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Shining Light on Nanotechnology to Help Repair and Regeneration

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Abstract

Phototherapy can be used in two completely different but complementary therapeutic applications. While low level laser (or light) therapy (LLLT) uses red or near-infrared light alone to reduce inflammation, pain and stimulate tissue repair and regeneration, photodynamic therapy (PDT) uses the combination of light plus non-toxic dyes (called photosensitizers) to produce reactive oxygen species that can kill infectious microorganisms and cancer cells or destroy unwanted tissue (neovascularization in the choroid, atherosclerotic plaques in the arteries). The recent development of nanotechnology applied to medicine (nanomedicine) has opened a new front of advancement in the field of phototherapy and has provided hope for the development of nanoscale drug delivery platforms for effective killing of pathological cells and to promote repair and regeneration. Despite the well-known beneficial effects of phototherapy and nanomaterials in producing the killing of unwanted cells and promoting repair and regeneration, there are few reports that combine all three elements i.e. phototherapy, nanotechnology and, tissue repair and regeneration. However, these areas in all possible binary combinations have been addressed by many workers. The present review aims at highlighting the combined multi-model applications of phototherapy, nanotechnology and, reparative and regeneration medicine and outlines current strategies, future applications and limitations of nanoscale-assisted phototherapy for the management of cancers, microbial infections and other diseases, and to promote tissue repair and regeneration.

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Conflict of Interest

The authors state no conflict of interest.

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Keywords

Liposomes; Low level laser (or light) therapy; Nanomaterials; Nanoparticles; Photodynamic therapy; Phototherapy; Repair and regeneration; Stem cells

1. Introduction

Photodynamic therapy (PDT) has emerged as an important therapeutic approach to treat infections, cancer and other diseases involving removal of unwanted cells or tissues. PDT has received approval for treating esophageal, lung, head and neck, skin, bladder cancer and other cancers (Chatterjee et al., 2008, Dougherty, 2002). It involves the use of either exogenous or endogenous non-toxic light-sensitive molecules called photosensitizers (PS) combined with harmless visible light of the appropriate wavelength to match the absorption spectrum of the PS. After photon absorption the PS reaches an excited state that can undergo reaction with ambient oxygen, resulting in the production of reactive oxygen species (ROS). The three principle components of PDT are the PS, visible light, and oxygen. These individually harmless components, when combined, yield potent ROS that produce cell inactivation and death through modification of intracellular components. Suitable PS has a high extinction coefficient in the farred or near infrared (NIR) spectral region and a high yield of the long lived triplet electronic state (formed from the excited singlet state by intersystem crossing). The triplet PS is able to react with surrounding molecular oxygen by one of two distinct pathways. The type 1 pathway involves electron transfer to or from the triplet PS, leading to the formation of ROS such as superoxide, hydroxyl radicals and hydroperoxides. The type 2 pathway relies on the fact that molecular oxygen is a triplet in its ground state and therefore has a spin-allowed interaction with PS triplet state producing both species (PS and oxygen) in the singlet state. Excited state singlet oxygen ($^1\text{O}_2$) is known to be a potent oxidizing agent (Huang et al., 2012, Sharma et al., 2011). Figure 1 shows a Jablonski diagram and the resulting type 1 and type 2 pathways. PDT can be considered to be a highly selective modality as (i) the PS can be targeted to the unwanted cells or tissues and, (ii) cell death is spatially limited to regions where light of the appropriate wavelength is applied.

Drug delivery is one of the main challenges in PDT remaining to be overcome. For effective and safe PDT, it is crucial that the PS should be delivered in therapeutic concentrations to the target cells (e.g. tumor or infected cells) with little or no uptake by non-target cells, thus minimizing undesirable side-effects in healthy tissues. As most highly effective PS are highly hydrophobic and insoluble in water, various encapsulation strategies have been studied to prevent the hydrophobic PS from forming inactive aggregates in an aqueous environment. Aggregation reduces the efficiency of the PS, which must be in monomeric form to be photoactive (Konan et al., 2002). Many of these delivery systems take the form of nanoparticles (NP) or other nanostructures. NP, with a size ranging from 1–100 nm, exhibits unique physical and chemical properties that can be exploited for drug delivery by conjugation with drugs such as PS. These emerging nanoscale drug delivery systems are being used to improve current treatment regimens in PDT (Box 1). Four different types of interactions between NP and PS have been reported: (i) PS embedded in polymeric NP, (ii) PS covalently bound to the surface of NP, (iii) PS non-covalently bound to NP and (iv) cases where the NP itself is the PS (Perni et al., 2011). This classification is consistent with that proposed by Chatterjee et al. (2008) (Chatterjee, Fong, 2008) of 'active' (NP as the PS) and 'passive' (the other three explained earlier) NP. Passive NP can be divided further as biodegradable (liposomes, polymeric nanomaterials) and non-biodegradable types (metallic NP, silica NP, quantum dots, CNT, fullerenes) (Fig. 2A–E). Although these nanocarriers tend to accumulate selectively in target sites, however they can be engulfed and destroyed

by cells of the reticuloendothelial system (RES). In order to reduce this undesirable uptake by phagocytic cells, long-circulating carriers that consist of surface modified carriers as 'actively-targeted' NP have also been developed (Derycke and de Witte, 2004, Konan, Gurny, 2002).

Box 1

Reasons to use nanoparticles (NP) as drug delivery vehicles for photosensitizers in PDT (Koo et al., 2007)

- NP can be produced in an optically transparent form.
- Improve solubility to facilitate parental drug administration.
- Sustained drug delivery, resulting in zero order release kinetics (Hoffman, 2008).
- Increased drug stability to reduce degradation and maximize drug action.
- Stopping the PS from dimerizing and aggregating as occurs in the free state, these forms are ineffective (Usacheva et al., 2003).
- If the PS is not soluble in water, the encapsulation can be used to enhance solubility (Schwartz et al., 2009).
- Owing to their subcellular and submicron size, NP PS can penetrate deep into the tissue.
- Increase the drug concentration at targeted site, achieved through either passive or active targeting, and reduce systemic effects of drug and its toxic sequel in non-targeted tissue.
- Larger concentrations of PS (critical mass) for the production of lethal reactive oxygen species.
- NP can be used for a range of accompanying therapies giving room for combination therapy (imaging, diagnosis and treatment in conjugation with other modalities).

A striking increase in the average age of the population especially in Western countries necessitates innovation in methods for tissue repair and regeneration. Stem cells and tissue engineering have emerged recently as alternative new modalities; however both technologies still have many limitations. Phototherapy (LLLT/ PDT) has begun to be used as an aid to improve the tissue repair and regeneration processes. LLLT accelerates repair, stimulates proliferation and promotes stem cell viability and differentiation. Nano-assisted devices on the other hand, have gained importance in the last few decades in the areas of reparative and regenerative medicine. Surfaces, structures and biomaterials with nanoscale features have the potential to mimic the natural microenvironment of cells (biomimetic); features which induce cell migration, adhesion, proliferation and differentiation. However both phototherapy and nanotechnology are still facing challenges on their own, while the usage of phototherapy in conjunction with nanotechnology has recently been suggested to overcome the limitations and create synergistic effects for repair and regeneration (Fig. 3) (Goldberg et al., 2007, Verma et al., 2011).

This review focuses on current knowledge on areas of phototherapy and nanotechnology for their potential applications in the treatment of cancers, microbial infections, and augmenting tissue repair and regeneration. Furthermore their synergistic effects when used in combination have been highlighted under four major headings: (i) nanomaterials used for

PDT, (ii) phototherapy mediated tissue repair and regeneration (iii) nanotechnology used for tissue repair and regeneration (iv) phototherapy in conjunction with nanotechnology used for tissue repair and regeneration. We also discuss the future trends and limitations of nano-assisted phototherapy applications.

2. Types of nanomaterials used for PDT

PDT uses the energy of light to destroy tumors, microbial infections and other unwanted cells. The efficacy of PDT is determined by the dosimetry of the activation light and the structure of the PS that together modulate the photodynamic reaction at depth in diseased tissue. The application of nanotechnology has opened a new realm of advancement in the field of PDT and has provided hope for the development of nanoscale delivery carriers for PS, which might improve the efficiency of photodynamic activity and can overcome many side-effects associated with classic PDT. The following NP includes the most commonly used types of NP in the field of PDT. Many more types exist but are not described here for the sake of brevity. The reader is referred to several excellent reviews that provide information about the broader range of NP in more detail (Chatterjee, Fong, 2008, Huang, Sharma, 2012, Paszko et al., 2011).

2.1. Lipid-based nanoparticles, micelles and nanoemulsions used for PDT

Lipid-based NP, such as liposomes, lipoplexes and nanoemulsions have been used extensively in recent decades as drug delivery vehicles for hydrophobic PS for the treatment of cancers and microbial infections (Derycke and de Witte, 2004, Huang, Sharma, 2012, Malam et al., 2009, Paszko, Ehrhardt, 2011). Liposomes are closed spherical vesicular nanostructures formed by a lipid bilayer composed of either synthetic or natural phospholipids and cholesterol molecules (structural components of cell membranes) characterized by extended, two-dimensional and clearly separated hydrophilic and hydrophobic regions (Fig. 2A) (Bangham, 1961, Boulaiz et al., 2011). The effectiveness of liposomes as drug vehicles is related to their pharmacokinetics and depends on the physicochemical conditions, e.g., size, surface charge, membrane lipid packing, steric stabilization, dose, and administration route (Maruyama, 2011). Liposomes can target specific tissues through both active and passive targeting strategies, since these can easily be manipulated by adding additional molecules to the surface of the lipid bilayer.

Micelles are so-called colloidal dispersions (particle size, 5–100-nm) of aggregates of surfactant molecules dispersed in a liquid colloid. Micellar nanocarriers demonstrate a series of attractive properties as drug delivery systems, such as improved bioavailability, enhanced permeability retention (EPR) effects, and substantial beneficial changes in drug biodistribution. Micelles can be classified in two groups based on the nature of the amphiphilic core i.e. polymeric micelles and lipid micelles (van Nostrum, 2004). Many studies have utilized liposomes, lipoplexes and nanoemulsions and micellar nanocarriers as delivery vehicles for PS, which can substantially improve the efficacy and safety of PDT (Table 1).

2.2. Polymeric nanocomposites used for PDT

The development of nano-formulated polymeric PS has emerged as a promising technique because of the biocompatibility and the accessibility for multi-functionalization of NP. Polymeric nano-based PDT drugs offer several advantages viz. ability to deliver a large amount of PS to the target area; flexibility toward surface modification for better efficiency; prevention of degradation in the living biological environment; ability to attach multiple components such as targeting ligands and diagnostic contrast agents; enhanced EPR effects (Gaumet et al., 2008); minimization of immunogenicity and side-effects of the drug (Lee

and Kopelman, 2011). Many polymeric nanocarriers have been used to create PDT agents. They are classified as natural polymers (chitosan, alginate, albumin, xanthum, collagen) (Chen et al., 2009, Deda et al., 2009, Khadair et al., 2008), and synthetic polymers like polyacrylamide (PAA), polylactidepolyglycolide co-polymers (PGLA) (PLGA), *N*-(2-hydroxypropyl)-methacrylamide (HPMA) (Konan et al., 2003, Tang et al., 2008, Zeisser-Labouebe et al., 2006). Table 2 presents a summary of potential polymeric nanocarriers used in PDT.

A dendrimer can be described as a highly branched polymeric 3D structure that provides a high degree of surface functionality and versatility (Fig. 2B). Li and Aida (2009) have reviewed the field of dendrimeric porphyrins, phthalocyanines, and other tetrapyrroles (Li and Aida, 2009). Fluorescein isothiocyanate-labeled poly-L-lysine encapsulated in polyion complex (PIC) micelle dendrimers have been used for the treatment of CNV-choroidal neovascularization in rats (Tamaki, 2009). PDT for corneal neovascularization was carried out using polymeric micelles encapsulating dendrimer porphyrins (Sugisaki et al., 2008). Photochemical internalization, (PCI), which is a specific technology based on PDT has been used in site-specific triggered drug/gene release to overcome drug-resistance using dendrimer phthalocyanine (DPc)-encapsulated polymeric micelle (DPc/m) in drug-resistant MCF-7 cells and a xenograft model (Lu et al., 2011). In another study, mice treated with DPc/m showed a much better tumor response than those with DPc-PDT (Nishiyama et al., 2009). Introducing a disulfide crosslinker in the polyion complex micelle showed improved *in vitro* PDT effect of DPc/m (Herlambang et al., 2011).

2.3. Carbon nanomaterials used for PDT

Carbon nanotubes (CNT) are allotropes of carbon with a cylindrical nanostructure. CNT are categorized as single-walled nanotubes (SWNT) and multi-walled nanotubes (MWNT). Most SWNT have a diameter of close to 1 nm, and have high aspect ratio making them highly reactive (Wang et al., 2009) and can be several millimeters long, significantly larger than any other material. Shi kam et al. (2004) have shown that CNT are internalized by mammalian cells through endocytosis (Shi Kam et al., 2004), however other mechanisms may also be involved (Kostarelos et al., 2007). Furthermore, CNT are excreted from the body rapidly and when solubilized seem to have no significant cytotoxicity (Liu et al., 2008). CNT can be chemically modified to carry covalently linked active agents or targeting groups. However, supramolecular modification is even more promising, since it would be much easier to carry out the required modifications, and multiple functionalities could be easily placed as a single addend through non-covalent association with the CNT. Banerjee et al. (2010) reported the design of antimicrobial nanocomposite films based on conjugates of MWNT and protoporphyrin IX (PpIX) that are highly effective against virulent *Staphylococcus aureus* (*S. aureus*) upon irradiation with visible light. MWNT-PpIX conjugates could effectively deactivate *S. aureus* cells in solution upon irradiation with visible light. These conjugates represent a facile strategy for the design of anti-microbial and anti-fouling coatings (Banerjee et al., 2010).

Graphene oxide (GO) consists of sheets sp²- and sp³-hybridized carbon atoms, including carboxyl, hydroxyl, and epoxide functional groups (Wang et al., 2011b). Dong et al. (2010) synthesized a PS carrier, methoxy-poly(ethylene glycol) modified nano-graphene oxide (NGO-mPEG) to target cancer cells for PDT (Dong et al., 2010). Zinc phthalocyanine (ZnPc) was loaded onto NGO-mPEG and no apparent cytotoxicity was observed towards MCF-7 carcinoma cell line. Huang et al. (2011) designed folic acid-conjugated GO and prepared as drug carrier to target site achieving higher specificity (Huang et al., 2011b). The second generation PS chlorin(e6) (Ce6) was effectively loaded into the system and showed high photodynamic efficacy on MGC803 cells upon irradiation (Huang, Xu, 2011b).

Fullerenes are a class of closed-cage, third allotropic form of carbon materials, C_n , where $n=60, 70, 72, 76, 84$ and so on up to one hundred C atoms (see Fig. 4F for an example). Fullerenes have been an important feature of the nanotechnology revolution. A great deal of attention has been focused on developing medical uses of these unique molecules especially when they are surface modified with functional groups to make them soluble and therefore able to interact with biological systems (Sharma, Chiang, 2011) (Table 3). The fullerenes are seen as potential PDT agents as they possess some of the characteristics that render them well-suited as a PS drug. Depending on the functional groups introduced into the molecule, fullerenes can effectively be used in photodynamic inactivation (PDI) either or both for pathogenic microbial and malignant cancer cells (Mroz et al., 2011) (Lu et al., 2010) (Fig. 4). The mechanism appears to involve superoxide anion (type 1 PDT) as well as 1O_2 (type 2 PDT), and under the right conditions fullerenes may have advantages over clinically applied PS (Mroz et al., 2007b, Sharma, Chiang, 2011).

2.4. Silica-based nanoparticles used for PDT

Silica is a robust material with tunable chemical properties. Its versatile polymerization has meant that silica is frequently used for the stabilization of PS and to functionalize various types of silica NP (SiNP) for biomedical applications (Brown, 2003, Couleaud et al., 2010, Jain, 2005, Weetall, 1970) (Fig. 2C). SiNP has been loaded with various PS such as protoporphyrin IX (PpIX), elsinochrome A, 2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide (HPPH), meta-tetra(hydroxyphenyl)chlorin (mTHPC), and methylene blue (MB) by encapsulation and covalent linkage (Brevet et al., 2009, Gary-Bobo et al., 2012). Organically modified silica (ORMOSIL) based NP have seen widespread use due to the flexible hydrophobic/hydrophilic properties, which can be tuned in accordance with the degree of hydrophobicity of the PS. Furthermore, ORMOSIL having functional groups added to the surface are also emerging as a promising vectors for PDT applications (Couleaud, Morosini, 2010).

The feasibility of using ORMOSIL as a carrier for PS was established by Roy et al. (2003) (Roy et al., 2003) and their formulation of NP overcomes many of the limitations of 'unmodified' SiNP. ORMOSIL loaded with fluorescent dyes like mTHPC was used to evaluate its effect on cancer cells *in vitro* using PDT (Compagnin et al., 2009) and functionalization of mesoporous silica NP (MSN) with mannose dramatically improved the efficiency of PDT on breast cancer cells (Brevet, Gary-Bobo, 2009). MB was encapsulated or covalently linked to ORMOSIL and reported to have a significant photo-cytotoxicity in tumor cells after PDT (Tang et al., 2005). HPPH, which is in phase I/II clinical trials was effectively encapsulated into ORMOSIL by co-polymerization of controlled hydrolysis of triethoxyvinylsilane and triethoxysilane-derivatized HPPH in micellar media. *In vitro* studies demonstrated the active uptake of drug-doped NP in the cytosol of cancer cells after PDT (Ohulchanskyy et al., 2007). Porous hollow silica nanospheres embedded with hypocrellin A (HA) were constructed and their *in vitro* efficacy showed higher photostability and higher 1O_2 generation together with active uptake of HA-doped NP compared to free HA in HeLa cells (Zhou et al., 2010). Similarly, encapsulation of PpIX in ORMOSIL showed improved PDT effects on HeLa cells (Qian et al., 2009). In another study, Simon et al. (2010) synthesized PpIX in ORMOSIL and reported intracellular accumulation of PpIX SiNP in the cytoplasm of HCT 116 and HT-29 colon cancer cells (Simon et al., 2010).

2.5. Metal nanoparticles used for PDT

Gold (Au) nanostructures are of particular interest in therapies such as PDT due to their biocompatibility, stability, size control and ease of surface functionality (Fig. 2D) (Daniel and Astruc, 2004). Khaing Oo et al. (2012) studied the size-dependent enhancement of ROS

by conjugating PpIX with Au NP of varying size (19, 66 and 106 nm). *In vitro* study on the ROS formation produced by PpIX-conjugated Au NP in human breast cancer cells (MDA-MB-231) revealed size-dependent enhancement of intracellular ROS formation, while the enhancement greatly depended on cellular uptake of Au NP (Khaing Oo et al., 2012). Cheng et al. (2008) found that PEGylated Au nanoconjugates are highly efficient drug vectors for PDT. The Au nanoconjugates act as a water-soluble and biocompatible cage allowing hydrophobic drugs to reach the site of PDT action. Further, they reported both *in vitro* and *in vivo* studies showing increased PDT efficacy (Cheng et al., 2008). *In vivo* studies in cancer-bearing mice indicated high efficiency in the delivery time of phthalocyanine (Pc4) i.e., less than 2 h when conjugated with Au NP, compared to 2 days for the free drug (Cheng, A, 2008). In another study, Au NP (Camerin et al., 2010) were studied the pharmacokinetic and phototherapeutic properties of a free phthalocyanine (C11Pc) and Au NP-bound phthalocyanine (C11Pc-Np) in a murine tumor model (amelanotic melanoma).

Molecular plasmonics is the field that deals with localized surface plasmon resonance effects and their interaction with molecular components (Csaki et al., 2011). The gold core can be used for plasmonic imaging and improved hyperthermal treatment. Fales et al. (2011) used a silica coating to encapsulate the MB in a shell around Au NP, which showed a significant increase in $^1\text{O}_2$ generation as compared with NP synthesized without MB. This increased $^1\text{O}_2$ generation showed a cytotoxic effect on BT549 breast cancer cells upon NIR laser irradiation (Fales et al., 2011). Khlebstov et al. (2011) used PDT to control the accumulation and biodistribution of a gold-silver nanocage core and MSN shell functionalized with the PDT sensitizer Yb-2,4-dimethoxyhematoporphyrin (Yb-HP) in mice bearing Ehrlich carcinoma tumors in a comparative study with intravenously injected free Yb-HP molecules (Khlebstov et al., 2011).

2.6. Semiconductor nanomaterials used for PDT

Some metal oxide semiconductor nanomaterials, like TiO_2 and ZnO NP, have the ability to act as direct PS and catalytically generate ROS after light irradiation with appropriate wavelength and fluence. Yamaguchi et al. (2010) discussed the *in vitro* study of TiO_2 /PEG nanoparticles on C6 rat glioma spheroids cells using UV irradiation (Yamaguchi et al., 2010). Each monolayer (TiO_2 /PEG) and C6 cells was co-incubated with varying concentrations of TiO_2 / PEG and UV irradiated. The cytotoxic effect was correlated with the concentration of TiO_2 /PEG and the energy dose of UV irradiation. The fact that TiO_2 NP are producers of hydroxyl radicals suggesting that killing of cells is mediated through type 1 PDT (Cai et al., 1992). Like TiO_2 , ZnO also shows photocatalytic activity. Li et al. (2010) have demonstrated ZnO NP size-dependent PDT on SMMC-7721 cancer cell proliferation (Li et al., 2010). Further, they observed smaller the ZnO nanoparticle size, the higher the cytotoxicity for cancer cells.

Quantum dots (QD) are semiconductor nanocrystals whose size (1–6 nm) regulates the band-gap that have drawn a lot of attention for luminescent based applications including PDT as a result of their intrinsic photophysical characteristics (Fig. 2E). Compared with molecular PS, some properties of QD in particular stand out: they can be target to specific pathological areas and made water soluble; their emission can be broadly tuned (UV to IR) simply by size and composition, and they are stable *in vivo* for very long periods of time without degradation into their potentially toxic elemental components (Bakalova et al., 2004, Smith et al., 2008). QD have been used as energy donors and acceptors in various biological studies based on FRET (fluorescence resonance energy transfer) in the combination with PS agents for PDT. One of the first reports on the generation of $^1\text{O}_2$ via FRET was by Samia et al. (Samia et al., 2003, Samia et al., 2006). Silicon-Pc4, a known PDT agent has undergone clinical trials for cutaneous neoplasms such as mycosis fungoides (Baron et al., 2010). Baron et al. (2010) demonstrated that QD conjugated to silicon-Pc4 via

an alkyl amino group can generate $^1\text{O}_2$ via FRET from QD nanocrystals to PS (Baron, Malbasa, 2010). Tsay et al. (2007) developed water stable peptide-coated QD PS to generate $^1\text{O}_2$ via FRET (Tsay et al., 2007). They conjugated Rose Bengal and chlorin(e6), PS which generated $^1\text{O}_2$ in high yield, with phytochelatin-related peptides, which in turn were conjugated to CdSe/CdS/Zn QD. Hydrophilic CdTe(S) QD conjugates to folate with different spacers (PEG or Jeffamine D-400) and examined phototoxicity against folate-receptor-positive or negative cancer cells. Morosini et al. (2011) obtained evidence for folate-receptor-mediated targeting and PDT effects using 515 or 658 nm light (Morosini et al., 2011). Christensen et al. (2011) recently reported the effect of classical CdSe/ZnS QD and novel spherical carbon dots on generation of $^1\text{O}_2$ and other ROS in *in vitro*. The dual properties exhibited by both these NP can be used for photodynamic and photocatalytic or antioxidant applications. (Christensen et al., 2011).

2.7. Actively-targeted nanomaterials used for PDT

Targeted drug-delivery systems are one of the strategies proposed to solve the problems which underline non-specific PS accumulation. Actively-targeted nanocarriers encompass the strategy of coupling a specific entity to the surface of NP, enhancing their selective interaction with target cells recognized by specific markers/ ligands such as monoclonal antibodies, folate, growth factors, lipoproteins, aptamers and transferrin (Huang, Sharma, 2012, Paszko, Ehrhardt, 2011). Table 4 is a summary of different actively-targeted nanocarriers being used for PDT.

2.8 Miscellaneous nanomaterials used for PDT

2.7.1 Upconversion nanoparticles—Upconverting nanoparticles (UCNP) have been looked upon to overcome the limitation of light penetration through skin and tissues (Fig. 5A). Upconversion is the phenomenon by which a material absorbs light in lower energy and emits light with a shorter wavelength corresponding to higher energy (usually in the visible range). This phenomenon can be taken advantage of by nanomaterials thus making it a potential strategy for PDT. These nanocrystals absorb near infrared (NIR) laser and emit radiation in the visible range. Chatterjee and Yong (2008) (Chatterjee and Yong, 2008) designed UCNP composed of sodium yttrium fluoride (NaYF_4) nanocrystals co-doped with Yb^{3+} and Er^{3+} with a polymeric coat of poly(ethylenimine) (PEI). ZnPc (PS) was superficially adsorbed to the UCNP. They were able to image cellular uptake by fluorescence imaging microscopy of PEI/ $\text{NaYF}_4:\text{Yb}^{3+}:\text{Er}^{3+}$ NP and they found significant human colon cancer cells death following irradiation with NIR laser. Wang et al. (2011) have shown excellent PDT efficacy in tumor-bearing mice upon ‘intratumoral’ injection of UCNP-Ce6 and the followed NIR light exposure (Wang et al., 2011a). They have also demonstrated that the NIR-induced PDT based on UCNP-Ce6 remarkably increased tissue penetration depth compared to the traditional PDT using visible excitation light, offering significantly improved treatment efficacy for deep tumors blocked by thick biological tissues.

Cui et al. (2012) studied *in vivo* therapeutic efficacies of the amphiphilic *N*-succinyl-*N'*-octyl chitosan modified UCNP carrying ZnPc (ZnPc-SOC-UCNP) in PDT NIR light. They showed the prominent anti-tumor effects of the ZnPc-SOC-UCNP on the S180 tumor-bearing mouse model demonstrate the promising prospect of SOC-UCNP as a potential drug delivery system for *in vivo* PDT-mediated cancer therapy based on the upconversion mechanism (Cui et al., 2012). Fadel et al. (2010) evaluated the photodynamic effects of the three ZnPc loaded PLGA NP in tumor-bearing mice and exhibited significantly smaller mean tumor volume, increased tumor growth delay and longer survival compared with the group injected with free ZnPc. Histopathological examination of tumor tissue from mice

PDT treated with PLGA ZnPc showed regression of tumor cells compared to free ZnPc (Fadel et al., 2010).

2.7.2 Two-photon excitation nanomaterials—In contrast to UCNP, simultaneous two-photon excitation requires extremely high power densities that can only be readily achieved with femtosecond (fs) lasers. Two-photon absorption (TPA) was originally proposed by Maria Göppert-Mayer in 1931 in her doctoral dissertation (M., 1931.), but it was not experimentally verified until Werner Kaiser in 1961 (W.K. Kaiser, 1961). Shen et al. (2011) demonstrated two photon excitation NP with fs laser illumination at 800 nm on HepG2 cancer cells (Shen et al., 2011). Gary-Bobo et al. (2011) prepared MSN containing a porphyrin for efficient TPA-PDT (Gary-Bobo et al., 2011). They functionalized MSN surface mannose derivative to target lectins, which are molecules overexpressed by breast cancer cells (MCF-7 and MDA-MB-231) and colon cancer cells (HCT-116). Two-photon excitation-PDT with these MSN was investigated *in vitro* on human breast and colon cancer cells. *In vivo* experiments were also performed on athymic mice bearing xenografted tumors from colon cancer cells (Gary-Bobo, Mir, 2011).

2.7.3 Self-illuminating nanoparticles—Chen and Zhang (2006) (Chen and Zhang, 2006) developed a novel class of nanomaterials for PDT that was termed ‘self-lighting’. It relied on the combination of conventional ionizing radiation (X-rays) for PDT. NP constructed from BaFBr/Eu²⁺ have three emission bands, peaking at around 400, 500, and 640 nm, respectively. The emission spectrum of these NP matches the absorption spectra of most porphyrins such as 5, 15-diphenyl-2-3-dihydroxychlorin, making it suitable for PDT. These NP upon exposure to X-rays emitted scintillation or persistent luminescence activating the PS to produce ¹O₂. This approach has the advantage of penetrating deep into the tumor tissue compared to the visible light; however this has not been much explored.

2.7.4 Magnetic nanoparticles—Magnetic nanoparticles (MNP) have been recognized as a promising tool for the site-specific delivery of drugs and diagnostic agents by an external magnetic field applied outside the body (McCarthy et al., 2007) (Fig 5B). MNP generally consist of a magnetic core (Fe₃O₄) coated with a biocompatible layer and the PS are covalently attached by linkers. Huang et al. (2011) co-precipitated FeSO₄ and FeCl₂ to form 10-nm MNP, functionalized them with amino groups using a silane, and conjugated Ce6 using carbodiimide chemistry (Huang et al., 2011a). Fluorescence microscopy showed uptake into human gastric cancer MGC803 cells, and phototoxicity was observed after 24-h incubation. MGC803 tumors grown in nude mice were imaged by both *in vivo* fluorescence and MRI after intravenous injection of 0.125 μmol Ce6 eq/kg up to 8 h post-injection. *In vivo* PDT was carried out at 8-h time point with 632.8 nm HeNe laser, and significant tumor destruction was observed (Huang, Li, 2011a).

2.7.5. Lipoprotein nanoparticles—Lipoproteins are naturally occurring NP composed of specific apoproteins, phospholipids, and cholesterol on the surface that encapsulate a mixture of cholesterol esters and triglycerides in the hydrophobic core (Wang, Tao, 2011a) (Fig 5C). Low-density lipoprotein (LDL) has innate cancer-targeting potential, as LDL receptors are overexpressed on malignant cells and has been used to incorporate diverse hydrophobic molecules and deliver them to tumors (Glickson et al., 2009, Maziere et al., 1991, Wang, Tao, 2011a). The studies on series of well-defined tetraphenylporphinesulfonates, (TPPS-1, 2A, and 4) and its interaction between LDL were carried out. It was found that compounds with at least two unsulfonated phenyl groups were well-associate with LDL for the effective PDT (de Smidt et al., 1993).

Benzoporphyrin derivative monoacid (BPD) complexed with human LDL was used in PDT of choroidal melanomas experimentally induced in rabbits. PDT treatment with BPD LDL indicated immediate photothrombosis after disintegration of endothelial membranes as revealed by angiography and histologic results (Schmidt-Erfurth et al., 1994). Marotta et al. (2011) encapsulated bacteriochlorins(e6) bisoleate (Bchl-BOA) in LDL to evaluate its PDT efficiency and found phototoxicity in xenografts of HepG2 tumors in nude mice (Marotta et al., 2011). Natural PS like hypericin exhibited influential properties for tumor diagnosis and PDT (Miskovsky, 2002, Sim et al., 2005). It generates a high yield of $^1\text{O}_2$ and other ROS which are associated with photo-oxidative cellular damage and the treatment of nasopharyngeal cancer *in vitro* and *in vivo* models (Olivo et al., 2006, Polo et al., 2002). The selectivity of tumor targeting can be increased by LDL containing PS like amphiphilic hematoporphyrin IX (Hp) and a hydrophobic Zn(II)-phthalocyanine (ZnPc) when combined with human LDL (Huntosova et al., 2010).

3. Clinically approved photosensitizers – stand-alone and nanoparticles

PDT is an excellent modality for the treatment of macular degeneration, cancers, microbial infections and other diseases. Great advances have been achieved in the past few decades in defining the role of PDT in the treatment of different types of cancers. PDT was first approved in 1993 in Canada, using the PS Photofrin for the treatment of bladder cancer. The development of Photofrin was a breakthrough in PDT research, and this PS holds the largest number of approvals for clinical use (Dolmans et al., 2003). Currently, PDT has been approved for clinical treatment in the United States, European Union (EU), Canada, Russia, China and Japan (Biel, 2006, Dolmans, Fukumura, 2003, Dougherty, 1996, Huang, 2005, 2008). United States Food and Drug Administration (FDA) has given approval for Photofrin mediated PDT for treatment of Barrett's esophagus, obstructing esophageal carcinoma and early and obstructing tracheobronchial carcinoma; Levulan (aminolevulinic acid) mediated PDT for actinic keratosis; and BPD mediated PDT for age-related macular degeneration. In the EU, the above noted indications have also been approved in addition to the treatment of early head and neck cancers and palliative treatment of head and neck cancer using the PS Foscan; and treatment of basal and squamous-cell skin cancers using the PS Metvix (Biel, 2006, Dolmans, Fukumura, 2003) (see Figure 6 for a graphical timeline). Several PS for PDT have been developed and approved in China and Russia but not in other countries (Biel, 2006, Huang, 2008). Table 5 summarizes potential PS as stand-alone or nano-assisted drug delivery platforms for different diseases have been approved by the FDA, Canada, UK, China and EU (Biel, 2006, Dolmans, Fukumura, 2003, Dougherty, 1996, Huang, 2005).

Nanomaterials are new class of materials that presents new challenges with changing size, shape, structure and their bio-efficacy. FDA maintains a *status quo* on these nanomaterials, which makes clinical usage of nano-PS not possible in the coming few years. In the mean time FDA approved PS encapsulated in the FDA approved polymers is possible, as this will make the approval process of going through pre-clinical, clinical and post-marketing phases faster (Bawa, 2011). The regulatory challenges faced by FDA in regulating the nanomedical products will be to fit the products into its classification scheme and in maintaining adequate expertise in the field (Miller, 2003). Considering the issues, in the next few years the authors expect FDA approved PS encapsulated in NP would be increasingly used in a clinical setting as well as newly developed third generation PS in nanoparticulate forms.

4 Phototherapy mediated tissue repair and regeneration

The tissue repair and regeneration process is a complex and well-orchestrated set of regular biological responses of the organism to various injuries. LLLT exhibits a variety of biostimulatory effects such as: it reduces pain, inflammation, prevents tissue death and

augments repair and regeneration (Table 6). Various studies have demonstrated that LLLT acts on multiple events of repair and regeneration (de Villiers et al., 2011, Fushimi et al., 2012, Tuby et al., 2007). It has been shown that LLLT induces cell proliferation (Fushimi, Inui, 2012, Hawkins and Abrahamse, 2006, Mvula et al., 2010, Saygun et al., 2012), promotes angiogenesis (Chen et al., 2008), collagen synthesis (Prabhu et al., 2012) and allows the wound site to close more quickly (Demidova-Rice et al., 2007, Fushimi, Inui, 2012). Additionally it was demonstrated that LLLT irradiation results in elevation of antioxidants in the blood and expression of heat shock proteins (Yaakobi et al., 2001). LLLT was also shown to stimulate the expression of multiple genes related to cellular migration, proliferation, anti-apoptosis and pro-survival elements responsive to NFkB (Chen et al., 2011) besides modulating the production of growth factors and cytokines (Fushimi, Inui, 2012, Hawkins and Abrahamse, 2006, Mvula, Moore, 2010, Peplow et al., 2011, Saygun, Nizam, 2012, Zhang et al., 2003).

The basic biological mechanism behind the effects of LLLT is thought to be through absorption of red and NIR light by chromophores, in particular cytochrome c oxidase which is contained in the respiratory chain located within the mitochondria (Karu and Kolyakov, 2005). It is assumed that this absorption of energy may cause photodissociation of inhibitory nitric oxide from cytochrome c oxidase (Lane, 2006) leading to increased enzyme activity and electron transport (Pastore et al., 1994). Furthermore, it has been demonstrated that LLLT enhances mitochondrial respiration, ATP production and increases proton gradient, leading to an increased activity of the Na⁺/H⁺ and Ca²⁺/Na⁺ antiporters, and of all the ATP driven carriers for ions, such as Na⁺/K⁺ ATPase and Ca²⁺ pumps (Karu, 1999). Changes in ion balance leads to a short-term increase in the intracellular pH (Δ pH) is one of the required components involved in transmission of mitogenic signals in cells (Pouyssegur et al., 1985). ATP is the substrate for adenylyl cyclase, and therefore the ATP levels control the amount of cAMP. Ca²⁺, K⁺ and cAMP are very important second messengers, influencing cell proliferation, and Ca²⁺ ions in particular have significant role in regulating gene expression and triggering mitosis (Friedmann et al., 1991). It is suggested that LLLT produces a shift in overall cell redox potential in the direction of greater oxidation (Karu, 1999). Several transcription factors are regulated by changes in cellular redox state such as redox factor-1 (Ref-1)-dependent activator protein-1 (AP-1) (Fos and Jun), NFkB (Chen, Arany, 2011), p53, activating transcription factor/cAMP-response element-binding protein (ATF/ CREB), hypoxia-inducible factor (HIF)-1 α , an HIF-like factor (Mucaj et al., 2012). In turn, LLLT by altering redox state can induce the activation of numerous intracellular signaling pathways, alter the affinity of transcription factors for DNA, increase RNA and DNA synthesis, regulate nucleic acid and protein synthesis, enzyme activation and cell cycle progression (Liu et al., 2005) (see Fig. 7 for a graphical illustration of the pathways).

Stem and progenitor cells have the ability to continuously self-renew, proliferate and give rise to one or more differentiated cell types and hence are being considered as a therapeutic option in reparative and regenerative medicine (Mvula et al., 2008, Verma, Domb, 2011). Due to the fact that stem cells grow and proliferate at very slow rates, and their harvest yields are low, new modalities which can increase their proliferation and still maintain their stem cell character is necessary (de Villiers, Houreld, 2011). Studies on LLLT and stem cells have demonstrated that LLLT can change metabolism of stem cells, promote their migration, proliferation, differentiation and increase their viability (de Villiers, Houreld, 2011). LLLT was also found to activate early cell cycle regulatory genes, mitogen activated protein kinase (MAPK) and extracellular signal regulated kinase (ERK) cascades in mesenchymal and cardiac stem cells (Tuby, Maltz, 2007). The hypoxic milieu of stem cells induces proliferation, migration, paracrine activities and survival of stem cells via generation of ROS (Jeong et al., 2012). It is thought that LLLT like hypoxia, by generating ROS may have photostimulatory effects on stem cells. Bioelectrical signals generated by ion channels

also have crucial roles in stem cells (Li and Deng, 2011). LLLT through modulation of activity of multiple ion channels present in stem cells might be responsible in promoting proliferation, migration and differentiation of stem cells (Li and Deng, 2011).

In PDT, administration of PS either topically or systemically and irradiation of wounds with laser light of suitable wavelength to excite PS in a way to give an optimum response, may offer a promising treatment modality to promote healing, especially in chronic wounds such as diabetic foot ulcers and decubitus ulcers (Peplow et al., 2012) (Table 6). Since healing of chronic wounds is compromised by infection, one reason for choosing PDT as a potential future treatment is its anti-microbial action (Peplow, Chung, 2012). However, it should be noted that, high concentrations of ROS cause mammalian cell death by ATP depletion and lipid peroxidation, and only controlled and relatively low concentrations of ROS can trigger cellular processes and proliferation (Peplow, Chung, 2012). This is true for both in case of LLLT and PDT. In LLLT, studies performed on different laser wavelengths and fluences showed that the effects of laser on tissue depend on irradiation dosage parameters and may cause either stimulation (2–16 J/cm²), zero bioactivation (20 J/cm²) or inhibition (24–28 J/cm²) (Silva et al., 2004b). In PDT, it is also essential to use the appropriate dosimetry of both the PS and the activating light source, since high doses cause cell death such as when used as a cancer therapy modality and low doses cause tissue regeneration (Peplow, Chung, 2012).

3. Nanotechnology used for tissue repair and regeneration

With a striking increase occurring in the world's aged population, the demands for various biomedical approaches or implants to repair injured, non-functional or lost-tissues are increasing. However, conventional tissue replacements such as synthetic products, auto-grafts and allo-grafts have their own limitations which cannot fully satisfy the demands of the patients. Consequently, tissue engineering emerged as a possible alternative to implants and current healing modalities. The combination of nanotechnology and tissue engineering creates a highly synergistic effect for regenerative medicine (Verma, Domb, 2011) (Table 7). With the aid of nanotechnology, the development of structures at the molecular level is enabled which, provides improved interactions between biomaterial surfaces and biological entities (Engel et al., 2008). To successfully achieve tissue regeneration, it is not only necessary to increase the number of cells constituting the tissue but it is also necessary to reconstruct a native like extra cellular matrix (ECM) called tissue scaffold that physically supports the cells and provides chemical and mechanical cues which will induce cells to exhibit migration, adhesion, proliferation and differentiation that is characteristic of the desired specific tissue. Surfaces, structures and biomaterials with nanoscale features produced by nanotechnology can mimic the natural environment of cells (biomimetic), provide dynamic control of soluble and surface-bound cytokines, cell to cell interactions, physico-chemical cues, furthermore increase mechanical strength in scaffolds which is necessary in structures such as bone and cartilage (Kim and Fisher, 2007, Martino et al., 2012). Two main applications of nanotechnology in tissue regeneration are: a) delivery of drugs, growth factors, genes and DNA, b) construction of tissue scaffolds through nanofibers. 3D scaffolds replacing the natural ECM made out of synthetic or natural biomaterials or in combination of both, are usually seeded with stem cells harvested from the patient and expanded with the help of growth factors, to be used as a tissue matrix to regenerate complex organs either *in vivo* or *in vitro*.

Nanotechnology plays a role in the migration, proliferation and differentiation of progenitor cells (Martino, D'Angelo, 2012). However, the process of harvesting and expanding stem cells is still faced with great efficacy and efficiency problems. While nanotechnology has the potential to overcome many limitations of tissue regeneration, challenges are still faced

in this field. The main goals of tissue engineering are: production of biodegradable nanomaterials, achieving cell viability, stimulating migration, proliferation, differentiation and controlling organization of cells, producing a vascularized growing cell mass and accomplishing proper cell function in the new construct (Vacanti, 2006). Surface chemistry, morphology, mechanical as well as electrical properties of the nanomaterials used, is main important factors in tissue engineering (Martino, D'Angelo, 2012). Cells are required to have some sort of cell-substrate adhesion in order to survive. Through alteration of surface roughness and/or chemical characteristics of nanomaterials, hydrophobicity can be induced and consequently different proliferation and adhesion rates of cells can be achieved (Dulgar-Tulloch et al., 2009, Elena Fadeeva, 2009, Fadeeva, 2010, Ranella et al., 2010). Right surface topography needs to be determined for stem cell differentiation towards each desired lineage (Martino, D'Angelo, 2012). Different cell types have been shown to react differently to various surface roughness and surface energies (Elena Fadeeva, 2009, Fadeeva, 2010, Ranella, Barberoglou, 2010). Cells on nanoscale surfaces have been found to exhibit less stress fibers and focal adhesions than those on microscale surfaces (Teixeira et al., 2004). Further, surface roughness of the scaffold was found to be more ideal on nanoscale than microscale surfaces (Fadeeva, 2010). Nanoscale grooves of varying periodicities and depths and the grain size of certain nanomaterials are also important factors in cell proliferation, adhesion and orientation (Dulgar-Tulloch, Bizios, 2009, Rebollar et al., 2008, Teixeira, Nealey, 2004). Formation of electrical charges through electroactive polymers were shown stimulate either the proliferation or differentiation of various cell types (Martino, D'Angelo, 2012). Additionally, mass transport is necessary to achieve a successful scaffold since without receiving nutrients and removing wastes; cells in a scaffold are not able to survive long-term. While in a native tissue vasculature is responsible for the mass transport, producing a vascularized system *in vitro* scaffold has been a challenge (Borenstein J.T.1, 2002, Karande et al., 2004). An alternative method has been tested by producing high porosity in scaffolds that enabled the required mass transport, but the trade-off between high porosity (80–90% porosity was found to be ideal) and mechanical integrity is a double edged sword. Producing a pre-vascularized scaffold is still the future goal since it will also aid in integration with the host tissue (Karande, Ong, 2004).

4. Phototherapy in conjunction with nanotechnology used for tissue repair and regeneration

The applications of light to nanomaterials is evident because many compounds have been synthesized to explore the potential to kill cancer cells and microbial infections and, more recently, to improve the tissue repair and regeneration processes (Primo et al., 2011, Silva, Lacava, 2004b). The wavelengths more commonly used for tissue repair and regeneration are in the red and NIR bands of the spectrum (Hourel and Abrahamse, 2010, Silveira et al., 2007). However, recent studies showing that other wavelengths may also have photostimulatory effects, such as blue (Adamskaya et al., 2011) and green (de Sousa et al., 2010, Fushimi, Inui, 2012) light. Different dosage regimens of light irradiation are required for different clinical applications, which have been recently reviewed by the World Association for Laser Therapy (Bjordan, 2012). Even though yet not so common, combined usage of phototherapy in conjunction with nanotechnology in tissue repair and regeneration (both for soft and hard tissues), often has synergistic and if not, complementary effects.

PS are responsible for transfer and translation of light energy, however maintaining a controlled release is a challenge which can be overcome by usage of nanocarriers (Primo, da Costa Reis, 2011). Recently, this method has been applied *in vitro* on 3D culture of fibroblasts and mesenchymal stem cells (MSCs) using nanoemulsion (with chloroaluminum phthalocyanine) and LLLT on a skin equivalent, which resulted in positive healing effects (Primo, da Costa Reis, 2011). In another study, LLLT (685nm, 35mW, 10 J/cm²)

demonstrated positive photobiomodulation effects as evidenced by stimulated vascularization, fibroblast proliferation and collagen deposition at its initial stages of bone tissue regeneration surrounding the nanomaterial based implants (poly-L-lactic/ polyglycolic acid) in the femur of rats (Freddo et al., 2009). Besides reducing inflammation, when LLLT (830nm, 100 mW/cm², 60 J/cm²) was used post-surgery for bone-fracture consolidation in tibias of osteoporotic rats, in conjunction with Biosilicate®, (a fully crystallized bioactive glass ceramic fillers), a significantly increased deposition of new bone tissue and improved biomechanical properties were observed compared to without nanomaterial-assisted device (Fangel et al., 2011). Nissan et al. (2006) demonstrated that GaAs laser-therapy (904nm, 0.72 J, 4 mW/cm²) resulted in new bone formation around hydroxyapatite implants and led to significant increase in the total bone area in rat mandibles by affecting calcium transport during new bone formation (Nissan et al., 2006). In another study, when efficacy of GaAlAs and HeNe laser irradiation in the treatment of bone defects in rat mandibles filled with biocomposite BCP/PLGA (bi-calcium phosphate/poly(lactide-co-glycolide)) was analyzed, the biostimulatory effect of LLLT on BCP/PLGA biomaterial osseointegration and early bone repair was reported. Furthermore, the presence of large amounts of collagen fibers on light irradiated tissue was observed, and this might be due to the positive photostimulatory effect of LLLT on bone repair, since several other studies also demonstrated increase collagen accumulation following LLLT (R. Obradovic, 2007).

Previously it has been mentioned that, in response to suitable microenvironments and/or stimuli, MSCs can be expanded and terminally differentiated into osteoblasts, chondrocytes, adipocytes, myoblasts, neural cells, etc. (Abramovitch-Gottlib et al., 2005). Synergistic effects of combined nano-assisted biomatrix and LLLT have been demonstrated in a few studies, where enhanced differentiation toward osteoblasts was observed (Abramovitch-Gottlib, Gross, 2005, Green et al., 2009). A brief 10 min daily nanoparticle-facilitated exposure of MSCs to nanosecond pulse laser-induced photoacoustic stimulation enhanced differentiation toward osteoblasts (Green, Longtin, 2009). Fibroblast-populated collagen lattices (FPCL) are frequently being used in tissue-engineered skin replacements however, their main drawback is that interaction of fibroblasts and ECM causes contraction of the lattice, reducing its original area. The effect of He-Ne laser (632 nm, 3 J/cm²) on the behavior of 3T3 fibroblasts seeded in collagen lattices containing 20% chondroitin-6-sulphate was investigated and the results indicated a controlled contraction of FPCL caused by LLLT (Ho et al., 2009).

5. Nanotoxicology

Although nanotechnology is promising, the use of nanomaterials is also controversial; some scientists support their use while others are against it. This is mainly due to the lack of information of the effect of NP on human health and environment. The exposure to NP is expected to increase and it is difficult to test and to estimate their effect on human health and their impact on environment. At present there are not available clear guideline(s) on the use of nanomaterials. The recent advancement in the nanotechnology field and the widespread biomedical and industrial applications of NP has led to increased interest in their possible deleterious effect; which further has given rise to a whole new area of nanotoxicology (Hubbs et al., 2011). Nanotoxicity can be caused through inhalation, ingestion, skin uptake, and injection of engineered NP. Importantly, NP toxicity depends on multiple factors derived from both individual NP physicochemical properties and environmental conditions, NP size, charge, concentration, outer coating bioactivity (capping material, functional groups), and oxidative, photolytic, and mechanical stability have each been shown to be determining factors in NP toxicity (Krug and Wick, 2011). The greater surface area per mass compared with larger-sized particles of the same chemistry renders NP more active biologically. The surfaces of nanostructures are involved in catalytic and oxidative reaction

that could induce cytotoxicity. Moreover, some NP contains metals or compounds with known toxicity and they could induce, in consequence to a breakdown, a similar toxic response from the cells. The main molecular mechanism of *in vivo* nanotoxicology is the induction of oxidative stress by free radical formation (Lanone and Boczkowski, 2006). Oxidative stress may have a role in the induction of inflammation by regulation of redox sensitive transcription factor (Dobrovolskaia and McNeil, 2007).

Nanoparticle toxicologists study the brain, blood, liver, skin, and gut in addition to the traditional concentration on lung toxicity (Meng et al., 2009). It has long been known that asbestos fibers cause the lung diseases asbestosis and mesothelioma when inhaled, and several authors have asked whether single-walled CNT (which have similar characteristics to asbestosis fibers) can have similar adverse effects (Palomaki et al., 2011). Reports that fullerenes exhibited toxicity toward various fish (Fraser et al., 2011, Jovanovic et al., 2011) have been discounted by other authors (Henry et al., 2011) and attributed to the presence of a tetrahydrofuran bio-product introduced during fullerene preparation (Kovochich et al., 2009). Furthermore, the possible use of materials such as CdSe (Khalil et al., 2011) and cadmium telluride (CdTe) (Tino et al., 2011) in QD construction has also led to concerns about heavy metal toxicity, which would preclude any clinical use. These studies concluded that QD could cause indirect *in vivo* genetic damage, which may be attributed to free radical-induced oxidative stress in mice. Possible phototoxicity is a key feature that must be taken into account when choosing the material to be used to facilitate healing, especially when it reacts with the presence of light (Yao et al., 2010).

Studies about the effect of NP after absorption, inhalation and ingestion have been carried out. Nanocrystalline magnesium oxide and titanium dioxide did not show dermal absorption (van der Merwe et al., 2009). In contrast, other studies demonstrated penetration and translocation of NP in the systemic vasculature (Lademann et al., 1999, Oberdorster et al., 2005). Although nanosilver-based dressings and surgical sutures have been approved for clinical application, their dermal toxicity is still little known. Some studies confirmed the dermal biocompatibility of nanosilver (Chen et al., 2006, Muangman et al., 2006) while other researchers have demonstrated the cytotoxicity of these materials (Paddle-Ledinek et al., 2006). Moreover other studies have demonstrated silver NP induced cytotoxicity to alveolar macrophage cells and epithelial lung cells (Soto et al., 2007). Few studies have been carried out on toxicity of NP post-ingestion. The acute toxicity of nanocopper was reported in some studies and also reported to damage liver, kidney and spleen (Chen, Meng, 2006). Chung et al. (2010) recently reported occurrence of systemic argyria after ingestion of colloidal nanosilver proves its translocation from the intestinal tract (Chung et al., 2010).

Before nanomaterials will be extensively used in human applications it will be highly important to have more consistent and comprehensive studies about their harmlessness. For this reason in the last few years many *in vitro* and *in vivo* studies have been focused on investigating and understanding the kinetics and toxicology of the NP to test their efficacy and also to establish guidelines and procedures to guarantee greater safety for manufacturing and regulating their use safely.

Conclusion and future perspectives

Phototherapy (LLLT/ PDT) has emerged as one of the important therapeutic options in management of cancer, macular degeneration, and microbial infection, while it also reduces inflammation, pain and augments tissue repair and regeneration. The enhancement of phototherapy using nanomaterials is a vibrant multi-modal research area. From photocatalysis and photovoltaics, to photodiagnosis and phototherapy, the interaction of light with NP is an area of research that is proving to be increasingly important. Recent

reports have described the potential use of nanocarriers with additional active complementary and supplementary roles in phototherapy. Still, both phototherapy and nanotechnology are facing challenges on their own; however, a combination of these two potential technologies clearly has developed as an emerging area in its own right. Approaches for a combination of nanotechnology in conjunction with phototherapy for tissue repair and regeneration have also been developed (Abramovitch-Gottlib, Gross, 2005, Green, Longtin, 2009). Nevertheless, basic aspects of synergism between nanotechnology and phototherapy remain to be investigated in more detail. Often the *in vivo* fate of NP is unknown; the problem of long-term effects has been noted and more importantly in the context of PDT, light effects are often neglected (Allison et al., 2008). Investigation into so-called nanotoxicity should focus on long-term exposure in humans, animals and the environment.

The interest in nanobiosensor technology is increasing. Nanobiosensors compared to the current tests used in diagnosis to identify potential markers of diseases can offer several advantages. Nanobiosensors can detect lower concentrations of molecules and also with high specificity, provide a rapid screening method using small samples of patient material with lower-cost (Bellan et al., 2011). Recently in the area of 'theranostics', NP have shown a great potential to be used as multi-modal tools with functionality as both a contrast agent for imaging and a therapeutic agent for treatment of infectious microorganisms and cancerous tissue (Lee and Li, 2011). Although the therapeutic functionality can take the form of attached cytotoxic drugs that are released from the NP (Janib et al., 2010) or therapeutic radioisotopes (Grimm and Scheinberg, 2011), many groups have constructed a light-activated modality into their multi-functional NP that is either photothermal or photodynamic (or even both at the same time). This property of multi-functionality is one of the areas where the concepts of nanotechnology will be critical in further progress. The use of active targeting moieties to direct PS-loaded NP to tumors or other biological targets is another rapidly growing area where nanotechnology may provide a distinct advantage over traditional conjugation methodology (Huang, Sharma, 2012, Paszko, Ehrhardt, 2011).

Nano-assisted regenerative medicine in conjugation with phototherapy has the potential to create a paradigm shift in the healthcare management in the future. Recently, studies have demonstrated the applications of phototherapy in conjunction with nanotechnology in tissue repair and regeneration, which often has synergistic and if not, additive effects. A multi-disciplinary approach will not only help to treat infection, cancer and other diseases which require removal of the unwanted tissue but it will also aid in tissue repair and regeneration. The advances in this combined field will have a direct consequence on whether or not the far-fetched dream of engineered repair and regeneration will be achieved.

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Fig. 1. Schematic illustration of photodynamic action (Jablonski diagram)

The PS initially absorbs a photon that excites it to the short-lived excited singlet state. This can lose energy by fluorescence, internal conversion to heat, or by intersystem crossing to the long-lived triplet state. This triplet PS can interact with molecular oxygen in two pathways, type 1 and type 2, leading to the formation of reactive oxygen species (ROS) and singlet oxygen (1O_2) respectively.

Fig. 2. Schematic representation of various nanoparticles with photosensitizer (PS) used in photodynamic therapy

(A) Liposome containing lipid soluble PS in hydrophobic lipid bilayer. (B) Polymeric dendrimer with PS. (C) Silica nanoparticle (SiNP) doped with PS. (D) Gold nanoshell encapsulated with PS. (E) Typical quantum dot (QD) with core shell, followed by a biocompatible polymer coating. PS is covalently attached to the QD that can take part in FRET with the emission of the QD being used to excite the PS.

Fig. 3. Combined applications of phototherapy, nanotechnology and, reparative and regenerative medicine
ECM, extra cellular matrix.

Fig. 4. Fullerene photodynamic therapy for disseminated peritoneal cancer in mice
(A) Novel skin flap model to allow illumination of the abdominal cavity with white light.
(B) Skin flap can be readily closed after illumination. **(C)** Non-invasive bioluminescence imaging of mouse colon carcinoma engineered to express luciferase after no treatment or after BF₄ fullerene-mediated PDT. **(D)** Quantification of bioluminescence signals from **(C)**. **(E)** Kaplan–Meier survival plot of mice treated with white, green, or red light (100 J/cm²) 24 h after injection of 5 mg/kg BF₄. **(F)** mono-pyrrolidinium fullerene (BF₄). (Reproduced with permission from reference (Mroz et al., 2011). Copyright 2011 Elsevier).

Fig. 5. (A) Schematic representation showing near-infrared irradiation on nanoparticle (NP) induces upconversion (UC) which emits visible light activating the coated photosensitizer (PS) to generate singlet oxygen ($^1\text{O}_2$). (B) Multi-functional NP with magnetite core (Fe_3O_4) coated with biocompatible layer and PS is covalently attached by linker. (C) Lipoprotein NP consists of hydrophobic PS, specific apoprotein, cholesterol esters, triglycerides and cholesterol used as a drug carrier for photodynamic therapy.

Figure 6.
Historical timeline of clinical approvals of photosensitizers used for photodynamic therapy

Figure 7. A possible mechanism of action of low-level laser (or light) therapy (LLLT) mediated repair and regeneration

Light is initially absorbed by mitochondrial chromophore (cytochrome c oxidase) and causes increase production of ATP, reactive oxygen species (ROS) and nitric oxide (NO), which in turn cause changes in cellular redox potential, Ca²⁺, K⁺, cAMP and pH levels and induce several transcription factors (Ref-1, AP-1, NFκB, HIF-1α). The photosignal transduction and amplification chain induced by light leads to an increase of cell proliferation (stimulation of DNA and RNA synthesis), cell motility, production of growth factors and extra cellular matrix accumulation.

Table 1

Potential lipid-based nanoparticles, micelles and nanoemulsions being used for photodynamic therapy and their results

Lipid-based nanocarriers	Type of nanomaterials used	<i>In vitro/ in vivo/ clinical</i>	Study findings	Ref
Verteporfin	Visudyne, a liposomal composition of benzoporphyrin derivative mono acid ring A (BPD) (a semi synthetic chlorin derived from protoporphyrin IX)	Clinical	Visudyne widely used in ophthalmology as PS in combination with transpupillary red laser for destroying neo-vasculature in the eye secondary to disease such as wet age-related macular degeneration (AMD)	(1999, Christie and Kompella, 2008, Cruess et al., 2009)
		<i>In vivo</i>	Approved by FDA (2000) for treatment of AMD; and pathological myopia (2001) A combination of Verteporfin PDT and immunosuppression has shown promise for inflammatory subfoveal choroidal neovascular degeneration (CNV) Liposomal BPD with PDT destroyed lymphatic vessels in tumors	(Hogan et al., 2005) (Tammela et al., 2011)
Liposomal zinc(II) phthalocyanine	Composed of palmitoyl-oleoyl-phosphatidylcholine and dioleoyl phosphatidylserine	Clinical	Tested in clinical trials of PDT for squamous cell carcinomas of the upper aero-digestive tract; but has not received approval from regulatory authorities	(Ochsner, 1996)
Liposomal mTHPC (Foslip)	DPPC/DPPG liposomal formulation of meso-tetra (hydroxyphenyl) chlorin (mTHPC)	Both	Potentially used as intratumoral injection of liposomal Foslip in mouse model of local recurrence of breast cancer; photothrombotic activity	(D'Hallewin et al., 2008)
Liposomal photofrin	Unilamellar vesicle of DPPC	<i>In vivo</i>	Exhibited enhanced uptake into tumor after PDT; prevent aggregation of photofrin	(Jiang et al., 1998)
Liposomal hypocrelin A (LHA)	Unilamellar liposome	<i>In vivo</i>	LHA achieved a higher tumor to-normal tissue ratio and higher maximal levels within the tumor cells after PDT	(Wang et al., 1999)
Liposomal photofrin (LPF)	Multilamellar liposomes	<i>In vivo</i>	Higher apoptotic index of tumor after PDT	(Igarashi et al., 2003)

Lipid-based nanocarriers	Type of nanomaterials used	In vitro/ in vivo/ clinical	Study findings	Ref
Liposomal fullerenes	Prepared via the fullerene exchange method from cyclodextrin	<i>In vitro</i>	Low toxicity in the dark and PDT activity against HeLa cells; C70 derivatives showed higher photodynamic activity than C60 derivatives	(Doi et al., 2008)
Porphyrin incorporated poly-cationic liposome	Cationic lipids such as <i>N</i> -[1-(2,3-dioleoyloxy)propyl]- <i>N</i> , <i>N</i> , <i>N</i> -trimethylammonium chloride	<i>In vitro</i>	Efficient anti-microbial PDI of MRSA	(Ferro et al., 2007)
Liposomal aluminum-chloride-phthalocyanine (AlClPc)	Cationic liposomes	<i>In vitro</i> clinical	Preferentially absorbed by bacterial cells compared to eukaryotic dental pulp cells; clinical trial showed significant reduction of bacterial load in dental caries lesions after PDT	(Longo et al., 2012)
Lipoplex with negatively charged chlorin e6 (Ce6)	Cationic lipoplex	<i>In vivo</i>	Selective targeting to tumor and improved therapeutic effects on cancer after PDT compared to free Ce6	(Shim et al., 2011)
Foscan nanoemulsion	Composed of <i>m</i> THPC in soy-phosphatidylcholine Epikuron 170/Tween 80 and nonionic surfactants	Both	Topical application of nanoemulsion PS to skin and other mucosal surface; PDT for skin cancer	(Primo et al., 2008a)
Magnetic nanoemulsion	Composed of biodegradable surfactants and biocompatible citrate-coated cobalt ferrite-based magnetic fluid to encapsulate Zn-phthalocyanine	<i>In vitro</i>	Used in synergistic PDT and magnetohyperthermia therapies of neoplastic cells	(Primo et al., 2008b)
Porphysome nanovesicles	Hybrid of self-assembled lipid NP and porphyrin (PS)	<i>In vivo</i>	Multi-modal potential; enabled the sensitive visualization of lymphatic systems using photoacoustic tomography; accumulated in tumors of xenograft-bearing mice; laser irradiation induced photothermal tumor ablation	(Lovell et al., 2011)
Polymeric micelles	PEG-PLA micelles encapsulating <i>m</i> THPC	<i>In vitro</i>	Used in PDT for head and neck cancer cells	(Ding et al., 2011)
pH-sensitive polymeric micelles	N-isopropylacrylamide (NIPAM) copolymer micelles encapsulating aluminum chloride phthalocyanine (Alps)	Both	Exhibited substantially low cell cytotoxicity and enhanced anti-cancer activity	(Le Garrec et al., 2002)
PEG-lipid micelles	tetraphenylporphyrin (TPP)-loaded PEG-phosphatidyl ethanolamine (PE) micelles additionally	<i>In vivo</i>	Improved anti-cancer effect after PDT against murine Lewis lung carcinoma	(Roby et al., 2007)

Lipid-based nanocarriers	Type of nanomaterials used	<i>In vitro/ in vivo/ clinical</i>	Study findings	Ref
	modified with tumor-specific monoclonal 2C5 antibody			

DPPC, dipalmitoylphosphatidylcholine; DPPG, dipalmitoylphosphatidylglycerol; NP, nanoparticle; PDI, photodynamic inactivation; PDT, photodynamic therapy.

Table 2
Potential polymeric nanocarriers being used for photodynamic therapy and their results

Polymeric nanocarriers	PS encapsulated	Natural/ synthetic	<i>In vitro/ in vivo / clinical</i>	Study findings	Ref
Marine atelocollagen/xanthan gum	5, 10, 15-triphenyl-20-(3- <i>N</i> methylpyridinium)porphyrin	Natural	<i>In vitro</i>	Exhibited enhanced photodynamic activity; polymeric nano carrier found more phototoxic than respective phosphatidylcholine lipid emulsion	(Deda, Uchoa, 2009)
Aerosol OT (AOT)-alginate	Methylene blue (MB)	Natural	<i>In vitro</i>	Encapsulated MB in AOT-alginate significantly enhanced intracellular ROS production, and overall cytotoxicity following PDT in human breast cancer cells (MCF-7) and mouse metastatic mammary tumor cells (4T1)	(Khdaif, Gerard, 2008)
Hydrophobically modified glycol chitosan (HGC); conjugated chitosans (GC)	Chlorine6 (Ce6)	Natural	Both	GC-Ce6 showed great potential in HT-29 tumor-bearing mice after PDT; exhibited <i>in vivo</i> burst release of drugs and NP stabilities for cancer therapy	(Lee et al., 2011)
Poly(lactic acid) (PLA)	Hypericin (Hy)	Synthetic	<i>In vitro</i>	Higher photoactivity exhibited by Hy-loaded PLA NP compared to free drug in NuTu-19 ovarian cancer cell	(Zeisser-Labouebe, Lange, 2006)
PLGA	Bacteriochlorophyll-a (BChl-a)	Synthetic	<i>In vitro</i>	PLGA-BChl-a in macrophage cell line showed enhanced drug delivery efficacy after PDT	(Gomes et al., 2007, Gomes et al., 2005)
Poly (d, l-lactic acid)-PLA	Porphyrins; m-TPP; m-TCPP; chlorin; Pheo-a; Ce6	Synthetic	<i>In vivo</i>	Photothrombotic activity in chick chorioallantoic membrane (CAM) model	(Pegaz et al., 2005)

Polymeric nanocarriers	PS encapsulated	Natural/ synthetic	<i>In vitro/ in vivo / clinical</i>	Study findings	Ref
Hyperbranched polyether-ester, (HPPEE)	Ce6	Synthetic	<i>In vitro</i>	Phototoxicity effect and uptake of HPPEE- ce6 towards human oral tongue cancer CAL-27 cells	(Li et al., 2012)
Polyacrylamide (PAA)	m-THPC	Synthetic	<i>In vivo</i>	Rat C6 glioma cells killed after PDT	(Gao et al., 2007)
PEG-PLL (poly-L-lysine-PEG)	Dendrimeric phthalocyanines (DPc); encapsulated polymeric micelle (DPc/m)	Synthetic	Both	Nude mice with A549 tumors (A549 human lung cancer cells) treated with DPc/m- PDT showed much better tumor response than DPc	(Nishiyama, Nakagishi, 2009)
Polymethacrylic acid (PMAA); N-sulfonato-N,O-carboxymethylchitosan (NOCCS)	meso-tetra (hydroxyphenyl)porphyrin (<i>m</i> -THPP)	Both	<i>In vitro</i>	mTHPP loaded NOCCS/PMMA exhibited more phototoxicity in 14C-carcinoma cells compared with free mTHPP and liposomal mTHPP	(Reza Saboktakin et al., 2011)
PEG coated PLA	Hexadecafluoro zinc phthalocyanine (ZnPcF16)	Synthetic	<i>In vivo</i>	ZnPcF16 loaded PEG-coated PLA compared to ZnPc on EMT-6 tumor bearing mice showed improved PDT response	(Allemann et al., 1996)
PAA	Photofrin	Synthetic	Both	Targeted detection and cancer treatment of glioma-bearing rats treated with NP followed by PDT showed significant improvement compared with administration of non-targeted NP or systemic photofrin after PDT	(Reddy et al., 2006)
PEG-PE	meso-tetraphenylporphine (TPP)	Synthetic	<i>In vivo</i>	Anti-cancer effect of TPP-loaded polymeric micelles with tumor-specific antibodies after PDT against murine Lewis lung carcinoma in C57BL/6 mice	(Roby, Erdogan, 2007)

Polymeric nanocarriers	PS encapsulated	Natural/ synthetic	<i>In vitro/ in vivo / clinical</i>	Study findings	Ref
PLGA	Zinc phthalocyanine (ZnPc)	Synthetic	<i>In vivo</i>	Significantly smaller mean tumor volume, increased tumor growth delay and longer survival compared with free ZnPc	(Fadel, Kassab, 2010)
PLGA	meso-tetra(<i>p</i> -hydroxyphenyl) porphyrin (<i>p</i> -THPP)	Synthetic	<i>In vivo</i>	<i>p</i> -THPP/PLGA showed enhanced photodynamic activity against mammary tumor cells compared to free drug on CAM model	(Vargas et al., 2004)
PLGA	<i>m</i> -THPP	Synthetic	<i>In vivo</i>	Smallest size NP (117 nm) exhibited highest rate of ROS production, fastest m-THPP release in CAM model after PDT	(Vargas et al., 2009)
PEGylated PLGA	Temoporfin	Synthetic	Both	Temoporfin encapsulated in PEGylated PLGA exhibited significantly reduced cellular uptake of NP; lower dark cytotoxicity; favorable <i>in vivo</i> tissue distribution after PDT	(Rojnik et al., 2012)
Conjugated glycol chitosan (GC)	Protoporphyrin IX (PpIX)	Both	<i>In vivo</i>	PpIX-GC-NP showed prolonged blood circulation, enhanced tumor targeting ability, and improved therapeutic efficiency in tumor-bearing mice, compared to free PpIX	[184]
PLGA and PLA	(<i>p</i> -THPP)	Synthetic	<i>In vitro</i>	<i>p</i> -THPP photocytotoxic efficiency of nanoparticles evaluated on EMT-6 mammary tumor cells was more compared to free drug	[26]
PEG-PE	TPP	Synthetic	Both	PDT on B-16 melanoma cells and	[192]

Polymeric nanocarriers	PS encapsulated	Natural/ synthetic	<i>In vitro/ in vivo / clinical</i>	Study findings	Ref
PEG-PE	TPP	Synthetic	<i>In vitro</i>	<p>melanoma-bearing C57BL/6 mice with laser light 9 h after the administration of the micellar TPP resulted in significant tumor inhibition</p> <p>PDT with PS, TPP-containing PEG-PE micelles, had anticancer effects against murine (LLC, B16) and human (MCF-7, BT20) cancer cells</p>	[193]

NP, nanoparticle; m-TCPP, meso-tetra-(4 carboxyphenyl)-porphyrin; m-THPC, *meta*-tetra(hydroxyphenyl)-chlorin; m-TPP, meso-tetraphenylporphyrin; Pheo-a, pheophorbide-a; PE, phosphatidyl ethanolamine conjugate; PEG, polyethylene glycol; PLGA, poly-(D,L-lactide-co-glycolide); PDT, photodynamic therapy; PS, photosensitizer.

Table 3
Potential fullerene derivatives as photosensitizer being used for photodynamic therapy and their results

Fullerene based nanomaterial	Nanomaterial description	Study findings	In vitro/ in vivo	Ref
C60-PEG	Polyethylene-Glycol	C60-PEG in mice carrying subcutaneous tumor showed increased anti-tumor effect with increasing irradiation power and C60 dose	In vivo	(Tabata et al., 1997)
C60-PEG-DTPA-Gd	C60-Polyethylene glycol-diethylenetriaminepentaacetic acid (DTPA)-gadolinium (Gd)	Significant increase in tumor PDT effect with increasing fluence	In vivo	(Liu et al., 2007)
BF1, BF2, BF3, BF5	-	Studies on cancer cell lines (J774, LLC and CT 26) showed killing at high fluences	In vitro	(Mroz et al., 2007a)
BF4	Mono-(dimethylpyrrolidinium) fullerene	Effect of PDT with fullerene to treat intraperitoneally disseminated metastatic colon cancer	In vivo	(Mroz, Xia, 2011)
BF6	Tris-(dimethylpyrrolidinium) fullerene	Synergistic therapeutic effect of PDT and suboptimal dose of the antibiotic tobramycin on mice infected with highly aggressive and invasive <i>P. aeruginosa</i> infection	In vivo	(Lu, Dai, 2010)
C6-(Glc)	Glycoconjugated-C60	Studies on human-melanoma (COLO679)-xenograft bearing mice showed tumor suppression with high doses of C6-(Glc)	In vivo	Otake, Sakuma et al. 2010
FC4S	Hexa(sulfo-n-butyl)-C60	Consistent inhibition of the tumor growth was found to be more effective using a lower wavelength (515 nm) laser was better absorbed than 633 nm)	In vivo	(Chi Y, 1999)
C60-PEG	C60-polyethylene glycol (PEG)	Infusion of a high concentration of fullerene- PEG followed by photoillumination did not result in suppression of atherosclerosis in but rather in a progression of the atherosclerosis lesion in rabbit model. However, this intervention showed no adverse effects on the normal iliac artery	In vivo	(30)

BF1-6, Buckminster fullerene cationic derivatives (1-6); PDT, photodynamic therapy.

Table 4

Potential actively-targeted nanocarriers being used for photodynamic therapy (PDT) and their results

Actively-targeted nanocarrier	Markers/ ligands	<i>In vivo/ in vitro</i>	Study findings	Ref
Antibody-phthalocyanine-Au NP conjugates	Monoclonal antibodies (MoAb)	<i>In vitro</i>	Selective targeting of breast cancer cells that overexpress HER2EGF	(Huang, Sharma, 2012)
Anti-EGFR MoAb-verteporfin loaded NP	MoAb	Both	A431 cells <i>in vitro</i> , tumor size <i>in vivo</i> experiments showed significant decrease compared with tumors treated with verteporfin-NP complex without the antibody	(Kameyama et al., 2011)
Fullerene hexa-adduct conjugated with MoAb rituximab	MoAb	<i>In vitro</i>	T-43 MoAb was used to demonstrate a MoAb-dependent enhancement of the uptake by a bladder tumor cell line of a pheophorbide-a liposomes	(Bergstrom et al., 1994, Rancanet al., 2007)
Bacteriophage MS2 virus modified Porphyrin	Aptamer	<i>In vitro</i>	MS2 was used to construct a targeted, multivalent photodynamic therapy vehicle for the treatment of Jurkat leukemia T cells.	(Stephanopoulos et al., 2010)
folic acid-conjugated GO	Folate	<i>In vitro</i>	Findings suggest that folic acid has great potential as an effective drug-delivery system in targeting PDT	(Huang, Xu, 2011b)
F3 peptide groups with MB-conjugated polymeric nanoparticles	Peptide	<i>In vitro</i>	Selective targeting to tumor cells and angiogenic vasculature	(Hah et al., 2011, Qin et al., 2011)
F3 peptide with multifunctional polymeric nanoparticles	Peptide	<i>In vivo</i>	significant improvement in survival rate in glioma-bearing rats	(Reddy, Bhojani, 2006)
Transferring conjugated with AlPcS4	PEGylated liposomes loaded with aluminum phthalocyanine tetrasulfonate	<i>In vitro</i>	The targeted formulation displayed cell killing on rat bladder carcinoma cells greater than 3-fold in the log scale compared to the control, non-targeted AlPcS4 liposomes cell killing greater than 3-fold in the log scale compared to the control, non-targeted AlPcS4 liposomes	(Derycke et al., 2004)
AlPcS 2	Photosan-3 (PS-3) and disulfonated phthalocyanine	<i>In vitro</i>	Both liposome encapsulation and	(Gupta et al., 2011)

Actively-targeted nanocarrier	Markers/ ligands	<i>In vivo/ in vitro</i>	Study findings	Ref
	(AIPcS 2) when modulated by encapsulation in liposomes and conjugation with monoclonal antibodies		antibody conjugation resulted in localization of sensitizers at sensitive targets and reduced accumulation	

NP, nanoparticle.

Table 5

Potential photosensitizers as stand-alone or encapsulated in nanocarriers approved for photodynamic therapy targeting different diseases

Photosensitizer	Drug (generic)	Potential indications	Wavelength absorption (nm)	Country
HPD (partially purified)	Photofrin HiPorfin	Bladder, lung esophagus cancers; Lung cancer; Esophagus, lung, gastric, cervical cancers, cervical dysplasias; Barrett's esophagus, obstructing esophageal carcinoma and early and obstructing tracheobronchial carcinoma; Early stage head and neck cancers;	630	Canada; EU; Japan; USA, UK; EU; EU China
BPD-MA (a semi-synthetic chlorin derived from protoporphyrin IX)	Verteporfin	Age-related macular degeneration (AMD), pathological myopia	689	USA
m-THPC	Foscan	Head and neck tumors, prostate and pancreatic tumors	652	EU
5-ALA	Levulan Aila	Basal-cell carcinoma, actinic keratosis, early stage head and neck, gynaecological tumors; Genital warts	635	USA, EU, China
5-ALA-methylester	Metvix	Basal and squamous-cell carcinoma, actinic keratosis	635	USA, EU
Methylene blue	Periwave	Periodontitis	660	Canada
Toluidine blue	Helbo blue FotoSan	Endodontitis, periodontitis	560	EU

5-ALA, 5-aminolevulinic acid; BPD-MA, benzoporphyrin derivative monoacid ring A; EU, European Union; HPD, haematoporphyrin derivative; mTHPC, metatetrahydroxyphenylchlorin; UK, United Kingdom; USA, United States of America.

Table 6
Summary of potential applications of phototherapy (LLLT/PDT) in tissue repair and regeneration

Type of tissue	Type of laser	Parameters	<i>In vitro</i> / <i>In vivo</i>	In combination with	Study findings	Ref
Cardiac stem cells	GaAs laser	$\lambda = 804$ nm, PO= 400 mW, PD= 50 mW/cm ² ED= 1 J/cm ² and 3 J/cm ²	<i>In vitro</i>	-	1–2 weeks post-LLLT (1 J/cm ²) demonstrated significant increase in the number of cells; energy density of 1 J/cm ² caused higher stimulation of cell proliferation than 3 J/cm ²	(Tuby, Maltz, 2007)
Adipose derived stem cells	Diode laser	$\lambda = 636$ nm, ED= 5 J/cm ²	<i>In vitro</i>	Epidermal growth factor (EGF)	Increased cell proliferations with EGF; significant increase at 48h compared with cells irradiated and not cultured with EGF; cells with EGF but not irradiated showed no change compared with cells that had been irradiated and without EGF	(Mvula, Moore, 2010)
Cardiac myocardium	GaAs laser	$\lambda = 803$ nm, PO= 400 mW, PD= 6 mW/cm ² , ED= 1.08 J/cm ²	<i>In vivo</i>	-	Epicardial laser irradiation of rat and dog hearts after chronic MI caused a marked reduction in infarct size	(Oron et al., 2001)
Bone marrow mesenchymal cells (BMSCs)	Diode laser	$\lambda = 635$ nm, PD= 6.61 mW/cm ² , PO= 0 to 200 mW ED= 0.5, 1.0, 2.0 and 5.0 J/cm ²	<i>In vitro</i>	Myogenic differentiation induced by 5-azacytidine (5-aza)	LLLT at different ED has positive stimulating effects on proliferation, GF secretion (VEGF, NGF); myogenic differentiation of BMSCs without cytotoxicity; optimal stimulating ED is different for the purpose of proliferation (0.5 J/cm ²), secretion (5.0 J/cm ²) and myogenic differentiation (5.0 J/cm ²)	(Hou et al., 2008)
Human adipose derived stem cells (hADSCs)	Diode laser	$\lambda = 636$ nm, PO= 78 mW, PD= 8.59 mW/cm ² , 5 J/cm ²	<i>In vitro</i>	Differentiation to smooth muscle cells induced by retinoic acid (RA)	After post-irradiation, hADSCs not showing morphological difference, laser did not induce differentiation, and there was an increase in viability and proliferation; hADSCs can be differentiated into SMCs within 14 days using retinoic acid	(de Villiers, Houreld, 2011, Mvula, Mathope, 2008)
Periodontal ligament fibroblasts	GaAlAs diode laser	$\lambda = 809$ nm, PO= 10 mW, CW mode, ED= 1.96–7.84 J/cm ²	<i>In vitro</i>	-	Irradiated cells revealed a considerably higher proliferation activity than non-irradiated	(Kreiser et al., 2003)
Periodontal ligament fibroblasts	Diode laser	$\lambda = 588$ nm, ED= 4J/cm ²	<i>In vivo</i>	Enamel matrix protein derivative (EMD)	LLLT improved effects of EMD; significant probing pocket depth reduction and clinical attachment level gain; less gingival recession, reduction in post-operative swelling	(Ozcelik et al., 2008)
Open skin wound healing	GaAlAs	$\lambda = 780$ nm, ED= 2J/cm ² , pulse frequency 2336 Hz	<i>In vivo</i>	-	Significant increase in number of fibroblasts, blood vessel endothelium; increase tensile strength; decrease number of macrophages	(Bayat et al., 2010)

Type of tissue	Type of laser	Parameters	<i>In vitro/</i> <i>in vivo</i>	In combination with	Study findings	Ref
Healthy and non-diabetic open skin wound	Diode laser	$\lambda = 890$ nm, ED= 0.2 J/cm ² Pulsed infrared= 80 Hz	<i>In vitro</i>	-	Significant acceleration of wound healing in both healthy and diabetic rats	(Dadpay et al., 2012)
Diabetic wound	HeNe laser	$\lambda = 632.8$ nm ED= 1.0 J/cm ²	<i>In vivo</i>	-	Accelerated collagen production, marginal increase in biomechanical properties	(Reddy et al., 2001)
Excision wound	PDT with diode laser	$\lambda = 685$ nm	<i>In vivo</i>	Chloroaluminum phthalocyanine (AlClPe) as a photosensitizer	Higher collagen accumulation, enhanced re-epithelialization, more pronounced connective tissue remodeling	(Peplow, Chung, 2012)
Full-thickness burn	Diode laser	$\lambda = 600$ nm, delivery fractionated protocol 1 J/cm ² on days 1,3,8 and 10 post-wounding, single dose 4 J/cm ² on day 1 immediately after injury	<i>In vivo</i>	-	Fractionated delivery had more neutrophils while single dose had more mononuclear cells on day 21; Both treatments accelerated angiogenesis, stimulated leukocyte chemotaxis	(Nunez et al., 2012)
Third-degree burn	PDT with diode laser	$\lambda = 685$ nm	<i>In vivo</i>	Toluidine blue-O as a photosensitizer	Shortened length of inflammatory stage, increased collagen, re-epithelialization, angiogenesis.	(Peplow, Chung, 2012)
Facial nerve	HeNe or argon pumped tunable dye laser	$\lambda = 361, 457, 514, 633, 720$, and 1064 nm, ED= 8.5 to 40 mW, ED= 162.4 J/cm ²	<i>In vivo</i>	-	Most effective laser treatment demonstrated by helium-neon (HeNe) or argon pumped tunable dye laser; increased the rate of regeneration of rat facial nerve following crush injury	(Anders et al., 1993)
Bone repair of surgical defects in femur	GaAlAs laser	$\lambda = 830$ nm PO= 40 mW, ED= 16 J/cm ²	<i>In vivo</i>	Defects filled with lyophilized bovine bone	Improved amount of collagen fibers at early stages of the bone repair (15 days); increased amount of well-organized bone trabeculae (30 days)	(Gerbi et al., 2005)
Osteoblasts	HeNe	$\lambda = 632$ nm, PO= 10 mW	<i>In vitro</i>	-	LLLT promoted proliferation and maturation in human osteoblasts	(Stein et al., 2005)
Liver hepatocytes	Ga-Al-As laser	$\lambda = 804$ nm	<i>In vivo</i>	-	Following acute hepatectomy, significant enhancement of liver regeneration conducive to both the formation of new hepatocytes and MSCs; angiogenesis in regenerating liver	(Oron et al., 2010)
Tendon repair	GaAlAs laser	$\lambda = 685$ and 830nm, PO= 60 mW, ED= 3 and 10 J/cm ²	<i>In vivo</i>	-	3 J/cm ² best dose in tenotomy compared with 10 J/cm ² in the 685 nm laser compared to 830 nm, which was more effective than sham group, following the same pattern, 3 J/cm ² was better than 10 J/cm ² using collagen bundle aggregation as parameter to measure the healing process in tendons; suggests the existence of wavelength tissue specificity and dose-dependency	(Carrinho et al., 2006)

Type of tissue	Type of laser	Parameters	In vitro/ in vivo	In combination with	Study findings	Ref
Satellite cells for muscle atrophy	GaAlAs	$\lambda=830\text{ nm}$ mW , $\text{ED}=$, $\text{Spot}=0.3\text{ cm}^2$ $\text{PO}=60$	<i>In vivo</i>		Different actions on deep and superficial gastrocnemius muscle showing re-growth of muscle fibers might be related to the proliferation of muscle satellite cells and angiogenesis associated with bFGF, respectively, using a suspension model for disuse	(Nakano et al., 2009)

ED, energy density; bFGF, basic fibroblast growth factor; GFs, growth factors; MSCs, mesenchymal stem cells; MI, myocardial infarction; PD, power density; PO, power output; LLLI, low-level laser irradiation.

Table 7
Summary of nano-assisted biomaterials with their potential applications in tissue repair and regeneration

Type of tissue	Nanostrategy	Type of nanomaterial used	In combination with	Mechanism of action	In vitro/ in vivo	Study findings	Ref
Bone osteoblast	Guided bone-regenerative membrane surface	Chitosan nanofibers	Bone morphogenetic protein-2 (BMP-2)	Provide prolonged bioactive surface, BMP-2 stabilized and localized for a longer time, at the surface of chitosan membrane.	<i>In vitro</i>	Increased osteoblastic cell attachment; increased cell proliferation; alkaline phosphatase activity, calcium deposition	(Park et al., 2006)
Bone Osteoblast	Nanophase of alumina, titania ceramics and hydroxyapatite	Hydroxyapatite (67 nm grain size)		Selectively absorbed vitronectin (a protein which subsequently enhances adhesion of osteoblasts).	<i>In vivo</i>	Enhanced osteoblast adhesion; inhibition of competitive fibroblast adhesion compared to conventional (179 nm) hydroxyapatite	(Webster et al., 2000)
Bone osteoblast	Nanoapatite coating	Calcium phosphate ceramics with apatite, with calcium and magnesium ions incorporated in the structure	Titanium	Apatite used closely mimics the apatite in bone in terms of composition, structure and the mechanism of formation.	Both	Enhanced bone ingrowth and apposition; influence on human osteoblast behavior	(Li, 2003)
Bone osteoblasts	Nano crystalline organoapatite	Biomimetic hydroxyapatite precipitated in the presence of poly (amino acids) thereby incorporating them into mineral phase	Titanium mesh	Low density of nanocrystalline character of organoapatite is more susceptible to natural remodeling process	<i>In vitro</i>	Accelerated migration and increased proliferation of osteogenic cells	(Spoerke and Stupp, 2003)
Bone osteoblasts	Addition of carbon nanotubes (CNT) into emulsions of polylactic acid and chloroform	Multi-walled CNT	Electrical stimulation (10 μ A at 10 Hz) for 6h/day for various periods of time	Current-conducting composite substrate for exposing osteoblasts to electric current stimulation	<i>In vitro</i>	Increased (46%) osteoblast proliferation after 2 days; increased (307%) concentration of extracellular calcium after 21 days; up-regulation of mRNA expression for collagen type-I	(Supronowicz et al., 2002)

Type of tissue	Nanostrategy	Type of nanomaterial used	In combination with	Mechanism of action	In vitro/ in vivo	Study findings	Ref
Bone	Silk fibroin fiber scaffolds containing BMP-2 and nanoparticles of hydroxyapatite prepared via electrospinning	Silk fibroin, poly(ethylene oxide) nano hydroxyapatite bnp-2(hydroxyapatite is a native component of bone)	Bone marrow derived mesenchymal stem cells (hMSCs)	High surface area coupled with high porosity formed by randomly oriented flexible fibers provide a 3D structure for cell interactions and nutritional/gas exchange; electrospun silk/PEO scaffolds were efficient as delivery carriers of BMP-2; nano-hydroxyapatite (a nanophase ceramic) used as a popular bone substitute, coating and filler material due to its ability to promote mineralization	<i>In vitro</i>	after both I and 21 days High calcium deposition; enhanced transcript levels of bone-specific markers for osteogenesis	(Li et al., 2006)
Cartilage	Heparin/poly (L-lysine) nanoparticles inside the PGL nanospheres	Heparin/poly L-lysine	TGF-β3 rabbit bone marrow-derived mesenchymal stem cells (MSC)	Heparin/PLL NP loaded with GFs coated onto PLGA nanosphere surfaces are excellent structures for cell adhesion and organized growth	<i>In vitro</i>	Chondrogenesis of mesenchymal stem cells in PLGA nanosphere constructs was well-organized; A round-shaped morphology of lacunae similar to native cartilage and also formed cell aggregates in nanosphere constructs indicated that cells were intact, moved together and well-interacted.	(Park et al., 2008)
Cartilage	Nanofibrous scaffold	Nanofibrous porous scaffold made of poly-caprolactone nanofibers (700 nm, diameter)	TGF-β1 MSC	Microstructure resemblance to a native collagen fibrillar matrix substrate enhances MSC chondrogenesis; increased substrate surface for ECM accumulation in highly porous scaffolds synthesize more sulfated glycosaminoglycan	<i>In vitro</i>	Display of thick, cartilage-like layers, suggesting efficient differentiation into chondrocyte-like cells and produced cartilaginous ECM	(Li et al., 2005)

Type of tissue	Nanostrategy	Type of nanomaterial used	In combination with	Mechanism of action	<i>In vitro/ in vivo</i>	Study findings	Ref
Urinary bladder and urothelial cells	Poly (glycolic acid) fiber scaffold partially submerged in a suspension of PA	Peptide-amphiphile (PA)	PA/PGA gel scaffold	Reconstruction of ECM of urinary bladder	<i>In vitro</i>	Nano-textured film used in cultured stem cells improved tissue regeneration via tissue engineering	(Harrington et al., 2008)
Full-skin thickness wound healing	Electrospun Chitosan-poly(vinyl alcohol) (C-PVA) nanofiber scaffold and 2D Chitosan-poly(vinyl alcohol) film	Chitosan-poly(vinyl alcohol) (C-PVA) nanofiber 2D Chitosan-poly(vinyl alcohol) film	Topical administered growth factor – R-Spondin 1 Mouse 3T3 fibroblast cells	To create a nanofibers PVA network impregnated with 3T3 fibroblasts scaffold	<i>In vivo</i>	C-PVA had significantly higher catalase and superoxide dismutase activity of the healing tissue; wound closure (98.6%) after 2 weeks of post-injury	(Sundaramurthi et al., 2012)
Neurons	Alignment CNT with polycarbonate urethane	CNT and polycarbonate urethane	Bone marrow and cortical neural stem cell from mouse embryo	CNT are conductive and have nanostructured dimensions that mimic the 3D structure of proteins found in ECM; it interact with various cell types of stem cells and selectively enhance survival of MSCs prefer to proliferate on CNT	<i>In vitro</i>	Nanospheres grown on CNT/polycarbonate urethane array support astrocyte and neuron differentiation; neuron-like structures adhere on CNT which showed that CNT are biocompatible for neural stem cell adhesion in nanospheres; assist in proliferation of MSCs and aid in differentiation of cortex-derived neuron stem cells; increased differentiated environment of neural stem cells to neuron	(Nho et al., 2010)
Neurons	Three dimensional (3D) nanofiber scaffold	Self-assembled peptide amphiphile molecules	Neural progenitor cells neurite-promoting laminin epitope Isoleucine-lysine-valine-alanine-valine IKVAV which promotes neurite	Mechanical support for cells; medium through which diffusion of soluble factors and migration of cells occur; nanofibers that form around cells in 3D present the epitopes at	<i>In vitro</i>	Very rapid differentiation of cells into neurons while discouraging the development of astrocytes	(Silva et al., 2004a)

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Neuron	CNT coated with 4-hydroxynonenal	CNT (diameter 20nm, length 20–100 µm)	sprouting and directs neurite growth	an artificially high density relative to a natural ECM In contrast to artificial growth substrates, CNT have diameter similar to those of neuritis and may therefore allow for kinds of localized molecular interactions required for formation of functional neuronal circuits	<i>In vitro</i>	Neurons can attach to and grow across surfaces of CNT; adhesion of substrates to CNT play an important role in the process of growth cone motility and neurite branching	(Mattson et al., 2000)
Nerve	Multiwalled carbon nanotubes (MWNT)-coated with electrospun poly (L-lactic acid-co-caprolactone) (PLCL) nanofibers	MWNT PLCL scaffolds	Bioactive molecule 4-hydroxynonenal – a molecule that effects neurite growth, – a polyethyleneimine – a substrate for neuronal growth embryonic rat hippocampal neurons	CNT change the surface properties of the PLCL biopolymer; coverage of CNT make the hydrophobic PLCL surface more hydrophilic; ultrahigh electrical conductivity of CNT alter the non-conducting nanofiber into an electrically conducting substrate	<i>In vitro</i>	Improved neurite outgrowth of rat dorsal root ganglia neurons and focal adhesion kinase expression of PC-12 cells; up-regulated focal adhesion kinase (FAK) (key signaling molecule in neurite outgrowth expression)	(Jin et al., 2011)
Corneal tissue	Residual dendrimer amine group modified with COOH group containing biomolecule [covalently immobilized laminin peptide Tyr-Ile-Gly-Ser-Arg (YIGSR)] which is a cell adhesion peptide into collagen scaffolds	YIGSR grafted to a collagen – acrylic copolymer scaffold	Immortalized human corneal epithelial cells	YIGSR attached dendrimers are used as collagen cross-linkers, incorporating the peptide into the bulk structure of the collagen gels; modification with the cell adhesion peptides rather than immobilization of the entire protein minimize potential for immunologic reactions and protein denaturation and also maximize surface uniformity and density since smaller molecular peptides are more accessible to the receptors (e.g. integrins) on the cell surface	<i>In vitro</i>	YIGSR incorporation into the bulk and YIGSR modification of surface promoted the adhesion and proliferation of human corneal epithelial cells, nerve cell density and neurite extension from dorsal root ganglia enhanced	(Duan et al., 2007)

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Adipose Tissue	Electrospun 3 dimensional nanofibrous scaffold made of poly L-lactic acid	Poly L-lactic acid scaffolds	Human bone marrow-derived mesenchymal stem cells (hMSC)	Electrospun 3D nanofibrous scaffolds share morphologic similarities to collagen fibrils and promote favorable biologic response of seeded cells; polymeric scaffold closely mimics the nanofibrous architectural scale of natural ECM for the in vitro engineering of adipose tissue	<i>In vitro</i>	Highly elevated levels of adipogenesis associated genes (1797-fold) for lipoprotein lipase, around 6-fold for PPAR γ ; presence of lipoprotein lipase vesicles-a characteristic feature of adipose tissue detected	(Shanti et al., 2008)
Vascular tissue	Scaffold formed by aligned poly(ϵ -caprolactone) (PCL) nanofibers by electrospinning grafted by gelatin molecules	Poly(ϵ -caprolactone) Gelatin molecules	Human coronary artery endothelial cell culture	Improved cell compatibility; provide a convenient strategy to obtain cells with controlled orientation by cell culturing without the requirement of a fluid system; gelatin grafting improves endothelial cell spreading and growth on PCL nanofibers.	<i>In vitro</i>	Expression of three characteristic markers: platelet endothelial cell adhesion molecule (PECAM-1), intracellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM 1); gelatin grafted PCL nanofiber able to control orientation of ECs in the direction of fibers; providing an effective way to control EC orientation without using a fluid field	(Ma et al., 2005)
Vascular tissue	Nanostructured surface in polymers	Poly-Lactic-Co-Glycolic Acid (PLGA)	Rat aortic endothelial cells	Increased adsorption of proteins such as albumin, laminin, collagen, fibronectin and vitronectin due to greater surface reactivity of nanophase compared with conventional materials	<i>In vitro</i>	Increased nanometer surface roughness; number of aortic smooth muscle cells and number of endothelial cells increased on nanostructured compared with conventional PLGA	(Miller et al., 2002)

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Cardiac myocytes	Electrospun non-woven poly(lactide) PLLA based scaffolds	Semi-crystalline poly(L-lactic acid) (PLLA)	Primary cardiomyocytes from ventricular portion of the heart	Electrospinning allows design of nanomaterials with reorganizable architecture for cell and tissue growth; cardiomyocytes have preference for hydrophobic surfaces and most hydrophobic PLLA scaffold provides best support for cardiac myocytes; nano-electrospun non-woven scaffold provides flexibility and guidance for cardiac myocytes growth	In vitro	PLLA scaffolds promoted better cell adhesion and mature cytoskeleton structure (compared to poly(glycolide) based scaffold) with well-defined periodic units in the sarcomeres (contractile machinery); reestablished cell-to-cell contracts and intercalated disks, which are typical for mature adult cardiac tissue	(Zong et al., 2005)

ECM, extra cellular matrix.