Modification of Existing Technology to Aid Research

Dr. Ed Schmidt

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Dr. Ed Schmidt is a scientific researcher interested in understanding intricate gene regulatory mechanisms of complex organisms. For the past 8 years he has worked with GeneSearch developing "The Dracula Pipette" recently renamed "The Embryo Cradle" to innovate and propel forward his line of work.

To aid our readers better understand your work, please tell us how your research background led to your interest in gene regulatory mechanisms?

Much of my career has focused on investigating mechanisms and consequences of gene regulation. My PhD work at Oregon State University investigated the gene regulatory mechanisms that shut-down a gene involved in producing the DNA precursors needed to replicate the genome as fetal cells destined to become muscle transitioned from a state of proliferative expansion to become mature contractile muscle. At the University of Geneva in Switzerland I investigated the gene regulatory programs underlying the unique stable functions of mature liver cells in rat and mouse livers. All of these studies, however, were based on describing the natural state of different cells under different conditions, and did not actually attempt to investigate the underlying mechanisms. I moved to the University of Utah to do a second postdoc to learn how to manipulate the mouse genome such that I might be better able to test the roles of specific genes in physiological processes. These technologies require very sophisticated embryo manipulations, and thus it was at this stage in my career that I first became actively involved in embryology technologies.

You've done a lot of research into redox biology as well as developing a mouse liver model. How has this previous work influenced your current R&D work for GeneSearch?

About 8 years ago my lab became interested in cellular antioxidant systems and how they regulate cellular physiological processes. For our studies, we need to occasionally develop novel mouse lines with specific mutations that are expected to help us understand these processes. This brings us back quite frequently to needing the sophisticated embryological manipulations that underlie development of novel mutant mouse lines. We generally out-source this work to committed experts who do nothing else and can therefore accomplish this much more quickly and efficiently than we could. However, it also made me wish that the technology could improve to the point that generating novel mouse lines could become more routine, and could be done in-house by less specialized labs. It was this latter desire that piqued my interest in the Dracula embryo manipulation tool.

You and GeneSearch have improved the original "Dracula Pipette" for embryo handling. Can you discuss the limitations of the original design?

In a word, "size"; however with this came very demanding technological hurdles. The original Dracula was designed for manipulating llama embryos. In scaling the Dracula tool down it was necessary to adapt it for use under a microscope, to drive the tool with advanced micromanipulators and hydraulic systems, and most importantly, to incorporate high performance materials that could provide the physical and optical properties needed for this tool on this small size-scale.

How does your new "Embryo Cradle" compare to the "Dracula Pipette?"

My understanding is that the name "Dracula" was chosen based on the utility of the tool for puncture-and-aspirate procedures. Now that our small-scale tools are becoming highly functional and showing great promise for applications in human clinical medicine, the name has become rather a handicap. In recognizing this, GeneSearch changed the name to the "Embryo Cradle" to emphasize the gentle way it holds the embryo as it preforms very delicate manipulations.

How will the new design benefit mouse and human embryo procedures?

For mouse, our goal is to make procedures easier, such that they can be performed easily and routinely by a broader cross-section of research scientists. For the human clinic, this tool holds great promise for developing improved technologies for no-harm pre-implantation genetic testing.

How do you expect your current research into embryo handling to affect your future work?

My goal is to make all of our mouse models in-house in the future, cutting time and costs needed to make novel mouse models, giving us more freedom to make and test some more cavalier allelic designs without worrying that investing in a non-useful design might bankrupt the lab.

Bringing Big Technology to Even the Smallest Of Laboratories

Advanced approaches for the genetic or epigenetic modifications of embryonic stem cells have revolutionised our ability to modify the cells from which novel mouse lines are produced. However, procedures for utilizing these cells in embryos have remained the same for decades. Dr. Ed Schmidt's work together with GeneSearch on "The Mouse Dracula Pipette" looks to amend this.

A MAJOR HURDLE IN A KEY PLACE

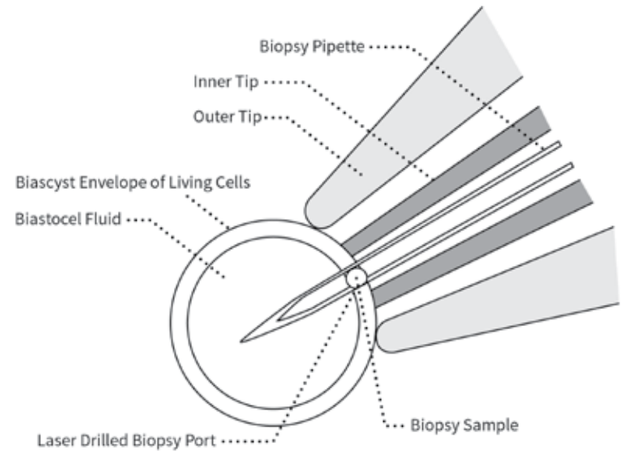
In the field of biomedical research mouse embryo cell based manipulation is a critical pillar for the foundation of any research. Such undertaking is very technologically and economically challenging. The cost and difficulties in generating chimeric mice is a major reason why many researchers avoid making novel mouse lines to test important hypothesis. Also, harm-free biopsy of embryonic stem cells remains difficult if not impossible using contemporary tools and technologies.

Mouse embryology goes far beyond just biomedical research. It also extends itself into human genetics as a "trial" or "prototype" for clinical research and for clinical procedures in both human genetics and reproduction clinics. Agriculture in the form of improved dairy animal management and conservation efforts for better reproductive management of endangered species are some other fields that can see themselves benefitted as well, however, these are fields with more cost prohibitive budgets and technical limitations that have up to now been left out of mouse embryology.

Its elegant coaxial design holds what I perceive to be great promise for putting highly sophisticated embryological procedures into the hands of a much broader cross-section of biomedical laboratories.

CURRENT MANIPULATION TOOLS

To this day the field of mouse embryo manipulation has been utilising pipettes for holding and pipettes for manipulation positioned by separate micromanipulators with 3 axes. In order to penetrate the embryo the injection pipettes and holding pipettes are presented in exactly opposite directions to create a conventional compression strategy. The problem with this is that the mouse oocyte and early embryo have a notoriously hard



zona pellucida, often requiring the use of a piezo hammer and sharp injection pipettes for dependable penetration. As the embryo expands, the zona is stretched thinner, has less resistance to deformation, and this strategy becomes increasingly difficult.

As a way to help with the penetration, other instruments such as chilled stage microscopes or piezo hammer can be brought in to help facilitate the penetration of the embryo with conventional equipment. These extra instruments and steps required for conventional methods to work add layers of economic and technical strain to the research. Laser or piezo assistance in the penetration of the zona pellucid can cost laboratories between 10,000 and 50,000 U.S. dollars per unit. It is also worth mentioning that dependency on such technology for this fundamental part in mouse embryo manipulation opens up the research to be susceptible to work schedule problems and delays that can arise and are more prevalent the more factors are introduced.

The technical prowess needed to manage mouse embryo manipulation with conventional methods and technology is extreme. Most embryological procedures require skilled expertise to perform; some, like those involving hatched or manipulated blastocysts, are inaccessible to even expert embryologists.

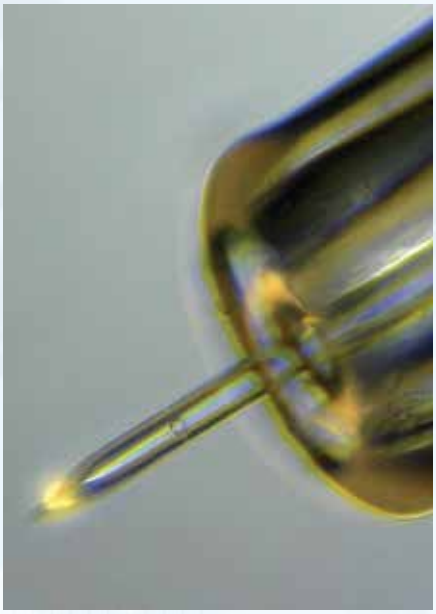
A DIFFERENT SPIN

The "Dracula Pipette" was originally conceived and developed as a tool for cryopreservation of llama embryos and it was used to produce the first live births from frozen/thawed lama embryos. Early version was hand operated. Human and mouse embryos are 1/1000th the volume of a llama embryo, to accommodate for this, diameters of all tips were scaled down to 1/10th of the original size.

The Mouse Dracula Pipette looks to be the solution to the problems that have hampered biomedical research for decades, bringing economic and manageable technology to the hands of all researchers.

THE INNOVATION THE MOUSE DRACULA OFFERS

The innovation of the Dracula system is the simple elegant coaxial design developed and continually refined by GeneSearch Inc. The Dracula has two concentric tips with separate



vacuum or pressure control for the space between the two tips, the space between the inner tip and the injection probe, and the space inside the injection probe, itself. Embryos are held firmly yet without damaging stresses by a ring of vacuum between the two tips. A brief pulse of vacuum inside the inner tip can aid in penetration as the injection probe is advanced. The Dracula system draws the injection surface taught, like the surface of a drum.

The Dracula Pipette has another unique ability, simultaneous injection and aspiration, or "flushing". Once the puncture has been made fluid contents of the embryo can be removed very quickly by applying a vacuum inside the inner tip. This causes fluid to flow out around the injection pipette, though the opening in the inner tip.

A biopsy port can be laser-drilled on the side of the probe. When the probe is in use it is pushed through the zona pellucida and trophoblast layers, the biopsy port is aligned with the trophectoderm layer and a vacuum is applied. This allows a few cells to be drawn into the probe and then sheared off of the trophectoderm layer as the probe is withdrawn.

Price looks to be another innovation for the Dracula system. The current cost production of a single Mouse Dracula RC-1, a versatile research tool with accessories including several sets of tips, biopsy probes and injection pipettes is ~$2,500 U.S. dollars. At present, GeneSearch is evaluating an all-plastic version of the Dracula device, which will cut this cost to ~$250 U.S. dollars. In the future, additional cost reductions may allow production of a single-use "Disposable Dracula" tool for human ART/ genetic diagnosis clinics.

A FIRM PLAN LOOKING FORWARD

The Mouse Dracula Pipette for all it is now has a steady aim on where it wants to be. A two phase plan each with two aims has been set up to be realized during the next three years.

Phase one of the plan looks to establish the effectiveness in the manipulation of mouse embryos, specifically through the optimization for the insertion or extraction of a small number of stem cells to/from the trophectoderm and the inner cell mass to improve chimera production. To do this the following Specific Aims must be fulfilled:

Phase One Aim One: optimize the Mouse Dracula for delivering embryonic cells into mouse embryos. The secure yet gentle handling by this tool allows even large probes to be easily inserted into the blastocoel of blastocysts.

Phase One Aim Two: optimize the performance of the Dracula system for no harm biopsy. The coaxial design of the Dracula allows delicate embryos to be handled.

In phase two of the plan the tool will be refined for more effective commercialization. This phase will initiate once aim one of phase one has been fulfilled. In phase two, the following will be fulfilled:

Phase Two Aim One: optimize ease of use and adaptability of the Mouse Dracula. This aim is to make the Mouse Dracula adapt to a large range of microscope and micromanipulator platforms.

Phase Two Aim Two: refine the Mouse Dracula for more effective commercialisation. Components are currently individually manufactured and hand assembled, this maximizes our ability to address our feedback and improve performance. Once we have met our desired requirements we look to adapt this process to higher volume production.

Researcher Profile

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Dr. Ed Schmidt received his Ph. D. in Biochemistry & Biophysics from Oregon State University. He is currently a Professor for the Department of Microbiology & Immunology, Montana State University. His primary research interests are to understand the intricate gene regulatory mechanisms that function in development and maintenance of complex organisms. He has been the recipient of numerous national and international grants and awards. For the past 8 years he has collaborated with GeneSearch, Inc. in "The Dracula Project".

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