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Pharmacytes: An Ideal Vehicle for Targeted Drug Delivery

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An ideal nanotechnology-based drug delivery system is a pharmacyte—a self-powered, computer-controlled medical nanorobot system capable of digitally precise transport, timing, and targeted delivery of pharmaceutical agents to specific cellular and intracellular destinations within the human body. Pharmacytes may be constructed using future molecular manufacturing technologies such as diamond mechanosynthesis which are currently being investigated theoretically using quantum *ab initio* and density-functional computational methods. Pharmacytes will have many applications in nanomedicine such as initiation of apoptosis in cancer cells and direct control of cell signaling processes.

Keywords: Apoptosis, Cell Signaling, Drug Delivery, Microbivore, Microrobot, Nanomedicine, Nanomotor, Nanorobot, Nanotechnology, Pharmacyte, Respirocyte.

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1. INTRODUCTION

What would an ideal drug delivery vehicle look like? To start with, it would be targetable not just to specific tissues or organs, but to individual cellular addresses within a tissue or organ. Alternatively, it would be targetable to all individual cells within a given tissue or organ that possessed a particular characteristic (e.g., all cancer cells, or all bacterial cells of a definite species, etc.). This ideal vehicle would be biocompatible and virtually 100% reliable, with all drug molecules being delivered only to the desired target cells and none being delivered elsewhere so that unwanted side effects are eliminated. The ideal vehicle would remain under the continuous control of the supervising physician, including post-administration. Even after the vehicles had been injected into the body, the doctor would still be able to activate or inactivate them remotely, or alter their mode of action or operational parameters. Once treatment was completed, all of the vehicles could be removed intact from the body, leaving no trace of their presence. Let's call this hypothetical ideal drug delivery vehicle a "pharmacyte."[1]

The application of advanced nanotechnology to medicine, or nanomedicine[1–3]—in particular, the future engineering discipline of medical nanorobotics—will eventually make possible the design, fabrication, and therapeutic deployment of pharmacytes. Pharmacytes will be self-powered, computer-controlled nanorobotic systems capable of digitally precise transport, timing, and targeted delivery of pharmaceutical agents to specific cellular and intracellular destinations within the human body. Drug molecules could be purposely delivered to one cell, but not to an adjacent cell, in the same tissue. To fully appreciate the scope of this future development it is helpful to briefly review some of the background and recent history of medical nanorobotics.

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2. FROM NANOPARTICLES TO NANOROBOTS

To bridge the gap in our knowledge between present-day nanoparticle-based technologies and future nanorobotic technologies, a great deal of research remains to be done. In the relatively near term, over the next 5 years, pre-nanorobotic nanomedicine can address many important medical problems by using nanoscale-structured materials and basic nanodevices for drug delivery that can already be manufactured today—most notably organic polymer or lipid-based systems such as polymeric micelles, liposomes and solid lipid nanoparticles, and various nanocrystal-based systems, many of which have already advanced to marketed products. Surveys of these technologies are available elsewhere,[2,3] so here we consider just a few selected examples of nanoparticle-related work that may exemplify early steps toward the more sophisticated capabilities that nanorobots will ultimately possess.

Kopelman's group at the University of Michigan has developed dye-tagged nanoparticles to be inserted into

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allowed to release a 0.001 μm^3 cytocidal payload into the targeted cell, while the remaining 99.9% of the nanorobots release nothing. After initiating cell death, unmetabolized free cytocidal molecules can be locally reacquired by the pharmacyte and subsequently transported out of the patient, thus minimizing any post-treatment collateral damage. Note that the strict size requirements for macromolecules to reach the leaky vasculature of a tumor and convectively enter its pores[34] may apply to passively-diffusing payload molecules which might be conveyed and released by pharmacytes, but these limits do not apply to the motorized active nanorobots themselves. Upon arriving in the vicinity of a tumor, the pharmacyte may deliver its payload either via direct nanoinjection[31k] (for tumor cells adjoining the vasculature) or by progressive cytopenetration[1h] through adjacent cells until the targeted tumor cell that awaits payload delivery is reached.

It is well-known that apoptotic cellular "death receptors" can be expressed on both normal and cancerous cells in the human body, so one challenge for conventional drug-based therapy is to find some way to activate death receptors selectively on cancer cells only.[35] With pharmacytes, such selectivity should be simple and routine using multiple chemosensors,[1j] a benefit that may be characteristic of most future nanorobot-based therapeutics. For example, if caspase cascade amplification is sufficient to permit single-site activation of the cascade, then in principle an extracellular nanorobot intending cytocide of a detected cancerous cell could press onto the outer surface of the target cell an appropriate ligand display tool. This tool might contain suitably exposed trimeric CD95L (aka. FasL) ligand (binds to the extracellular domains of three CD95 death receptors), TNF or lymphotoxin alpha (binds to CD120a), Apo3L ligand aka TWEAK (binds to DR3), or Apo2L ligand aka TRAIL (binds to DR4 and DR5).[35,36] The binding event would then activate a single death receptor complex, potentially triggering the entire irreversible cytocidal cascade. If necessary, multiple such display tools could be employed. This technique avoids much of the storage requirement for bulky consumables aboard the medical nanorobot. As another approach, molecular sorting pumps on the pharmacyte surface could be used to selectively extract from the cytoplasm of a target cell specific crucial molecular species of IAPs (Inhibitors of Apoptosis) that normally hold the apoptotic process in check. Examples include survivin, commonly found in human cancer cells,[37] the transcription factor NF-κB, and Akt, which delivers a survival signal that inhibits the apoptosis induced by growth factor withdrawal in neurons, fibroblasts, and lymphoid cells. Conversely, decoy receptors (DcRs)[38] that compete with DR4 and DR5 for binding to Apo2L could be saturated with intrinsically harmless but precisely engineered intracellular "chaff" ligands. With IAPs removed or DcRs blockaded, apoptosis may be free to proceed.

Pharmacytes could also tag target cells with biochemical substances capable of triggering a reaction by the body's natural defensive or scavenging systems, a strategy called "phagocytic flagging".[1k] For example, novel recognition molecules are expressed on the surface of apoptotic cells. In the case of T lymphocytes, one such molecule is phosphatidylserine, a lipid that is normally restricted to the inner side of the plasma membrane[1m] but, after the induction of apoptosis, appears on the outside.[39] Cells bearing this molecule on their surface can then be recognized and removed by phagocytic cells. Seeding the outer wall of a target cell with phosphatidylserine or other molecules with similar action could activate phagocytic behavior by macrophages, which had mistakenly identified the target cell as apoptotic. Loading the target cell membrane surface with B7 costimulator molecules also permits T-cell recognition, allowing an immunologic response via the immunological synapse.[40] These tagging operations should work well against cells that have an apoptotic response that can be triggered by cytotoxic T cells—such as human cancer cells and cysts.

A second major application area of pharmacytes would be the control of cell signaling processes. As a trivial example, Ca^{++} serves as an intracellular mediator in a wide variety of cell responses including secretion, cell proliferation, neurotransmission, cellular metabolism (when complexed to calmodulin), and signal cascade events that are regulated by calcium-calmodulin-dependent protein kinases and adenylate cyclases. The concentration of free Ca^{++} in the extracellular fluid or in the cell's internal calcium sequestering compartment (which is loaded with a binding protein called calsequestrin) is $\sim 10^{-3}$ ions/nm^3. However, in the cytosol, free Ca^{++} concentration varies from 6×10^{-8} ions/nm^3 for a resting cell up to 3×10^{-6} ions/nm^3 when the cell is activated by an extracellular signal; cytosolic levels $> 10^{-5}$ ions/nm^3 may be toxic, e.g., via apoptosis. To transmit an artificial Ca^{++} activation signal to a typical $(20\ \mu m)^3$ tissue cell in $\sim$1 msec, a single pharmacyte stationed in the cytoplasm must promptly raise the cytosolic ion count from 480,000 Ca^{++} ions to 24 million Ca^{++} ions. This is a transfer rate of $\sim 2.4 \times 10^{10}$ ions/sec which may be accomplished using $\sim$24,000 hull-mounted molecular sorting pumps[1a] across a total nanorobot emission surface area of $\sim 2.4\ \mu m^2$. Onboard storage volume of $\sim 1\ \mu m^3$ can hold up to $\sim$20 billion calcium atoms, enough to transmit up to $\sim$1000 artificial Ca^{++} signals into the cell even assuming no reabsorption and recycling of the ions.

Properly configured *in cyto* pharmacytes could also modify natural intracellular message traffic according to preprogrammed rules or by following external commands issued by the supervising physician. In the case of steroids and thyroid hormones, this may involve the direct manipulation of the signaling molecules themselves (after they have passed through the cell membrane) or their bound receptor complexes. However, most signaling molecules are absorbed at the cell surface, initiating a

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signal cascade which may be modulated by manipulating second-messenger molecules or other components of the *in cyto* signal cascade. A few basic examples of signal modifying action involving cAMP would include:

(A) *Amplification.* A single epinephrine molecule received by a beta adrenergic receptor at a cell surface transduces the activation of dozens of G-protein alpha subunits, each of which in turn activates a single adenylate cyclase enzyme which cyclizes hundreds of ATP molecules into cAMP molecules. The intracellular population of cAMP (in muscle or liver target cells) is normally $<10^{-6}$ M or $\sim$5 million molecules for a typical (20 μm)^3 tissue cell. Stimulation by epinephrine raises the cAMP population to $\sim$25 million molecules in a few seconds. However, upon detecting this rising tide of cAMP during the first few msec, each *in cyto* pharmacyte could quickly amplify this existing chemical signal by releasing 20 million cAMP molecules (occupying a storage volume of $\sim$0.01 μm^3) from onboard inventories in $\sim$1 msec—thus accelerating cellular response time by several orders of magnitude.

(B) *Suppression.* Similarly, upon detection of rising cAMP levels in target cells, resident pharmacytes could use molecular pumps to rapidly remove cAMP from the cytosol as quickly as it is formed, even under maximum adrenal stimulation. The diffusion-limited intake current at the basal concentration ($\sim 6 \times 10^{-7}$ molecules/nm^3) for a cAMP-absorbing spherical nanodevice 1 μm in radius is $\sim$4 million molecules/sec,[1n] so a single such device could probably keep up with natural cAMP production rates and thus completely extinguish the response by preserving a flat basal concentration even in the face of a maximum stimulus. (As a practical matter, it may be more efficient to control epinephrine generation at its glandular source unless it is desired to interface with just a single tissue type.) Simultaneously, the cAMP-absorbing nanorobot may hydrolyze the stored cAMP in the manner of the cAMP phosphodiesterases, then excrete these deactivated AMP messenger molecules back into the cytosol. Similar methods might be useful in ligand-gated ion channel desensitization or in disease symptom suppression—as, for example, in suppressing the prolonged elevation of cAMP in intestinal epithelial cells associated with the cholera toxin, that produces severe diarrhea by causing a large influx of water into the gut.

(C) *Replacement.* Combining suppression and amplification, an existing chemical signal could be eliminated and replaced by a different—even an opposite—message pathway using pharmacyte mediators. Alternative pathways may be natural or wholly synthetic. Novel responses to existing signals may be established within the cell to enhance functionality or to improve stability or controllability. For instance, detection of one species of cytokine by a pharmacyte could trigger rapid specific absorption of that cytokine and a simultaneous fast release of another (different) species of cytokine in its place. Such procedures must of course take into account the many redundant signaling pathways and backup systems (e.g., developmental signals, immune system, blood clotting) that exist within the cell. Medical nanorobots can allow the replacement of many redundant pathways with more refined and specific responses.

(D) *Linkage.* Previously unlinked signal cascades may be artificially linked using *in cyto* nanorobots. As a fanciful example, the receipt of epinephrine by pharmacytes located in the capillaries of the brain could trigger these devices to suppress the adrenalin response while simultaneously releasing chemical messengers producing message cascades that stimulate production of enkephalins or other opioids, thus encouraging a subjective state of psychological relaxation rather than the "fight or flight" response to certain stressful conditions.

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