

Fashioning of Polymer Multi-functional Nanovector Microparticle Delivery System by High Definition Microextrusion (HDME)

By

Peter D. Gabriele, Andy Hogan, John D'Ottavio, Mike Flemmens, and Matt Weir

717-227-5920

Fax 717-227-2743

ARmark Authentication Technologies, LLC

ABSTRACT

Considerable advances have recently been achieved in the development of nano-scale particles for cancer diagnostics and therapies. ARmark Authentication Technologies, LLC has developed a new technology platform to produce stable multifunctional polymeric microcarrier delivery vehicles for nanoparticles and bioagents. High definition microextrusion (HDME) offers microvector precision control of spherical geometry. Particles produced by this method show a narrow particle size distribution. The uniformity of shape and size is significant to microparticle precision pharmacokinetics. The HDME process allows the active pharmaceutical ingredient (API) to be directly loaded into spatially resolved domains throughout the particle geometry. Sensitive bioagents can likewise be incorporated through a post-absorption enhancement step. The microvector design is consistent with Ferrari's stable-core-constituent multicomponent targeting strategy in nanobiotechnology delivery. In this study, a model biomimetic radio frequency (RF) enhanced "microvector" was fashioned under high definition microextrusion (HDME). The HDME technology provides options to alternative platform design and delivery system architecture, for instance, targeted hyperthermia and diagnostic imaging in cancer treatment. The proprietary @mark microvector platform can also be constructed into other geometric forms such as ellipses, cylinders or microneedles.

@MARK MICROVECTOR TECHNOLOGY: **A MICROPARTICLE EXTRUSION** **TECHNOLOGY**

The @mark microvector technology is based on ARmark Authentication Technologies proprietary high definition microextrusion (HDME). Unlike traditional chemical methods of encapsulation, (emulsion and coupling technologies) particle size control and distribution under

HDME produces delivery platforms that can biomimic cells, create cross section architecture, as well as carry customized shaped charge payloads. Figure 1 is an example of a multifunctional nanocarrier and drug delivery vehicle, which is also responsive to microwave radio frequency stimulation.

Cancer is complex.¹ Many cancers, particularly solid tumors, are still untreatable by conventional therapies such as radiation, immunotherapy, surgery or chemotherapy². Recovery is often complicated by the fact that some of the most effective treatments have an adverse impact on surrounding healthy tissue and cells. As a result, more recent efforts have moved toward targeted therapies that attempt to kill only unhealthy cells.

The general premise of this study is to address nanocarrier and microvector weakness in order that will create a stable carrier with an emerging polymer technology, high-definition microextrusion (HDME). This multicomponent targeting strategy described by Ferrari³ is considered a single, stable, multifunctional FDA component composed of a core constituent derived from compliant drug delivery materials with the potential for carrying a plurality of sensitizers and/or bioactive compounds that function in concert as a therapeutic device.

¹ Nature 11 September 2008 Hayden, E.C., Cancer complexities slows quest for cures.

² **Baronzio, G.F., and E.D. Hager** 2006. Preface in *Hyperthermia in Cancer Treatment*. G.F. Baronzio and E.D. Hager (ed.). Landes Biosciences, Georgetown, TX.

³ **Ferrari, M.** 2005. Cancer Nanotechnology: Opportunities and Challenges. *Nature Review/Cancer*. **5**: 161-171.

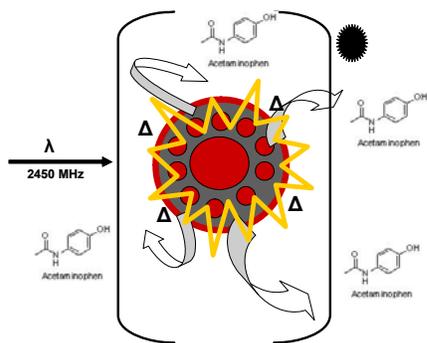


Figure 1 above is the microcell-microvector flat-cell concept that is the extended focus of this study. The superparamagnetic nanoparticles (SPIONs) are spatially resolved as a red core structure surrounded by a series of smaller circular red structures or islands. The red islands and core are SPION compounded in a master batch base of Ethocel®. The “interstitial sea” of gray is Ethocel compounded with acetaminophen. The entire structure is 75μ in diameter and 10μ in thick. All materials are FDA compliant. Sizes for this study were chosen for cost.

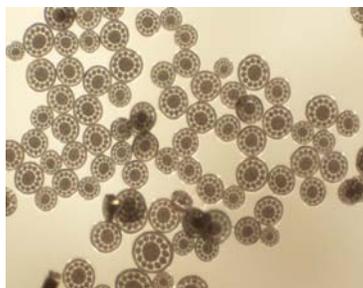


Figure 2 above is a selection of 20μ to 75μ diameter flat-cell microcell-microvector co-processed with superparamagnetic iron oxide nanoparticles particles.

HIGH DEFINITION MICROEXTRUSION (HDME): A NEW TECHNOLOGY FOR NANOCARRIER DESIGN

The prospect of nanomedicine providing new solutions to cancer therapy has heightened interest and research in therapeutic targeted delivery. The multifunctional vector described by Ferrari is now a standard concept that is expected to advance nanomedicine. However, traditional and state-of-the-art artificial cell delivery systems still fall short of a complete solution to vector design problems, primarily the result of poor stability and monolithic character.

The significance of this work is the established platform technology that meets a number of the critical needs in nanocarrier construction; such as the capacity to harbor more than one functional nano-bioagent in a stable core constituent matrix and deliver a functional benefit. This

technology is based on high definition microextrusion (HDME).

The fundamental feature of HDME is the ability to *simultaneously* process active agents into a compliant stable core constituent drug delivery polymer; segregate or commingle active agents in spatially resolved domains; and architecturally design polymer gradients for controlled bio-erosion, biodegradation or matrix-diffusion. This technology will allow the researcher to formulate and engineer stable microvector delivery designs that can evade the epithelial-endothelial and reticuloendothelial systems (RES) while conserving the activity of active agents nestled within the spatially resolved interior or surface domains. HDME was developed to create microparticles within the 10μ to 75μ diameter particle size range for targeted delivery and are derived from traditional FDA compliant drug release polymers. This is not a solution technology.

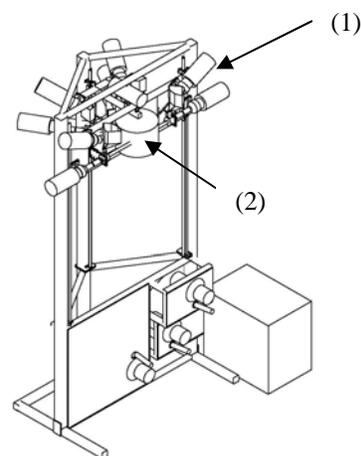


Figure 3 shows the HDME “quad” extruder used to create the microparticle. The quad-channels (1) allow the simultaneous co-extrusion of four or more separate material sources. The extruders are fed into the rendering chamber (2) where the contents from each feed-throat are directed through microfluidic channels for mixing, commingling, spatial resolution or segregation. Once in the rendering chamber the microfluidic extrudate is resolved into 21,000 nanofibrils that orient and conformally constrain the polymer structure. The energy of constraint drives spherulization.

There are several distinct ways to envision the utility of the microvector. First, the microvector as a device tool can be designed as a single self contained unit cell having monolithic action such as ferrying a drug or superparamagnetic iron oxide nanoparticles (SPIONs) to the tumor site to support targeted radio frequency hyperthermia (TRFH). Secondly, the nanocarrier microvector can be a dual focused controlled release therapeutic delivery vehicle comprised of an active pharmaceutical ingredient (API) with nano and/or bioagent payloads; thus, having dual drug delivery and nanoparticle transport activity. And finally the nanocarrier microvector can also be designed as a

multifunctional combination device confining both active agents and focusing biotechnology such as immunospecific agents designed to respond to external or remote manipulation.

Another benefit of the stable multifunctional microvector nanocarrier approach is the local delivery of concentrated API materials to the pathology rather than dependence on systemic fluid dynamics and critical blood level concentration. In the $\text{\textcircled{R}}$ mark microvector approach an otherwise toxic API can circumvent sensitive organ systems as a result of confinement of the API in the stable polymer core until the microvector reaches its target. The “drop-off” release concept could involve either a nanoparticle inclusion with a target specific antibody adjunct that could support the process of tumor cell invasion as the carrier core constituent polymer erodes either passively or by RF enhancement. The idea of a “hay wagon” or Trojan horse delivery of particles over time that can be released by deliberate remote controlled polymer erosion or enhanced matrix-diffusion flux augmented with targeted antibody focusing is ideal.

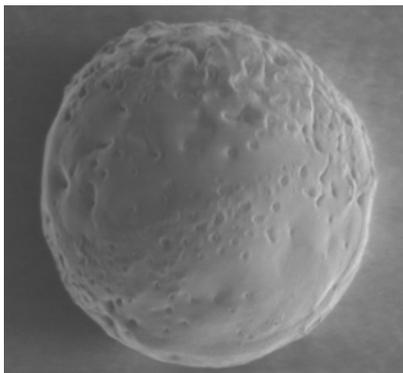


Figure 3 above is a scanning electron micrograph of a 10µm modified microvector microsphere. The spheres can be customized to biomimic circulatory cells and disposed to carry a number of payloads. Intra-particle diffusion and flux from drug loaded microparticles follows first order kinetics. Ideal drug delivery follows “zero order kinetics”, wherein drug-flux and interstitial fluid concentrations achieve steady state and remain constant. Zero order kinetics is difficult to achieve. Diffusion and mass transfer variables govern a non-linear flux. For microparticle diffusion flux in the x, y and z directions, can be modeled using Fick’s second law. Important to note is that in all solutions of the second law a key variable is the driving force for flux which is simply the difference in concentration between two spatially located points.

HDME MICROVECTORS FASHIONED FOR RADIOFREQUENCY HYPERTHERMIA THERAPY

Cancer cells are sensitive to heat. In a proof of concept study, we demonstrated the utility of HDME as a method to

produce a model multifunctional nanocarrier. In this study a 75µ X 10µ model “microvector” formed by HDME was comprised of acetaminophen as the active pharmaceutical ingredient (API), a hyperthermia agent superparamagnetic iron oxide nano-particle (SPION), in Ethocel® a drug delivery polymer matrix. RF stimulated microvectors established the hyperthermia clinical range, accelerated the release of the API, and yeast cell viability plate count showed early cell death when the yeast and the microvectors were co-exposed to low-dose microwave. Additionally, yeast cells targeted adsorption to microvectors that were treated with anti-Saccharomyces antibody to simulate focusing potential. Microvector microspheres can also be designed from 5µ to 50µ in diameter with uniform particle size distribution.

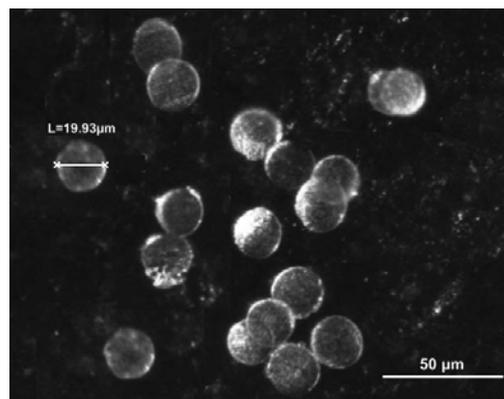


Figure 4 above is a micrograph that shows the uniformity of 20µm microsphere particles produced by HDME. Modeling shows the effect of micro-sphere size on resulting drug flux as well as the effect of various intra-sphere concentration profiles on resulting drug flux. It appears that the key problem encountered to achieve zero order release is the decreasing concentration of drug in the microsphere. HDME process allows the API to be directly loaded into spatially resolved domains throughout the particle geometry while providing for precise control of the particle geometry. These characteristics provide for a controlled and targeted volume (V) to area (A) ratio. The controlled V/A ratio, maximized for the case of spherical particles, provides the capability to tailor the capacity to carry API (V) versus the capacity to deliver the API (A), since surface area is proportional to drug flux.

SELECTED POSTER RESULTS

In this poster session we will show the fundamentals of HDME and some of the microparticle results. We have successfully created microspheres using several compliant FDA biodegradable (polylactic acid) and matrix-diffusion (Ethocel) polymer formats. It is not only the intention of this research to demonstrate the extrusion option but to emphasize the benefits of creating microvectors from compliant materials. The purpose is to arrive at a therapeutic option using materials of construction that have a predicate

history with the FDA; thereby, avoiding a protracted pre-clinical health and safety evaluation.

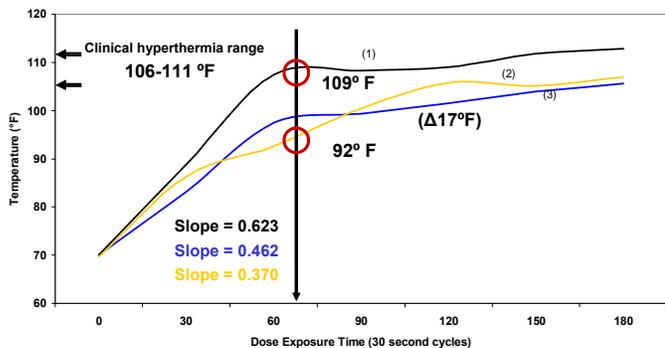


Figure 5 above illustrates the comparative 2450 MHz microwave aqueous heating profile of microcells-microvector at 138 watts (P1) with SPION (1) and without SPION (2). Line numbered (3) is the heating profile of neat water. Microcell-microvectors without SPION only reached 92°F; whereas, microcells-microvectors with SPION reached 109°F for the same exposure. The 106°F to 111°F is the clinical hyperthermia range.

The RF pumping in figure 7 shows the relative recovery comparison of RF enhanced diffusion of API from SPION containing microcell-microvector compared to passive diffusion at 98°F in a convection oven. Microwave heating (or RF “pumping”) of the microcell-microvector with SPION showed accelerated elution of the API from the microcell matrix in 10 minutes to nearly the same level as the 24 hours passive matrix-diffusion sample.

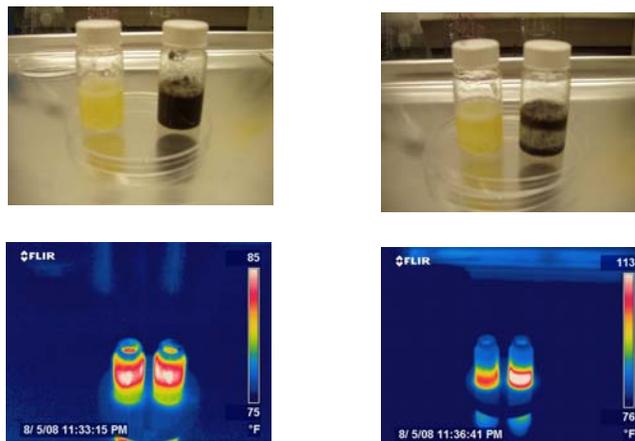


Figure 8 above shows the IR thermography results of the co-exposure of 10 ml-albumin dispersed in 500 mg-microcell-microvectors with SPION (black) and without SPION (yellow). This IR thermography shows before (left side) and after (right side) exposure to 2450 MHz microwave at 138 watts power for 60 seconds. Note the whitening in the sample in the upper right picture. The thermographic response in the lower right picture shows that the microcell-microvector solution in albumin reached hyperthermia therapeutic temperature range.

DISCUSSION

High Definition Microextrusion (HDME) provides a powerful tool to manage characteristic flux profiles of high initial release followed by a decaying rate of flux to be enhanced with RF remote stimulation. HDME process allows the active pharmaceutical ingredient (API) to be directly loaded into spatially resolved domains throughout the particle geometry while providing for precise control of the particle geometry. These characteristics provide for a controlled and targeted volume to area ratio in microspheres. The controlled V/A ratio, an maximized for the case of spherical particles, provides the capability to tailor the capacity to carry API (V) versus the capacity to deliver the API(A), since surface area is proportional to drug flux. The ability to spatially resolve domains of API also yields the capability to mitigate the effect of decreasing driving force via non uniform loading of API. However, the HDME process also has the capability to produce biomimetic RF enhanced micro particles. In this scenario, the nano-featured particles can be selectively heated.

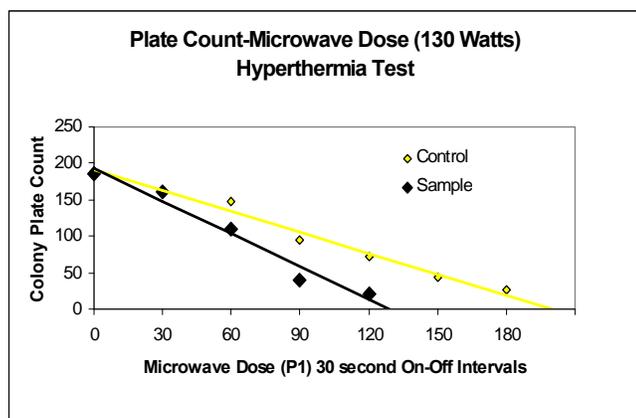


Figure 6 above illustrates comparison of SPION responsive early cell death by yeast cell viability plate count. Co-cultures are exposed to low-dose microwave energy for 30 second on-off intervals at P1 130-watts. Black line indicates microcells-microvectors with SPION initiated early death. Yellow is without SPION.

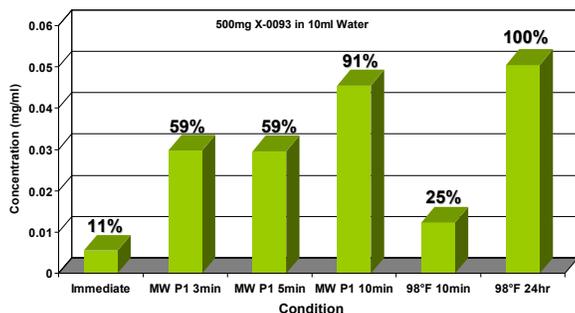


Figure 7 above shows RF “flux” pumping relative to passive matrix-diffusion.