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Utilizing a fiber-like platform to investigate the biophysical regulators of metastasis in the tumor microenvironment

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Metastatic cancers are responsible for 90 percent of all cancer related deaths, and the tumor microenvironment (TMEN) is a critical regulator of the metastatic dissemination of cancer cells. The TMEN is a dynamic microcosm which imposes a unique combination of chemical and physical cues to direct cell behavior, and recent studies have begun to uncover the important role of physical cues imposed by the extracellular matrix (ECM) in guiding cells out of the primary tumor. As observed in breast cancer, during metastasis protein fibers are re-organized to act as a highway cancer cells use to more efficiently escape the tumor, and the physical confinement imposed by the fibers alter gene expression to support distinct modes of invasive cell migration. By microcontact printing high aspect ratio ECM stripes onto flat substrates to mimic fibers, key aspects of confined fibrillar migration seen in vivo can be recapitulated. Different modes of cell migration are highly context dependent, thus micropatterned fiber-like surfaces represent a model system in which to study cell behavior in a more physiologically relevant environment. In this system, key regulators of cytoskeletal dynamics can be perturbed to reveal the biophysical underpinnings of invasive cell migration and metastasis.

Thermoresponsive Lipid Nanoparticles for Glioblastoma Chemotherapeutical Delivery

Mubashar Rehman, Di Shi, Thomas J. Webster, Asadullah Madni, and Ayesha Ihsan

Lipid nanoparticles of solid and liquid lipids have been widely used for bioavailability enhancement and the sustained drug release of water insoluble drugs. We have previously shown that fine tuning of liquid content produces lipid mixtures that exhibit a melting point (MP) required for low temperature sensitive drug delivery. The objective of the present study was to comprehensively characterize the thermoresponsive nature of thermoresponsive lipid nanoparticles (TLN) in vitro for treating glioblastoma, the most aggressive form of brain cancer. For this, TLN were prepared by microemulsification of a lipid mixture, consisting of solid and liquid fatty acids, that show solid-liquid phase transition at 39˚C as shown by a change in viscosity and transmittance of light. Drug release studies were performed by the dialysis bag method and a modified electrochemical method for real time detection of thermoresponsive drug release at 37-39˚C. Next, cytotoxicity of the TLN were evaluated using an in vitro blood-brain barrier model. Results showed that unloaded TLN were non-toxic to endothelial and glioblastoma cells, whereas paclitaxel, a potent chemotherapeutic agent, loaded TLN were cytotoxic to glioblastoma cells after 24 hrs. In summary, this study shows much promise for the use of the presently fabricated TLN for treating glioblastoma.

CXCR4 binding peptide density targets triple negative breast cancer and hinders metastasis

Daxing Liu, Craig McCarthy, Peng Guo, Debra Auguste

CXCR4 is a key regulator in breast cancer metastasis; inhibition of CXCR4 via antibody targeting hinders cancer cell migration and reduces tumor burden in vivo. An acquired resistance to antibody therapy can limit the number of patients responsive to treatment and the long-term benefit. In this study, we synthesized liposomes bearing the D-enantiomer of a CXCR4 binding peptide DV1. Triple negative breast cancer (TNBC) cell lines (MDA-MB-231 and MDA-MB-436) exhibited stronger binding to liposomes functionalized with surface density of DV1 (24k molecule/um2) (L-DV1-24k) in vitro relative to non-neoplastic cells. An ex vivo study indicated that L-DV1-24k can discriminate between metastatic breast cancer cells and surrounding tissues, which means the early diagnosis characterization of the neoplastic transformed cancer site, as well as the circulating tumor cells. An in vivo lung metastatic model study indicated that L-DV1-24k can reverse the early metastatic trend of MDA MB 231-luc to lung by tracking CTC cells, early arrest and extravasation, and arising anoikis after administrating 2 times per week. Finally L-DV1-24k delayed MDA-MB-231 cells metastasis to lung for 3 weeks and kept 84.6% of tumor suppression rate for at least 31 days totally.

The Therapeutic Effect of Epigenetic Drug-encapsulating Lipid Nanoemulsions for Triple Negative Breast Cancer Cells

Bumjun Kim, Debra Auguste

Epigenetic therapy has emerged as an alternative to conventional chemotherapy due to its selectivity toward cancer cells that often present aberrant epigenetic patterns such as hypermethylation and histone deacetylation. However, the stability issues of epigenetic drugs have to be addressed for the efficient treatment. Herein, we present LPAR-targeted-lipid nanoemulsions(LNEs) encasulating two epigenetic drugs, Decitabine (DAC) and Panobinostat (PAN). Our result showed that the uptake of LNEs were dependent on the LPAR expression in triple negative breast cancer(TNBC) cell lines. DAC and PAN encapsulated LNEs(DAC/PAN-LNEs) effectively killed a variety of TNBC cell lines and suppressed the growth of breast cancer. We also investigated the working mechanism of DAC/PAN-LNEs in a molecular level. DAC/PAN-LNEs was effective in inhibiting the growth and the migration of mesenchymal breast cancer cells that overexpress mFOXM1 by restoring mCDH1/E-cadherin and suppressing mFOXM1 expression, while ineffective to epithelial breast cancer cells that inherently express low mFOXM1 and high mCDH1. Overall, we successfully LPAR-targeted LNEs encapsulating DAC and PAN that can selectively treat mCDH1(low)/mFOXM1(high) TNBC cell lines.

A Cross–Reactive Array Based on Spectroscopically Encoded Polymers for the Classification of Biomolecules

Jessica Fitzgerald and Hicham Fenniri

The detection of biologically or environmentally significant analytes has employed sensor technology based on two major approaches. The first and most common sensing mechanism is based on the lock-and-key approach, where a strong and specific interaction between the sensory element and the analyte leads to a measurable signal. The second and more recent approach uses

sensor arrays that emulate the mammalian gustatory or olfactory systems. The latter takes advantage of a series of physical and/or chemical changes resulting from specific or non-specific interactions of the analyte with each element of the sensor array. The collective changes in the array can then be extracted using multivariate data analysis, and plotted to show unique patterns for a given species. Earlier reports described cross-reactive sensor arrays (CRSA) with different sensory elements, including organometallic compounds, dyes, metalloporphyrins, nanoparticles, composite materials, enzymes, or lymphocytes. The changes registered by different types of CRSAs upon exposure to a given analyte include changes in electrical properties, acoustic waves, surface plasmon resonances, color, radioluminiscence, vapoluminiscence, and fluorescence. Herein we demonstrate a new approach to an artificial tongue based on the vibrational properties of spectroscopically encoded polymers, and its application to the classification of small and large biomolecules.

A Double Look: Using Biomaterials as an Art Platform

Tess Torregrosa, Abigail Koppes, and Ryan Koppes

Both the arts and STEM fields have always looked to push human thinking. At the intersection of art and STEM, artists have integrated new technology to be a medium and inspiration for their work. Some broad examples broad of exposing this intersection has been the through 3D printing, theater, and costume. This work will focus on two creations outside of Northeastern and a project from our lab, the Laboratory for Neuromodulation and Neuromuscular Repair (LNNR). Utilizing 3D printing, Amy Karle was able to create Regenerative Reliquary, a new media art, by printing stem cells and a scaffold to build bone. The play Orchids to Octopi by Melinda Lopez, commissioned by the National Institutes of Health, teaches the audience about evolution. Lastly, our idea of a nervous system inspired sculptural costume piece that incorporates our fiber technology normally used to stimulate neurons into wearable items. These projects involve both artists and scientists coming together to make something more than what either one could have achieved alone. Perhaps through art, we as scientists can bridge the gap between the STEM community and the public and excite a broader audience about new and novel ideas.

Injectable hydrogels for microfluidic applications

Aslihan Kazan, Seref Akay, Rabia Onbas, Ozlem Yesil-Celiktas

Injectable hydrogels are unique materials developed for diverse life science applications such as drug delivery, tissue, regenerative and biomedical engineering. In the scope of this study, silica, alginate and alginate-silica hybrid hydrogels were synthesized where gelation was induced by different mechanisms. Formulated gels were characterized by SEM, BET, FTIR and EDS/mapping analysis particularly for the hybrid counterpart. Hydrogels were injected in spiral coil single channel glass-silicon-glass, S-shaped single channel PDMS and 8-channel glass-silicon-glass microsystems. Amino and D-glucono-δ-lactone functionalization for silica and alginate improved the mechanical properties and increased surface area and enzyme activity, respectively. Formulated hybrid gels exhibited a homogeneous structure although aggregation of silica is an important problem leading to loss of porous structure. The results indicated that alginate and hybrid hydrogels exhibited remarkable enhancement for immobilizing enzymes into microsystems and will contribute to the design of new injectable hydrogels particularly for microfluidic applications.

Acknowledgements
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Direct Mechanical Stimulation of MSCs Encapsulated in a Bionanocomposite Magneto-responsive Hydrogel Scaffold via an External Magnetic Field

Adedokun Adedoyin and Adam K. Ekenseair

Injuries sustained to the articular cartilage tissue do not heal naturally due to the tissue's innate avascular structure. Modern advances in the field of tissue engineering have shown that hydrogels, capable of responding on-demand to changes in their local environment, are promising candidates as injectable scaffolds for osteochondral tissue regeneration applications. This work focuses on the development of an injectable, magneto-responsive pNiPAAm-based hydrogel capable of responding to changes in an external magnetic field to guide the regeneration of damaged cartilage in situ. The magnetic hydrogel scaffold was created by chemically-crosslinking amine-functionalized superparamagnetic iron (III) oxide nanoparticles into the polymer network. Differences in magnetic field stimulation patterns were generated by developing a unique Arduino-controlled electromagnet cell culture system. Results show that these magnetic hydrogels are capable of delivering and maintaining viable stem cell populations. Furthermore, the mechanical stimulation forces created within the hydrogel as a response to changes in the magnetic field did not have any adverse effects on cell viability when compared to our experimental control. Moreover, over a three-week time-period, there was an increase in hydrogel calcification and ALP expression across all experimental conditions. Thus, magneto-responsive hydrogels were shown to be promising candidates as injectable scaffolds for tissue regeneration.

Engineering an elastic hydrogel as a sprayable wound healing patch with antimicrobial properties

Devyesh Rana, Ehsan Shirzaei Sani, Roberto Portillo Lara, Nasim Annabi

Chronic wounds affect 2% of the U.S. population. Healing of chronic wounds is often impaired by the occurrence of bacterial infections. Therefore, there is a need for wound dressings to promote wound healing and prevent infections. In this study, we engineered novel elastic and sprayable hydrogels with antimicrobial properties, through photopolymerization of various ratios of methacrylated tropoelastin (MeTro) and gelatin methacryloyl (GelMA) for treatment of wound. MeTro/GelMA hydrogels were rendered antimicrobial through the conjugation of the broad spectrum antibacterial peptide (AMP) Tet213. The engineered hydrogels were shown to mimic the mechanical properties of the native tissue and to strongly adhere to native skin. Our results alos demonstrated that 70/30 MeTro/GelMA hydrogels at 15% (w/v) final polymer concentration and 0.01% (w/v) AMP could efficiently inhibited the growth of both Gram positive (Methycilin Resistant Staphylococcus Aureus) and Gram negative (Escherichia Coli) bacteria. MeTro/GelMA hydrogels were shown to promote the proliferation and spread of 3D encapsulated 3T3 fibroblasts in vitro. In addition, they could biodegrade in vivo without eliciting any significant inflammatory response when implanted subqtenously in rats. Our engineered

MeTro/GelMA-AMP hydrogel represent a novel sprayable adhesive patch for the clinical management of chronic wounds.

Cell-Laden Gelatin/Tropoelastin Hydrogel Composites for Peripheral Nerve Repair and Anastomosis

Jonathan Soucy, Ehsan Shirzaei Sani, David Diaz Vera, Roberto Portillo Lara, Felipe Dias, Suzanne Mithieux, Anthony Weiss, Abigail Koppes, Ryan Koppes, Nasim Annabi

A completely or partially severed nerve can cause a patient complete/partial loss of sensation, chronic pain, and even permanent disability. Current guidelines recommend repairing severed nerves with sutures; however, sutures hinder tissue regeneration and often result in scar tissue formation, further preventing functional recovery. To limit the need for sutures, fibrin glue has been proposed as a material to reattach nerve endings, but current formulations are not mechanically robust to significantly reduce the need for sutures. In this report, we engineered photocrosslinkable composite hydrogels from two naturally derived proteins, gelatin and tropoelastin, to be used as a glue for nerve anastomosis. These composites were prepared by conjugating methacrylate groups to gelatin and tropoelastin, then using free-radical chemistry to crosslink the two polymers into an adhesive with tunable physical properties. An in vitro cell study demonstrated that the engineered adhesives supported the growth of both glial and neuronal cells. Furthermore, results from in vivo subcutaneous implantation models demonstrated that the composite is biodegradability and biocompatible. Finally, we confirm that a severed nerve anastomosed by this composite had adhesion strength greater than the commercially available fibrin glue. Taken together, these results demonstrate the potential of gelatin/tropoelastin composites for use in nerve repair.

Prediction of urea cycle disorders via liver-on-a-chip simulation

Fatemeh Sharifi, Bahar Firoozabadi

Liver has many vital functions without which, human body can survive no more than few days. The role of liver in protein metabolism is probably the most important of its metabolic functions. One major part of this protein metabolism is urea generation. Liver helps to remove Nitrogen groups, Ammonia, from amino acids by generation of urea from amino acids. Ammonia is a very toxic material and if it is not rapidly and efficiently removed from the blood circulation, it will result in central nervous system disease. In this study, first ammonia elimination and urea production was simulated in a microchannel. Then, Twelve rate equations were also solved in order to obtain the concentration of each metabolites participating in urea cycle. Two disorders related to urea cycle i.e. Hyperammonemia and Argininosuccinicaciduria were simulated. Results show a mild decrease and a sharp increase in concentration of the metabolites relating to carbamoyl phosphate synthesis for hyperammonemia type I and II, respectively. Concentration of Argininosuccinate is increased nearly 10 fold in Argininosuccinicaciduria disorder. Predicted results are useful in better understanding, control and managing the effects of metabolite deficiencies, drugs and their side effects on overall metabolic behavior in hepatocytes' urea cycle.

Effect of pH-Varied Cerium Oxide Nanoparticles on the Growth of Gram-Positive and Negative Bacteria

Ece Alpaslan, Benjamin M. Geilich, Hilal Yazici, Thomas J. Webster

The antibacterial activity of dextran-coated nanoceria (DCN) was examined against Pseudomonas aeruginosa and Staphylococcus epidermidis in terms of a dose, time and pH dependent manner. DCN was much more effective at killing P. aeruginosa and S. epidermidis at basic pH (pH=9) compared to an acidic pH environment (pH=6) due to a smaller size and positive surface charge at pH 9. After 6 hours of incubation with nanoceria at pH 9, P. aeruginosa showed drastic morphological changes as a result of cellular stress. This study provides significant evidence for the use of nanoceria for a wide range of anti-infection applications without resorting to the use of antibiotics.

Biogenic silver nanoparticles perform dual function as Berberine Carrier and Anticancer Agent against breast cancer

Ramasamy Bhanumathi, KaruppaiyaVimala, and Soundarapandian Kannan

Biologically amalgamated silver nanoparticles (AgNPs) have been used as a drug carrier and determined the anticancer potentiality against breast cancer. Syzygium cumini (S. cumini) seed extract was used to formulate the monodispersed biogenic AgNPs. In the present investigation, a natural anticancer potential, Berberine (BHC) was loaded on the surface of biogenic AgNPs and were characterized by UV–visible spectroscopy, Fourier Transform Infrared Spectroscopy (FT-IR), X-Ray Diffraction (XRD), Zeta Potential, Dynamic Light Scattering (DLS), and Transmission Electron Microscopy (TEM). We evaluated the synthesized biogenic AgNPs, free BHC and BHC loaded AgNPs, they showed a dose-dependent growth inhibition of both MCF-7 and MDA-MB-231 breast cancer cells holds at 1.0 ± 0.2 μg/mL, 0.5 ± 0.01 μg/mL and 0.25 ± 0.02 μg/mL in 48 h. The cytotoxicity effect of biogenic AgNPs, free BHC and BHC loaded AgNPs were tested against HBL-100 cells and revealed that they do not have significant cytotoxicity in the normal cell when compared to cancer cells. The Western blot analysis authenticate that there is a down regulation of anti-apoptotic protein bcl-2 with elevated expression of p53 and Bax in treated cancer cells. The in vivo analysis witnessed that there is no significant toxicity in the vital organs of the BHC loaded AgNPs treated murine mice model system. These results suggest that biogenic AgNPs has dual function as an effective anticancer agent cum drug carrier for treatment of breast cancer. Thus, the present study not only poise a strongly undoubted evidence for the anticancer effect of BHC, which is pharmacologically accepted antidiabetic drug but also has improved anticancer effect along with AgNPs.

Nano-Drug Delivery System for Improved Synergistic Chemotherapy of Colon Carcinoma

Kathirvel Rayappan, Shenbagamoorthy Sundarraj, Roberto Portillo Lara, Nasim Annabi and Soundarapandian Kannan

Herein, we demonstrate the synthesis and biofunctional significance of hollow mesoporous silica nanoparticle (HMSNs). The data described in this study authenticate that the mesochannels of HMSNs served as nano-reservoirs for payloads of anti-cancer drug, β-sitosterol. Further, the β-sitosterol conjugated into the hollow core whereas, cisplatin was packed into the carboxyl groups modified pore walls via coordinated interaction. The fabricated HMSN nano-drug system is

chemically modified with 3:1 ratio of two chemical branches, the first one was pore wall modification by means of poly lactic acid (PLA)-polyethylene glycol (PEG) with carboxylic group terminus tagged cisplatin drug and the other was Mal-PEG conjugated tumor biomarker, folic acid (FLOR) for selective targeting tumor cells. The surface modified HMSNs sustenance the internalization of cisplatin from HMSNs due to Cl^- ion release through the cleavage of the coordination interaction. Further, β-sitosterol release depends on the discharged rate of cisplatin and the pH of the cell microenvironment. The delivered drugs can able to induce apoptosis through caspase activation, cell cycle arrest and formation of reactive oxygen species (ROS), which are expected to instantaneously envisage the therapeutic proficiency in HT-29 colon cancer cells. Thus, the developed nano-drug system based targeting may offer effective treatment strategies for colon cancer.

Antimicrobial Peptide-Enriched Nano-Vesicles for the Treatment of Antibiotic-Resistant Infections

Nicole Bassous and Thomas J. Webster

The integration of immune cell mimics together with modern medical intervention will substantiate the concept of a "smart" vaccine. The objective of the current study is to fabricate tunable polymersomes (Ps) that combat antibiotic-resistant bacterial infections. Ps are polymer-based, biocompatible vesicles that self-assemble via the hydrophobicity interactions of admixed aqueous and organic substances. In this study, Ps are conjugated to include antimicrobial peptides (AMPs) along the peripheral hydrophilic region and silver nanoparticles inside the hydrophobic corona. Initial results indicate that finely tuned treatment concentrations of AMP and silver nanoparticles in Ps synergistically inhibit the growth of diverse bacterial colonies. While low concentration AMP/Ps demonstrate strong toxicity towards colonies of multi-drug resistant E. coli in vitro, AMP/Ag nanoparticle/Ps combination is needed to completely curb methicillin-resistant S. aureus growth. This bacteriostatic activity, coupled with minimal cytotoxicity towards native human cells, extends the potential for AMP/Ag nanoparticle/Ps therapies to replace antibiotics in the clinical setting.

Surface Modification of Titanium Orthopedic Implants to Reduce Infection and Inflammation

Garima Bhardwaj and Thomas J. Webster

A large population of the world is affected by a number of orthopedic problems. According to the American Association of Orthopedic Surgeons (AAOS); between the years 2009 and 2012, there has been an 83.72% increase in total hip and knee replacement procedures. Despite the high success rates of these surgeries, about 20% of these implants fail due to infection within 10-20 years. In the pathogenesis of infection around implants, the initial adhesion of bacteria onto biomaterial surfaces is a critical first step. There is a race for the surface between healthy cells and bacteria. An important strategy in the reduction of orthopedic infections is to develop implant materials that prevent initial bacteria adhesion onto implant surfaces. Surface coatings of different sizes and geometries to reduce infection and inflammation can be achieved by various methods, one of which is Electrophoretic Deposition (EPD). The aim of this research is to modify the surface of titanium by coating it with nanoscale hydroxyapatite, nanoscale ceria and

nanoscale selenium using electrophoretic deposition with a DC current to impart anti-bacterial,anti-inflammatory, and pro-bone growing responses – all in the same material without the use of pharmaceutical drugs which has detrimental side effects in the body.

Permeability Maps for GelMA Under Different Photo-Crosslinking Times and Degrees of Methacrylation

Hossein Goodarzi Hosseinabadi, Amir K. Miri, Reza Bagheri, and Ali Khademhosseini

We proposed a simple and efficient spherical indentation-based testing method to estimate permeability of gelatin methacrylate (GelMA) fabricated by different mass concentrations and photo-cross-linking conditions. Numerical simulation of the experiment along with Biot theory of poroelasticity was used to analyze and further calibrate the experimental data. The roles of the UV exposure time and the degree of methacrylation on the achieved hydraulic permeability of the hydrogel system are particularly illustrated. Beside the SEM microstructural evaluations, we validated the proposed approach by predicting the release response in GelMA. Present results provide a data map for future use of GelMA based hydrogels in tissue engineering and drug delivery applications.

Photo-thermally driven refreshable microactuators based on graphene oxide doped paraffin materials

Sichao Hou, Miao Wang, Ruiqing Huo, Shouwu Guo, and Ming Su

Actuators based on phase change materials (paraffin) can simultaneously produce large stroke length and large force due to thermal expansion, but the low thermal conductivity of paraffin requires high power input and long actuation time. This article reports a graphene oxide (GO) doped paraffin dynamic actuator, which has shown superior thermal conductivity, and can be photo-thermally driven due to its strong light absorbing ability. The actuator shows rapid responses upon light adsorption, and can be cycled many times without fatigue. The thermal properties of GO-paraffin composites with varied loading amount from 0.1 to 1.0% are characterized to optimize the loading amount. A multi-cell phase change actuator was fabricated and a corresponding computer-aided optically-controlled system was built to separately control the actuation of each cell. The infrared camera was used to monitor the actuation time and height by recording the cell temperature as a function of time. A promising application of this phase change actuators is in refreshable Braille displays that contain haptic devices for the blind and partially sighted, translating text from a computer into readable characters.

Cold Atmospheric Plasma (CAP) Modified Core-shell Nanofibers for Bone Tissue Engineering Applications

Yangfang Zhou, Mian Wang and Thomas J. Webster

Coaxial electrospinning is a novel technique for producing core-shell nanofibers which combine the advantages of having a robust structure and the ability of delivering hydrophilic bioactive agents. Core-shell nanofibers were fabricated by polyvinyl alcohol (PVA) and poly-l-lactide (PLLA) and had diameters from 1200 nm to 1500 nm. In order to mimic the properties of an extracellular matrix, scaffolds were modified by cold atmospheric plasma (CAP) which not only increased surface roughness of the nanofibers [1], but also changed the release profile of water-soluble agents that were loaded into the core. Experimental results showed that samples modified

by CAP for 90 seconds released drugs faster than those with less modification time, which means that CAP modification affected the release speed. Moreover, samples treated by CAP for longer times had better osteoblast adhesion and proliferation; in other words, the cytocompatibility of the scaffolds improved. In conclusion, CAP modification can play an important role in enhancing the cytocompatibility of scaffolds as well as in controlling drug release, and, thus should be further studied for numerous bone tissue engineering applications.

Reference:
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Single Cell Array for Population-based Subcellular Toxicity Assay

Junfei Xia, Yuting Qiu, Xiaojie Xun, Ming Su

Single cell assay aims to identify the function and behavior of individual cells in a population of cells. As there are no simple techniques available to distinguish the contributions of individual cells from a population of cells, information that describes an individual cell should be accompanied by information that describes the average of other cells. Therefore assays that can obtain information about individual cells and large numbers of cells simultaneously are highly useful. This type of design can be best represented by an ordered array of individual cells that is confined on a planar substrate. The order in the array makes it easy for microscopic observation of single cells. Such a design eliminates the need to perform single-cell assay and multiple-cell assay side by side.

In this work, we present a low cost and robust method for fabricating a single cell array at centimeter scale. The method features a combination of soft lithography and layer-by-layer assembly. We used the single cell array to investigate cellular responses at both the individual and population level using multiple commonly used toxicity assays. This method is an efficient tool for analyzing individual cellular functions in a population and is highly adaptable to a variety of cell-based assays.

Simultaneously Generating Highly Ordered Genomic DNA Microarrays and Nanostrands

Junfei Xia, Jingjiao Guan, Ming Su

Patterning DNA in the form of ordered arrays holds great potential for high throughput analysis of unknown genes. Among methods for analyzing single DNA molecules, stretching DNA is the most effective way especially because it allows for precisely locating genes on DNA. An array of methods are available for stretching DNA including molecular combing, flow field stretching and electric field stretching. In molecular combing, DNA molecules are stretched upon movement of meniscus on tethered DNA. Based on this mechanism, a topologically-patterned hydrophobic surface such as PDMS can be used for generating ordered DNA nanostrands.

In this report, we present a novel method for patterning DNA microarray and stretched DNA molecules simultaneously. Human genomic DNA were absorbed and stretched on a wettability patterned surface. DNA molecules were electrostatically absorbed onto a positive charged pattern while DNA nanostrands were stretched on the hydrophobic substrate from each

hydrophilic island due to surface tension at the receding contact line. Formed DNA micro-patterns were readily available for gene analysis without need for second transfer. This method enjoys low-cost, easy to operate and widely applicable to a variety of wettability patterned surface.

Cationic Self-Assembling Peptide Amphiphiles as Antibacterial Agents Against Drug-Resistant Bacteria

Kanny (Run) Chang and Thomas J. Webster

To develop a self-assembled peptide molecule that can kill bacteria, antibacterial cationic peptide amphiphiles (ACA-PAs) were designed to self-assemble into a cylindrical supramolecular structure in water. The hydrophobic interactions of the hydrocarbon tail group served as the driving force of self-assembly, and the β-sheet stacking directed the aggregated structure into a cylindrical shape. As a result, the cationic polar heparin-binding group was exposed on the surface of the self- assembled structure to interact with anionic bacteria membranes.

The novel self-assembled structure of the ACA-PAs was observed by transmission electron microscopy. In bacterial studies, Gram-positive methicillin-resistant Staphylococcus aureus (MRSA, ATCC#43300) and Gram-negative multidrug-resistant Escherichia coli (MDR E. coli, ATCC#BAA-2471) were used to test antibacterial activity. Also, cytotoxicity of the ACA-PAs was determined with human dermal fibroblast (HDF) cells using a MTS assay. Our results showed that the self-assembled ACA-PAs can self-assemble into nanorods, and have excellent antibacterial effects against antibiotic-resistant bacteria. In particular, results of this study showed that the self-assembly of ACA-PAs is important for killing Gram-negative bacteria. The self-assembled nanorods also exhibited significantly lower toxicity towards HDF cells than bacteria. In conclusion, these self-assembled peptides could be promising materials to combat infectious disease caused by bacteria drug-resistance.

Atomic Layer Deposition of Nanocrystalline TiO2 Thin Films for Orthopedic Applications

Luting Liu and Thomas J. Webster

Titanium (Ti) and its alloys have been extensively used as implant materials in orthopedic applications. Nevertheless, implants may fail due to a lack of osseointegration and/or infection. The aim of this in vitro study was to endow an implant surface with favorable biological properties by the dual modification of surface chemistry and nanostructured topography. The application of a nanostructured titanium dioxide (TiO2) coating on Ti-based implants has been proposed as a potential way to enhance tissue-implant interactions while inhibiting bacterial colonization due to its chemical stability, biocompatibility, and antimicrobial properties. In this paper, atomic layer deposition (ALD) is introduced for the first time to provide unique nanostructured TiO2 coatings on Ti. The effect of nanoscale TiO2 coatings on human fibroblast and osteoblast functions and bacterial activities was investigated. In vitro results indicated that the TiO2 coating stimulated osteoblast adhesion and proliferation significantly while suppressing fibroblast adhesion and proliferation compared to uncoated materials. In addition, the inherent photocatalytic activity of TiO2 along with increased nanoscale roughness, was shown to inhibit Gram-positive bacteria (Staphylococcus aureus), Gram-negative bacteria (Escherichia coli), and antibiotic-resistant bacteria (Methicillin-resistant Staphylococcus aureus or MRSA) adhesion all

without resorting to the use of antibiotics. In summary, results from this study demonstrate the strong potential of using ALD-grown TiO2 coatings for improving numerous orthopedic implants.

Silver-coated Gold Nanorods for Anti-microbial Applications

Junyan Zhang and Thomas J. Webster

Gold nanoparticles, such as gold nanorods, have attracted much attention due to their unique optical properties resulting from the surface plasmon resonance (SPR) effect and can be applied for various biomedical applications including imaging, medical diagnostic and cancer treatment. Among different gold nanoparticles, gold nanorods have been of interest due to their tunability and large red shift towards the near infrared region. Silver has a long history as an anti-microbial agent either in its bulk or nanoparticle form. In this study, silver-coated gold nanorods were synthesized and tested as a novel anti-microbial material. Moreover, the coated nanorods absorb in the near-infrared region (NIR) and have thus the potential to be used in photothermal therapy for cancer. Therefore, silver-coated gold nanorods can serve as promising candidates for the combined therapy of tumors and bacterial infection.

DNA-Peptide Nanotubes As Artificial Extracellular Matrices For Bone Tissue Engineering

Gujie Mi, Di Shi and Thomas J. Webster

The extracellular matrix (ECM) is a complex network composed of collagen, fibronectin and other macromolecules that dictates how cells attach, behave and interact with one another and thus plays a key role in tissue morphogenesis, differentiation and repair. In this context, the construction of artificial matrices that mimic the structure of the naturally occurring ECM and display biological signals might prove to be highly promising in controlling tissue regeneration. In the present study, DNA nanotubes were constructed based on double-crossover motifs and were further functionalized with a BMP-7 derived peptide due to its role in regulating the proliferation, differentiation and apoptosis of bone cells. The objective of the present study was to develop an extracellular microenvironment that can mimic the structural and regulatory characteristics of natural ECMs. Results showed that the DNA nanotube coated glass coverslips revealed a tangled network of fiber-like materials that resemble the morphology of natural ECM. Cell studies further demonstrated dramatic increases in osteoblast adhesion, proliferation and bone-related gene expression, suggesting DNA-peptide nanotubes could be a promising candidate as artificial extracellular matrices. Further experiments in vitro are still need to fully investigate their effects on cell functions.

Thermosensitive Liposomes for Brain Tumor-Targeted Drug Delivery

Di Shi, Gujie Mi and Thomas J. Webster

Currently, targeting of malignant brain tumors such as glioblastoma multiforme (GBM) and the delivery of therapeutic agents into the brain remains a big obstacle because of the difficulties in developing candidates that can cross the BBB and specifically target GBM. In this study, superparamagnetic iron oxide nanoparticles (SPIONs) loaded thermosensitive liposomes (ferri-TSLs) were developed for BBB penetration and targeting of the brain glioma under mild hyperthermia. To specifically target GBM, an innovative cell-penetrating peptide and an anti-glioma antibody were also conjugated to the liposome surface. In addition, an in vitro BBB

model was established using mouse brain endothelial cells (bEnd.3) and astrocytes (C8D1-A) to indicate the permeability of these ferri-TSL samples. With the encapsulation of SPIONs and doxorubicin (DOX) inside the liposomes, ferri-TSL showed thermo-triggered DOX drug release and relatively high BBB permeability. These results suggest that ferri-TSL is an excellent candidate for brain tumor drug delivery and lay the foundation for further optimization of the liposomes to improve glioma targeting efficiency.

Lanthanide as a Cyto-protectant

Maximilian Bizanek, Francielli Silva Genier, Eric Dahl, Thomas J. Webster, Amit K. Roy

Constantly challenged by various environmental stressors, the body's cells experience corresponding metabolic changes, induced by factors that upregulate stress-response related genes. Sources of cellular stress include heat, radiation, chemicals, and nutrient deprivation. The accumulated damage from exposure to these stimuli has been postulated as an important cause of aging, cancer, and degenerative diseases. Recent studies from our group and others have shown that nanoparticles (NP's) can efficiently mitigate the negative effects of cellular stress. We investigated the cytoprotective effects of Ceria (Ce3+) nanoparticles on nutrient-deprived human dermal fibroblast cells (HDF), and demonstrated that pre-incubation with these NP's attenuate cell death as shown with MTS assays. Further investigation into the effects and mechanisms of NP's on stressed cells are underway to evaluate the use of NP's as therapeutic treatments.

The aim of our study was to investigate how the mechanism of this cytoprotective effect presented itself, and quantify that effect with MTS assays. These preliminary findings demonstrated that NP's promote cell survival and regeneration in nutrient deprived HDF cells. While these findings are promising, and do point to possible therapeutic applications of Ce-NP's in the future, further investigations are necessary.

A comparison between electro and rotary-jet spinning to produce polymeric fibers with incorporated nanohydroxyapatite and carbon nanotubes: Reduced bactericidal activity and greater cell viability

Mirian M. M. de Paula, Fernanda R. Marciano, Thomas J. Webster and Anderson O. Lobo

Recently, surface modification of FDA approved polymers to improve biological affinity (specifically bone regeneration) and reduce bacterial growth has been gaining momentum. Carbon nanotubes and hydroxyapatite have also been used to improve the mechanical, bioactivity and bactericidal properties of FDA approved polymers. In this study, electro and rotary-jet spinning were used to produce nano/micro fibers with different amounts of incorporated nanoparticles. Herein, the efficacy of nano/micro fibers with incorporated nanohydroxyapatite (nHAp) and carbon nanotubes (CNT) were investigated in terms of their bactericidal properties and cell viability. Polycaprolactone (PCL) fibers with different amounts of CNT, nHAp and a mixture of them (1%) were synthesized using electro and rotary jet spinning. SEM micrographs show that the method used controlled composite morphology and roughness. Comparatively, the rotary-jet spun nano/micro fibers were thickener and more porous than those that were electrospun. However, all the fibers showed no cell toxicity. Interestingly, the rotary-jet spun fibers containing different amounts of CNT, nHAp and CNT/nHAp presented

more bactericidal activity than those that were electrospun. In summary, this study provides significant promise for the use of rotary-jet spinning to obtain porous and micro/nano fibers with incorporated nanoparticles for reducing implant infections.

Stable encapsulation of triangular silver nanoplates with a silica shell for targeted and photothermal antibacterial treatment

Chih-Sheng Chiang, Kanny Chang, Mian Wang and Thomas J. Webster

The relentless emergence of antibiotic resistance found in many bacteria strains has become a major health problem, resulting in increasing mortality rates and excess medical expenses for infected patients. The urgent requirement for a new treatment to combat antibiotic-resistant bacteria promotes the development of nanomedicine-based strategies. To reduce the possibility for bacteria to develop resistance, triangular silver nanoplates (TNP) which can produce multiple mechanisms of action towards killing bacteria were synthesized. However, TNP is extremely unstable in a chloride-containing environment, which largely limits its application. Therefore, we report here for the first time modified TNP (TNP@SiO2) encapsulated by a silicate shell with tunable thickness via the Stöber method. The silicate shell not only provides TNP@SiO2 with high compatibility in a chloride-containing environment, but retains its optical properties, allowing high photo-thermal conversion efficiency under NIR irradiation. In addition, TNP@SiO2 was functionalized with a targeting ligand to selectively induce toxicity towards bacteria while enhancing cytocompatibility in mammalian cells. Together, the ligand-immobilized TNP@SiO2 can achieve a targeted photothermal therapy and controlled release of silver ions based on a nanomedicine-derived strategy to effectively kill bacteria.

Exploring the antibacterial effects of polymerosome nanomaterials embedded with silver nanoparticles and doxorubicin

Keerthana Subramanian, Kanny (Run) Chang and Thomas J. Webster

The World Health Organization recently stated that antibiotic resistant “superbugs” pose a massive threat to human health(1). Needless to say, the need for different approaches to combat these infections is higher now than ever.

The objective of this study was to explore the effects of a polymerosome nanocarrier embedded with silver nanoparticles (Ag) and the anti-cancer drug doxorubicin towards the inhibition of bacteria. Utilizing polymers (PDLLA-PEG-COOH) to create delivery vehicles, or polymersomes (Ps), is a feasible option because the polymer has excellent biodegradability and biocompatibility.

Studies here indicated that while Ps only containing silver nanoparticles (AgPs) had no effect on bacterial growth, the anti-cancer drug doxorubicin aids in bacterial inactivation. Through this exploratory study, we hope to find the ideal silver-to-doxorubicin ratio that aids in the effective treatment of both gram-positive and gram-negative bacteria.

(1) https://www.nytimes.com/2017/02/27/health/who-bacteria-pathogens-antibiotic-resistant-superbugs.html?rref=collection%2Ftimestopic%2FWorld%20Health%20Organization&action=c

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Designing nanofibers based on polyesters, hydrogels and peptides for cartilage repair

Paria Ghannadian, Siddhi Kanakiya, Akhil Agarwal, Mirian Michelle Machado de Paula, Thomas J. Webster and Anderson de Oliveira Lobo

Cartilage damage is very common due to aging or disease such as arthritis. Cartilage has poor regeneration and repair due to its limited vascularization. In this research, we are fabricating the next generation of improved cartilage materials composed of synthetic and natural hydrogels with polycaprolactone (PCL) nanofibers produced by electrospinning or nanoparticles such as hydroxyapatite (HA) and carbon nanotubes (CNT) in them. Here, we synthesized hydrogels (composed of 2.5% Gelatin and 10% PVA, electrospun PCL mats using different concentrations (10-20% w/v)) and modified the surface of the PCL nanofibers by reagents to disperse them into the hydrogels. After this, we used three freeze thaw cycles to make the hydrogels porous for cell ingrowth. Our next step is to determine their mechanical properties compared to cartilage (using testing such as tensile tests and compression tests) and characterize their surface properties via SEM to obtain a better comprehension between the synthesis processes and resulting properties. Lastly, we will add peptides and other bioactive compounds (such as Methionine) as well as mesenchymal stem cells to quickly regenerate cartilage at the site of injection.

Antibacterial Selenium Nanoparticle Coatings for Field Hospitals

James Moxley and Thomas J. Webster

While advancing antibacterial resistance has recently come to demonstrate a looming threat to the health-care infrastructure of developed nations, many areas throughout the developing world still suffer persistently high mortality rates from commonly treatable bacterial strains. Humanitarian efforts in these regions, primarily seen today in the form of non-governmental organizational (NGO) outreach as well as peace-keeping military operations, are significantly strained by an incapability to reproducibly produce and maintain sterile conditions in the field. Injured personnel are often required to be rapidly transported out of the region in order to receive safe medical treatment, incurring large financial costs while demonstrating severe risks to the individuals in the process. In order to address this issue, antibacterial selenium nanoparticulate coatings were prepared for application in rapidly constructed field hospitals operating in diverse environments. These coatings have demonstrated antibacterial properties when applied to wall paneling suspended in various bacterial cultures. Ongoing work includes determining the effects of local weather (principally, heat and humidity) and transportation (principally, vibrational and shock disturbances) conditions on the persistence of applied coatings. New means of rapid, on-site administration of antibacterial nanoparticulate coatings, onto both medical instrumentation and house-keeping materials, are also currently under development.

Self Assembled DiBlock polymersomes for the treatment of mitochondrial diseases

Jakob Farnham, Nicole Bassous and Thomas J. Webster

This project focuses on the delivery of mitochondria to human cells with the aim of treating cells that have a deficiency in Adenosine triphosphate (ATP), the primary source of cellular energy. To protect and selectively deliver the mitochondria, they are encapsulated by stable polymersome vesicles which endure high pressures and temperatures inside a physiological environment. The polymer designed to form the "shell" protecting the mitochondria is mPEG-PDLLA, which self-assembles into a hydrophilic inner/outer membrane and hydrophobic core when injected into an aqueous solution. By this time, we have successfully incorporated our vesicles with mitochondria and defined an expected size range of the polymersomes based on transmission electron microscopy (TEM), fluorescence microscope imaging, and dynamic light scattering (DLS). Now that we have effectively proved the viability of this treatment method, we can move on to in vitro studies. In particular, we will compare the survivability of mitochondria when released into a high temperature or pressure environment with mitochondria that are encapsulated in our polymersomes. We will also analyze the ability of the polymersomes to release the mitochondria, and the potential for targeted cells to then incorporate these mitochondria into their own system via endocytosis.

Developing Multifunctional Theranostic Vehicles for Cancer Stem Cell Therapy

Halla Laufey Hauksdóttir, Thomas J. Webster and Már Másson

Iron oxide nanoparticles (IONP) already have an established use within the medical field. Most of the products available utilize their superparamagnetic properties to improve MRI contrast. However, these IONP also present a number of possibilities with multifunctional roles in cancer treatment. IONP offer possibilities in therapy such as drug carriers and in hyperthermia therapy. In addition, selenium is a trace mineral and is an essential nutrient considered to have both cancer preventive and cancer treatment properties. The purpose of this study is to determine the effects of selenium and IONP on cancer stem cells (CSCs). CSCs are cells within tumors with capabilities of self-renewal, differentiation and potential to proliferate extensively. CSCs are essential for tumor growth and could explain the high diversity of malignancies and even cancer therapy resistance. Current treatments are based on killing as many cancer cells as possible leading to tumor reduction. Failure to eliminate CSCs causes likely tumor recurrence and metastasis. Targeting CSCs in therapy should lead to a more effective eradication of tumors and reduce the risk of cancer relapse and metastasis. The ultimate goal of this research is to develop composite nanovehicles of iron oxide and selenium for targeted cancer therapy.

The effects of piezoelectric nanoparticle/polymer scaffolds on cell proliferation

Yuan Li and Thomas J. Webster

Owing to their piezoelectric properties, piezoelectric nanomaterials have been used to enhance cell growth and functions. For this purpose, ZnO/poly(vinylidene fluoride) (PVDF) nanoparticle/polymer scaffolds were synthesized and investigated here. These materials demonstrated piezoelectricity at normal temperatures and good biocompatibility properties to human cells. Thus, they were hypothesized to be good candidates to stimulate human bone cell functions.

In this experiment, ZnO nanoparticles were produced by Sigma Aldrich. From the TEM pictures, most ZnO nanoparticles had polygon crystal shapes with a size of 50 nm. ZnO/PVDF scaffolds were fabricated by electrospining. According to the SEM picture, the ZnO/PVDF scaffolds were formed by electrospun fibers. Most fibers had a diameter ranging from 400-600 nm.
Human osteoblasts and fibroblasts were seeded on these piezo-materials in vitro. The results indicated that these piezoelectric materials were non-toxic to fibroblasts and osteoblasts. Compared to the control group, both osteoblast and fibroblast proliferation were not affected by ZnO/PVDF scaffolds when ZnO concentration was below 1 mg/ml in the electrospining polymer solution.
In conclusion, these piezoelectric nanomaterials are good candidates to stimulate cell proliferation in the ongoing work. Future studies will investigate cell functions on ZnO/PVDF scaffolds under a piezo-excited treatment.

Role of the microstructure topology on the mechanical properties of an elastic extra cellular matrix

H.G. Hosseinabadi, Reza Bagheri and V. Altstadt

The microstructure of the extracellular matrix (ECM) can be defined based on the regularity in the shape of pores together with the variations of the pore size. However, the distinct roles of the pore shape and the variations of their size on the mechanical response of the ECM are less understood. Here, the role of the shape and the size of pores on the mechanical response of an ECM is systematically investigated. Finite element approach is used to capture the behavior of a 2D infinite porous environment. Similar densities, cell wall material properties and topological coordination number is used in the models. The results show that the pore shape regularity and the variations of the pore size may not distinguishably influence the elasticity of the ECM at adequately small deformations. However, the instability response of the ECM at higher deformations can be highly influenced by the regularity of the pore shape in the system. On the other side, increasing the variations of the pore size may not influence the instability response but can deteriorate the mechanical strength of the ECM in a macroscopic scale by introducing a more vulnerable microstructure to bucking and bending moments.

Engineering an immunomodulating and adhesive hydrogel for the diabetic wound treatment

Bahram Saleh, Harkiranpreet Kaur Dhaliwal, Ehsan Shirzaei Sani, Mansoor Amiji and Nasim Annabi

Diabetes mellitus is an important health problem that affects millions of people worldwide. The key processes that are impaired in diabetic wound healing are angiogenesis and controlling inflammation. The goal of this project is to engineer an immunomodulating, bioactive, elastic and adhesive gel that will enhance diabetic wound healing.
In this study, we have synthesized a visible light crosslinkable gelatin-based hydrogels with mechanical properties and degradation rate suitable for skin regeneration. To control inflammation and further improve wound healing, we incorporated hyaluronic acid-poly ethylene amine/polyethylene glycol (HA-PEI/PEG) nanoparticles within the engineered hydrogels to deliver immunomodulatory RNAs to the wound healing sites. These RNAs are able to modulate

macrophages polarization from pro-inflammatory M1 to anti-inflammatory/tissue healing M2 phenotype. It was found that the mechanical properties of hydrogels do not change after incorporation of 0.5% (w/v) of RNAs encapsulated HA-PEI/PEG nanoparticle (average particle size: 115.0 ± 10.2) inside the hydrogel. Furthermore, the release of RNA from the hydrogels showed 87% cumulative intact RNA release after 24 h, which could modulate macrophages polarization in vitro from M1 to M2 phenotype.
Our results demonstrate that the engineered hydrogel can be used as a multifunctional sprayable wound patch with the ability to control inflammation and induce vascularization and tissue healing. In addition, its strong adhesion to skin can prevent bacterial infection and eliminate the need for suturing.

Engineering conductive fibrous patches for cardiac tissue regeneration

Brian Walker, Chu Hsiang Yu and Nasim Annabi

Cardiac tissue engineering (TE) approaches have been applied for the treatment for patients suffering from heart failure after myocardial infarction (MI). Different types of materials have been used as scaffolds for cardiac tissue regeneration. Electrospinning is a technique utilized for engineering fibrous scaffold for tissue engineering applications. This technique implements an electrical field to create thin fiber mats out of polymer materials to form fibrous scaffolds, which can be seeded with cells. This technique may be utilized to engineer biomimetic cardiac patches using biocompatible, conductive, and mechanically robust materials. This project proposes to form fibrous scaffolds based on gelatin methacryloyl (GelMA) as the base material, due to its tunable mechanical properties, favorable biodegradation, and the presence of cell binding sites, which promote cell adhesion and proliferation. Further, we will introduce electrical conductive properties to the engineered fibrous GelMA based scaffold through the conjugation of a bio-ionic liquid (Bio-IL) in a one-step free-radical polymerization process, using the visible light photoinitiator Eosin Y. Lastly, we will study the mechanical and conductive properties of the engineered scaffolds. We will also evaluate the ability of the engineered biomaterial to support the growth of primary cardiomyocytes and determine its potential for cardiac tissue regeneration.

Magnetic assembly of biomimetic conductive muscle fiber constructs

Asel Primbetova, Andrew Spencer, Chu Hsiang Yu, Ryan Koppes and Nasim Annabi

The design of biomaterials with properties that can mimic native tissues as well as complex, multiscale architecture remains an ongoing challenge in tissue engineering. Skeletal muscle tissue is hierarchical, organized into aligned fascicles of myofibrils within multi-nucleated muscle cells capable of controlled contraction. The study presented here describes a method to fabricate and assemble a filamentous, muscle cell-laden hydrogel. Fibers were produced by wetspinning a mixture of GelMA and PEDOT:PSS into a solution of calcium chloride, followed by secondary photocrosslinking, producing conductive fibers laden viable C2C12 myoblasts. Fibers were subsequently sectioned and the ends were magnetized with a magnetically responsive radical molecule and organized into aligned multi-fiber constructs with rare earth magnets. The use of a magnetic radical enables subsequent neutralization of the radical via the addition of vitamin E, a well known biocompatible free radical scavenger. Application of electrical stimulation on the fibers is expected to promote three-dimensional cell alignment,

differentiation, and maturation into striated myofibrils. This work establishes a new method to generate conductive cell-laden fibers and direct cell spreading, proliferation and differentiation, which may be used to recapitulate the complex architecture of hierarchical, fibrous tissues such as ligaments, tendons, or peripheral nerves.

Engineering Antimicrobial and Osteoinductive Biomaterials for the Bone Tissue Regeneration

Ehsan Shirzaei Sani, Seyed Hossein Bassir, Roberto Portillo Lara, Giuseppe Intini and Nasim Annabi

The clinical management of infected bone defects and peri-implant diseases still constitute significant challenges in the orthopedic field. In these cases, the self-healing and regeneration capability of bone tissue can be significantly affected by the large size of bone defects, bacterial infection and inflammation. Conventional methods for treatment of infected bone defects include bone grafting materials such as autografts and allografts, combined with local administration of antibiotics. However, these treatment methods generally are time consuming and require complex surgical procedures. Therefore, we aimed to engineer new antimicrobial and osteoinductive hydrogel adhesives, which could prevent bacterial growth and promote bone regeneration for the clinical management of infected bone defects. The engineered hydrogels are based on gelatin methacryloyl, and can be rapidly crosslinked in situ using visible light. We incorporated the antimicrobial peptide (AMP) Tet213, as well as osteoinductive silicate nanoparticles (SNs) to the engineered hydrogels. Our in vitro characterization demonstrated that SN-loaded GelMA-AMP hydrogels exhibited high biocompatibility and high antimicrobial activity. Furthermore, the hydrogel precursor could be readily delivered and photocrosslinked in situ to seal calvarial bone defects in mice for up to 14 days. The engineered hydrogel adhesives constitute an effective strategy to prevent bacterial infection and promote bone regeneration.

Hybrid Modality of Protein Biopharmaceuticals: A Chemo-enzymatic Site-specific Bioconjugation Mediated by Transglutaminas

Shanshan Liu, Kevin Moulton and Zhaohui Sunny Zhou

Nature is not perfect: although natural peptides and proteins have myriad functions, much more is to be desired for improved properties. Traditional protein engineering relies on recombinant technology, but is limited to amino acid-based scaffold. Herein we present a new approach, hybrid modality engineering of peptides and proteins, in which chemo-enzymatic site-specific bioconjugation of non-peptide functional groups are introduced for new properties and activities. Bioconjugation can be achieved via a combination of enzymatic transformation with tailored substrate specificities and chemical reactions of functional selectivity. For example, existing glutamine-containing protein (including natural products) can be site-specifically modified using transglutaminase (TGase). In another example, N-terminus of peptides and proteins are selectively converted into ketones by pyridoxal-5-phosphate (PLP). Further derivatizations were achieved via orthogonal chemistry towards the newly introduced functional groups.
Our platform enables the introduction of a broad range of modifiers (e.g., drug conjugates, fluorophores, affinity tags and photo-caging groups) to existing proteins, including biotherapeutics. These modifications may confer improved or new activities, such as pharmacokinetic (PK) and pharmacodynamics (PD) profiles. The new derivatives can be used for therapeutic applications; it can also be used for biological applications, such as functional

probes and biomaterials.

Temperature Dependent Incorporation of Fluorescently labeled Type I Collagen Molecules into Native Bovine Sclera Fibrils

Seyed Mohammad Siadat and Jeffrey W. Ruberti

Understanding the self-assembly behavior of extracellular matrix (ECM) is essential to design effective reparative therapies for a number of connective tissue pathologies. However, the formation, growth, and remodeling of collagen fibrils – the main building block of ECM – are poorly understood. To improve our understanding of ECM assembly, we sought to develop a method to visualize individual collagen molecule dynamics during association with collagen fibrils while limiting the effect of the observation method on the assembly kinetics.
Type I telocollagen was tagged at the N-terminus α-amino group and at the lysine ε-amino group with Alexa Fluor 488 producing 2-10 fluorophores per molecule. The labelling only marginally affected self-assembly kinetics and fibril morphology (D-banding) as confirmed spectrophotometrically and by transmission electron microscopy, respectively.
Native collagen fibrils were trypsin extracted from 1-10 day old bovine sclera and stored in phosphate buffered saline at 4°C. The incorporation rate of labelled collagen into sclera fibrils was measured as 0.25, 0.5, and 0.88 Intensity per mm2 at 25, 30, and 35°C, respectively. The reaction of the monomers with the fibril surfaces had a kinetic signature similar to de novo collagen assembly and the results confirm that collagen molecules actively interact with native collagen fibril surfaces.

Effect of formulation variables on the preparation of intra-articular oxaceprol loaded nanoparticles

Emine Alarçin, Oya Kerimoğlu, Çağlar Demirbağ and Seher Karslı-Çeppioğlu

Oxaceprol has been used for the treatment of degenerative and inflammatory joint diseases such as osteoarthritis and rheumatoid arthritis for several years. In the present study, oxaceprol loaded poly(lactide-co-glycolide) (PLGA) biodegradable nanoparticles were developed for intra-articular injection, with the aim of improving its bioavailability and targeting drug to specific site. Oxaceprol loaded PLGA nanoparticles were prepared by the double emulsion (W1/O/W2) solvent evaporation method using various ratios of drug to polymer (1:2, 1:5, 1:10). The prepared nanoparticles were characterized on the basis of particle size, zeta potential, drug loading and release. Particles were found to be spherical with particle size ranges between 126-352 nm. Entrapment efficiency was between 51% and 73%. Oxaceprol released from the nanoparticles up to 30 days. Oxaceprol loaded PLGA nanoparticles could be used for further in vivo experiments.

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The Effect of Epoxide Functional Group Content on Cell Viability and Behavior

Meryem Pehlivaner and Adam Ekenseair

In this study, pendant epoxide rings were introduced into a poly(N-isopropylacrylamide) (NiPAAm)-based in situ forming hydrogel via copolymerization with glycidyl methacrylate (GMA), and the effect of epoxide ring content on the viability and behavior of fibroblasts was investigated by DNA, Alamar Blue, and Live/Dead assays and immunofluorescent staining. The impact of GMA incorporation on hydrogel physical and chemical properties was explored via 1H-NMR, DSC, and swelling tests. Copolymerization with GMA resulted in decreased swelling ratios and lower critical solution temperatures (LCSTs) of the hydrogel due to the increase in overall hydrophobicity of the polymer network. Cells encapsulated in GMA-modified hydrogels showed higher viability and proliferation compared to cells encapsulated in base pNiPAAm hydrogels. Moreover, incorporation of epoxide groups was found to improve biocompatibility of the hydrogels through chemical binding of serum proteins onto the polymer network structure, which led to increased cell attachment, proliferation, and cluster formation. These results demonstrated that free epoxide addition could be a powerful tool to alter cell behavior through controlling material chemistry with applications in tissue engineering and regenerative medicine.

One-pot Growth of Silver Nanoparticles on Self-Assembled PEGylated Rosette Nanotubes: Design, Preparation, and Antimicrobial Properties

Yiwen Fan and Hicham Fenniri

With the emerging antibiotics resistance problems for the treatment of bacterial infectious diseases, researchers are constantly seeking alternative treatment modalities. For instance, silver nanoparticles (AgNPs) have been widely utilized as effective antimicrobials due to their long-term antibacterial activity, low cytotoxicity and lower bacterial resistance. Recent studies have demonstrated that the bactericidal effects are strongly dependent on the size and dispersion of AgNPs. Therefore, preparing AgNPs with defined dimensions is an important aim to enhance the reactivity of nanoparticles with bacteria. We report here that self-assembled supramolecular rosette nanotubes (RNTs) consisting polyethylene glycol spontaneously reduce the silver ions and subsequently generate stable monodisperse AgNPs in aqueous solution. We have also found that the RNTs/AgNPs system has antimicrobial activity and significantly low cytotoxicity toward human dermal fibroblast cells.

Molecular Modeling of Fluorescent Organic Nanotubes

Arthur Gonzales, Belete Adefris Legesse, Takeshi Yamazaki and Hicham Fenniri

Rosette nanotubes (RNTs) are soft organic nanomaterials self-assembled guanine-cytosine (GΛC) hybrid building blocks. The material's substantial design flexibility is partly attributed to their diverse surface functionalization and a chemically/physically tunable channel for guest molecule loading. With novel applications in mind, a new tricyclic GΛC motif was synthesized self-assemble into fluorescent RNT in solution. In this work, molecular modeling techniques were applied to aid in the characterization of this new RNT and to predict its structure and self-assembly.

MD and the 3-dimensional reference interaction site model (3-D-RISM) theory were applied to

determine the most stable conformations and thermodynamics of the RNT, and to simulate the growth of the RNT in silico.
The results suggest that this tricyclic GΛC motif can either form helical coils (HC) or ring stacks (RS). The thermodynamics analysis of both conformations suggests that RS is slightly more stable than HC. The self-assembly studies showed that π-π interactions drive the formation of aggregates at high temperatures. And for the first time, the growth of the RNT was observed in silico with a stable helical coil template driving the alignment of free GΛC motifs to conform to the rest of the RNT. Experiments are underway to verify our predictions.

Design and fabrication of a microfluidic-based liver-on-a-chip platform

Fatemeh Sharifi, Ozlem Yesil Celiktas, Bahar Firoozabadi, Yu Shrike Zhang and Ali Khademhosseini

Advances in tissue engineering, biomaterials and micro-fabrication have led to the rapid growth in organ-on-chip platforms which can mimic organs physiology in more similar conditions to those exist in human body. Liver is one of the fundamental organs which has a major role in body metabolisms and detoxification. Accordingly, liver attracts the attention of researchers in two major fields of diseases related to organ itself and those caused by detoxification. In this research, the aim is to design and fabricate a microfluidic device in order to investigate a hepatic disease. The design of the chip is optimized with the software using finite element method. Shear stress and flow rate inside the chip was calculated based on the threshold of average blood velocity in the liver sinusoid. Results obtained from static cultures showed that cells have very good viability up to three weeks. As a next step, hepatocytes will be cultured inside the chip and simulation will be validated under dynamic conditions. Cell viabilities will be monitored day by day. Having obtained the suitable viability duration, the chip will be ready for modeling various diseases and testing different drugs.