NIRT: Ink-Jetting of Nanostructured Matrices for Controlled Gene Delivery

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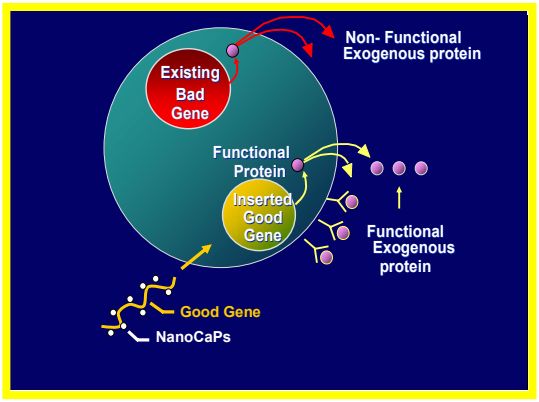
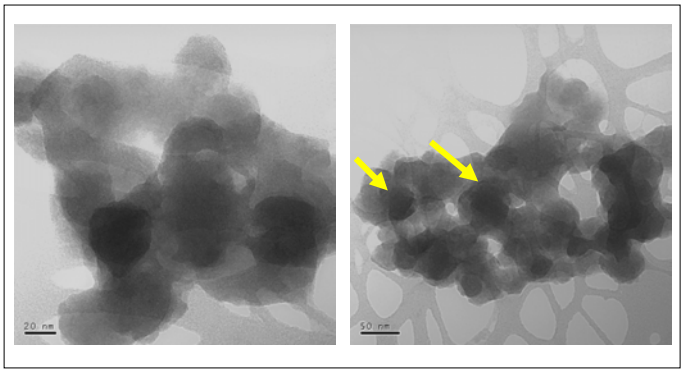
Overall Vision: Tissue engineering is based on the use of generating biocompatible and biodegradable template structures for integrating new tissue and vasculature necessary for ultimately replacing a traumatized organ. Thus there is a need for materials that are compatible to cells of various tissues. Bone is a complex material composite that harbors both organic and inorganic components making it a true nanocomposite structure interwoven in a 3-D matrix exhibiting structural and biological characteristics. Generation of bone and integration with tissue require materials that are not only biocompatible and biodegradable but also exhibit the ability to augment and modify the surface structure permitting cell growth, adhesion and other complex functions. Gene therapy has tremendous potential for replacing defective organs and tissue in tissue engineering, while also being useful for the treatment of a myriad of genetically transferred diseases such as muscular dystrophy and cystic fibrosis. At present, this has been realized by viral therapy, which has various shortcomings, including limited DNA carrying capacity, high cost and production problems. On the other hand, a non-viral (plasmid) gene delivery system is very attractive due to its significant benefits. At Carnegie Mellon University and the University of Pittsburgh, we have been successful in developing nano-sized bioceramic carriers for plasmid DNA. At the same time, we have succeeded in engineering a novel ink jet printing system for fabricating in situ 3D biomimetic matrices to guide wound healing in bone defects. There are therefore two major goals of this collaborative research projects. Accordingly, our project is divided into the following technical tasks: (i) Synthesis; (ii) Characterization; (iii) Molecular transfection; (iv) ink jet printing of matrices and analysis of controlled gene delivery.

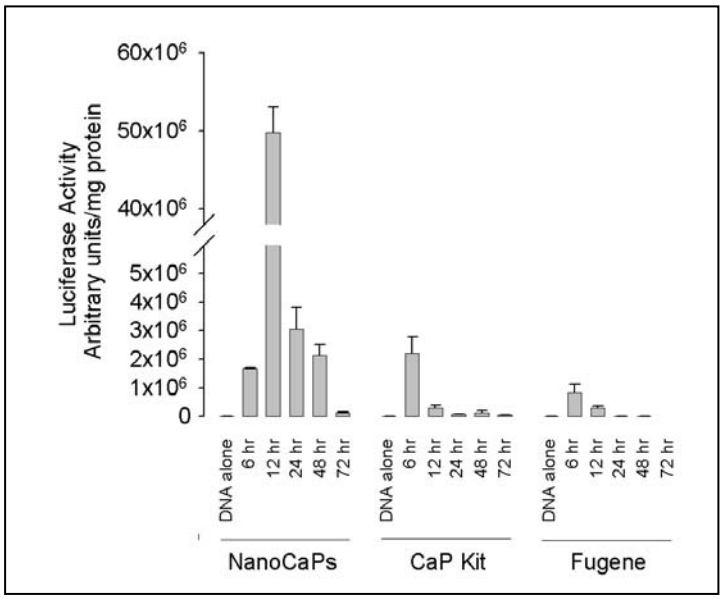


Synthesis and Transfection of Nano-Sized Bioceramic Carriers[1,2]

One of the most important aspects of this research is to synthesize carriers for binding non-viral, plasmid DNA and effectively carry it through the plasma membrane of a living cell and transfect the gene into the nucleus. Bioceramic particles synthesized in a nanostructured form can provide a large number of binding sites (~10^5) for plasmid DNA. The nano-sized materials can also easily degrade once inside the cytoplasm thus permitting safe and effective transport of the plasmid DNA into the nucleus. Our primary goal



has been to synthesize nanosized calcium phosphates called (NanoCaPs) for effectively binding plasmid DNA. We have developed a novel aqueous approach for synthesizing stoichiometric nanosized (~10-20nm) hydroxyapatite (HA) particles.



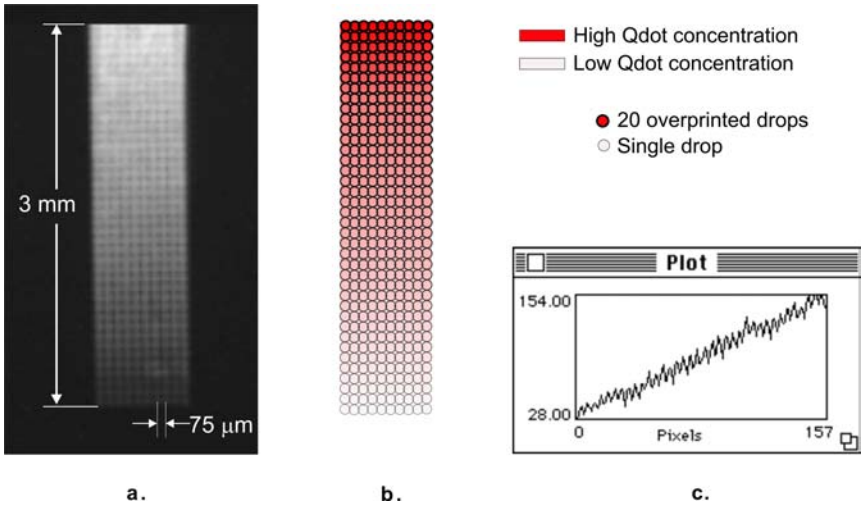
To demonstrate the transfection properties of NanoCaPs, a novel synthetic protocol was developed in which stoichiometric HA was synthesized under an invariant pH condition of 7.5. Using this novel synthetic protocol for generating the NanoCaPs, we designed a series of experiments to compare the transfection efficiency of NanoCaPs™ to the commercially available calcium phosphate kit CalPhos (Clontech) and FuGENE (Roche). 4μg of DNA was determined to be the optimal concentration to achieve the highest transfection efficiency using the CalPhos kit as indicated by the manufacturer and 1μg of DNA was optimal for FuGENE. The NanoCaPs™ assays were therefore performed using 4μg of DNA. The NanoCaPs experiment was carried out in the following fashion: the DNA was first added to the calcium precursor and then the phosphate precursor was added to the mix. This mixture was allowed to incubate for 15 minutes and then added to the media of MG63 (osteoblastic) cells. Luciferase activity was assessed at 6, 12, 24, 48, 72 and 96 hours using a luciferase assay system (Promega) and a luminometer to determine the transfection efficiency. The same experiment was repeated with NIH3T3 cells which yielded similar results. The transfection efficiency of the NanoCaPs™ was compared to the calcium phosphate (CaP) kit and FuGENE by determining the luciferase activity. At 12 hours the NanoCaPs show a 25 fold increase in transfection efficiency compared to the CaP kit. This data indicate that the NanoCaPs have excellent potential as a gene delivery system[3].

Inkjet Printing of Nanostructured Matrices

The overall goal is to be able to fabricate specific patterns of NanoCaP/Plasmid conjugates in 3D matrices in order to study cell responses to engineered designs. In particular, an ink jet printing system is being developed to manufacture 3D fibrin matrices with specified patterns of signaling molecules including the NanoCaP/plasmids. The printer is enclosed within a custom-built hood to maintain a sterile environment and prevent any bacterial contamination. Collaboration was initiated with a Pittsburgh-based ink-jet printing company (Matthew's International, Inc.) who provided an alternative microsolenoid valve technology. The new valves are able to reliably print fibrinogen with minimal-to-no clogging problems. The microsolenoid valves appear to perform better in comparison to the standard traditional valves used.

Ink-jet Printing of Controlled Gradients

Initial *in vitro* studies are focused at studying cell response to 2-D patterns of signaling molecules. As a first step toward this milestone we successfully printed *controlled gradients* of surrogate nanoscale particles called quantum dots (Qdots) which are tiny semiconductor crystals that emit light brightly in a range of sharp colors. Qdots are fluoropores with broad band excitation, but narrow band emission. The emission wavelength is proportional to the Qdot size, which are nominally around 10 to 14 nanometers for our application. The gradient in the figure was printed using 0.2 mM 605 nm emission streptavidin quantum dots in 10 mM sodium borate buffer pH 8.2 with 1% bovine serum albumin deposited onto nitrocellulose-coated glass slides. The printed concentrations of Qdots were modulated by overprinting each location within the pattern. Thus, the deposited concentration increases proportionate to the number of overprinted drops as demonstrated in the measured image intensity plot until the number of binding locations on the nitrocellulose saturate.



Efforts are currently in progress to develop an efficient, low-cost, but highly sensitive method to assess transfection *in vivo* prior to conducting studies in animal models. For this purpose we have successfully developed a novel CAM assay based on Qdot visualization technology. In particular, this is a shell-free CAM assay that is a minimally invasive, non-destructive, reliable, accurate, and sensitive methodology for assessing transfection. It is planned to incorporate use these as tracking agents for analyzing the transfection pathways.

References:

[1] D. Choi and P.N. Kumta, "Novel Aqueous Approach for Synthesis of Nanostructured Hydroxyapatite", submitted to *J. Materials Science, Materials in Medicine*.

[2] P. N. Kumta, C. Sfeir, D. Choi, J. Hollinger, L. Weiss and P. Campbell, "Methods for manufacturing Hydroxyapatite Structures", Patent Pending.

[3] D. Olton, C.Sfeir, J. Li and P.N. Kumta, "Nanostructured Calcium Phosphate Carriers for Plasmid Gene Delivery", submitted to *J. Biomedical Materials Research*.