Mechanical properties of mammalian single smooth muscle cells
II. Evaluation of a modified technique for attachment of cells to the measurement apparatus

J. J. GLERUM* and R. VAN MASTRIGT
Departments of Urology and Biomedical Physics and Technology, Erasmus University Rotterdam, The Netherlands.

Received 27 June 1989; accepted 18 April 1990

Summary
A method is described for attaching isolated single smooth muscle cells to an apparatus designed for measuring the longitudinal forces developed passively and actively by the cell upon straining, electrical or pharmacological stimulation. Primary attachment of the cell is based on its natural negative surface charge in combination with a positive surface charge on the micro-tools used for attaching. Definite attachment is obtained by a knotting technique. Results show that this method of attachment is reliable and strong enough to withstand forces exceeding those necessary to break or tear the cell.

Although this method allows relatively short cells to be attached (L > 80 μm) alternative methods e.g. gluing, are necessary to attach the shortest smooth muscle cells.

Introduction
In the field of muscle contractility research, especially on smooth muscle, there is general agreement on the value and necessity of single cell contractility measurements (Fay et al., 1976, Glerum et al., 1987). Amongst the essentials needed for such measurements are methods for single cell isolation, a sensitive microforce transducer and a method for cell attachment. In the first paper on single cell measurements Fay (1977) introduced a method for knotting a single cell to micro-tools. A number of other methods of smooth muscle cell attachment have been published, e.g. a method of sucking the cell ends into micro-pipettes (Ishii & Takahashi, 1982). This introduces the problem of maintaining compliance and stability in the measurement apparatus as the necessary vacuum has to be created, transduced, maintained and controlled separately from the actual transducer. In the quoted references it was shown that this problem can be solved by optically measuring the degree of bending of the tip of the micro-pipette the cell is sucked into, but due to a relatively long optical path, the long term stability of such a measurement method is poor and calibration of each new pipette tip is necessary (Ishii, 1988, personal communication). Another disadvantage of methods involving the measurement of the bending of a pipette tip, is the non-optimal axial pull on the smooth muscle cell. The cell either has to bend sharply at both ends, as in Ishii's method (Ishii et al., 1988), or has to be folded in the middle as in the methods of Tung (1986) or Van Dijk-Looyaard (Van Dijk et al., 1984). In this last method cells are clamped to the bottom of the cell incubator so that the stability of the attachment depends on the friction between the cell and the bottom of the incubator and also between the cell and the micro-tips. As both levels of friction are generally low, slipping of the attachment does occur in this method.

The best way of attaching a muscle cell would simply be to glue it to the micro-tools in use, but except for the use of fibrin glue (Copelas et al., 1987), almost all attempts to do so have failed thus far. The major problem of gluing, besides finding a bio-compatible glue, lies in the far more difficult control of glue solutions in micro-pipettes and injectors and the controlling and positioning of a significantly increased number of micro-manipulators.

In this article we report our experiences with the knotting technique as originally published by Fay (1977) and described in detail by Warshaw & Fay (1983), applied to pig urinary bladder and human term pregnant uterus smooth muscle cells. Several fundamental changes in this method were necessary, which led to significant improvements.

*To whom all correspondence should be addressed at: Department of Urology, Erasmus University, Room EE 1630, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.
Materials and methods

Single smooth muscle cells from pig urinary bladders were isolated by enzymatic digestion of strips of bladder tissue collected at the local slaughterhouse. Single smooth muscle cells from a term human uterus were obtained likewise from biopsies excised at Caesarean sections. Both procedures were carried out as described previously (Glerum et al., 1987), except for an additional overnight incubation in collagenase at 4°C for both types of tissue and an additional trypsinisation of the pig bladder tissue.

To be able to select vital cells during the experiments a Fluorecine Di-Acetate vital staining technique was used: After isolation the cells were incubated in a freshly prepared diluted FDA solution for 15 min., next the FDA was removed by centrifugation and the cells were resuspended in 2 ml of Krebs-Hepes buffer and 1 ml of MEM/FCS. This cell suspension was immediately poured into the cell incubator, the vital cells thus showing a clear green intracellular fluorescence. Detailed description of the procedure is available on request.

During experiments the cells were incubated in a specially constructed cell incubator with a bottom of optical glass mounted on a Zeiss inverted microscope equipped with phase contrast and incident light fluorescence optics. Conditions of incubation were kept at temperature 37°C, Po2 150 mm Hg and Pco2 38 mm Hg approximately, thus resulting in pH 7.35 (Glerum et al., 1987). Fluid evaporation was prevented with a thin layer of Klearol (Van Dijk et al., 1984).

Cell attachment micro-tools were drawn as micro-pipettes from Clark Medical capillary glass tubes (outer diameter 3.0 mm, type: GC200-15), with a tip diameter of approximately 50 μm at the base of the tip and 1-2 μm at the end of the tip, the tip diameter thus conically declined over a length of approximately 5 mm. After drawing, the micro-pipettes were bent in a Z like manner (Fig. 1.), in order to avoid a non-horizontal working plane of the transducer and length displacement instrument.

Cells were attached between a fixed micro-pipette mounted to a Märzhauser three-dimensional electromechanical micro-manipulator (type STM 3), fixed onto the microscope object table, and a similar pipette connected to an opto-mechanical micro force transducer, developed in our own laboratory, with a sensitivity of 10 μN V⁻¹, a linear range of ± 140 μN, a resolution better than 0.1 μN and a long term drift of approximately 2 μN h⁻¹ (Glerum & Van Mastrigt, 1990). This transducer was mounted on a specially designed vertical movement stage connected directly to the side of the cell incubator. Figure 1 shows a cross-section through the central parts of the measurement apparatus.

Prior to the attachment procedure the tips of the micro-pipettes were coated by dipping them for 5 min in a 0.1 mg ml⁻¹ solution of Poly-L-Lysine (mol. weight 100 000, Sigma, P 1274), and subsequent rinsing for a few seconds in distilled water.

The smooth muscle cell selected by means of the fluorescence technique was first, at one of its ends, approached by the

Fig. 1. Cross-section of the cell measurement apparatus. The cell is attached between two Z-bend micro-pipettes. The right pipette (FTP) is connected to an ultrasensitive force transducer, the left pipette (LTP) is connected to a length displacement apparatus. The cell is submerged in a temperature controlled cell incubator mounted on an inverted microscope.