Nontoxic and durable salt bridges using hydroxyethylmethacrylate hydrogels

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KINDLER, DEAN D., AND PETER R. BERGETHON. *Nontoxic and durable salt bridges using hydroxyethylmethacrylate hydrogels.* J. Appl. Physiol. 69(1): 373–375, 1990.—Polyhydroxyethylmethacrylate polymers (poly-HEMA) form hydrogels that provide an excellent alternative to agar in the production of salt bridges for use in bioelectrochemical experiments. A method for the simple production of poly-HEMA salt bridges is described. The poly-HEMA bridges were compared with agar bridges of similar geometry. Whereas poly-HEMA salt bridges have a conductivity that is 20 times lower than that of agar bridges of a similar geometry, poly-HEMA bridges are capable of dissipating twice the power compared with agar bridges. The mechanical properties of the poly-HEMA bridges make them easy to manufacture, store, and sterilize. When agar bridges were compared with poly-HEMA bridges in long-term cell culture experiments, the failure rate of the agar bridges was found to be ~10% per week vs. a virtually nonexistent failure rate for the poly-HEMA bridges. Because poly-HEMA salt bridges are reliable, durable, and nontoxic to cells, they are a practical alternative to agar salt bridges in certain experimental designs.

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IN BIOLOGICAL EXPERIMENTS that require the passage of electrical current as a means of stimulation, it is imperative to separate the metallic electrodes from the biological system to prevent contamination by electrolysis products. The standard method used to achieve this isolation involves employing a salt bridge composed of agar and a chemically and biologically compatible electrolyte solution (3, 4, 7, 8). Agar bridges are nontoxic and form a gel of an electrolyte solution; thus they carry current at a relatively low electrical resistance but effectively prevent significant diffusion of contaminants. Despite good electrical characteristics, agar bridges are fragile and fail with a high frequency during lengthy experiments or in experiments that require the movement and bending of the bridges. Because salt bridges are used extensively in this laboratory in stimulation experiments of IMR-90 lung fibroblasts and neonatal rat aortic smooth muscle cells, it was found necessary to design a system that significantly improved on the durability limitations of the time-honored agar salt bridge.

Hydrogels made of hydroxyethylmethacrylate (HEMA) have been used in cell culture systems as a nontoxic and durable matrix for cell growth (1, 2, 5). It is the nature of the hydrogel polymers to imbibe large quantities of aqueous solution, and in the case of HEMA hydrogels water can account for 30–40% of the final volume of the matrix (6). By taking advantage of these characteristics, a method to make biocompatible salt bridges using HEMA as the gelling matrix for the electrolyte solution was developed and tested.

METHODS

Materials. Tris(hydroxymethyl)aminomethane (Tris), sodium chloride, ammonium persulfate, and sodium bisulfite were purchased from Sigma Chemical, St. Louis, MO. 2-HEMA was purchased from Aldrich Chemical, Milwaukee, WI. Polyvinyl chloride (PVC) tubing, 3.175 mm (⅛ in.) ID by 6.35 mm (¼ in.) OD, was purchased from VWR Scientific, San Francisco, CA.

Preparation of hydrogel bridges. Hydrogels were prepared by polymerizing the monomer in the presence of the electrolyte solution. The hydrogel bridges of polyhydroxyethylmethacrylate were formed by mixing a 1:1 (vol/vol) mixture of monomer and an aqueous buffer of either 0.15 M NaCl or 0.05 M Tris·HCl in 0.15 M NaCl, pH 7.44. After degassing under low pressure and while the solutions were on ice, 0.1 ml of 12% sodium bisulfite and 0.1 ml of 6% ammonium persulfate solution (per 1 ml of monomer) were added to the mixture. A syringe was attached to one end of a 15-cm length of PVC tubing, and the monomer-aqueous solution was drawn into the tubing. With the syringe attached to one end and the open end of the tube dipped in excess polymer mixture, the bridges were placed at 37°C for 2 h at which time polymerization was completed inside the tubing. The syringe was cut from the tubing and the lengths of the bridges were reduced to a standard length of 12 cm. To remove residual monomer and catalyst, the polymerized bridges were then exhaustively dialyzed against the aqueous solution used in their preparation. The bridges were sterilized by treating each end with 70% ethanol before use in cell culture experiments.

Preparation of agar salt bridges. The agar-salt solution

used to make the salt agar bridges consisted of 3 g/100 ml agar in 0.15 M sodium chloride. This agar solution was brought to a boil with constant stirring. The molten agar was then drawn up into 15-cm lengths of PVC tubing as described for hydrogel bridge preparation. Sterile agar salt bridges were prepared with the following modification. After molten agar was prepared it was dispensed into a 250-ml beaker. The ends of the PVC tubing were submerged in the agar, and the agar and tubing were then autoclaved and allowed to cool. During cooling a partial vacuum is created that draws the sterilized agar up through the tubing where it gels and forms a sterile bridge.

Conductance measurements of hydrogel and agar bridges. The conductance of each bridge was measured with a Yellow Springs Instruments model 31 conductivity bridge. The salt bridges were connected to the circuit by a pair of platinum black electrodes immersed in an aqueous solution that matched the composition of the aqueous solution in the gel. Each platinum electrode was fixed in position, and a consistent volume of buffer was maintained surrounding the electrodes. The cell constant of this bridge-conductivity cell was determined by standardization to a primary reference conductivity electrode. The conductivity of each salt bridge was expressed in S/m.

Power dissipation measurements of hydrogel and agar bridges. Measurement of the power dissipation of the hydrogel and agar salt bridges was studied by the stepwise increase of potential across the bridges with a constant voltage source. The changes in current for each step over time were recorded. Power consumption was followed for a minimum of 10 min per step to ensure that the power curve was unchanging before the next potential step was applied. A Bio-Rad Laboratories model 500 power supply was the current source. A Fluke 8050A digital multimeter (DVM) was used to measure current. The voltage steps for the hydrogel bridges were 50, 100, 200, 300, 350, and 400 V. Voltage steps for the agar salt bridges were 5, 20, 50, and 100 V.

In use failure rate determination of hydrogel and agar bridges. The failure rate of the bridges was tested by connecting a group of five bridges in parallel between two beakers filled with 0.15 M sodium chloride solution and connected to the power supply through platinum electrodes. A constant voltage source maintained a potential across the system throughout the experiment. The voltage was chosen to force the bridge to dissipate power at a level judged to be the upper tolerance limit for the bridge. In the case of the agar bridges the voltage was 20 V, which set the initial power level at 60 mW per bridge. The potential chosen for the HEMA bridges was 175 V, which provided a power level of 400 mW per bridge. Each bridge class (HEMA vs. agar) was tested separately. Because each bridge drew essentially the same current under constant voltage, by measuring the total current through the circuit with the DVM, the failure of a bridge could be monitored by the decrease of current in the total circuit. All trials were performed in an environmental chamber maintained at 37°C.

RESULTS

On polymerization within the PVC tubing, the HEMA forms a uniform translucent matrix of electrolyte and polymer. The gels are fully hydrated at the completion of polymerization with any of the aqueous solutions, and the salt bridge is extremely flexible and durable. As long as both ends of the bridge remain in a solution, the tubing protects the gel from contamination and desiccation almost indefinitely.

Bridges made with HEMA were conductive, but their conductance was a magnitude lower than that of salt bridges made from agar. For comparison, the average conductance of a 12-cm-long (3.175 mm ID) agar salt bridge was 1.61×10^{-4} S compared with a conductance of 8.5×10^{-6} S for a HEMA bridge of equivalent dimension. If normalized for a bridge with an area of 1 cm^2, the agar bridge has a conductivity of 1.81 S/m compared with the HEMA bridge conductivity of 0.095 S/m. Because of their higher conductivity, the agar bridges were capable of carrying a higher current at a given voltage compared with the HEMA bridges (Fig. 1); however, the HEMA bridges were capable of dissipating far more power than their agar counterparts. On the average, the HEMA bridges could handle 8 W of power (20 mA at 400 V) for 10 min before failing, whereas the agar bridges could handle only 4.1 W (41 mA at 100 V) for 10 min without failing. As Fig. 1 also shows, the conductivity (represented by the slope of the current vs. voltage curve) is stable at power levels below 60 mW for agar bridges and 500 mW for the HEMA bridges. With time, however, the conductivity starts to rise secondary to joule heating at power levels above these limits.

Experience with manufacturing and use as well as

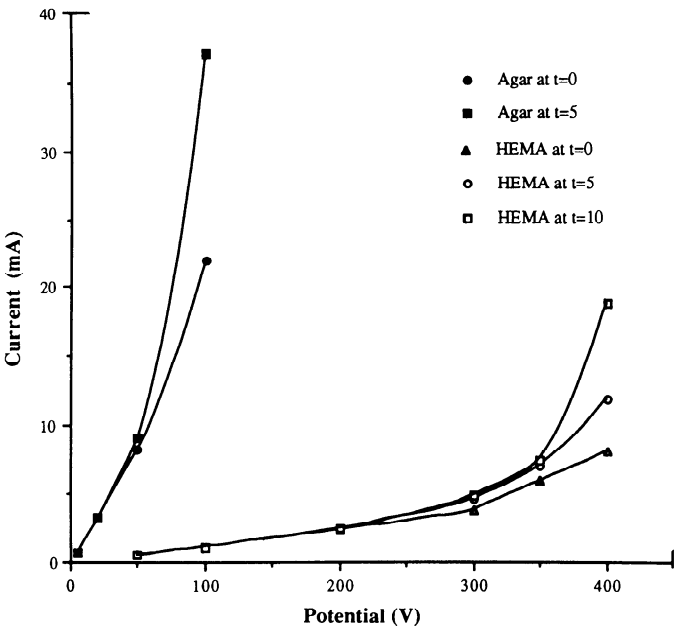

FIG. 1. Representative current vs. voltage curves for 12-cm-long salt bridges composed of 0.15 M NaCl and either agar or poly-HEMA as described in text. Each data point represents average of 3 measurements on 5 equivalent bridges. Change in conductivity secondary to joule heating is indicated by increasing steepness of slope of curves with increasing time of power dissipation. t = 0, Initial time of power dissipation; t = 5 and 10, 5 and 10 min after power dissipation, respectively.

experiments to induce failure demonstrate differences in the durability and reliability of the HEMA and agar salt bridges. The rate of production of defective bridges during these experiments was nearly 30% for the agar bridges whereas the defective rate for the HEMA bridges was <2%. These rates are similar to those experienced during routine experimental protocols. In a provocative test performed at 37°C, at initial power levels of 60 mW for the agar bridges and 400 mW for the HEMA bridges, both types of bridges showed a steady rise in conductivity secondary to joule heating. The rise in conductivity led to a progressive increase in current drawn from the power source and hence increased power consumption by the bridge. Under these conditions all of the HEMA bridges failed within 24 h, and all of the agar bridges similarly failed within 48 h. Importantly, during experimental use in which a constant current source was used and the power dissipation was well below the tolerance limit of the bridges (0.2 mW for HEMA bridges and 0.01 mW for agar bridges), failure rates favored the use of the HEMA bridges. For HEMA bridges the failure rate was 0% (0/40 failures over an 8-mo period). Twenty of these bridges were used for 14 days in cell culture experiments at 37°C. Alternatively, the use of agar bridges in similar experimental designs was associated with a failure rate of ~1 in 10 failures per week during long-term experiments.

DISCUSSION

The salt bridge constructed of a matrix of HEMA and an electrolyte solution offers a reasonable alternative to the agar salt bridge. HEMA bridges are inexpensive and easy to make, sterilize, and store. They are very reliable, durable, and nontoxic to cell systems. However, because the conductivity of the HEMA bridge material is significantly lower than that of agar, HEMA-based bridges do require a higher voltage than their agar counterparts to maintain a set current. If necessary, this limitation on conducted current can be overcome easily by altering the geometry and dimensions of the bridge, i.e., by increasing the diameter.

Our experience has proved that the HEMA salt bridge provides an excellent tool in low-current electrical stimulation experiments where it is necessary to eliminate electrolysis products. Although both HEMA and agar bridges are sensitive to destruction from runaway joule heating if a power dissipation is chosen that incorporates an adequate safety margin, the HEMA bridges maintain structural and electrical integrity longer and better than the agar salt bridge. This durability is probably due to the physical property of extreme flexibility of the HEMA bridges. HEMA bridges can be drastically bent, looped, or twisted with little fear of disrupting their continuity. The agar salt bridge will almost always crack and tear under the same manual strain, with the subsequent loss of its electrical integrity. Although it is unlikely to ever replace the agar bridge in all applications, the HEMA salt bridge provides a viable and effective alternative to the agar salt bridge and is worth adding to the bioelectrochemist's and electrophysiologist's trick bag.

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