UNIVERSITY OF
BIRMINGHAM

NOVEL MICROMANIPULATION STUDIES OF BIOLOGICAL AND NON-BIOLOGICAL MATERIALS

By

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A thesis submitted to The University of Birmingham for the degree of **DOCTOR OF SCIENCE**

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The University of Birmingham

March 2015

Abstract

Many biological and non-biological materials in the form of microscopic particles (or microparticles) are used to produce functional products for a wide range of industrial sectors including pharmaceutical and medical, chemical, agrochemical, food and feed, personal and household care. Understanding their mechanical properties is essential for predicting their behaviour in manufacturing and processing, and for maximising their performance in end-use applications. However, it had not been possible to determine the mechanical properties of single microparticles until the author, as the main contributor, developed a novel micromanipulation technique at the University of Birmingham. The technique is capable of determining the mechanical properties of both biological and non-biological particles as small as 400 nm in diameter, and can be used for obtaining force-displacement data of single microparticles at large deformations, including those corresponding to rupture. The technique was enhanced by mathematical modelling and finite element analysis in order to allow intrinsic material properties to be determined, for example, the particle (or particle wall) elastic modulus, viscoelastic and plastic properties, and stress/strain at rupture. For biological materials, applications of this technique include understanding mechanical damage to animal cells in suspension cultures, yeast and bacterial disruption in downstream processing equipment, biomechanics of chondrocytes and chondrons for tissue engineering, and adhesion and cohesion of biofilms and food fouling deposits. For non-biological materials, applications include understanding and controlling particle breakage in processing equipment, and the formulation of microcapsules with optimum mechanical strength to achieve controlled release and targeted delivery of functional active ingredients.

The research on micromanipulation has been sponsored by BBSRC, EPSRC, DEFRA, DTI, EU, the Royal Society K C Wong Fellowships and 19 national and international

companies, and has resulted in more than one hundred academic publications. The knowledge generated has also assisted these companies to commercialise particulate functional products.

Dedication

To my daughter Melanie and wife Ping

Author’s Declaration and Acknowledgements

This thesis details the author's research work on micromanipulation studies of biological and non-biological materials at the School of Chemical Engineering, University of Birmingham, UK from 1989 to 2013. His research interests and achievements through reference to the selected publications (SP) are presented, which are numbered with a prefix SP in the body of the text. Other references are cited by authors' surnames. The author believes his contributions to the selected publications were significant, particularly to those with him as the first author and/or corresponding author.

A number of PhD students, postdoctoral associates/fellows, internal and external collaborators are gratefully acknowledged for their various scientific contributions, who have authored or co-authored the selected publications. In particular, I would like to thank Professor Colin Thomas for supervising my earlier research work on developing a novel micromanipulation technique for determining the mechanical properties of mammalian cells and microbial cells, who has then become a long-term mentor, collaborator and close friend.

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1. Micromanipulation Studies of Biological Materials

1.1. Development of a Novel Micromanipulation Technique for Measuring the Bursting Strength of Single Mammalian Cells

In the 1980s, there was a great deal of research on the culture of mammalian cells in bioreactors to produce high-valued diagnostic and therapeutic products, such as monoclonal antibodies and interferons. Mammalian cells require nutrients and oxygen to grow in bioreactors such as stirred tanks, and they need to be well mixed in order to eliminate possible gradient in temperature, pH and concentrations of nutrients. Oxygen transfer to mammalian cells was achieved mainly via sparging and agitation in bioreactors. However, mammalian cells such as mouse-mouse hybridoma tended to be mechanically damaged by sparging, particularly bubble disengagement at the gas-liquid interface. Agitation caused gas dispersion, which reduced bubble size and resulted in greater damage. In order to minimise or eliminate the damage, it was important to understand the mechanical properties of such cells. Since they have a typical diameter of 10 μm, appropriate experimental techniques were not available in this period.

In Sept. 1989, the author joined the School of Chemical Engineering, University of Birmingham, UK as an honorary research fellow funded by a Sino-British Scholarship, and began to develop a new experimental technique in order to measure the mechanical strength of single mammalian cells.

The first prototype of a micromanipulation rig was based on the compression of single mammalian cells between two horizontal probes with flat ends, which were made of single rigid optical fibres having a diameter of approximately 50μm, see Figure 1 [SP1].

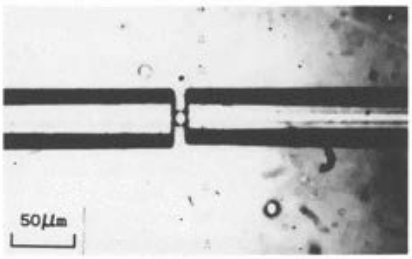


Figure 1: A single mouse-mouse hybridoma cell held by two horizontal probes before being compressed [SP1].

When a cell was compressed at a given speed, the force imposed on the cell and the probe displacement were recorded. The cell was initially deformed, and then burst. From the force-displacement data, the cell diameter and bursting force were determined. It was found that mouse-mouse hybridoma cells in a population had varying bursting force. Therefore a number of cells from a continuous (steady state) culture were measured in order to give statistically representative results. Generally, the bursting force increased with increasing cell diameter. Moreover, several cells in a same growth stage from a synchronous culture were examined in order to minimise the effect of their change in physiology on cell bursting force. It was found that the cells of similar size had very similar bursting forces, which validated the novel micromanipulation technique [SP1]. This novel technique opened a new avenue to study the mechanical properties not only of mammalian cells but also of other cells and non-biological micro-particles since understanding such properties is generically important.

The technique was then used to measure the bursting force of single hybridoma cells of different ages in a batch culture (Zhang et al., 1992) and the effect of a medium additive Pluronic F68 on their bursting force [SP4]. It was found that the addition of Pluronic F68 increased the bursting force of the cells by approximately 60%, but the increase was not fully responsible for the effective protection of cells by this polymer in sparged bioreactors. The results provide direct evidence to explain the main protective mechanisms of the additive,

which moderated the hydrodynamics in the reactors (Handa-Corrigan et al., 1989) and inhibited adhesion of the cells to the bubble surfaces (Chattopadhyay et al., 1995).

Moreover, the micromanipulation technique has been used to investigate the bursting force of different cells lines (Zhang et al., 1993) and the susceptibility of hydridoma cells in different cell cycles to hydrodynamic forces [SP6] in order to determine the optimal strategy for producing monoclonal antibodies.

The bursting force data of single mammalian cells of a given diameter although useful are not intrinsic material properties, i.e. are not independent of the method of measurement. Therefore, a mathematical model was developed to determine intrinsic mechanical properties including bursting membrane tension and compressibility modulus [SP2].

Modelling of cell-hydrodynamic interactions was then undertaken to predict cell disruption in different flow fields. Using the intrinsic mechanical properties of single cells determined by micromanipulation and the hydrodynamics in each field, this modelling work resulted in successful predictions of cell disruption in laminar flow generated in a cone-plate viscometer [SP3], and good estimation of cell disruption in turbulent flow in a capillary [SP5]. This work also demonstrated that the typical hydrodynamic forces generated by agitation in a closed stirred –tank bioreactor without sparging were not sufficient to disrupt mammalian cells (Thomas et al., 1994), whilst the main mechanism of cell disruption was due to shear and elongational flows generated by bubble disengagement at gas-liquid interfaces. The micromanipulation studies of mammalian cells provided their mechanical properties for the first time, which led to understanding the damage mechanisms in different flow fields.

1.2. Mechanical Characterisation of Microbial Cells

Microbial cells, including yeast cells and bacteria can also be used to produce diagnostic and therapeutic proteins. The cells need to be disrupted i.e. broken open if the products are

intracellular and cannot be secreted to the culture medium. Therefore, understanding the mechanical properties of microbial cells is essential if cell disruption in processing equipment is to be predicted and optimised. However, yeast and bacteria are significantly smaller than animal cells. Because of alignment difficulties, it was not possible to use two horizontal probes to capture single microbial cells from fermentation broth and then compress them to bursting or breaking. Therefore, the micromanipulation rig was modified significantly. Instead of using two horizontal probes, one vertical probe was used to compress single cells in suspension against the base of a glass chamber. The corresponding optical and imaging systems were also been modified in order to observe yeast cells (Mashmoushy et al., 1998) and bacteria (Shiu et al., 1999) for micromanipulation measurements.

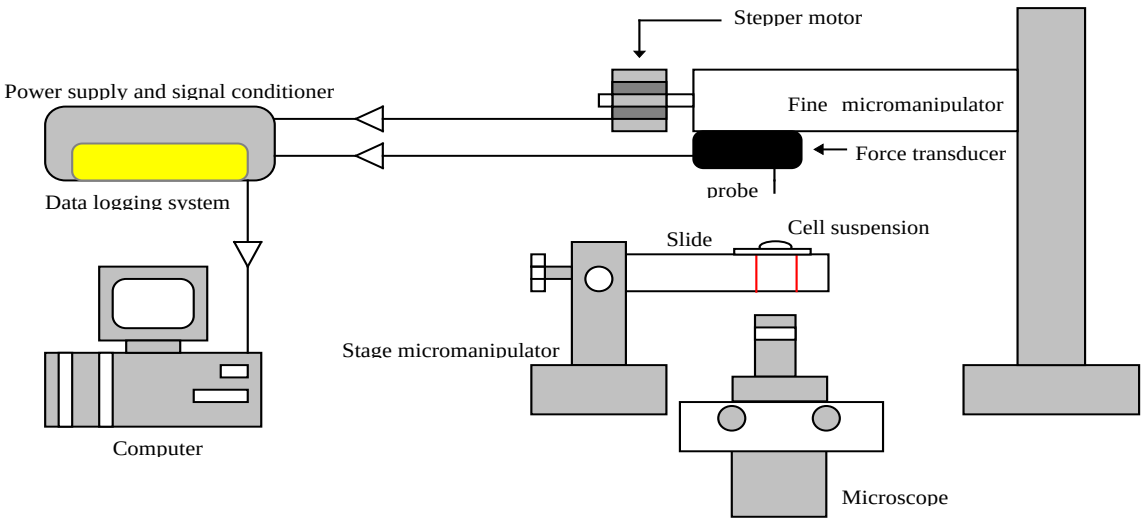


Figure 2: Schematic diagram of the micromanipulation rig used to test microbial cells (Adapted from Mashmoushy et al., 1998).

The mechanical properties of yeast cells (*Saccharomyces cerevisiae)* in a suspending liquid at various osmotic pressures were investigated using the modified micromanipulation technique [SP10]. The bursting force, nominal deformation at bursting, and cell diameter were quantified at different compression speeds (strain rates). It was found that these mechanical

properties depended on the osmotic pressure, but were independent of the compression speed. The bursting force linearly increased with the deformation at bursting but did not vary with the cell diameter. Moreover, the cells deformed elastically up to strains corresponding to bursting. Finite element analysis (FEA), see Section 3.1, was then used to determine intrinsic material properties of the cell wall including the Young's modulus, which combined with the deformation at rupture provided a useful failure criterion that can be used to predict whether given hydrodynamics in processing equipment can cause effective cell rupture.

1.3. Micromanipulation of Other Biological Materials

1.3.1 Keratinocytes

Understanding the mechanical properties of keratinocytes in relation to their structure and chemical composition can assist in the development of more efficacious skin-care products. The cornified cell envelope in keratinocyte is an important marker of stratum corneum maturation in healthy and dry skin. In collaboration with a group of scientists led by Mr Clive Harding in Unilever, UK, the mechanical properties of resilient and fragile cornified cell envelopes, formed by transglutaminase-mediated epsilon-(gamma-glutamyl)lysine cross-linking of specialized corneocyte proteins, were characterised using the micromanipulation technique (Harding et al., 2003). The relationship between the morphological and physical changes with varying degrees of maturation of the cell envelopes during the terminal differentiation and their mechanical properties was established. These studies demonstrated that during the normal process of cell envelope maturation, there was an actual strengthening of this protective structure and that the impairment of this process resulted in poor quality of the stratum corneum.

1.3.2 Chondrocytes and Chondrons

Chondrocytes in healthy cartilage produce a hydrated pericellular matrix (PCM), and the assembly of the cell with PCM is called a chondron. Chondrocytes can respond to mechanical loading via mechanotransduction. Understanding their mechanical properties is a pre-requisite to the study of this process. The mechanical properties of single chondrocytes and chondrons isolated from bovine articular cartilage were quantified using the micromanipulation technique (see Figure 2). It was found that there was a significant difference between chondrocytes and chondrons in their elastic limit, viscoelasticity, bursting force and nominal deformation at rupture. Chondrons were generally stiffer than chondrocytes and showed less viscoelastic behaviour than chondrocytes. Thus, the results demonstrated the mechanical significance of the PCM [SP44] and provided a guide for formulating scaffolds to be used in tissue engineering.

The gene expression profiles of enzymatically isolated single chondrocytes and chondrons in response to dynamic compression were investigated in collaboration with a research group led by Professor Alicia El-Haj, Institute of Science and Technology in Medicine at Keele University [SP34; SP42]. Following dynamic compression, chondrocytes and chondrons showed variations in gene expression profiles. Aggrecan, Type II collagen and osteopontin gene expressions were significantly increased in chondrons, lubricin gene expression decreased in both chondrons and chondrocytes, SOX9 gene expression was unchanged. The work shows a clear role of the PCM in interfacing the mechanical signalling in chondrocytes in response to dynamic compression, and significantly improves understanding of cartilage mechanobiology.

1.3.3. Pollen Grains

Pollen grains (e.g. from bee pollens) contain nutraceutical ingredients including amino acids, vitamins, carbohydrate and pigments. A pollen grain has a rigid wall with a two-layer structure of exine and intine, which is composed of sporopollenin lignin, cellulose, and semi-cellulose. To harvest the nutraceutical ingredients from pollens, it is necessary to disrupt the wall, which is often achieved by mechanical methods such as milling, crushing, pulverization, and shear plane degradation[19]. For such operations, understanding the mechanical properties of pollen grains is essential for predicting and optimising the disruption behaviour, similar to yeast cells or bacteria. The mechanical properties of desiccated ragweed pollen grains were determined using micromanipulation. Moreover, single dry pollen grains were modelled as capsules with air-filled cores and impermeable walls. A constitutive equation based on the Hookean model was used to determine the material property, Eh (product of the Young's modulus E and wall thickness h). Such data with further modelling of the stress-strain relationship up to rupture may be used to predict the disruption behaviour of ragweed pollen grains in processing equipment.

1.3.4. Fouling Deposits

1.3.4.1. Biofilm

A biofilm is composed of bacterial cells embedded in a network of exopolysacharides (EPS), which tends to adhere to any available surface. The formation of a biofilm can be desirable, e.g. to immobilise cells for biotransformations, or detrimental in the case of surfaces of domestic appliances and medical tools. The micromanipulation rig used for measuring the bursting strength of single mammalian cells using two horizontal probes [SP1] (see Section 1.1) was modified to measure directly the adhesive strength of biofilms formed in pipe flows [SP7]. A T-shaped probe connected to a force transducer was specially designed

to scape *Pseudomonas fluorescens* biofilms away from the inner surface of a pipe to which they were attached, see Figure 3. The probe was positioned 1μm above the surface of the test stud, and the force as a function of the probe displacement was recorded. Strictly, the force is related to the adhesion and deformation of the biofilm. Therefore, the apparent adhesive strength between a biofilm and the substratum is defined as the work required to remove the biofilm per unit area from the substratum. It was found that the fluid velocity in which the biofilms were grown had a significant impact on the structure and apparent adhesive strength. The higher the fluid velocity, the more compact the biofilm structure and the greater the apparent adhesive strength.

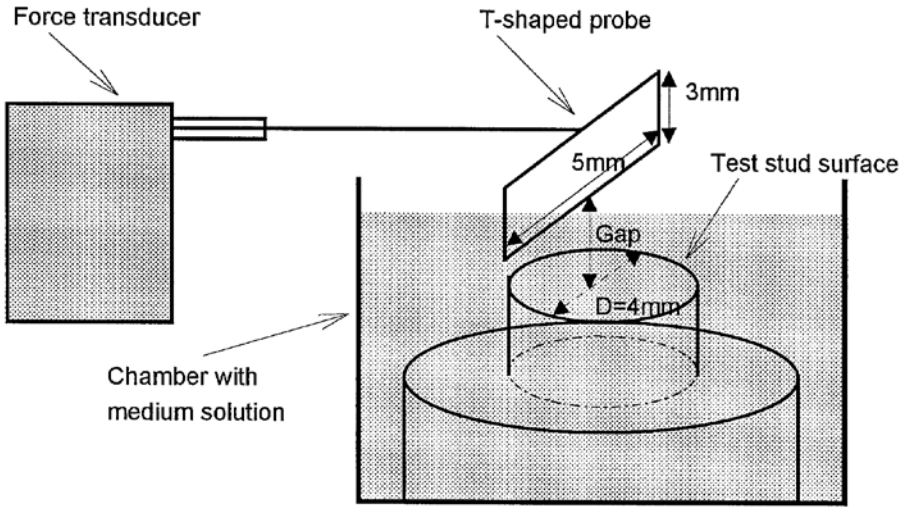


Figure 3 Schematic diagram illustrating a test stud and T-shaped probe used to measure the apparent adhesive strength of biofilm by micromanipulation [SP7].

Micromanipulation was further applied to measure the apparent adhesive strength of *Pseudomonas fluorescens* biofilms on a substratum under various growth conditions in order to develop antibiofouling strategies [SP22]. It was found that the apparent adhesive strength of the biofilms on glass, which were developed in pipe flows, depended on the biofilm age,

nutrient concentration, suspended cell concentration, pH, and surface roughness of the substratum.

The micromanipulation technique and a flow chamber technique were then applied to assess the adhesive and cohesive integrity of the immobilised bacterial populations (biomass) of *Pseudomonas fluorescens (*Garrett et al., 2008). The micromanipulation data show that the apparent adhesive strength of the biomass was greater than the apparent cohesive strength, and have been used to develop a deeper understanding of the mechanism of biofilm formation in the early stage and to interpret the removal behaviour of the biomass from the flow chamber.

1.3.4.2. Food Fouling Deposits

The micromanipulation rig developed for biofilms was then extended to investigate the apparent adhesive and cohesive strengths of different food fouling deposits including tomato paste [SP16], whey protein [SP25], bread dough and egg albumin on stainless steel [SP26-27]. The research was led by Professor Peter Fryer at the University of Birmingham. It was found that tomato paste, bread dough and egg albumin deposits had a smaller adhesive than cohesive strength, whilst whey protein had a smaller cohesive than adhesive strength, which indicates that they could be removed in different modes by a fluid flow. The former deposits were removed as a large fragment, whilst the latter was removed layer by layer, which has been validated by experiments. Moreover, a range of coated surfaces was used to study the effects of surface energy on the force required to remove tomato deposits, and the surface energy required to minimise the adhesive strength was identified. Furthermore, the apparent adhesive strength data for toothpaste on glass, stainless steel and fluorinated surfaces obtained by micromanipulation were compared with those directly measured by atomic force microscopy (AFM) using functionalized tips in force mode [SP40]. The results obtained from

using the two techniques are consistent, and a relationship between the deposit-substratum interactions at the nano-scale and micro-scale has been established, which can be used to develop a new strategy for cleaning process equipment with the minimal consumption of cleaning chemicals, water and energy.

2. Micromanipulation Studies of Non-Biological Materials

Many speciality products (e.g. pharmaceutical, nutraceutical, food and feed, agrochemical) contain particulate components, including microcapsules, microspheres, granules, agglomerates, and aggregates, which offer certain functions at end-use applications. For example, microcapsules can assist in the stabilisation of active ingredients, and achieve their controlled release or targeted delivery. However, these particles should have desirable physical, structural and mechanical properties. Characterisation of these properties is essential to the development of such products in order to ensure their functionalities.

2.1. Microcapsules

The micromanipulation rig, as illustrated in Fig. 2, was applied to the measurement of the bursting (rupture) force of single microcapsules as small as 1 μm in diameter, which were made of different formulations [SP13], including those with a shell of melamine formaldehyde (MF) [SP8; SP13] and a core of different oils [SP8; SP13; SP15]. The MF microcapsules showed elastic-plastic behaviour, followed by bursting. The elastic limit, the bursting force, nominal deformation at rupture and their relationships with diameter were established. These properties were used to define the volume-weighted mean fracture strength of microcapsules for industrial applications and to compare this parameter for microcapsules made of different formulations and processing conditions (Smets et al., 2013).

MF microcapsules using different amounts of formaldehyde were prepared and their rupture strength was investigated in order to identify the minimum quantity of formaldehyde that can provide sufficient mechanical strength to the microcapsules [SP39], since there are legal limits for using it in commercial consumer products. MF microcapsules with a narrow size distribution were made using membrane emulsification followed by *in-situ* polymerisation [SP51] and it was found that the microcapsules also had a narrower strength (indicated by the rupture force) distribution in comparison with those made using emulsification in a conventional stirred vessel or homogeniser.

In order to reduce leakage of perfume from microcapsules in liquid detergents during storage, microcapsules with double shells of melamine formaldehyde and calcium carbonate were made [SP43; SP54]. The mechanical strength of such microcapsules was dominated by that of the calcium carbonate shell. The leakage of perfume from the microcapsules with the double shells was reduced by a factor of 20 compared with MF microcapsules with only a single shell [SP43].

New microcapsules, which are more user-friendly than MF, including poly(methyl methacrylate) (PMMA) microcapsules with a perfume core [SP55] and silica microcapsules with silicone oil cores were also formulated in collaboration with Professor Brian Vincent's group at the University of Bristol, UK [SP38], and their structure and mechanical properties were characterised. These studies have laid a foundation for future developments that should produce a new generation of microcapsules with an oil-based core for industrial applications.

2.2. Microspheres

Hydrogels have the potential to be used as drug carrier, artificial organ and scaffold for tissue engineering. The mechanical properties of a range of hydrogels (calcium alginate, calcium alginate coated with chitosan [SP18], and alginate/calcium phosphate (Alg/CP)

composites [SP48; SP52] in the form of microspheres were investigated using micromanipulation. Generally, these hydrogel microspheres exhibited viscoelastic properties and the force imposed on them for a given deformation increased with the compression speed [SP18, SP23]. The Hertizian model modified for describing viscoelastic behaviour (Mattice et al., 2006) was used to determine the instantaneous Young modulus, the relaxed Young's modulus and relaxation times of agarose microspheres for small deformations [SP36; SP48].

The mechanical properties of hydrated dextran microspheres used as a drug carrier were extensively studied, and a relationship between the Young's modulus of microspheres determined by micromanipulation and that of macrogels measured using a controlled stress rheometer was established [SP12; SP20], in collaboration with Professor Win Hennik's group in Utrecht University, The Netherlands. The values of the Young's modulus were used to estimate the pore size of methacrylated dextran (dex-MA) microspheres based on the statistical theory on rubber elasticity, which was correlated with the maximum release of model protein drugs: myoglobin, ovalbumin, bovine serum albumin and bovine gamma globulin [SP12]. The mechanical properties of hydroxyl ethyl methacrylated dextran (dex-HEMA) microspheres have also been evaluated, which are related to their rate of degradation in phosphate buffer, to mimic physiological liquid, over a period of 6 months [SP28].

Calcium shellac microspheres as a potential carrier of carbamite peroxide for tooth whitening were prepared using two different methods: extrusion followed by gelation [SP32], and emulsification followed by gelation [SP37], and their size, structure and mechanical strength were characterised. The carriers may be incorporated into chewing gum or toothpaste, which allows release of the active ingredient (hydrogen peroxide) using a trigger of mechanical force. The results generated a lot of interests from Philips Research Laboratories, UK, which has been sponsoring a postdoctoral research fellow at Birmingham

since March 2011for a project on controlled release of active ingredients with applications to oral care.

Different microspheres of pharmaceutical excipients were mechanically characterised, and the mechanical properties such as the nominal single particle rupture stress were correlated with their compaction behaviour, i.e. in terms of the dependence of the compaction pressure of the powder bed on the strain [SP31]. The results were used to identify excipients that can be applied to making probiotic (Huckle et al, 2008) and nutraceutical (Law and Zhang, 2007) products in tablet form, which requires the production of rigid tablets at low compaction pressure.

In collaboration with TOSOP, Germany and Millipore, UK respectively, mechanical characterization of microparticles used for chromatography media were undertaken [SP24; SP29]. The mechanical properties of the microspheres in relation to their formulation and processing conditions were used as a basis for preparing chromatography media with desirable surface properties, structure, and sufficient mechanical strength for industrial applications.

In collaboration with a group of material scientists at the University of Bristol, UK and Imperial College, UK, led by Professor Jason Riley**,** macroporous copper with a triply periodic minimal surface (TPMS) was produced by replicating the remarkable form of a sea urchin skeletal plate using templated electrochemical deposition [SP30]. The mechanical testing of this material was conducted using a Lloyd material testing machine, which operates on the same principle as micromanipulation but can be used to handle macro-materials. It was found that the TPMS structure leads to a material with a high compressive strength. The methodology inspired from nature is generally applicable and can be extended to generate a range of composite materials with high mechanical strength.

2.3. Nanomanipulation of Sub-Micron Particles

Particles at sub-micro scale are also used in functional products. For such small particles, conventional optical microscopy does not provide sufficient resolution to visualise them. In collaboration with Professor Athene Donald, FRS in Cavendish Laboratory at the University of Cambridge, a nanomanipulation device was developed, which was accommodated in the chamber of an environmental electron microscope [SP21; SP29]. This device allows measurement of force as a function of displacement for single particles as small as 400 nm, and identification of the rupture mode of single microcapsules with a core-shell structure [SP33]. The contact area and lateral extension of single melamine formaldehyde microcapsules under compression were measured from the obtained SEM images with high magnification [SP41], which provide additional data to validate modelling of the intrinsic stress- strain relationship of the material, see Section 3.2.

2.4. Adhesion of Single Microcapsules to Surface

In collaboration with Dr Kuo-Kang Liu and his colleagues in Nanyang University in Singapore, the adhesion of single urea formaldehyde microcapsules to a flat glass substrate in response to an osmotic change was studied. Inspired by the micromanipulation technique developed at Birmingham, a microscope visualization instrument was developed to measure the microcapsule–substrate contact area and inflated microcapsule volume [SP14]. Later, high-resolution reflection interference contrast microscopy (HR-RICM) and phase-contrast microscopy were applied to probe the adhesion contact area (Liu et al., 2002), which significantly improved the resolution of measurement. A theoretical model was developed to describe the adhesion energy in relation to the contact area and osmotic inflation of the cell volume, which was validated by the experimental data. The research represents an important

step in extending the micromanipulation technique to study cell-substrate interactions, which are extremely important for tissue engineering.

In collaboration with Procter & Gamble, Belgium, perfume microcapsules in detergents were developed, which requires the microcapsules to deposit on a fabric surface in laundry process. The adhesion of single melamine formaldehyde (MF) resin microparticles and MF microcapsules containing liquid perfume to a flat fabric film made of cellulose under varying environmental conditions was directly measured using AFM in order to understand their fundamental interactions (Liu et al 2013; He et al., 2014). It was found that there was little adhesion between MF microparticles or microcapsules and a cotton film in an aqueous solution. However, when the fabric surface was functionalised with a polymer chitosan, the adhesion between MF microcapsules and the cotton film increased significantly, which depended on pH, ionic strength and the surfactant concentration in the solution. The main mechanism for the polymer to enhance the deposition was found to be due to molecular bridging between the microcapsules and fabric surface. The work has provided a new approach to improve formulation of liquid detergents in order to enhance deposition and retention of perfume microcapsules on fabric surfaces in laundry processes.

2.5. Adhesion between a Pair of Particles

Sponsored by Unilever, UK, the micromanipulation technique initially developed for measuring the bursting strength of single mammalian cells was modified to measure directly the adhesive force between ice particles in air and in a sucrose solution [SP17]. Ice particles were generated in the chamber of a heating/cooling stage under a microscope, see Figure 4. The size of the ice particles ranged from ten to several hundreds of microns.

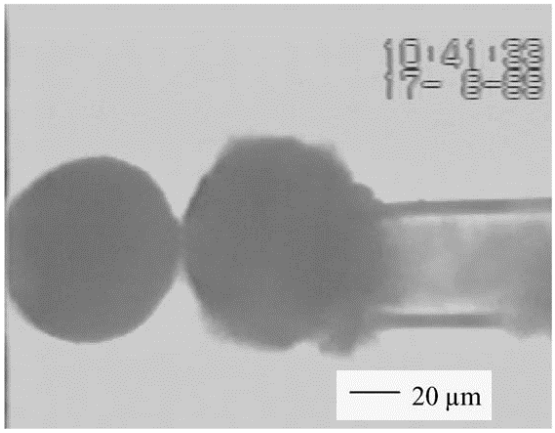


Figure 4: Images of ice particles on the probe tips before their adhesion was measured (adapted from SP17).

The effects of particle size, contact time on the adhesion in air and sucrose solution were investigated, and the results were useful in the determination of appropriate formulation and processing conditions to control ice crystal growth in ice cream, which can have an impact on its texture and mouth feel.

3. Finite Element Analysis to Determine Intrinsic Material Properties of Single Particles

From the compression of single spherical particles using the micromanipulation technique described above, the force as a function of displacement to failure (bursting, breaking or rupture) is useful for comparison of the relative mechanical strength of the particles prepared under different conditions. In order to determine the intrinsic material properties from such data, the stress-strain relationships need to be determined. This requires finite element analysis (FEA), particularly for large deformations of the particles to failure.

3.1. Young's Modulus of the Yeast Cell Wall

Compression of single yeast cells was first modelled based on finite element analysis (FEA) by Professor Anton Middelberg's group in University of Adelaide, Australia using a

commercial software package (Phenics). It was found that the yeast cells were elastic up to breaking, and the Young's modulus, stress and strain at breaking of the cells were determined using FEA [SP11] based on the experimental data and the cell diameter measured using the micromanipulation technique, cell wall thickness using transmission electron microscopy (TEM) and initial stretch ratio independently measured [SP10].

The Young's modulus for yeast cells *Saccharomyces cerevisiae* from the exponential phase of a batch fermentation, determined by micromanipulation and FEA is 112 ± 6 MPa [SP10]. However, the Young's modulus value of the yeast cells determined by AFM using sharp indenters was in the range of 0.2–1.6 MPa [SP56]. In order to explain the difference, the yeast cell wall was modelled further as a double layer consisting of a soft external layer of mannoproteins and a stiff inner layer of β-glucan fibres and chitin. From the FEA of the double layer, it was demonstrated that previous AFM studies give reasonable estimates of the Young's modulus of the external layer whilst micromanipulation, which generates large deformation of the yeast cells provides the total stiffness of the cell wall, which is dominated by that of the inner layer.

3.2 Viscoelasticity of Hydrogel Microspheres

A commercial software package ABACUS was used to model the compression of single calcium alginate microspheres in liquid suspension [SP35]. The volume change of the microspheres during compression and relaxation was determined using a high-speed micro-camera followed by image analysis. It was found that force relaxation occurred during holding after compression, which might be due to water loss from the microspheres under compression or their viscoelasticity. In order to determine which mechanism is dominant, a poroelastic material model was first used to evaluate the possible effect of water loss from the microspheres during holding, and it was found to be negligible. Following this, a

compressible, isotropic and homogeneous linear viscoelastic material model with two relaxation times was adopted to fit experimental compression and relaxation data. They were found to be in good agreement, resulting in the determination of the instantaneous elastic modulus, long-term elastic modulus and the two relaxation times. Deformation parameters of the compressed microspheres including the contact radius and central lateral extension were also obtained from finite element modelling, and were compared with experimental data, which also show close agreement. This confirms the validity of the model for the microspheres under test. The general approach can be applicable to modelling other hydrogel microspheres.

3.3 Strain-dependent Viscoelasticity of Chondrocytes and Chondrons

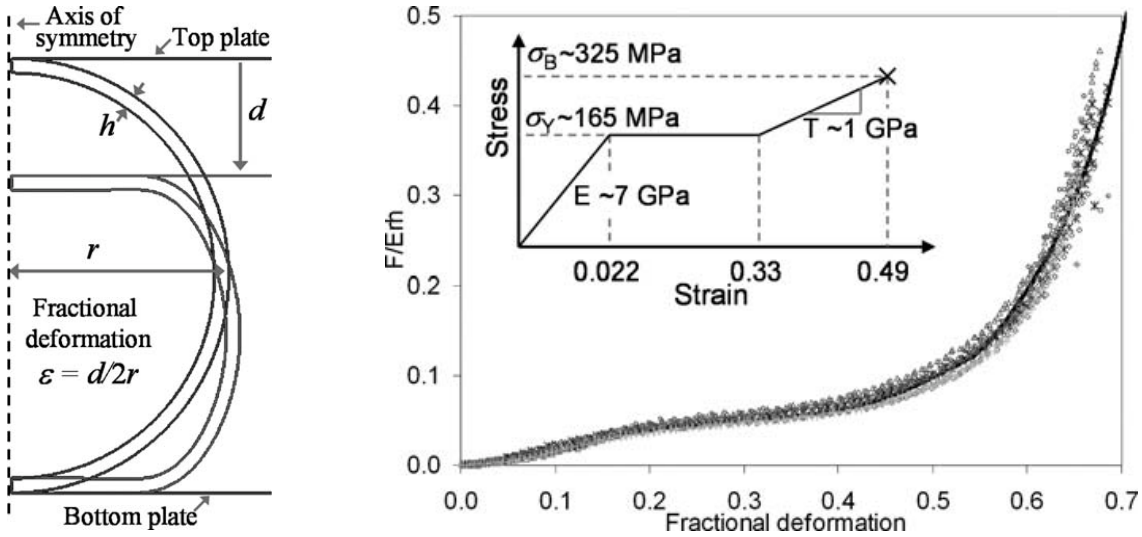
From micromanipulation measurements, single chondrocytes and chondrons isolated from bovine articular cartilage were found to be elastic up to a nominal deformation of 30% and viscoelastic from 30% to 50% [SP42]. ABACUS was applied to simulate the experimental force-displacement/time data from the diametrical compression of single chondrocytes and chondrons [SP44]. Because of the large deformations (strains) in the cells, a nonlinear elastic model was used for simulations of compression to a nominal deformation of 30% and a nonlinear viscoelastic model for 50%. The Young's modulus value corresponding to nominal deformations up to 30%, and viscoelastic properties (the instantaneous elastic modulus, long-term modulus and an apparent viscosity) for larger deformations of 30%to 50% for chondrocytes and chondrons were determined respectively. The results show that chondrons were generally stiffer and exhibited less viscoelastic behaviour than chondrocytes. This implies that the PCM significantly influenced the mechanical properties of the cells. The viscoelastic behaviour might be due to the mechanical response of the cell cytoskeleton and/or nucleus at higher cell deformations. The finding in combination with gene expression of cells

after being compressed to different deformations [SP34] can assist in developing a more effective strategy to stimulate the cells mechanically, resulting in improved tissue engineering of cartilage constructs.

3.4. Elastic-plastic Properties and Failure of Microcapsules

Compression of single microcapsules with a core of hexylsalicylate and shell of melamine–formaldehyde between two parallel surfaces to large deformations beyond the shell thickness up to rupture was modelled using ABACUS, which allows characterization of the contributions of bending and stretching to the force resisting deformation. Initially a linear-elastic model was developed that enables the determination of the shell thickness and Young's modulus of microcapsules from their force- displacement data in the elastic region. The shell thickness value is in close agreement with independent measurements using TEM [SP45]. Corresponding to moderate deformations beyond the elastic region, the shell material was assumed to be perfectly plastic and its yield stress was determined by FEA and the experimental data [SP46]. FEA was then extended to large deformations where the shell material shows strain hardening up to failure. The failure of the microcapsules is considered to result from the maximum strain in the shell exceeding a critical value. Based on the FEA results and experimental data, the failure strain and stress of the microcapsules were determined [SP47], see Figure 5.

FEA was also applied to modelling the compression of inorganic tetraethoxyorthosilane-methyltrimethoxysilane (TEOS-MTMS) microcapsules with a core of oil that ruptured at small deformations within the elastic regime [SP53], and user-friendly PMMA microcapsules with a wide range of shell thickness [SP55]. The shell thickness, Young's modulus and the rupture stress of these microcapsules were quantified.



(a) (b)

Figure 5. Illustration of modelling the stress-strain relationship of the shell of MF microcapsules based on FEA [SP47]. (a). Schematic diagram showing compression of a microcapsule with a liquid core between two parallel surfaces (the radius of the microcapsules is denoted by *r*, the thickness of the shell is *h* and the displacement of the top surface is *d*); (b). Micromanipulation data of 6 different MF microcapsules fitted by the simulation results of FEA (continuous line) (*E* is the Young's modulus, σ_Y is the yield stress, T is the strain hardening modulus and σ_B is the stress at bursting).

The FEA of single microcapsules with varying size, shell thickness and stress-strain relationship up to failure under compression presents a general methodology to enable calculation of the intrinsic material properties from micromanipulation measurements, which is paramount to fully understand their mechanical behaviour for end-use applications, particularly to control the release of their core active ingredients using a trigger of mechanical forces.

4. Summary of Main Achievements and Future Perspectives

A novel micromanipulation was initially developed to directly measure the bursting force of single mammalian cells, which was then modified to characterise the mechanical property of a wide range of biological and non-biological materials, including yeast cells, bacteria,

keratinocytes, chondrocytes, chondrons, adhesion and cohesion of biofilms and food fouling deposits on surface, microcapsules made of different core and shell materials, microspheres of different composition and structure, and adhesion of single particles on surface and adhesion between a pair of particles. For each type of material, the relationship between its chemical composition, structure and mechanical properties has been established. FEA of spherical chondrocytes and chondrons, different types (MF, PMMA and silica) of microcapsules and calcium alginate microspheres under compression has been undertaken, which combined with micromanipulation measurements has determined their intrinsic material properties [SP57-58]. The micromanipulation technique and FEA have provided a very powerful tool and novel approach to study the mechanical properties of particles particularly at the micro-scale, which has found ever increasing applications in a number of industrial sectors.

The micromanipulation technique has generated considerable interest from scientific communities, as evidenced by a large number of national and international collaborators from physics, chemistry, material science, biology, medicine, pharmacy and engineering.

In addition, the micromanipulation technique has been used to characterise the mechanical properties of numerous samples of microcapsules, microspheres, granules and agglomerate supplied by a number of national and international companies, including Arjo Wiggins, UK; Unilever UK and The Netherlands; Zeneca Agrochemicals; Bayer, Germany; Merck Shape and Dohme, UK; Roche, Switzerland; Bavarian Nordic, Germany; Tosoh, Biosep Gmbh, Germany; Rhodia, France; Appleton Paper Inc., USA; Millipore Bioprocessing Ltd., UK; ICI, UK; National Starch, USA; Firmenich SA Corporate R&D, Switzerland; Givaudan Schweiz AG, Switzerland and UK; Micropore Technologies Ltd., UK; International Flavors and Fragrances, USA; Cytec, UK; and Procter & Gamble, USA, UK, Belgium, China and Japan. The data have helped these companies develop commercial products containing

the micro-particles. For example, the technique has been used to characterise the fracture strength of perfume microcapsules, which assisted Procter & Gamble in commercialising perfume microcapsules in detergents of five major brands, which have reached one billion consumers and generated a total sales value of more than five billion US dollars. P&G research fellow Johan Smets stated: “The contributions made by Birmingham have enabled us to develop superior products with perfume microcapsules, significantly improved our competitiveness and secured our leading position in the market of North America and Western Europe”. An entry based on an application of using the micromanipulation technique to measure the fracture strength of perfume microcapsules in detergents was submitted by University of Birmingham and Procter & Gamble, Belgium to the Institution of Chemical Engineers for its Global Awards 2014 in the category of Innovative Product, and was highly commended.

In future, with ever increasing demands from consumers for more healthy food, enhanced well-being and health care, more and more functional products with particles are required. Understanding of the interrelationships between chemical composition, structure, mechanical properties and functionalities of particles, particularly at micro-scale will become essential. The micromanipulation technique with further development, e.g. to control the environmental conditions of samples during testing and to achieve automatic measurement can provide a pivotal role in mechanical characterization of these particles in order to ensure these products have desirable functionalities, are user-friendly, economically viable and sustainable.

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