropyl acrylamide), poly (2-hydroxyethyl methacrylate), and polyvinyl alcohol[10]. We have therefore, studied alginic acid as a bioadhesive and biocompatible material without harmful effects on tissues for drug-loaded matrices that can adhere to the surface of a metal electrode[7].

Alginic acid is a natural anionic polysaccharide found in the surface of seaweeds such as laver and marine brown alga. Alginate solution quickly forms a gel matrix in the presence of a divalent cation such as Ca^{2+}, and gelation occurs primarily at junctions ("egg-box junctions") in G-G-sequence-rich chain regions (G-blocks) rather than in M-blocks[11]. In contrast to other gels such as agar, gelatin, and carrageenan, alginate calcium gel is thermostable (thermoirreversible gel). It is amenable to fabrication into various shapes, e.g., rods, disks, films, and microparticles, depending on the intended application and site of administration[12]. Its viscosity and intensity can also be controlled during its preparation.

It is biologically safe because it is of natural origin. Additionally, alginic acid is less expensive and easy to handle and prepare.

Alginate calcium gel is expected to be used as a vehicle for drug delivery because drugs or other polysaccharides can be incorporated within the matrix[11]. The formation of a netlike lattice between the cation and the alginate within the gel is responsible for the slow release of embedded drugs[8]. Owing to these unique features, the use of alginate calcium gel is evaluated as the matrix for the incorporated drugs.

Nowadays, alginate is used as a medicine, e.g., as a stomachic, as a nutrient[13], and for wound dressing[14,15]. In dentistry, it is widely used as an impression material. As an impression material, its adhesiveness to the mucous membrane is the first priority. Alginate is also utilized as a food additive, in cosmetics, and in other products. Alginate is expected to be used in future pharmacological applications, particularly in a drug delivery system[8].

Thus, we considered that calcium alginate gel can be used as a possible matrix for the electrodes in IOP applied directly to the oral mucous membrane or skin because of its unique properties, such as bioadhesiveness, capability to contain an appropriate amount of drugs, nontoxicity, and ease of handling.

4.2 Mechanism of lidocaine release from calcium alginate gels

Our study showed that lidocaine was released from calcium alginate gels following AC application. The mechanism of lidocaine release from calcium alginate gels is considered to involve one or more processes such as passive diffusion, electrorepulsion, electroosmosis, and syneresis from gels. Except for passive diffusion, they are affected by electric force. The positively charged lidocaine is considered to be loaded through its interaction with the acidic residues of alginic acid or to be physically absorbed into the gel layer[7]. The electric field applied to the gels electrorepulsively releases lidocaine ions out of the alginic acid gel layer, and lidocaine ions are transported to the cathode side.

As for syneresis, its two factors (time dependence and pH change) account for the partial release of water from calcium alginate gel, resulting in the transport of a large amount of lidocaine.

In this study, we observed no marked shrinkage of calcium alginate gels. This was because the application time was relatively short (1 h), the voltage was relatively low (5 V), and water was constantly replenished through the cellophane membrane.

4.3 Lidocaine concentration

Our study showed that a larger amount of lidocaine was transported at gels with lower alginic acid concentrations than at gels with higher alginic acid concentrations. This indicates that alginic acid blocks the lidocaine transport, probably owing to the netlike lattice between the cation and the alginate within the gel. The alginic acid concentrations used in our study ranged from 0.5 to 1.5%. When calcium alginate gel is used as the matrix of electrodes, it should be soft enough that it can conform with the uneven surface of the skin or mucous membrane. At the same time, it should be hard enough that it can maintain its position and does not flow from its site of application. At concentrations lower than 0.5%, the gels were very soft and delicate, making it difficult to place them into the experimental cells. Therefore, the appropriate concentration of alginic acid for use as a matrix of electrodes should be more than 0.5%.

Our study also showed that the amount of lidocaine transported increased when a high voltage was applied. Our previous study revealed that lidocaine transport depends on voltage[6], and the same dependence for calcium alginate gel and lidocaine hydrochloride solution was observed in this study.

In the 5 V group, lidocaine concentration increased significantly compared with that in the passive diffusion group; however, the lidocaine concentration in the 2.5 V group did not significantly differ from that in the passive diffusion group. This suggests that at voltages higher than 2.5 V, a significant amount of lidocaine can be transported from calcium alginate gel. Because significant amounts of lidocaine were transported in all groups with AC application from 40 to 60 min compared with the passive diffusion group, this indicates that the transport from calcium alginate gel started between 30 and 40 min after AC application.

4.4 Temperature changes

There was no significant difference in temperature between the passive diffusion group and the voltage-applied group, which suggests that the temperature of the gel was not affected by AC application. Therefore, thermal burns would not be a concern in its clinical use.

4.5 pH changes

The pH increased significantly when a concentrated alginic acid was added to the gels. The highest pH (10.76 ± 0.11) was observed in 1.5% alginic acid gels at 5 V of AC application.

The pH increase at 5 V indicates that the distilled water in the receptor chamber was electrolyzed, resulting in the production of OH^- ions. Our previous study showed that the reaction rate of electrolysis increases depending on the voltage and duty cycle[6].

On the other hand, the pH decreased during passive diffusion. This pH decrease associated with passive diffusion indicates that the dissociated lidocaine hydrogen ions ($C_{14}H_{22}N_2O\text{-}H^+$) spontaneously permeate from the donor chamber to the receptor chamber without water electrolysis, resulting in the accumulation of H^+ ions.

The unchanged pH in the 2.5 V group suggests that lidocaine H^+ ions transported from the donor chamber following electric application were cancelled by OH^- ions produced by electrolysis.

pHs higher than 12.0 likely cause skin damage with a high probability, whereas those lower than 11.0 cause less damage to the skin[16]. The Organization for Economic Cooperation and Development (OECD) test guidelines (OECD, 1992) state that the corrosiveness of a substance is observed at $pH < 2$ or $pH > 11.5$[17]. The pH increased significantly when 5 V was applied in all the calcium-alginate-gel-containing groups. However, serious chemical burns would not be a concern because the pH was below 11.0 on average.

For the minimum sequences of AC application to the skin or mucous membrane, shorter duty cycles, lower voltages, and alteration of frequency should be considered for clinical use. Furthermore, addition of a pH regulator to the gel or drugs could be an option.

4.6 Bipolar square-wave with 80% duty cycle

Lidocaine transport induced by a bipolar square wave with the 80% duty cycle was enhanced significantly compared with the case of passive diffusion. The findings show that lidocaine from the calcium alginate gel electrode was transported by the bipolar square wave with the 80% duty cycle.

Our previous study showed that the 60% at 20 V or 70% at 10 V duty cycle are good conditions for transport. More than 90% duty cycle induces larger pH changes[6]. However, we had to set the voltage much lower than that in the previous study, because the high voltage shrinks calcium alginate gel and the contact between the gel and the metal electrode is lost. Accordingly, we set the duty cycle at 80% for transport energy.

5. Conclusions

We confirmed that lidocaine was released from calcium alginate gels used as a matrix for IOP electrodes by square-wave AC application. The lidocaine transport depended on the alginic acid concentration in the gels. The temperature of the gels did not increase. pH increased, but it was below 11.0, showing a low possibility of serious chemical burns. Consequently, the calcium alginate gel can be used as a possible matrix for IOP electrodes.

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