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Nanoemulsion Formulations Improve Efficacy of Cancer Treatment

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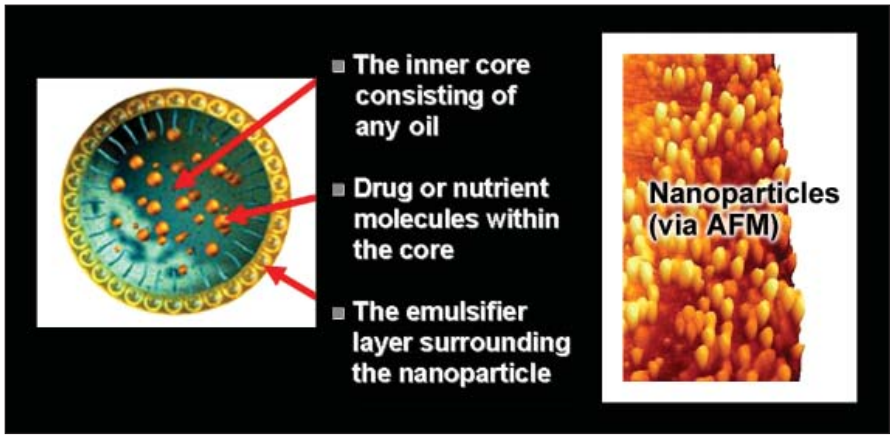

Figure 1. Illustration of a nanoemulsion.

The efficacy of pharmaceuticals, nutraceuticals, and cosmeceuticals depends not only on the chemical composition of the active ingredients, but also on the formulation of the product and the method of delivery.

Nanoemulsions—formulations that encapsulate drug or nutrient molecules in sub-micron-sized oil droplets suspended in water—have been found to increase the rate and level of absorption into the body, and offer other benefits including the mitigation of side-effects as well as an antiviral and antibacterial action. With an emulsifier layer surrounding the droplet, nanoemulsions exhibit high levels of stability for extended periods of six months or more (Figure 1).

Researchers at the Center for Health and Disease Research at the University of Massachusetts, Lowell, Department of Clinical Laboratory and Nutritional Sciences, have conducted *in vitro* and *in vivo* studies demonstrating that nanoemulsion formulations dramatically improve the efficacy of delivering vitamin E and omega-3, anti-inflammatory drugs (like aspirin), and more significantly, cancer treatments.

The Center's extensive laboratory studies have demonstrated that nanoemulsions can be a highly-effective delivery mechanism through various potential routes of administration such as topical cream, inter-muscular injection and oral ingestion.

As participants of the newly-founded Advocacy Program developed by Microfluidics Corporation of Newton, Mass., which works with leading doctors and scientists worldwide, Dr. Nicolosi and doctoral students at the Center have utilized Microfluidics' high shear Microfluidizer® processors to create nanoemulsion preparations consisting of particles ranging from 50–100 nm. The high surface/volume ratio of the small particles improves bioavailability and efficacy. For poorly-soluble drugs in particular, the nanoemulsion system offers an appealing substitute delivery mechanism that not only improves bioavailability, but also can diminish adverse side-effects.

Increased Efficacy of Anti-Cancer Drugs

Improving drug efficacy and reducing side-effects is critically important for anti-cancer medications. Tamoxifen, for example, is currently the world's largest selling breast cancer treatment, but this lipid-soluble drug is associated with significant side-effects and can act as partial agonist on the endometrium, which has been linked to the development of endometrial cancer in some women.

A study of tumor growth in nude mice by a research team led by Jean-Bosco Tagne, a doctoral student in Dr. Nicolosi's program and a researcher at the Whitehead Institute for Biomedical Research, has shown that the nanoemulsion formulation of tamoxifen prepared with donated Microfluidizer processor equipment dramatically improves the

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efficacy of the drug when administered by subcutaneous injection and by topical application (Figure 2).

In addition, the research which was presented at the October 2006 MicroFluidics Technology Fair in Boston found that nanoemulsion formulations also improved the efficacy of dacarbazine, an antineoplastic chemotherapy drug commonly used in the treatment of various cancers like malignant melanoma and Hodgkin's lymphoma. Nanoemulsions of dacarbazine prepared with Microfluidizer processor increased tumor reduction by twofold when administered topically and by tenfold when administered via intermuscular injection (Figure 3).

The study suggests that the side-effects of tamoxifen and dacarbazine can be reduced by administering nanoemulsions in conjunction with cell targeting techniques. The researchers applied genome-wide location analysis (GWLA) microarray techniques and expression analysis to find targets of E_2F_4, a cell cycle regulatory factor that is involved in tumor growth regulation. The researchers concluded that these new technologies (nanoemulsion formulations and the use of GWLA) could contribute to a better understanding of the prevention and treatment of certain cancers. In a separate study, Fongshu Kuo, a doctoral student at Dr. Nicolosi's lab, found that nanoemulsion formulations of an anti-oxidant synergy formulation (ASF) reduces tumor growth rate in neuroblastoma-bearing nude mice. Neuroblastoma is the most common solid tumor in children. The nanoemulsion formulation consisted of simple soybean oil, the surfactant polysorbate 80 and water.

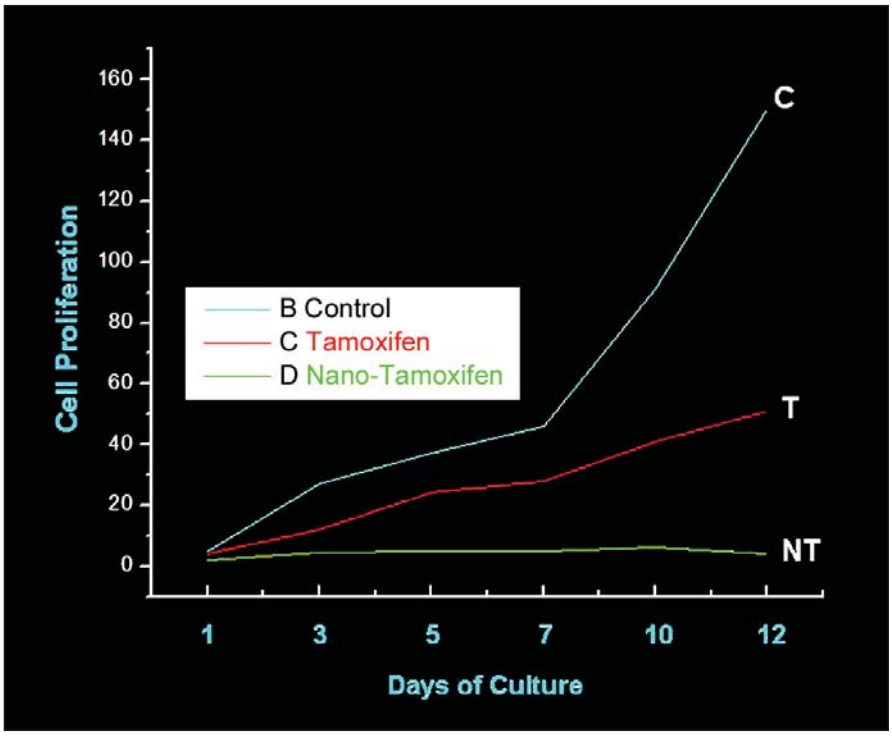

Figure 2. The effect of Tamoxifen (T) and a nanoemulsion preparation of Tamoxifen (NT) on cell proliferation.

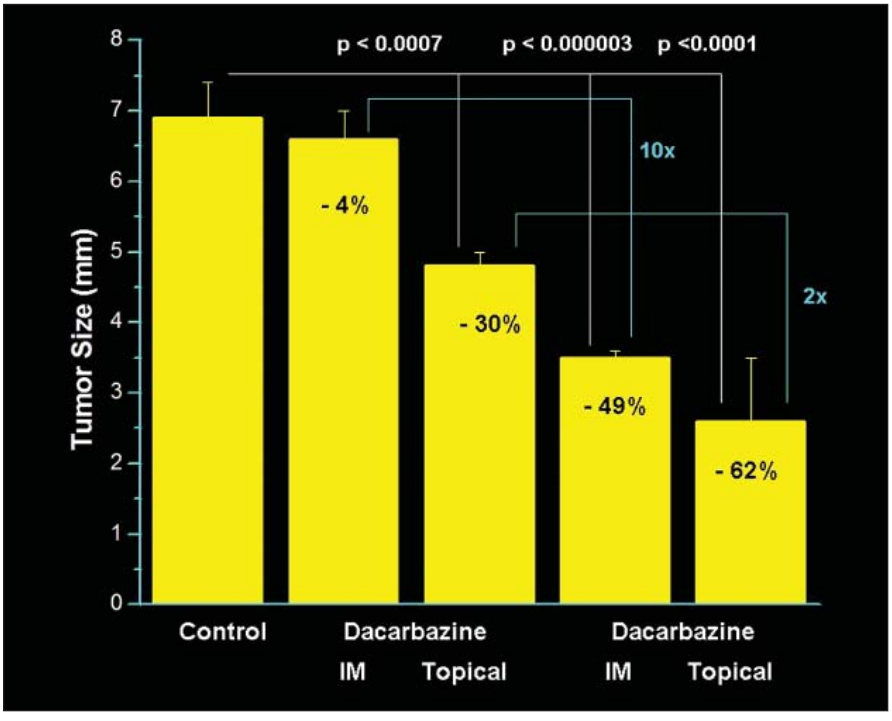

Figure 3. Tumor reduction with nano preparations of dacarbazine.

The study results, which will appear in the *Journal of Experimental Therapeutics and Oncology*, indicate that ASF nanoemulsions prepared with Microfluidizer processor technology caused a reduction of tumor size by an average of 65% when applied either by subcutaneous injection or transdermal application, whereas suspensions of ASF formulated with a conventional homogenizer (Polytron Model PT 10/35, Brinkmann Instruments, Inc., Westbury, NY) were ineffective in decreasing tumor size (Figure 4).

Microfluidizer Processor Technology

Research into nanoemulsions and cancer treatment has become a major focus of the UMASS Lowell Center for Health and Disease Research. "When we installed the Microfluidizer processor, it spawned a new area of research for my lab which had historically been known for studies of cardiovascular disease,"

Dr. Nicolosi explained.

"We are now investigating a variety of nanoemulsion applications for pharmaceuticals, nutraceuticals, and cosmeceuticals, but it is in the area of cancer treatment where we see some of the most exciting opportunity. Quite frankly, we would not have achieved the results that we have without access to Microfluidizer processor technology in our lab."

The lab uses the Microfluidizer processor model M-110EH-30, which is specifically designed to operate reliably and efficiently at up to 30,000 psi process pressure and is ideal for biopharmaceutical research laboratories. A relatively new method of particle reduction, Microfluidizer high shear fluid processing is favored by many research laboratories and pharmaceutical and biotechnology companies because of its unparalleled ability to produce extremely small, uniform particles in very few product passes.

Utilizing Microfluidics' fixed geometry interaction chamber technology, the M-110EH-30 is capable of generating shear rates within the product stream that are orders of magnitude greater than any other commercially available fluid processing or mixing equipment.

Equipped with a single-acting intensifier pump that amplifies the hydraulic pressure at the simple turn of a knob, the M-110EH-30 is able to drive the product stream through precisely defined fixed-geometry microchannels within the interaction chamber. Shear rate, which is directly proportional to the process pressure setting, imparts the required energy directly to the product stream, reducing particles and droplets to nanoparticles. The unit installed in Dr. Nicolosi's lab has been modified to process volumes as small as 10 ml.

The fixed geometry of the microchannels not only ensures that the processing conditions are identical for all product passing through a single machine, but are also identical for all machines using a particular interaction chamber design and pressure setting, regardless of flow-rate capacity. Essentially, this ensures that once a high shear fluid processor achieves a successful result with a small laboratory system like the M-110EH-30, then the same interaction chamber and pressure specifications can be used in the design of a full-scale production system that produces significantly larger volumes. Because of the ability to scale-up production seamlessly, many users of Microfluidics' high shear fluid processors are able to skip the usual pilot stage and move directly from the laboratory to full-scale commercial production capacity.

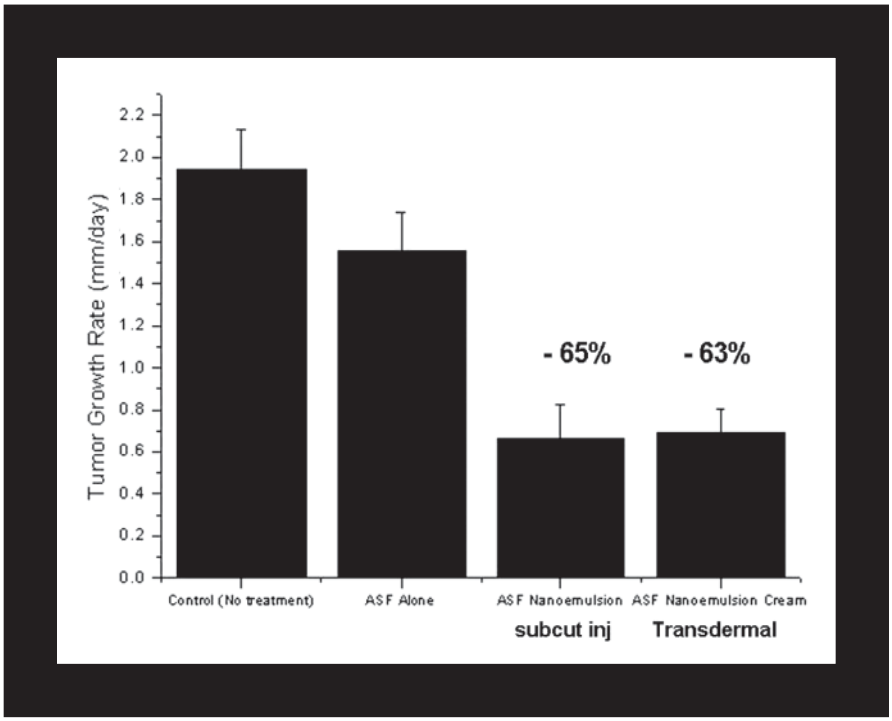

Figure 4. Subcutaneous injection vs. topical delivery of ASF-containing nanoemulsions on neuroblastoma-induced tumor growth rates.

Self-Assembling Method

Prior to using the Microfluidizer processor technology, Dr. Nicolosi's lab was also involved with producing and evaluating created nanoemulsions and nanospheres with "self-assembling" technology that involved mixing polymers and solvents with an oil solution and water to form the dispersed spherical droplets. In the process of mixing, small particles of the drug were added and became encapsulated within the spherical droplets.

Two self-assembling technologies have been employed at the lab. "The self-assembling technologies produce good quality nanoemulsions and nanospheres with a relatively uniform particle size of 40-50 nm," said Dr. Nicolosi, "but there are a number of disadvantages to this technology, the most important of which can be the use of solvents and polymers. Moreover, only a small amount of drug material can be loaded into each droplet, considerably increasing dosage size."

The self-assembling procedure is also time-consuming and cumbersome, requiring between 24 and 48 hours for each batch. And the process chemicals can be costly and hazardous. Scale-up to production volumes could also be prohibitively expensive. "The Microfluidizer processor technology can overcome many of the limitations of the self-assembling methods," said Dr. Nicolosi. "The Microfluidizer processor from Microfluidics allows us to quickly and easily prepare high quality, uniform nanoemulsions. We are able to load as much as ten times more drug and nutrient molecules into each droplet while maintaining the same droplet size."

Dr. Nicolosi and the Center for Health and Disease Research now use the Microfluidizer processor almost exclusively, and cannot overstate the importance the equipment plays in nanoemulsion research.