Prof. Rimona Margalit’s Research

**1. Micro- and nano-particulate drug delivery technologies for topical, regional and systemic applications**

Our efforts in the drug delivery arena focus on two drug delivery technologies, one veteran and one novel, that are inventions of our group. These technologies each yield, micro and nano sized particles that are composed of biomaterials with particular emphasis on naturally occurring adhesion macromolecules as integral components for drug delivery and targeting. Drug carriers are designed to overcome deficiencies of treatment with free drugs, that frequently lead to failed therapies and poor therapeutic responses. Existing carriers and many of those under development still suffer from many limitations. The two technologies we developed have the potential to resolve limitations of the other carriers. The veteran technology is named Bioadhesive Liposomes (BAL). These are drug-encapsulating liposomes that have been surface-modified by covalent binding of target-recognition agents such as hyaluronan, collagen, EGF or gelatin to their surface. Developed originally for local (topical and regional) drug therapies, recent studies have shown that nano (unilamellar) hyaluronan-liposomes are also suitable for therapies that require systemic administration. The novel technology (currently in the process of patenting), while also belonging to the particulate class of drug carriers, is an entirely new conceptual approach; it is different from liposomes and other lipid-based particles, as well as from nanospheres and microspheres, be they from biological or synthetic materials. It has a wide scope of therapeutic applications, and is suitable for both local and systemic applications. The nano species of the novel carriers are long circulating and can be targeted to specific cell surface receptors that are over-expressed by many tumors. These novel carriers are, furthermore, non toxic. In all cases tested, and for both carrier technologies, the therapeutic outcomes with carrier-mediated therapy are significantly increased as compared to free drugs. It would be naive to assume that a single technology of drug delivery would fit the multitude of pathologies where carrier-mediation is a must or can provide significant improvement. We therefore plan to continue with both technologies, assuming – based on current findings and on intrinsic carrier properties – that the novel technology will be superior in most cases, but that there will be niche applications where the situation will be reversed. For example, as shown in figures 1 and 2 respectively, Bioadhesive Liposomes localize at the cell membrane and do not seem to participate actively in drug entry into the cell, whereas under similar conditions the novel carriers actively mediate drug entry into the cells.



**Figure 1:** Confocal microscopy of B16F10.9 cells incubated with the following fluorescein formulations: A - free fluorescein B - fluorescein encapsulated regular unilamellar liposomes C – fluorescein encapsulated in unilamellar hyaluronan-liposomes. Unencapsulated fluorescein was removed from each of the liposomal formulations prior to incubation with the cells, and all formulations were equal in fluorescein concentration, 15 µM. Incubation was for one hour at room temperature.

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| http://www.tau.ac.il/lifesci/departments/biochem/members/margalit/image002.gif | **Figure 2:** Confocal microscopy of P388/ADR cells incubated with the following doxorubicin formulations: A - free doxorubicin. B – doxorubicin encapsulated in the new carrier. Unencapsulated doxorubicin was removed from the carrier prior to incubation with the cells. Free and encapsulated doxorubicin were of equal concentration, 5 µM. Incubation was for one hour at room temperature. In each panel, the cells are seen in the upper part and the corresponding fluorescence on the bottom part. |

Our conceptual approach, for any project is to start at the molecular level and proceed systematically to studies in cell cultures, and then to animal studies. At the molecular level we investigate structural, physicochemical and biochemical properties. The studies in cell cultures explore cell–carrier interactions with particular emphasis on kinetics and thermodynamics of cell-carrier binding, on cellular localizations of carrier and drug, on the mechanisms by which carrier-mediation affects drug entry into cells, and on therapeutic activity. The in vivo studies focus on pharmacokinetics, drug and carrier biodistributions, adverse effects, and therapeutic responses.

Current projects with the Bioadhesive Liposomes include: (1) Continued physicochemical and biochemical characterization of the Bioadhesive Liposomes, in particular those that have hyaluronan or collagen on their surface, focusing on: cryoprotection; the effects of the natural enzymes hyaluronidase and collagenase on bioadhesivity and on retention at the cell membrane; the effects of temperature and endocytotic inhibitors on liposome-cell interactions, for small and large liposomes, regular and bioadhesive. (2) Chemical defense: Development of bioscavenger formulations for protection from Organophosphates (OP), the toxic components of nerve gases and of many insecticides. The systems consist of enzymes – several different enzyme species that scavenge OP in a variety of mechanisms – each enzyme species encapsulated in Bioadhesive Liposomes, that will be positioned in the airways and lungs and on skin, to act there as protective layers between the OP and inner body system. Molecular level studies are at an advanced stage. In vitro, ex vivo and in vivo feasibility studies are underway. (3) Tumor chemotherapy with small hyaluronan liposomes encapsulating the chemotherapeutic drugs doxorubicin and (separately) mitomycin C, focusing on formulation aspects, in vitro cytotoxicity and in vivo studies in syngeneic mouse tumor models.

Current projects with the novel technology include: (1) Structural characterization, in particular size, shape, surface properties and mapping of internal domains. (2) Formulation studies of large and small drugs, hydrophilic and hydrophobic. (3) In vitro and in vivo studies in tumor chemotherapy, focusing on syngeneic and on nude mouse tumor models. (4) Gene transfection (in vitro and in vivo) and (5) Drug delivery for the treatment of neurodegenerative diseases, especially exploring (in vivo) BBB bypass approaches.

**2.** **Novel chemosensitizers for overturning multidrug resistance (MDR) to tumor chemotherapy**

Chemotherapy frequently fails cancer patients due to inherent or acquired multidrug resistance (MDR). In the dominant mechanism, intracellular levels of cytotoxic drugs are reduced below their lethal thresholds due to active extrusion of the cytotoxic drug(s) from the tumor cell, operated by ATP-dependent pumps such as P-glycoprotein (Pgp), Multidrug Resistance-associated Protein (MRP), Lung Resistance-related Protein (LRP) and others. Reducing, or better yet, abolishing the extrusion, by pump inhibition, is a main approach to overturning MDR. The search for effective inhibitors (also named chemosensitizers, MDR modulators, MDR reversal agents) is now into the third generation. 1st -generation candidates were drugs already approved for other non-cancerous indications, with the obvious advantage that, being already approved, they could speed up the track to the clinic for MDR reversal. Unfortunately, although successful in vitro, patients could not benefit from these chemosensitizers since their clinically-relevant doses exceed safety limits resulting in unacceptable adverse effects and toxicity. Poor solubility was an additional frequent limitation. Chemical derivatization of 1st-generation molecules and combinatorial chemistry lead to 2nd and 3rd generation chemosensitizers. These were more potent and less toxic than 1st-generation compounds, yet many were still prone to adverse effects, to unfavorable changes in plasma pharmacokinetics (PK) of the anticancer drugs, and to poor solubility. Few are currently in early clinical trials.

Our approach, which we see as 4th-generation chemosensitizers, returns to the concept of the 1st-generation in that we focus on drugs already approved for other non-cancer indications. But, in critical difference, we focus only on molecules that require doses that are distinctly well-below their human safety limits for MDR reversal. We have already identified one molecule that meets the criteria for 4th-generation chemosensitizers and have verified its performance as an MDR reversal agent in vitro and in vivo.

Current projects in the lab include: (1) Extension of the in vivo studies with the identified molecule, to include a wide repertoire of anticancer drugs and tumor types. (2) Exploration of additional approved drugs from the same family of the identified one, for their potential in MDR reversal. (3) Studies on the mechanism(s) by which this new class of chemosensitizers modulates MDR. (4) Investigation of potential benefits from combinations of carrier-mediated chemotherapy and chemosensitizers.