Elsevier Editorial System(tm) for Trends in Biotechnology
Manuscript Draft

Manuscript Number: TIBTECH-D-09-00124R2

Title: Liposomal Gene Delivery Mediated by Tissue-Engineered Scaffolds

Article Type: Review

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Abstract: In the absence of a single ideal gene delivery carrier even with the recent explosion of newer ones, the recent trend is to explore the complementary synergy promised by the combination of delivery systems such as the liposomes; the most widely researched versatile non-viral carriers and tissue-engineered scaffolds; the macrostructures with de ined architecture comprised of natural or synthetic macromolecules. Here, we discuss the recent advances in liposomal gene delivery and the bene its of the combined liposome-scaffold approach such as long-term expression, enhanced stability, reduction in toxicity and ability to produce spatio-temporal expression patterns. This approach is generating signi icant impact in the ield due to its potential for enhanced extended localised gene delivery for application in a variety of clinical conditions.

Liposomal gene delivery mediated by tissue-engineered scaffolds

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Abstract

In the absence of any ideal gene delivery carrier despite the recent explosion of novel carrier systems, the current trend is to explore the complementary synergy promised by a combination of delivery systems such as liposomes, which are the most widely researched versatile non-viral carriers and tissue-engineered scaffolds with macrostructures of defined architecture comprised of natural or synthetic macromolecules. Here, we discuss the recent advances in liposomal gene delivery and the possible benefits of a combined liposome-scaffold approach, such as long-term expression, enhanced stability, reduction in toxicity and ability to produce spatio-temporal expression patterns. This approach is generating significant impact in the field due to its potential for extended localised gene delivery for applications in a variety of clinical conditions.

Gene Delivery Systems

The bottleneck in the success of gene therapy has been the development of a safe and efficient gene delivery system[1]. Viral carriers are on one end of the spectrum, with very high transfection efficiency, but also the potential risks of toxicity or immunogenicity, in addition to their other disadvantages, such as difficulty of large scale production and limited capacity to carry DNA beyond a certain size. Naked plasmid DNA is on the other end of the spectrum, exhibiting a very attractive safety profile, but extremely low efficiency. Non-viral carriers, which include liposomes and polymers, lie in middle of the spectrum with a moderate efficiency and safety profile. Research in the field of delivery systems over the past few decades has focused mainly on the development of an optimal

delivery system, aiming to increase transfection efficiency towards the viral end of the spectrum, while reducing toxicity to exhibit a superior safety profile and reduce immunological concerns. This review focuses on non-viral carriers, which due to their superior safety profile and their broad acceptance have been considered as reliable treatment options for a wide variety of medical indications and we will provide a snapshot of the versatility of liposomes as the most widely researched non-viral carriers. We will also highlight recent progress in the field, including crucial modifications to the liposomal formulations, which have enabled them to overcome major barriers in systemic delivery or intracellular obstacles to improve their efficiency of gene delivery.

Another focus of this review is tissue-engineered scaffolds. These are being widely used as control release systems to deliver drugs, cells and/or bioactive agents, such as growth factors and genes. These slow-release systems can lengthen gene expression without risk of insertional mutagenesis as is the case in some of the viral carriers. However, the delivery of plasmid DNA from tissue-engineered scaffolds still poses the problem of low efficiency. Given the lack of a single superior delivery system that addresses all clinical requirements with high efficacy and a convincing safety profile, an increasing amount of research in recent years has focused on improving the delivery systems through a combinatorial approach, whereby each component complements the others and thus might lead to an optimized outcome.

In conclusion, we will illustrate how the combinatorial approach of using liposomal systems together with tissue-engineered scaffolds has been employed and has lead to

synergistic effects in three main areas of safety, efficiency and extended expression in a variety of applications and highlight future trends and the promising potential for clinical translation.

Liposomes: multifaceted and versatile non-viral delivery systems

Liposomes are spherical lipid bilayers of diameters in the range of 50–1000 nm that have proven useful as convenient delivery vehicles for biologically active compounds[2]. Liposomal systems, despite being the oldest of the non-viral gene-delivery vehicles, still remain attractive amid a surge of newer non-viral gene carriers. Their persisting popularity is not only due to advantages, such as their unlimited load carrying capacity, relative safety and ease of large-scale production, but can also be attributed to their versatile nature in terms of possible functionalization and formulations. Despite the lower transfection rates of conventional liposomal systems (typically requiring 1,000 to 10,000 times more particles to achieve successful genetic modification of cells compared with viral counterparts), their potential for targeted delivery through functionalisation, for example by conjugation with antibody (or fragments), peptides, sugars and so-called 'stealthing' i.*e.* polyethylene glycol (PEG)-ylation of lipids, and for escape from the reticuloendothelial system (RES) and, consequently, long-term circulation has proven to be a great advantage. Early progress during the 1970s and 1980s has lead to the development of "stealth" liposomes with long circulation times after intravenous administration and decreased uptake by macrophages. These stealth liposomes are able to extravasate out of vasculature and to accumulate in other target tissues, such as lung, kidney and liver, in therapeutically effective doses without rapid clearance from the

blood stream, thus improving their bio-distribution[3]. Moreover, liposomes can be labeled with fluorescent tags for traceability *in vivo*. A variety of stimuli, such as pH, temperature, ultrasonic waves, magnetic fields and light are currently being investigated for improved gene delivery in various settings[4-7]. In Tables 1 and 2, we list only the recent, major advances in gene delivery applications (for more extensive and complete versions, the readers are referred to the online supplementary material). Co-application of liposomes with other polymers such as polyethyleneimine provides an avenue for improved transfection efficiency[8-10].

Successful liposomal gene delivery: stumbling blocks and solutions

There are a number of extracellular and intracellular barriers to successful non-viral gene delivery. In systemic delivery, serum instability and sequestration by the RES due to uptake by macrophages are major problems. Various factors such as size, charge and surface hydration of the liposomes play important roles here. Cellular membranes pose another barrier to liposome uptake. The cationic liposomes show high transfection efficiency, which can partly be attributed to interactions with negatively charged cell membranes. The structure and properties of cationic lipids, lipoplex (see Glossary) assembly and endocytosis of lipoplexes have recently been described in detail[11]. The internalisation of liposomes occurs most commonly by endocytosis, in which the genetic material is subjected to degradation upon acidification in endolysosomes. Thus, the efficiency of liposome uptake largely depends on their ability to escape the endosomal environment and to deliver their DNA/RNA content safely into the cytosol. This is also the reason why many research efforts have been directed towards enhancing endosomal

escape. Transfection efficiencies can be predicted from the structural phases of lipids and the morphologies of lipoplexes. For example, studies have shown that the presence of a non-bilayer-phase-preferring lipid, such as dioleoylphosphatidylethanolamine (DOPE) or cholesterol, promotes transition of liquid crystalline phase (L^{C}_{α}) to inverted hexagonal phase (H^{C}_{II}) and hence membrane fusion, indicating that increasing the weight fraction of DOPE might result in higher transfection efficiencies [12].

Peptides for intracellular delivery

Recently, peptides have been increasingly used with the aim to aid the intracellular delivery of the genes. Tat peptide (TATp) is by far the most commonly used cell penetrating peptide, or so-called protein transduction domain (PTD), and is derived from the transcriptional activator protein encoded by human immunodeficiency virus type 1 (HIV-1). Its mechanism has recently been elucidated as macropinocytosis, a nonclathrin noncaveolar endocytosis brought about by formation of large vacuoles that are generated by actin filaments[13]. TATp-mediated delivery of liposomes and DNA has recently been reviewed[13, 14]. Octaarginine is another commonly used PTD that is thought to use cell surface heparin sulfate proteoglycans as non-specific receptors for uptake. Octaarginine-modified liposomes have been used for enhanced cellular uptake and controlled intracellular trafficking of plasmid DNA[15]. Apart from cell penetration, peptides are also being utilized for endosomal escape, which in turn results in higher transfection efficiency. Another cell-penetrating peptide is GALA, (a 30-amino acid synthetic peptide with a glutamic acid–alanine–leucine–alanine repeats), a fusogenic pH-sensitive peptide developed by Szoka and co-workers that aids cytosolic delivery by facilitating the

disruption of endosomal membrane and release of DNA in cytoplasm. Kobayashi et al. and Sasaki et al. demonstrated enhanced endosomal escape of macromolecules via GALA and its derivatives[16, 17]. While fusogenic peptides act upon acidification in endosomes, it has been recently shown that a stearylated INF7 peptide derivative enhanced gene expression in a fusion-independent manner and was able to rupture artificial membranes, both at acidic and neutral pH, extending the time the liposomes could escape endosomal degradation[18]. Once successful delivery of liposomes to the cytosol has been accomplished, liposome-mediated gene delivery faces additional obstacles, such as the requirement of intracellular trafficking to the nucleus and uptake into the nucleus via the nuclear pore complexes. The potential of using nuclear localisation signals (NLSs) for targeting to the nucleus has been studied by several groups and it was found that the efficiency of nuclear targeting depended on the valency (positive charges) associated with plasmid DNA and the number of NLS associated with a cargo, such as plasmid DNA, proteins, liposomes and nanoparticles[19]. Different NLSs will bind to different receptors on nuclear membranes, such as to importins[20] or farnesoid X receptor (FXR)[21] , either directly or indirectly by forming complexes with other cytoplasmic proteins. A significant increase in gene expression that was mediated by liposomes and the means of using a NLS has been shown, both *in vitro* and *in vivo*[20, 21]. Attempts have also been made to utilize the biological responses against liposomes, such as cytokine production, for their increased uptake. Tumor necrosis factor-α (TNF-α), which is induced by lipoplexes, is known to activate transcription factor nuclear factor κB (NF-κB), and NF-κB upon activation can aid nuclear transfer of DNA. It has been

reported that if NF-κB binding sequences are incorporated in plasmid DNA, lipoplex-mediated transgene expression can be enhanced [22].

Short term expression and toxicity related to liposomes

Short-term expression following liposomal gene delivery constitutes a major problem in clinical applications that require sustained levels of transgene expression over months and years. Short-term expression is due to the cargo being either not integrated into the host genome, or only unstably, and this limitation can be addressed with repeated doses of the gene, a practice, which however is not always feasible and practical. Here, gene delivery via release systems with an extended effect, such as tissue-engineered scaffolds, presents the opportunity of controlled DNA release over a long period of time as required for long-term expression. The toxicity of cationic lipids is another concern as these are frequently inflammatory. This toxicity is dose-dependent and is based on to the exposure of the liposome to and its interactions with immune cells. The use of tissue-engineered scaffolds could address these issues as a topical delivery of liposomes via a tissue scaffold would reduce their exposure to immune cells.

Tissue-Engineered Scaffolds

The view of tissue-engineered scaffolds as gene delivery systems is a relatively novel concept. Initially, scaffolds were proposed for applications in tissue engineering and considered solely as inert structural support for tissue repair and regeneration. Over the last few years, this view has changed dramatically and scaffolds are no longer seen only as dynamic tools for mimicking biological environments, but now are also regarded as

delivery vehicles for cells and/or bioactive agents. They provide a multitude of advantages, such as safe profile, protection of cargo, and enhanced and extended gene expression and the ability to control a localized delivery of cargo, as depicted in Figure 1.

Tissue-engineered scaffolds as depots and controlled-release systems

Tissue-engineered scaffolds can be designed in order to physically and/or chemically control the release pattern of any incorporated bioactive agents. A controlled release of DNA will not only lead to extended periods of gene and thus protein expression, but will also minimize the risk of under- or over-dosing of the expressed protein. The major advantage of using natural scaffolds, such as collagen and fibrin, in addition to their safety profile, is their tunable degradation, which can be readily achieved either by varying the concentration of monomers and/or crosslinking agents and thus controls the long-term release of bioactive agents. Similar to extracellular matrix (ECM), fibrin-based biomaterials could also act as temporary depots for the sustained release of substances [23], which could be readily optimized by varying the concentrations of the fibrinogen and thrombin components[24]. The release profile of bioactive agents from collagen/gelatin scaffolds can also be further optimized by appropriate choice of crosslinking agents, such as microbial transglutaminase or *N*-ethyl-*N*-(3-diethylaminopropyl)-carbodiimide/*N*-hydroxysuccinimide (EDC/NHS), as well as the degree of crosslinking.

As an alternative to natural scaffolds, synthetic scaffolds have also been suggested as they are highly flexible in that they can be manufactured in any desired shape and size, with a tightly defined architecture and relevant parameters such as porosity. Some of

these synthetic scaffolds, such as polylactic acid (PLA), also have the additional advantage of a degradation within the body, which will only leave behind harmless breakdown products such as lactic acid[1]. By crosslinking of PLA with PEG, or by using co-polymers such as poly(lactic-*co*-glycolic acid) (PLGA), their degradation in the body can be further controlled[1].

Need for further enhancement

Although, as depicted in Figure 1, the enhanced and sustained localized gene delivery that could be achieved via tissue-engineered scaffolds is certainly superior to that of naked plasmid delivery, further enhancement is required for therapeutic benefit. Towards this goal and as opposed to delivering naked plasmid DNA through scaffolds, a number of researchers have utilized different transfection reagents such as liposomes to first complex the DNA and to subsequently deliver these complexes via the scaffolds, as outlined below.

A combined liposome–scaffold approach

As mentioned above, the combination of liposomal gene delivery systems with scaffold technologies is now being considered as being complementary. Table 3 summarizes a number of studies that have investigated tissue-engineered scaffold-mediated liposomal gene delivery. The various aspects and intrinsic benefits offered by this combined liposome-scaffold approach are discussed in detail below.

Long-term expression

By far the most important advantage of combining tissue-engineered scaffolds with liposomal gene delivery is the possibility and flexibility of a well-controlled sustained delivery. This allows to overcome an only short-term gene expression following liposomal delivery, although the release kinetics of the used lipoplexes would depend on various factors, such as their size and net charge, as well as their biomolecular and chemical interactions.

The combination of tissue-engineered scaffolds with liposomes has already been utilized for sustained delivery of drugs. For example, a single application of fibrin-enmeshed tobramycin-bearing liposomes had a similar effect on reducing pseudomonas colonies when treating pseudomonas keratitis compared to 24 hourly doses of fortified topical tobramycin[25]. Subsequently, a number of studies have described sustained release systems using liposomes loaded with proteins or drugs and that had been incorporated in fibrin[26-29]. The biomedical applications of collagen, including a combination of liposomes with collagen for drug delivery, have been reviewed elsewhere [30].

The extended release of lipoplexes, and consequently the long-term expression of their delivered genes, has been demonstrated by a number of groups[31-35]. To achieve sustained delivery, different approaches were possible. For example, lipoplexes could be merely entrapped physically within the scaffolds by tailoring certain parameters, such as the degree of crosslinking and pore sizes. Alternatively, lipoplexes were specifically bound to components of the scaffolds. Recently, we described a fibrin-lipoplex system making use of naturally-occurring interactions between liposomes and the fibrinogen components of the scaffold, which obviated the need for chemical conjugation[32].

Another approach for creating a sustained delivery system is the adsorption of lipoplexes on the surface of scaffolds [36-38]. To facilitate lipoplex adsorption, scaffold surfaces have been coated with various ECM proteins, and this has led to being able to transfect a higher number of cells, while at the same time reducing the amount of DNA required. Several other strategies have been developed to associate lipoplexes or DNA complexes with the scaffold surface, including the specific binding of complexes to the scaffold through biotin–avidin interaction, gelatin entrapment, or by nonspecific adsorption [36].

Maintaining lipoplex stability

Another major advantage of the combining liposomes with scaffolds is that this approach maintains lipoplex stability with a consequently prolonged bioactivity. An increased liposomal stability has been demonstrated in fibrin-encapsulated liposomes that were used as protein delivery system[39], as well as in biophysical studies of collagen-lipid interactions[40]. The local delivery of lipoplexes from a biomaterial scaffold, such as fibronectin-coated PLG, could have the ability to maintain lipoplex stability and therefore could increase the number of transfected cells and transgene expression[34]. In a spinal cord injury model, high transgene expression has been achieved by implanting fibronectin-coated-PLG bridges with multiple hollow channels that had been immobilised with lipoplexes[34]. However, the fabrication of the scaffold can also adversely affect the stability of lipoplexes. Therefore, special processing techniques, such as cryopreparation or carbohydrate stabilization as well as mild processing conditions need to be adopted to avoid any detrimental effect on lipoplex stability, and thus the activity of incorporated DNA complexes[41]. On the other hand, if lipoplexes are immobilized on the surface of a

scaffold, the need for careful processing steps is obviated as the lipoplexes are immobilised after the scaffold fabrication. [42].

Moderating lipoplex toxicity

Although considered safer than viral delivery systems, lipoplexes are frequently associated with some degree of toxicity, typically in the form of inflammatory responses. The enhanced transgene expression observed via the combined liposome-scaffold approach reduces the required dose and this indirectly reduces dose-related toxicity. However, the cellular toxicity seen in direct bolus delivery of lipoplexes has been shown to be reduced significantly when they are delivered via gene activated matrix (GAM)[31]. This can be explained by the fact that, at any given time, only those lipoplexes that are released from and that are only a fraction of the total amount incorporated in the scaffold, are exposed to the immune cells. This apparent 'fooling' of the immune system helps to reduce the observed toxicity of lipoplexes. Another postulation that can explain the observed reduction in toxicity is that the specific interaction of cells with scaffold material such as fibrin can lead to suppression of the caspase pathway, which is involved in cell apoptosis and that of reactive oxygen species, which are typically activated by liposomes[43]. Also, when compared to bolus delivery, this approach has been shown to transfect cells, which are otherwise hard to transfect such as primary cells, and with improved cellular viability[36]. Thus, with regard to toxicity, embedding the lipoplexes within the scaffold appears a particular useful approach, whereas surface adsoption is beneficial in terms of flexibility of fabrication and stability. In addition, the possibility of

a localised therapy as afforded by the use of scaffolds significantly reduces the occurrence of systemic toxicity.

Multiple gene delivery and spatio-temporal patterning

Combining liposomes with scaffolds also provides several additional advantages, such as the possibility to deliver multiple genes simultaneously[32], or to create spatial[44] and temporal patterns of gene delivery. Recently, we demonstrated the successful simultaneous delivery of two reporter genes by means of a fibrin-lipoplex model system [32]. Such a system might prove particularly useful in diseases in which multiple genes are involved, or in which the local restoration of a specific gene function can result in therapeutic benefit, e.g. the compromised wound healing seen in diabetes mellitus. In most tissues in the body, a highly orchestrated spatio-temporal control of gene expression is established, particularly in neural and vascular networks. Recently, spatially-patterned expression of nerve growth factor (NGF) was achieved using lipoplexes that had been immobilised in microfluidic networks of polydimethylsiloxane (PDMS) and this patterned expression of NGF led to neurite outgrowth and guidance [44]. Another means to control spatial gene expression, is to immobilize lipoplexes to specific regions of the scaffold, which is the basis for transfected cell-arrays used in high-throughput functional genomics studies[38]. Here, patterned deposition of lipoplexes can be achieved by various techniques, such as spotting, printing, pinning and microfluidics[45]. Additionally, temporal control over gene expression can be achieved in a number of ways, such as by layer-by-layer assembly[46] of the scaffold with lipoplexes incorporated only in certain layers. Cell-controlled temporal expression patterns are also possible[43], in which the lipoplexes are

confined within the scaffold, and only become available for transfection only upon cell-mediated degradation of scaffold. Another approach could be to simply mixing polymer scaffolds that have different degradation profiles[45] or to fabricating a complex scaffold consisting of predetermined regions with different degradation rates, different porosity or different density of "homing" agents, such as antibodies or peptide ligands. The success of spatial patterning depends largely on the stability and activity of DNA complexes after they have been deposited on or embedded in the scaffold, and thus, the differential concentration achieved on the pattern as against the non-patterned region of the scaffold [45].

On the other hand, lipoplexes have also been shown to enhance the transfection efficiency that can be achieved using only tissue-engineered scaffolds as demonstrated for the delivery of lipoplexes based on fibrin-scaffolds based in skin wound healing [47]. The authors of this study showed a significantly higher skin flap survival, when it was treated with a fibrin-lipoplex system carrying vascular endothelial growth factor plasmid (pVEGF) as compared to using a fibrin gel carrying pVEGF. The enhanced gene expression upon liposome-scaffold delivery was synergistic and not merely additive. Their claim could be substantiated by observations of six to seven-fold increase in protein production compared to control levels as long as two weeks after treatment of rat mesenchymal stem cells with a porous sponge-like collagen scaffold that had been embedded with lipoplexes carrying glial cell line-derived neurotrophic factor (GDNF) gene[48].

Potential for clinical translation

One of the most promising aspects of combining liposome with scaffold-based delivery is its potential for clinical translation in the near future. A range of tissue-engineered scaffolds have already been approved for human use and this list is ever-increasing and some relevant examples are summarized in Table 4. Currently, over a hundred clinical trials addressing liposomal gene delivery are underway and are at different phases of completion. Considering the advantageous regulatory status of tissue-engineered scaffolds and of liposomal approaches, a clinical realization of combined liposome-scaffold delivery could be anticipated within the next two decades.

Future perspectives

The full potential of a combined liposome-scaffold approach remains to be investigated as research to date has mainly focused on providing proof of concepts. It is anticipated that the future of a combined liposome-scaffold approach would be centered around two goals: making optimal use of the progress in individual fields and the understanding derived thereof, and utilizing and manipulating interactions between the liposomes and the scaffold material as depicted in Figure 2.

In particular, the versatility of liposomes has not yet been tested in the context of their inclusion into tissue engineered-scaffolds. The recent advances in liposomal gene delivery are yet to be applied in the combined approach. A recent study has already pointed out the need for focusing research on designing lipoplexes with the aim to increase the cellular internalization of DNA for enhancing gene delivery from scaffold surface [49]. It is therefore highly likely that the successful application of combination therapy will depend on advances in liposomal gene delivery with regard to targeted

delivery, enhanced intracellular trafficking and nuclear localization. The combined strategy can also be approached from the scaffold using so called "smart" biomaterials, such as stimuli-responsive polymers, or polymers that are cell interactive and based on "click" chemistry. Shape memory polymers could be micropatterned with lipoplexes, then compacted for ease of handling and for reaching the injury site, and upon implantation would return to their original shape containing micropatterned lipoplexes. This approach could be particular useful in areas of nerve regeneration, surgical sutures [50] and vascular stenting [51, 52]. *In situ* gelling systems with lower critical solution temperature (see Glossary) at body temperature could be employed to carry lipoplexes, which after application to wounds would form a gel scaffold and subsequently release lipoplexes in a sustained manner. Also, research in the field of whole tissue organ regeneration can be geared up through micropatterned lipoplexes in 3-D scaffolds for the creation of a highly controlled spatio-temporal gene expression, mimicking the natural embryonic development. A thorough understanding of interactions between liposomes and the scaffold might pave the way towards fathoming the release mechanisms and adding additional levels of control.

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Table 1: Stimuli-responsive liposomes: prominent recent studies

Stimuli-responsiveelement in liposomes	Additional modifications	*In vitro* / *in vivo*	Applications	References
		pH-sensitive liposomes		
Hydrazone	PEG on TATp liposomes via pH/ non-pH sensitive bonds	*In vitro* and *in vivo*	Tumor specific intracellular gene delivery	[4]
		Ultrasound-sensitive liposomes		
Lipidshelled decafluorobutane microbubbles	-	*In vivo*	Therapeutic arteriogenesis	[5]
		Magnetic field-sensitive liposomes		
Magnetite in cationic liposomes	Varying concentrations of magnetite	*In vitro* and *in vivo*	Enhanced gene transfer under influence of a magnetic field	[6]
		Light-sensitive liposomes		
Hollow gold nanoshells (near infrared light)	Different coupling methods, hollow gold nanoshells tethering, encapsulation or in free suspension outside the liposomes	*In vitro*	Remote triggering of liposome release by near infrared light	[7]

Table 2: Targeted liposomes in gene delivery, prominent recent examples

Targeting moiety	Targeted tissue / Cells / Receptors	Application	Reference
Endothelium-specific antibody (273-34A)	Mouse lung endothelial cells	Targeted delivery of oligodeoxynucleotides	53
Galactosylated cationic liposomes	Liver / parenchymal cells	siRNA delivery	54
DSPE-PEG-anisamide	Human lung cancer cells	Liposome-polycation-DNA nanoparticles for tumor targeting	55
Mannosylated cationic liposomes	Melanoma/ Mannose receptors	DNA vaccination	56
Monoclonal antibody: rat 8D3	Mouse transferring receptor	Gene delivery to Brain	57
Monoclonal antibody: FIB504	Gut mononuclear leukocytes/ B_7 integrins	Systemic leukocyte-directed siRNA delivery	58
CRPPR peptide	Heart endothelium	Targeting of heart and dynamic imaging	59
Fab' fragments of recombinant humanised monoclonal antibody, HuCC49	TAG-72-overexpressing cancer cells	Systemic gene delivery to human colon cancer cells	60
DSPE-PEG2000-anisamide	B16F10 cells / Sigma receptor	siRNA delivery to metastatic tumors	61
K16GACSERSMNFCG	Lung/ human airway epithelial cell lines/(ICAM-1)	Cystic fibrosis gene therapy	62
DSPE-PEG-anisamide	Sigma receptor over-expressed in the B16F10 melanoma cells	siRNA delivery to tumors	63
Monoclonal antibody: 2G4	Myocardium/ myosin	Gene delivery to ischemic myocardium	64
Monoclonal antibody: 8D3	Brain/ transferrin receptor	Targeted delivery to brain	65

Table 3: Combined liposome-scaffold approach for gene delivery

Scaffold/ Substrate	Liposomes	DNA	Application	References
Serum-coated tissue culture polystyrene	Lipofectamine ™ 2000	Plasmid- luciferase/ EGFP	Substrate mediated delivery	[36]
Porous poly (D,L-lactide) disks	FuGene® 6 lipophilic transfection reagent; 20 mM DOTAP: cholesterol (1:1) liposome	Plasmid- GFP	Bone repair	[31]
PLG matrices	Lipofectamine ™ 2000	Plasmid- luciferase/β-galactosidase/NGF/ NGF-GFP dual expression	Nerve regeneration	[35]
Type II collagen glycosaminoglycan scaffolds	GenePORTER® Reagent	Plasmid- insulin-like growth factor (IGF)-1	Cartilage repair	[33]
Fibrin	Lipofectamine™ and Plus Reagent	Plasmid- VEGF	Wound healing	[47]
Collagen	GenePORTER® Reagent	Plasmid- GDNF	Brain injury	[48]
Tissue culture polystyrene	Lipofectamine ™ 2000	Plasmid- EGFP-luciferase	Substrate mediated delivery	[49]
Polystyrene plate	Lipofectamine™	Plasmid- luciferase	Substrate mediated delivery	[37]
Fibrin	Lipofectin ®Reagent	Plasmid- EGFP; luciferase + β-galactosidase	Simultaneous delivery of multiple genes to wound bed	[32]
ECM coated Multiple channel PLG bridges	TransFast™ Transfection Reagent	Plasmid- firefly luciferase and β-galactosidase	Spinal cord injury	[34]
Polydimethylsiloxane cured on patterned molds using photolithography	Lipofectamine ™ 2000	Plasmid- EGFP + luciferase; NGF	Nerve repair	[44]

Table 4: Examples of tissue-engineered scaffolds approved for human use

Major component	Clinical use	Market name	Marketed by
Collagen	Skin repair	TransCyte	Advanced Biohealing (http://www.advancedbiohealing.com/)
		Apligraf	Organogenesis (http://www.organogenesis.com/)
		Dermagraft	Advanced Biohealing (http://www.advancedbiohealing.com/)
		INTEGRA dermal regeneration template	Integra Lifesciences (http://www.integra-ls.com/home/)
	Bone repair	Infuse bone graft	Medtronic (http://www.medtronic.com/)
		OP-1	Stryker (http://www.stryker.com/en-us/index.htm)
		VITOSS Scaffold FOAM	Orthovita and Kensey Nash (http://www.orthovita.com/ http://www.kenseynash.com/index.asp)
		FortrOss	Pioneer Surgical (http://www.pioneersurgical.com/)
		BioSet-RTI	Pioneer Surgical and Regeneration Technologies (http://www.pioneersurgical.com/ http://www.rtix.com/)
	Nerve conduit	NeuraGen	Integra (http://www.integra-ls.com/home/)
	Cartilage repair	Menaflex	Regenbiologics (http://www.regenbio.com/usa/en/)
		CaReS	Arthro Kinetics (http://www.arthro-kinetics.com/)
Fibrin	Sealant for wound management	TISSEEL	Baxter International (http://www.baxter.com/)
Gelatin	Bone repair	Regenafil	Exactech (http://www.exac.com/)

Glossary:

Click Chemistry: an approach described by K. Barry Sharpless wherein substances are generated by joining small units together with heteroatom links (C- X- C). The reaction to be termed as click chemistry based, certain criteria must be met: the reaction must be modular, wide in scope, give very high yields, generate only inoffensive byproducts that can be removed by non-chromatographic methods and be stereoscopic[66]

Lipoplexes: complexes consisting of liposomes and nucleic acids. Cationic lipids, on account of their positive charge, readily complex with negatively charged nucleic acids. Nucleic acids can be compacted in these particles. Lipoplexes facilitate the entry of nucleic acids within cells and protect their degradation by nucleases.

Lower critical solution temperature: temperature below which a mixture is miscible in all proportions

Tissue engineered scaffolds: fundamental components of tissue engineering, made up of natural or synthetic macromolecules. Scaffolds are 3-D macrostructures and can take a variety of architectural forms such as gels, hydrogels, foams or sponges, with defined parameters such as pore size, mechanical strength and degradation rate.

Figures Legends:

Figure 1. Schematic depiction of the role of tissue-engineered scaffolds in gene delivery. Tissue-engineered scaffolds can be employed either as reservoirs, or as sustained delivery systems. If they are used as reservoirs, the host tissue will integrate with the scaffold material and the contents of the scaffolds will exhibit their intended function in the context of the host tissue. In sustained delivery systems, the contents of the scaffolds are delivered as and when the scaffold material degrades. This results in long-term gene expression when compared with delivery by lipoplexes alone. As depicted in the graph shown below, tissue-engineered scaffold-mediated sustained gene delivery enhanced gene expression and a synergistic effect is observed when tissue-engineered scaffolds delivered lipoplexes (yellow curve) as opposed to naked plasmids (purple curve).

Figure 2. Schematic illustration of potential future developments of tissue-engineered scaffold-mediated liposomal delivery. Breakthroughs in the near future will most likely be based on the full utilization and application of recent advances in individual biomaterials, including stimuli-responsive materials, shape memory polymers, and interactive polymers. Advancements in liposome technology, such as the development of stimuli-responsive and functionalized formulations, will also contribute to further progress together with the advent of innovative release strategies.

Figure 1
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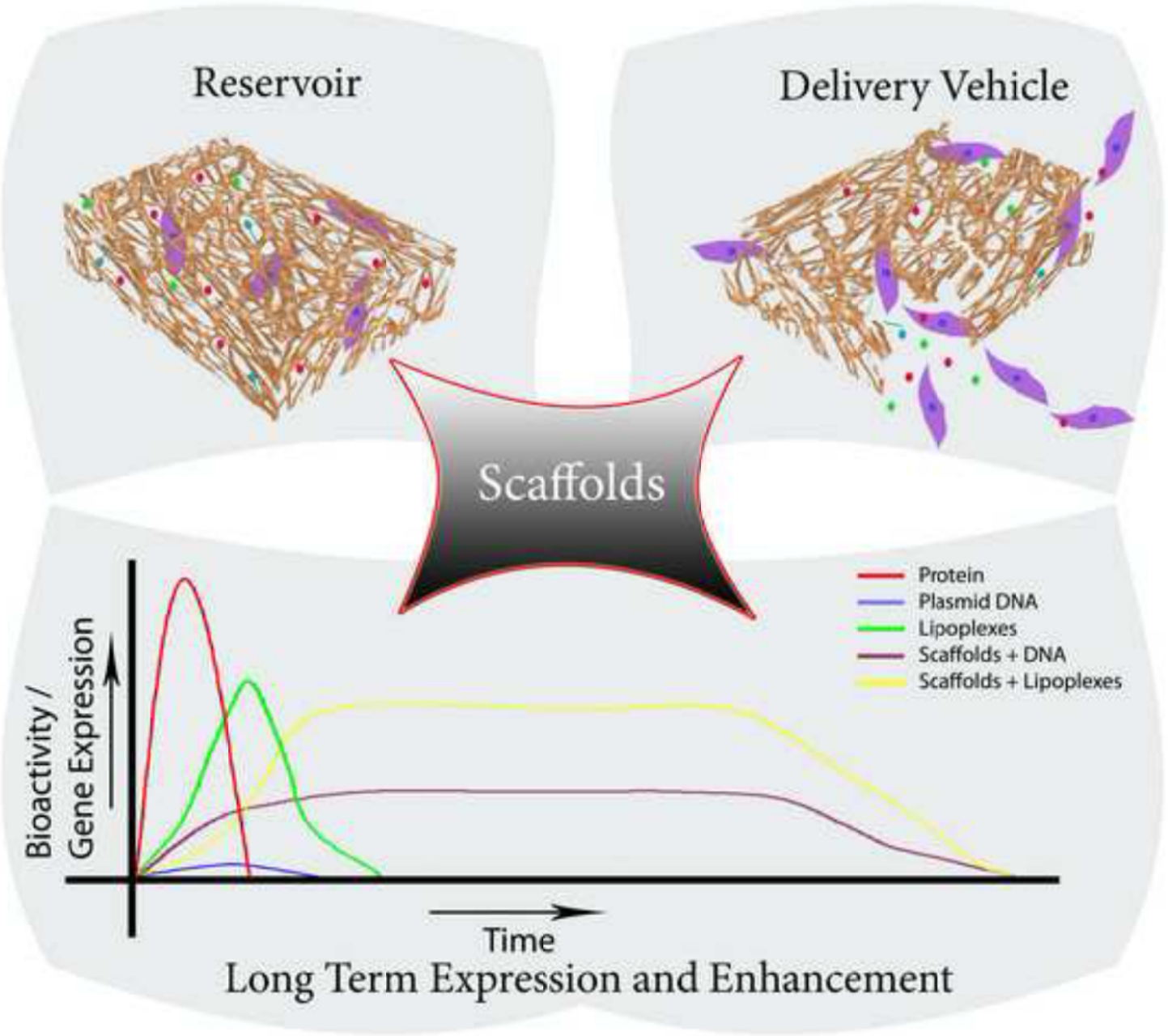

Figure 2
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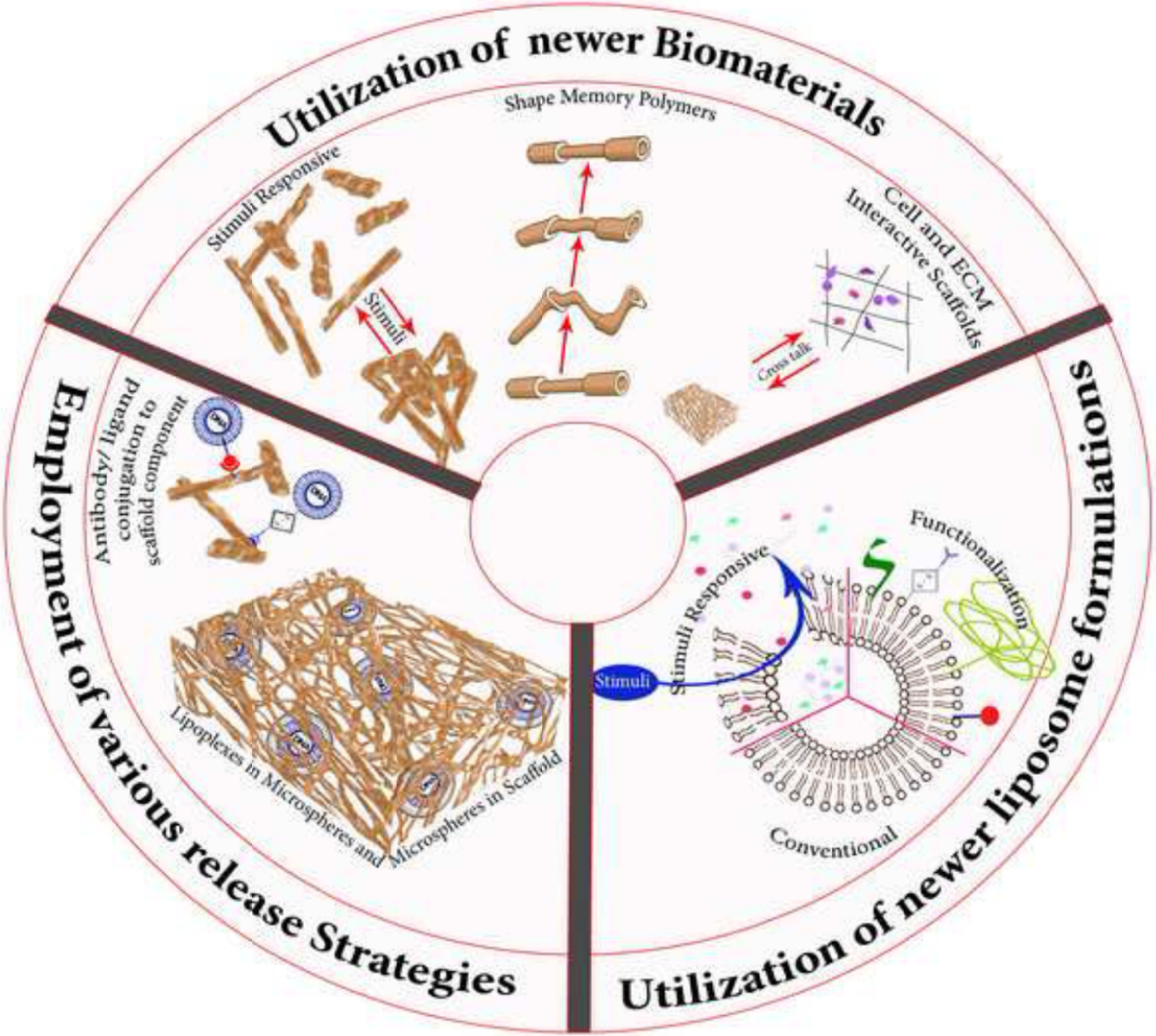

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