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PROSPECTS

Application of nanomaterials in three-dimensional stem cell culture

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Abstract

Petri dish cultured cells have for long provided scientists an aperture to understanding cell's behavior both in normal and disease states as well as in vitro and in vivo. But recent advances have brought to light how the architecture and composite nature of the immediate environment within which the cell is proliferated can profoundly influence its phenotypic features and functions, thus making obvious, limitations of the conventional two-dimensional cell culture despite it cost effectiveness. Fortunately, the transition to three-dimensional (3D) cell culture has occurred concurrently with expanded knowledge of nanoscience and materials, thereby lending significant impetus for innovative research. This review is focused on the application of nanoparticles in 3D stem cell breeding, recent trends and developments in medical sciences for improved drug delivery, and treatment approaches to some human diseases. We also reviewed prevailing challenges and concerns of nanotoxicity as it continues to impede and delay clinical applications as well the ongoing concerted and multidisciplinary efforts to overcome them.

KEYWORDS

nanomedicine, nanoparticles, stem cell

1 | INTRODUCTION

Scientists and researchers in recent decade have intensified attention to studies relating to the field of nanoscience and its vast range of applications. Publications of results and findings from these studies have revealed great potentials and benefits for overall improvement in the quality of life or human survival, thereby making these endeavors even more promising and attractive, hence the huge traction attributable to the multidisciplinary research with nanoscale materials.[1] Scientists, however, are still locked in debate on the future implication of nanotechnology, given its manifest capabilities to transform many frontiers and breakthroughs that were hitherto considered a dead end.[2] In its broad sense, nanotechnology has been defined as the manipulation of matter (atoms and molecules) with at least one of its dimensions within the range of 1 to 100 mn.[3] Accordingly, nanomaterial or nanoparticle is defined as a material or particle either naturally

occurring or artificially fabricated whose external dimension, internal and/or surface structure, falls within the nanoscale range of 1mn to 100 nm. It is worthy to note that 1 nm is equivalent to 10^{-9} m (1 billionth of a meter); in essence, the emphasis is on the size.[4-6]

The dimensionality of nanomaterials plays an important role in shaping their physical, chemical, and biological characteristic behaviors. Consequently, nanomaterials have been categorized into 0, 1, 2, and 3 dimensional (D).[7] The impact of utilization of nanomaterials is already being felt across various specializations. In healthcare, for instance, nanomedicine or nanobiotechnology has found profound application in targeted drug delivery and therapeutics, gene therapy, regenerative medicine, stem cell research, diagnostic tools, and surgical aids amongst several biomedical devices.[4]

2 | DIMENSIONAL STEM CELL CULTURE

Stem cells are the "master cells" of tissues and organs of the body, which undergo differentiation to serve mainly for restoration or replacement of worn out, injured, or diseased cells of various tissues and organs[8,9] such as heart, bone marrow, brain, muscle tissues, skin, and so on, and have been adapted for their regenerative roles.[10,11] There are two major types of stem cell in mammals: embryonic stem cells (ESC) which are somatic cells capable of differentiating to become part of all cell types found in tissues and organs of the body such as heart, nervous, immune system, etc, therefore, have been termed is pluripotent.[12] ESC originates from the inner mass cells of a blastocyst.[10] Whereas adult stem cells, on the other hand, are multipotent. This means that they can differentiate into more than one types of cells of the tissues and organs of the body but are more limited compared to ESC, given their confinement to the family of the parent tissue they are derived from (same lineage).[13] They are also described as nonpluripotent, but with the aid of advanced technologies, they have successfully been induced via genetic engineering to become pluripotent cells and are thus called induced pluripotent cells.[14-17]

Generally, cell culture has a long history in cell biology and, in our modern era, it plays a vital role in the detailed understanding of both theoretical and experimental knowledge of cell biology and disease studies. A cell in its native physiological environment (in vivo) exists in a 3D form, which allows it to interact freely with its surrounding. It has been reported that the 3D culture models closely mimic the cell natural microenvironment in tissues and organs than cells grown in the conventional in vitro 2D approaches. Although 2D models are easy and simple to execute, their major limitations are overactivation of proliferation signals, which is the direct result of cells' adhesion to plastic petri dish and inadequate interface or interaction with other cells. Based on these inherent disadvantages of the 2D culture system, the need for the 3D model thrived basically for its advantages of presenting the growing cells an enabling environment to form patterned growth such as suspension, adherent or multicellular clusters (spheroids) occasioned by cell-cell, and cell-matrix interaction, nutrient and oxygen circulation, waste removal as well as migration, as they would normally do in vivo.[18]

The 3D culture system can be grouped into two broad types: scaffold and scaffold-free type. Scaffold provides a form of support for the proliferating cells and has been shown to confer physiochemical influences on the cells propagated in it.[19,20] Fortunately, technological advancement has occurred concurrently with expansion in knowledge of stem cell biology, pathology and nanomedicine, thereby lending commendable impetus for state-of-the-art research in diagnosis, vaccine development, targeted drug delivery designs, as well as other applications.

3 | APPLICATION OF NANOMATERIAL IN 3D STEM CELLS CULTURE

Utilization of nanomaterials in stem cell research has been demonstrated to be a veritable and useful tool for immense advances in therapeutic studies, targeted drug delivery and pharmacological interventions, furthering molecular understanding of human physiology as well as regenerative medicine in general.[4]

Some of the established areas where nanomedicine is making encouraging headways, especially in stem cell application, are discussed below in brief.

3.1 | Stem cell labeling

3.1.1 | Quantum dot

Quantum dots (Qdot) are light-emitting nanocrystals usually 2 to 10 nm in size. They are mostly made from groups II-VI (such as CdSe, CdTe, CdS, or ZnSe) or group III-V elements of the periodic table (Figure 1). They have a wide range of usage for long-term stem-cell labeling, monitoring and tracking of cell survival, location, and differentiation. Qdots were first reported for bio-labeling in 1998.[21,22] Due to their acknowledged photostability and long term fluorescence intensity, Qdot has gained

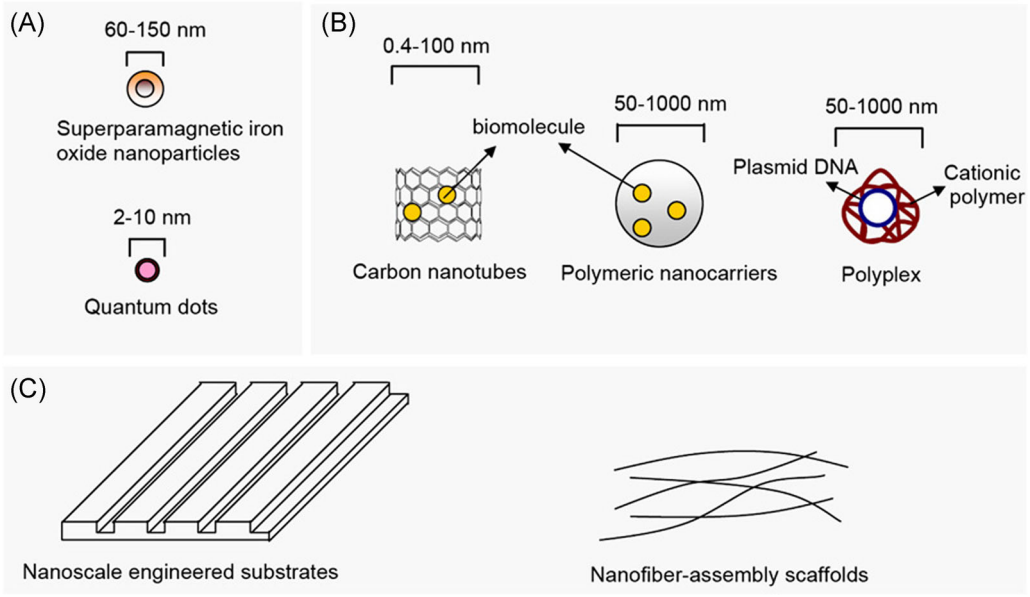

FIGURE 1 Nanoparticles and scaffold for stem cell research. This image adapted from[1] with copyright permission

more acceptance over organic dyes in immuno-labeling.[23,24] Furthermore, they have larger surface areas than conventional chromophores, thereby making them suitable for conjugation with bio-recognition molecules such as nucleic acid, peptides, antibodies, or small-molecule ligands. For instance, Qdots are particularly suitable for labeling of certain proteins, especially during investigations of heart tissue injury to eliminate autofluorescence.[25] Similarly, colloidal quantum dots have found a unique application as a contrast agents for cell imaging both in vitro and in vivo (Figure 2).

3.1.2 | Superparamagnetic iron oxide

Superparamagnetic iron oxide (SPIO) is a magnetic nanoparticle consisting of an inner iron oxide (Fe_3O_4) core with a dextran- or carboxydextran-coated exterior surface. The surface coating material guarantees its solubility in an aqueous environment and avoids aggregation of nanoparticles.[27,28] SPIO is used to improve the contrast of magnetic resonance imaging of cellular targets. Similar to other nanoparticle labeling techniques, SPIO can be deployed by either surface attachment to stem cell or internalization by endocytosis or phagocytosis[29] (Figure 3). But Walczak et al[30] suggested that electroporation is a much faster labeling method compared to SPIO. However, it is important to note that surface labeling with SPIO is most suitable for in vitro studies compared to in vivo studies. This has been attributed to the rapid detection of SPIO labeled cells and subsequent clearance by reticulo-endothelia apparatus.[31] Whereas cellular uptake of SPIO through endocytosis is well documented, it may be aided with a transfection agent for in vivo cell tracking and for yielding good

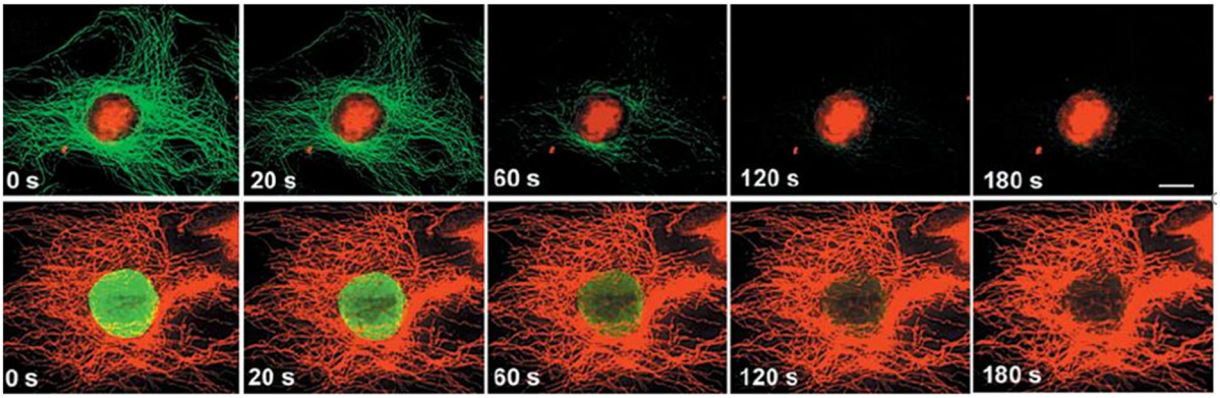

FIGURE 2 Cell labeling with quantum dots and illustration of quantum dot photostability, compared with the dye Alexa 488. In the upper panels, the nucleus is stained red with quantum dots and the actin fibers are stained green with the dye. In the lower panel, the labeling is reversed. This image adapted from (Alivisatos, Paul., 2004)[26] with copyright permission

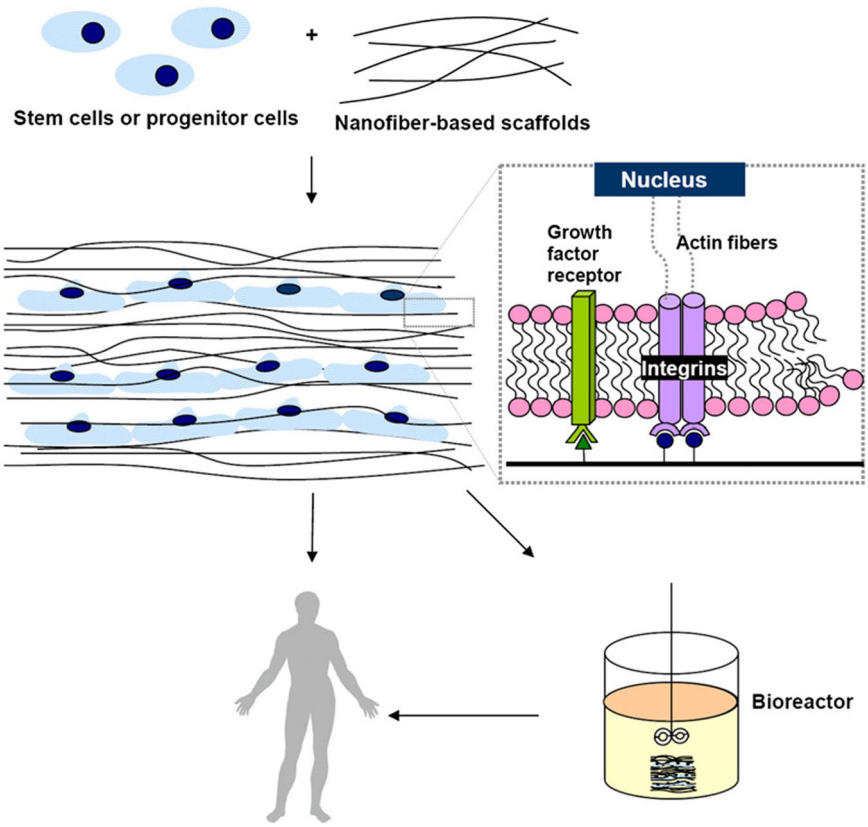

FIGURE 3 Nanofiber-assembly scaffolds for tissue engineering. This image adapted from[1] with copyright permission

magnetic resonance cell imaging, as reported in human mesenchyme stem cell (hMSC)[32] and neural stem cell[33] labeled culture studies.

4 | NANOPARTICLE DRUG AND GENE DELIVERY

Nanoparticles are designed and used for gene delivery through the process of transfection. The major advantages of this approach over viral vector delivery methods are that the risk of viral infection, toxicity, mutagenesis, and immunogenicity is completely eliminated or drastically reduced to tolerable levels.[34,35]

Successful nanoparticle-aided gene delivery has been reported in ESCs with apatite nanoparticles, which were coated with fibronectin and E-cadherin with a significant 3-fold higher gene expression.[36,37] Also, a similar successful delivery was also achieved in mESC with nanoscale-constructed cell-substratum cultured on a silicon nanowire array, and interestingly cell viability was not impacted.[38] Furthermore, Bianco et al reported the use of carbon nanotubes (CNTs) for the delivery of drugs and other essential biomolecules, such as nucleic acid and proteins, into mammalian cells in vitro before scaffold seeding and culturing. But the prospect of carbon nanotube utilization in stem cell research is being explored; however, most recent studies and surveillance work are focused at assessing its cytotoxicity.[1]

Another promising revelation for nanoparticle application is its potential for the treatment of atherosclerotic plaques. Recent novel research demonstrated that modified gold nanoparticles delivered with adult stem cells were used to clear atherosclerosis plaques in the arteries of pig and also facilitated regeneration of the arteries.[4] It further stated that the combination of nanoparticles with stem cells yielded a better effect compared to nanoparticles alone, hence underscoring the need for further exploration of the potential of nanomaterials in stem cell research approaches. In a related development, a 3D cell culture (spheroid) was reportedly used as a model to examine nanoparticle penetration or uptake in solid avascular tumors of lungs, breast liver, etc. The study used nanoshell coatings made of polyethylene glycol and phosphatidylcholine to conceal silica and citrate linked gold nanoparticles from immune detection, thereby reducing immune triggered reactions and system-wide toxicity. Although 3D culture model does not possess all the features of the tumor microenvironment, the result revealed the valuable basis for understanding the behavior of nanoparticles and possible utilization for cancer therapy, such as targeted drug delivery or thermal[39]

5 | TISSUE ENGINEERING

Nanostructured biomaterials for both hard (bone) and soft (cartilages, connective) tissue engineering have been to be one of the focal hotspots for extensive research in nanomedicine and regenerative medicine. Due to the nature of these tissues, designs of enabling ESM or scaffold must take into account their special requirements, such as structural support, high porosity (for cellular proliferation and diffusion), and biodegradability.[40-42] Interestingly, a suitable ESM or scaffold is one of the primary requirements for 3D cell culture and bioprinting. In this regard, CNTs have been extensively used to fabricate various scaffolds with varying percentages of nanocomposite materials. Also, scaffolds that would allow and promote angiogenesis are another major factor for consideration.[43] As reported by Jell et al [44] polyether urethane–reinforced CNTs demonstrated significant results for vascularized bone tissue formation. Similar success have been recorded in cartilage tissue engineering studies with CNTs in the presence of electrical stimulation.[45-47] In the case of connecting tissue such as the skin[48], cells cultured on a 3D nanofibrous composite scaffold yielded favorable results, leading to the conclusion of its potential deployment for soft tissue regeneration and repair (Figure 3).[49]

6 | 3D NANOSCAFFOLD DEVELOPMENT

The scientist has successfully used nanofabrication technologies to design 3D scaffolds for stem cell proliferation. These biodegradable scaffolds closely mimic the cell's natural microenvironment, thus allowing them to grow within them while depositing their own matrix as the scaffold is gradually degraded. Using a peptide nanofiber scaffold[50], a group of researchers succeeded in synthesizing 18 different peptides with mouse neural stem cells.[11] A similar approach was adopted to study cartilage repair with an electrospun, porous 3D nanofiber scaffold.[51] Nanofibrous structured scaffolds has been shown to favorably support bone tissue (osteoblast, osteoclast, and fibroblast) proliferation and differentiation in 3D cell cultures for possible implantation.[52,53] In a recent report on breast cancer, it was revealed that a 3D nanofiber scaffold exerted significant influence on CD44+/CD24− by limiting their migration and invasion tendencies thus further highlighting the importance of cell-extracellular matrix interaction in stem cell proliferation, which could be exploited for designing therapeutic interventions with better efficacies.[54,55]

7 | CLINICAL APPLICATIONS OF SOME NANOMATERIALS

7.1 | Allergic asthma treatment

A number of nanomaterials have been deployed for clinical treatment of a certain illness, which are hard to treat with conventional therapeutics. Chitosan nanosphere has been shown as a viable alternative for allergic asthma treatment compared to traditional drugs, such as theophylline, methotrexate, cyclosporine, and azathioprine with established limitations and side effects.[56,57] Chitosan is a derivative of chitin developed through N-deacetylation to form a positively charged biopolymer with properties such as biodegradability, biocompatibility, minimal toxicity, and low immunogenicity.[58] It is also reported to form stable DNA complexes called polyplexes (see Figure 1) and efficient intracellular gene delivery associated with its mucosa adhesive and poly-cationic nature.[59]

7.2 | Application in cardiovascular diseases therapy

Nanocomposite fabrications have been found to demonstrate potential to meet the delicate requirements for heart tissue regeneration, mechanical forte and physiological suitability such as antiplatelet properties and hematological compatibility, hence hold promising chances for application in the treatment of heart-related diseases.[60] In the area of heart patches, recent research has shown that nanocomposite constructions of zein protein fused into electrospun PSG, resulting in better water uptake and rigidity of the cardiovascular patch.[61-63] A similar improvement in vascularization was reported in a fabrication involving a decellularized heart tissue matrix and silk material incorporation.[62] Furthermore, carbon nanofiber reinforced with polylactic-co-glycolic acid (PLGA) was reported to have enabled cardiomyocyte differentiation, migration, and matrix attachment in culture environment, making it another prospective heart patch option for repairing and regenerating tissues after myocardial infarction.[64] Similarly, a 3D culture of heart tissues using a scaffold fabricated from gold nanoparticles, poly fibers of polycaprolactone, and gelatin demonstrated amplified contraction, and significant cardiac activities, suggesting its potential suitability for use as a cardiac patch and regenerative interventions.[65] Likewise, nanoparticles fashioned into 3D scaffolds have been used to attempt making artificial heart valves. An example of these was a valve fabricated from a nanomaterial called polyhedral-oligomeric-silsesquioxanes poly(carbonate-urea) urethane (POSS-PCU) strengthened with polyurethane and

polycarbonate fragments reportedly displaying encouraging properties as a workable heart valve.[66] These nanocomposites and nanoparticles have shown considerable opportunities for utilization in the treatment of cardiac diseases but more studies are required to address the concerns of their long term effectiveness and improve overall performance.[67]

7.3 | Toxicity of nanoparticles

Although nanomaterial applications in stem cell studies has revealed promising results, issues relating to its biocompatibility and toxicity have failed to reach a consensus. A number of studies have revealed conflicting conclusions about the cytotoxicity, neurotoxicity, and genotoxicity of nanoparticles.

Studies conducted by Shah[68] and Chakraborty[69] stated that no adverse effect was noticed in the overall differentiation and morphological features of ESC cultured with Qdots, but another study reported some abnormalities in the proliferation pattern of the stem cell. However, some researchers have projected that the cytotoxicity of Qdots may have resulted from their cellular degradation. Oxidative degradation of Qdots produces Cd^{2+} ion, which is known to cause cell poisoning through binding to the sulfhydryl group of mitochondria.[70-72] Furthermore, Dubertret et al[73] also reported a collection of Qdots in the nucleus of cells.

In the case of SPIO nanoparticles, reports of extensive investigations of cultured hMSC, mouse ESC, did not establish any cytotoxic effect after internalization of the nanoparticle by stem cells.[32,74-76] This can be attributed to the fact that it is composed mainly of biodegradable iron, which can be recycled and used by other biochemical pathways.[77] However a study conducted by Bulte et al[78] in 2004 stated Fe from SPIO disrupted differentiation of hMSC but Arbab et al[79] in a later study attributed it to transfection agents and not SPIO nanoparticle.

The cytotoxicity of carbon nanotubes in hESCs, mESCs, and hMSCs have been assessed in a number of studies.[1,80,81] It was unanimously reported that carbon nanotube toxicity may be a function of its concentration, exterior coating, as well as its physical features such as size and shape.[82] In the same manner, the genotoxicity of multiwalled carbon nanotubes (MWNTs) was highlighted for its induction of apoptosis in mMSCs, tumor gene–suppression abilities, mutagenesis,[83] as well as generation of reactive oxygen species.[84] In view of these issues, further investigation would be necessary to establish its toxicity mechanism and hence determine its biocompatibility in stem cell works.

8 | CONCLUSION

The synergy between cell culture and nanoparticle is rapidly evolving with promising potentials for improved health care delivery. Nanomaterial applications in 3D cultures appear to have drawn a new chapter in stem cell studies informing changes in approach to tissue engineering, drug and vaccine targeting, cellular imaging, and tracking. Interestingly, some of these findings are now being utilized for preventive and therapeutic interventions in areas of respiratory disorders, cancer and tumor treatment, tissue transplantations. Besides, its physicochemical properties being used for these therapeutic purposes, nanomaterials have significantly advanced the fabrication of suitable 3D scaffolds or matrices for cell culturing, especially the composite or reinforced scaffold, which facilitates noticeable and distinctive topographies of cell proliferated on it as would naturally occur in vivo. On the basis of the proven low immunogenicity and toxicities of some nanoparticles, the future of nanomedicine is beaming very brightly. Worthy of note in this perspective is the payload capacity of these fabricated 3D nanostructures. Applicability for targeted delivery of therapeutic agents such as proteins, genes, engineered cells or tissues, attenuated microbial (viral) particles is bound to be more precise, thereby enhancing their effectiveness on cancerous or tumor cells with assured minimal side effects to normal cells of other tissues and organs of the body as against the system-wide side effects of current cancer therapies. With this growing realization of the biological advantages of 3D cell culture, we thus can envisage the 2D approach embarking on a relegation journey to the obsolete realm in the near future.

Although significant progress has been made in advancing the prospect of nanoparticles in medicine, challenges of nanotoxicity, however, have lingered over the years. This being one of the major concerns has hindered approval from relevant biosafety and ethical regulatory bodies, therefore, truncating the transition of many of these laudable achievements to clinical phase. Further studies are required to fully understand the neurotoxic impacts, possible biochemical complications of these materials over long periods with a view of addressing such side effects to pave the way for the much-needed nanomedicine products for beneficiaries.

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CONFLICT OF INTERESTS

The authors report no conflict of interests. The authors alone are responsible for the content and writing of the paper.

AUTHOR CONTRIBUTIONS

SG planned the study, wrote the protocol, collected the data and drafted the manuscript and accepted the final draft. MPA and KK planned and designed the study and collected the data. OO analyzed the data and critically revised the draft and finally approved the manuscript.

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