



## Review

## Application of mesenchymal stem cells combined with nano-polypeptide hydrogel in tissue engineering blood vessel

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## ABSTRACT

At present, the vascular grafts used in clinic are mainly autologous blood vessels, but they often face the dilemma of no blood vessels available due to limited sources. However, synthetic blood vessels made of polytetrafluoroethylene (ePTFE), which is commonly used in clinic, are prone to thrombosis and intimal hyperplasia, and the long-term patency rate is poor, so its effectiveness is severely limited, which is far from meeting the clinical needs. With the development of nano-materials, stem cells and 3D bio-printing technology, people began to explore the preparation of new endothelialized vascular grafts through this technology. Nano-peptide materials have excellent biocompatibility, can be compounded with different bioactive molecules, and have unique advantages in cultivating stem cells. It has been reported that self-assembled nano-polypeptide hydrogel was successfully constructed, mesenchymal stem cells were correctly isolated and cultured, and their transformation into blood vessels was studied. It was confirmed that the 3D bio-printed nano-polypeptide hydrogel tissue ADMSCs still had strong vascular endothelial differentiation ability. The application of mesenchymal stem cells and nano-polypeptide hydrogel in tissue engineering blood vessels has gradually become a research hotspot, and it is expected to develop a new type of transplanted blood vessel that meets the physiological functions of human body in terms of vascular endothelialization, cell compatibility and histocompatibility, so as to realize the customized and personalized printing of the endothelialized transplanted blood vessel according to the shape of the target blood vessel, which has attractive prospects and far-reaching social and economic benefits.

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Cardiovascular disease is one of the most common serious diseases that threaten people's health. The treatment methods mainly include drug therapy, interventional therapy and surgical treatment. When the local blood vessels of human body are seriously diseased, which can not guarantee the normal supply of blood and is not suitable for drug therapy and interventional therapy, surgical vascular transplantation is needed.

At present, the vascular grafts used in clinic are mainly autologous blood vessels, such as replacing diseased blood vessels with autologous great saphenous vein or internal thoracic artery. Although autologous blood vessel surgery is effective, it is often faced with the problem of no blood vessels available due to limited resources. Therefore, people have to focus on artificial blood vessel substitutes. At present, the artificial blood vessels available in clinic are mainly synthetic blood vessels made of expandable polytetrafluoroethylene (e-PTFE), but there are some problems, such as thrombosis and intimal hyperplasia, poor long-term patency rate, etc., whose effectiveness is severely limited and far from meeting the clinical needs. Based on the above limitations, it is an urgent problem to develop a new type of autoendothelialized artificial blood vessel without immunogenicity and apply it in clinic.

### **1. Mesenchymal stem cells can be used as seed cells of tissue engineering blood vessels**

The research shows that the preparation of tissue-engineered blood vessels must have three elements: suitable seed cells, excellent scaffold materials and growth factors with clear functions. The differentiation of stem cells is not only related to the pre-existing programming in the cell itself, but also regulated by the microenvironment in which the cells are located. The ways of regulating the differentiation of stem cells mainly occur between cells and between cells and extracellular matrix, and cytokines play the role of information transmission and nutrition between them. Therefore, the related influencing factors of stem cells' directional endothelial differentiation are mainly cells, extracellular matrix and microenvironment in which cells are located [1,2].

Mesenchymal stem cells are pluripotent stem cells with self-renewal and multi-directional differentiation potential, which exist in various tissues *in vivo*. Because of its characteristics of easy isolation and amplification, multi-directional differentiation and low immunogenicity, it has been studied and applied as an ideal seed cell in tissue engineering and regenerative medicine [3].

At present, bone marrow-derived mesenchymal stem cells are mostly used as seed cells in basic research and clinical application. However, bone marrow-derived mesenchymal stem cells are limited in practical application, for example, they need invasive operations such as bone marrow puncture and limited access, so it is necessary to find a more advantageous stem cell to replace [4].

It is found that adipose tissue-derived mesenchymal stem cells (ADSCs) can meet all the criteria as seed cells, and it has great advantages as a new seed cell for tissue engineering. Compared with bone marrow mesenchymal stem cells, it has the characteristics of abundant sources, convenient materials, low trauma, easy separation and culture, stable biological characteristics and so on (It has been reported that adipose-derived mesenchymal stem cells (ADMSCs) have been isolated, cultured and identified, and can differentiate into endothelial cells, osteoblasts, chondrocytes, adipocytes and cardiomyocytes under specific induction conditions.) Zuk et al. [5] isolated, cultured and identified adipose tissue-derived mesenchymal stem cells in 2001. Under specific induction conditions, they can differentiate into osteoblasts, chondrocytes, adipocytes, cardiomyocytes, endothelial cells, etc., making them the seed cells of great concern [3,4,6]. Therefore, mesenchymal stem cells

have the ability to transform into vascular endothelial cells, and can be used as seed cells of tissue engineering blood vessels.

At present, there are many kinds of seed cells that can be used in vascular tissue engineering. Mature cells such as endothelial cells and smooth muscle cells used in previous studies as seed cells can't be used on a large scale because of their weak expansion ability *in vitro* and difficulty in obtaining materials. Nowadays, many researchers use embryonic stem cells, mesenchymal stem cells, etc. as seed cells, and prove that they can successfully differentiate into endothelial cells needed for vascular tissue engineering, or successfully construct tissue-engineered blood vessels under the influence of vascular endothelial cell growth factors [7–9]. For example, some studies have confirmed that bone marrow mesenchymal stem cells can differentiate into vascular endothelial cells *in vitro* under the combined induction of vascular endothelial growth factor and basic fibroblast growth factor [10]. Some studies have used low serum medium to induce adipose-derived stem cells to differentiate into endothelial-like cells, and form branched lumen structures. Using immunohistochemical method, -CD31, a specific surface marker of endothelial cells, can be detected. It has been transplanted into the hind limb ischemia model of mice and achieved certain therapeutic effect, and the local blood flow and capillary density have increased. In addition, it has been reported that adipose-derived stem cells can release cytokines such as hepatocyte growth factor and vascular endothelial growth factor through paracrine mechanism to promote angiogenesis [11,12]. It can be seen that adipose-derived stem cells can be induced to differentiate into endothelial cells and promote angiogenesis under certain conditions.

In order to prove that ADMSCs have the ability to transform into vascular endothelial cells, some researchers have carried out primary culture and passage of ADMSCs, and studied their transformation into blood vessels [7,13]. The results showed that the primary cultured ADMSCs showed spindle-shaped and fibroblast-like morphology under inverted microscope, and the morphology of ADMSCs after several passages had certain stability. The immunophenotype identification by flow cytometry showed that admcs expressed CD29 and CD44, but did not express CD31 and CD34, which was consistent with the characteristics of mesenchymal stem cells. Then, we induced the differentiation of ADMSCs towards endothelial cells, and detected its immunophenotype by flow cytometry. It was found that the endothelial cell support solution containing vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (b-FGF) had the best effect of inducing differentiation, suggesting that ADMSCs can be transformed into vascular endothelial cells under the stimulation of appropriate growth factors, and can be used as seed cells for constructing tissue engineering blood vessels [7,8,13].

### **2. Self-assembled nano-polypeptide hydrogel can be used as a good scaffold material for three-dimensional culture of stem cells**

Because the differentiation of stem cells is not only related to the pre-existing programming in the cell itself, but also regulated by the microenvironment in which the cells are located, the ways of regulating the differentiation of stem cells mainly occur between cells and between cells and extracellular matrix, and cytokines play the role of information transmission and nutrition among them, so the related influencing factors of the directional endothelial differentiation of stem cells are mainly cytokines, extracellular matrix and microenvironment in which the cells are located.

The material of vascular tissue scaffold should have good biocompatibility, strong plasticity, certain tensile strength and no

immunogenicity. At present, commonly used materials for preparing scaffolds include natural materials such as alginate, polysaccharide and collagen, and artificial degradable materials such as polylactic acid, polyglycolic acid and PLGA. However, these two types of biomaterials have certain limitations, and it is difficult to use these biomaterials for completely controllable research in clinic.

Therefore, if nano-scale biomaterials can be synthesized by polypeptide molecules with completely known components, the disadvantages of the above two materials can be avoided, and it may become an ideal scaffold material for tissue engineering. As a matter of fact, it has become a research hotspot in the field of tissue engineering to obtain novel nano-frame materials based on the natural self-assembly of polypeptide molecules. This kind of nano-polypeptide framework material has better biocompatibility and degradability, and the more outstanding advantage is that it has biological activity, and can easily compound different bioactive molecules according to different requirements, which endows the scaffold material with “biological intelligence” characteristics. At present, a number of world-renowned research groups have started the research of biological nano-scaffold materials based on polypeptides, and achieved many gratifying results in their respective research [14,15].

Some researchers have selected the complementary self-assembled polypeptide RADA16-I sequence as the basic unit, compounded with KLT polypeptide (which can regulate the differentiation of stem cells into endothelial cells) and RGD short peptide sequence (which can promote the attachment and connection of cells), and successfully constructed a functional self-assembled nano-polypeptide hydrogel, which proved that the self-assembled polypeptide hydrogel was a frame structure formed by the polymerization of nanofibers, and its gap size was close to that of most cells, so that cells could grow and migrate in it. At the same time, it is confirmed that ADMSCs cultured in three-dimensional polypeptide gel with endothelial inducing solution can be seen to be interconnected and easier to form endothelial-like structures [16]. It can be seen that the self-assembled nano-polypeptide hydrogel has the characteristics of “biological intelligence” and can be used as a good scaffold material for three-dimensional culture of stem cells.

### 3. Mesenchymal stem cells play a paracrine role in promoting angiogenesis in three-dimensional culture of nano-polypeptide hydrogel

Stem cells can express, synthesize and secrete cytokines, growth factors and other bioactive factors, such as VEGF, HGF, bFGF and TGF- $\beta$  [17]. Hypoxia is an important factor affecting the secretion of bioactive factors by stem cells [18]. It is found that the concentration of VEGF in cell supernatant is low under ordinary conditions, which can not meet the treatment requirements. Therefore, culturing mesenchymal stem cells *in vitro* and enhancing the secretion of bioactive factors are the key to solve the problem of stem cell treatment [19,20]. The cultivation of ADMSCs by microsphere method leads to the increase of bioactive factors secreted by stem cells, which is closely related to the hypoxia of the cell environment [21].

Studies have proved that ADMSCs can induce differentiation into blood vessels in the three-dimensional structure of self-assembled nano-polypeptide hydrogel. The mechanism of its differentiation into blood vessels is not only that the nano-polypeptide hydrogel is compounded with KLT polypeptide which can regulate the differentiation of stem cells into endothelial cells, but also directly related to the fact that stem cells release cytokines such as VEGF and hepatocyte growth factor (HGF) through paracrine mechanism to promote endothelial formation [11,12]. Xi Liu

reported that ADMSCs were cultured with polypeptide hydrogel as scaffold, and it was found that the cytokines such as VEGF and HGF secreted by ADMSCs increased in three-dimensional culture [22].

Under the condition of three-dimensional culture, cells are more similar to the internal environment and can be better influenced by the physical environment and biological environment. Therefore, functional self-assembled nano-polypeptide hydrogel is needed as a biological framework for three-dimensional culture of stem cells. The self-assembled polypeptide RADA16-I can spontaneously form nanofibers with extremely rich water content under physiological conditions, which is very similar to extracellular matrix (ECM) and can be used as a biological framework for three-dimensional cell culture [23]. Peptide RGD is the key integrin of cell adhesion, which can promote cell adhesion [24]. Peptide KLT is an excitatory factor of VEGF [25].

When ADMSCs are exposed to hypoxia, even ordinary two-dimensional culture will lead to the increase of angiopoietin, but the increase range is obviously lower than that of three-dimensional culture. Heme oxygenase (HO) widely exists in the microsomal enzyme system in the body, which can be activated by a variety of oxidative stress factors, and plays an important role in antioxidation, anti-inflammatory response and immune regulation [26,27]. Therefore, under the condition of three-dimensional culture, ADMSCs are in anoxia state, which leads to the increase of angiopoietin secretion. This may be due to the activation of protein kinase B (Akt) signaling pathway in three-dimensional culture, which leads to the up-regulation of hypoxia gene expression in ADMSCs, and the increase of angiogenesis promoting factors secreted by ADMSCs.

Sun Nianfeng and other scholars have also studied the role of ADMSCs in promoting paracrine under the condition of three-dimensional culture of nano-polypeptide hydrogel. ELISA showed that VEGF and HGF secreted by cells increased under hypoxia and three-dimensional culture conditions, but the increase was more obvious under three-dimensional culture conditions. The results of Western-Blot showed that the intracellular expression of HO-1 in the three-dimensional culture group was about twice as much as that in the ordinary culture group, which proved that ADMSCs had obvious paracrine effect in the three-dimensional culture of nano-polypeptide hydrogel [28,29].

### 4. Application of mesenchymal stem cells combined with nano-polypeptide hydrogel in 3D bioprinting tissue engineering blood vessel

In recent years, with the rise and rapid development of 3D printing technology, researchers began to apply 3D bio-printing technology to explore new tissue engineering [30,31]. Its accurately printed polymer materials such as “bone joints” are constantly being applied in clinic. Because it can quickly and accurately prepare personalized medical materials according to the needs of different patients, it has been more and more explored and studied in the field of tissue engineering, especially in the cultivation and repair of tissues and organs [32].

At present, most of the methods used in 3D bio-printing are hydrogel as scaffold material, “bio-ink” cells are stacked layer by layer in culture vessels, and then the required tissues are obtained through subsequent bio-culture [33]. The raw material of 3D bio-printer is human cells. The main component of so-called bio-paper is hydrogel, which can be used as a scaffold for cell growth. Because it uses cells from patients themselves, it will not produce rejection reaction. At present, some scientific research institutes in China have taken the lead in realizing 3D printing of biomaterials and cells under aseptic conditions, and the survival rate of the printed human living cells is as high as 90%. At present, a small

proportion of human ear cartilage tissue, liver unit, kidney unit and other tissues have been successfully printed [34].

It has been reported that 3D bio-printing technology has successfully printed out a small proportion of human ear cartilage tissue, liver unit, kidney unit and other tissues [35–37]. However, the “bio-ink” used by different research institutions in 3D bio-printing tissues and organs will be different. Professor NOROTTE of Columbia University and others [38] developed a 3D printing technology of bio-gel spheres based on 3D automatic computer-aided deposition, which was applied to stent-free small-diameter angioplasty, showing the advantages of rapid prototyping technology such as rapidity, repeatability and quantification. SKARDAL et al. printed hyaluronic acid hydrogel and crosslinked it with tetrahedral polyethylene glycol tetraacrylate to prepare vascular repair material [39]. German scholars printed agarose gel in liquid fluorocarbon, and got the hollow 3D structure like branched vessels [40]. MILLER, University of Pennsylvania, USA, used casting method to compound cell-loaded hydrogel to form a tubular blood passage [41,42]. SKARDAL et al. [39] University of Utah, USA, used an instrument purchased by Fab@home Company to print hyaluronic acid hydrogel and crosslinked it with tetrahedral polyethylene glycol tetraacrylate to prepare vascular repair materials. Some scholars have printed out a piece of human tissue, which contains skin cells and some functional “blood vessel”-like structures [41,42]. The above research suggests that as long as the right seed cells and extracellular hydrogel scaffold materials are selected, the application of 3D bio-printing tissues and organs will become wider and wider.

On this basis, Sun Nianfeng and other scholars reported that self-assembled nano-polypeptide hydrogel composite growth factor and ADMSCs can be used as “bio-ink” for 3D bio-printing of various tissues and organs. They used 3D bio-printing technology to print out relevant tissue models and induce the differentiation of ADMSCs into vascular endothelium. It is found that the 3D printed tissue is a transparent cylinder with a cavity structure printed in 3D, which is beneficial to the material exchange between cells and culture medium and plays an important role in the proliferation and differentiation of internal ADMSCs cells. After phalloidin staining, we can see that stem cells are stained, arranged tightly and orderly, with normal shape and good growth. Cells are in full contact with hydrogel, adhere tightly and have strong proliferation ability. Results It was confirmed that ADMSCs grew well in 3D printed tissues, and still had strong ability to differentiate into vascular endothelium [43,44].

The above research has made some preliminary explorations in tissue engineering blood vessels, and studied the feasibility of seed cells (ADMSCs), extracellular matrix (self-assembled nano-polypeptide hydrogel) and self-assembled nano-polypeptide hydrogel combined with ADMSCs as “biological ink” for 3D printing biological tissues.

According to this, some scholars have synthesized the previous theoretical results of functional self-assembled nano-polypeptide hydrogel, growth factors and stem cells, and put forward the plan of preparing new tissue-engineered blood vessels by using 3D bio-printing technology with self-assembled nano-polypeptide hydrogel combined with ADMSCs and growth factors promoting angiogenesis, which is expected to realize personalized customized printing of new artificial blood vessels and improve patency and effectiveness of transplanted blood vessels.

If we can make progress in these studies, it is expected to develop biological blood vessels that meet the index specifications in vascular endothelialization, cell compatibility and histocompatibility, so as to realize a new type of endothelialized artificial blood vessel that can be customized and printed according to the diseased blood vessels, which has attractive prospects and great social and economic benefits.

## Authors' contributions

Ailing Tian and Xin Yi performed the interpretation of the data and wrote the manuscript. Nianfeng Sun designed the study and revised the manuscript. All authors read and approved the final manuscript.

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## Consent for publication

All authors of this manuscript agreed to publication.

## Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Declaration of competing interest

The authors declare that they have no competing interests.

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