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Forisome based biomimetic smart materials

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Abstract. With the discovery in plants of the proteinaceous forisome crystalloid (Knoblauch, *et al*. 2003), a novel, non-living, ATP-independent biological material became available to the designer of smart materials for advanced actuating and sensing. The in vitro studies of Knoblauch, *et al*. show that forisomes (2-4 micron wide and 10-40 micron long) can be repeatedly stimulated to contract and expand anisotropically by shifting either the ambient pH or the ambient calcium ion concentration. Because of their unique abilities to develop and reverse strains greater than 20% in time periods less than one second, forisomes have the potential to outperform current smart materials as advanced, biomimetic, multi-functional, smart sensors or actuators. Probing forisome *material properties* is an immediate need to lay the foundation for synthesizing forisome-based smart materials for health monitoring of structural integrity in civil infrastructure and for aerospace hardware. Microfluidics is a growing, vibrant technology with increasingly diverse applications. Here, we use microfluidics to study the surface interaction between forisome and substrate and the conformational dynamics of forisomes within a confined geometry to lay the foundation for forisome-based smart materials synthesis in controlled and repeatable environment.

Keywords: smart materials; smart actuators; biomimetic materials; microfluidics.

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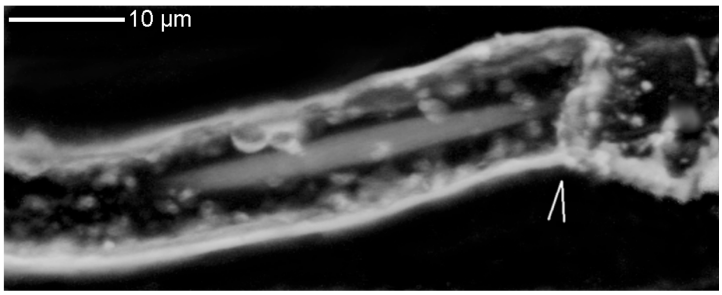

Fig. 1 Forisome in a living sieve tube of the broad bean, *Vicia faba*. In this false-color image produced by confocal laser scanning microscopy, the forisome is shown in dark gray, while the sieve tube appears in light gray. The arrowhead highlights a so-called sieve plate, a perforated cross wall found at regular intervals in this natural microfluidics system. The forisome is seen in the elongate low-calcium state, permitting mass flow through the sieve tube. Taken by Knoblauch and Peters (2005).

1. Introduction

Biomimetics is the field of the engineering sciences in which inspiration is sought from biological systems for the design of novel materials and structures. Natural systems have the capacity to sense their environment, process this data, and respond. For example, the Venus flytrap, a carnivorous plant, has prey-capturing behaviour and can execute repeatable reversible mechanical actions very swiftly. The scientific underpinnings to permit direct engineering of biomimetic machines analogous to a living plant, such as the Venus fly trap, do not yet exist since they require the integrated functioning of exceedingly complex biochemical systems, including but not limited to lipid bilayer membranes, ion channels, aquaporins, and metabolically-powered hydrogen ion transporters (Mann 1995, Fleurat-Lessard, *et al.* 1997, Leopold and Jaffe 2000, van Volkenburgh 1999).

With the discovery of behaviour of the plant protein bodies called forisomes (Knoblauch, *et al.* 2003), a novel, non-living, smart, ATP-independent[1] biological material capable of advanced sensing and actuating became available to the designer of smart materials. In the living plant, forisomes act as reversible valves in the sieve-tube network, a natural microfluidics system serving the distribution of photosynthetic ally produced sugars throughout the plant body (Fig. 1 of Knoblauch and Peters 2004a). The *in vitro* studies of Knoblauch, *et al.* (2003) show that, by the application of shifts of pH or calcium concentration, forisomes (2-4 µm wide and 10-40 µm long) can be repeatedly stimulated to contract and expand anisotropically and swiftly (on the order of ms). The calcium-inducible contraction by some 30% along the longitudinal axis is accompanied by a doubling of the forisome diameter (Fig. 2b). Forisomes exert similar mechanical forces in expansion and contraction. Furthermore, forisomes can be isolated, freeze-dried, rehydrated, and employed *in vitro* as desired. No functional metabolic apparatus is required for forisome-based smart actuation. These features make forisomes a prime candidate to outperform current smart materials, such as piezoelectric materials, shape-memory alloys, and synthetic hydrogels/polymers in functioning as advanced smart valves and actuators. Despite their obvious potential, our understanding of forisomes still is limited. In this paper, we utilize microfluidic devices to probe surface interaction between forisomes and substrates and to study forisome conformation

[1]ATP (adenosine triphosphate) is a molecule that consists of the nitrogenous base adenine linked to the sugar ribose and has a chain of three phosphate groups attached to the ribose in a linear fashion. ATP, which is present in all living cells, is the fuel which generates electricity in nerves and contraction in muscles.

kinetics within a confined geometry, to lay the foundation for forisome-based smart materials synthesis.

1.1. Comparison of forisomes with other exisiting materials

Smart materials systems are nonliving systems that integrate the functions of sensing, actuation, logic, and control to respond repetitively to external stimuli (Shahinpoor and Thompson 1995, Culshaw, 1995, Mavroidis and Dubey 2003). Often the system response can convert one form of the energy to another. We will focus on the materials component and review some current smart materials and biomimetic motor proteins, followed by a table comparing **forisomes** with these materials. Due to space limitations, references are limited mainly to review articles.

1.1.1. Piezoelectric materials

Piezoelectric ceramics/polymers exhibit swift, linear shape changes in response to applied electric fields. The electricity makes the material expand or contract almost instantly. Piezoelectric ceramic materials are fabricated from components that exhibit piezoelectricity in their crystalline form. Single-crystal piezoelectric materials can only be polarized down a particular axis and often exhibit spontaneous polarization. The advantages of piezo-ceramics include high efficiency, fast response, and compactness. A commonly used piezo-ceramic is PZT (Giurgiutiu and Rogers 1997, Lin and Chang 2002). PZT patches/ paints (Egusa and Iwasawa 1998, Ma 2003) are often used as both sensors and actuators for vibration control. PVF (polyvinylidene fluoride) is a well known piezoelectric polymer that can be easily handled and has a good strain to voltage conversion efficiency, but requires high voltages to function as an actuator.

1.1.2. Shape memory alloys (SMA)

Shape memory alloys can change shape in response to heat or cold. When an SMA is below its transformation temperature, it has a very low yield strength and can be deformed easily into any new shape (Tadaki, *et al.* 1998, Baz 2001). When the material is heated above its transformation temperature it undergoes a change in crystal structure, which causes it to return to its original shape. If the SMA encounters any resistance during this transformation, it can generate large forces. This phenomenon provides a unique mechanism for remote actuation. However, it is hard to control shape memory alloys precisely due to their nonlinear features. The most common shape memory material is an alloy of nickel and titanium called Nitinol.

1.1.3. Stimuli sensitive materials (SSM)

Stimuli-sensitive materials cover a broad spectrum of materials that respond to parameters such as pH, temperature, and electric/magnetic fields (Shahinpoor 2003, Kuhn, *et al.* 1950, Yu, *et al.* 2001, Cohen 2001). These materials show promise in applications ranging from pumps and valves, to shock tube instrumentation. Electrostrictive materials are similar to piezoelectric materials, with slightly greater strain capability which depends on the temperature. Magnetostrictive materials (Kellogg and Flatau 2004) such as Terfenol-D elongate when exposed to a magnetic field. Conducting polymers and ionic polymer-metal composites (IPMCs) (Millet 1990, Nemat-Nasser 2002) are among those electro-active polymer materials that have shown tremendous potential to make efficient actuators. The electroactive polymers (EAPs) can be deformed repetitively by applying external voltage and can quickly recover their original configuration upon reversing the polarity of the applied voltage.

Hydrogels have been developed recently (Yu, *et al.* 2001) to achieve autonomous flow control in microdevices.

1.1.4. Smart fluids

There are two main classes of smart fluids: electrorheological (ER) and magnetorheological (MR) fluids (Melek and Heming 1999, Zukoski 1993). ER fluids generally consist of semi-conducting particles suspended in a dielectric oil, whereas MR fluids use magnetizable particles suspended in a non magnetizable carrier liquid. The excitation of the fluid by the appropriate field (electric or magnetic) causes polarization and subsequent alignment of the particles suspended within the liquid. The resulting chain structure is held in place by the applied field, and hence resists fluid flow. MR and ER fluids are being developed for use in car shocks, valves, and damping structures (Yalcintas and Coulter 1998).

1.1.5. Biomimetic protein actuators

ATP (adenosine triphosphate) is the universal "energy currency" of all living cells. Cells maintain the ratio of the concentrations of ATP and its components, ADP (adenosin-di-phosphate) and phosphate, far from equilibrium by metabolic processes. This arrangement allows the chemical energy stored to be utilized as a driving force for unrelated, ATP-consuming reactions such as muscular contraction or the maintenance of signaling capability along nerve fibers. ATP-driven motor protein molecules maintain order and polarity in cells by carrying cargoes (molecules or cell organelles) along the febrile tracks of the cytoskeleton to their appropriate positions (Bray 2001). While these motors possess promising characteristics, their dependence on strictly defined chemical environments certainly is a disadvantage for technological applications (Knoblauch and Peters 2004b). The number of currently known biological polymers (other than the protein elastin; Urry, *et al.* 2002) that may serve as models for ATP-independent smart actuators is small (Knoblauch and Peters 2004b). For example, nematode sperm cells crawl despite lacking a functional cytoskeleton, utilizing forces that result from the reversible, probably pH-regulated polymerization of the unique main sperm protein MSP (Bottino, *et al.* 2002). Forisomes (Knoblauch and Peters 2004a) in the sieve tube system of leguminous plants undergo reversible, swift contraction-expansion cycles, and the contraction can be generated by electrically induced pH waves.

The table below compares the performance parameters of forisomes with some existing smart materials. We observe that most materials excel in some measures of performance but are unsatisfactory in others. For instance, piezoelectric materials have fast response (>100 Hz) but only allow for a maximum strain of 0.2%; hence PZT is not attractive for applications where large displacement motion is essential. Many smart materials can be embedded in composite structures and utilized for active shape control for

Table 1 Comparisons of different materials properties, from Culshaw (1995)

Material type	Max strain (%)	Young's modulus (MPa)	Energy density (MJ/m^3)	Bandwidth (Hz)	Power density (MW/m^3)
Electroactive P(VDF-TrFE)	7	300	0.7	> 2 (est.)	> 5 (est.)
Polyacrylate elastomer	29	0.13	0.02	> 2	> 0.04
Piezo-ceramic	1	nonlinear	0.03	> 1	> 0.1
Shape memory alloy	8	30000	100	< 0.25	100
Forisome	**30-200**	**0.04 (est.)**	**0.6 (est.)**	**> 1**	**10 (est.)**

structural health monitoring. The use of forisome-inspired proteins as a building block opens the prospect of constructing a material that is smart with large strain, stiff (integrate forisome with hard composites) with quick response, large anisotropic deformation under various stimuli for health-monitoring applications.

Here, bandwidth is used in the sense of strain repetition rate. Energy density is calculated as Young's modulus times fractional strain (except for forisomes). For forisomes, the Young's modulus is defined as (maximum force)/[(cross-sectional area of condensed phase)(longitudinal strain in dispersed phase)] to give 0.04 MPa. To estimate the energy density of forisomes, we use elementary electromagnetics: in its condensed phase, a forisome is rather like a loosely bound ionic crystal which would account for its stiffness under compression. Supposing that the calcium-driven phase transition depends upon calcium ions penetrating the crystal and turning selected –+ ionic bonds into a ++ sites of electrostatic repulsion, we then calculate Coulomb energy stored in the ++ couple upon its formation and estimate the energy density to be 0.6 MJ/m^3.

1.2. Material properties of single forisomes

The *in vitro* studies of Knoblauch, *et al.* (2003) suggested that the forisome is a natural prototype of new smart materials and technical molecular motors. In its native contracted state in the favabean sieve tube, the forisome apparently is tethered stably within the translocation stream and, when triggered by an increase in the concentration of free calcium, performs a sudden phase transition into its expanded conformation. In the process, its shape changes from elongated spindle-like to ovoid, with a concomitant tripling in volume. The swollen forisome then blocks translocation in the sieve tube; in fact, rapid reversible blockage of phloem is well known (Pickard and Minchin 1990, Lang and Minchin 1986). The forisome is a proteinaceous crystalloid which is not membrane-bound (see Fig. 2), is non-living, and does not require a functional metabolic machinery for its successful operation. By appropriate chemical or electrical stimuli, forisomes isolated from the living organism can be caused to undergo phase transitions between the contracted and the expanded state.

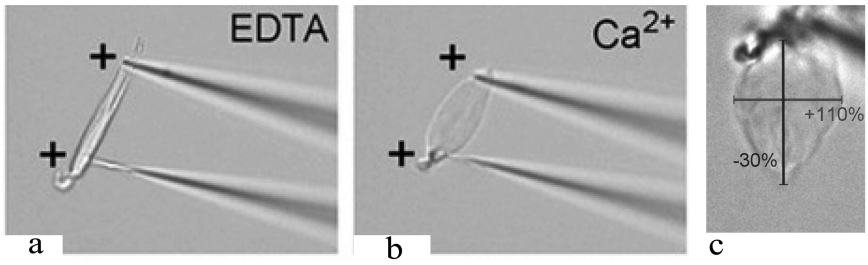

Fig. 2 (a and b): Ca^{2+}-induced change of shape in an isolated forisome fixed on two glass micropipettes. The forisome is seen in the longitudinally elongated, low-calcium state (a: Ca^{2+}-levels were kept at submicromolar levels by the chelator EDTA), and in the radially expanded high-calcium conformation (b). Note that the glass pipettes are bent by the contracting forisome (black crosses are at identical positions for comparison in both images). (c): Forisome in the longitudinally contracted state; relative changes of length and diameter as compared to the expanded state are indicated.

1.2.1. Single forisome conformation kinetics

After extraction from *Vicia faba* sieve tubes, single forisomes are transferred to Ca^{2+} free medium in a test chamber that allows microscopic observation. The following key observations were made by Knoblauch, *et al.* (2003) when forisomes were subjected to the external stimuli.

1. Forisomes swell and deswell with Ca^{2+} and pH variations. In contraction, forisomes shorten longitudinally by 30% and lengthen in diameter by 120%, resulting in a more than three-fold volume increase.
2. The anisotropy of the response to Ca^{2+} occurs only in elongated, undamaged specimen. Forisomes that had been turned into more or less spherical masses due to mechanical damage during the isolation procedure, swelled and deswelled isotropically.
3. Forisomes generate mechanical forces during their conformational changes. Preliminary experiments showed that when the tips of a forisomes are attached to flexible glass pipettes, the pipettes become bend during Ca^{2+}-induced forisome contraction (Fig. 2a and 2b). The minimum force generated by forisomes in such tests was estimated to be 0.1 μN; the maximum force has still to be determined. Intriguingly, similar forces appear to be generated in contraction and expansion.

2. Probing forisome properties using microfluidic devices

Microfluidics is a growing, vibrant technology with increasingly diverse applications. Microfluidics enables biological processes to be examined on a few micron scale at which physical processes such as osmotic movement and surface interactions become important. To synthesize forisome based smart materials, we need to understand the binding properties between forisome and substrates, and the conformation kinetics of forisomes inside a hosting matrix at small length scales. Microfluidics involves the manipulation of fluid flows at a small length scale which has been useful in studying the modification of fluid motion by surface effects. The precision offered by microfluidic systems is of proven utility in fundamental studies of chemical processes, material properties, and cellular ultrastructure. Microfluidic flows can be manipulated using external fields, such as pressure, electrical current, acoustic forces, magnetic fields, and capillary forces. At small length scales, the forces involving the surface area to volume ratio play an important role (Stone and Kim 2001). Since our ultimate goal is to synthesize forisome based composites, we need to understand how forisomes behave inside a small confined geometry and surface interaction between forisomes and different material substrates. Investigating forisomes conformation kinetics inside microfluidic devices will provide a good benchmark for this purpose.

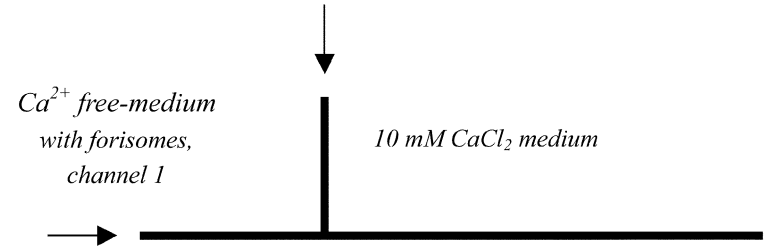

Fig. 3 Initial microchannel geometry and setup. Microchannels range from 100-1000 microns wide

2.1. Methods

The microfluidic channels are etched into a PDMS slab (polydimethylsiloxane) deposited upon a glass slide and were prepared using standard soft lithograph techniques (Xia and Whitesides 1998). The geometry used is simply a T-intersection with 100--1000 micron size wide channels. More complicated structure can be designed using any graphic software (See Fig. 3). A dilute forisome suspension (forisomes in a Ca^{2+} free-medium (10 mM ethylenediaminetetraacetic acid [EDTA] in aqueous solution with 100 mM KCl, 10 mM trizma buffer and HCl, pH 7.3) is injected into one channel arm at flow rates between 1.5-3.0 mL/h; 10 mM $CaCl_2$ solution is injected a solution into another arm.

While the task of depositing forisomes into microchannels is not a trivial one, the potential benefits is well worth the effort. Calcium solution can be rapidly introduced to and removed from the forisome. This process can also be done reproducibly. Over 100 repetitions of swelling and de-swelling have been observed with a single forisome in less than 10 minutes. The flow of calcium solution relative to Ca^{2+} free-medium can be easily controlled within the channels. Using the conventional technique involving micropipettes, it is difficult to complete large repetitions of swelling and de-swelling in a timely fashion.

Technical difficulties arise during the forisome deposition process inside the microchannel. In order to get the forisomes to stay within the channel, two important factors must be considered. First, the flow of Ca^{2+} free-medium containing forisomes must be fast enough that the forisomes do not collect preferentially near the entry point of the channel. Secondly, the flow must be slow enough so that the forisomes will bind to the glass in the channel and will not simply wash straight through. Essentially, there is a delicate balance that must be achieved in order for the forisomes to stick in a useful position within the microchannels.

When using the 100 μm microchannel, it is quite difficult to get forisomes to adhere properly within the channel: depending upon the flow rate, the forisomes either collected near the entry point, or washed straight through the channel. Better success was achieved using the same geometry but with channels up to 1 mm in width. Many forisomes still appeared near the entry point, but in contrast to the narrower channels, forisomes were also found in all regions of the microchannel. At low flow rates (0.5

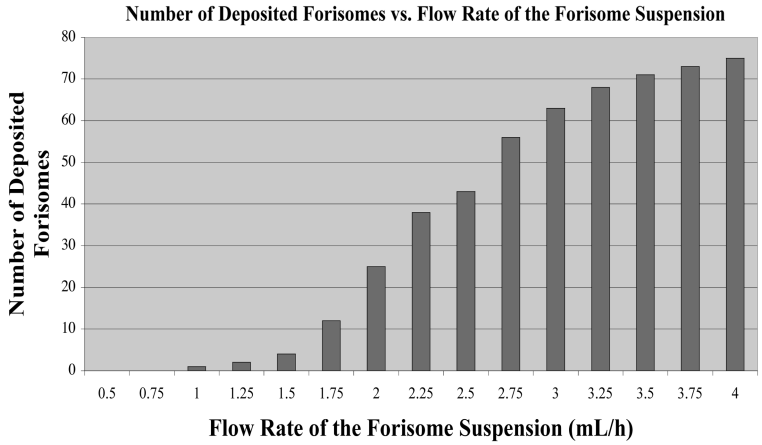

Fig. 4 Number of forisomes tethered to the microchannel walls of a 1 mm section of a microchannel (1 mm wide, 0.1 mm deep) as a function of the flow rate of the forisome suspension in the channel

mL/h--1.5 mL/h), the forisomes tended to collect near the entry point and did not travel very far down the channel. Obviously this hinders the ability to control the calcium solution when swelling and de-swelling the forisomes. At higher flow rates, forisomes continue to collect at the entry point, but forisomes can also be seen further down the channel. Using the wider channels, and flow rates of above 1.5 mL/h of Ca^{2+} free-medium with forisomes, forisomes can be found in all regions of the microchannel, and their conformation can be controllably by the calcium solution. At very high flow rates, the forisomes simply flow straight through the channel and are not able to bind to the glass. When testing different flow rates of the medium, the calcium solution was set at a flow rate of 0.25 mL/h so it had little effect on the net flow rate within the main channel. Fig. 4 shows the number of forisomes tethered to the channel walls with varying flow rates of the forisome suspension.

The above observations illustrate that the tethering of forisomes with a solid substrate is dependent on the hydrodynamics of the forisome suspension inside the microchannels, and the surface interaction between forisomes and the substrate. Simple rheological testing showed that forisome suspension at low volume fraction is a Newtonian fluid. For slow viscous flow in a pipe or open channel, simple dimensional analysis suffices to show that,

$$\dot{\gamma} \propto \frac{Q}{R^3}, \dot{\gamma}[s^{-1}] \text{ is the shear rate at a boundary point}$$

Q [m^3s^{-1}] is the volumetric flow rate, and R [m] is a characteristic lateral dimension. Obviously, higher rates of flow should produce higher boundary shears and carry forisomes farther along a channel before they stick. From Fig. 4, we observe that the higher shear rates enhance the forisome deposition at a certain regime.

Furthermore, the microscale mixing of Ca^{2+} free-medium and the calcium solution is important for the forisome tethering. Mixing inside microchannels has been studied extensively in recent years (Stroock, *et al.* 2002). Since the Reynolds number is small in our case, the mixing is diffusion controlled. The Peclet number is on the order of 100. Gobby, *et al.* (2001) performed computational fluid dynamics simulations of mixing behavior inside a T-shape microchannels and showed that the mixing length increases with the fluid speed and is influenced by the mixer aspect ratio. We plan to perform more studies with varying flow rates based on Gobby's numerical results to correlate forisome's tethering and conformation kinetics.

Regarding the surface interaction of forisomes and microchannel walls, it is necessary for the forisomes to bind to the glass to stay in the channel, this adhesion has adverse effects when trying to swell and deswell the forisomes. When a forisome binds entirely to the glass, it no longer exhibits the contraction and expansion displayed by forisomes in other environment. This situation is somewhat analogous to fixing a piece of elastic to a plank of wood. Under these circumstances, the piece of elastic will no longer stretch and remains fixed to the wood.

Many forisomes in the channels do not completely bind to the glass. From the experiments completed so far, it is observed that only the ends of many forisomes bound to the glass. In certain situations, one end of the forisome will stay in place while the rest of the forisome contracts towards that point. At no time during the introduction of calcium solution does the bound end move. The contraction of a forisome within the channels occurs in two distinct fashions: one, the forisome is bound at both ends to the glass and appears to shimmer or "wiggle"; or two, the forisome is bound at one end to the glass, and the rest of the forisome contracts towards the bound point. We observe that broken forisomes always contract as there is only one end able to adhere to the glass. Overall, in the observed forisomes that were stuck to the glass at only one end, the increase in width was consistently between 20%-65%, and decrease in length ranged from 6%-15%. Fig. 5 shows the above trend, the data shown in each bar is

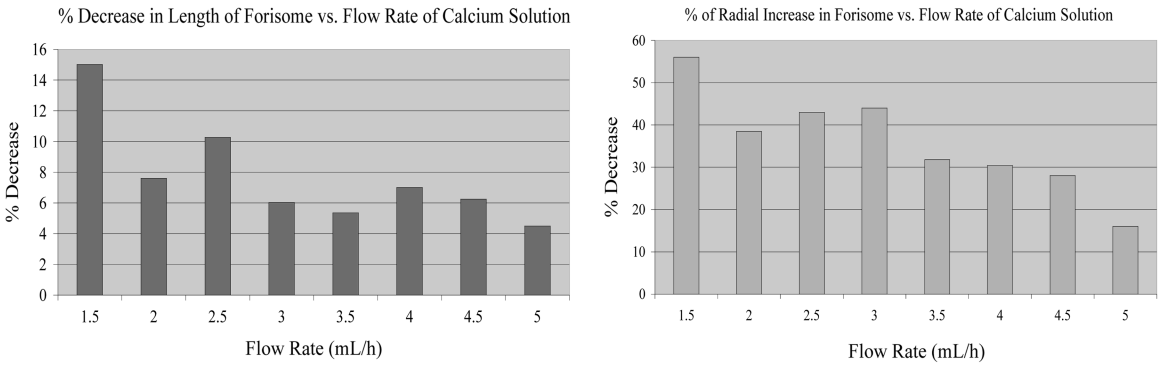

Fig. 5 Relative Ca^{2+} induced longitudinal contraction (left) and radial expansion (right) of forisomes tethered to the walls of a microchannel as a function of flow rate in this channel. While the relative changes of length and diameter tended to decrease with increasing flow velocity, they are generally lower than the values reported from forisomes that were kept suspended in test solutions.

based on the average value of 50 samples. The contraction process does indeed happen rapidly inside microchannels. The average contraction occurred in 0.41 seconds. The expansion after the contraction seemed to occur slightly quicker in most forisomes, with an average time of 0.22 seconds. More experimental data is needed to confirm any assumption about the contraction and expansion speeds, as one would expect the two to occur on similar time scales. Also, the length and width of a forisome after undergoing a contraction and expansion cycle proved consistently to be almost identical.

3. Discussion

The use of microfluidic devices to probe forisome materials properties has a number of significant advantages. First, the volume of microchannels is very small, so that the amount of forisome suspension required for experimentation is minute. The soft lithography techniques used to construct microfluidics devices are inexpensive and amenable to elaborate designs. Such devices offer straightforward solutions for fundamental investigations into forisome adhesional properties and deformation kinetics within a geometrically confined reaction space. Forisomes confined within a small geometry with a steady flow condition under calcium ion concentration variations, contracted markedly less than forisomes freely suspended in a solution. The Reynolds number (inertia/viscous forces) and Weber number (inertia/capillary forces) proved to be critical to determine the hydrodynamic interaction of the forisome suspension of the deposition process to the substrates. Furthermore, the surface interaction of forisomes and substrate (microchannel walls) also determines the forisome conformation change inside a confined geometry. For example, forisomes adhere very swell to glass surfaces but much less so to the PDMS walls (hydrophobic) in the channel. When using a food dye (Brand of Schilling) with the calcium solution, it is interesting to note how quickly the dye is absorbed and washed out of the forisome. The forisomes absorb the dye, and then return to their original color immediately when placed in the original Ca^{2+} free-medium solution again.

One possible disadvantage of using the microchannels is that the direct accessibility of the forisome is lost once it is inside the channel. Forisomes are not readily repositioned once they are in a microchannel, and without advanced techniques such as optical tweezers, experimental manipulations

within the channel appear impracticable. Despite this drawback, the ease with which swelling can be induced and subsequently reversed within the channels is a marked improvement over any methods used before. Once a forisome is in the channel, it might be maintained and kept functioning for days or even weeks if oxidation is prevented. This will allow for further experiments on the durability of the forisomes.

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