Bioactive molecules: current trends in discovery, synthesis, delivery and testing

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Abstract: Important bioactive molecules are molecules that are pharmacologically active derived from natural sources and through chemical synthesis. Over the years many of such molecules have been discovered through bioprospective endeavours. The discovery of taxol from the pacific yew tree bark that has the ability in stabilising cellular microtubules represents one of the hallmarks of success of such endeavours. In recent years, the discovery process has been aided by the rapid development of techniques and technologies in chemistry and biotechnology. The progress in advanced genetics and computational biology has also transformed the way hypotheses are formulated as well as the strategies for drug discovery. Of equal importance is the use of advanced drug delivery vehicles in enhancing the efficacy and bioavailability of bioactive molecules. The availability of suitable animal models for testing and validation is yet another major determinant in increasing the prospect for clinical trials of bioactive molecules.

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Introduction

The search for bioactive molecules has been an ongoing task as long as we believe that pharmacologically active molecules are the answers for various human ailments and diseases. The development in the field has seen numerous advancements both in its science as well as techniques and enabling technologies that have been driving the pace of discovery and synthesis. The recent progresses in bioinformatics, chemical genetics and computational biology have changed the way and direction in which bioactive molecules are classified and discovered. Advances in these areas together with combinatorial chemistry, have transformed the landscape of modern scientific discovery of bioactive molecules, thus making their availability to consumers more certain. This review will briefly revisit the chemistry of what makes molecules biologically active. Thereafter, the currently advanced technology for the identification, detection, synthesis and delivery of pharmacologically active molecules will be discussed. Finally, an overview of a suitable and reliable animal model for the high throughput screening of bioactive molecules is presented.

The Chemistry of Bioactive Molecules

The natural world is full of plant and other material resources in which bioactive molecules are obtained. Sources of these bioactive materials include from plant and animal sources, marine and also those that are semi-synthetically derived. The table below lists a fraction of those bioactive molecules that have been identified and used in various ways:

Table 1: Bioactive molecules and their biological properties.

Chemicals	Benefits	References
Tea phenolics	Antioxidant effects	1
Quercetin	Proliferation inhibition ability through tyrosine kinase	2
Beta-carotene and lycopene	Prostate cancer prevention	3
Rutin	Antioxidant	4
Isoflavones	Prostate cancer prevention	5
Ascorbic acid	Prevention of advanced malignancy	6
Epigallocathecin gallate	Anticancer	7
Curcumin	Anticancer and anti-inflammatory	8
Ellagic acid (Pomegranate juice)	Prostate cancer	9
Oroanthocyanidin (Cranberry juice)	Urinary tract infections	10
Quercetin and apigenin	Cardiovascular risk	11
Omega-3 and -6 fatty acids, green tea, licorice, quercetin, shark cartilage, curcumin	Anticancer	12, 13
Podophyllotoxin (Mayapple)	Breast cancer	14

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In addition to those identified in Table 1, IMU researchers have also numerous bioactive molecules whose pharmacological role had been identified through chance observations, experiments and ethnopharmacological information handed down through the ages. The sources of these bioactive compounds are numerous, and usually these are plant-based sources. Some of these compounds include cardiac glycosides classes, in which an important representative is digoxin. The anticholinergic class of drugs are another example where atropine is a representative. Unlike digoxin, which had not been improved upon by synthetic substitutes, the anticholinergic drugs have been enriched by a wide representation of substitutes, which have improved properties compared to the original alkaloid.

Cocaine is another alkaloid that is famously obtained from the coca plant and a lot of studies have been conducted on this parent compound in order to reduce its addictive properties. Much of the local anaesthetics available these days are a result of the manipulation of the complex structure of cocaine. Besides cocaine, there are also the classes of antibiotics from the penicillins, cephalosporins, tetracyclins and actinomycins which are all obtained from microorganisms. These antibiotics have been explored extensively through the years and have provided mankind with an armoury of weapons against infectious diseases. Although these antibiotics have had a long history and have been used extensively, research is still being done to modify their structure in an attempt to improve the activity or to mitigate against acquired resistance by microorganisms.

Besides bioactive molecules which are sourced from the natural world, much research attention is spent on hormones and neurotransmitters. Synthetic modification of the parent or original compound is done with the aim of getting a specific activity or an improved antagonistic response. Some of these bioactive molecules include acetylcholine, histamine, cortisone/hydrocortisone, and phenoxyacetic acids.

In addition to the search for modified bioactive molecules with selective activity or those with a particular antagonistic response, there is extensive work done on bioactive molecules with selective toxicity to specific organisms, target organs or receptors. Examples of selective toxicity are the trimethoprim/methotrexate; both drugs target dihydrofolate reductase, but the former targets bacterial dihydrofolate reductase whereas the latter targets mammalian enzymes and hence can be used in cancer chemotherapy.

Compound Library Design in Drug Discovery

Compound and chemical library is a collection of real and/or virtual chemical compounds. The real compound library contains real chemical compounds with associated information such as the chemical structure, purity, quantity and physicochemical characteristics of the compound. The virtual compound library consists of 2-dimensional (2D) or 3-dimensional (3D) representations of chemical compounds that are used for diverse purposes using computational methods.

The logical compound library designs of both types are often similar to one another. The experimental methods (for real compound libraries) and computational methods (for virtual compound libraries) are often complementing one another in the drug discovery development process.

Compound libraries are mainly used for high-throughput screening (HTS) in drug discovery. HTS is a process consisting of activity testing of a large number of chemical compounds against few assays and/or targets. In drug discovery campaigns, both real and virtual compound libraries are commonly run in parallel and the results of one compared to another. The main aim in designing a compound library is the discovery of promising novel drug leads. Two decades ago, the compound libraries typically consisted of enormous numbers of small-molecule chemical structures; by contrast, the current compound library design is more advanced and focuses on the approaches used for selecting compound membership.

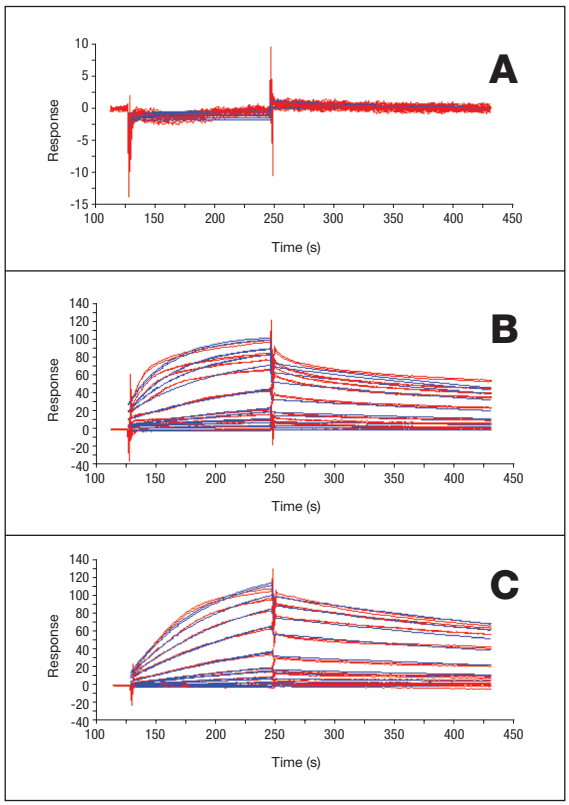
The selection of compounds is frequently based on two widely used library design strategies: diversity oriented design and target oriented design. The objective of diversity oriented design strategy is to create libraries with an extremely diverse set of chemical compounds built for example, on skeletal diversity – a strategy where the scaffold elements of chemical compounds are chosen to maximise their variation in 3D structure and physicochemical properties. A physicochemical property diversity method include hydrogen bond donors/acceptors, polarisable groups, charge distributions, hydrophobic and hydrophilic fragments, rotatable bonds and several other properties. Statistical techniques, such as cluster and principal component analysis are used to measure the diversity of the libraries. In contrast to diversity oriented design, the pursuit of target-oriented design is to generate libraries that are dedicated to specific chemotypes, molecular species, or classes of compounds. Target-oriented compound library design results in focused libraries with a limited number of well-defined structures. To generate focused libraries shape, electrostatics, pharmacophore models, Quantitative Structure Activity Relationship (QSAR) models, molecular descriptors and target binding site are used. In general, it is easier to design targeted libraries than designing diverse chemical libraries.

Advanced Method of Detection: Surface Plasmon Resonance

Sensitive detection of ultra-low concentration of biomolecules is of great importance in bioactive molecules research and has numerous applications. In the past, the use of conventional radio-labelling was a much sought-after technique but has now been superseded by label-free quick detection system using the advanced surface plasmon resonance (SPR). SPR is a biosensing technique in which biomolecules are capable of binding to specific analytes or ligands. The initial step is the immobilisation of one component to a metallic (gold) film. Light (laser) from the opposite side of the film is used to excite the surface plasmons. Plasmons refer to the oscillations of free electrons propagating along the film's surface. The refractive index of light reflecting off this surface is measured and compared to the incidence (on the uncoated) refractive index dynamically. The binding of immobilised biomolecules to their ligands, will alter the surface plasmons that are sensitised in the opposite side of the film, in a manner that is directly proportional to the change in bound, or adsorbed, mass (Figure 1). The strength of binding can also be found based on its disassociation potential.

Recently, SPR techniques have been improvised and integrated with the existing techniques described above. Studies involving the protein–protein and lipid–protein interactions that modulate affinity with less than 1.0 μM, can be studied using SPR, complex surface molecules like integrin and its characteristic binding at the cell surface are studied for it potential binding properties with its specific targets.[15] Another improvisation is the localised SPR (lSPR), which is a technique based on SPR. This inprovisation allows greater signal detection and has been used for the detection of biomarkers in blood of patients with ovarian cancer at the picomolar (pM) range.[16] Another method was used to measure synaptic T-cell receptors binding to MHC directly by employing SPR and microcalorimetry.[17] In other areas, SPR has also been employed to investigate the binding of biomolecules to tumour-specific receptors, in the detection of biomarkers in pre-term babies as well as infectious agents.[18-25] All these underscore the importance of SPR as an indispensable tool for studying bimolecular interactions.

Figure 1: Nonspecific protein fails to bind to the heparin chip A. Band C had the domains for Heparan binding[16]



Advanced Delivery Methods of Bioactive Molecules

The effectiveness of a bioactive molecule is enhanced when a suitable and optimal delivery system is available. Much of these delivery systems have been utilised for the delivery of molecules derived from biological origin that exhibit diverse properties that include anti-oxidants, immune modulatory compounds, antimicrobials and prebiotics.

Dendrimers

Dendrimers are highly branched, monodisperse molecules having a large number of end groups and can be synthesized in either a divergent or convergent manner. They adopt a globular shape and are polyvalent, two features that are often seen in naturally occurring systems. Therefore, dendrimers are regarded as promising candidates for use in biomedical applications.[26] Polyester-based dendrimers are biodegradable, whereas traditional dendrimers like poly (amidoamine) (PAMAM) and poly (propylene imine) (PPI) dendrimers show much lower biodegradability under physiological conditions.

Dendrimers consist of a group of branched materials with diverse functions that can be constructed with defined architectural and chemical structures. When decorated with bioactive ligands made of peptides and saccharides through peripheral chemical groups, dendrimer conjugates are turned into nanomaterials possessing attractive binding properties with the cognate receptors. At the cellular level, bioactive dendrimer

conjugates can interact with cells with avidity and selectivity, and this function has particularly stimulated interests in investigating the targeting potential of dendrimer materials for the design of drug delivery systems. In addition, bioactive dendrimer conjugates have so far been studied for their versatile capabilities to enhance stability, solubility and absorption of various types of therapeutics.[27] In recent years, dendritic scaffolds have been explored for their use in biomedical applications such as drug delivery, synthetic vaccination, magnetic resonance imaging or tissue engineering.

Dendrimers can be used as potential carriers for (targeted) drug delivery in at least two ways. Biologically active molecules can be either physically entrapped inside the dendritic structure, or covalently attached to the periphery of the dendrimer to yield dendrimer-drug conjugates. The dendritic cavity of an appropriately designed dendritic structure could be used for trapping drugs with the possibility of subsequent controlled release.[28]

Hydrogels

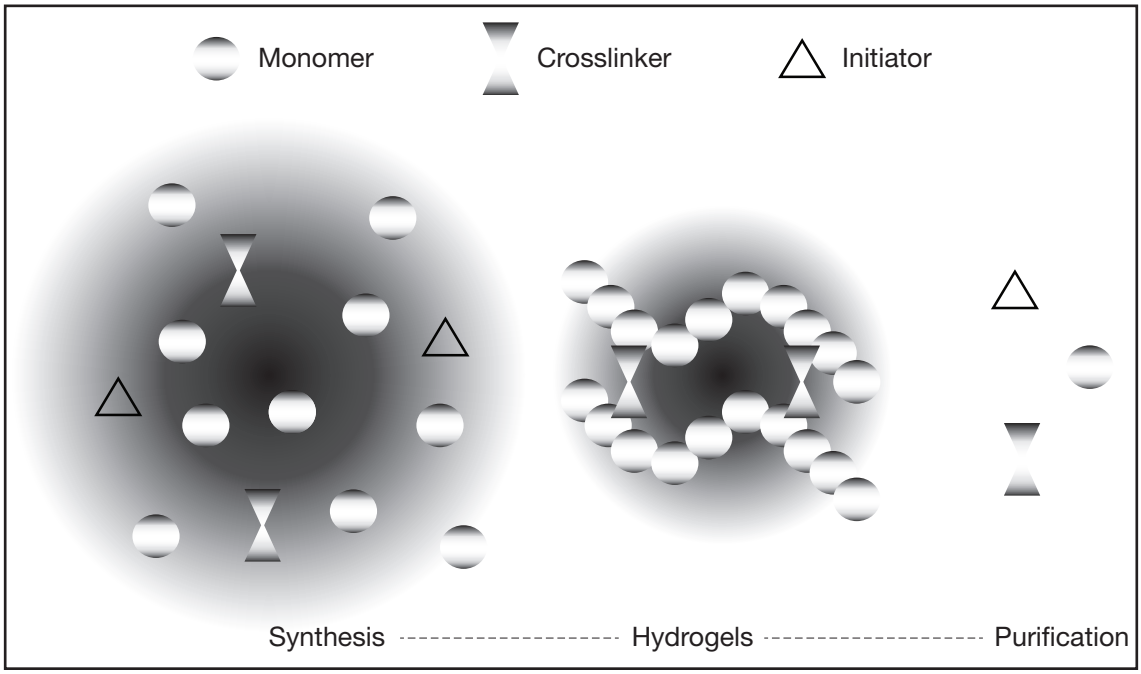
Hydrogels are cross-linked polymeric networks with hydrophilic nature, which have the capability to hold liquid portions i.e. water within the spaces available among the polymeric chains due to the presence of hydrophilic, amino, carboxyl and hydroxyl groups, in the polymer chains. The hydrogels have been widely used in several biomedical applications, viz. cell carriers, drug delivery and/or entrapment, and tissue targeting.[29]

Hydrogels can be classified into two groups i.e. permanent hydrogels and physical hydrogels based upon their nature of the crosslinking reaction. If the crosslinking reaction involves formation of covalent bonds, then the hydrogels are termed as permanent hydrogels.[30] (Eg. poly (methyl methacrylate), poly (hydroxyethyl methacrylate)). Physical hydrogels are formed due to the physical interactions, viz. molecular entanglement, ionic interaction and hydrogen bonding, among the polymeric chains[31] (polyvinyl alcohol-glycine hydrogels, gelatin gels and agar-agar gels).[37]

Hydrogel synthesis is made up of three integral parts i.e. monomer, initiator, and cross linker. To control the heat of polymerisation and the final hydrogel properties, diluents such as water or other aqueous solutions can be used. After the synthesis, the hydrogel mass needs to be washed to remove impurities left from the synthesis process. These include non-reacted monomers, initiators, cross linkers, as well as unwanted products produced via side reactions (Figure 2). The hydrogel properties can be modulated by varying the synthetic factors, such as reaction vessel, time, temperature, monomer type, type of cross linker, cross linker-to-monomer ratio, monomer concentration, and type and amount of initiator.[32]

Hoffman *et al*[29] reported that the diffusional exponent (n) can give relative information about the release behaviour of the bioactive agent from the hydrogel systems. He classified delivery systems based on the release profile of the bioactive agent from the system which include the Fickian system.

Figure 2: Synthesis of hydrogels[33]



Microspheres

Biodegradable polymer microspheres are one of the most common techniques for controlled release drug delivery and hold several advantages. Microspheres can encapsulate the drugs including small molecules, proteins, and nucleic acids and are easily administered through a syringe needle. They are generally biocompatible, can provide high bioavailability, and are capable of sustained release for long periods of time.[34] Microspheres pose a great opportunity to be used as reservoirs for drugs and carriers of bioactive molecules on their surface.

Microsphere drug delivery systems have been developed by a variety of techniques including combinations of phase separation or precipitation, emulsion/solvent evaporation, and/or spraying methods.[35,36] Drugs may be incorporated into the particles in several different ways depending on the nature of the drug. Hydrophobic active materials may be co-dissolved with the polymer in a solvent such as ethyl acetate or methylene chloride. Hydrophilic active materials, including proteins, may be suspended in the organic phase as a finely ground dry powder. Alternatively, an aqueous solution of a hydrophilic drug/active material may be mixed with the organic polymer solution to form a water-in-oil emulsion. The emulsion-solvent extraction/evaporation methods are most commonly used, especially at the lab scale. In these processes, a solution containing the polymer (and possibly the drug to be encapsulated) is emulsified in a non-solvent phase (the continuous phase) containing a stabiliser. The emulsion can be prepared with any of a variety of physical methods including homogenisation and sonication.[37]

The use of new, degradable polymers that have predictable degradation kinetics *in vivo* will be of great use for controlled drug delivery, especially of large bioactive molecules. Small sized drugs are easily

incorporated in microspheres, although the loading efficiency is still variable. Large sized bioactive molecules are difficult to soak into microspheres, and when incorporated into a microsphere, have difficulty diffusing out. Therefore, controlled degradation of microspheres, preferably by surface erosion, instead of the bulk erosion of poly (lactic-co-glycolic acid) (PLGA) spheres, will be required to deliver such drugs in a controlled and orderly fashion.

DUROS Technology

DUROS (Alza Corporation, CA, USA) is one type of implant technology, which provides an alternative for the delivery of a wide range of therapeutic compounds, including peptides, proteins, and other bioactive macromolecules.[38] These implants are miniature titanium cylinders designed to provide continuous osmotically driven delivery of drugs within the body for up to one year. Following implantation, DUROS implants enable continuous, precise delivery of the therapeutic compound at rates as low as 1% of a drop of water per day. The cylinder is manufactured from titanium because of the material's tolerability to human tissue and its long use in medical devices such as implantable defibrillators and joint replacements. The cylinder protects therapeutic agents from degradation in the body and enables a drug to remain stable for extended periods of time.

ProLease and Medisorb

ProLease and Medisorb are two patented and proprietary processes for creating injectable sustained release products lasting from days to months. ProLease is specifically designed for complex and fragile bioactive molecules such as proteins, and Medisorb is designed for traditional small molecules and peptides. Both technologies are microsphere based delivery systems composed of the desired bioactive molecule incorporated into a matrix of poly-(DL-lactide-coglycolide), a common biodegradable medical polymer. Microspheres are packaged in vials as a dry free-flowing powder. Before administration the microspheres are suspended in an aqueous vehicle and administered by subcutaneous or intramuscular injection. Release profiles can be adjusted by manipulation of formulation parameters and by control of the fabrication process.[38]

Liposomal Technologies

A liposome is a tiny vesicle consisting of an aqueous core entrapped within one or more natural phospholipids forming closed bilayer structures. Liposomes have been extensively recognized as a drug carrier system, due to their unique characteristics such as ability to incorporate hydrophilic and hydrophobic drugs, good biocompatibility, less toxicity, lack of immune system activation and targeted delivery of bioactive compounds to the particular site of action.[39,40]

Even though liposomes have been successfully proven as promising carriers for therapeutically active compounds, some of the drawbacks for liposomes used in pharmaceuticals are the rapid degradation due to the reticuloendothelial system (RES) and inability to achieve sustained drug delivery over a prolonged period of time. To overcome this problem, two polymeric approaches have been suggested as follows: 1) modification of the surface of liposomes with hydrophilic polymers such polyethylene glycol (PEG) and 2) integration of the pre-encapsulated drug-loaded liposomes within depot polymer-based systems.[41]

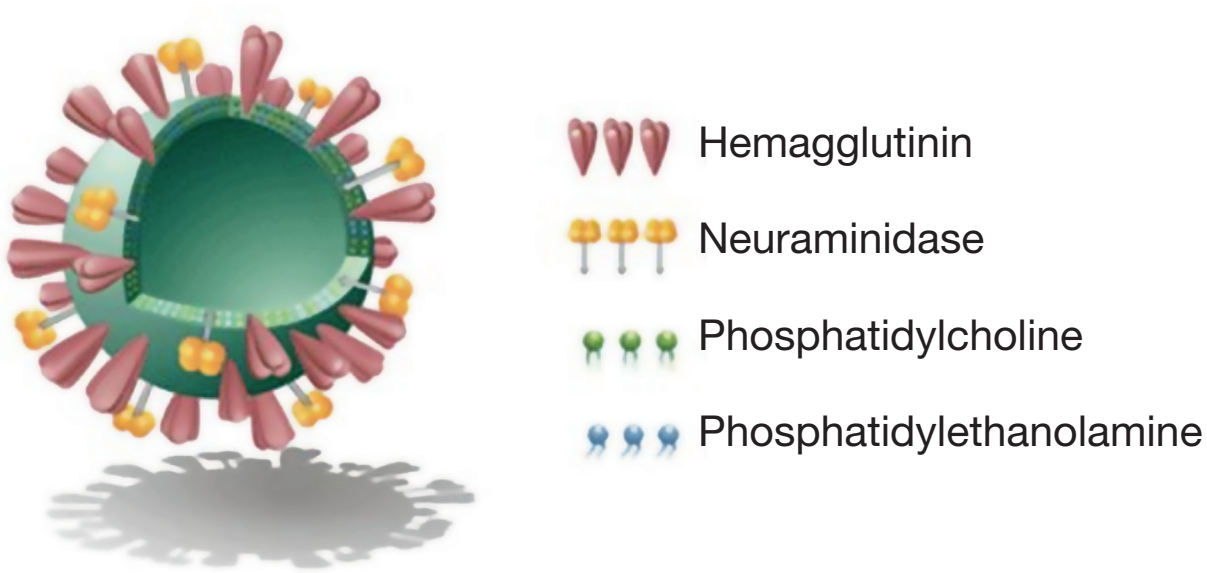
The polymer-based systems are more stable and have more sustained delivery than liposome-based systems. However, one of the major setbacks is poor biocompatibility which is associated with loss of bioactiveness (i.e. the drug) during fabricating conditions such as heat of sonication or exposure to organic solvents. The benefits of a composite system, however, include improvement of liposome stability, the ability of the liposome to control drug release over a prolonged period of time, and preservation of the bioactiveness of the drugs in polymeric-based technology. In addition, increased efficacy may be achieved from this integrated delivery system when compared to that of purely polymeric-based or liposome-based systems.[42,43]

The lipid components of liposomes are predominantly phosphatidylcholine derived from egg or soybean lecithins.[44] Liposomes are biphasic, a feature that renders them the ability to act as carriers for bioactive molecules. Drug molecules are located differently in the liposomal environment and depending upon their solubility and partitioning characteristics, they exhibit different entrapment and release properties. Lipophilic drugs are generally entrapped almost completely in the lipid bilayers of liposomes and since they are poorly water soluble, problems like loss of an entrapped drug on storage are rarely encountered. Hydrophilic drugs may either be entrapped inside the aqueous cores of liposomes or be located in the external water phase.[45-47]

Virosomes

Virosomes are the new type of liposome generation that have been used to increase bioactive molecule delivery to the cytoplasm by escape endosome. A virosome (Figure 3) is another type of liposome formulation. It comprises non-covalent coupling of a liposome and a fusogenic viral envelop. A stimuli-sensitive liposome is a type of liposome that generally depends on different environmental factors in order to trigger drug, protein and gene delivery. Studies conducted by Schroeder *et al.*, Liu and co-workers and Lentacker and co-workers demonstrated that the exposure of the liposome loaded with per fluorocarbons gas to ultrasound waves triggered drug and gene delivery into the cytoplasm of the targeted cells through cell membrane pores.[48-50] Their data demonstrated that the liposome-loaded magnetic agents triggered drug delivery to the specific site *in vivo*, using an externally applied magnetic field. The enhancement of endosomal release of drug-loaded liposome into the cytoplasm was also reported to be influenced by the utilization of pH-sensitive liposomes or by attachment of pH-sensitive fusogenic peptide ligands.[51]

Figure 3: A schematic representation of a virosome[51]



Injectable Polymeric Scaffolds

The strategy for generating an ideal depot for an active compound or bioactive molecule-loaded liposome with the benefit of in local drug retention and sustained release over prolonged time has recently received much attention in both pharmaceutical and bioengineering research.[52] The in-situ forming injectable polymer was among the most successful models, since it was able to encapsulate protein and/or bioactive molecules or function as a pre-encapsulated drug-loaded liposomal formulation that was in liquid form. This solution or suspension mixture could then be injected into the target organ with a needle to form a semisolid scaffold and finally an implant. The success in shifting from liquid formulation to semisolid and finally to an implant was a result of various desirable polymeric properties and stimulating agents such as water, light, temperature, and pH, that facilitated such processes within the polymer such as precipitation, cross-linking, and polymerisation.[53] Since the majority of hydrogels were composed of natural or synthetic biodegradable polymers, bioactive molecules were released via passive diffusion, matrix pore formation, or polymeric degradation. Furthermore, semisolid implant formation was reported as being dependant on the polymeric state such as phase inversion, low-glass transition temperature, or on hydrogels that formed by the aid of cross-linking reagents and chemo- or thermosensitisation.[54] In addition, the system could deliver drugs directly or indirectly to the targeted sites, through subcutaneous injection and/or intratumoral injection. Overall, the semisolid temporary depots offer several advantages such as enhanced local drug retention, sustained release, and potential for long-term storage. However, repeated injections and passive drug release are still a factor that limits their use as ideal pharmaceutical carriers.[55]

Zebrafish as the Ideal *In Vivo* Model for Bioactive Compound Discovery

HTS in the discovery of therapeutic lead compounds from nature challenges scientists as a tedious yet highly essential task. To establish the efficacy of chemicals from natural sources, both *in vitro* (cell culture) and *in vivo* (animal model) studies are implicated to form the basis of ensuing clinical trials. *In vivo* models were selected based on the genetic and physiological similarity with humans, ease of handling, and reproductive frequency, among others. The mouse (*Mus musculus*), for instance, is a preferred laboratory animal for scientific investigations ranging from genetics to drug toxicity.[56] However, limitations in mammalian models revealed the need to establish an alternative vertebrate species for research.

In recent years, the zebrafish (*Danio rerio*) from the minnow family Cyprinidae, emerged as a highly-sought after experimental model for natural product screening.[57,58] Initially introduced as a robust model for developmental and genetic studies,[59] the unique characteristics of the zebrafish embryo catapulted the species to the forefront of *in vivo* research. Further on, the organism became a validated model for toxicity,[60-62] immunity[63] and drug discovery.[59,64-66] This review elucidates the reasons behind employing the unassuming zebrafish as the ideal screening organism for drug discovery from bioactive molecules.

Characteristics of *Danio rerio*

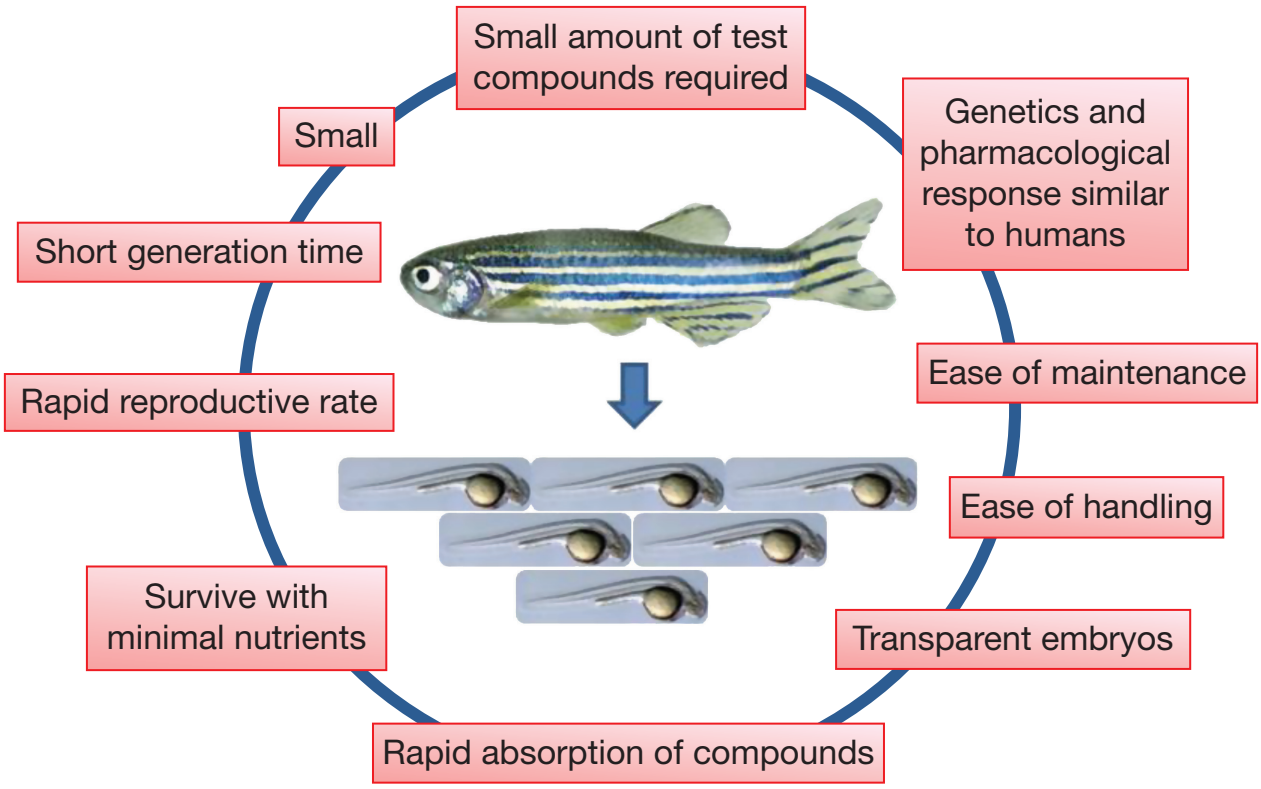
The zebrafish exhibits a number of features that support HTS for bioactive extracts or molecules from natural compounds (Fig. 4. Even relatively small laboratories have the capacity to house large populations of 1-inch zebrafish. The fish has a very rapid reproductive rate, whereby females can lay up to 200 eggs with every mating cycle within the span of a few days. The miniscule embryos, measuring only 1 – 4 mm long and exhibiting rapid extrauterine growth, can survive for days in multi-well plates primarily on the nutrients from its yolk sac.[58] Simply adding test compounds into the bathing medium elicit easy absorption through the gills, skin and digestive tract 72 hours post-fertilisation.[58,67] Furthermore, the assays do not necessitate the use of large quantities of compounds commonly required

with conventional animal models. This facilitates fast, inexpensive screening of extracts and compounds in a wide range of parameters formerly only possible in cell culture systems.

The embryos are also optically-transparent, thus enabling direct visualisation of internal viscera. The characteristic enables researchers to track internal molecular targets with fluorescent or coloured dye and measure its response to chemical compounds.[68] The animal's small size and ease of handling led to the development of whole-animal bio-imaging assays in 96-well plates using zebrafish.[69] The fact that zebrafish is a vertebrate links it with the well-studied genetics of *Drosophila/Caenhorhabditis elegans* and mammalian systems. Approximately 87% of the zebrafish genome is homologous to the human genome,[70] and this evolutionary proximity to humans permit significant correlations between zebrafish and clinical studies.

Perhaps more pertinent to drug screening is the similarity of *Danio rerio's* pharmacological response to that of humans. It would be impractical to establish elegant experimental models only to discover that the results are not translatable. Studies demonstrated a high predictability of zebrafish drug response compared to expected clinical effects and a highly conserved drug metabolism[71,72] reinforcing the rationale of using the model in drug/bioactive compound screening.

Figure 4: Characteristics of the *Danio rerio* model that enhances the efficiency of bioactive molecule discovery from natural products[58]



Zebrafish in Natural Product research

With the distinct advantages of the zebrafish model, it is unsurprising to observe an influx of bioactive compound screens using this system.[73] Some studies have even compared its efficiency to that of *in vitro* applications,[74,75] underlining the model's vast potential in establishing economical large-scale high-content screens. In a classic example, Crawford and his team developed a zebrafish bioassay-guided fractionation approach to isolate angiogenic inhibitors from East African medicinal plants.[65] Numerous other studies involving the use of zebrafish have surfaced, including the isolation and validation of potential anti-angiogenic, neuroactive lead compounds, adding on to the expanding collection (Table 2).

Table 2: Bioactive compound discovery from natural products using the zebrafish model

Study	Source	Bioactive compound	Reference
Angiogenesis	*Oxygonum sinuatum, Plectranthus barbatus*	Emodin, coleone A lactone	(65)
	Angelica sinensis	(extract)	(76)
		n-butylidenephthalide	(77)
	Panax notoginseng	(extract)	(78)
	Radix astragali	(extract)	(79)
		Astragaloside IV	(80)
	Dang Gui Long Hui Wan	Indirubin	(81)
	Citrus fruits	Nobiletin	(82)
	Orthosiphon stamineus	Sinensetin	(83)
	Alpinia oxyphylla	(extract)	(84)
	Rehmannia glutinosa	Norviburtinal	(85)
	Boesenbergia rotunda	Panduratin A	(86)
	Glycyrrhiza uralensis Fisch	Isoliquiritigenin, isolicoflavonol	(87)
Neuroactivity	*Herba epimedii*	Icaritin	(88)
	Radix astragali	Calycosin	(79,89)
	Plant flavonoid	Quercetin	(90)
	Curcuma longa	Bisabolene sesquiterpenoids	(91)
	Valeriana officinalis	(extract)	(92)
	Eriocaulon buergerianum	(extract)	(93)
	Fructus Alpinia oxyphylla	(extract)	(94)
Wound healing	*Angelica sinensis*	SBD.4	(95)
	Radix astragali	(extract)	(96)
	Radix Rehmanniae		
Hearing loss	Green tea	Epicatechin	(97)
Haematology	*Zingiber officinale*	10-gingerol	(98)
Metabolism	Green tea	(extract)	(99)
	Spongia (Heterofibria)	Heterofibrin A1	(100)
	Cinnamon, clove	(extract)	(101)
Melanogenesis	*Trifolium pratense*	Biochanin A	(102)
	Ecklonia cava (EC) and Sargassum silquastrum (SS)		(103)
Oxidative stress	Plant flavonoid	Quercetin 3-O-methyl ether	(104)
UV-protection	Broccoli, cauliflower	(extract)	(105)
	Macrocystis pyrifera, Porphyra columbina, Sarcothalia radula, Gigartina skottsbergii	(extract)	(106)

Future Directions

The lure of a brighter future in bioactive compound discovery highly influences work on zebrafish-natural product screening applications. The imminent outlook of zebrafish models in HTS inclines towards automated applications to increase productivity, efficiency, and minimise technical errors of human origin. These systems are already being established. For instance, robotic liquid handling in combination with automated microscopy and compatible software allows for a less laborious investigation of inflammatory response.[67] Another platform for automation involves mechanical tools for embryo dispensation and other processes, working with clockwork accuracy to identify anti-angiogenic compounds.[107]

Automated systems extend their reach to anti-cancer studies as well, with a quantitative bio-imaging system coupled to image analysis algorithms tracking the spread of malignant cells.[69] The novel Automated Reporter Quantification in vivo (ARQiv) HTS method developed by Walker and colleagues (2012) is a versatile complement to conventional 'high-content' whole-organism screening methods.[108]

Conclusion

There is a current perception that bioactive compounds are obtained primarily through synthetic, computer-modelling or high-throughput screening and other drug discovery methods. Although these techniques are important in producing bioactive molecules, the exploration of bioactive molecules from the natural product sources still remains important and continues to be a stable source of newly discovered compounds.

Bioactive molecules, both natural and synthetic, need to fulfil a variety of criteria in order to be pharmacologically relevant. For instance, Lipinski's rules place limits on molecular weight, the number of hydrogen bond donors and acceptors, the number of rotatable bonds and aqueous solubility. Applying Lipinski's rules in library design acts as a molecular property filter; drug designers effectively restrict the set of compounds to those with drug-like characteristics.

The need for superior vertebrate models for HTS in drug discovery has steered the advent of the zebrafish system. The unique features of this simple organism allows for rapid and cost-effective studies deemed improbable in traditional in vivo assays. With such a robust laboratory species, limitations that once plagued natural product scientists may be replaced by an encouraging prospect – a system that blurs the line between in vitro culture and whole-animal assays.

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