BIOMIMESIS IN DRUG DELIVERY

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INTRODUCTION

This short review highlights recent activities in the field of biomimetic systems and their application in intelligent therapeutics and drug delivery. Biomimetic materials and systems are exceptional candidates for various controlled drug delivery applications and have enormous potential in medicine for the treatment of disease **[1]**. Biomimesis is the process of coordinating molecular recognition and interactions to design biological, biohybrid, and artificial materials that can be structurally similar to and/or function in similar ways as biological structures. This includes topics with emphasis in therapeutic agent delivery which involve novel materials consisting of (i) natural biological molecules such as proteins, oligonucleotides and polynucleotides, and/or unnatural biomolecules that have been assembled/synthesized by biological systems; (ii) hybrid structures of synthetic (e.g., polymeric chains, metal particles, etc.) and natural biological molecules (i.e., conjugated biomaterials); or (iii) materials consisting of man-made and in-vitro building blocks, such as synthetic polymers, unnatural amino acids, aptamers, α-helical coiled coils, materials from configurational biomimesis or molecular imprinting methods, polymerosomes, micelles, etc.

BIOMIMETIC SYSTEMS

To clarify the context and definition of biomimetic systems, Figure 1 describes the intersection of these systems and their sub-classifications. Biomimetic systems may be classified as (i) biological, (ii) biohybrid, and (iii) synthetic structures. To create biomimetic systems, one can utilize and mimic biological processes and interactions where the underlying molecular principles are understood.

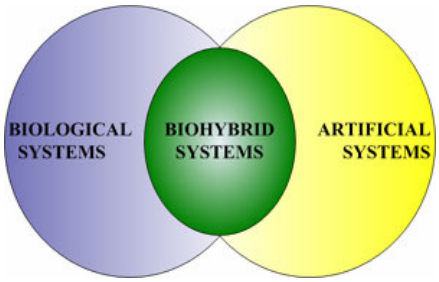

Figure 1. Classification of Biomimetic Systems. Biomimetic systems can be classified as biological, synthetic, or biohybrid. Biohybrid systems reside at the intersection of artificial and biological systems.

Biological structures denote materials consisting of natural biological molecules such as proteins, DNA, RNA, and/or unnatural biomolecules that have been assembled/synthesized by biological systems, such as unnatural amino acids prepared via genetic engineering methods. Biohybrid structures are comprised of materials that combine synthetic structures (e.g., polymeric chains, metal particles, etc.) and natural biological molecules. Synthetic structures represent materials based on man-made building blocks, such as synthetic polymers and peptides (i.e., prepared in-vitro by solid phase peptide synthesis).

The recent 2004 Gordon Research Conference entitled Drug Carriers in Medicine and Biology discussed exciting topics of research involving drug delivery which included biomimetic strategies **[2]**. It is evident that the study of biology and biological processes is creating novel and successful strategies for a number of drugs and carriers in terms of delivery issues such as persistence, biodistribution, penetration, metabolism, systemic clearance, as well as imaging to treat a number of disease or abnormal cellular states. Because controlled drug delivery systems can improve safety, efficacy, convenience, and patient compliance, novel delivery methods are a major focus of pharmaceutical companies.

BIOHYBRID STRUCTURES

Biologically active molecules can be incorporated into polymer networks (e.g. physically or chemically entrapped) to produce conjugated biomaterials, which have applicability in targeted and controlled delivery of therapeutic compounds **[3]**. In a recent review **[4]**, the field of stimuli-responsive polymers and their bioconjugates is detailed, and their potential applications in drug delivery are highlighted. A prime example of biohybrid targeting is via glycoprotein mimics that function as a synthetic multivalent ligand able to facilitate site-specific drug delivery **[5]**. Also, there has been progress on receptor-mediated endocytosis where therapeutics are targeted to the internal machinery of the cell. For example, Lackey et al. **[6]** demonstrated a functional biomimetic system which involved the use of a pH-responsive polymer as a membrane-disrupting agent that aided the transport of antibody conjugates from the endosome to the cytoplasm.

Recently, Daunert and coworkers **[7]** have produced stimuli–responsive hybrid materials consisting of hydrogels and genetically engineered protein. The stimuli-responsive hydrogel exhibited three specific swelling stages induced by conformational changes and binding affinities of the protein in response to various ligands. The authors showed gating and transport of biomolecules across a polymer network, demonstrating a large potential in microfluidics and drug delivery.

Wang and coworkers **[8]** have prepared stimuli-sensitive hydrogels with well-defined volume transitions based on pH and temperature changes in the environment which are copolymers of metal-chelating hydrophilic monomers and thermo-sensitive coiled-coils. The coiled-coil components are part of the sequence of the microtubule-motor protein, Kinesin, which induces cytoskeletal gliding through ATP hydrolysis.

Tirrell and coworkers **[9]** have prepared thermally reversible hydrogels containing polyethylene glycol and proteins obtained from chimeric genes. The switching of these gels can be controlled by crafting custom sequences, which highlighs great potential in controlled release.

Using oligonucleotide bridges, dendrimers can be used as imaging and therapeutic agents. Baker and collaborators **[10]** have designed a DNA-driven dendrimer assembly. Dendrimers conjugated to different biofunctional moieties were linked together using complementary DNA oligonucleotides which produced clustered molecules which targeted cancer cells that overexpress the high-affinity folate receptor.

In the early 1990's, pioneering work demonstrated that large libraries of synthetic RNA molecules could be screened in vitro to identify and capture ligands with high binding selectivity and affinity for a specific target **[11,12]**. These ligands, termed aptamers, have proven to be valuable therapeutic agents with some enhanced properties relative to antibodies **[13]**. These structures can be easily tagged with proteins **[14]**, fluorophores and antibiotics, and can be chemically ligated with cytotoxic agents and radionucleides for therapy and diagnosis of aberrant cells **[15]**. Perhaps the greatest implications of aptamers will be felt in gene regulation and ribozyme function where the natural aptamer domain can be mutated for regulating protein expression.

SYNTHETIC STRUCTURES

Synthetic structures also have major roles in biomimetic design. Today, polymer scientists and engineers can synthesize a wide variety of macromolecules with precise control of their molecular structure and physiochemical properties. Synthetic biological building blocks can take advantage of flexibility in design and use various motifs that have programmable

conformational states, which can produce novel carriers for drug delivery, designed therapeutics, and sensing.

Alpha-helical coiled-coils are ubiquitous structural motifs in the proteome, and abound in G proteins, motor proteins, chaperones, transcription factors, cytoskeletal proteins, and viral fusion proteins, etc. The terminals of coiled-coils are quite amenable to modification, and have been used for solubilization during protein crystallization. Kim and collaborators used the Fos/Jun Leucine zipper pair to assemble α and β chains of DQ2, a class II MHC molecule, which presents gliadin epitopes to CD4 single-positive T-cells. The crystal structure obtained can be used to design therapeutics for curing Celiac Disease, a chronic autoimmune digestive disease due to the ingestion of gluten (i.e., a protein found in wheat, rye, and barley) that damages the duodenum and limits nutrient absorption **[16]**.

Coiled coils have also been used to design more stable antigen-binding sites. Arndt et al have stabilized the traditionally unstable Fv (Fragment Variable) antibodies using the coiled-coil WinZip A2B1 **[17]**. In general, coiled coils have well-defined structure and properties and have demonstrated great potential as peptide based drug delivery vehicles.

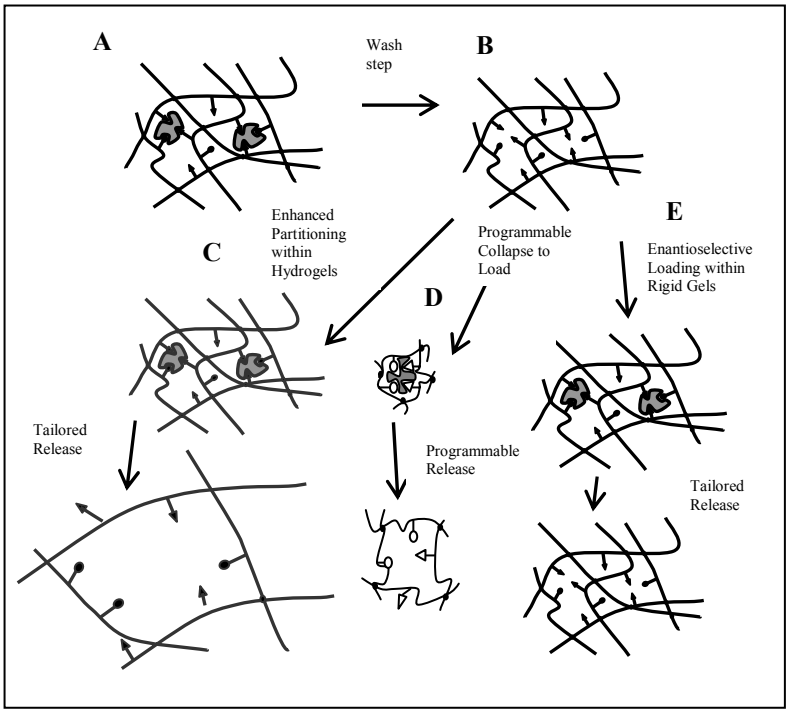

FIGURE 2: Configurational Biomimesis in the Production of Recognitive Networks. A: The formation of the network (imprinting process). **B:** Wash step where original template is removed. **C:** Networks can be designed to increase partitioning or uptake within hydrogels and swellable materials. **D.** Programmable collapse can lead to loading as well as programmable release. Modulation of binding events can occur via pH, temperature, or other stimuli sensitive mechanisms, etc. **E.** Within higher crosslinked matrices, the affinity can alter the release profile and extend release. It can also lead to enantioselective loading of mixtures.

By designing the molecular functionality and structure of a synthetic polymer network to mimic biological systems, three-dimensional network structures exhibiting structural similarities can be created with tailorable drug delivery properties **[18,19]**. In essence, biomimetic assemblies provide a molecular platform that mimic biological systems with applicability in encapsulating and delivering drug molecules.

Synthetic networks that can be designed to recognize and bind biologically significant molecules can be prepared using template-mediated polymerization techniques (e.g. molecular imprinting). Only recently, researchers have applied imprinting methodology in the design of polymers for recognition of biologically significant molecules and application as controlled drug delivery systems **[20-23]**. Of particular interest, a recent review **[24]** highlights the wide applicability of these polymer systems in controlled drug delivery such as sustained release, enhanced loading capacity, and enantioselective loading or release are discussed (Figure 2). The review also discusses the future of designed recognition, configurational biomimesis within polymeric gels, problems to be solved in the design of synthetic recognition-

based networks, and recent efforts toward integrating imprinted polymers in controlled drug delivery systems and sensing devices.

Strict control of molecular structure, which is an inherent advantage of synthetic systems, is of high importance in the design of materials with reproducible and consistent properties. Deming has pioneered the use of transition metals as initiators for the ring-opening polymerization of N-carboxyanhydrides. The use of organonickel as initiator effectively suppresses the chain termination and transfer reactions, giving block copolypeptides of highly controlled chain lengths which produce novel polypeptide hydrogels **[25]**. Variation of copolymer chain length and composition produced various hydrogels, characterized by confocal microscopy and TEM. At the molecular level, familiar conformations of poly peptides were obtained with poly-(L) giving α-helices and poly-(V) giving β-sheets **[26]**.

Maysinger and coworkers have synthesized micelles from poly (caprolactone)-b-poly (ethylene oxide), for the delivery of hydrophobic drugs **[27]**. The block copolymer was tagged with a fluorophore to study the intracellular distribution of the drug. Confocal microscopy demonstrated that they were endocytosed and localized within various organelles such as the mitochondria and Golgi apparatus, but not the nucleus. It was also shown that a non-covalently bound agent to the micelles would be internalized more than the agent alone. Subsequently, the uptake of these micelles in pluripotent P19 mouse embryonal carcinoma cells was studied **[28]**. It was shown that extent of internalization was markedly reduced at low temperatures and pH, and in the presence of drugs like chlorpromazine. Such micelles could be used in delivery of androgens and estrogens during hormonal imbalances.

BIOLOGICAL STRUCTURES

Biological structures, by themselves are leading to a number of advances. Ghadiri et al. **[29,30]** synthesized nanotubes from the ring arrangement of cyclic peptides, which show antimicrobial **[31]** and ion channel properties **[32]**. A recent success includes the demonstration that cyclic peptides, when used as adaptors in β-barrel shaped α-hemolysin, can be used for single channel detection and quantification of molecules.

Wu et al. **[33]** utilized a single-chain, single-gene approach to produce genetically engineered antibodies (i.e., chimeric structures) to provide immunotherapeutic treatment of cancer. Novel fragmented antibody constructs such as single chain Fv (scFv) with human IgG1 hinge and Fc regions (scFv-Fc dimers) are designed to produce molecules for the delivery of radionucleotides to tumors with reduced immunogenicity, increased circulation life, and gained effector functions **[34]**.

Over the last twenty years, bioadhesion has been a focus area of drug delivery research **[35-38]**, allowing for enhanced control over drug delivery (e.g., site-specific adhesion and increased residence time). Recently, there has been a particular focus on integrins, which are cellular adhesion molecules **[39]**. Yu et al. **[40]** have created peptides for studying interactions between amino acids, for testing them as biomaterial coatings and for use as drug delivery devices. Melanoma cells spread indiscriminately on carboxyl-coupled RGD amphiphiles and did not spread on amino-coupled RGD amphiphiles, showing that they are effective cellular recognition agents.

One of the biggest revolutions in genetics is RNA interference, which holds immense potential in gene annotation and drug therapy. As important as it is to find quick and efficacious methods of therapy, it is also essential for us to scrutinize development biology to obtain details about differentiation. Lum et al **[41]** used siRNA-based screening to find key genes involved in Hedgehog signaling in *Drosophila*, which is responsible for cellular differentiation during early embryonic development. Unrestrained Hedgehog signaling leads to cancer.

Hedgehog pathway is a series of repressive steps wherein Hedgehog protein (*Hh*) binds to the Patched protein (*Ptc*), which gets inactivated. Thus, Ptc can no longer suppress Smoothened protein (*Smo*), which has a suppressing action on the Cytoplasmic Complex (*CC*). The *CC* is now incapable of suppressing the transcription factor Cubitus Interruptus (*CI*). The group used luciferase luminescence assays and created a genetic map which included known genes and four new genes- Dally-like protein (Dlp), CK1α, caupolican and CG9211. Dlp was shown to be required for reception of the Hh signal. It was proved to be upstream of *Ptc* when its requirement was voided on RNAi of *Ptc.* CK1α and caupolican were shown to be regulators. Most importantly, the authors found homologous murine and human genes which have developmental defects when their Hedgehog signaling goes awry. The homologue of CK1α could be implicated in colon cancer, basal cell carcinoma, and medulloblastoma. In a surprising lead, the group has discovered that reckless Hedgehog signaling is responsible for the maintenance of metastasis in prostate cancer cell lines. In particular, using cyclopamine, a drug which target *Smo*, the group showed that the cell lines stopped differentiating **[42]**. This is logical as active *CC* can then suppress *CI*. Even more interestingly, normal prostate tissue does not contain any *Smo* mRNA, but cancerous tissue has increased levels with increasing activity, leading to the conclusion that *Smo* expression is the trigger for Hedgehog signaling. Future remedies for prostate cancer may be cyclopamine-based therapeutics. This is an excellent example of RNAi based systematic identification and annotation of genes, leading to clinical strategies.

CONCLUSIONS

In the last two decades, there have been significant developments in advanced drug delivery formulations, facilitating the creation of systems that do not simply release a drug at a specific rate but release the drug in a way that the designer has designed. The role of incorporating biological structures, cues, or structural homology has accelerated these efforts. Only recently, the potential of biomimetic systems as intelligent biomaterials and in therapeutic applications has begun to be realized.

Of continued importance to biomimetic developments will be rational design of the constituent chemistry and subsequent linking of various synthetic and biological counterparts. If certain strategies to produce or obtain biological structures are insurmountable and/or inefficient, researchers have used a number ingeniously crafted methods based on fundamental biology to obtain novel biological or hybrid structures. However, the challenge lies in building precise structural alignment as well as tunable or switchable functionality into materials as well as optimizing delivery profiles and release constraints.

The future holds much promise for systems based on biology, and we will begin to see the tailoring of therapeutics based on an individual's genetic predisposition toward disease (i.e., individualized therapeutics).

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