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TURNING POINT ARTICLE

THE DEVELOPMENT OF THE BIOLISTIC PROCESS

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I have been asked to provide some personal insights into how the biolistic process came to be developed. I am happy to do this, and would ask the indulgence of the reader to permit me to share some personal philosophical perspectives, in addition to outlining the mechanistic history of the progression of the research. This account is, to the best of my ability, accurate. As this history touches on things that go back at least 17 years, there will doubtless be some recollections that are imperfect and people that I have failed to fully acknowledge. For these mistakes, I sincerely apologize in advance. Likewise, there were numerous scientists who worked independently of me and who contributed to early biolistic research. I do not attempt to represent their work here. There are now hundreds of scientists doing biolistic research, and I make no effort here to survey or review the literature on the subject of biolistics (this has been done by others). Rather, this short history only touches on some of the highlights of my personal involvement concerning the development of the biolistic process.

Background

When I was a graduate student in the late 1970s, my dream was to contribute to the scientific breakthroughs that would help to feed a hungry world. There were grave concerns about possible mass starvation in large parts of the world, which was commonly thought to be imminent due to the 'population bomb'. Largely for that reason, I went into plant breeding, and earned my Ph.D. in 1980. Since that time plant breeding has been a significant factor (one of several) resulting in increased food production worldwide, and world hunger has been reduced – or at least held in check. However, my own contributions to the field of plant breeding were modest at best. Ironically, the area where I was able to have some impact was in the field of genetic engineering – a field where I had virtually no training and no pre-inclination.

At the beginning of the 1980s, plant genetic engineering was still within the realm of science fiction. Some scientists were already proposing its possible practical application, but there was considerable skepticism and resistance – especially from conventional breeders (myself included). Nonetheless, as a young faculty member at Cornell University, the possibilities of engineering plants captivated my imagination. As an 'old-fashioned' plant breeder, with no molecular training and no lab experience (in fact, no laboratory or funding to speak of), I should have known that I could not possibly participate in this hot new field. If I had known how many brilliant, well trained, and well equipped scientists were

way ahead of me in embarking in this direction, perhaps I would not have even bothered. However, it was my extreme good fortune to be both naive and rather poorly informed. And so, believing all things were possible, I plunged ahead. In retrospect, I believe my ignorance served me well.

An obvious prerequisite for plant genetic engineering is the ability to transform plants. In the early 1980s there was no method for obtaining transformed plants. Until one can deliver new genes into the plant genome, there is no point in talking about plant engineering. At first my lab focused on pollen transformation – a natural avenue for a plant breeder who lacked tissue culture and regeneration capabilities. The concept of pollen transformation had already been put forward by Drs. K. K. Pandey (New Zealand) and D. Hess (Germany). My first graduate student (Yan San Chyi) and I did a great deal of research looking for ways to utilize pollen to transmit foreign DNA into natural zygotes and embryos, thereby producing transgenic seed directly. These methods included use of irradiated donor pollen; direct uptake of DNA into pollen; electroporation of pollen; and agro-infection of pollen (Chyi et al., 1984; Sanford et al., 1984a–c; Chyi and Sanford, 1985; Sanford and Skubik, 1985). The results of all this work were a series of putative transgenics, which at first looked very promising but later proved to be non-reproducible aberrations. These aberrant plants probably reflected the mutagenic or physiological effects of the DNA treatments, rather than genuine transformants. (This work was greatly hampered by lack of good marker genes, and might be interesting to repeat now.) One of my last efforts aimed at pollen transformation involved the use of a microlaser beam to cut holes in the cell wall of individual pollen grains – with the purpose of letting DNA diffuse through the opening in the wall (Sanford, 1982). Although the microlaser allowed me to cut 1 μm holes in the walls of living pollen tubes, it became obvious to me that this approach was clearly not going to be practical.

While presenting my work with microlasers to colleagues at Cornell, someone suggested that I contact Ed Wolf, a professor of electrical engineering and the Director of the National Submicron Facility. The Submicron Facility at Cornell specializes in the use of electron and ion beams to modify the surface of materials for such purposes as microcircuitry. The idea was that perhaps such beams could be used to cut holes in cell walls. I met with Ed and we discussed this idea. Ed explained to me some of the technical limitations to this approach. However, Ed was intrigued by potential biological applications for his facility's technologies, and was eager to discuss other physical means for delivering DNA into cells. I remember Ed calling up and asking if we couldn't shoot in 'beams' of the DNA molecules themselves (they would have much more momentum than particles such as electrons). I indicated that I doubted the DNA could be accelerated as a free molecule, or that it would survive impact against the cell, or that it would have sufficient momentum to penetrate a cell wall. However, I remember later mulling over that idea, and then one of us (I think me) called back and proposed using a larger solid particle as the 'bullet', with the DNA going in with it. As I recall, we both liked that idea, but Ed did the calculations and concluded that his facility's technology (electromagnetic accelerators) would not be powerful enough to accelerate solid particles ('macroparticles') big enough to puncture a cell wall (i.e. large enough to create a hole about 1 μm in diameter, as with the microlaser). We continued to mull this over for some time. I recall Ed asked me how fast I thought a micron-sized particle would have to be moving to penetrate a cell wall. My intuitive response was – the speed of a 'BB' or pellet from a 'BB' or pellet gun (I had been engaged in a personal battle with marauding squirrels that fall). Ed immediately realized that such speeds (less than 1000 ft s^{-1}) could be achieved using a variety of relatively unsophisticated technologies, and certainly did not require ion beams and electrostatic accelerators! We determined to look at simple mechanical acceleration systems to test our concept, to see if we could accelerate 'macro' particles into cells. In fact, Ed wanted to test the BB-gun idea immediately, and within days bought one of these toy pistols from Fay's drug store. Little did we realize that a toy pistol would lead to the 'gene gun', which would be a valuable tool for a wide range of biologists. At Ed's initiative and insistence we would soon embark on our first BB gun experiments.

First Experiment

Ed happened to have some 4 μm tungsten spherical particles on hand, which were perfect in that they were a high-density metal (more momentum), and were large enough to be easily seen through a microscope. Ed then asked Nelson Allen, the head machinist at the Submicron Facility, to modify the pistol so as to facilitate easy loading and firing of the tungsten powder. Nelson drilled a hole in the gun barrel for loading powder, and made a deflector plate to reduce the gas blast to the target. Ed and Nelson asked me what we should use as our test subject, and I determined that onion epidermal cells should be our best model system – being the largest plant cell type and offering readily accessible cells that could be easily observed under a microscope. During the Christmas break 1983, the three of us went into the Submicron Facility (which required all of us to don white gowns, booties and hats), and surrounded by various multi-million dollar ion beam accelerators, we proceeded to blast whole onions with our sophisticated 'Macroparticle Accelerator'. We would pump up the pistol and load a bit of tungsten powder into the end of the barrel, aim, close our eyes, and fire. Up close, the gas blast was violent enough to blow a hole in the onion's surface (the air reeked of onion – onion juice and bits of onion were all over our sophisticated high-tech frocks). From a distance of one foot, the tungsten powder was launched by the gas blast and gently settled onto the onion's surface – microscopic examination revealed no cell penetration. However, with the right load and the right distance, the damage to the tissue was less than catastrophic, and when the epidermal cell layer was peeled off and examined microscopically, tungsten particles could clearly be seen inside some cells. This told us that velocities characteristic of a simple blast of air (i.e. less than 1300 ft s^{-1}) could be used to accelerate particles into cells. We went home that night amused by the contrast between the sophistication of the Submicron Facility and our very primitive and seemingly foolish antics with toy guns and raw onions, but we were encouraged and cautiously optimistic.

Early Years of Research

Ed and Nelson playfully mounted the first gene gun in a fancy pistol case labeled 'Macro-particle Accelerator', and presented it to me. I took it back to my lab, along with the tungsten particles, and began to experiment with onion. Through a series of experiments I was able to show that onion cells could survive particle penetration,

and that foreign material, including DNA, could be delivered into such living cells (Sanford et al., 1987). With those data, I began to write a patent application for Cornell and a grant proposal to Cornell's Biotechnology Institute. The Institute was supposed to be committed to funding high-risk, highly innovative research (the stuff breakthroughs are made of). Long afterward, I learned that the evaluating panel's first response was laughter and ridicule (the idea of 'shooting' DNA into cells with any sort of a gun)! As a personal aside, I have learned that 'divergent thinking' or 'upside-down thinking' (a skill I learned from my friend Stephen Johnston) is crucial for most scientific breakthroughs – but consistently generates hostility and ridicule. At any rate, I am told a single individual spoke up during the review and pointed out that this was exactly the type of work the Institute had been mandated to support (bless him for his courage and insight). And so we received a small grant with which we could get the gene-gun research off the ground. With those funds I hired Ted Klein. Ted played a critical role in taking biolistics from a very primitive proof of concept (Klein et al., 1987) to a working gene delivery system. Ironically, like my own, Ted's background (majoring in botany as an undergraduate and microbiology in graduate school) did not directly fit. He had no special training in plant genetics, plant cell biology, molecular biology, physics, or engineering (in the same vein, Ed Wolf's Ph.D. was in physical chemistry). The ability of 'outsiders' to bring fresh ideas and new capabilities to a field is something I have seen repeatedly, and this has been a recurrent theme in both the development of biolistics and all the other aspects of my career.

Ted and I soon realized that simple gas-blast devices (by now we had purchased several types of pellet guns) were too violent when used up close, and were too slow when used at greater distances. We clearly needed gentler and more sophisticated acceleration mechanisms. I proposed a gunpowder-driven plunger device with stopping plate – which Nelson Allen then built. The nature of the plunger and stopping plate had to be optimized experimentally, as initially we were generating very high-velocity debris that was both dangerous to us and disastrous for the target plant samples. We settled on HMW polyethylene for the plugger (macroprojectile), and lexan for the stopping plate. This new device was an important improvement. Using that gun, Ted was eventually able to demonstrate the first biolistic transformations of plants (Klein et al., 1988c). Initially Ted used RNA from the TMV virus, then used transient expression of the CAT reporter gene (Klein et al., 1988a). However, this new device still delivered too much gas blast and, as we increased distance to the target (to reduce injury to the plant tissue), we still were rapidly losing particle velocity/penetration due to air resistance. For these reasons, I requested that Nelson build for us an airtight chamber in which we could enclose both the gun and target tissue, allowing us to then evacuate the entire system with a vacuum pump. We found firing through a vacuum greatly reduced damage and increased penetration. However the resulting device was very cumbersome to use, took a long time to set up and fire, and was very poorly suited for maintaining sterile conditions. Ted and I then requested an improved design which addressed these problems, and Nelson built a very beautiful unitized gene gun which became instrumental for the next several years of gene-gun research (that gun is now the property of the Smithsonian Museum). It was with this device that Ted was able to achieve stable plant transformation, using the *npt*II selectable marker gene. I will always remember Ted's early years working on the gun – how the lab smelled like both McDonald's Restaurant (due to all the blasted onions) and a firing range (due to the gunpowder). Ted would set up the gun on a ring stand and carefully 'cock' the device. He would then tie a string to the firing mechanism, step out of the lab, close the door, and pull the string from the hallway. There would be a tremendous blast, and occasionally debris flying through the air. People throughout the second floor of Hedrick Hall would be jumping out of their seats at the sound of sporadic gunfire in the lab (before we went to vacuum). Naturally, the general consensus in the department was that we were crazy. It seems to me that neither Ted nor I really minded that. The unusual nature of what we were doing, and the humor ('lets give it a shot', etc.), generally made it really very much fun.

Once we had a rudimentary biolistic gene delivery device in operation, we had to (a) determine the best particles for use as microprojectiles; (b) select appropriate reporter genes and vectors; and (c) work out effective methods for attaching the DNA to the microprojectiles. We looked at many different types of prospective microprojectiles, including dried bacteria, glass, silicon crystals, nickel, platinum, gold, and tungsten powders. Although all of the particles in the 1 μm size range worked to some degree, the best particles were high density, especially gold or tungsten. For non-medical purposes, M10 tungsten particles seemed most practical, and have been the standard for plant work ever since those early days. Ted also worked out highly effective methods for precipitating nucleic acids onto gold and tungsten particles. Once he had a working biolistic device, and the right particles coated with the right DNA, he had to systematically optimize the physical parameters of the bombardment process, including velocity, degree of vacuum, distance to target, target pre-conditioning, etc. It took several years of this type of developmental work to get to the point where Ted could prove stable biolistic transformation of a plant (Klein et al., 1988c). I am especially grateful to Ted, not just for the pivotal role he played in making biolistic technology practical, but for his dedication to the project and his belief that it would eventually work on a practical level. I remember that at one point several years into the work, when the plant cells consistently died in a series of bombardment experiments, I felt extremely discouraged and expressed to Ted my doubts that we were on a path leading toward success. I suggested that he might want to start to look for a less shaky career path. Ted responded that he believed the technology could be made to work, and that he would like to see it through. I am so glad he did! It was not long after that things started to fall into place and the technology really took off. Ted and I were soon to participate in a series of 'firsts', including the first transformation of a chloroplast (Boynton et al., 1988; Daniell et al., 1990); the first transformation of mitochondria (Johnston et al., 1988), and the first transformation of corn (Klein et al., 1988b).

Other Gun Concepts

Ted and I looked at a wide variety of acceleration devices in addition to the gunpowder-driven design. The challenge was to bring microscopic particles up to the velocity of a rifle bullet (about the speed of sound) in a way that is not overly traumatic to the target tissue. We experimented with gas-blast systems, macroprojectile (plunger) systems, transferred impulse (drumhead) mechanisms, and centripetal acceleration devices, and investigated the prospects for electrostatic acceleration systems. Nelson Allen was a proponent

of the transferred impulse concept and made an experimental unit. Ed Wolf very generously provided some of his discretionary funds to allow testing of some of these various ideas, including paying for some of Nelson's shop time. Nelson also put in long hours in fabricating various prototype units or components in his own time. Of the systems we investigated, the macroprojectile mechanism was the most practical, and is still the mechanism which is overwhelmingly the biolistic mechanism of choice. Once our biolistic idea had been published (Klein et al., 1987), other labs began to design and build their own biolistic devices. Most notable would be the macroprojectile and gas-blast devices developed by Dennis McCabe and colleagues at Agracetus, Inc. However, all gene guns employed to date have employed the basic features outlined in the patent application of 1984 (Sanford et al., 1990).

Biolistics, Inc.

By mid-1986 the biolistic process was starting to show some promise. Ed Wolf approached me and suggested we buy the rights to our own invention from Cornell University. At that time it still seemed unlikely to me that the technology would ever have any commercial value. For example, *Agrobacterium* had just been shown to be an effective tool for transforming tobacco, and it was not clear that any other method was needed. Nonetheless, we decided to see if Cornell would even consider the notion of a license. To our surprise, Cornell was very receptive to the idea, and so Ed and Marlene Wolf, and John and Helen Sanford became partners and formed Biolistics, Inc. Incidentally, the term 'biolistics' (derived from biology and ballistics) was coined by the Wolf family – we needed a better name for the process, as well as a name for the corporation. Biolistics, Inc. then licensed the biolistic patent from Cornell. As we did not anticipate any commercially relevant activities for Biolistics for at least several years, Ed and I both proceeded to take our respective sabbaticals as we had previously planned. Little did we know how different the whole picture would appear before we returned several months later. During my sabbatical, because of collaboration between my lab and scientists at Pioneer Hi-Bred International, Inc., Pioneer approached me and indicated an interest in obtaining their own biolistic apparatus. Representing Biolistics, Inc., I negotiated with Pioneer a collaborative research agreement that would supply Pioneer with access to the first gene gun to be available to researchers outside of my Cornell lab. Because we were still on our sabbaticals, Ed and I had to arrange to travel back to Ithaca, where we contracted with Nelson Allen (as an independent contractor) to make the first commercially available gene gun. We came up with a design that would be easier to use, more appropriate for sterile techniques, and would have numerous safety features to protect the user (Sanford and Wolf, 1994). Nelson took this design to a subcontracting machine shop outside of Cornell University. There Stan Rumsey and Dale Loomis soon began to routinely fabricate gene guns for Biolistics, Inc., bringing great ingenuity and artistry to the custom manufacture of a series of gene-gun prototypes. As Stan's health began to fail (God rest his soul), Dale Loomis increasingly became our key partner in terms of prototype development. Dale displayed a striking genius for design enhancement, fabrication, and the testing of diverse gene guns and other research apparatus. Dale has a profound and amazing ability to quickly turn a vague concept or pencil sketch into a beautiful and fully functional machine.

Because of newly-breaking technical breakthroughs coming from collaborations between my lab and various collaborators (described below), there suddenly arose an overwhelming demand for gene guns. In two short years, Biolistics, Inc. entered into research agreements with virtually every major plant biotech company. Ed and I hired Dean Shea (Ed's son-in-law), who admirably served as Biolistics' manager and only real employee. Our tiny company rapidly became so hot that it felt quite overwhelming. Ed and I were retaining our Cornell responsibilities, and we were conscientiously trying to avoid real or perceived conflicts of interest or time. We worked on Biolistics, Inc. primarily evenings and weekends, using our own energies, facilities, and resources. As the business issues heated up, Ed took an extended leave of absence from Cornell to work for Biolistics, Inc. As a low-energy person, I soon realized I needed out of it – and I increasingly felt it was starting to control us, rather than us controlling it. I believe Ed felt the same way, and was torn between our exciting little business and his major responsibilities as Director of the Submicron Facility. Therefore Ed and I began to look for an exit strategy. In 1989, just about two and a half years after incorporating Biolistics, we sold off the entire technology rights of the company to DuPont (except for the ornamental rights, which were retained by Sanford Scientific, Inc., a consulting structure I had formed several years earlier). This set aside to SSI was graciously agreed to by Ed and Marlene, and was acceptable to Cornell and DuPont. Cornell was a party and major beneficiary of that sale, having taken an equity position in Biolistics at the time of licensing the technology.

Technical Breakthroughs

The nearly overnight success of Biolistics, Inc. was only possible thanks to several unanticipated technical breakthroughs that happened shortly after its formation, which were made possible through key collaborations. In the fall of 1986, a collaboration was initiated involving Ted Klein and myself at Cornell and Dwight Tomes and Arthur Weissinger at Pioneer Hi-Bred, which within several months led to the first effective transformation of corn (Klein et al., 1988b). Corn transformation had been for many years an unobtainable 'Holy Grail' of plant biotechnology – a pivotal roadblock/milestone for plant genetic engineering. Shortly after that collaboration was initiated, I took a sabbatical in Stephen Johnston's lab at Duke University to continue our collaborative work on parasite-derived resistance. While I was at Duke, Stephen encouraged me to apply biolistics to the challenge of mitochondrial transformation, another unobtainable Holy Grail in terms of transformation. Simultaneously, John Boynton and Nick Gillham at Duke entered into a collaboration with me and my lab, aimed at the first-ever chloroplast transformation. These two collaborations, involving Ted Klein at my Cornell lab and various Duke scientists, led simultaneously to two big breakthroughs, which were published back-to-back in the journal *Science* (Boynton et al., 1988; Johnston et al., 1988). Even as these breakthroughs were in progress, our only funding for biolistic work was cut out from under us. For reasons that still defy my understanding, the USDA terminated the small grant that was supporting Ted Klein – our only gene-gun funding. Even as the world of genetic researchers was beginning to beat a path to my lab's door, clamoring to collaborate and gain access to the gene gun, we were being denied funding and faced the possible collapse of the lab's biolistic research program. I was

profoundly disgusted and discouraged, to the point of wanting to entirely abandon science and all the gamesmanship that seems to go with it. My friend and colleague, Stephen, graciously scraped together some joint funding to do yeast mitochondrial transformation, which kept my lab afloat during this crisis period. However, for some time we found ourselves working less with plants, and more wherever we could find basic research funding: mostly with algae and fungi (Armeleo et al., 1990; Cummings et al., 1990; Shark et al., 1991; Smith et al., 1992).

Research After the DuPont Buyout

Part of the DuPont purchase agreement included a 2-year, $400 000 grant to Cornell to support further biolistic research. For the first time in my career I had sufficient funding to establish a substantial research team and do research without major constraints in terms of equipment, facilities or funding. I was also freed of having to worry about Biolistics, Inc. During those 2 years numerous major advances were made. In collaboration with Stephen Johnston and co-workers, and thanks to prototype development by Dale Loomis, my lab developed an improved helium-driven gene gun (Sanford et al., 1991), which quickly replaced the (instantly obsolete) gunpowder units. This new design was amenable to reconfiguration as a hand-held medical device suitable for bombardment of animal skin. Biolistic animal cell transformation was soon demonstrated (Johnston et al., 1991; Williams et al., 1991). This allowed Stephen and I to test our concept of genetic vaccination, which Tang et al. (1992) soon showed to be a valid and highly effective new type of vaccination. Under the guidance of two post-docs in my lab, Julie Russell (now Julie Russell Kikkert) and Franzine Smith, extensive research was conducted aimed at optimizing the physical and biological parameters which affect biolistic efficiency (Russell et al., 1990, 1992a,b). Biolistic transformation rates were increased by several orders of magnitude through this optimization, and a great deal was learned about factors that limit the process. With the improved apparatus and optimized process, many firsts were soon achieved, which demonstrated the broad utility of the gene-gun process. These included biolistic transformation of microbes (*E. coli*, yeast, other fungi and bacteria, and algae), diverse plant species, and diverse types of animal cells and tissues. Many research applications for the biolistic process were also demonstrated, including transient gene expression assays, viral inoculation, biolistic infection of plants with *Agrobacterium*, delivery of biological projectiles such as bacteria (Rasmussen et al., 1994) or viral particles (Kikkert al., 1999), and genetic vaccination (Tang et al., 1992).

Present Status

There are now hundreds of commercially available gene guns in use, as well as a large number of custom-made (home-made) guns. Gene guns are being widely used in academic and commercial labs. They are used as research tools in the areas of microbiology, zoology, botany, genetics, and medicine. I believe it is accurate to say that most of the presently grown transgenic crop acreage in the entire world was created through use of the biolistic process – having been originally transformed with the gene gun. There are now excellent prospects in medicine for the gene gun, which is currently the most promising delivery system for genetic vaccines. Future biolistic research that is needed includes better apparatus, better particles, better understanding of medical uses, and better understanding of gun-mediated chloroplast transformation for plants. In my opinion the most pressing need in the area of biolistic technology is the development of a medical gene gun suitable for extensive clinical use. It must be safe, reproducible, rapid-firing, trauma-free, and non-intimidating for both clinician and patient. Dale Loomis, Joe Celeste, and I have developed such a device (Loomis et al., 1999), and will begin making it available to interested researchers for evaluation.

This short history serves as a closure for my career in science. After a gradual and prolonged withdrawal from academia, I have finally reduced my university obligation to zero (retaining only a courtesy appointment at Cornell). At the same time, I have sold Sanford Scientific, Inc., a company I had formed and which has been employing the biolistic process for the engineering of ornamental plants. My only remaining goal in the area of science is to transfer the medical gene gun which Dale and I have developed to someone who can run with it. Beyond that, I hope to work in any way that God calls me in His service. I have established a very modest endeavor, Feed My Sheep Foundation, as a possible mechanism for such service. Its mission is to contribute to feeding His children, both physically and spiritually. The Foundation owns rights to several plant genetic engineering technologies, and plans to make those technologies freely available to international researchers for use in the developing world, while selling off those rights to industry for use in the developed world, in order to create operating funds for the Foundation.

Acknowledgments

I believe it is a fairly rare thing for a researcher to make an impact that generates very much special notice. Most scientists contribute a lifetime of excellent work and receive little recognition and minimal reward. Yet for some reason I was especially fortunate in this respect. I was blessed with a dream come true – an opportunity to contribute to a series of milestone developments that appear to have had a positive impact on the world. Although it was never my primary goal, I also received academic recognition and financial gain. I understand how little merit I brought to all this, and I am deeply aware that there were a large number of scientists who were more intelligent, more skilled, better trained, and harder working than I was. As I ponder this, I do not know why I was blessed in all these ways. Of course, most people would just call it luck. I guess I would rather call it mercy. I have become more and more certain of the Biblical truth that all our blessings are unmerited, and come from God through His mercy. Therefore I must first and foremost credit the Lord for anything good or worthwhile that may have come from my work. Professional success has been a profound blessing, yet it is the least that He has done for me. If He asks me, I will gladly give all of it back to Him – as long as I might still call Him Father.

My debt to the Lord in no way diminishes my indebtedness to a large number of friends and colleagues who were indispensable to the development of the biolistic process. First and foremost I would like to thank my wife, Helen, who has been my partner in life for 25 years, who bore with me throughout my entire career in science, and who was my active partner and fellow-officer in both Biolistics, Inc. and Sanford Scientific, Inc. Secondly, I would like to give thanks to

all my partners in biolistic research. I would like to give special recognition and thanks to Ed Wolf, Nelson Allen, Ted Klein, Dale Loomis, and Stephen Johnston, each of whom made very major contributions and who were my most pivotal partners in our joint development of gene-gun technology. Some of my other partners in biolistic research to whom I am most deeply indebted and wish to thank include (alphabetically): Henry Daniell, Mike Devit, Peter Harpending, Andy Humiston, Julie Russell Kikkert, Sandra McElligott, Janet Rasmussen, Mihir Roy, Stan Rumsey, Kathy Shark, Dean Shea, Franzine Smith, Joyce VanEck, Pat Wallace, Sandy Williams, and Guangning Ye. Lastly, although I never had the opportunity to work with them directly, I would be seriously in error if I did not acknowledge Dennis McCabe and Brian Martinell, whose foundational work at Agracetus, Inc. contributed so greatly to the implementation of biolistics in both agriculture and medicine.

God bless you all!

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