Dynamics of rebounding *Bacillus subtilis* spores determined using image-charge detection

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Abstract A novel image-charge detection technique was used to investigate the mechanical elasticity of bare bacterial spores during high-velocity impact. Spores of *Bacillus subtilis* introduced to vacuum using electrospray and aerodynamic acceleration impacted and rebounded off of a glass plate. A dual-stage, asymmetric image-charge detector measured the velocity and direction of each spore both before and after impact with the glass surface. Two ranges of impact velocity were investigated, with average initial velocities of 197 ± 17 and 145 ± 12 m/s. Impacts were strongly inelastic, with most of the translational kinetic energy lost upon impact, similar to polystyrene particles of similar size under similar impact velocities. Specifically, $69\% (\pm 16\%)$ and $74\% (\pm 11\%)$ of initial kinetic energy was lost in impacts at the two velocity ranges, respectively. The average coefficients of restitution for the two velocity regimes were 0.53 ± 0.15 and 0.49 ± 0.12 . There was no statistically significant difference in the fractional kinetic energy loss between these two populations. The variance of these results is much larger than experiments using polystyrene spheres of comparable size. These results imply significant plastic deformation of the spore—a striking result given that spores of this strain of *B. subtilis* are known to survive impacts on glass at these velocities. Triboelectric charge transfer during impact was also observed. Although much is known about spore elasticity from static measurements, this is the first study to investigate the elastic properties of bacterial spores in a dynamic scenario, as well as the first demonstration of an image charge detector for measurements of rebounding particles.

Keywords High-velocity impact · Coefficient of restitution · Bouncing dynamics · Image charge detection · Triboelectric effect

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1 Introduction

Bacterial spore coats present unique elastic properties of interest to both fundamental science and to applications in nanomaterials and nanotechnology. For instance, proteins have varying elastic properties and represent an interesting system as structural templates for nano-materials [1]. Nanoparticles based on biological models of proteins and DNA have been successfully demonstrated for potential use in nano-medicine [2]. Bacterial spores present another potential model for use in the development of different types of nanomaterials.

During periods of extreme nutrient deprivation, certain species of bacteria produce endospores. These spores are a mostly dehydrated product of the vegetative cells that are extremely resistant to potentially damaging environmental conditions. Bacterial spores are composed of three primary layers [3]. The innermost section of the spore contains the chromosome surrounded by two membranes. The next layer, the cortex, consists of a densely packed network of proteins and carbohydrates or “peptidoglycan”. Surrounding the cortex, on the exterior of the spore, is the spore coat. This coat consists of a network of different interlocking proteins. The structure, as a whole, is extremely resilient to potentially damaging conditions and events.

The mechanical properties of bacterial and fungal cells have been extensively investigated. Most studies have focused on the properties of vegetative cells, but some work has been done with endospores as well [4, 5]. Researchers that chose to focus primarily on vegetative cells have investigated both fungal cells [6, 7] and species of Gram-negative bacteria [8, 9]. These studies have sought to determine two elastic properties of these cells, the Young’s modulus and the cellular spring constant.

The tolerance of cell walls and membranes to the steadily increasing force of an AFM (atomic force microscopy) stylus has been used to generate force–distance curves for each type of cell or spore studied. Values for the Young’s modulus and the spring constant can be derived from these plots. Heinz and Hoh [10] first envisioned using AFM to explore the intermolecular interactions in biological structures including bacteria. They sought to understand how the intermolecular forces that bind biological systems together are specifically distributed in the space between the molecules that make up the surface of a biological target. In AFM, a mechanical spring attached to a stylus is used to measure force as a function of distance. This technique was developed primarily as an imaging tool that would provide more finely detailed surface mapping than other methods that were available at the time. The AFM has a sharp tipped stylus (around 10–100 nm) that is well suited for molecular work as the radius of curvature of the AFM tip is on the scale of the molecular interactions that it is used to probe. Data generated using the AFM technique can be used to determine values for the different mechanical properties of a cell.

The previous studies primarily investigated the mechanical properties of cells as a function of their resistance to a steadily increasing force. It is valuable to compare cell response to this type of stress to the mechanical reaction that a cell has to a sudden high velocity impact. Both energy regimes are necessary to more completely understand how cell walls and membranes respond to stress. We recently reported on experiments in which intact bacterial spores survived impact at velocities as high as 299 ± 28 m/s onto a glass surface [11]. It was assumed in these experiments that the spores bounced upon initial impact with the glass, and may have bounced one or more additional times before coming to rest inside a canister, from which spores were collected for culturing. The number of bounces, and whether the spores bounce at all, depends in part on the amount of kinetic energy lost to heat, plastic deformation, and other modes, both within the spore and on the impact surface, during the impact event.

The kinetic energy loss of rebounding microparticles has been studied for many systems including micron-sized polystyrene (PS) spheres [12–14], glass [15], ammonium fluorescein [16], and club-moss spores and plant pollen [17], impacting at velocities ranging from less than 1 m/s to a few hundreds of m/s on a variety of dense and semi-dense surfaces. Much of the underlying theory has been presented by Jordan [18] and by Dahneke [12]. Briefly, particles approach a surface with an initial kinetic energy dependent on mass and velocity. Near the surface there is an interaction energy (due to intermolecular attractive forces) resulting in a potential well. Particles impacting with sufficiently low velocity will stick to the surface due to this interaction energy. Particles with greater incoming velocity will rebound. The potential well due to the interaction energy has no net effect on particle velocity at a large enough distance from the surface. Rebounding particles always have a lower rebound velocity than the incoming velocity. A portion of the particle's incoming translational kinetic energy is lost to several modes, including plastic deformation, internal friction, wave phenomena in the target and particle, and flexure work within thin/flexible targets [12, 17]. Based on both theoretical and experimental work, plastic deformation is the dominant mechanism for fast impacts on solid, rigid surfaces [12].

Rebound dynamics can be characterized in terms of the coefficient of restitution (COR), which is defined as the ratio of velocities before and after an impact:

$$e = \frac{v_2}{v_1}. \quad (1)$$

In this equation, e represents the COR, v_2 is the velocity post-impact, and v_1 is the velocity pre-impact. COR values between 0 and 1 are expected for collisions that are neither perfectly elastic nor completely inelastic.

Dahneke reported [14] that PS microspheres impacting glass, quartz, polystyrene, and thick gold surfaces at normal incidence angles gave COR values ranging from 0.90–0.96 at velocities where the surface interaction energy was negligible (faster than ~4 m/s). At higher velocities, the COR of PS on quartz dropped gradually due to plastic deformation, reaching as low as 0.15 for impacts at 370 m/s, the highest reported. For impact velocities of 125–240 m/s (the range of speeds in the present work), the COR ranged from 0.3 to 0.6, with lower COR for higher velocities. For ammonium fluorescein particles, Wall et al. [16] reported that the COR dropped off much more quickly with velocity, presumably because of the lower plastic deformation limit of this material compared with PS. They concluded that the plastic deformation was occurring in the particle and not the glass target due to the higher elastic yield limit of glass. Lifshitz and Kolsky [19] demonstrated that surface roughness contributes to lower COR values due to increased plastic deformation of local protuberances.

The COR is commonly used to represent the rebounding of macroscopic systems [20–23], and has been reported for nanoparticles (bismuth particles on silicon surfaces) [24]. To date, no studies have been done investigating the COR of microorganisms. Measurement of the COR for bacterial endospores compliments previous studies that have focused on other mechanical properties of these spores.

In this study, the rebounding behavior of *Bacillus subtilis* spores was measured as a function of the change in velocity of individual spores upon impacting a hard glass surface. The fractional kinetic energy loss due to impact was also determined. Initial spore velocities fell into two ranges, with individual impacts spanning 125–240 m/s. These velocities were measured using a dual-stage asymmetric image-charge detector that was designed expressly for this study. Experiments were performed in vacuum.

2 Experimental design

An overview of the experimental setup is shown in Fig. 1. Spores of *Bacillus subtilis* were electrically charged and aerosolized using electrospray [11, 25] and were subsequently swept into a differentially pumped vacuum system. The potential difference between the negatively biased electrospray tip and the grounded system entrance guided the charged spores into a pinhole at the entrance to the vacuum system. The velocity of the spores was increased as they passed through a series of system elements designed to reduce system pressure from the system entrance to the detector and to focus the spores into a beam. This focusing guided the spores through a beam tube and a skimmer cone before passing through the image-charge detector. After passing through the detector once, the spores impacted a glass surface and bounced back through the detector. The two-stage, asymmetric charge detector recorded the velocities and directions of each spore both before and after impact. A modification in the vacuum setup (turning off one turbopump) allowed some variation of initial velocity, and two velocity ranges were studied, providing continuous coverage from 125 to 240 m/s. The COR and the fractional kinetic energy change upon impact were tested for both ranges of initial velocities.

All tests were performed with spores of *Bacillus subtilis* (1A308, ATCC, Manassas, VA, USA). Vegetative cells were subjected to a standard sporulation protocol in which the vegetative cells were deprived of nutrients over a period of time, inducing the cells to enter the dormant endospore form [26]. At the completion of this process, the spores were collected and suspended in sterilized HPLC-grade water (Thermo Fisher, Waltham, MA, USA) at a concentration of 10^6 CFU/ml (colony forming units per ml). The sporulation was carried out with the aim of increasing the resistance of the cells to mechanical stress. Cells undergo a number of physiological changes as they become spores. Primarily, they are almost completely dehydrated and they develop a thick outer coat comprised of a network of protein and glycan. The altered composition of the spores enables them to survive extremely harsh environmental conditions and stresses.

Electrospray was performed using the *B. subtilis* spore suspension described above. The solution was pumped through a custom-made electrospray tip (35-gauge hypodermic tubing) at 100 μ l/h using a KD Scientific (Holliston, MA, USA) syringe pump. A negative high voltage was applied to the needle (negative-mode electrospray) for these experiments. Negative-mode electrospray was selected due to the fact that a larger percentage of electrosprayed spores were detectable in negative mode than in positive mode experimentally. The electrospray tip was biased at -3500 V using a 5-kV, 25-W high-voltage power supply (PS350, Stanford Research Systems, Sunnyvale, CA, USA). After being electrosprayed, the spores entered the vacuum system in a highly charged state, enabling them to be easily detected by the charge detector. Note that the electrical charge on the spores is not used for acceleration, but only for detection.

Because of the lack of nutrients in the water used to store or suspend these spores, the spores remained metabolically inactive for the duration of the experiment. This was verified by serial dilution assays of the stock solution and suspension before and after experiments. No reduction of the viable counts of the electrospray suspensions was observed before or after experiments. In addition, no significant reduction in the viable counts of the stored solutions (kept under refrigeration) was observed over a period of 2 years.

The charged spores were introduced into the vacuum system via a 150- μ m pinhole. They were then swept through a beam tube and a skimmer cone by the flow of gas through the

Fig. 1 Scale drawing of experimental setup for spore bouncing experiments. Spores are electrosprayed (*top*) and pass down through a beam tube and a skimmer cone into vacuum. Spores then pass through the detector, bounce off of the glass surface and pass back through the detector again

vacuum system. Evaporation of the solvent took place both before and within the beam tube. Experiments using only solvent (no spore) showed no observable signals in the charge detector, indicating that evaporation was either complete or mostly complete. It was not possible to verify that the spores were completely desolvated, however. Through the use of the skimmer cone as well as two turbo-molecular pumps, the pressure was reduced from ambient conditions to approximately 8.6×10^{-4} Torr at the charge detector. A second beam velocity was obtained by turning off the first turbopump—the different gas flow rates and pressures before the skimmer resulted in slower spores. In that case, the system pressure was

only 6.8×10^{-2} Torr at the impact surface. Given the spore velocities, the relaxation distances at these pressures were approximately 95 m and 88 cm, respectively, so gas-based acceleration or deceleration of the spores is expected to be minimal within the charge detector.

Spore velocity and direction were determined using an image charge detector with two stages (Fig. 2). The charge detector consisted of two printed circuit boards (PCBs) patterned with metal electrodes [27]. The two identical plates were placed facing one another, parallel to the flight path of the incoming charged spores. Each plate consisted of two sensing regions interspersed by three grounded regions. The two sensing regions were of different length, one of them being 0.64 cm long while the other was 1.91 cm long. The differing length of the sensing regions enabled unambiguous determination of the direction that the spore was traveling. Traveling from the system entrance towards the system terminus, spores passed the shorter sensing region first, followed by the longer sensing region. The signal produced by a spore thus traveling appeared as two closely spaced peaks followed by two peaks that were spaced further apart. Once the spore impacted the hard glass surface and rebounded into the detector, it passed through the two sensing regions in reverse order. Two more widely spaced peaks were followed by two more closely spaced peaks. This design allowed for the signal from a rebounding spore to be easily distinguished from the signal that was produced by two spores traveling closely together but in the same direction. In contrast, a single or multi-stage charge detector with sensing regions all of the same length cannot be used to determine the direction that a charged particle is traveling. In such case, the signal produced by two spores traveling closely together would not be readily distinguishable from the signal produced by a spore that had rebounded. The present design also provides two separate measurements of velocity before bounce and two after bounce (one for each stage), and provides additional verification that bounce occurred based on the distance from the PCBs to the glass impact plate and the timing between incoming and rebounding signals.

PCB plates were designed using Eagle Layout Editor 6.4.0 and were fabricated by Quick Turn Circuits (Salt Lake City, UT, USA). The two sensing stages on facing plates were connected to each other as well as to a charge-sensitive pre-amplifier (A250, Amptek, Bedford, MA, USA) by wires that were connected to vias on each plate. The charge-sensitive pre-amplifier was in turn connected to three differentiating and shaping amplifiers (A275, Amptek). The grounded regions on both plates were also wired to each other as well as to outside ground. The plates were spaced 4 mm apart using ceramic spacers.

A square of pane glass that was 15×15 mm served as the impact surface. This surface was positioned at the terminus of the charge detector, normal to the flight path of the spores. This provided a smooth, flat surface that increased the likelihood that impacted particles would rebound back into the detector after impact. In order to measure the fractional change in kinetic energy due to impact, the velocity of the particles both before and after bouncing was measured. The signal generated by the charge detector provided the time that it took a spore to pass through each stage of the detector. By dividing the known length of the detector stage that corresponded to this signal by the transit time that the signal indicated, particle velocities were calculated. By comparing the velocities of each spore both before and after impact, the COR and fractional change in kinetic energy were calculated for each spore.

As illustrated in Fig. 2, rebounding spores can be detected within a range of angles off of normal, limited by the geometry of the PCBs and detection electrodes. Assuming that the incoming spores are aligned to the center of the PCBs, rebounding spores can deviate by up to 13 degrees of normal (parallel to the plane of the PCBs) and still be detected by both detection elements. The rebound velocity of such spores would be underestimated by no more than 2.8% due to this effect.

Fig. 2 Above one of the two boards that make up the PCB-based image-charge detector. Due to the differently sized detection stages, the direction of the traveling particle is unambiguously determined. Below illustration of spore bouncing and detection

The image-charge detector used for these experiments consisted of two sensing regions, one much longer than the other. The different electrode dimensions were designed such that the direction of travel for a detected particle could be determined. Each time a charged spore passed through a sensing region, a pair of peaks was produced, the first when the particle first began to pass through the detecting region and the second when the particle exited the detection region. Thus, a particle passing through the dual-stage asymmetric charge detector should generate four peaks. A particle passing through the detector, bouncing, and passing all the way back through the detector again typically produced a total of eight peaks. This pattern consisted of a pair of two closely spaced peaks followed by two pairs of widely spaced peaks. If, after impact, a particle bounced back into the detector at an angle, it is possible that it would be detected by the longer detecting region but not the shorter, thus producing a total of six peaks. This type of signal was likely generated by a particle re-entering the detector at an angle such that it passed only over the first detection region but not the second. Depending on the angle, the particle then passed out of the side of the detector or collided with the grounded region between the two sensing regions. Due to the error in velocity that would result from such a large angle, the few signals that showed only six peaks were rejected from the analysis. By dividing the known length of the sensing region by the transit time between each pair of peaks, each particle's velocity was calculated.

An additional test was performed to confirm that a particular signal was produced by a bouncing spore. The calculated velocities of each spore both before and after impact were used to generate a theoretical transit time against which a measured transit time was compared. This transit time indicated the time during which the spore traveled from the end of the detector to the impact surface and then rebounded back into the detector. For half of this distance, the spore was traveling at its initial velocity while for the other half of the distance the spore was traveling at the post-impact velocity. With the recorded time that it took to travel this distance, and the two measured velocities, it was possible to calculate what this intermediate bouncing time should have been. By comparing this theoretical value with the observed value it was possible to further confirm that a true bouncing signal was being observed.

$$\left(\frac{0.0141 \text{ m}}{v_1} + \frac{0.0141 \text{ m}}{v_2} \right) = t_t. \quad (2)$$

In this equation, 0.0141 m is the distance between the end of the final sensing element and the impact surface, v_1 is the pre-impact spore velocity, and v_2 is the post impact spore velocity. The variable t_t is the theoretical time that it should take the spore to travel from the end of the sensing element, to the impact surface, and back into the final sensing element based on the two recorded velocities. A small fraction of apparent bouncing signals had more than 10% variance between the measured and theoretical times—these signals were excluded from analysis. This confirmation was in addition to the confirmation provided by the apparent peak spacing due to the detector design.

3 Results and discussion

A typical signal from a bouncing spore is shown in Fig. 3. Visible are the four peaks corresponding to the incoming spore (one signal each from entering and exiting each of the two sensing stages), and four signals as the spore, upon rebound, passed again through the two

Fig. 3 Signal produced by a charged *B. subtilis* spore passing through the charge detector at 130 m/s, bouncing, and then passing back through again at 69 m/s. Due to triboelectric charge transfer upon impact, the peak area for a peak produced by the spore on the return trip is somewhat smaller than the peak area corresponding to the initial passage through the detector. The rebound peaks are also wider due to lower velocity of entering and exiting the charge sensing stages. Velocities derived from peak spacing were used to verify that signal produced by a bouncing spore was being observed

sensing regions. Boxcar averaging was used to reduce high-frequency noise. This particular spore had an incoming velocity of 130 m/s and rebound velocity of 69 m/s. Uncertainty in velocity measurement is 5.5% as determined by the variance among the pairs of peaks as each spore passes through the two sensing elements before and after bounce.

The average spore velocity for experiments performed at a pressure of 8.6×10^{-4} Torr was 197 ± 17 m/s. The average fractional kinetic energy loss after impact (Fig. 4) was 0.69 ± 0.16 , meaning that most of the translational kinetic energy was lost in the impact. For the experiments performed at the vacuum pressure of 6.8×10^{-2} Torr, the average initial spore velocity was 145 ± 12 m/s. The average fractional kinetic energy loss after bouncing at this velocity was 0.74 ± 0.11 . The average coefficients of restitution for the two velocity regimes were 0.53 ± 0.15 and 0.49 ± 0.12 , respectively. The CORs for the two velocity ranges were not statistically different, indicating that the fraction of energy lost at impact was not dependent upon the initial velocity of the spore impacting the surface. The measured COR values for spores are very similar to PS spheres, the latter spanning the range 0.6 to 0.3 for this range of velocities. However, the results are in contrast to PS studies which show a gradual decrease in COR with increasing velocity. The distribution of energy losses around the average value for both initial velocities show large variances, so likely any gradual trend in COR with velocity is obscured by the variance in the data.

The kinetic energy loss of these spores is comparable to the KE loss of polystyrene microspheres at similar velocities on similar surfaces. In the case of PS spheres, the KE loss was attributed largely to plastic deformation, which is presumably also the loss mechanism for the spores. However, in previous experiments, we have shown that spores of this particular strain survive impacts on glass at velocities up to 299 m/s with very high survival rates. This implies that the spores can survive in spite of the plastic deformation occurring in the impact. Based on the average mass and density of *B. subtilis* spores, the kinetic energy loss in impact, as measured in these experiments, averages 5×10^{-12} J.

Fig. 4 Distribution of fractional kinetic energy loss for the bacterial spores upon impacting a glass surface over the two ranges of impact velocity studied. Each bin shows the number of observed spores with fractional KE lost greater than or equal to the bin label, i.e., a loss of $0.30 \leq x < 0.40$ falls in the 0.3 bin

A significant proportion of the signal that was collected showed only the four peaks of a pre-bounce spore, but no post-impact signal. These were likely produced by a spore traveling through the detector once but rebounding off of normal with an angle such that the spore did not pass back through the charge detector. The spore shape may be the reason that the bouncing signal was not more frequent. *B. subtilis* spores are elongated, with average dimensions of $1.07 \pm 0.09 \mu\text{m}$ length and $0.48 \pm 0.03 \mu\text{m}$ width [28]. The orientation of the spore upon impact was not controlled, and may have resulted in many impacts with off-normal rebounds. Approximately one or two out of every ten recorded signals for a given experiment demonstrated that a bounce had occurred. The aspheric spore geometry and roughness of spore surface may also explain the large variance in the measured COR values.

In these experiments, we assumed that all solvent had evaporated from the spores prior to impact. However, this cannot be verified using this technique. It is possible, therefore, that a small layer of solvent, or perhaps other species, was still present on the spore surface during impact. The properties of these surface materials would likely be very different from the spore coat proteins, and may have introduced additional uncertainty in the presented results.

It is apparent from all signals observed, as can be seen in Fig. 3, that the areas of the peaks produced by the charged spore post-impact was somewhat smaller than the areas of the peaks corresponding to the initial pass through the detector. Each peak area is directly proportional to the charge of the particle that produced the peaks, although the charge detector used was not calibrated for absolute charge. The object that produced the second set of peaks must have had a smaller charge than the object that produced the first set of peaks. It has already been confirmed that the signals observed were indeed produced by a rebounding object. It is likely that the loss in peak area after each bounce was due to a triboelectric transfer of charged species between the spore and the glass surface during impact, although the type of charge carrier cannot be determined from these experiments. In all cases, the post-bounce charge was less than the pre-bounce measurement, although generally only 15–30% of the charge was lost in impact.

4 Conclusions

The dynamic elastic properties of *B. subtilis* spores have been investigated. A dual-stage asymmetric charge detector has been introduced that was specifically designed to monitor bouncing particles. By using this detector to measure the velocities of impacting spores, both before and after impact, it was possible to determine how the kinetic energy of a traveling spore is affected by impact with a glass surface. Using this change in velocity, the coefficient of restitution was also determined for the bouncing spores. Data showed a large variance in the coefficient of restitution and also the fractional kinetic energy loss upon impact, but the average values compare well with polystyrene spheres impacting at similar velocities. The shape of the spores likely played a role in the dynamics of these impacts. Some of the electrical charge on the spores was transferred from the surface of a charged spore to the glass at the time of impact. The work presented here will serve to supplement other investigations into the mechanical properties of bacterial spores.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest related to this work.

References

- Gosline, J., Lillie, M., Carrington, E., Guerette, P., Ortlepp, C., Savage, K.: Elastic proteins: biological roles and mechanical properties. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* **357**, 121–132 (2002)
- Talekar, M., Tran, T.-H., Amiji, M.: Translational nano-medicines: targeted therapeutic delivery for cancer and inflammatory diseases. *AAPS J.* **17**(4), 813–827 (2015)
- Driks, A.: Maximum shields: the assembly and function of the bacterial spore coat. *Trends Microbiol.* **10**(6), 251–254 (2002)
- Zhao, L.M., Schaefer, D., Marten, M.R.: Assessment of elasticity and topography of *Aspergillus nidulans* spores via atomic force microscopy. *Appl. Environ. Microbiol.* **71**(2), 955–960 (2005)
- Nakanishi, K., Kogure, A., Fujii, T., Kokawa, R., Deuchi, K.: Development of method for evaluating cell hardness and correlation between bacterial spore hardness and durability. *J. Nanobiotechnol.* **10**, 22 (2012)
- Svaldo Lanero, T., Cavalleri, O., Krol, S., Rolandi, R., Gliozzi, A.: Mechanical properties of single living cells encapsulated in polyelectrolyte matrixes. *J. Biotechnol.* **124**(4), 723–731 (2006)
- Stenson, J.D., Hartley, P., Wang, C.X., Thomas, C.R.: Determining the mechanical properties of yeast cell walls. *Biotechnol. Prog.* **27**(2), 505–512 (2011)
- Yao, X., Jericho, M., Pink, D., Beveridge, T.: Thickness and elasticity of Gram-negative murein sacculi measured by atomic force microscopy. *J. Bacteriol.* **181**(22), 6865–6875 (1999)
- Vadillo-Rodriguez, V., Beveridge, T.J., Dutcher, J.R.: Surface viscoelasticity of individual Gram-negative bacterial cells measured using atomic force microscopy. *J. Bacteriol.* **190**(12), 4225–4232 (2008)
- Heinz, W.F., Hoh, J.H.: Spatially resolved force spectroscopy of biological surfaces using the atomic force microscope. *Trends Biotechnol.* **17**(4), 143–150 (1999)
- Barney, B.L., Pratt, S.N., Austin, D.E.: Survivability of bare, individual *Bacillus subtilis* spores to high-velocity surface impact: implications for microbial transfer through space. *Planet. Space Sci.* **125**, 20–26 (2016)
- Dahneke, B.: The capture of aerosol particles by surfaces. *J. Colloid Interface Sci.* **37**(2), 342–353 (1971)
- Dahneke, B.: Measurements of the bouncing of small latex spheres. *J. Colloid Interface Sci.* **45**(3), 584–590 (1973)
- Dahneke, B.: Further measurements of the bouncing of small latex spheres. *J. Colloid Interface Sci.* **51**(1), 58–65 (1975)

15. Kreibel, H.W., Chilton, E.G.: The Drag of Cylinders in Steady Particle-Laden Gas Flow. Defense Atomic Support Agency, Washington, DC (1962)
16. Wall, S., John, W., Wang, H.-C., Goren, S.L.: Measurements of kinetic energy loss for particles impacting surfaces. *Aerosol Sci. Technol.* **12**, 926–946 (1990)
17. Paw U, K.T.: The rebound of particles from natural surfaces. *J. Colloid Interface Sci.* **93**(2), 442–452 (1983)
18. Jordan, D.W.: The adhesion of dust particles. *Brit. J. Appl. Phys. Suppl.* **3**, S194 (1954)
19. Lifshitz, J.M., Kolsky, H.: Some experiments on anelastic rebound. *J. Mech. Phys. Solids* **12**, 35–43 (1964)
20. Muller, P., Heckel, M., Sack, A., Poschel, T.: Complex velocity dependence of the coefficient of restitution of a bouncing ball. *Phys. Rev. Lett.* **110**, 254301 (2013)
21. Marinack Jr., M.C., Jasti, V.K., Choi, Y.E., Higgs III, C.F.: Couette grain flow experiments: the effects of the coefficient of restitution, global solid fraction, and materials. *Powder Technol.* **211**(1), 144–155 (2011)
22. Mueller, P., Antonyuk, S., Tomas, J., Heinrich, S.: The normal and tangential coefficient of restitution of different types of granules. *Chem. Ing. Tech.* **83**(5), 638–642 (2011)
23. Goldsmith, W.: *Impact: the Theory and Physical Behaviour of Colliding Solids*, Dover Publications Mineola, N.Y. (2001)
24. Ayesh, A. I.; Brown, S. A.; Awasthi, A.; Hendy, S. C.; Convers, P. Y.; Nichol, K., Coefficient of restitution for bouncing nanoparticles. *Phys. Rev. B* **81**(19), 195422 (2010)
25. Pratt, S., Austin, D.E.: Bacterial spores survive electrospray charging and desolvation. *J. Am. Soc. Mass Spectrom.* **25**(5), 712–721 (2014)
26. Nicholson, W.L., Munakata, N., Horneck, G., Melosh, H.J., Setlow, P.: Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. *Microbiol. Mol. Biol. Rev.* **64**(3), 548–572 (2000)
27. Barney, B.L., Daly, R.T., Austin, D.E.: A multi-stage image charge detector made from printed circuit boards. *Rev. Sci. Instrum.* **84**(11), 114101 (2013)
28. Zandomeni, R.O., Fitzgibbon, J.E.; Carrera, M.; Steubing, E.; Rogers, J.E.; Sagripanti, J.-L. Spore Size Comparison Between Several *Bacillus* Species, Edgewood Chemical and Biological Center, US Army, Aberdeen Proving Ground, MD. Report date 01 Oct (2005).