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Influence of Photoactivated Tetra Sulphonatophenyl Porphyrin and TiO_2 nanowhiskers on Rheumatoid Arthritis infected Bone Marrow Stem Cells proliferation *in vitro* and oxidative stress biomarkers *in vivo*

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Photodynamic therapy (PDT) is mostly used to induce apoptosis or necrosis in the benign and malignant tumors, along with other microbial infections and suppression of autoimmune diseases including rheumatoid arthritis (RA). The bone marrow stem (BMS) cells are also in focus in translational medicine, tissue engineering and as an autoimmune diseases suppressant. In this study we used Tetra Sulphonatophenyl Porphyrin (TSPP) with TiO_2 nanowhiskers for RA PDT and evaluated their effect on stress biomarkers (CAT, SOD, GPX, GR, TAO and MDA) *in vivo* and BMS cells proliferation *in vitro*. We compared four murine groups, three of which had Collagen Induced Arthritis as TP-L (illuminated), TP-nL (dark) and CIA (control), whereas the other group was normal without disease and treatment. All anti-oxidative enzymes and biomarkers were significantly ($p < 0.01$) affected by the treatment except TAO ($p > 0.05$). Moreover, we also evaluated the growth proliferating effect of TSPP-TiO_2 (TP) PDT on the *in vitro* RA infected BMS cells i.e. 25 µl had highest cell count ($12.33x10^6$ cells/well) and 33% more growth rate in photoactivated TP when compared with 50 and 100 µl treatment groups. Herein, we report that photoactivated TSPP-TiO_2 for RA PDT may be safer than photosensitizers without the titanium nanomaterials in terms of reduced oxidative stress and also promotion of RA BMS cells growth *in vitro as* novel finding.

Introduction

Stem cells are specialized, immature cells with prolonged capacity of self-renewal and plasticity to various specialized cell types, i.e., osteoblast, chondrocytes, adipocytes, neurons, myocytes under certain favorable conditions or differentiation medium[1, 2]. Almost all the vital organs and tissues in the body contain stem cells. The Bone Marrow Stromal or Stem (BMS) cells are named after their origin, i.e., from the bone marrow of long bones, which was first time reported by Friednstein et al[3]. BMS cells are colonogenic and have the potential to proliferate *ex vivo* without any structure or functional deformation and differentiate to various types of specialized cells[4]. On the bases of these vital properties BMS cells have been explored for potential cure of various maladies[5], cancers[6], tissue engineering[7] and autoimmune diseases; notably rheumatoid arthritis (RA)[8, 9].

RA is an autoimmune progressive joint inflammatory disease in humans with unknown etiology[10]. So far, multiple triggers have been attributed to the onset of RA, e.g., age, gender, lifestyle, and genetic makeup of the individual[11]. The worst feature of RA is persistent chronic inflammation that leads to 50-70 % disability in the patients in 10-15 years[12]. TNF-α is the most commonly investigated biomarker for RA and considered as key proinflammatory cytokine in RA synovial milieu[13]. To date, only empirical therapy is commonly employed to suppress the clinical signs in RA patients and no proper treatment is available[14].

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Photodynamic therapy (PDT) is therapeutic procedure which consists of Photosensitizer (PS), visible light and biologically available oxygen[15]. Porphyrin derivatives are the most popular PS for cancers and infectious diseases[16], despite adverse effects including accumulation of the PS in vital organs, neurotoxicity and phototoxicity. These adverse effects have limited biomedical applications of the porphyrin derivatives[17, 18]. PDT has been reported as a successful remedy for various neoplastic and non-neoplastic maladies[19]. When the PS is photoactivated with visible light it will generate singlet oxygen ($^1O_2$)[20] as the main cyctotoxic agent, although other ROS such as hydroxyl radical and other radicals are also produced. These ROS and $^1O_2$ will interact with cellular signaling pathways and induce apoptosis or necrosis[21]. However, the intracellular antioxidant enzyme systems will be activated to neutralize these ROS and protect the cells from injury[21]. Superoxide dismutase (SOD), Glutathione Peroxidase (GPX), and Glutathione reductase (GR) are among the vital anti-oxidative enzymes as ROS scavengers while Malondialdehyde (MDA) is an oxidative stress bio-marker[22].

Titanium is second most abundantly used nanomaterial for human consumption; either as a food additive, environmental scavenger, in sunscreens and also in various biomedical applications[23]. In biomedical applications it is commonly used in prosthetic orthopedic implants, nano drug delivery systems, sonodynamic therapy, and photodynamic therapy[24]. Nano Titanium dioxide (TiO_2) became popular for cancer theranostics after the first introduction by Fujishima et al almost two decades ago[25]. The use of TiO_2 nanowhiskers for cancer therapy was already reported by Li et al[26]. And the biomedical applications of TiO_2 nanowhiskers combined with TSPP were also reported recently, which demonstrated higher efficacy of Tetra Sulphonatophenyl Porphyrin (TSPP) combined with TiO_2 nanowhiskers during RA PDT as compared to TSPP and TiO_2 alone[27]. It is observed that the TSPP-TiO_2 nanocomposites had excellent theranostics effect by successfully lowering the TNF-α and IL-17, i.e., major proinflammatory factors and biomarkers for RA and bio-imaging the subclinical RA that was confirmed at the onset of clinical signs. Similarly, the TSPP-TiO_2 nanocomposites had protective effect on circulatory and excretory system in murine models by lowering the relevant liver (i.e. AST, ALT and LDH) and kidneys (i.e. BUN and CRT) functional biomarkers in comparison to TSPP and TiO_2 alone during PDT[28].

Therefore, considering the above observations and the importance of the cellular antioxidant enzyme systems herein, we report for the first time, the effect of TSPP-TiO_2 on isolated RA BMS cells and various stress enzymes during PDT *in vivo* in murine models.

Materials and methods

Chemicals and Animal selection

Male Sprague-Dawley (SD) rats were selected due to their ability to produce excellent model of Collagen induced arthritis (CIA). All the animals were provided standard pallet feed and water ad-libitum with a 12/24 hours daily light cycle. At the beginning of the experiments the average animal weight was 220±20 grams and eight weeks of age. All the experiments involving animals were conducted under the guidelines of Animal Research Ethics Board of Southeast University and were approved by the National Institute of Biological Science and Animal Care Research Advisory Committee of Southeast University, Nanjing, China. All chemicals used in cell culture experiments were purchased from HyClone Laboratories, Inc. Utah, USA, whereas the Collagen type II and Adjuvant were obtained from Chondrex, Inc. The chemicals used for differentiation medium, i.e., β-Glycerophoshate and L-Ascorbic acid were purchased from Sigma-Aldrich Co. LLC., Dexamethasone from Adamas-Beta, and TNGβ1 was supplied by PeproTech, Inc. Rocky Hills, NJ, USA.

Experimental layout

To evaluate the in vivo stress biomarkers, all the animals were divided into three main groups (i.e. treatment (TP), control (CIA) and normal (NORM)), containing three animals in each group except treatment, which was further divided into two groups illuminated (TP-IL) and non- illuminated (TP-NL) three animals each. The treatment and control comprised of CIA models, whereas normal was kept without CIA and treatment. Similarly, the TP group was subcutaneously injected with TSPP-TiO_2, whereas control was injected placebo and normal had no CIA and no treatment. One hour post-injection the animals were further exposed to 500-550 nm visible LED light (5mW/dm^2 light intensity) for one hour duration with whole body exposure. This light dose is sufficient to achieve the photoactivation through TSPP-TiO_2 nanocomposites. Experiment was continued for two weeks with daily treatment.

The *in vitro* cell culture experiments were performed on primary BMS cells culture obtained from the CIA models. Initially, $3x10^3$ CIA BMS cells ml^{-1} were cultured in six well plates, and after 24 hours various TSPP-TiO_2 concentrations (0, 25, 50, 100 µl of 0.1 mM TSPP + 0.6 mM TiO_2) were added to BMS cells culture. Then after two hours, one group was exposed to 500-550 nm light for five minutes (i.e., with same intensity as mentioned earlier) and named as TPiL-CIA, whereas the other group was kept non illuminated and named as TPnL-CIA. Similarly, normal BMS cells from healthy rats were obtained and group was named as TPiL-N for illuminated and TPnL-N for non-illuminated TSPP-TiO_2 treatments. Post 72 hours incubation cells were trypsinized and counted by Hemocytometer according to procedure described earlier.[29, 30]

Photosensitizer preparation

The tetra sulphonatophenyl porphyrin (TSPP) was supplied by ABI chemicals and TiO_2 nanowhiskers were generously provided by Dr. Xiao Hua Lu from College of Chemical Engineering, Nanjing University of Technology, Nanjing China. (Fig. 1) TSPP and TiO_2 nanocomposites were separately dissolved in ultrapure deionized water to achieve concertation of 0.1 mM TSPP and 0.6 mM, respectively. TSPP has excellent hydrophilic properties and can readily dissolve in deionized distilled water, whereas TiO_2 readily becomes super hydrophilic when photoactivated[31]. Then TSPP was physically adsorbed on the porous surface of TiO_2 nanowhiskers after mixed in aqueous solution for overnight. The porous nature of TiO_2 nanowhiskers and ionic bonding between the TSPP and TiO_2 provides effective scaffolds for successful delivery and slow release of TSPP from TiO_2 nanowhiskers in desired site. Afterwards, 0.4 ml of TSPP-TiO_2 nanocomposites (0.1 mM TSPP + 0.6 mM TiO_2) were injected to the TP group. Subsequently, different amount of TSPP-TiO_2 were added to various BMS cells culture for *in vitro* experiments.

Arthritis models

Collagen Type II and Freund's adjuvant were mixed together by sonication method to form 1mg ml^{-1} insoluble emulsion as described earlier[27]. Then immediately within one hour 0.3 ml of emulsion was injected at the base of tail to all animals as subcutaneous parenteral. At day 18 to 21 post injection, all the rats showed obvious clinical signs of CIA. Booster doses were repeated when required.

BMS cells isolation and culture

The long bones (femur and tibia) were collected from CIA and normal rats. Then by using the cold PBS (pH 7.4) at 4°C with 2% FBS and 1mM EDTA, all the bones were mechanically crushed and bone marrow was obtained for primary cell culture. The bone marrow containing medium was sieved through 70 µm filter and centrifuged at 3000 rpm for 5 minutes to remove the PBS as supernatant. Then the pallet was dispersed and cultured in DMEM/F12 standard medium containing 10% FBS and 1% Penicillin-Streptomycin solution at 37°C temperature, 5% CO_2 and 95% humidity. After three days only sticking cells remained in the tissue culture bottle and non-sticking cells were removed by changing medium and washing with PBS.

***In vitro* BMS cells proliferation and confirmation**

After 72 hours, BMS cells were passaged to 75 cm^2 tissue culture-flasks as passage one and after one week cells were trypsinized (0.25% trypsin) for further experiments. The BMS cells had typical spindle shaped appearance and their plasticity was confirmed by differentiation to chondrocytes and osteocytes as reported earlier[32]. Briefly, cells were trypsinized to 24 well plates as $5x10^4$ cells $well^{-1}$ and incubated for 24 hours to allow cells sticking to the bottom of culture-plate. The standard medium was replaced with Chondrogenic differentiation medium supplemented with L-ascorbic acid 50 µg ml^{-1} and TGFβ1 1 ng ml^{-1}. Post one week incubation cells were washed with PBS, fixed with 3.7% paraformaldehyde and stained with 0.05% alcian blue stain. For Osteogenic differentiation cells were incubated in standard medium supplemented with ascorbic acid 50 µg ml^{-1}, Na β-glycerphospahte 10 mM and dexamethasone 10^{-8} M for two weeks and then stained with alizarin red 1% after washing and fixation as mentioned earlier[33]. (Fig. 2)

Serum sampling

Blood samples were collected from all treatment groups via 3 ml 21 gauge needle syringe intracardiac injection, under general anaesthesia of isoflurane[34]. Then the serum was separated by centrifugation at 3000 rpm for 5 minutes and stored at -20 °C for further analysis.

Oxidative stress biomarkers estimation

All antioxidant enzymes and biomarkers were estimated by spectrophotometric methods and the kits were supplied by Nanjing Jiancheng Institute of Bioengineering (Nanjing, Jiangsu, China). The assays were performed according to the manufacturer instructions. The procedure for estimation of CAT, SOD, GPX, TAO and MDA activity was measured by the same procedure as reported earlier[35]. Briefly, the GPX activity was measured as the amount of enzymes that will oxidize the 1 µmol L^{-1} GSH per minute at 37 ^{o}C in reaction system for 100 µl of serum. Similarly, the CAT activity was measured by the disappearance rate of H_2O_2 at 340 nm in 100 µl serum sample. The SOD activity was measured by the inhibition rate of hydroxylamine oxidation by 50 percent in coupled system using xanthine and xanthine oxidase. The TOA were measured as the reduction of ferric ions by the agent and formation of Fe^{2+} TPTZ (2,4,6-tri (2 pyridyl)-s-triazine) blue complex that was treated with phenanthroline for generation of a stable complex the absorption of which was measured at 520 nm. The MDA results were expressed as thiobarbituric acid reactive substance in µmol 100^{-1} µl of malondialdehyde[36].

Statistical analysis

Data were statistically analyzed by SPSS version 18 for analysis of variance (ANOVA) of BMS cells proliferation *in vitro* and t-test was performed for serum biomarkers evaluation. The probability value <0.05 was considered as significant.

Results

Oxidative species generated by porphyrin derivatives is essential for PDT of any target tissue. In this study we evaluated the effect on the extent of oxidative stress due to the presence of TSPP-TiO_2 nanocomposites for PDT. Subsequently, PDT will lead to drop in oxygen content of the subject system that will influence the BMS cells proliferation rate. Therefore, experiments were performed to find the relationship between the stress enzymes mean during PDT with TSPP combined with TiO_2 nanowhiskers and the growth rate of BMS cells from the RA infected murine models.

***In vivo* stress biomarkers**

The TSPP-TiO_2 nanocomposites have been successfully used to ameliorate the RA during PDT after evaluating their protective effect on the circulatory and excretory system in the *in vivo* murine experimental models[27,28]. Since in PDT, the PS generates ROS and $^1O_2$ for ablation of tumor or amelioration of other diseases, the relevant stress biomarkers could be further explored in other than targeted tissues. Therefore, the stress biomarkers mean values were estimated from the blood serum after TSPP-TiO_2 photoactivation and values expressed are the mean values per 100 µl of various groups.

All the mean values for CAT, SOD, GR, GPX, and MDA were significantly ($p < 0.01$) affected by the TSPP-TiO_2 either illuminated or dark, whereas the value of total antioxidants remained non-significant with probability value more than 0.05.

The CAT mean value in CIA was 11.72±0.75 (SD) U/100 µl, which was lower than TP-NL (13.08±1.70) and TP-L (13.35±1.26), whereas in normal group its mean remained 14.39±1.53 (SD) U/ 100 µl (Fig. 3a). Similarly, the SOD mean value was higher in TP-L (127.74±4.5 (SD) U/100 µl), followed by TP-NL (118.11±7.87 (SD) U/100 µl) and CIA (112.77±2.43 (SD) U/100 µl), whereas the normal group value was highest i.e. 137.44±3.45 (SD) U/100 µl. (Fig. 3d)

The mean value of GPX observed in TP-L (157.90±5.15) was highest among the CIA and TP-NL was 154.03±3.74 (SD) U/100 µl and 149.714474±7.34 (SD) U/100 µl, respectively, whereas the normal group serum GPX value was 192.89±11.52 (SD) U/100 µl (Fig 3b). The GR serum mean value remained almost the same among all treated groups of CIA, TP-L and TP-NL, i.e., 17.904100±1.48, 17.71±2.16, 17.71±2.167 (SD) U/100 µl respectively, whereas the normal rats serum value estimate for GR was 25.63±1.56 (SD) U/100 µl. Moreover, the lowest TAO level was observed in the TP-L group (9.78±1.60 (SD) U/100 µl) as compared to the CIA (13.06±1.69 (SD) U/100 µl), TP-NL (13.29±2.13 (SD) U/100 µl) and normal group (12.15±1.85 (SD) U/100 µl). (Fig. 3a) The MDA mean concentration value was lowest in TP-L (123.88±10.41 (SD) U/100 µl) among treated groups, i.e. TP-NL(169.41±6.09 (SD) U/100 µl) and CIA (143.31±9.97 (SD) U/100 µl), respectively, and the normal group mean serum concentration was 75.06±7.68 (SD) U/100 µl (Fig. 3c).

***In vitro* BMS cells growth effect**

The BMS cells proliferation was significantly affected by various concentrations of TSPP-TiO_2 ($p < 0.01$) and illumination ($p < 0.05$). Similarly, the proliferation rate

of BMS cells was also significantly ($p < 0.01$) affected by the CIA, as compared to normal BMS cells count mean.

In the post treatment highest cell number mean± standard deviation (SD) was observed in 25 µl TPiL-BMS cells group ($12.33x10^6$±2.72 (SD)), that is 33.79 % higher as compared to TPnL-BMS cells, i.e., $8.17x10^6$±1.17 (SD). The cell count mean for 50 µl, 100 µl and control group concentration of nanocomposites TSPP-TiO_2 treatment was $8.8x10^6$± 2.37 (SD), $5.67x10^6$±0.57 (SD), $9.33x10^6$± 3.93 (SD), respectively, in comparison with TPiL-BMS cells group, i.e., $7.2x10^6$±2.16 (SD), $5x10^6$±1.73 (SD), $6.67x10^6$±0.57 (SD), respectively. The percent change in cell proliferation was 18.2, 11.7 and 28.5% in 50 µl, 100 µl and control group, respectively. (Fig. 4)

The illumination effect on mean in normal BMS cells was $3x10^6$±1.67 (SD), $1.17x10^6$±1.0 (SD), $3x10^6$±3.4 (SD), $6.67x10^6$±3.7 (SD) for 25, 50, 100 µl of nanocomposites TSPP-TiO_2 treatment and control group, respectively; whereas $2.60x10^6$±1.7 (SD), $2.67x10^6$±1.3 (SD), $2x10^6$±1.0 (SD), $4x10^6$±1.0 (SD), respectively, was mean count in TPnL-N group.

Discussion

PDT is considered one of the most efficient therapy for many superficial malignant and benign tumors, in addition to other microbial infections[37]. PDT has been more extensively used to induce apoptosis or necrosis in neoplastic tissues; however, in some cases like Hypericin PDT has been reported to coincide with growth enhancement effect on human neoplasms[38]. Similarly, Aluminum-Phthalocyanin mediated PDT has also been associated with growth promoting effect on osteoblast cells[39]. The role of PDT is of interest in cures of autoimmune diseases especially RA[40]. In previous studies we reported a new therapeutic effect of photoactivated TSPP-TiO_2 nanocomposites on RA[27, 28]. This discovery led us to the new domain in theranostics; therefore we extended its potential applications to the BMS cells in the CIA infected models and evaluated the stress enzymes profile as oxidative stress biomarkers in TSPP-TiO_2 nanocomposites treated animal models.

During PDT the ROS and $^1O_2$ are generated from the photoactivated nanocomposite TSPP-TiO_2 in the presence biologically available molecular oxygen. These ROS and $^1O_2$ will interfere with cellular signal pathways; meanwhile, the cell in response will neutralize these ROS by activating intra and inter-cellular anti oxidative cellular enzymes system[41]. ROS play a vital role in intra and inter-cellular signaling. However, the uncontrolled generation of ROS will disturb the oxidative and anti-oxidative equilibrium in the cell and lead to lipid peroxidation that is directly proportional to cellular SOD, GPX, CAT, GR and inversely proportional to the MDA level[22]. Moreover, the oxidative enzyme activity is lowered due to inhibitory effect of nanocomposites on mRNA expression of these enzymes[22]. The disturbance of the ROS equilibrium within the cell is referred to as oxidative stress which leads to oxidation of DNA, and degradation of cellular organelles, proteins and lipids[21].

Porphyrin derivatives are well known for oxidative stress during PDT[15, 42], although when TSPP was combined with TiO_2 nanowhiskers, the oxidative stress effect was mitigated[28]. This may do due to the fact that TSPP is adsorbed in the pores of TiO_2 nanowhiskers and released slowly during a long time period. The $^1O_2$ quantum yield Φ^{Δ} value for only TSPP was earlier reported as 0.64[43], while we determined a somewhat lower value of 0.44[27] for the nanocomposite material TSPP:TiO_2 with a ratio of 1:2 (by mass). This quantum yield is still sufficiently high for PDT, consistent with our results presented in this paper. Moreover, the size and type of nano titania also affect the cellular anti-oxidative enzymes, i.e., nano TiO_2 with smaller size are more cytotoxic than larger size, similarly, TiO_2 nanoparticles are reported more cytotoxic than TiO_2 nanowhiskers[44]. In our earlier study the MTT assay results also revealed that when TSPP was combined with TiO_2 nanowhiskers, the cell viability remained 100% and 80% in lower and higher concentration of TSPP-TiO_2 nanocomposites, respectively, as compared to the TSPP alone, i.e., less than 70%[28].

Our results showed a higher level of SOD when TSPP-TiO_2 nanocomposites were photoactivated *in vivo*, while the CAT level remained almost same as dark TSPP-TiO_2 nanocomposites and control group. H_2O_2 has potential to penetrate cell membranes and can lead to lipid peroxidation. Generally, SOD, GPX and CAT neutralize H_2O_2 to stable alcohols and water to avoid damage to biomolecules[45]. Additionally, almost the same concentration mean of CAT in nanocomposites TSPP-TiO_2 treated groups vouches its inertness to the normal body cells. Moreover, the highest level of GR also demonstrates the least ROS generation from the photoactivated TSPP-TiO_2 nanocomposites. The

primary role of GR is to reduce the oxidized glutathione by the help of NADPH[21].

The MDA is a major product of lipid peroxidation and used as prototype biomarker for cell membrane oxidative damage[46]. The MDA least mean was observed in photoactivated nanocomposites TSPP-TiO_2 groups. The lower MDA level shows a protective effect of TSPP-TiO_2 nanocomposites on lipid peroxidation. Our results are in contrast with findings reported in the literature due to the fact that the previous studies used single and pristine TiO_2 nanoparticles only in the brain, which are known to be more cytotoxic[22, 47]. We used TiO_2 nanowhiskers, which were reported to be safer than nanoparticles[48] and also combined these whiskers with TSPP to evaluate their stress on the whole body system. Nevertheless, Porphyrin in combination with Mn (iii) (MnTM-2-PyP^{5+}) has been reported by Benov and Batinic-haberle for lowering the overall MDA level in streptozotocin diabetic rats[49]. Their results are in agreement with our findings.

TAO is the measure of estimated antioxidant capacity of the body and its lower value is indicator of overall oxidative stress[35]. Generally, the TAO estimates the chain-braking antioxidants in liquid (thiols, urates, bilirubin, and ascorbate) and lipid phase (flavonoids, α-tocopherol, and carotenoids)[50]. We found almost the same TAO mean value in all treated groups, which indicate the safety of TSPP-TiO_2 nanocomposites.

CIA BMS cells proliferation rate was found surprisingly higher in photoactivated TSPP-TiO_2 nanowhiskers treated group. In RA, the synovial milieu has unique pathophysiological environment, i.e., hypoxia and comprised of various stress enzymes and biomarkers[51, 52]. Jimenez-Boj et al reported that Rheumatoid joint tissue gets bilateral insult, i.e., inflammation in the synovial milieu, and elevated pro-inflammatory cytokines activities and inflammatory (T&B) cells aggregates inside the bone marrow[53]. Therefore, we can also assume that hypoxic condition exist inside the bone marrow. Since PDT utilizes biologically available oxygen to generate ROS[54], this will lead to a further drop in oxygen concertation and relatively higher stress enzyme level may have some beneficial effect on the growth rate of CIA BMS cells. Moreover, it has already been proven that lower oxygen level promotes growth rate of hematopoietic stem cells[55], neural crest cells[56] and survival of embryonic stem cells[57]. Similarly, Yamanaka et al. also reported that hypoxic conditions (5%) promote the growth rate in induced pluripotent stem cells[58].

It has been proven that during PDT the bioavailable oxygen at ground state is photochemically consumed by PS to generate $^1O_2$[15]. This will result in rapid reduction of oxygen bioavailability in the subject tissue as reported earlier[59, 60]. Therefore, the TSPP-TiO_2 nanocomposites PDT provides scaffolds for the hypoxic environment by utilizing biologically available oxygen to generate ROS and $^1O_2$ in the rheumatoid joint milieu and bone marrow in the vicinity, which is helpful for BMS cells proliferation to cope with RA either by tissue repair[61] or by suppressing the autoimmunity[62].

Conclusion

In summary, from the above results we conclude that the photoactivated TSPP-TiO_2 nanocomposites are safer in terms of anti-oxidative biomarkers during RA PDT, and can be used for biomedical applications. Moreover, in CIA the ex vivo BMS cells treated with photoactivated TSPP-TiO_2 nanocomposites can increase the proliferation rate, which is helpful in lowering the autoimmune reactions and ameliorate tissue injury.

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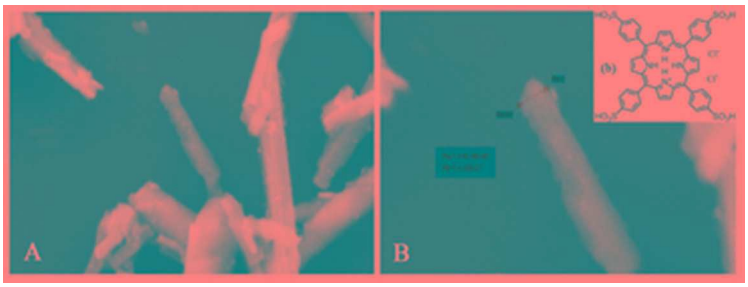
Scheme 1 Bone Marrow Stem Cells isolation and in vitro proliferation from collagen induced arthritis murine models

Fig. 1 SEM micrograph of TiO_2 nanowhiskers (A), average size (B) and Tetra Sulphonatophenyl Porphyrin structural formula (b) (scale 200 nm)

Fig. 2 Collagen induced arthritis Bone Marrow Stem Cells; black arrow head clearly demonstrate characteristic typical spindle shaped cells (A), Cells differentiated to Chondrocytes (red circles) and arrow head shows cartilaginous beads (arrow head) (B) and typical osteoblast (in red circles) with mineral deposition (arrow heads) (C).

Fig. 3 (a) Showing blood serum level of CAT (black line), GR (red line) and TAO (yellow line); **(b)** GPX mean value; **(c)** MDA level and **(d)** is demonstrating SOD in treatment group CIA (collagen induced arthritis) as control, TP-NL (TSPP-TiO_2 group without illumination), TP-L (TSPP-TiO_2 illuminated) and Normal group. Whereas, CAT stand for catalase, GR for glutathione reductase, TAO for Total Anti-Oxidant Count, GPX for glutathione peroxidase, MDA for malondialdehyde and SOD for Super Oxide Dismutase. For all oxidative stress bio-markers probability value was < 0.01 except TAO ($p > 0.05$)

Fig. 4 Collagen induced arthritis rat bone marrow stem cells proliferation rate in vitro after treatment with various concentrations of Tetra Sulphonatophenyl Porphyrin and TiO_2 nanocomposites illuminated and dark. ($p < 0.01$)



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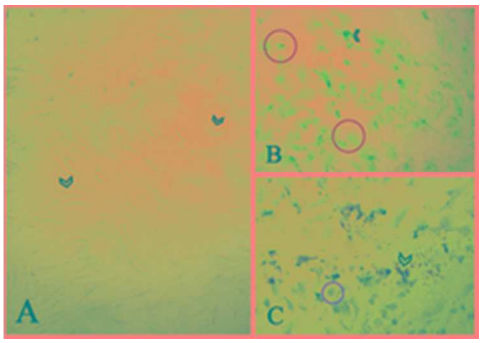

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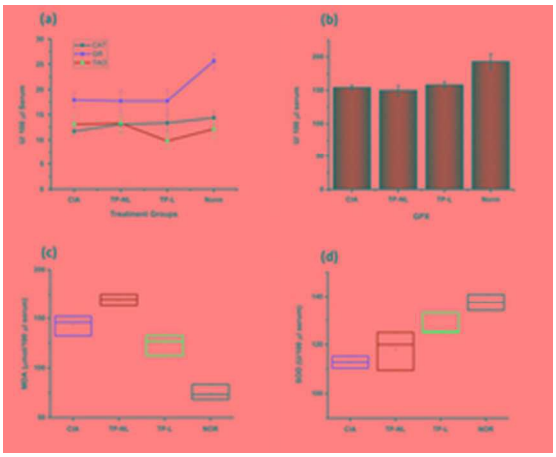

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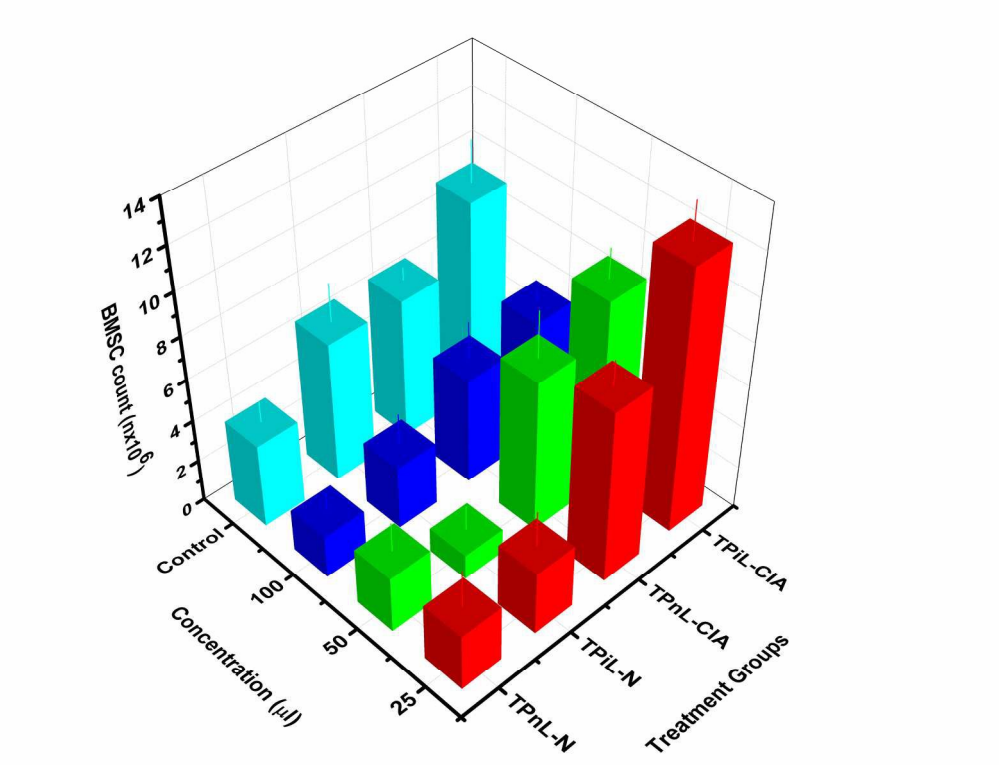

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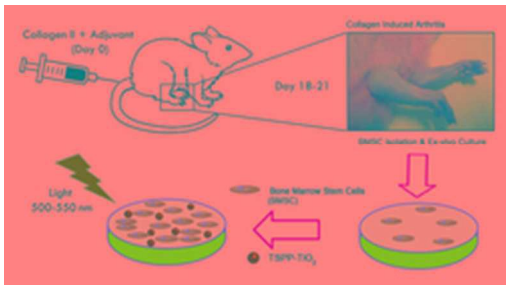

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